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Synthesis of spermidinylcholestanol and spermidinylcholesterol, squalamine analogues

Bassima Choucair,[a] Michel Dherbomez,[b] Cristos Roussakis[c] and Laïla El Kihel[a,†,*]

[a]*Université de Caen, Cyceron, LRA, Av. Becquerel, 14000 Caen, France*
[b]*IUT de La Rochelle, Rue François de Vaux de Fauletier, 17000 La Rochelle, France*
[c]*Faculté de Pharmacie de Nantes, Laboratoire de Pharmacologie Marine, 44035 Nantes cedex, France*

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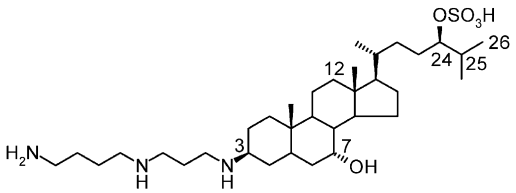
Abstract—Several novel squalamine-related polyaminosterols are reported. The synthesis of 7α-*N*-[3*N*-(4-aminobutyl)aminopropyl]aminocholestanol **I**, 6α-*N*-[3*N*-(4-aminobutyl)aminopropyl]aminocholestanol **II**, 7α and 7β-*N*-[3*N*-(4-aminobutyl)aminopropyl]aminocholesterol (**III** and **IV**), was accomplished from cholesterol, they provide the first examples in which spermidine is introduced in the B steroidal ring. These molecules showed comparable antibacteria and fungi activities to squalamine, and were cytotoxic on a human non-small cell bronchopulmonary carcinoma line (NSCLC-N6). Therefore, these molecules with antibiotic and cytotoxic activities are promising for immune-compromised patients in cancer chemotherapy.

1. Introduction

Squalamine is a novel aminosterol isolated from the tissues of the dog fish shark, *Squalus acanthias*. This compound has an unusual chemical structure, its a 5α-cholestane with 7α-hydroxy, 3β-spermidinyl and 24*R*-sulfate groups (Fig. 1).[1] Squalamine was the first example of a natural product which is a steroïdal polyamine (spermidine). Squalamine exhibits antimicrobial activity against a wide variety of microorganisms (Gram negative bacteria, Gram positive bacteria and fungi).[2] Its also demonstrates promise as an antiangiogenic agent, preventing the formation of new blood vessels in developing malignant tumors.[3] Squalamine also inhibits tumor growth in multiple animal models and is currently in phase II clinical trial for treatment of advanced non-small cell lung cancer.[4] The combination of squalamine and cisplatin has shown high activity against human cancer cells.[5] More recently, seven new aminosterols related to squalamine were discovered from the liver of the dog fish shark *S. acanthias*. These compounds exhibited a relatively invariant cholestane skeleton with a trans A/B ring junction, with spermidine or spermine attached equatoriallly at C3. The side chain had several variations: The sterol side chain had a hydroxyl or a hydroxymethyl group at the C24 position, and a sulfate group at either the C24 or C26 position. One of these aminosterols was dessulfated, but had a ketone function at C24 and a double bond Δ25–26, while another had a hydroxyl group at the C12 position. These compounds had a broad spectrum of antimicrobial activity comparable to squalamine.[6]



squalamine

Figure 1. Squalamine.

However, only trace amounts of these molecules were present in the different tissues of the shark. Chemical syntheses of squalamine has been accomplished from the expensive starting materials, 3β-acetoxy-5-cholenic acid,[7] 5-cholenic acid[8] and desmosterol.[9] Inexpensive starting materials used include stigmasterol,[10–12] methyl 3-keto-5α-chenodeoxy cholonate,[13] 3-keto-23,24-bis-norchol-4-en-22-ol[14] and methyl chenodeoxycholonate.[15] Fourteen to seventeen steps are needed to synthesize this compound, but these syntheses are accomplished with a low overall yield (1.9–19%). For these reasons, several squalamine analogues

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* Corresponding author. Tel.: +33 2 31 93 64 58; fax: +33 2 31 93 34 73; e-mail: elkihel@pharmacie.unicaen.fr
† Present address: Centre d'Etude et de Recherche sur le Médicament de Normandie, UFR des Sciences Pharmaceutiques, 5 rue Vaubénard 14032 Caen cedex, France.

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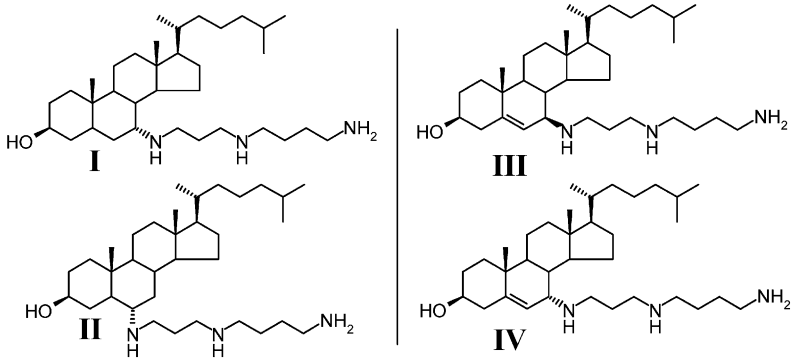
were synthesized. Only one analogue has been described with a spermidine moiety at the side chain,[16–17] but the other analogues have spermidine attached at the C3 position.[18–19] Selinsky's groups has synthesized squalamine analogues with a 24*R*,*S*-hydroxy or 24*R*,*S*-amino, with or without the 7-hydroxy group. Their aim was to explore the relationship between the functional groups and antimicrobial activity. Some of these analogues exhibit antimicrobial activity comparable to squalamine.[20]

Biological studies of squalamine and analogues led to the following conclusions. The sterol side chain could be dessulfated. The 7α-hydroxyl group could be suppressed. The structure of the polyamine on the steroid could be varied. The steroid can be have other functions on the side chain.

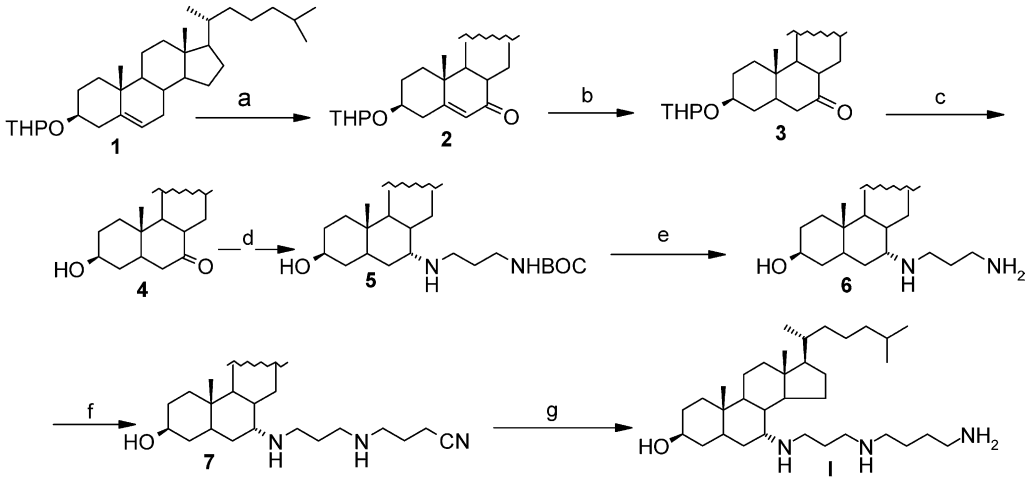
In this way, our approach was to develop new analogues of squalamine. Squalamine has a spermidine unit at C3 and a hydroxyl at the C7 position of the steroidal skeleton. We describe analogues with spermidine at C7 and a hydroxyl group at C3 position. These analogues were synthesized from cholesterol, an inexpensive starting material. The hydroxyl group at the C3 position of this steroid was conserved and the asymmetric polyamine (spermidine) was introduced at the C7 position. Therefore, the synthesis steps were minimized. 7α-Polyaminocholestanol (**I**), 6α-polyaminocholestanol (**II**) 7α- and 7β-polyaminocholesterols (**III** and **IV**) were synthesized (Scheme 1).

2. Results and discussion

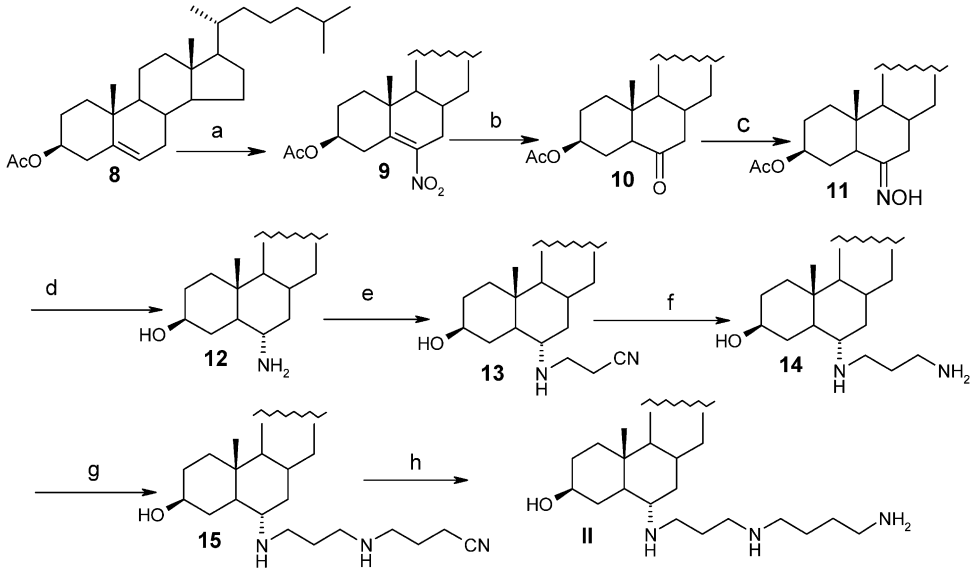
7α-*N*-[3*N*-(4-Aminobutyl)aminopropyl]aminocholestanol **I** was prepared from cholesterol. As depicted in Scheme 2, the hydroxyl group of cholesterol was protected with a tetrahydropyranyl group **1** (99%). Oxidation with chromium trioxide–pyridine complex afforded the allylic ketone **2** in 62% yield. Selective reduction of the Δ5 double bond under Birsh conditions, with lithium/ammonia at −78 °C, gave the A/B trans junction in 82% yield (compound **3**). Alcohol functionality of this compound was deprotected to give compound **4**. The intermediate **5** was obtained in 84% yield via a reductive amination of ketone **4** with 3*N*-(tertiobutylcarbonyl)aminopropane utilizing sodium cyanoborohydride (pH 5–6 was ajusted with acetic acid) as the reducing agent. Probably due to steric hinderance, only the



Scheme 1. Analogues of squalamine.



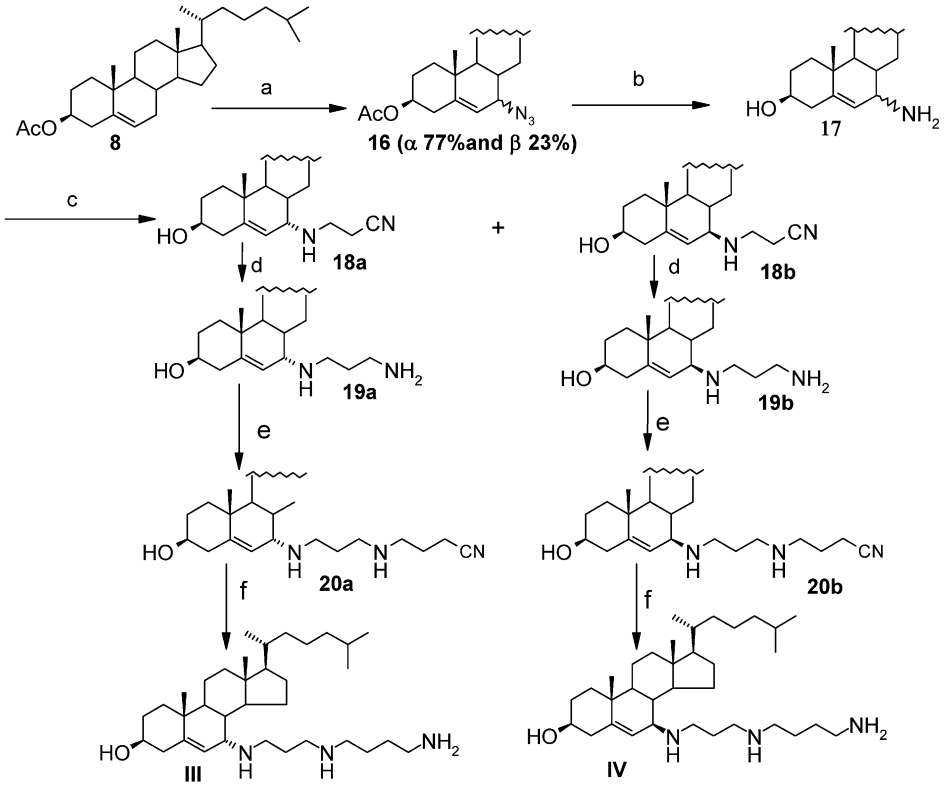
Scheme 2. Synthesis of 7α-spermidinylcholestanol. Reagents and conditions: (a) CrO_3-2py, CH_2Cl_2, RT; (b) Li/NH_3, THF, −78 °C; (c) PPTS, ethanol, reflux; (d) $NH_2(CH_2)_3NHBOC$, $NaBH_3CN$, AcOH pH 5–6; (e) TFA, CH_2Cl_2; (f) Br–$(CH_2)_3$–CN, DMF, 60 °C; (g) $LiAlH_4$, $NiCl_2$, $6H_2O$ THF, reflux.



Scheme 3. Synthesis of 6α-spermidinylcholestanol. Reagents and conditions: (a) CH_3COOH, HNO_3; (b) CH_3COOH/Zn, reflux; (c) $HONH_2.HCl$, py, reflux; (d) $LiAlH_4$, THF, reflux; (e) acrylonitrile, MeOH; (f) $LiAlH_4/NiCl_2,6H_2O$, THF, reflux; (g) $Br–(CH_2)_3–CN$, DMF, 60 °C; (h) $LiAlH_4/NiCl_2$, $6H_2O$, THF, reflux.

α epimer was observed. The amine function was deprotected **6** (79%) and alkylated by 4-bromobutyronitrile to give compound **7** in 51% yield. Nitrile reduction of **7** was achieved with lithium aluminium hydride in presence of nickel chloride hexahydrate giving the polyaminosterol **I** in 30% yield.

6α-*N*-[3*N*-(4-Aminobutyl)aminopropyl]aminocholestanol **II** was prepared as shown in Scheme 3 cholesteryl acetate was treated with high density ($d=1.53$) nitric acid in anhydrous acetic acid to give the 6-nitro derivative **9** in 74% yield. Ketone **10** was treated with zinc in acetic acid. Oxime derivative **11** was obtained from the ketone by reaction with hydroxylamine hydrochloride in pyridine and reduced by lithium aluminium hydride in tetrahydrofuran, to 6α-aminocholestanol (**12**). Probably due to steric hinderance, only the α epimer was observed. Acrylonitrile was reacted in methanol with aminosterol (**12**) giving the



Scheme 4. Synthesis of 7α- and 7β-spermidinylcholesterol. Reagents and conditions. (a) Lead IV acetate, $(CH_3)_3SiN_3$, CH_2Cl_2; (b) $LiAlH_4$, THF reflux; (c) CH_2═CH–CN, MeOH; (d) $LiAlH_4$,/$NiCl_2$, $6H_2O$, THF, reflux; (e) $Br–(CH_2)_3–CN$, DMF, 60 °C, 72 h, (f) $LiAlH_4$,/$NiCl_2$, $6H_2O$, THF, reflux.

6α-*N*(2-cyanoethyl)aminocholestanol (**13**). The nitrile function was reduced by lithium aluminium hydride (compound **14**) and the amine obtained was alkylated by 4-bromobutyronitrile to give compound **15** in 51% yield. Nitrile reduction of **15** was achieved with lithium aluminium hydride in presence of nickel chloride hexahydrate giving the polyaminosterol **II** in 35% yield.

The polyaminosterols **III** and **IV** were synthesized steroselectively from 7α and 7β-aminocholesterol as a key intermediate[21]. A convenient novel synthetic route was developped. Polyaminocholesterols (**III**) and (**IV**) were easily prepared from epimeric mixture, 7α,β-aminocholesterol. This epimeric mixture was obtained in two steps from cholesteryl acetate (Scheme 4): cholesterol was acetylated by using acetic anhydride and pyridine to give the acetate **8** (90%), and the azido group was introduced directly by trimethylsilyl azide action in presence of lead IV acetate on allylic position C-7 of cholesteryl acetate. α/β Epimeric mixture **16** (77%α and 23%β) was obtained in 68% yield. Reduction of epimeric azides **16** was acheived with lithium aluminium hydride to give amine **17**. Acrylonitrile was reacted in methanol with amine epimeric mixture **17** required the 7α- and 7β-*N*(2-cyanoethyl)aminocholesterol (**18a** and **18b**) easily separated by chromatography on silica gel column. Each nitrile function was reduced by lithium aluminium hydride in presence of nickel chloride hexahydrate (**19a** and **19b**) and the amine obtained was alkylated by 4-bromobutyronitrile to give compounds **20a** and **20b**. Finally, each nitrile was reduced with lithium aluminium hydride in presence of nickel chloride hexahydrate to give squalamine analogue **III** in 46% yield and **IV** in 48% yield.

Minimum inhibitory concentrations (MIC) of these aminosterols (**I**, **II**, **III** and **IV**) were determined against fungi (*S. cerevisiaie* and *C. albicans*), and both Gram-positive (*S. aureus* and *E. hirae*) and Gram-negative (*E. coli*) bacteria (Table 1).

The activity on *C. albicans* was compared to clinical references products (Amphotericin B, Econazole, Nystatin and 5-fluorocytosine, Table 2).

The antiproliferative activity of these squalamine analogues was studied in vitro on a human non-small cell bronchopulmonary (NSCLC-N6). The cytotoxicity determinations (inhibitory concentration: IC_{50}) showed clearly strong antiproliferative properties on this cell line ($IC_{50} <$ 3.3 μg/mL; significative activity when $IC_{50} < 10$ μg/mL).

3. Conclusion

There are two a key elements in our synthesis of squalamine analogues. Its the first example, to our knowledge, in which spermidine is introduced into the B steroid ring. Cholesterol was chosen as the starting material in the synthesis of these squalamine analogues. An inexpensive material allowing preparation of significant quantities of material following a multistep synthetic process. Analogues **I** and **II** were prepared in seven and eight steps, respectively. Aminosterols (**III**) and (**IV**) were easily prepared from epimeric mixture, 7α,β-aminocholesterol in six steps.

The results of these studies clearly show the antiproliferative effect on the cloned NSCLC-N6 cell line and the antimicrobial activity against a broad spectrum of microorganisms. 7α- and 7β-aminospermidinylcholesterol were active against Gram negative bacteria. No activity with analogues **I** and **II** was observed on Gram negative bacteria. The stereochemistry of spermidinyl moiety at the steroid seemed to have small affect on antimicrobial activity.

Our results suggest that introduction of spermidine at B steroid ring is a key target of biological activites for polyaminosterols. Therefore, these molecules with antibiotic and cytotoxic properties are promising for immune-compromised patients in cancer chemotherapy.

4. Experimental

4.1. General procedure

All melting points are uncorrected The IR spectra were recorded on a Perkin Elmer 1600 FT-IR spectrometer. Nuclear magnetic resonance (^{1}H NMR and ^{13}C NMR) spectra were obtained on a Brucker DPX 250 MHz or a Jeol JNM 400 MHz. The chemical shift values (parts per million) are relative to tetramethylsilane as solvent reference and coupling constant (*J*) values are expressed in Hertz. The mass spectra were recorded Jeol-GC mate (GC–MS system). The optical rotations were measured with Perkin Elmer 343 polarimeter. Thin-layer chromatography was run on Merck silica gel 60 F254. Developed plates were

Table 1. Determination of MIC (μg/mL) values after 48 h incubation. NI: no inhibition with MIC > 50 μg/mL

Strain	*S. cerevisiaie* ATCC28383	*C. albicans* CIP1180-79	*S. aureus* CIP 54127	*E. hirae* CIP 5855	*E. coli* 54127
Analogue **I**	6.25	25	6.25	6.25	NI
Analogue **II**	1.56	6.25	3.12	1.56	NI
Analogue **III**	3.12	6.25	3.12	3.12	6.25
Analogue **IV**	3.12	3.12	6.25	6.25	3.12
Squalamine	—	4–8	1–2	—	1–2

Table 2. Determination of MIC (μg/mL) values after 48 h incubation

Compounds	Amp B	Econazole	Nystatin	5-Fc	**I**	**II**	**III**	**IV**
C. albicans CIP1180-79	0.4	1.5	6	> 50	25	6.25	12.5	3.12

visualized by upon spraying with sulfuric acid/ethanol 2:8 and heating.

4.1.1. 3β-[(Tetrahydropyran-2*R*,*S*-yl)oxy]cholest-5-ene 1. To a solution of cholesterol (10 g, 24 mmol) in methylene chloride (50 mL) was added 3,4-dihydropyrane freshly distilled (3.18 mL, 36 mmol) and pyridinium *para*-toluene sulfonate (0.59 g, 2.4 mmol). The mixture was stirred at room temperature and under nitrogen atmosphere for 1 h 30 min. The solution was washed with water (50 mL), and the organic layer was dried over sodium sulfate and evaporated to give the crude product, which was purified by chromatography on silica gel column (hexane/ethyl acetate 9.5:0.5) to afford compound **1** as a white solid (11.29 g, 99%).

^{1}H NMR ($CDCl_3$, 250 MHz), δ: 0.68 (s, 3H, Me 18), 0.86 (d, 6H, J=6.5 Hz, Me 27 and Me 26), 0.92 (d, 3H, J=6.5 Hz, Me 21), 0.97 (s, 3H, Me 19), 3.49–3.54 (m, 2H, H-6′ of THP), 3.92 (m, 1H, H3α), 4.71 (br s, 1H, H-2′ of THP), 5.33 (td, 1H, J=1.01, 6.8 Hz, H6). ^{13}C NMR ($CDCl_3$): δ: 12.1, 18.7, 19.4, 19.7, 21.2, 22.7, 23.9, 24.1, 25.4, 28.1, 28.3, 29.6, 31.2, 32.0, 32.1, 35.9, 36.2, 37.5, 38.0, 39.1, 39.6, 40.0, 42.4, 50.3, 56.0, 56.8, 63.0, 77.3, 96.8, 122.0, 140.5. MS-EI: m/z=470 (10%, $M^{+\cdot}$), 386 (14%), $M^{+\cdot}$−tetrahydropyranyl), 368 (28%, 386 –H_2O), 85 (100%).

4.1.2. 3β-[(Tetrahydropyran-2*R*,*S*-yl)oxy]-7-oxo-cholest-5-ene 2. Pyridine (16.2 mL, 200 mmol) was added to a solution of chromium anhydride (10.61 g, 106 mmol) in methylene chloride (100 mL). The solution was stirred at room temperature and under nitrogen atmosphere for 2 h. The protected cholesterol **1** (5 g, 10 mmol) was added, and the mixture was stirred for 12 h. The solution was filtered, washed with 0.1 N HCl (100 mL), 5% $NaHCO_3$ (100 mL) and with water 100 mL). The organic layer was dried over sodium sulfate and evaporated to give the crude product, which was purified by chromatography on silica gel column (hexane/ethyl acetate 8:2) to afford pure compounds **2** (3 g, 62%). IR: ν: 2944 (CH_2), 1673 (C=O allylic ketone), 1651 (C=C alkene). ^{1}H NMR($CDCl_3$, 250 MHz), δ: 0.68 (s, 3H, Me 18), 0.85 (d, 6H, J=6.5 Hz, Me 27 and Me 26), 0.90 (d, 3H, J=6.5 Hz, Me 21), 0.93 (s, 3H, Me 19), 3.45–3.49 (m, 2H, H-6′ of THP), 3.55–3.64 (m, 1H, H3α), 4.72 (br s, 1H, H-2′ of THP), 5.68 (d, 1H, J=1.02 Hz, H6). ^{13}C NMR ($CDCl_3$): δ: 12.3, 17.7, 19.2, 20.2, 21.5, 22.7, 24.1, 25.7, 26.7, 28.1, 28.3, 28.9, 29.7, 31.5, 36.0, 36.5, 38.8, 39.1, 39.8, 40.4, 43.4, 45.; 50.3, 55.1, 63.2, 75.3, 97.4, 126.4, 165.9, 202.5. MS-EI: m/z=400 (19%, $M^{+\cdot}$−tetrahydropyranyl), 344 (75%), 85 (100%).

4.1.3. 3β-[(Tetrahydropyran-2*R*,*S*-yl)oxy]cholestan-7-one 3. Tetrahydrofuran (10 mL) was cooled to −78 °C with dry ice/acetone, and ammonia was then collected to a total volume of approximately 20 mL. Lithium wire (0.2 g, 28 mmol) was added in small pieces to the solution with vigorous stirring. A deep blue solution was obtained after the lithium was completely dissolved. Ketone **2** (3.5 g, 7.22 mmol) was dissolved in THF (50 mL) and added to the flask in a steady stream from an addition funnel. The solution was stirred for 40 min and then quenched by the addition of ammonium chloride and ethanol unitil the blue coloration dissipated. The ammonia was allowed to evaporate at room temperature, and the residue was dissoved in ethyl ether (30 mL) and washed with 0.1 N HCl (25 mL) and water (25 mL). The organic layer was dried over sodium sulfate and evaporated. The crude product was purified by chromatography on silica gel column (hexane/ethyl acetate 8:2). The desired product **3** (2.89 g, 82%) was obtained as a white solid. IR: ν: 2940 (CH_2), 1707 (C=O ketone). ^{1}H NMR ($CDCl_3$, 250 MHz), δ: 0.65 (s, 3H, Me 18), 0.86 (d, 6H, J=6.5 Hz, Me 27 and Me 26), 0.90 (d, 3H, J=6.4 Hz, Me 21), 0.93 (s, 3H, Me 19), 3.46–3.48 (m, 1H, H-6′ of THP), 3.57–3.59 (m, 1H, H3α), 3.88–3.90 (m, 1H, H-6′ of THP), 4.68 (br s, 1H, H-2′ of THP). ^{13}C NMR ($CDCl_3$): δ: 12.1, 18.8, 19.7, 21.7, 22.7, 22.9, 23.8, 25.0, 25.4, 26.8, 28.1, 28.5, 33.2, 34.8, 35.1, 35.7, 36.2, 36.5, 39.6, 42.5, 44.2, 45.1, 45.7, 48.9, 52.1, 56.1, 63.0, 77.6, 96.5, 211.5. MS-EI: m/z=486 (24%, $M^{+\cdot}$), 385 (10%, $M^{+\cdot}$−tetrahydropyranyl), 367 (32%, 385 –H_2O).

4.1.4. 7-Oxo-cholestan-3β-ol 4. Pyridinium *para*-toluene sulfonate (0.6, 2.4 mmol) was added to a solution of compound **3** (1.2 g, 2.4 mmol) in ethanol (25 mL) and heated under reflux for 12 h. The solvent was evaporated and the residue was dissolved in ethyl ether/ethyl acetate (5:5). The mixture was washed with brine, dried over sodium sulfate and evaporated to give the crude product, which was purified by chromatography (hexane/methylene chloride 5:5) and crystallized from ethanol/ethyl ether (5:5) to afford product **4** (0.89 g, 85%) as a white solid. Mp 140 °C. IR: ν: 3419 (OH alcohol), 2948 (CH_2), 1706 (C=O ketone). ^{1}H NMR ($CDCl_3$, 250 MHz), δ: 0.65 (s, 3H, Me 18), 0.86 (d, 6H, J=6.5 Hz, Me 27 and Me 26), 0.89 (d, 3H, J=6.4 Hz, Me 21), 0.91 (s, 3H, Me 19), 3.57–3.59 (m, 1H, H3α). ^{13}C NMR ($CDCl_3$): δ: 12.1, 19.1, 22.2, 22.7, 24.1, 25.3, 28.3, 28.7, 29.7, 31.3, 36.0, 36.3, 36.4, 36.5, 38.2, 39.1, 39.8, 42.8, 46.4, 47.2, 49.2, 50.3, 55.4, 55.6, 70.9, 212.5. MS-EI: m/z=402 (18%, $M^{+\cdot}$), 385 (100%, $M^{+\cdot}-{}^{\cdot}OH$).

4.1.5. 7α-*N*-(3*N*-tert-Butoxycarbonyl-aminopropyl)aminocholestan-3β-ol 5. To a solution of 3*N*-(tertiobutylcarbonyl)aminopropane (0.921 g, 5.29 mmol) and compound **4** (1.29 g, 2.64 mmol) in anhydrous methanol (10 mL) was added sodium borohydride (0.25 g, 3.96 mmol) and the pH was ajusted with acetic acid to 5–6. The mixture was stirred at room temperature and under argon atmosphere for 48 h. The solution was treated with 0.1 N HCl (30 mL), 5% $NaHCO_3$ (30 mL) and extracted with methylene chloride. The organic layer was washed with, brine and dried over sodium sulfate. The solution was evaporated and the crude product was purified by chromatography (ethyl acetate/hexane 5:5 and methylene chloride/methanol 9:1) to afford product **5** (1.467 g, 84%) as amorphous solid. ^{1}H NMR ($CDCl_3$, 250 MHz), δ: 0.65 (s, 3H, Me 18), 0.84 (d, 6H, J=6.5 Hz, Me 27 and Me 26), 0.91 (d, 3H, J=6.4 Hz, Me 21), 1.02 (s, 3H, Me 19), 1.45 (s, 9H, HN–CO_2–C(CH_3)$_3$), 2.48 (m, 1H, St–HN–$CH_aH_{a'}$–$(CH_2)_2$–NH–), 2.57 (m, 1H, H7β of α epimer), 2.72 (m, 1H, HN–$CH_aH_{a'}$–$(CH_2)_2$–NH–), 3.21 (t, 2H, J=5.1 Hz, –CH_2–NH–BOC), 3.63 (m, 1H, H3α), 5.80 (br s, 1H, HN–CO_2–C(CH_3)$_3$, D_2O exchange). ^{13}C NMR ($CDCl_3$): δ: 12.1, 15.1, 18.8, 21.8, 22.7, 23.9, 26.3, 27.1, 28.1, 28.2, 28.3, 29.7, 33.6, 33.8, 35.2, 35.9, 36.3, 37.1, 39.5, 39.6, 39.8, 41.0, 42.9, 43.0, 46.5, 47.6, 54.4, 56.3, 61.9, 71.6, 78.8, 155.9. MS-EI: m/z=560 (25%,

$M^{+\cdot}$), 416 (25%, $M^{+\cdot}-CH_2$=CH–NHBoc), 402 (64%, 416 $-{}^{\cdot}CH_3$), 387 (22%, St^+), 293 (100%).

4.1.6. 7α-*N*-(3*N*-Aminopropyl)aminocholestan-3β-ol 6. To a solution of compound **5** (1.55 g, 2.75 mmol) in methylene chloride (20 mL) was added dropwise trifluoroacetic acid (1.92 mL, 24.8 mmol). The mixture was stirred at room temperature for 3 h. The mixture was treated with 5% $NaHCO_3$ (20 mL) and washed with water (20 mL). The organic layer was dried over sodium sulfate and evaporated. The crude product was purified by chromatography (methylene chloride/methanol/ammoniaque 8:1:1) to give product **6** (1.0 g, 79%) as amorphous solid. ^{1}H NMR ($CDCl_3$, 250 MHz), δ: 0.65 (s, 3H, Me 18), 0.85 (d, 6H, J= 6.5 Hz, Me 27 and Me 26), 0.91 (d, 3H, J=6.4 Hz, Me 21), 1.02 (s, 3H, Me 19), 2.63 (m, 1H, H7β of α epimer), 2.74 (m, 1H, St–HN–CH_aH$_{a'}$–$(CH_2)_2$–NH_2), 2.87 (m, 1H, St–HN–$CH_aH_{a'}$–$(CH_2)_2$–NH_2), 3.25 (t, 2H, J=7.5 Hz, –HN–$(CH_2)_2$–CH_2–NH_2), 3.66 (m, 1H, H3α), 8.91 (br s, 1H, St–N*H*–$(CH_2)_2$, D_2O exchange). ^{13}C NMR ($CDCl_3$): δ: 12.1, 15.2, 19.0, 21.5, 22.7, 24.0, 26.3, 28.1, 28.3, 31.7, 33.6, 33.8, 36.0, 36.2, 36.4, 36.9, 37.7, 38.2, 39.2, 39.7, 39.9, 42.9, 43.2, 46.6, 47.6, 51.1, 56.3, 61.9, 71.4. MS-EI: m/z=443 (16%, $M^{+\cdot}-{}^{\cdot}CH_3$), 428 (12%, $M^{+\cdot}-{}^{+}CH_2$–NH_2), 416 (47%, St–NH–CH_2^+), 402 (7%, St–NH^+), 387 (100%, St^+).

4.1.7. 7α-*N*-[3*N*-(3-Cyanopropyl)aminopropyl]aminocholestan-3β-ol 7. To a solution of amine **6** (0.330 g, 0.71 mmol) in DMF (5 mL) was added sodium hydrogenocarbonate (0.09 g, 1.07 mmol). After stirring at room temperature for 15 min, 4-bromobutyronitrile (0.14 mL, 1.43 mmol) was added and the mixture was heated at 60 °C for 36 h. The solution was diluted with water and extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate and evaporated. The residue was purified by chromatography (hexane/ethyl acetate 5:5) afforded product **7** (0.190 g, 51%) as a oil. IR: ν: 2932 (CH_2), 2254 (CN). ^{1}H NMR ($CDCl_3$, 250 MHz): δ: 0.65 (s, 3H, Me 18), 0.86 (d, 6H, J=6.5 Hz, Me 27 and Me 26), 0.91 (d, 3H, J=6.5 Hz, Me 21), 1.1 (s, 3H, Me 19), 1.19 (t, 2H, J=6.7 Hz, –NH–CH_2–CH_2–CH_2–CN), 2.47–2.53 (t, 4H, J_1=7.06 Hz, J_2=7.13 Hz, –CH_2–CN and –NH–CH_2–$(CH_2)_2$–CN), 2.60 (ddd, 1H, $J_{7\beta-8}$=4.3 Hz, $J_{7\beta-6}$= 5 Hz, H7β of α epimer), 2.68 (m, 1H, St–HN–CH_aH$_{a'}$–$(CH_2)_2$–NH–), 2.82 (m, 1H, St–HN–$CH_aH_{a'}$–$(CH_2)_2$–NH–), 3.38 (m, 1H, H3α), 3.75 (t, 2H, J=5.9 Hz, St–HN–$(CH_2)_2$–CH_2–NH–), 8.98 (br s, 1H, St–N*H*–, D_2O exchange). ^{13}C NMR ($CDCl_3$): δ: 12.0, 14.1, 15.2, 18.8, 21.5, 22.7, 24.0, 24.6, 25.6, 26.3, 28.1, 28.4, 31.6, 36.0, 36.2, 36.4, 36.9, 37.6, 38.0, 39.1, 39.6, 39.8, 42.9, 43.0, 43.2, 46.5, 51.0, 54.4, 56.3, 56.5, 60.5, 71.3, 120.0. MS-EI: m/z=487 (2%, St–NH–$(CH_2)_3$–NH–CH_2–CH_2^+), 416 (46%, St–NH–CH_2^+), 402 (7%, St–NH^+), 387 (8%, St+), 369 (5%, 386 – H_2O), 169 (100%).

4.1.8. 7α-*N*-[3*N*-(4-Aminobutyl)-3-aminopropyl]aminocholestan-3β-ol I. A solution of nitrile **7** (0.500 g, 0.9 mmol) in dry THF (5 mL) was added dropwise to a suspension of lithium aluminium hydride (0.21 g, 5.6 mmol) and nickel chloride hexahydrate (0.213 g, 0.9 mmol) in dry THF (20 mL) under argon. The mixture was refluxed for 6 h. The reaction mixture quenched with $Na_2SO_4.10H_2O$, filtered through a pad of celite, and washed with ethyl ether/n butanol (6:4). Removal of the solvent in vacuo and purification by flash chromatography (dichloromethane/methanol/ammoniaque 8:1.5:0.5) to afford a pure product **I** as amorphous solid (0.15 g, 30%). ^{1}H NMR ($CDCl_3$, 250 MHz): δ: 0.68 (s, 3H, Me 18), 0.85 (d, 6H, J=6.5 Hz, Me 27 and Me 26), 0.91 (d, 3H, J=6.5 Hz, Me 21), 1.03 (s, 3H, Me 19), 2.44 (m, 1H, St–HN–CH_aH$_{a'}$–$(CH_2)_2$–NH–), 2.60 (m, 1H, H7β of α epimer), 2.67 (m, 1H, St–HN–$CH_aH_{a'}$–$(CH_2)_2$–NH–), 2.81 (t, 2H, J=5.7 Hz, –HN–$(CH_2)_3$–CH_2–NH_2), 3.11–3.23 (t, 4H, J_1=9.5 Hz, J_2=7.6 Hz, St–HN–$(CH_2)_2$–CH_2–NH– and –NH–CH_2–$(CH_2)_3$–NH_2), 3.58 (m, 1H, H3α), 7.15 (br s, 2H, –NH2, D_2O exchange), 8.10 (br s, 1H, –NH–, D_2O exchange), 8.32 (br s, 1H, –NH–, D_2O exchange). ^{13}C NMR ($CDCl_3$): δ: 12.1, 15.2, 18.8, 21.8, 22.7, 23.9, 25.6, 25.7, 26.3, 28.1, 28.2, 29.5, 29.8, 33.6, 33.8, 35.2, 35.9, 36.3, 37.1, 39.6, 39.7, 40.0, 41.0, 42.9, 43.0, 46.1, 46.6, 47.6, 47.7, 54.2, 56.3, 62.0, 71.6. MS-EI: m/z=473 (3%, St–NH–$(CH_2)_3$–NH–CH_2^+), 430 (5%, St–NH–CH_2–CH_2^+), 416 (50%, St–NH–CH_2^+), 402 (100%, St–NH^+), 387 (8%, St^+), 369 (5%, 387 –H_2O). Anal. Calcd for $C_{34}H_{65}N_3O$: C 76.77; H 12.32; N 7.90. Found: C 76.75; H 12.39; N 7.92.

4.1.9. 3β-Acetoxy-6-nitro cholest-5-ene 9. To a solution of nitric acid (9.6 mL, 197 mmol) in acetic acid (36 mL) was added dropwise cholesteryl acetate (12.5 g, 29 mmol). The mixture was stirred for 2 h. The ice was added to the solution. The product was filtered, washed with water and crystallized from ethanol to afford product **9** (10.3 g, 74%) as a yellow solid. Mp 110 °C. ^{1}H NMR ($CDCl_3$, 250 MHz), δ: 0.68 (s, 3H, Me 18), 0.86 (d, 6H, J=6.5 Hz, Me 27 and Me 26), 0.91 (d, 3H, J=6.5 Hz, Me 21), 1.03 (s, 3H, Me 19), 2.17 (s, 3H, CH_3–CO), 4.6–4.68 (m, 1H, H3α). ^{13}C NMR ($CDCl_3$): δ: 11.9, 18.7, 19.2, 21.0, 22.7, 23.2, 23.9, 24.4, 27.8, 28.1, 28.3, 30.4, 30.5, 33.7, 35.4, 35.9, 36.2, 38.7, 39.6, 39.8, 42.1, 51.8, 55.8, 56.4, 72.8, 130.2, 131.3, 171.2. MS-EI: m/z=473 (27%, $M^{+\cdot}$), 426 (20%, $M^{+\cdot}-HNO_2$), 398 (46%, $M^{+\cdot}-(CH_3COOH+.CH_3)$), 383 (100%).

4.1.10. 3β-Acetoxy-cholestan-6-one 10. To a solution of compound **9** (0.890 g, 1.87 mmol) in acetic acid (20 mL) was added zinc (2 g, 30 mmol) and the solution was refluxed for 4 h 30 min. The solution was filtered, diluted with water and extracted with methylene chloride. The organic layer was dried over sodium sulfate and evaporated. The crude product was purified by chromatography (Hexane/ethyl acetate 9.8:0.2) and crystallized from ethanol (0.60 g, 72%). Mp 136 °C.

IR: ν: 1730 (C=O/ester), 1718 (C=O/ketone). ^{1}H NMR ($CDCl_3$, 250 MHz), δ: 0.66 (s, 3H, Me 18), 0.76 (d, 6H, J= 6.5 Hz, Me 27 and Me 26), 0.90 (d, 3H, J=6.5 Hz, Me 21), 0.95 (s, 3H, Me 19), 2.02 (s, 3H, CH_3–COO), 4.67 (m, 1H, H3α). ^{13}C NMR ($CDCl_3$): δ: 12.3, 13.4, 19.0, 21.7, 21.8, 24.1, 24.3, 26.4, 27.2, 28.3, 28.4, 36.0, 36.4, 36.7, 38.3, 39.2, 39.8, 41.3, 43.2, 43.3, 47.0, 54.1, 56.4, 56.8, 73.2, 170.0, 210.7. MS-EI: m/z=444 (25%, $M^{+\cdot}$), 384 (100%, $M^{+\cdot}-CH_3COOH$), 369 (27%, 384 $-{}^{\cdot}CH_3$).

4.1.11. 3β-Acetoxy-6*N*-hydroxyiminocholestane 11. Ketone **10** was added to a solution of hydroxylamine chloride (0.751 g, 10 mmol) in pyridine (8 mL). The

mixture was refluxed under argon atmosphere for 5 h. The solution was diluted with water and extracted with methylene chloride. The organic layer was treated with 0.1 N HCl (15 mL), 5% $NaHCO_3$ (15 mL) and washed with water. The solution was dried over sodium sulfate and evaporated. The product was purified by chromatography (hexane/ethyl acetate 9:1) to afford product **11** (2.38 g, 76%) as a white solid. IR: ν: 2950 (O–H alcohol), 1734 (C=O/ester), 1712 (C=N–OH). ^{1}H NMR ($CDCl_3$, 250 MHz), δ: 0.65 (s, 3H, Me 18), 0.85 (d, 6H, J=6.5 Hz, Me 27 and Me 26), 0.91 (d, 3H, J=6.5 Hz, Me 21), 0.99 (s, 3H, Me 19), 2.02 (s, 3H, CH_3–COO), 3.32 (dd, 1H, J=9.0 Hz, C_5–*H*), 4.67 (m, 1H, H3α), 7.73 (br s, 1H, –C=N–O–*H*). ^{13}C NMR ($CDCl_3$): δ: 12.4, 18.9, 19.0, 21.7, 22.9, 24.2, 24.4, 24.5, 24.8, 27.4, 27.9, 28.3, 28.5, 30.0, 36.1, 36.2, 36.3, 36.5, 39.3, 39.8, 40.0, 43.3, 49.7, 56.5, 57.0, 73.6, 159.9, 171.0. MS-EI: m/z=459 (11%, $M^{+\cdot}$), 442 (17%, $M^{+\cdot}-{}^{\cdot}OH$), 399 (79%, $M^{+\cdot}-CH_3COOH$), 384 (100%, 399 $-{}^{\cdot}CH_3$).

4.1.12. 6α-Aminocholestanol 12. Under nitrogen atmosphere, solution of **11** (1.50 g, 3.27 mmol) in tetrahydrofuran (8 mL) was added dropwise over 10 min to a stirred suspension of lithium aluminium hydride (0.744 g, 19 mmol) in tetrahydrofuran (20 mL) at 0 °C. The mixture was refluxed for 4 h, after which it was cooled and quenched by careful addition of saturated aqueous sodium sulfate. The solution was filtered, dried and evaporated under reduced pressure. The crude product was purified by column chromatography (methylene chloride/methanol/triethylamine (8:1.8:0.2) crystallized from ethanol/ethyl ether (8:2) to give amine **12** (0.900 g, 70%) as a white solid. Mp 127–128 °C. IR: ν: 3500–3000 (OH alcohol and NH_2 amine). ^{1}H NMR ($CDCl_3$, 250 MHz), δ: 0.69 (s, 3H, Me 18), 0.86 (d, 6H, Me 27 and Me 26), 0.91 (d, 3H, J=6.5 Hz, Me 21), 1.02 (s, 3H, Me 19), 2.32 (br s, 2H, NH_2, D_2O exchange), 2.95 (dd, 1H, H6β of α epimer, 3.64 (m, 1H, H3α). ^{13}C NMR ($CDCl_3$): δ: 12.5, 16.9, 19.0, 21.4, 22.9, 24.2, 24.6, 28.4, 28.5, 30.0, 30.4, 31.8, 35.9, 36.1, 36.3, 36.5, 39.5, 39.9, 40.3, 43.0, 47.0, 52.4, 54.7, 56.4, 56.7. MS-EI: m/z=403 (16%, $M^{+\cdot}$), 386 (24%, $M^{+\cdot}-NH_3$), 248 (100%).

4.1.13. 6α-*N*-(2*N*-Cyanoethyl)aminocholestanol 13. A solution of amine **12** (0.72 g, 1.8 mmol) and acrylonitrile (1.1 mL, 16 mmol) in methanol (10 mL) was stirred at room temperature for 24 h. The solvent was evaporated giving the crude product which was purified by chromatography (hexane/ethyl acetate 8:2) and crystallized from ethanol/ethyl ether (8:2) to afford (0.760 g, 93%) of nitrile **13** (β) as a white solid. Mp=136 °C. IR: ν: 3391–3000 (OH alcohol and NH amine), 2248 (nitrile). ^{1}H NMR ($CDCl_3$, 250 MHz), δ: 0.68 (s, 3H, Me 18), 0.84 (dd, 6H, J_1=6.6 Hz, J_2=0.8 Hz, Me 27 and Me 26), 0.91 (d, 3H, J=6.5 Hz, Me 21), 1.04 (s, 3H, Me 19), 2.44 (m, 2H, –HN–CH_2–CH_2–CN), 2.62 (m, 1H, H6β of α epimer), 2.74 (m, 1H, St–HN–C$H_a$$CH_{a'}$–$CH_2$–CN), 2.92 (m, 1H, St–HN–$CH_a$$H_{a'}$–$CH_2$–CN), 3.6–3.7 (m, 1H, H3α). ^{13}C NMR ($CDCl_3$): δ: 11.6, 18.6, 18.7, 19.7, 22.5, 22.8, 23.9, 28.0, 28.2, 31.4, 35.8, 36.2, 36.9, 37.4, 39.5, 42.1, 43.0, 43.8, 49.5, 52.8, 55.8, 58.2, 58.3, 58.9, 59.0, 59.3, 59.9, 71.6, 118.9. MS-EI: m/z=456 (12%, $M^{+\cdot}$), 416 (30%, St–NH–CH_2^+), 301 (100%).

4.1.14. 6α-*N*-(3*N*-Aminopropyl)aminocholestanol 14. A solution of nitrile **11a** (0.40 g, 0.87 mmol) in dry THF (5 mL) was added dropwise to a suspension of lithium aluminium hydride (0.19 g, 5.25 mmol) and nickel hexahydrate (0.206, 0.87 mmol) in dry THF (10 mL) under argon. The mixture was refluxed for 2 h. The reaction mixture quenched with $Na_2SO_4\cdot10H_2O$, filtered through a pad of celite, and washed with ethyl ether/*n*-butanol (6:4). Removal of the solvent in vacuo and purification by flash chromatography (methylene chloride/methanol/ammoniaque 7:2.5:0.5) to afford a pure product **12a** (0.18 g, 41%) as amorphous solid. ^{1}H NMR ($CDCl_3$, 250 MHz), δ: 0.68 (s, 3H, Me 18), 0.86 (d, 6H, J=6.5 Hz, Me 27 and Me 26), 0.91 (d, 3H, J=6.5 Hz, Me 21), 1.04 (s, 3H, Me 19), 2.70 (m, 2H, St–NH–CH_2–CH_2–), 2.74 (m, 1H, St–NH–C$H_a$$H_{a'}$–$CH_2$–), 2.80 (m, 1H, H6β of α epimer), 2.87 (m, 1H, St–HN–$CH_a$$H_{a'}$–$(CH_2)_2$–), 3.00 (t, 2H, J=12 Hz, St–HN–$(CH_2)_2$– CH_2–NH_2), 3.66 (m, 1H, H3α). ^{13}C NMR ($CDCl_3$): δ: 12.1, 15.3, 18.7, 20.9, 22.7, 23.9, 24.1, 28.1, 28.2, 30.3, 33.4, 33.6, 34.1, 35.0, 35.8, 36.2, 36.5, 39.6, 40.0, 40.6, 44.4, 45.6, 49.5, 52.0, 56.3, 56.9, 60.2, 73.1. MS-EI: m/z=460 ($M^{+\cdot}$, 13%), 443 (12%, $M^{+\cdot}-NH_3$), 402 (100%, St–NH^+).

4.1.15. 6α-*N*-[3*N*-(3-Cyanopropyl)aminopropyl]aminocholestanol 15. To a solution of amine **14** (0.270 g, 0.580 mmol in DMF (4 mL) was added sodium hydrogenocarbonate (0.073 g, 0.871 mmol). After stirring at room temperature for 35 min, 4-bromobutyronitrile (0.115 mL, 1.161 mmol) was added and the mixture was heated at 60 °C for 36 h. The solution was diluted with water and extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate and evaporated. The residue was purified by chromatography (hexane/ethyl acetate 6:4) afforded product **15** (0.180 g, 59%) as a oil. IR: ν: 3500–3000 (OH alcohol and NH amine); 2254 (nitrile). ^{1}H NMR ($CDCl_3$, 250 MHz), δ: 0.65 (s, 3H, Me 18), 0.85 (d, 6H, J=6.5 Hz, Me 27 and Me 26), 0.90 (d, 3H, J=6.5 Hz, Me 21), 1.08 (s, 3H, Me 19), 2.49 (t, 2H, J=7.2 Hz, –HN–$(CH_2)_2$–CH_2–CN), 2.59 (td, 1H, J_1=6.1 Hz, J_2=3.9 Hz, H6β of α epimer), 2.64 (m, 1H, St–HN–C$H_a$$H_{a'}$–$(CH_2)_2$–NH), 2.85 (m, 1H, St–HN–$CH_a$$H_{a'}$–$(CH_2)_2$–NH), 3.44 (m, 1H, H3α), 3.61 (t, 2H, J=5.7 Hz, –HN–CH_2–$(CH_2)_2$–CN), 4.30 (t, 2H, J=6.2 Hz, St–NH–$(CH_2)_2$–CH_2–NH–), 8.69 (br s, 1H, St–N*H*–, D_2O exchange). ^{13}C NMR ($CDCl_3$): δ: 12.5, 15.4; 16.7, 19.0; 20.9, 22.9, 23.2, 24.1, 24.6, 25.6, 28.4, 28.2, 30.8, 33.4, 33.6, 35.0, 35.8, 36.0, 36.5, 39.9, 43.0, 44.8, 46.1, 46.6, 48.3, 49.5, 52.0, 56.3, 56.9, 60.2, 72.2, 120.3. MS-EI: m/z=487 (20%, St–NH–$(CH_2)_3$–NH–CH_2–CH_2^+), 473 (4%, St–NH–$(CH_2)_3$–NH–CH_2^+), 416 (29%, St–NH–CH_2 $^+$), 402 (14%, St–NH^+), 315 (100%).

4.1.16. 6α-*N*-[3*N*-(4-Aminobutyl)aminopropyl]aminocholestanol II. Nitrile **15** (0.070 g, 0.132 mmol) was reduced in the same manner as **7** to yield (0.025 g, 35%).^{1}H NMR ($CDCl_3$, 250 MHz): δ: 0.67 (s, 3H, Me 18), 0.84 (d, 6H, J=6.6 Hz, Me 27 and Me 26), 0.91 (d, 3H, J=6.5 Hz, Me 21), 0.95 (s, 3H, Me 19), 2.45 (m, 1H, St–HN–C$H_a$$H_{a'}$–$(CH_2)_2$–NH–), 2.54 (m, 1H, H6β of α epimer), 2.69 (m, 1H, St–HN–$CH_a$$H_{a'}$–$(CH_2)_2$–NH–), 2.79 (t, 2H, J=6.3 Hz, –HN–$(CH_2)_3$–CH_2–NH_2), 3.15 (t, 2H, J=9.2 Hz, St–HN–$(CH_2)_2$–CH_2–NH–), 3.62 (m, 1H, H3α), 7.12 (br s, 2H, –NH2, D_2O exchange), 7.89 (br s, 1H, –NH–,

D_2O exchange), 8.25 (br s, 1H, –NH–, D_2O exchange). ^{13}C NMR ($CDCl_3$): δ: 12.0, 16.2, 18.6, 21.0, 22.5, 22.8, 23.8, 24.4, 24.8, 28.0, 28.2, 30.4, 30.5, 31.6, 35.6, 35.8, 36.0, 36.1, 38.7, 38.9, 39.5, 40.0, 42.5, 42.6, 47.3; 47.8, 48.9, 50.6, 51.1, 54.8, 56.3, 59.2, 71.9. MS-EI: m/z (%)=473 (4%, St–NH–$(CH_2)_3$–NH–CH_2^+), 444 (5%, St–NH–$(CH_2)_2$–CH_2^+), 430 (10%, St–NH–CH_2–CH_2^+), 416 (40%, St–NH–CH_2^+), 402 (100%, St–NH^+), 387 (72%, St^+). Anal. Calcd for $C_{34}H_{65}N_3O$: C 76.77; H 12.32; N 7.90. Found: C 76.72; H 12.38; N 7.93.

4.1.17. 3β-Acetyl-7α,β-azidocholesterol 16. Trimethylsilyl azide (15 mL, 104.9 mmol) was added dropwise to a solution of cholesteryl acetate (4.5, 10.5 mmol) and lead (IV) acetate (9.45 g, 21 mmol) in methylene chloride (40 mL). The mixture was stirred for 2 h. the solution was diluted with water and the precipitate lead (II) azide was removed by fitration, and decomposed with sodium nitrite/dilute hydrochloric acid. The organic layer was dried over sodium sulfate and evaporated. The crude product was purified by chromatography (hexane/ethyl acetate 8:2) to give 3.4 g (68% of epimeric mixture: α epimer 77% and β epimer 23%) of **16** as colorless oil. IR: ν: 2103 (N_3 azide) and 1727 (C=O ester). ^1H NMR ($CDCl_3$, 250 MHz): δ_H=0.67 (s, 3H, 18-Me), 0.87 (dd, 6H, J=6.6, 1.92 Hz, $CH(CH_3)_2$), 0.92 (d, 3H, J=6.6 Hz, 21-Me), 1.03 (s, 3H, 19-Me), 2.04 (s, 3H, CH_3–COO), 3.27 (ddd, 0.23H, $J_{7\alpha-8}$=8.5 Hz, $J_{7\alpha-6}$=1 Hz and $J_{7\alpha-4}$=1.5 Hz, H-7α of β epimer), 3.5 (ddd, 0.77H, $J_{7\beta-8}$=4.5 Hz, $J_{7\beta-6}$=5 Hz and $J_{7\beta-4}$=1.5 Hz, H-7β of α epimer), 4.66 (m, 1H, 3-H), 5.30 (dd, 0.23H, $J_{6-7\alpha}$<1 Hz and J_{6-4}=1 Hz, 6-H of β epimer), 5.56 (dd, 0.77H, $J_{6-7\beta}$=5.1 Hz and J_{6-4}=1.6 Hz, 6-H of α epimer). ^{13}C NMR ($CDCl_3$): δ: 12.4, 12.7, 19.0, 21.8, 22.9, 23.2, 24.2, 24.5, 28.0, 28.4, 28.5, 36.1, 36.2, 36.5, 39.2, 39.9, 40.0, 43.2, 49.7, 54.6, 54.3, 58.1, 72.3, 120.2, 149.3, 171.0. MS-EI: m/z=469 (5%, $M^{+\cdot}$), 441 (100%, $M^{+\cdot}-N_2$).

4.1.18. 7α,β-Aminocholesterol 17. Under nitrogen atmosphere, solution of **16** (1 g, 2.13 mmol) in tetrahydrofuran (10 mL) was added dropwise over 10 min to a stirred suspension of lithium aluminium hydride (1 g, 2.13 mmol) in tetrahydrofuran (20 mL) at 0 °C. The mixture was refluxed for 4 h, after which it was cooled and quenched by careful addition of saturated aqueous sodium sulfate. The solution was filtered, dried and evaporated under reduced pressure. The crude product was purified by column chromatography (methylene chloride/methanol/ammoniaque 8:1.8:0.2) to give 7α,β-aminocholesterol (0.93 g, 70%) as amorphous white solid. IR: ν: 3445–3000 (OH alcohol and NH_2 amine). ^1H NMR ($CDCl_3$, 400 MHz), δ: 0.69 (s, 3H, Me 18), 0.86 (d, 6H, J=6.5 Hz, Me 27 and Me 26), 0.92 (d, 3H, J=6.5 Hz, Me 21), 1.05 (s, 3H, Me 19), 3.49 (m, 1H, H3),, 3.54 (ddd, 1H, $J_{7\alpha-8}$=8.5 Hz, $J_{7\alpha-6}$=1.2 Hz, $J_{7\alpha-4}$=1.5 Hz, H7α of β epimer), 3.58 (ddd, 1H, $J_{7\beta-8}$=4.8 Hz, $J_{7\beta-6}$=5.3 Hz, $J_{7\beta-4}$=1.2 Hz, H7β of α epimer), 4.62 (br s, 2H, NH_2, D_2O exchange), 5.55 (dd, 1H, $J_{6-7\beta}$=5.2 Hz, J_{6-4}<1 Hz, H6 of α epimer), 5.26 (dd, 1H, $J_{6-7\alpha}$=1.2 Hz, J_{6-4}<1 Hz, H6 of β epimer). ^{13}C NMR ($CDCl_3$): δ: 12.1, 15.2, 18.8, 21.8, 22.7, 23.9, 26.2, 28.1, 28.3, 29.8, 35.9, 36.2, 37.1, 37.4, 39.6, 39.8, 41.8, 42.9, 43.5, 46.5, 52.2, 49.6; 53.5; 56.3, 71.6, 128.6, 141.0. MS-EI: m/z=401 (71%, $M^{+\cdot}$), 384 (4%, $M^{+\cdot}-NH_3$), 351 (29%), 289 (100%). Anal. Calcd for $C_{27}H_{47}NO$ (401.68): C 80.7, H 11.7, N 3.5. Found: C 80.3, H 11.3, N 3.2.

4.1.19. 7α-*N*-(2-Cyanoethyl)aminocholesterol 18a and 7β-*N*-(2-cyanoethyl)aminocholesterol 18b. A solution of amine **17** (0.714 g, 1.77 mmol) and acrylonitrile (0.8 mL, 15 mmol) in methanol (5 mL) was stirred at room temperature for 24 h. The solvent was evaporated giving the crude product which was purified by chromatography (hexane/ethyl acetate) to afford 0.5 g (66%) of **18a** and **18b**, respectively, 70% (α) 30% (β) as amorphous solid.

Compound **18a**. IR: ν: 3481–3314 (OH alcohol and NH amine); 2254 (CN). ^1H NMR ($CDCl_3$, 250 MHz): δ: 0.68 (s, 3H, Me 18), 0.86 (d, 6H, J=6.5 Hz, Me 27 and Me 26), 0.91 (d, 3H, J=6.5 Hz, Me 21), 1.05 (s, 3H, Me 19), 2.40 (m, 2H, St–NH–$CH_aH_{a'}$–CH_2–CN), 2.69 (dd, 1H, $J_{7\beta-6}$=7.25 Hz, $J_{7\beta-8}$=4.2 Hz, H7β of α epimer), 2.77 (m, 1H, St–NH–$CH_aH_{a'}$–CH_2–CN), 3.08 (m,1H, St–NH–$CH_aH_{a''}$–CH_2–CN), 3.49 (m, 1H, H3α), 5.55 (dd, 1H, $J_{6-7\beta}$=5 Hz, J_{6-4}<1 Hz, H6 of α epimer). ^{13}C NMR ($CDCl_3$): δ: 12.1, 19.1, 19.4, 19.8, 22.9, 23.2, 24.1, 26.7, 28.1, 28.3, 29.9, 35.9, 36.3, 37.1, 39.6, 39.8, 41.0, 42.9, 43.0, 44.2, 47.6, 54.4, 56.3, 56.4, 60.2, 71.6, 119.2, 124.0, 143.5. MS-EI: m/z=454 (22%, $M^{+\cdot}$), 385 (100%, St^+).

Compound **18b**. IR: ν: 2934 (CH_2), 2249 (CN). ^1H NMR ($CDCl_3$, 250 MHz): δ: 0.68 (s, 3H, Me 18), 0.86 (d, 6H, J=6.5 Hz, Me 27 and Me 26), 0.93 (d, 3H, J=6.5 Hz, Me 21), 1.02 (s, 3H, Me 19), 2.39 (m, 2H, St–NH–CH_2–CH_2–CN), 2.66 (m, 1H, St–NH–$CH_aH_{a'}$–CH_2–CN), 2.77 (ddd, 1H, $J_{7\alpha-8}$=8.3 Hz, $J_{7\alpha-6}$=1.2 Hz, H7α of β epimer), 2.91 (m,1H, St–NH–$CH_aH_{a'}$–CH_2–CN), 3.46 (m, 1H, H3α), 5.21 (d, 1H, $J_{6-7\alpha}$=3 Hz, H6 of β epimer). ^{13}C NMR ($CDCl_3$): δ: 13.7, 18.6, 18.8, 20.8, 21.3, 22.7, 24.3, 25.1, 28.0, 29.7, 33.4, 37.4, 37.7, 37.9, 38.5, 39.7, 40.7, 41.2, 43.7, 45.0, 51.3, 54.2, 54.8, 58.5, 73.1, 120.6, 125.5, 144.7. MS-EI: m/z=454 (14%, $M^{+\cdot}$), 385 (100%, St^+).

4.1.20. 7α-*N*-(3-Aminopropyl)aminocholesterol 19a. A solution of nitrile **18a** (0.2 g, 0.44 mmol) in dry THF (5 mL) was added dropwise to a suspension of lithium aluminium hydride (0.14 g, 2.64 mmol) and nickel chloride hexahydrate (0.105 g, 0.44 mmol) in dry THF (15 mL) under argon. The mixture was refluxed for 1 h. The reaction mixture quenched with $Na_2SO_4.10H_2O$, filtered through a pad of celite, and washed with ethyl ether/n butanol (6:4). Removal of the solvent in vacuo and purification by flash chromatography (methylene chloride/methanol/ammoniaque 7:2:1) to afford a pure product **19a** (0.4 g, 69%) as amorphous solid. IR: ν: 3434 (NH_2 amine). ^1H NMR ($CDCl_3$, 250 MHz): δ: 0.66 (s, 3H, Me 18), 0.86 (d, 6H, J=6.5 Hz, Me 27 and Me 26), 0.91 (d, 3H, J=6.5 Hz, Me 21), 0.99 (s, 3H, Me 19), 2.17 (m, 1H, St–NH–$CH_aH_{a'}$–$(CH_2)_2$–NH_2), 2.30 (m, 2H, St–NH–CH_2–CH_2–CH_2–NH_2), 2.55 (m, 1H, St–NH–$CH_aH_{a'}$–$(CH_2)_2$–NH_2), 2.74 (m, 1H, H7β of α epimer), 2.81 (t, 2H, J=5.29 Hz, St–NH–$(CH_2)_2$–CH_2–NH_2), 3.56 (m, 1H, H3α), 5.66 (dd, 1H, $J_{6-7\beta}$=4.85 Hz, J_{6-4}<1 Hz, H6 of α epimer). ^{13}C NMR ($CDCl_3$): δ: 12.0, 19.0, 19.7, 21.0, 22.9, 23.1, 24.2, 27.0, 28.3, 31.6, 34.6, 35.9, 36.5, 37.0, 37.4, 39.6, 39.8, 40.0, 42.1, 42.4, 42.6, 43.6, 47.6, 54.3, 56.3, 61.4, 71.2, 125.3, 142.9.

4.1.21. 7β-*N*-(3-Aminopropyl)aminocholesterol 19b. Nitrile **18b** (0.250 g, 0.55 mmol) was reduced in the same manner as **18a** to yield (0.180 g, 72%) of product **19b** as

amorphous solid. IR: ν: 3500–3430 (OH alcohol and NH_2 amine), 2961 (CH_2). ^{1}H NMR ($CDCl_3$, 250 MHz): δ: 0.68 (s, 3H, Me 18), 0.86 (d, 6H, J=6.5 Hz, Me 27 and Me 26), 0.90 (d, 3H, J=6.5 Hz, Me 21), 1.03 (s, 3H, Me 19), 2.63 (m, 1H, St–HN–CH_aH$_{a'}$–$(CH_2)_2$–NH_2–), 2.74 (m, 1H, H7α of β epimer), 2. 87 (m, 1H, St–NH–$CH_aH_{a'}$–$(CH_2)_2$–NH_2), 3.25 (t, 2H, J=5.8 Hz, St–NH–$(CH_2)_2$–CH_2–NH_2), 3.66 (m, 1H, H3α), 5.63 (br s, 1H, St–NH–, D_2O exchange). ^{13}C NMR ($CDCl_3$): δ: 13.7, 18.6, 18.8, 20.8, 21.3, 22.7, 24.3, 25.1, 28.0, 29.7, 33.4, 33.8, 37.4, 37.7, 37.9, 38.5, 39.7, 40.6, 40.7, 41.2, 43.7, 45.6, 51.3, 54.2, 59.0, 58.5, 73.1, 120.6, 122.8, 139.0.

4.1.22. 7α-*N*-[3*N*-(3-Cyanopropyl)aminopropyl]aminocholesterol 20a. Compound **20a** was obtained from amine **19a** (0.46 g, 1 mmol) as described in the preparation of compound **7** to yield (0.22 g, 42%) as a oil. IR: ν: 2935 (CH_2), 2249 (CN). ^{1}H NMR ($CDCl_3$, 250 MHz): δ: 0.68 (s, 3H, Me 18), 0.86 (d, 6H, J=6.5 Hz, Me 27 and Me 26), 0.92 (d, 3H, J=6.5 Hz, Me 21), 0.99 (s, 3H, Me 19), 2.40 (t, 2H, J=7.7 Hz, –CH_2–CN), 2.50 (m, 1H, St–NH–CH_aH$_{a'}$–$(CH_2)_2$–NH–), 2.62 (ddd, 1H, $J_{7\beta-8}$=4.3 Hz, $J_{7\beta-6}$=5 Hz, H7β of α epimer), 2.95 (m, 1H, St–NH–$CH_aH_{a'}$–$(CH_2)_2$–NH–), 3.5 (m, 1H, H3α), 5.62 (dd, 1H, $J_{6-7\beta}$=1.8 Hz, J_{6-4}<1 Hz, H6 of α epimer). ^{13}C NMR ($CDCl_3$): δ: 12.0, 15.2, 19.0, 19.1, 21.2, 22.9, 23.2, 24.1, 24.3, 27.2, 28.1, 28.3, 36.2, 36.6, 37.3, 37.7, 37.8, 39.4, 39.7, 40.7, 40.8, 43.0, 44.5, 46.1, 46.3, 46.8, 53.4, 56.3, 65.4, 72.0, 119.9, 121.1, 143.0. MS-EI: m/z=471 (6%, St–NH–$(CH_2)_3$–NH–CH_2^+), 428 (16%, St–NH–CH_2–CH_2^+), 400 (12%, St–NH^+), 385 (100%, St^+).

4.1.23. 7β-*N*-[3*N*-(3-Cyanopropyl)aminopropyl]aminocholesterol 20b. Amine **19b** (0.180 g, 0.40 mmol) was alkylated in the same manner as **19a** to yield 0.10 g (50%) of product **20b** as a oil. IR: ν: 2935 (CH_2), 2249 (CN). ^{1}H NMR ($CDCl_3$, 250 MHz): δ: 0.68 (s, 3H, Me 18), 0.86 (d, 6H, J=6.5 Hz, Me 27 and Me 26), 0.90 (d, 3H, J=6.5 Hz, Me 21), 0.93 (s, 3H, Me 19), 2.45 (t, 2H, J=7.0 Hz, St–HN–$(CH_2)_3$–NH–$(CH_2)_2$–CH_2–CN), 2.53 (m, 1H, St–NH–CH_aH$_{a'}$–CH_2–), 2.74 (ddd, 1H, $J_{7\alpha-8}$=8.7 Hz, $J_{7\alpha-6}$=1.2 Hz, H7α of β epimer), 2.86 (m, 1H, St–NH–$CH_aH_{a'}$–CH_2–), 3.53 (m, 1H, H3α), 5.31 (d, 1H, $J_{6-7\alpha}$=3.0 Hz, H6 of β epimer). ^{13}C NMR ($CDCl_3$): δ: 12.4, 15.4, 19.1, 19.4, 21.7, 22.9, 24.1, 26.8, 27.0, 28.4, 28.8, 28.9, 30.0, 32.0, 36.1, 36.4, 36.5, 37.4, 39.8 40.1, 42.4, 43.6, 44.6, 46.8, 48.3, 48.8, 55.7, 57.2, 60.4, 71.8, 120.2, 124.6, 142.6. MS-EI: m/z=471 (3%, St–NH–$(CH_2)_3$–NH–CH_2^+), 428 (26%, St–NH–CH_2–CH_2^+); 400 (21%, St–NH^+).

4.1.24. 7α-*N*-[3*N*-(4-Aminobutyl)aminopropyl]aminocholesterol III. Compound **III** (0.09 g, 0.16 mmol) was prepared in the same manner as **I** to yield (0.04 g, 46%) as amorphous solid. ^{1}H NMR ($CDCl_3$, 250 MHz): δ: 0.68 (s, 3H, Me 18), 0.86 (d, 6H, J=6.5 Hz, Me 27 and Me 26), 0.91 (d, 3H, J=6.5 Hz, Me 21), 0.99 (s, 3H, Me 19), 2.66 (m, 1H, St–NH–CH_aH$_{a'}$–$(CH_2)_2$–NH–), 2.74 (ddd, 1H, $J_{7\beta-8}$=4.3 Hz, $J_{7\beta-6}$=5.0 Hz, H7β of α epimer), 2.80 (t 2H, J=5.4 Hz, –HN–$(CH_2)_3$–CH_2–NH_2), 2.92 (m, 1H, St–NH–$CH_aH_{a'}$–$(CH_2)_2$–NH–), 3.00–3.48 (m, 4H, St–NH–$(CH_2)_2$–CH_2–NH– and NH–CH_2–$(CH_2)_3$–NH_2), 3.63 (m, 1H, H3α), 5.66 (dd, 1H, $J_{6-7\beta}$=1.8 Hz, J_{6-4}<1.0 Hz, H6 of α epimer), 7.23 (br s, 2H, –NH2, D_2O exchange), 8.15 (br s, 1H, –NH–, D_2O exchange), 8.44 (br s, 1H, –NH–, D_2O exchange). ^{13}C NMR ($CDCl_3$): δ: 12.1, 18.6, 18.8, 21.7, 22.7, 23.9, 25.4, 25.6, 26.3, 28.1, 28.2, 29.5, 31.7 35.9, 36.3, 37.0, 37.4, 39.6, 39.7, 40.0, 40.7, 40.8, 43.0, 46.1, 46.6, 46.8, 47.6, 54.2, 56.3, 65.4, 71.5, 122.2, 139. MS-EI (m/z)= 471 (3%, St–NH–$(CH_2)_3$–NH–CH_2^+), 442 (2%, St–NH–$(CH_2)_2$–CH_2^+), 400 (100%, St–NH^+), 385 (24%, St^+). $[\alpha]_D^{20}$ −41° (C=0.2 M in MeOH). Anal. Calcd for $C_{34}H_{63}N_3O$: C 77.35, H 11.66, N 7.96. Found: C 77.29, H 11.69, N 7.95.

4.1.25. 7β-*N*-[3*N*-(4-Aminobutyl)aminopropyl]aminocholesterol IV. Compound **IV** (0.040 g, 0.78 mmol) was prepared in the same manner as **I** to yield (0.02 g, 48%) as amorphous solid. ^{1}H NMR ($CDCl_3$, 250 MHz): δ: 0.69 (s, 3H, Me 18), 0.86 (d, 6H, J=6.5 Hz, Me 27 and Me 26), 0.90 (d, 3H, J=6.5 Hz, Me 21), 0.93 (s, 3H, Me 19), 2.37 (m, 1H, St–NH–CH_aH$_{a'}$–$(CH_2)_2$–), 2.42–2.47 (m, 2H, –HN–$(CH_2)_3$–CH_2–NH_2 and –HN–CH_2–$(CH_2)_3$–NH_2), 2.70 (m, 1H, St–NH–$CH_aH_{a'}$–$(CH_2)_2$–), 2.75 (ddd, 1H, $J_{7\beta-8}$= 4.3 Hz, $J_{7\beta-6}$=5 Hz, H7α of β epimer), 2.79 (t, 2H, J= 8.3 Hz, St–NH–$(CH_2)_2$–CH_2–NH–), 3.5 (m, 1H, H3α), 5.30 (d, 1H, J=3.0 Hz, H6 of β epimer), 7.19 (br s, 2H, –NH_2, D_2O exchange), 8.13 (br s, 1H, –NH–, D_2O exchange), 8.39 (br s, 1H, –NH–, D_2O exchange). ^{13}C NMR ($CDCl_3$): δ: 12.1, 18.6, 18.8, 21.7, 22.7, 23.9, 25.4, 25.6, 26.3 28.1, 28.2, 29.5, 31.7, 35.9, 36.3, 37.0, 37.4, 39.6, 39.7, 40.0, 40.7, 40.8, 43.0, 46.1, 46.6, 46.8, 47.6, 54.2, 56.3, 59.0, 71.5, 122.8, 139.0. MS-EI: m/z=471 (1%, St–NH–$(CH_2)_3$–NH–CH_2^+), 400 (33%, St–NH^+), 385 (100%, St^+). $[\alpha]_D^{20}$ +41° (C=0.2 M in MeOH). Anal. Calcd for $C_{34}H_{63}N_3O$: C 77.35, H 11.66, N 7.96. Found: C 77.27, H 11.71, N 7.94.

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