SYNTHESIS OF 24-(PIPERIDIN-1-YL, MORPHOLIN-4-YL AND 4-METHYLPIPERAZIN-1-YL)-5β-CHOLAN-3α-OLS AND FOUR HYDROXYLATED 23-(4,5-DIHYDROIMIDAZOL-2-YL)-24-NOR-5β-CHOLANES

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Lithocholic (**1a**), chenodeoxycholic (**1b**), deoxycholic (**1c**) and cholic acid (**1d**) were used for the synthesis of the title compouds. Reactions of *O*-acetyllithocholic acid chloride with piperidine, morpholine and 1-methylpiperazine gave the corresponding amides **2a–2c** which were reduced with lithium aluminium hydride to 24-(piperidin-1-yl)-5β-cholan-3α-ol (**3a**) and analogues **3b** and **3c**. Heating of the acids **1a–1d** with ethylenediamine monotosylate afforded 23-(4,5-dihydroimidazol-2-yl)-24-nor-5β-cholan-3α-ol (**4a**) and analogues **4b–4d**. Compound **4a** was similarly obtained from 3α-acetoxy-24-nor-5β-cholane-23-carbonitrile. Identity of the products was corroborated by spectral characterization. Some of the products (in the form of salts) were tested for cancerostatic and antimicrobial activities *in vitro* with partially promising results.

Key words: 24-Heterocyclylcholanes; Synthesis.

Derivative of bile acids substituted with a heterocyclic moiety in the side chain represent a new class of hypolipemic, cholelitholytic and antibacterial agents[1–4]. As a continuation of our previous synthetic study[5], we recently have prepared from the bile acids **1a–1e** several steroidal side chain heterocycles derived from 5β-cholan-3α-ol and from polyhydroxylated 24-nor-5β-cholanes; the description of this work forms the subject of the present paper.

O-Acetyllithocholic acid chloride[5], prepared from the acid **1a**, was reacted at room temperature with piperidine, morpholine and 1-methylpiperazine in benzene under for-

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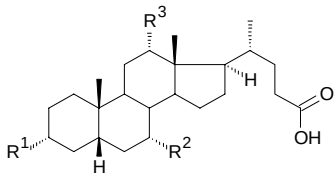
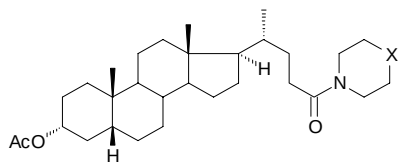
mation of the amides **2a–2c** which were reduced with lithium aluminium hydride in tetrahydrofuran to the title amines **3a–3c** under simultaneous deacetylation.

The Oxley and Short method[6,7] of synthesis of 4,5-dihydroimidazoles from nitriles was applied to 3α-acetoxy-24-nor-5β-cholane-23-carbonitrile[8] which was heated with ethylenediamine monotosylate to 200–230 °C and afforded under simultaneous deacetylation the steroidal 4,5-dihydroimidazole **4a** in a yield of 78%. It was found that the bile acids **1a–1d** with free hydroxyl groups reacted similarly (for analogy, *cf.* ref.[9]): their heating with ethylenediamine monotosylate resulted in homogeneous melts and the reactions proceeded quickly without stirring. The products were the 4,5-dihydroimidazoles **4a–4d**. It was observed that the 7α-hydroxyl group was easily eliminated at temperatures higher than 230 °C and unseparable mixtures of products were formed (presence of double bonds in the crude products was confirmed by ^{1}H NMR and ^{13}C NMR spectra); in cases of reactions of chenodeoxycholic (**1b**) and cholic (**1d**) acid it was thus advisable to work at temperatures not exceeding 225 °C.

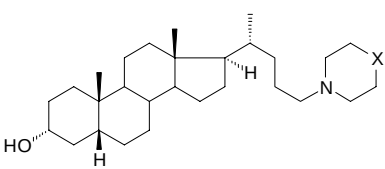
For increasing the hydrophilicity of the products, they were transformed to salts



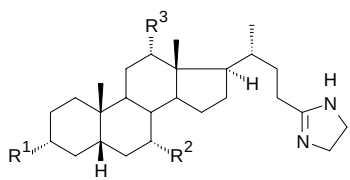
1	R^1	R^2	R^3
a	OH	H	H
b	OH	OH	H
c	OH	H	OH
d	OH	OH	OH
e	OAc	H	H



2	X
a	$-CH_2-$
b	$-O-$
c	$-N(CH_3)-$



3	X
a	$-CH_2-$
b	$-O-$
c	$-N(CH_3)-$



4	R^1	R^2	R^3
a	OH	H	H
b	OH	OH	H
c	OH	H	OH
d	OH	OH	OH

(hydrochlorides, methanesulfonates). All of the products were characterized by spectral data. IR spectra of the amides **2a–2c** show clearly a band at 1 621, 1 631, and 1 632 cm^{-1}, respectively, corresponding to the carboxamide. The IR spectra of the dihydroimida-

zoles **4a–4d** are typical by strong absorption bands in the region of 1 610–1 630 cm^{-1}, corresponding to the C=N vibration. Chemical shifts of the signals of the methylene groups in positions 2 and 6 in the heterocyclic moiety of amides **2a** and **2c** and of the methylene groups in positions 3 and 5 of the amide **2b** in their ^1H NMR spectra differ due to the proximity of the carbonyl group: they appear as two differentiated triplets near δ 3.5 ppm. With amines **3a–3c**, these methylene groups appear as a single triplet. The methylene groups in positions 4 and 5 of the dihydroimidazole ring, due to tautomeric proton exchange, are equivalent and form a singlet in the ^1H NMR, as well as in the ^{13}C NMR spectra. The dominant peaks in the mass spectra of the amides, amines and imidazolines may be explained by the rearrangement and cleavage.

The salts of compounds **3a–3c** and **4a–4d** (concentration of 10 μmol l^{-1}) were tested for inhibition of growth of leukemia and carcinoma cells *in vitro*. The growth of the mouse leukemia L1210 cells was inhibited by compounds **3a** (hydrochloride) and **3c** (hydrochloride or methanesulfonate) to 12% of the control value, by **3b** (methanesulfonate) to 70%. The growth of the murine L929 cells was totally inhibited by hydrochlorides and methanesulfonates of **3a** and **3c**, by **3b** (hydrochloride and methanesulfonate) to 33 and 47%, respectively. The growth of the human cervix carcinoma HeLa S3 cells was inhibited by **3c** (hydrochloride and methanesulfonate) to 19 and 32%, respectively. Hydrochlorides of compounds **3a–3c** were also subjected to *in vitro* anti-HIV screening but were found inactive. Methanesulfonates of compounds **4a–4d** were tested for antimicrobial activity *in vitro* (approximate IC_{50} values in μg/ml for inhibition of growth of *Escherichia coli* given): **4a**, 70; **4b**, 35; **4c**, 46; **4d**, 42.

EXPERIMENTAL

Melting points were determined on a Kofler block and were not corrected. Optical rotations were measured at 20 °C with the automatic instrument ETL-NPL (Bendics–Ericson) with the accuracy of ±2 °C, solvent chloroform (unless stated otherwise). IR spectra (ν, cm^{-1}) were taken in chloroform with a Perkin–Elmer 684 instrument, unless otherwise stated. ^1H and ^{13}C NMR spectra (δ, ppm; *J*, Hz) were recorded on a Varian Unity 200 spectrometer (200 MHz for ^1H and 50.3 MHz for ^{13}C). Deuteriochloroform (unless otherwise stated) was used as solvent and tetramethylsilane as internal standard. Mass spectra were run with an Incos 50 instrument, energy of the ionizing electrons 70 eV, ionizing current 200 mA, temperature of the ionic source 150 °C. The extracts were dried with Na_2SO_4.

General Procedure for Preparation of Amides **2a–2c**

A solution of *O*-acetyllithocholic acid[10] (**1e**, 3.0 g, 7.2 mmol) in $SOCl_2$ (15 ml) was allowed to stand at room temperature for 4 h, the mixture was evaporated *in vacuo* to dryness and then twice with benzene (15 ml). The residue was dissolved in benzene (20 ml), the stirred solution of the acid chloride was treated with the corresponding amine and the clear solution obtained was allowed to stand for 2 h at room temperature. The mixture was evaporated *in vacuo*, the residue dissolved in chloroform (250 ml), the solution was washed with water and dried. Evaporation and crystallization of the residue from a mixture hexane–ether (9 : 1) gave the product.

N-(O-Acetyllithocholyl)piperidine (**2a**). Reaction with piperidine (3.0 ml, 30.3 mmol) afforded 3.4 g (97%) of **2a**, m.p. 175–178 °C; $[\alpha]_D$ +44.4° (*c* 0.34). IR spectrum: 1 718 (C=O of acetyl); 1 621 (CON); 1 252 and 1 024 (C–O of ester). ^{1}H NMR spectrum: 0.65 s, 3 H (3 × H-18); 0.93 s, 3 H (3 × H-19); 0.94 d, 3 H, *J* = 5.7 (3 × H-21); 2.03 s, 3 H ($COCH_3$); 2.27 m, 2 H (2 × H-23); 3.39 t, 2 H and 3.54 t, 2 H, *J* = 5.4 (2 × H-2 and 2 × H-6 of piperidinyl); 4.72 m, 1 H (H-3β). ^{13}C NMR spectrum: 12.0 (C-18), 18.3 (C-21), 20.8 (C-11), 21.4 (CH_3 of acetyl), 23.3 (C-19), 24.2 (C-15), 24.6 (C-4 of piperidinyl), 25.5 (C-3 and C-5 of piperidinyl), 26.2 (C-3), 26.6 (C-7), 27.0 (C-6), 28.1 (C-16), 31.5 (C-22), 32.2 (C-4), 34.5 (C-10), 35.0 (C-1), 35.3 (C-20), 35.7 (C-8), 40.1 (C-12), 40.4 (C-9), 41.8 (C-5), 42.7 (C-13), 46.7 (C-2 and C-6 of piperidinyl), 56.2 (C-17), 56.5 (C-14), 74.0 (C-3), 170.5 (24-CO), 171.8 (CO of acetyl). Mass spectrum (*m/z*, %): 485 (M^+, $C_{31}H_{51}NO_3$, 0.1), 426 (6.4), 215 (6.5), 168 (7.7), 161 (4.5), 140 (54.9), 127 (100), 112 (14.1), 107 (12.7), 95 (14.1), 93 (13.4), 86 (12.7), 84 (16.2), 81 (14.9), 69 (12.4), 67 (11.3), 55 (15.5), 43 (15.5). For $C_{31}H_{51}NO_3$ (485.8) calculated: 76.65% C, 10.58% H, 2.88% N; found: 76.43% C, 10.75% H, 2.70% N.

N-(O-Acetyllithocholyl)morpholine (**2b**). Reaction with morpholine (3.0 ml, 34.4 mmol) afforded 3.4 g (97%) of **2b**, m.p. 194–196 °C (aqueous acetone); $[\alpha]_D$ +44.4° (*c* 0.27). IR spectrum: 1 719 (C=O of acetyl); 1 632 (CON); 1 251 and 1 027 (C–O of ester); 1 115 (C–O–C of morpholine). ^{1}H NMR spectrum: 0.65 s, 3 H (3 × H-18); 0.93 s, 3 H (3 × H-19); 0.94 d, 3 H, *J* = 5.7 (3 × H-21); 2.03 s, 3 H ($COCH_3$); 2.29 m, 2 H (2 × H-23); 3.48 m, 2 H (H-2 and H-6 of morpholinyl); 3.65 m, 6 H (H-2, 2 × H-3, 2 × H-5 and H-6 of morpholinyl); 4.72 m, 1 H (H-3β). ^{13}C NMR spectrum: 12.0 (C-18), 18.4 (C-21), 20.8 (C-11), 21.4 (CH_3 of acetyl), 23.3 (C-19), 24.2 (C-15), 26.3 (C-2), 26.6 (C-7), 27.0 (C-6), 28.2 (C-16), 31.3 (C-22), 32.2 (C-4), 34.5 (C-10), 35.0 (C-1), 35.6 (C-20), 35.7 (C-8), 40.1 (C-12), 40.4 (C-9), 41.8 (C-5), 42.7 (C-13), 46.0 (C-2 and C-6 of morpholinyl), 56.0 (C-17), 56.5 (C-14), 66.6 and 66.9 (C-3 and C-5 of morpholinyl), 74.3 (C-3), 170.5 (CO of acetyl), 172.2 (CON). Mass spectrum (*m/z*, %): 487 (M^+, $C_{30}H_{49}NO_4$, 0.1), 428 (4.2), 341 (1.6), 257 (2.0), 230 (1.8), 215 (5.8), 201 (3.3), 187 (2.7), 175 (3.9), 173 (3.5), 170 (7.1), 161 (6.1), 158 (5.2), 142 (44.4), 129 (100), 121 (12.0), 118 (11.0), 114 (8.2), 107 (22.5), 105 (15.5), 95 (22.5), 93 (26.8), 87 (33.8), 81 (28.2), 67 (21.8), 57 (23.9), 55 (28.2), 43 (28.3). For $C_{30}H_{49}NO_4$ (487.7) calculated: 73.88% C, 10.13% H, 2.87% N; found: 73.69% C, 10.30% H, 3.00% N.

1-(O-Acetyllithocholyl)-4-methylpiperazine (**2c**). Reaction with 1-methylpiperazine (3.0 ml, 27.0 mmol) afforded 3.2 g (89%) of **2c**, m.p. 130–135 °C (aqueous acetone), $[\alpha]_D$ +42.7° (*c* 0.25). IR spectrum: 1 710 (C=O of acetyl); 1 631 (CON); 1 252, 1 026 and 1 002 (C–O of ester and C–N). ^{1}H NMR spectrum: 0.65 s, 3 H (3 × H-18); 0.93 s, 3 H (3 × H-19); 0.94 d, 3 H, *J* = 5.7 (3 × H-21); 2.03 s, 3 H ($COCH_3$); 2.29 m, 2 H (2 × H-23); 2.31 s, 3 H (NCH_3); 2.38 m, 4 H (2 × H-3 and 2 × H-5 of piperazinyl); 3.48 t, 2 H and 3.68 t, 2 H, *J* = 5.05 (2 × H-2 and 2 × H-6 of piperazinyl); 4.72 m, 1 H (H-3β). ^{13}C NMR spectrum: 12.0 (C-18), 18.4 (C-21), 20.7 (C-11), 21.4 (CH_3 of acetyl), 23.2 (C-19), 24.1 (C-15), 26.2 (C-2), 26.5 (C-7), 26.9 (C-6), 28.2 (C-16), 30.2 (C-23), 31.3 (C-22), 32.1 (C-4), 34.5 (C-10), 34.9 (C-1), 35.6 (C-8), 35.7 (C-20), 40.0 (C-12), 40.3 (C-9), 41.3 and 45.4 (C-2 and C-6 of piperazinyl), 41.8 (C-5), 42.6 (C-13), 45.9 (NCH_3), 54.6 and 55.1 (C-3 and C-5 of piperazinyl), 56.0 (C-17), 56.4 (C-14), 74.2 (C-3), 170.4 (CON), 171.9 (CO of acetyl). Mass spectrum (*m/z*, %): 500 (M^+, $C_{31}H_{52}N_2O_3$, 3.5), 485 (2.9), 215 (2.8), 183 (5.3), 171 (2.0), 155 (66.2), 142 (48.6), 127 (7.8), 121 (4.2), 119 (4.2), 112 (7.0), 109 (4.9), 107 (9.2), 105 (7.0), 101 (29.6), 99 (29.6), 98 (16.9), 95 (9.2), 93 (11.3), 91 (7.0), 85 (45.1), 83 (60.6), 72 (26.8), 70 (100), 58 (76.1), 57 (49.3), 56 (12.1), 55 (12.1), 44 (12.1), 43 (28.1). For $C_{31}H_{52}N_2O_3$ (500.8) calculated: 74.35% C, 10.47% H, 5.59% N; found: 74.58% C, 10.29% H, 5.32% N.

General Procedure for Preparation of Amines **3a–3c**

Amide **2a–2c** (3.0 g) was dissolved in tetrahydrofuran (50 ml) and $LiAlH_4$ (0.50 g, 13.2 mmol) was added to the stirred solution over 5 min. The mixture was refluxed for 10 h and after cooling was decomposed with methanol. The solid was filtered off, the filtrate was evaporated to volume of 20 ml and the residue was diluted with chloroform (200 ml). The solution was washed with water, dried and evaporated. The residue was dissolved in benzene (200 ml) and the solution was saturated for 1 h with gaseous HCl. The precipitated solid was filtered, washed with benzene and dried. It was dissolved in methanol (15 ml), the solution was made alkaline with 10% aqueous NaOH and extracted with chloroform. The extract was washed with water, dried and evaporated. Crystallization from ether afforded the product.

*24-(Piperidin-1-yl)-5*β*-cholan-3*α*-ol* (**3a**). Amide **2a** (3.0 g, 6.2 mmol) gave 1.86 g (70%) of amine **3a**, m.p. 134–135 °C, $[\alpha]_D$ +29.8° (*c* 0.34). IR spectrum: 3 609 (O–H); 1 031 (C–N); 1 011 (C–O). ^{1}H NMR spectrum: 0.64 s, 3 H (3 × H-18); 0.91 d, 3 H, *J* = 6.0 (3 × H-21); 0.92 s, 3 H (3 × H-19); 2.24 t, 2 H, *J* = 7.7 (2 × H-24); 2.37 m, 4 H (2 × H-2 and 2 × H-6 of piperidinyl); 2.67 s, 1 H (OH); 3.60 m, 1 H (H-3β). ^{13}C NMR spectrum: 11.9 (C-18), 18.6 (C-21), 20.7 (C-11), 23.2 (C-23), 23.3 (C-19), 24.2 (C-15), 24.1 (C-4 of piperidinyl), 25.8 (C-3 and C-5 of piperidinyl), 26.4 (C-7), 27.2 (C-6), 30.5 (C-2), 33.8 (C-22), 34.5 (C-10), 35.4 (C-1), 35.7 (C-20), 35.8 (C-8), 36.4 (C-4), 40.1 (C-12), 40.4 (C-9), 42.1 (C-5), 42.6 (C-13), 54.5 (C-2 and C-6 of piperidinyl), 56.1 (C-17), 56.4 (C-14), 60.1 (C-24), 71.4 (C-3). Mass spectrum (*m/z*, %): 429 (M^+, $C_{29}H_{51}NO$, 2.5), 414 (1.2), 154 (1.3), 149 (1.1), 124 (6.9), 98 (100), 86 (16.6), 69 (4.2), 55 (6.6), 41 (4.2). For $C_{29}H_{51}NO$ (429.7) calculated: 81.06% C, 11.96% H, 3.26% N; found: 81.21% C, 12.13% H, 3.04% N.

Hydrochloride, m.p. 230–231 °C (methanol). For $C_{29}H_{52}ClNO$ (466.2) calculated: 74.72% C, 11.24% H, 7.60% Cl, 3.00% N; found: 74.67% C, 11.29% H, 7.79% Cl, 2.85% N.

Methanesulfonate, m.p. 259–261 °C (methanol). For $C_{30}H_{55}NO_4S$ (525.8) calculated: 68.53% C, 10.54% H, 2.66% N, 6.10% S; found: 68.68% C, 10.69% H, 2.43% N, 5.89% S.

*24-(Morpholin-4-yl)-5*β*-cholan-3*α*-ol* (**3b**). Amide **2b** (3.0 g, 6.2 mmol) gave 1.97 g (75%) of **3b**, m.p. 107–111 °C (ether), $[\alpha]_D$ +26.6° (*c* 0.32). IR spectrum: 3 610 (O–H); 1 115 (C–O–C of morpholinyl); 1 031 (C–N); 1 011 (C–O). ^{1}H NMR spectrum: 0.64 s, 3 H (3 × H-18); 0.91 d, 3 H, *J* = 6.3 (3 × H-21); 0.92 s, 3 H (3 × H-19); 2.29 t, 2 H, *J* = 7.6 (2 × H-23); 2.44 t, 4 H, *J* = 4.5 (2 × H-2 and 2 × H-6 of morpholinyl); 3.61 m, 1 H (H-3β); 3.73 t, 4 H, *J* = 4.6 (2 × H-3 and 2 × H-5 of morpholinyl). ^{13}C NMR spectrum: 12.0 (C-18), 18.6 (C-21), 20.8 (C-11), 23.0 (C-23), 23.3 (C-19), 24.2 (C-15), 26.4 (C-7), 27.2 (C-6), 28.3 (C-16), 30.5 (C-2), 33.6 (C-22), 34.5 (C-10), 35.3 (C-1), 35.6 (C-20), 35.8 (C-8), 36.4 (C-4), 40.1 (C-12), 40.4 (C-9), 42.0 (C-5), 42.6 (C-12), 53.7 (C-2 and C-6 of morpholinyl), 56.0 (C-17), 56.5 (C-14), 59.7 (C-24), 66.9 (C-3 and C-5 of morpholinyl), 71.6 (C-3). Mass spectrum (*m/z*, %): 431 (M^+, $C_{28}H_{49}NO_2$, 1.8), 416 (1.0), 156 (1.60), 126 (7.8), 100 (100), 98 (3.4), 95 (3.9), 93 (3.9), 87 (30.5), 81 (4.6), 69 (4.2), 66 (3.9), 57 (3.8), 56 (3.8), 55 (6.5), 43 (4.2), 41 (4.6). For $C_{28}H_{49}NO_2$ (431.7) calculated: 77.90% C, 11.44% H, 3.24% N; found: 77.76% C, 11.56% H, 3.10% N.

Hydrochloride, m.p. 228–230 °C (methanol). For $C_{28}H_{50}ClNO_2$ (468.2) calculated: 71.84% C, 10.76% H, 7.57% Cl, 2.99% N; found: 71.98% C, 10.52% H, 7.62% Cl, 3.20% N.

Methanesulfonate, m.p. 280–281 °C (methanol). For $C_{29}H_{53}NO_5S$ (527.8) calculated: 65.99% C, 10.12% H, 2.65% N, 6.07% S; found: 66.13% C, 10.00% H, 2.78% N, 6.21% S.

*24-(4-Methylpiperazin-1-yl)-5*β*-cholan-3*α*-ol* (**3c**). Amide **2c** (3.0 g, 6.0 mmol) gave 1.88 g (71%) of **3c**, m.p. 109–110 °C (ether). $[\alpha]_D$ +24.1° (*c* 0.29). IR spectrum: 3 607 (O–H); 1 031 (C–N); 1 011 (C–O). ^{1}H NMR spectrum: 0.64 s, 3 H (3 × H-18); 0.91 d, 3 H, *J* = 5.8 (3 × H-21); 0.92 s, 3 H (3 × H-19); 2.28 s, 3 H (N-CH_3); 2.28 m, 2 H (2 × H-24); 2.47 s, 8 H (2 × H-2, 2 × H-3, 2 × H-5 and 2 × H–6 of piperazinyl); 2.90 s, 1 H (OH); 3.60 m, 1 H (H-3β). ^{13}C NMR spectrum: 11.9 (C-18), 18.6 (C-21), 20.7 (C-11), 23.4 (C-23), 23.5 (C-19), 24.1 (C-15), 26.4 (C-7), 27.1 (C-6), 28.2 (C-16), 30.5 (C-2),

33.6 (C-22), 34.5 (C-10), 35.3 (C-1), 35.6 (C-20), 35.7 (C-8), 36.4 (C-4), 40.1 (C-12), 40.3 (C-9), 42.0 (C-5), 42.6 (C-13), 45.9 (N-CH_3), 53.0 and 55.0 (C-2, C-3, C-5 and C-6 of piperazinyl), 56.0 (C-17), 56.4 (C-14), 59.2 (C-24), 71.3 (C-3). Mass spectrum (*m/z*, %): 444 (M^+, $C_{29}H_{52}N_2O$, 2.5), 429 (1.1), 400 (0.4), 388 (0.4), 209 (0.8), 171 (0.7), 169 (1.6), 161 (0.6), 152 (0.6), 139 (5.9), 113 (100), 100 (30.6), 81 (4.6), 70 (27.0), 58 (8.5), 55 (7.2), 43 (9.1). For $C_{29}H_{52}N_2O$ (444.7) calculated: 78.32% C, 11.79% H, 6.30% N; found: 78.59% C, 11.65% H, 6.42% N.

Hydrochloride, m.p. 229–230 °C (methanol). For $C_{29}H_{53}ClN_2O$ (481.2) calculated: 72.38% C, 11.10% H, 7.37% Cl, 5.82% N; found: 72.52% C, 11.23% H, 7.21% Cl, 5.71% N.

Methanesulfonate, m.p. 258–259 °C (methanol). For $C_{30}H_{56}N_2O_4S$ (540.9) calculated: 66.62% C, 10.44% H, 5.18% N, 5.93% S; found: 66.81% C, 10.33% H, 5.33% N, 6.20% S.

General Procedure for Preparation of Imidazolines **4a–4d**

A mixture of acid **1a–1d** (1.0 mmol) and ethylenediamine monotosylate (700 mg, 30 mmol) was heated under nitrogen to 220–225 °C for 45 min. The cooled melt was treated with 5% aqueous Na_2CO_3 (20 ml) and after standing for 30 min, the precipitated solid was filtered, washed with water and dried. The product was dissolved in methanol and the solution was filtered through a layer of Dowex 2 × 8 (100–200 mesh) in the HO^- cycle. The filtrate was evaporated and the residue was recrystallized.

*23-(4,5-Dihydroimidazol-2-yl)-24-nor-5*β*-cholan-3*α*-ol* (**4a**). Acid **1a** (380 mg) gave 350 mg (87%) of **4a**, m.p. 217–220 °C (chloroform–methanol 1 : 9), $[\alpha]_D$ +20.4° (*c* 0.25). IR spectrum: 3 606 (OH); 3 443 (NH); 1 616 (C=N); 1 033 (C–O, C–N). ^{1}H NMR spectrum (hexadeuteriodimethyl sulfoxide): 0.64 s, 3 H (3 × H-18), 0.90 s, 3 H (3 × H-19); 0.93 d, 3 H, *J* = 8.1 (3 × H-21); 3.48 m, 1 H (H-3β); 3.48 s, 4 H (2 × H-4 and 2 × H-5 of dihydroimidazolyl); 3.96 bs, 2 H (OH and NH). ^{13}C NMR spectrum (hexadeuteriodimethyl sulfoxide): 11.7 (C-18), 18.1 (C-21), 20.4 (C-11), 23.1 (C-19), 23.8 (C-15), 25.6 (C-23), 26.1 (C-7), 26.9 (C-6), 27.8 (C-16), 30.1 (C-2), 32.4 (C-22), 34.2 (C-10), 35.1 (C-1), 35.2 (C-20), 35.4 (C-8), 36.1 (C-4), 39.9 (C-9), 40.1 (C-12), 41.6 (C-5), 42.3 (C-13), 49.2 (C-4 and C-5 of dihydroimidazolyl), 55.5 (C-17), 56.1 (C-14), 70.1 (C-3), 168.4 (C-2 of dihydroimidazolyl). Mass spectrum (*m/z*, %): 400 (M^+, $C_{26}H_{44}N_2O$, 2.3), 385 (10.0), 343 (18.3), 151 (2.3), 137 (2.8), 125 (10.6), 107 (4.2), 105 (4.2), 98 (23.9), 97 (78.8), 95 (14.4), 93 (7.0), 91 (6.8), 84 (100), 83 (9.9), 82 (12.7), 79 (7.8), 69 (6.3), 67 (9.9), 55 (14.4), 44 (9.9), 41 (9.2). For $C_{26}H_{44}N_2O$ (400.7) calculated: 77.94% C, 11.07% H, 6.99% N; found: 77.79% C, 10.89% H, 7.12% N.

Methanesulfonate, m.p. 143–147 °C (methanol–ether). For $C_{27}H_{48}N_2O_4S$ (496.8) calculated: 65.28% C, 9.74% H, 5.64% N, 6.45% S; found: 65.35% C, 9.88% H, 5.49% N, 6.33% S.

*23-(4,5-Dihydroimidazol-2-yl)-24-nor-5*β*-cholane-3*α*,7*α*-diol* (**4b**). Acid **1b** (390 mg) gave 400 mg (97%) of amorphous **4b**, $[\alpha]_D$ +21.4° (*c* 0.21, methanol). IR spectrum (Nujol): 1 618 (C=N); 1 048 (C–O, C–N). ^{1}H NMR spectrum (hexadeuteriodimethyl sulfoxide): 0.61 s, 3 H (3 × H-18); 0.84 s, 3 H (3 × H-19); 0.87 d, 3 H, *J* = 5.9 (3 × H-21); 3.19 m, 1 H (H-3β); 3.40 s, 4 H (2 × H-4 and 2 × H-5 of dihydroimidazolyl); 3.64 s, 1 H (H-7β); 4.31 bs, 3 H (2 × OH and NH). ^{13}C NMR spectrum (hexadeuteriodimethyl sulfoxide): 11.8 (C-18), 18.5 (C-21), 20.5 (C-11), 22.9 (C-19), 23.3 (C-15), 25.6 (C-23), 28.0 (C-16), 30.7 (C-2), 32.5 (C-9), 32.6 (C-22), 34.9 (C-6 and C-10), 35.4 (C-20), 35.6 (C-1), 39.3 (C-8), 39.7 (C-4), 40.1 (C-12), 41.6 (C-5), 42.1 (C-13), 49.3 (C-4 and C-5 of dihydroimidazolyl), 50.2 (C-14), 55.7 (C-17), 66.3 (C-7), 70.5 (C-3), 168.1 (C-2 of dihydroimidazolyl). Mass spectrum (*m/z*, %): 416 (M^+, $C_{26}H_{44}N_2O_2$, 0.3), 402 (1.1), 401 (2.0), 383 (0.4), 359 (0.6), 166 (1.2), 125 (9.2), 105 (4.2), 98 (23.9), 97 (82.4), 95 (9.9), 93 (6.3), 91 (4.2), 84 (100), 82 (8.5), 81 (7.9), 79 (7.3), 67 (9.2), 55 (15.5), 44 (12.7), 43 (10.0). For $C_{26}H_{44}N_2O_2$ (416.7) calculated: 74.95% C, 10.64% H, 6.72% N; found: 75.21% C, 10.41% H, 6.81% N.

Methanesulfonate, amorphous substance (washed with ether). For $C_{27}H_{48}N_2O_5S$ (512.8) calculated: 63.25% C, 9.44% H, 5.46% N, 6.25% S; found: 63.19% C, 9.38% H, 5.22% N, 6.47% S.

23-(4,5-Dihydroimidazol-2-yl)-24-nor-5β-cholane-3α,12α-diol (**4c**). Acid **1c** (390 mg) gave 400 mg (92%) of product **4c**, m.p. 133–136 °C (acetone–methanol), $[\alpha]_D$ +37.5° (*c* 0.24, methanol). IR spectrum (Nujol): 1 623 (C=N); 1 049 (C–O, C–N). ^{1}H NMR spectrum (hexadeuteriodimethyl sulfoxide): 0.59 s, 3 H (3 × H-18); 0.84 s, 3 H (3 × H-19); 0.93 d, 3 H, *J* = 5.4 (3 × H-21); 3.35 s, 4 H (2 × H-4 and 2 × H-5 of dihydroimidazolyl); 3.40 m, 1 H (H-3β); 3.80 s, 1 H (H-12β); 4.34 bs, 3 H (2 × OH and NH). ^{13}C NMR spectrum (hexadeuteriodimethyl sulfoxide): 12.6 (C-18), 17.2 (C-21), 23.3 (C-19), 23.7 (C-15), 25.7 (C-16), 26.3 (C-7), 27.2 (C-23), 27.4 (C-6), 28.8 (C-11), 30.4 (C-2), 32.7 (C-22), 33.1 (C-9), 34.0 (C-10), 35.4 (C-1 and C-20), 35.9 (C-8), 36.5 (C-4), 41.8 (C-5), 46.2 (C-13), 46.3 (C-17), 47.6 (C-14), 49.2 (C-4 and C-5 of dihydroimidazolyl), 70.1 (C-3), 71.2 (C-12), 168.1 (C-2 of dihydroimidazolyl). Mass spectrum (*m/z*, %): 416 (M^+, $C_{26}H_{44}N_2O_2$, 1.5), 401 (1.6), 383 (2.0), 359 (0.8), 235 (1.6), 125 (15.5), 105 (7.7), 98 (100), 95 (15.5), 93 (11.3), 91 (10.6), 84 (86.6), 82 (12.7), 81 (16.3), 79 (14.1), 67 (16.9), 55 (15.4), 44 (15.5), 43 (16.5), 41 (19.7). For $C_{26}H_{44}N_2O_2$ (416.7) calculated: 74.95% C, 10.64% H, 6.72% N; found: 75.10% C, 10.61% H, 6.94% N.

Methanesulfonate, m.p. 162–165 °C (acetone). For $C_{27}H_{48}N_2O_5S$ (512.8) calculated: 63.25% C, 9.44% H, 5.46% N, 6.25% S; found: 63.14% C, 9.58% H, 5.35% N, 6.08% S.

23-(4,5-Dihydroimidazol-2-yl)-24-nor-5β-cholane-3α,7α,12α-triol (**4d**). Acid **1d** (410 mg) gave 220 mg (51%) of product **4d**, m.p. 215–218 °C (acetone–methanol), $[\alpha]_D$ +22.8° (*c* 0.29, methanol). IR spectrum (Nujol): 1 609 (C=N); 1 011 (C–O). ^{1}H NMR spectrum (hexadeuteriodimethyl sulfoxide): 0.59 s, 3 H (3 × H-18); 0.81 s, 3 H (3 × H-19); 0.94 d, 3 H, *J* = 6.0 (3 × H-21); 3.17 m, 1 H (H-3β); 3.39 s, 4 H (2 × H-4 and 2 × H-5 of dihydroimidazolyl); 3.62 s, 1 H (H-7β); 3.78 s, 1 H (H-12β). ^{13}C NMR spectrum (hexadeuteriodimethyl sulfoxide): 12.5 (C-16), 17.3 (C-21), 25.7 (C-19), 26.4 (C-9), 27.5 (C-16), 28.7 (C-11), 30.5 (C-2), 30.9 (C-22), 32.8 (C-10), 34.6 (C-4), 35.1 (C-6), 35.5 (C-1), 35.6 (C-20), 39.4 (C-8), 41.6 (C-5 and C-14), 46.0 (C-17), 49.1 (C-4 and C-5 of dihydroimidazolyl), 66.5 (C-7), 70.7 (C-3), 71.3 (C-12), 168.1 (C-2 of dihydroimidazolyl). Mass spectrum (*m/z*, %): 432 (M^+, $C_{26}H_{44}N_2O_3$, 0.6), 417 (0.7), 399 (0.8), 375 (0.5), 207 (1.9), 125 (12.7), 105 (7.0), 98 (33.1), 97 (95.8), 85 (23.9), 84 (100), 81 (13.4), 79 (12.4), 67 (16.9), 55 (26.8), 44 (15.5), 43 (15.5). For $C_{26}H_{44}N_2O_3$ (432.7) calculated: 72.18% C, 10.25% H, 6.47% N; found: 72.31% C, 10.11% H, 6.35% N.

Methanesulfonate, amorphous substance (washed with ether). For $C_{27}H_{48}N_2O_6S$ (528.8) calculated: 61.33% C, 9.15% H, 5.30% N, 6.06% S; found: 61.49% C, 9.01% H, 5.17% N, 6.30% S.

23-(4,5-Dihydroimidazol-2-yl)-24-nor-5β-cholan-3α-ol **(4a)**

A mixture of 3α-acetoxy-24-nor-5β-cholane-23-carbonitrile[8] (2.0 g, 5.0 mmol) and ethylenediamine monotosylate (2.3 g, 10 mmol) was heated under nitrogen to 200–230 °C for 1 h under stirring. After cooling, the melt was mixed with water (10 ml), the precipitated solid was filtered, washed with water and dried. It was dissolved in a mixture of chloroform (14 ml) and methanol (1 ml) and the solution was filtered through a layer of aluminum oxide (2.0 g) and silica gel (5.0 g). Elution with chloroform gave 0.35 g of 3α-hydroxy-24-nor-5β-cholane-23-carbonitrile (formed by deacetylation of the starting material), m.p. 192–194 °C (methanol); ref.[8] gave m.p. of 195 °C. Elution with methanol resulted in 1.56 g (78%) of **4a**, m.p. 218–220 °C (chloroform–methanol 1 : 9), identical with the product described above.

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REFERENCES

1. Hylemon P. B., Fricke R. J., Mosbach E. H.: Steroids *40*, 347 (1982).
2. Mosbach E. H., Ayengar N. K. N., Mc Sherry C. K. (Beth Israel Medical Center): U.S. 4,460,509 (1984); Chem. Abstr. *101*, 211558 (1984).
3. Enhsen A., Glombik H., Kramer W., Wess G. (Hoechst A.-G.): Eur. Pat. Appl. 624,596; Chem. Abstr. *122*, 187872 (1995).
4. Marchi E., Milani M. R., Piani S., Roda A., Cainelli G. (Alfa Wassermann S. P. A.): Eur. Pat. Appl. 676,410; Chem. Abstr. *124*, 9100 (1996).
5. Nguyen T. T. H., Urban J., Klinotova E., Sejbal J., Protiva J., Drasar P., Protiva M.: Collect. Czech. Chem. Commun. *60*, 257 (1995).
6. Oxley P., Short W. F.: J. Chem. Soc. *1947*, 497.
7. Antonini I., Cristalli G., Franchetti P., Grifantini M., Martelli S.: J. Heterocycl. Chem. *17*, 155 (1980).
8. Ercoli A., De Ruggieri P.: Farmaco, Ed. Sci. Tec. (Pavia) *6*, 438 (1951); Chem. Abstr. *46*, 8131 (1952).
9. Waldmann E., Chwala A.: Ber. Dtsch. Chem. Ges. *74*, 1763 (1941).
10. Reindel F., Niederländer L.: Ber. Dtsch. Chem. Ges. *68*, 1969 (1935).