Isolation of Plakinamine I: A New Steroidal Alkaloid from the Marine Sponge *Corticium* sp. and Synthesis of an Analogue Model Compound

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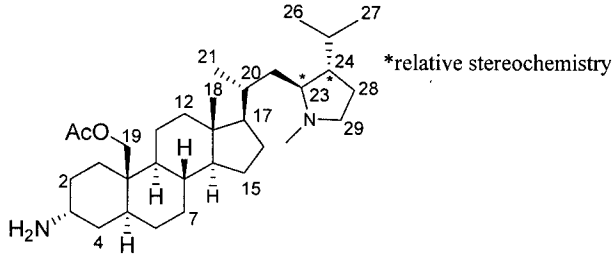
A new cytotoxic steroidal alkaloid, plakinamine I, with an unprecedented 3α-amino-19-acetoxy nucleus was isolated from a *Corticium* sp. sponge. The structure has been elucidated by spectroscopic analysis and secured by the synthesis of the model 19-acetoxy-3α-aminocholestane. The evaluation of the cytotoxic activities of several synthetic derivatives, intermediates of the above synthesis, allowed us to explore the influence of the different functions on the cytotoxicity observed for natural plakinamine I.

Introduction

Steroidal alkaloids are well-known metabolites of certain terrestrial plants, but only a few examples are reported from marine organisms. The marine sponge *Corticium* sp. represents a prolific source of new steroidal alkaloids:[1–7] the majority are structurally related to plakinamine A, as they possess an amino group at C-3 and a cyclic imine functionality on the side chain of a C_{29} steroidal framework. From the same sponge, two new derivatives featuring an unusual B(9a)-homo-19-nor steroidal nucleus have also been isolated.[3]

A re-examination of the polar extracts of the sponge *Corticium* sp., collected in Vanuatu, led to the isolation of a very minor component, plakinamine I (**1**), which represents the first example of the 19-acetoxy-3α-amino steroid from natural sources. In this paper we report the isolation and the structural elucidation of this new derivative, together with the synthesis of the model compound, 19-acetoxy-3α-amino-5α-cholestane, which is useful for the complete spectral characterization of the natural compound and for preliminary structure–activity relationship studies.



Plakinamide I (**1**)

Results and Discussion

The molecular formula $C_{32}H_{56}N_2O_2$ of plakinamine I (**1**) was deduced from an HR ESI-MS measurement of the $[M + H]^+$ pseudomolecular ion at m/z = 501.4412 [calcd. for $C_{32}H_{57}N_2O_2$ (501.4420)]. An initial analysis of ^{1}H NMR and COSY data revealed a molecule that was steroidal in nature. The ^{1}H NMR spectrum in CD_3OD features one 3H singlet at δ = 0.71 ppm assigned to the C-18 angular methyl group. Three doublets at δ = 0.91 (J = 6.6 Hz), 0.97 (J = 6.6 Hz) and 0.98 ppm (J = 6.3 Hz) are assigned to C-26, C-27, and C-21 secondary methyl protons, respectively. The absence in the ^{1}H NMR spectrum of the signal arising from the angular C-19 methyl group, together with the presence of two mutually coupled diastereotopic doublets at δ = 4.28 and 4.44 ppm (J = 12.1 Hz) and one singlet acetyl signal at δ = 2.07, indicate the presence of a 19-acetoxy function. Further, a 3α-amino group was assigned on the basis of the chemical shift and of the coupling constants of the 3β-H

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Table 1. ^{1}H and ^{13}C NMR chemical shift data for plakinamine I (**1**) and synthetic 19-acetoxy-3α-amino-5α-cholestane (**7**) [CD_3OD].[a]

Position	**1** δ_H[b]	δ_C	**7** δ_H[b]	δ_C
1	1.90, 1.52	27.7	1.95, 1.52	27.7
2	1.51, 1.66	29.8	1.51, 1.66	30.0
3	3.24 br. s	47.2	3.24 br. s	47.2
4	1.71, 1.32	35.3	1.71, 1.33	35.0
5	1.59	40.4	1.53	40.4
6	1.21	30.5	1.21	30.6
7	1.02, 1.74	33.0	0.97, 1.67	33.0
8	1.44	37.2	1.44	37.2
9	0.93	55.4	0.83	55.4
10	–	39.8	–	39.8
11	1.11, 1.65	23.2	1.31, 1.56	23.2
12	2.01 br. d (11.4), 1.21	41.8	1.93, 1.03	41.8
13	–	43.9	–	43.8
14	1.12	58.5	0.97	58.3
15	1.53, 1.03	25.1	1.53, 1.03	25.1
16	1.18, 1.77	28.4	1.18, 1.77	29.2
17	1.27	58.3	1.50	57.8
18	0.71 s	12.6	0.74 s	12.6
19	4.28 d (12.1), 4.44 d (12.1)	63.2	4.28 d (11.8), 4.42 d (11.8)	63.2
20	1.45 m	35.1	1.43 m	37.1
21	0.98 d (6.3)	19.5	0.92 d (6.6)	19.2
22	1.29, 1.51	42.5	0.93, 1.29	37.3
23	2.22	67.9	1.23, 1.10	24.9
24	1.77	53.4	1.06	40.8
25	1.74 m	31.4	1.44 m	29.2
26	0.91 d (6.6)	22.7	0.88 d (6.5)	23.1
27	0.97 d (6.6)	17.7	0.88 d (6.5)	23.0
28	1.65,1.79	25.2	–	–
29	2.23, 2.93	57.4	–	–
$N(CH_3)$	2.32 s	40.9	–	–
CH_3CO	2.07	21.2	2.04 s	21.1
CH_3CO	–	171.3	–	171.3

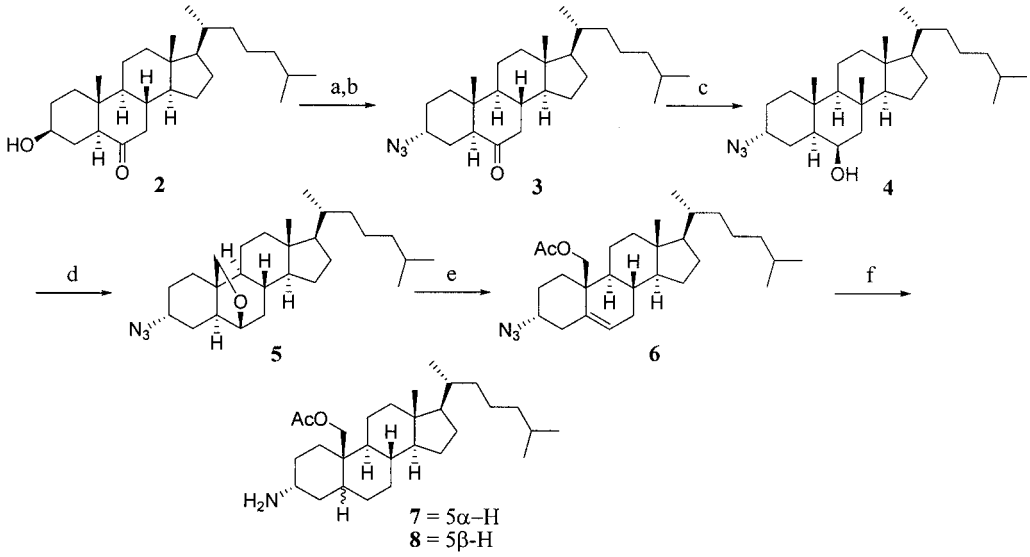
[a] The assignments were based on COSY, TOCSY, HMQC and HMBC experiments. [b] Coupling constants (in Hz) are given in parentheses.

signal (δ = 3.24 ppm, br. s). The characterization of the side chain was achieved by analysis of the COSY and HMBC data and by comparison of the spectroscopic data of related steroidal alkaloids.[6,7] Interpretation of the COSY data (Table 1) reveals a spin sequence from C-20 to C-29 that is indicative of a stigmastane skeleton with a nitrogen atom linking C-23 to C-29 to form a saturated pyrrolidine ring. In the HMBC spectrum the *N*-methyl signal at δ_H = 2.32 ppm shows ^{3}J correlation to both C-29 (δ_C = 57.4 ppm) and C-23 (δ_C = 67.9 ppm), which suggests that this group is on the side chain pyrrolidine ring. ROESY correlations between H-23 and Me-26 and Me-27 establish the *anti* relationship between H-23 and H-24, securing the relative configuration of the pyrrolidine ring of plakinamine I (**1**). Taken together, these data are consistent with a 19-acetoxytetrahydroplakinamine A structure for compound **1**, even though not all the ^{13}C NMR resonances could be unambiguously assigned, because of the scarcity of the material.

Compound **1** shows cytotoxic activity on human tumor MCF7 cell lines with an IC_{50} value of 3.9 μg/mL. The good cytotoxicity exhibited by the compound together with the need to obtain a reliable assignment of the ^{13}C NMR resonances for all carbon atoms of plakinamine I with an unprecedented steroidal backbone prompted us to undertake the synthesis of the model 19-acetoxy-3α-amino-5α-cholestane.

We selected the commercial available 3β-hydroxycholestan-6-one (**2**) as starting material and elaborated a six-step strategy (Scheme 1), which relies on the early introduction of the nitrogen function in order to reduce the synthetic steps and minimize the use of protecting groups.

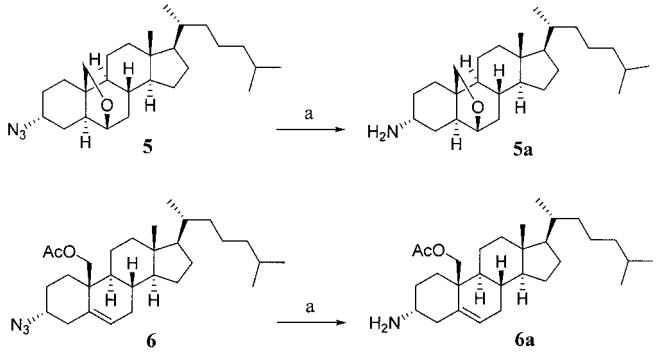
3β-Hydroxycholestan-6-one (**2**) was transformed in the α-azido derivative **3**, which was stereoselectively reduced to 6β-alcohol (**4**) with $LiAlH(tBuO)_3$. Oxidation[8] of the alcohol with lead tetraacetate/iodine (1:1) afforded 3α-azido-6β,19-epoxy-5α-cholestane (**5**) in 80% yield. Lewis acid cleavage of the tetrahydrofurane ring in **5** proved to be very sensitive to the reaction conditions: the preferential formation of the product **6** containing a Δ^5 double bond was observed only when freshly distilled acetic anhydride was used, whereas the presence of acetic acid caused the formation of the 3α-azido-6α,19-diacetoxycholestane derivative as a side product, which becomes the main product when an acetic anhydride/acetic acid 1:1 ratio is used. Finally, the hydrogenation in a Parr apparatus with H_2/Pd(OH) afforded 19-acetoxy-3α-amino-5α-cholestane (**7**) as a 4:6 diastereoisomeric mixture with the corresponding 5β-



Scheme 1. Reagents and Conditions: a) MsCl, ET_3N, dry Et_2O. b) NaN_3, dry DMSO, quantitative. c) $LiAlH(OtBu)_3$, dry THF, quantitative. d) $Pb(OAc)_4/I_2$, dry benzene, 80%. e) $BF_3{\cdot}OEt_2/Ac_2O$, dry ether, 70%. f) H_2 Pd(OH), 95% EtOH, 3 atm.

isomer **8**. Catalytic hydrogenation of 19-hydroxy steroidal derivatives has been extensively investigated[9,10] and this hydrogenation usually affords the 5β-H dihydro compound as major product, especially when a 3α function is present. The different stereochemical outcome observed in the hydrogenation of **6** could be explained by the influence of the α-nitrogen function. The two diastereoisomeric derivatives were separated by HPLC, and the structures determined by NMR analysis. ^{13}CNMR chemical shifts of all nuclei of the tetracyclic nucleus of **7** were almost superimposable with the corresponding nuclei of plakinamine I (**1**), thus allowing a reliable assignment of the NMR resonances of the natural compound.

Having gained synthetic access to a small group of steroidal derivatives with an oxygenated C-19 and a 3α-nitrogen function and also through the reductive transformations depicted in the Scheme 2, we decided to investigate the specific role of the above functionalities on the observed cytotoxicity. Table 2 reports the IC_{50} values (μg/mL) obtained for the synthetic derivatives **5–8**. The good cytotoxicity exhibited by compounds **7** and **8** relative to the natural plakinamine I indicates that the heterocyclic side chain plays only a marginal role, whereas the 3α-amino group (**5** versus **5a** and **6** versus **6a**) appears essential for the observed cytotoxicity.



Scheme 2. Reagents and Conditions: a) $(Ph)_3P$, THF/H_2O, 80%.

Table 2. IC_{50} values [μg/ml] of human breast tumor MCF7 cell lines of synthetic derivatives **5–8**.

Compound	IC_{50} [μg/ml]
5	>100
5a	12
6	>100
6a	3
7	3
8	20

Experimental Section

General Remarks: High-resolution ESI-MS spectra were performed with a Micromass QTOF Micro mass spectrometer. ESI-MS experiments were performed on an Applied Biosystem API 2000 triple-quadrupole mass spectrometer. NMR spectra were obtained on a Varian Mercury-400 and Inova-500 NMR spectrometers (^{1}H at 400 and 500 MHz, ^{13}C at 100 and 125 MHz) equipped with a Bruker X-32 hardware, δ (ppm), J in Hz, spectra referenced to $CHCl_3$ and CD_2HOD as internal standards. Optical rotations were measured on a Perkin–Elmer 243B polarimeter. HPLC was performed using a Waters Model 6000 A pump equipped with U6K injector and a differential refractometer, model 401. All reagents were commercially obtained (Aldrich, Fluka) at highest commercial quality and used without further purification except where noted. Benzene, ether, tetrahydrofurane, and triethylamine were distilled from calcium hydride immediately prior to use. All reactions were monitored by TLC on silica gel plates (Machery, Nagel). Crude products were purified by column chromatography on silica gel 70–230 mesh. All reactions were carried out under argon using flame-dried glassware.

Isolation of Plakinamine I (1): The sponge was collected at Efatè, Port Havanah, Vanuatu, South Pacific in 1996 and identified as

Corticium sp. (order Homosclerophorida, family Plakinidae) by John Hooper of Queensland Museum, South Brisbane, Australia. The animals were freeze-dried, and the lyophilized material (550 g) was extracted with methanol (3 × 2.5 L) and filtered. The extracts were combined and partitioned according to the modified Kupchan[11] procedure as follows. The methanol extract (88 g) was dissolved in a mixture of MeOH/H_2O containing 10% H_2O and partitioned against *n*-hexane. The water content (%v/v) of the MeOH extract was adjusted to 20% and 40%, and partitioned against CCl_4 and $CHCl_3$, respectively. The aqueous phase was concentrated to remove MeOH and then extracted with *n*BuOH. The bioactive chloroform extract (6.6 g) was fractionated in six runs by DCCC using $CHCl_3$/MeOH/H_2O (7:13:8) in the ascending mode (the lower phase was the stationary phase). Fractions (6 mL each) were collected and examined by TLC on SiO_2 with $CHCl_3$/MeOH/H_2O (80:18:2) as eluent. Fractions 15–16 were purified by HPLC on a C18 μ-Bondapak column (30 cm × 3.9 mm i.d.) eluting with MeOH/H_2O (95:5, containing 0.5% TEA) as eluent to yield 0.4 mg of pure compound **1.**

Plakinamine I (1): Colorless gum. $[\alpha]_D^{25}$ = –18.3 (*c* = 0.03, MeOH). ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$): *δ*, see Table 1. ESI-MS: *m*/*z* (%) = 501.6 (100) [M + H]$^+$. HRMS (ESI): $C_{32}H_{57}N_2O_2$: calcd. 501.4420; found 501.4412 [M + H]$^+$.

3α-Azido-5α-cholestan-6-one (3): Et_3N (7.6 mL, 54.6 mmol) and methanesulfonyl chloride (3.38 mL, 49.6 mmol) were sequentially added to a solution of 3β-hydroxy-5α-cholestan-6-one (**2**, 1.0 g, 2.48 mmol) in dry Et_2O. The reaction mixture was cooled to –10 °C for 10 min and was then stirred at room temperature for 3 h. The mixture was diluted with water and extracted with $CHCl_3$. The organic phase was dried ($MgSO_4$), and the solvents evaporated to dryness. The residue was chromatographed over silica gel (98:2, *n*-hexane/ethyl acetate) to afford the intermediate 3α-methylsulfoxy-5α-cholestan-6-one (1.19 g, quantitative). A solution of 3α-methylsulfoxy-5α-cholestan-6-one (500 mg, 1.04 mmol) in dry dimethylsulfoxide (30 mL) along with sodium azide (676 mg, 10.4 mmol) was stirred at 70 °C for 7 h. The reaction mixture was washed with water and extracted with ethyl acetate. The combined organic phases were dried with anhydrous magnesium sulfate and filtered, and the filtrate was concentrated to give pure 3α-azido-5α-cholestan-6-one **3 (**444 mg, quantitative) which was analyzed without further purification. $[\alpha]_D^{25}$ = –131.0 (*c* = 0.33, MeOH). ^{1}H NMR ($CDCl_3$, 400 MHz): *δ* = 0.68 (s, 3 H, 18-CH_3), 0.77 (s, 3 H, 19-CH_3), 0.92 (d, *J* = 7.0 Hz, 6 H, 26-CH_3, 27-CH_3), 0.95 (d, *J* = 6.5 Hz, 3 H, 21-CH_3), 3.98 (t, *J* = 2.6 Hz, 1 H, 3-CH) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): *δ* = 12.2 (CH_3), 12.8 (CH_3), 18.8 (CH_3), 21.3 (CH_2), 22.8 (CH_3), 23.0 (CH_3), 24.0 (CH_2), 24.1 (CH_2), 25.0 (CH_2), 25.1 (CH_2), 28.2 (CH_2), 28.2 (CH), 32.5 (CH_2), 35.9 (CH), 36.3 (CH_2), 38.2 (C), 39.7 (CH_2), 39.7 (CH_2), 41.6 (CH), 43.1 (CH_2), 43.2 (C), 51.0 (CH), 52.6 (CH), 56.3 (CH), 57.0 (CH), 57.4 (CH), 212.0 (C) ppm. ESI-MS: *m*/*z* (%) = 428.5 (100) [M + H]$^+$. HRMS (ESI): $C_{27}H_{46}N_3O$: calcd. 428.3641; found 428.3620 [M + H]$^+$.

3α-Azido-5α-cholestan-6β-ol (4): $LiAlH(OtBu)_3$ (366 mg, 1.41 mmol) was added to a solution of **3** (400 mg, 0.94 mmol) in dry THF, and the solution was stirred for 5 h at room temperature. Water was carefully added to decompose the excess of hydride. The mixture was treated with a saturated NaCl solution and extracted with $CHCl_3$. The combined organic phases were dried with anhydrous magnesium sulfate and filtered, and the filtrate was concentrated to give a crude product which was chromatographed on silica gel (97:3, hexane/ethyl acetate) to give pure **4 (**403 mg, quantitative). $[\alpha]_D^{25}$ = –8.5 (*c* = 0.17, MeOH). ^{1}H NMR ($CDCl_3$, 400 MHz): *δ* = 0.68 (s, 3 H, 18-CH_3), 0.92 (d, *J* = 7.0 Hz, 6 H, 26-CH_3, 27-CH_3), 0.96 (d, *J* = 6.5 Hz, 3 H, 21-CH_3), 1.04 (s, 3 H, 19-CH_3), 3.74 (br. s, 1 H, 6-CH), 3.99 (t, *J* = 2.6 Hz, 1 H, 3-CH) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): *δ* = 12.2 (CH_3), 15.2 (CH_3), 18.7 (CH_3), 20.5 (CH_2), 22.5 (CH_3), 22.8 (CH_3), 23.8 (CH_2), 24.2 (CH_2), 25.6 (CH_2), 28.0 (CH_2), 28.2 (CH), 30.1 (CH_2), 30.3 (CH_2), 34.6 (CH), 35.8 (CH_2), 35.9 (CH), 36.2 (C), 39.5 (CH_2), 39.8 (CH_2), 39.8 (CH), 42.6 (CH_2), 42.7 (C), 54.0 (CH), 56.1 (CH), 56.3 (CH), 58.5 (CH), 71.8 (CH) ppm. ESI-MS: *m*/*z* (%) = 430.4 (100) [M + H]$^+$. HRMS (ESI): $C_{27}H_{48}N_3O$: calcd. 430.6896; found 430.6776 [M + H]$^+$.

3α-Azido-6β,19-epoxy-5α-cholestane (5): **L**ead tetraacetate (2.06 mg, 4.65 mmol) and iodine (1.17 mg, 4.65 mmol) were added to a solution of **4** (400 mg, 0.93 mmol) in dry benzene, and the reaction mixture was stirred at room temperature for 2 h and then quenched by addition of a 10% solution of potassium iodide (100 mL) and a 15% solution of sodium sulphite (100 mL) until a yellow solution was formed. The solution was extracted with diethyl ether, and the combined organic phases were dried with anhydrous magnesium sulfate and filtered, and the filtrate was concentrated to give a crude product which was chromatographed on silica gel (99.5:0.5, hexane/ethyl acetate) to give pure **5** (317 mg, 80%). $[\alpha]_D^{25}$ = –6.1 (*c* = 0.42, MeOH). ^{1}H NMR ($CDCl_3$, 400 MHz): *δ* = 0.71 (s, 3 H, 18-CH_3), 0.92 (d, *J* = 7.0 Hz, 6 H, 26-CH_3, 27-CH_3), 0.94 (d, *J* = 6.5 Hz, 3 H, 21-CH_3), 3.68 (d, *J* = 8.6 Hz, 1 H, 19-CHa), 3.73 (d, *J* = 8.6 Hz, 1 H, 19-CHb), 3.93 (d, *J* = 5.1 Hz, 1 H, 6-CH) 3.96 (t, *J* = 2.6 Hz, 1 H, 3-CH) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): *δ* = 12.6 (CH_3), 18.8 (CH_3), 22.0 (CH_2), 22.8 (CH_3), 23.0 (CH_3), 23.8 (CH_2), 24.0 (CH_2), 25.6 (CH_2), 28.2 (CH_2), 28.2 (CH), 28.5 (CH_2), 29.9 (CH), 34.9 (CH), 36.0 (CH), 36.4 (CH_2), 37.6 (CH_2), 39.7 (CH_2), 39.8 (CH_2), 40.1 (C), 43.0 (C), 44.1 (CH), 54.2 (CH), 55.6 (CH), 56.3 (CH), 57.4 (CH), 70.0 (CH_2), 81.1 (CH) ppm. ESI-MS: *m*/*z* (%) = 428.2 (100) [M + H]$^+$. HRMS (ESI): $C_{27}H_{46}N_3O$: calcd. 428.3641; found 428.5531 [M + H]$^+$.

19-Acetoxy-3α-azidocholest-5-ene (6): $BF_3{\cdot}OEt_2$ (594 μL) was added to a solution of acetic anhydride (4.0 mL) in dry ether. After stirring for 5 min, a solution of **5** (300 mg) in dry ether (8.0 mL) was added. The mixture was stirred for 8 h at room temperature and was then treated with a saturated $NaHCO_3$ solution, extracted with diethyl ether, and the combined organic phases were dried with anhydrous magnesium sulfate and filtered. The filtrate was concentrated to give a crude product which was chromatographed on silica gel (92:8, hexane/ethyl acetate) to give **6** (259 mg, 70%). $[\alpha]_D^{25}$ = 26.5 (*c* = 0.60, MeOH). ^{1}H NMR ($CDCl_3$, 400 MHz): *δ* = 0.69 (s, 3 H, 18-CH_3), 0.86 (d, *J* = 6.8 Hz, 6 H, 26-CH_3, 27-CH_3), 0.91(d, *J* = 6.8 Hz, 3 H, 21-CH_3), 2.04 (s, 3 H, CH_3COO), 3.91 (m, 1 H, 3-CH), 3.96 (d, *J* = 11.4 Hz, 1 H, 19-CHa), 4.45 (d, *J* = 11.4 Hz, 1 H, 19-CHb), 5.64 (t, *J* = 3.0 Hz, 1 H, 6-CH) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): *δ* = 11.9 (CH_3), 18.7 (CH_3), 21.1 (CH_2), 21.4 (CH_3), 22.5 (CH_3), 22.8 (CH_3), 23.8 (CH_2), 24.1 (CH_2), 26.3 (CH_2), 28.0 (CH_2), 28.2 (CH), 28.3 (CH_2), 30.1 (CH_2), 31.2 (CH_2), 32.8 (CH_2), 35.7 (CH), 36.4 (CH_2), 39.5 (CH_2), 39.8 (CH_2), 40.3 (C), 42.4 (C), 50.0 (CH), 56.1 (CH), 57.2 (CH), 57.9 (CH), 64.4 (CH_2), 127.3 (CH), 132.8 (CH), 171.0 (C) ppm. ESI-MS: *m*/*z* (%) = 470.3 (100) [M + H]$^+$. HRMS (ESI): $C_{29}H_{48}N_3O_2$: calcd. 470.3747; found 470.3784 [M + H]$^+$.

19-Acetoxy-3α-aminocholestane (7 and 8): A solution of **6** (50 mg) in ethanol (15 mL) was hydrogenated in a Parr apparatus (3 atm, room temperature) in the presence of palladium hydroxide (Degussa type E101). The catalyst was removed by filtration, and the filtrate was evaporated to dryness. The residue was chromatographed on silica gel with $CHCl_3$ to give 40 mg of a mixture of 5α-

and 5β-dihydro derivatives. The mixture was further fractionated by HPLC on a Phenomenex LUNA 3-μ C18 column (15 cm × 4.6 mm i.d.) eluting with MeOH (0.5% TEA) to yield pure compound **7** (10 mg) along with diastereoisomeric compound **8** (12 mg).

19-Acetoxy-3α-amino-5α-cholestane (7): $[\alpha]_D^{25}$ = –25.0 (*c* = 0.09, MeOH). ^{1}H NMR (CD_3OD, 400 MHz): *δ*, see Table 1. ^{13}C NMR ($CDCl_3$, 100 MHz): *δ*, see Table 1. ESI-MS: *m/z* (%) = 446.6 (100) $[M + H]^+$. HRMS (ESI): $C_{29}H_{52}NO2$: calcd. 446.3998; found 446.3922 $[M + H]^+$.

19-Acetoxy-3α-amino-5β-cholestane (8): $[\alpha]_D^{25}$ = +18.5 (*c* = 0.08, MeOH). ^{1}H NMR (CD_3OD, 400 MHz): *δ* = 0.64 (s, 3 H, 18-CH_3), 0.84 (d, *J* = 6.6 Hz, 6 H, 26-CH_3, 27-CH_3), 0.89 (d, *J* = 6.7 Hz, 3 H, 21-CH_3), 2.01 (s, 3 H, 3-CH_3COO), 3.22 (t, *J* = 2.6 Hz, 1 H, 3-CH), 4.01 (d, *J* = 11.1 Hz, 1 H, 19-CHa), 4.34 (d, *J* = 11.1 Hz, 1 H, 19-CHb) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): *δ* = 12.5 (CH_3-18), 19.8 (CH_3-21), 20.9 (CH_3CO), 22.0 (CH_2-11), 22.9 (CH_3-26), 23.1 (CH_3-27), 24.9 (CH_2-23), 25.1 (CH_2-15), 27.1 (CH_2-1), 27.7 (CH_2-2), 29.1 (CH_2-25), 29.3 (CH_2-16), 29.3 (CH_2-6), 30.5 (CH_2-7), 36.8 (CH_2-4), 37.1 (CH-20), 37.2 (CH-8), 37.3 (CH_2-22), 39.0 (C-10), 40.7 (CH_2-24), 41.3 (CH-5), 41.7 (CH_2-12), 43.8 (C-13), 49.6 (CH-9), 51.9 (CH_2-3), 57.7 (CH-17), 58.2 (CH-14), 68.5 (CH_2-19), 171.3 (CH_3*C*O) ppm. ESI-MS: *m/z* (%) = 446.4 (100) $[M + H]^+$. HRMS (ESI): $C_{29}H_{52}NO_2$: calcd. 446.3998; found 446.3956 $[M + H]^+$.

Biological Assays: The cytotoxicity of the molecules was estimated on human breast cancer cells (MCF7).[11] Cell lines were cultured in DMEM F12 (BioWhittaker, Cambrex, Verviers, Belgium) with 5% fetal bovine serum (BioWhittaker). When the cells were confluent, they were trypsinized (Trypsin EDTA, BioWhittaker) and seeded (10^4 cells/well) on 96-well plates. The molecules were added at various concentrations, and cell growth was estimated by [^{3}H]-Hypoxanthine (ICN Chemicals, Paris, France) incorporation after a 48-h incubation. IC50 values (concentration inhibiting 50% of the cell growth) were then determined graphically on concentration versus inhibition percentage curves.[12] For each molecule, every concentration was tested three times, and three independent experiments were performed. The control consisted of doxorubicine (2 μM and 4 μM, Sigma).

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