SYNTHESIS OF A SCAFFOLD FOR THE CREATION OF NON-PEPTIDE LIBRARIES*

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5β-Cholanic acid derivative *XVII* was prepared as a skeleton for the creation of non-peptide libraries. In positions 3, 7 and 12, different functional groups were designed in order to provide for the attachment of various building blocks linked to them.

Recently, Ernest et al.[1] used a naphthalene derivative as a scaffold for the construction of protein β-turn mimics, later for the same purpose Hirschmann[2,3] employed a disubstituted steroid derivative as a scaffold. Disubstituted steroid molecule was employed successfully for the construction of libraries of synthetic receptors[4]. We have conceived the use of a scaffold for the generation of non-peptide libraries (for discussion of various library approaches see e.g.[5–7]) which would contain a rigid nucleus with three amino or pro-amino groups and a spacer for binding the molecule to a support. We have chosen cholic acid (*I*) as the most convenient starting material. Its synthetic potential was recognized by Hirschmann[2] who pointed to two major reasons for the choice: the well known rigidity of the steroid molecule, securing distinct reactivity of individual functional groups, and the wealth of accumulated knowledge of partial transformations of individual hydroxy groups in the classical steroid literature.

For practical reasons (polarity, solubility), it was considered convenient to carry out the whole sequence with an esterified carboxylic group (compound *II*, ref.[8]) which would only be regenerated at the end (Scheme 1). The trihydroxy ester *II* has been reported to be selectively acylated or oxidized first in the position 3, then 7 and eventually 12. The former reaction was most conveniently carried out by means of ethyl chloroformate in pyridine under the formation of a dihydroxy ester *III* (ref.[9]; partial acetylation by acetanhydride was less selective). For partial oxidation of diol *III* in

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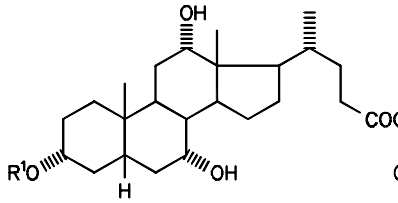
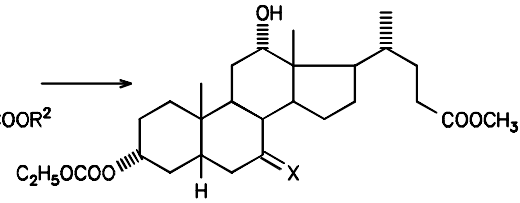
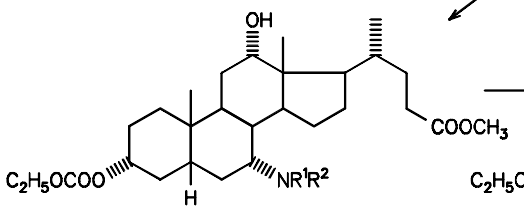
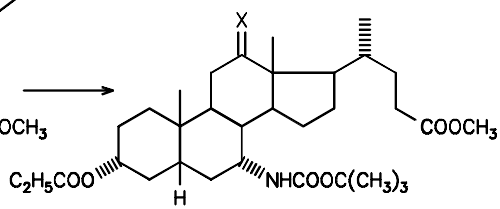




I, $R^1 = H$; $R^2 = H$
II, $R^1 = H$; $R^2 = CH_3$
III, $R^1 = C_2H_5OCO$; $R^2 = CH_3$

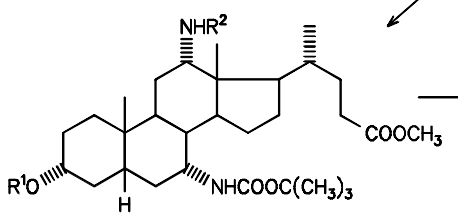
IV, X = O
V, X = NOH

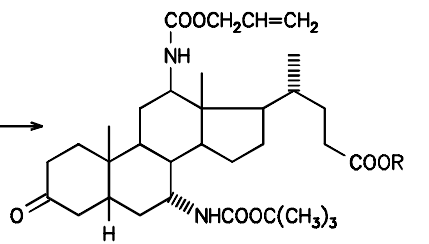




VI, $R^1 = H$; $R^2 = H$
VII, $R^1 = H$; $R^2 = OH$
VIII, $R^1 + R^2 = CO$
IX, $R^1 = H$; $R^2 = COOCH_3$
X, $R^1 = H$; $R^2 = COOC(CH_3)_3$

XI, X = O
XII, X = NOH





XIII, $R^1 = C_2H_5OCO$; $R^2 = H$
XIV, $R^1 = C_2H_5OCO$; $R^2 = COOCH_2CH=CH_2$
XV, $R^1 = H$; $R^2 = COOCH_2CH=CH_2$

XVI, R = CH_3
XVII, R = H

SCHEME 1

position 7, *N*-bromoacetamide in acetone[10] was most effective, the yield of 7-ketone *IV* was over 80%.

For the conversion of the 7-oxo group into the 7-amino group, sodium borohydride reduction of oxime *V* was considered first. The reduction, however, was so slow that the ester groupings present were partly affected. Eventually 7-amino derivative *VI* was prepared by catalytic hydrogenation. As expected[10,11], the amino group occupied the axial position (see the narrow signal of 7β-proton in the ^{1}H NMR spectra of *VI* and of its derivatives).

In some runs the hydrogenation stopped[12] at the stage of intermediary hydroxylamino derivative *VII*, the further reduction of which being the slowest step. Eventually, experimental conditions were worked out for the reductive amination[13] of ketone *IV* with ammonium acetate in the presence of sodium cyanoborohydride; stereospecificity of the amine formation was lower in this case (65.5% of *VI*, 3.1% of another amine; the desired 7α-isomer *VI* could be best purified after conversion to a Boc derivative *X*).

Because of solubility problems, acylation of amine *VI* was first attempted in a non-aqueous solution (pyridine). The desired carbamate *X* was only isolated by chromatography in 46% yield. A side product (yield 49%) was formed as well, its IR spectrum (ν_{as} 2 267 cm^{-1}) and elemental analysis prompted the structure of an isocyanate *VIII*. Its structure was confirmed by methanolysis leading to methyl carbamate *IX*. The same reaction with *tert*-butyl alcohol was very slow (10 days at reflux temperature) and afforded compound *X* besides products of other reactions (e.g., of alcoholysis of the 3-carbonate group).

Later Boc derivative *X* was cleanly produced by the Schotten–Baumann[14] modification using a two-phase system: aqueous potassium hydroxide and a tetrahydrofuran solution of amine *VI* and di-*tert*-butyl dicarbonate.

The same sequence of reactions (oxidation to *XI*, oximation to *XII*, reduction to *XIII*) was used in the conversion of the 12α-hydroxy group into an amino group. The equatorial configuration of amino group at carbon 12 was again confirmed by the ^{1}H NMR spectrum of *XIII* (doublet of doublets of the 12α-proton at δ 3.24, J = 9.7 and 1.2 Hz), in this case the approach of hydrogen from the β-side was hindered by the angular methyl group and an equatorial amino group was created. The above described reductive amination of ketone *VI* was applicable in this case (ketone *XI*) as well though an even higher temperature had to be used.

The 12β-amino derivative *XIII* was converted into corresponding allyloxy carbamate *XIV* by treatment with allyl chloroformate in pyridine. The 3α-acyloxy derivative *XIV* was hydrolyzed by potassium carbonate which recovered the 3α-hydroxy group. Compound *XV* was oxidized to 3-ketone *XVI* and only then the carboxyl in the side chain was regenerated by lithium hydroxide hydrolysis. ^{1}H NMR and IR spectra of product *XVII* verify that both amine protecting groups survived the reaction conditions. The compound *XVII* will be bound to beads through the carboxyl and then the third amino

group will analogously be created in the position 3. The use of the scaffold for the generation of non-peptide libraries will be published elsewhere.

EXPERIMENTAL

Melting points were determined on a micro melting point apparatus Boetius and are uncorrected. Optical rotations and infrared spectra (wavenumbers in cm^{-1}) were measured in chloroform unless stated otherwise. ^1H NMR spectra were recorded on a Varian UNITY-200 (200 MHz, FT mode) spec-

TABLE I
Characteristics parameters of 200 MHz ^1H NMR spectra of compounds *I–XVII* in deuteriochloroform; for other conditions see Experimental

Compound	H-18[a]	H-19[a]	H-3[b]	H-7[c]	H-12[d]	Other signals
I	0.65	0.85	3.59	4.01	4.13	0.96[e]
II	0.67	0.88	3.44	3.85	3.96	0.98[e], 3.66[f]
III	0.69	0.91	4.44	3.98	3.85	0.98[e], 1.29[g], 4.16[h], 3.67[f]
IV	0.68	1.19	4.54	–	4.00	0.97[e], 1.29[g], 3.67[f], 4.16[h]
V	0.69	1.06	4.55	–	4.00	0.98[e], 1.29[g], 3.11[i], 3.66[f], 4.16[h], 7.31[j]
VI	0.69	0.92	4.41	3.08	3.97	0.98[e], 1.29[g], 3.67[f], 4.16[h]
VII	0.67	0.94	4.44	3.08	3.97	0.96[e], 1.30[g], 3.67[f], 4.17[h]
VIII	0.69	0.91	4.45	3.90	3.98	0.97[e], 1.31[g], 3.67[f], 4.18[h]
IX	0.71	0.97	4.45	3.70	4.02	0.99[e], 1.33[g], 3.68[f], 3.69[f], 4.20[h], 4.91[k]
X	0.69	0.94	4.43	3.67[k]	3.91	0.97[e], 1.30[g], 1.44[l], 3.67[f], 4.18[h]
XI	1.03	1.06	4.43	3.67[k]	–	0.84[e], 1.31[g], 1.43[l], 3.66[f], 3.83[m], 4.18[h]
XII	0.92	1.03	4.45	3.67[k]	–	0.93[e], 1.30[g], 1.43[l], 3.33[n], 3.66[f], 4.18[h]
XIII	0.73	0.94	4.33	3.67[k]	3.24[o]	0.97[e], 1.31[g], 1.44[l], 3.33[m], 3.67[f], 4.18[h], 4.86[m]
XIV	0.81	0.95	4.44	3.69[k]	4.06[o]	0.88[e], 1.30[g], 1.42[l], 3.69[f], 4.17[h], 4.45[p], 5.28[q], 5.29[r], 5.90[s], 5.10[m], 5.54[m]
XV	0.81	0.93	3.50	3.68[k]	4.05[o]	0.88[e], 1.45[l], 3.68[f], 4.59[p], 5.24[q], 5.32[r], 5.92[s], 4.95[m]
XVI	0.85	1.04	–	3.72	4.13[o]	0.91[e], 1.42[l], 3.63[f], 4.55[p], 5.22[q], 5.28[r], 5.89[s], 5.69[m]
XVII	0.82	1.01	–	3.45	4.13[o]	0.90[e], 1.47[l], 4.33[t], 4.77[u], 5.15[q], 5.23[r], 5.86[v], 6.78[m]

[a] s, 3 H; [b] m, *W* = 39; [c] m, *W* = 16; [d] t, *J* = 6 and 6; [e] d, 3 H, *J* = 6.1 (H-21); [f] s, 3 H (OCH_3); [g] t, 3 H, *J* = 7.0 (CH_3CH_2O); [h] q, 1 H, *J* = 7.0 (CH_3CH_2O); [i] dd, 1 H, *J* = 13.4 and 1.5 (H-6α); [j] m, 1 H, (C=NOH); [k] overlapping signals; [l] s, 9 H ($COOC(CH_3)_3$); [m] m, 1 H, *W* = 32 (NH); [n] dd, 1 H, *J* = 8.8 and 2.4 (H-11α); [o] dd, 1 H, *J* = 9.7 and 1.2 (H-12); [p] d, 2 H, *J* = 5.6 ($COOCH_2CH=CH_2$); [q] d, 1 H, *J* = 10.3 (*cis*-$COOCH_2CH=CH_2$); [r] d, 1 H, *J* = 16.9 (*trans*-$COOCH_2CH=CH_2$); [s] tdd, 1 H, *J* = 5.6, 10.3 and 16.9 ($COOCH_2CH=CH_2$); [t] dd, 1 H, *J* = 13.6 and 4.9 ($CHCH=CH_2$); [u] dd, 1 H, *J* = 13.6 and 5.8 ($CHCH=CH_2$); [v] m, *W* = 45, 1 H ($CH_2CH=CH_2$).

trometer in deuteriochloroform with tetramethylsilane as internal standard. Chemical shifts are given in ppm (δ-scale), coupling constants and width of multiplets in Hz. Selected parameters are given in Table I. Thin-layer chromatography was performed on silica gel (ICN Biochemicals). For column chromatography silica gel 60–120 μ was used. Methyl 3α,7α,12α-trihydroxy-5β-cholanate (*II*) was prepared according to ref.[15].

Methyl 3α-Ethoxycarbonyloxy-7α,12α-dihydroxy-5β-cholanate (*III*)

Ethyl chloroformate (30 ml, 107 mmol) was slowly added to a solution of compound *II* (45.2 g, 107 mmol) in pyridine (130 ml) under stirring at 0 °C. The mixture was kept at 0 °C for an additional 45 min and then poured onto ice (800 ml), the precipitate was filtered off, dissolved in chloroform and washed with water. Evaporation of the solvent and crystallization from methanol yielded compound *III* (44.8 g, 85%). M.p. 151–153 °C, $[\alpha]_D$ +41° (*c* 1.1) (ref.[10] records 176–177 °C).

Methyl 3α-Ethoxycarbonyloxy-12α-hydroxy-7-oxo-5β-cholanate (*IV*)

N-Bromoacetamide (2.5 g, 18.5 mmol) and then acetic acid (5.0 ml) were added to a solution of compound *III* (5.0 g, 10.1 mmol) in aqueous acetone (190 ml, 20% of water). After 1 h the mixture was poured into water (500 ml), the precipitate was filtered off and washed with water. TLC (25% ether in benzene): R_F 0.40, traces of lipophilic impurities were removed by crystallization. M.p. 183–185 °C (methanol, 4.05 g, 81.3%), ref.[10] records 157–159 °C.

Methyl 3α-Ethoxycarbonyloxy-12α-hydroxy-7-oximino-5β-cholanate (*V*)

Hydroxylamine hydrochloride (7.0 g, 100.7 mmol) was added to a solution of ketone *IV* (20.0 g, 40.6 mmol) in pyridine (50 ml) and the mixture was kept at 90 °C under stirring. After 8 h the solution was poured onto ice (300 ml) and oily product was separated, dissolved in chloroform and washed with water. TLC (chloroform treated with ammonium hydroxide, 5% of acetone): R_F 0.25, no starting material (R_F 0.50) was present (when neutral solvent was used, e.g., 50% ether in benzene, compounds *IV* and *V* exert identical R_F 0.51). IR spectrum: 3 600, 3 400 (OH); 3 592, 3 288 (N–OH); 1 732 (C=O); 1 653 (C=N); 1 271, 1 259 (C–O). Product (16.2 g, 79%) crystallized from toluene, m.p. 156–157 °C, $[\alpha]_D$ +21° (*c* 1.0). For $C_{28}H_{45}NO_7$ (507.7) calculated: 66.25% C, 8.93% H, 2.76% N; found: 66.28% C, 9.05% H, 2.85% N.

Methyl 7α-Amino-3α-ethoxycarbonyloxy-12α-hydroxy-5β-cholanate (*VI*)

A) Mother liquors from the preparation of compound *VII* (2.89 g) were shaken with chloroform (20 ml) and ammonium hydroxide (4 ml), the organic phase was washed with water and dried over anhydrous sodium sulfate. Chloroform was evaporated in vacuo and the residue (2.35 g, 67%, based on oxime used) crystallized from toluene. M.p. 189–192 °C, $[\alpha]_D$ +32° (*c* 0.9). IR spectrum: 3 618, 3 444 (OH); 1 733, 1 438 (COOR); 1 620 (NH_2); 1 272, 1 261 (C–O). For $C_{28}H_{47}NO_6$ (493.7) calculated: 68.12% C, 9.60% H, 2.84% N; found: 68.17% C, 9.69% H, 2.76% N.

B) A mixture of ketone *IV* (128 mg, 0.26 mmol), ammonium acetate (160 mg, 2.07 mmol) and sodium cyanoborohydride (40 mg, 0.64 mmol) in methanol (3 ml) was stirred at 50 °C. The reaction was complete within 18 h (TLC), the reagent was decomposed by brine (1 ml). The mixture was diluted with toluene (6 ml) and concentrated in a vacuum to halve the volume. The mixture was separated between brine (10 ml) and toluene (10 ml), the organic layer was washed with water, dried and evaporated. Thin layer chromatography (2 plates, 200 × 200 × 1 mm, 96% of chloroform treated

with ammonia and 4% of methanol). The major component (85 mg, 66%, m.p. 189–192 °C) was identical in all respects with amine *VI*, a less polar admixture (4 mg) was not studied further.

Methyl 7α-(*N*-Hydroxyl)amino-3α-ethoxycarbonyloxy-12α-hydroxy-5β-cholanate (*VII*)

A suspension of platinum dioxide, hydrate (200 mg, 0.88 mmol) and oxime *V* (3.6 g, 7.1 mmol) in acetic acid (30 ml) was stirred in hydrogen atmosphere for 60 h. The solvent was evaporated and the product (710 mg, 20%) crystallized from toluene. M.p. 203–206 °C (methanol); $[\alpha]_D$ +32° (*c* 1.1). IR spectrum: 3 619 (OH); 3 588 (NH); 1 733, 1 271 (COOR). For $C_{28}H_{47}NO_7$ (509.7) calculated: 65.98% C, 9.29% H, 2.75% N; found: 66.08% C, 9.47% H, 2.67% N.

Methyl 3α-Ethoxycarbonyloxy-12α-hydroxy-7α-isocyanato-5β-cholanate (*VIII*)

Collected lipophilic fractions from the preparation of compound *X* (8.23 g, 49% based on amine *VI*) were crystallized from ether–heptane, m.p. 135–137 °C, $[\alpha]_D$ +23° (*c* 1.3). IR spectrum: 3 620 (OH); 2 267 (N=C=O); 1 733, 1 270; 1 260 (COOR). For $C_{29}H_{45}NO_7$ (519.7) calculated: 67.03% C, 8.73% H, 2.70% N; found: 67.02% C, 8.76% H, 2.79% N.

Methyl 7α-(*N*-Methoxycarbonyl)amino-3α-ethoxycarbonyloxy-12α-hydroxy-5β-cholanate (*IX*)

A solution of isocyanate *VIII* (135 mg, 0.23 mmol) in absolute methanol (13.5 ml) was heated to reflux for 6 h; TLC (25% ether in benzene): R_F 0.20, no starting material (R_F 0.65). The solution was concentrated in vacuo and the residue was chromatographed on three silica gel plates (200 × 200 × 1 mm) in benzene–ether (1 : 1). Product (123.5 mg, 86%) crystallized from ether, m.p. 167–169 °C, $[\alpha]_D$ +21° (*c* 1.4). IR spectrum: 3 621, 3 509 (OH); 3 452 (NH); 1 731 (COOR); 1 689, 1 506 (CONH); 1 270, 1 261 (C–O). For $C_{30}H_{49}NO_8$ (551.7) calculated: 65.31% C, 8.95% H, 2.54% N; found: 65.26% C, 8.95% H, 2.39% N.

In analogous treatment of isocyanate *VIII* with *tert*-butyl alcohol (120 h), most of the starting compound (ca 60%) remained unchanged, however, the desired compound *X* was accompanied by a more polar product with a free 3α-hydroxy group (^{1}H NMR spectrum).

Methyl 7α-(*N*-*tert*-Butoxycarbonyl)amino-3α-ethoxycarbonyloxy-12α-hydroxy-5β-cholanate (*X*)

A) Di-*tert*-butyl dicarbonate (19.0 g, 87.1 mmol) was added to a solution of amine *VI* (16.1 g, 32.6 mmol) in pyridine (55 ml) and toluene (55 ml) under stirring at 20 °C. After 20 min, the solution was diluted with toluene (100 ml) and washed with aqueous potassium hydrogen carbonate and water. The solution was dried with anhydrous sodium sulfate, concentrated in vacuo to one tenth of its volume and applied on a column of silica gel (600 g). Mixture toluene–ether (9 : 1) eluted the lipophilic component (8.23 g, see the preparation of compound *VIII*) and 25% ether in toluene eluted compound *X* (8.91 g, 46%), m.p. 181–182 °C (heptane); $[\alpha]_D$ +25° (*c* 1.6). IR spectrum: 3 619, 3 529 (OH); 3 455, 3 404 (NH); 1 734 ($COOCH_3$); 1 705 (COO); 1 495 (amide II); 1 270, 1 059, 1 024 (C–O). For $C_{33}H_{55}NO_8$ (593.8) calculated: 66.76% C, 9.34% H, 2.36% N; found: 66.58% C, 9.48% H, 2.29% N.

B) Aqueous potassium hydroxide (10%, 1.4 ml, 2.5 mmol) was added to a solution of amine *VI* (1.2 g, 2.4 mmol) in tetrahydrofuran (15 ml). The mixture was shaken with di-*tert*-butyl dicarbonate (880 mg, 4.0 mmol) for 30 min and then it was acidified with aqueous citric acid (10%, 10 ml, 5.2 mmol). The mixture was diluted with ether (50 ml), washed with brine and dried over anhydrous magnesium sulfate. TLC of the product revealed the presence of amide *X* only. Yield: 1.1 g (76%), m.p. 181–182 °C (heptane).

Methyl 7α-(*N-tert*-Butoxycarbonyl)amino-3α-ethoxycarbonyloxy-12-oxo-5β-cholanate (*XI*)

Compound *X* (6.79 g, 13.8 mmol) was oxidized in acetone (30 ml) at 0 °C with Jones reagent. After 5 min the excess of the reagent was reduced by a few drops of methanol and the mixture was poured into aqueous potassium hydrogen carbonate (5%, 100 ml). The product was extracted with chloroform, washed with water and dried over anhydrous sodium sulfate. Product *XI* (6.56 g, 97%) crystallized from acetone and heptane, m.p. 104–106 °C; $[\alpha]_D$ +111° (*c* 1.1). IR spectrum: 1 734 (COOR); 1 706 (NCOOR); 1 495 (amide II); 1 393, 1 367 ($C(CH_3)_3$); 1 269, 1 255 (C–O). For $C_{33}H_{53}NO_8$ (591.8) calculated: 66.98% C, 9.03% H, 2.37% N; found: 67.14% C, 9.23% H, 2.26% N.

Methyl 7α-(*N-tert*-Butoxycarbonyl)amino-3α-ethoxycarbonyloxy-12-oximino-5β-cholanate (*XII*)

Compound *XI* (6.4 g, 10.8 mmol) was dissolved in pyridine (15 ml) and hydroxylamine hydrochloride (3.0 g, 43.2 mmol) was added. The mixture was stirred at 80 °C for 2 h, the cool reaction mixture was poured onto ice (200 ml) and left in a refrigerator overnight. The solid product (6.4 g, 98%) was collected by filtration, dissolved in chloroform and washed with water. TLC (chloroform treated with ammonia and 5% of acetone): R_F 0.50, no starting ketone (R_F 0.67). M.p. 116–117 °C (ether); $[\alpha]_D$ +126° (*c* 1.1). IR spectrum: 3 589, 3 271 (N–OH); 3 455 (NH); 1 732 (COOR); 1 706 (NCOOR); 1 494 (amide II); 1 392, 1 367 ($C(CH_3)_3$); 1 270, 1 257 (C–O); 937 (N–O). For $C_{33}H_{54}N_2O_8$ (606.8) calculated: 65.32% C, 8.97% H, 4.62% N; found: 65.27% C, 9.18% H, 4.53% N.

Methyl 12β-Amino-7α-(*N-tert*-butoxycarbonyl)amino-3α-ethoxycarbonyloxy-5β-cholanate (*XIII*)

A) Oxime *XII* (3.3 g, 5.4 mmol) was hydrogenated according to the procedure used for the preparation of amine *VI*. The crude product was purified by column chromatography (250 ml of silica, chloroform with 2.5% of methanol and 1% of acetic acid). The major component (2.8 g, 79%, acetic acid salt of amine *XIII*, melting at 103–109 °C) was shaken in chloroform (20 ml) with ammonium hydroxide (5 ml) and then with water. Free base *XIII* melts at 180–182 °C (benzene–heptane); $[\alpha]_D$ +20° (*c* 1.1). IR spectrum: 3 455 (NH); 1 734 (COOR); 1 705 (NHCOOR); 1 492 (amide II); 1 270, 1 170 (C–O). For $C_{33}H_{56}N_2O_7$ (592.8) calculated: 66.86% C, 9.52% H, 4.73% N; found: 66.89% C, 9.42% H, 4.50% N.

B) A mixture of ketone *XI* (1.0 g, 1.69 mmol), ammonium acetate (1.2 g, 15.6 mmol) and sodium cyanoborohydride (245 mg, 3.9 mmol) in *tert*-butyl alcohol (6 ml) was stirred at 75 °C in nitrogen atmosphere. The reaction was complete within 5 h (TLC), the reagent was decomposed by aqueous citric acid (10%, 10 ml). The mixture was separated between brine (40 ml) and ethyl acetate (40 ml), the organic layer was washed with water, dried and evaporated. The product was chromatographed on a column of silica gel (50 g). A mixture of toluene, ether and methanol (10 : 10 : 1) eluted 380 mg (38%) of an oily product, ^{1}H NMR spectrum of which was identical with that of compound *XIII*.

Methyl 12β-(*N*-Allyloxycarbonyl)amino-7α-(*N-tert*-butoxycarbonyl)amino-3α-ethoxycarbonyloxy-5β-cholanate (*XIV*)

A solution of allyl chloroformate (0.8 ml, 7.5 mmol) in toluene (2 ml) was added to a solution of amine *XIII* (1.44 g, 2.4 mmol) in pyridine (3 ml) within 10 min under stirring at 0 °C. After 1 h the mixture was poured onto ice, the product separated was dissolved in chloroform and washed with water and dried over sodium sulfate. On evaporation, the residue (1.5 g, 91%) crystallized from methanol at –18 °C. M.p. 130–133 °C, $[\alpha]_D$ +85° (*c* 1.0). IR spectrum: 3 452, 3 399 (NH); 1 730, 1 392, 1 367 (COOR); 1 706, 1 268, 1 250 (NCOOR); 1 528, 1 511 (amide II). For $C_{37}H_{60}N_2O_9$ (676.9) calculated: 65.65% C, 8.93% H, 4.14% N; found: 65.37% C, 8.85% H, 4.01% N.

Methyl 12β-(*N*-Allyloxycarbonyl)amino-7α-(*N-tert*-butoxycarbonyl)amino-3α-hydroxy-5β-cholanate (*XV*)

A) Potassium carbonate (130 mg, 0.94 mmol) in water (1 ml) was added to a solution of compound *XIV* (640 mg, 0.94 mmol) in methanol (50 ml) and the mixture was kept at 20 °C for 18 h and then at 50 °C for 30 min. The solution was concentrated in vacuo to volume 5 ml, brine was added and the precipitate filtered and washed with water. The product *XV* (464 mg, 81%) crystallized from acetone, m.p. 214–216 °C; $[\alpha]_D$ +65° (*c* 0.9). IR spectrum: 3 609 (OH); 3 452, 3 347 (NH); 3 088, 1 654 (H–C=C); 1 705, 1 392, 1 367, 1 243, 1 067 (COOR); 1 528, 1 513 (amide II). For $C_{34}H_{56}N_2O_7$ (604.8) calculated: 67.52% C, 9.33% H, 4.63% N; found: 67.47% C, 9.28% H, 4.49% N.

B) Compound *XII* (1.0 g, 1.69 mmol) was treated with ammonium acetate (1.27 g, 16.5 mmol) and sodium cyanoborohydride (245 mg, 3.9 mmol) as described above (preparation of *XIII*, procedure *B*). The crude product was acylated with allyl chloroformate (0.8 ml, 7.5 mmol) in pyridine (1 ml) and toluene (1 ml) at 0 °C. The crude product was hydrolyzed in a solution of potassium carbonate (250 mg, 1.8 mmol) in aqueous methanol (98%, 110 ml) as described above. The product was chromatographed on a column of silica gel (100 g) using 50% ether in toluene. The major component (449 mg) crystallized from acetone (4.5 ml) yielding 349 mg of compound *XV* (34% from ketone *XII*), m.p. 206–211 °C, $[\alpha]_D$ +68° (*c* 1.6). Further crystallization yielded a product identical with the product described under *A* (identity of IR spectra).

Methyl 12β-(*N*-Allyloxycarbonyl)amino-7α-(*N-tert*-butoxycarbonyl)amino-3-oxo-5β-cholanate (*XVI*)

Hydroxy derivative *XV* (1.52 g, 2.5 mmol) was oxidized according to the procedure used for the preparation of compound *XI*. The product (1.4 g, 92%) crystallized from acetone–heptane, m.p. 217–218 °C; $[\alpha]_D$ +82° (*c* 1.1). IR spectrum: 3 369 (NH); 3 100, 1 650 (H–C=C); 1 704, 1 247, 1 164 (COOR); 1 524 (amide II). For $C_{34}H_{54}N_2O_7$ (602.8) calculated: 67.74% C, 9.03% H, 4.65% N; found: 67.70% C, 9.11% H, 4.39% N.

12β-(*N*-Allyloxycarbonyl)amino-7α-(*N-tert*-butoxycarbonyl)amino-3-oxo-5β-cholanic Acid (*XVII*)

Ester *XVI* (2.09 g, 3.5 mmol) was dissolved in methanol (80 ml) and lithium hydroxide monohydrate (1.5 g, 40.5 mmol) in water (7 ml) was added. The mixture was kept at 50 °C for 1 h. Alkalinity of the mixture was suppressed by the addition of citric acid (2.7 g, 14.0 mmol) and the volume was reduced one half on a vacuo evaporator. Brine (100 ml) was added and the precipitate was filtered off and washed with water and dissolved in chloroform. The extract was washed with water, dried over anhydrous sodium sulfate and evaporated in vacuo. The residue crystallized from acetone–heptane, m.p. 217–218 °C (1.4 g, 69%). $[\alpha]_D$ +177° (*c* 0.9). IR spectrum: 3 628 (OH); 3 450 (NH); 1 708 (NHCOOR); 1 648, 1 009 (H_2C=CH–CH_2O); 1 502 (amide II); 1 270, 1 198 (C–O). For $C_{33}H_{52}N_2O_7$ (588.8) calculated: 67.32% C, 8.90% H, 4.76% N; found: 67.14% C, 8.95% H, 4.55% N. Mother liquors were purified by chromatography on silica gel column in chloroform–acetone (9 : 1), yielding an additional 269 mg of the product (total yield 82%).

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