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Studies on the synthesis of cholane derivatives containing a mercapto group and their dimers with disulfide spacers. Part 2. 3α-Mercapto-5β-cholane-7α,12α,24-triol and its C(3)-C(3') disulfide dimer

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Studies on the synthesis of cholane derivatives containing a mercapto group and their dimers with disulfide spacers. Part 2. 3α-Mercapto-5β-cholane-7α,12α,24-triol and its C(3)–C(3′) disulfide dimer

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Two new sulfur-containing cholane derivatives were obtained from cholic acid: 3α-mercapto-5β-cholane-7α,12α,24-triol **2** and its C(3)–C(3′) disulfide dimer **3** as a potential supramolecular host.

Keywords: bile acids; cholane; disulfides; thiols; supramolecular chemistry

1. Introduction

As a continuation of our previous studies on cholic acid-derived supramolecular hosts containing disulfide spacers (*1*), we now report the synthesis of 3α-mercapto-5β-cholane-7α,12α,24-triol **2** along with its C(3)–C(3′) disulfide dimer **3**, potentially suitable for molecular or ionic recognition (Scheme 1).

2. Results and discussion

The steroidal iodide **5**, prepared from alcohol **4** by S_N2-type reaction with iodine-triphenylphosphine complex in benzene/acetonitrile solution (*2*), was used as a substrate in our first attempts at the nucleophilic substitution of iodide by SH^-. In the reaction of **5** with $NaSH{\cdot}2H_2O$ in different solvents (absolute ethanol, DMSO, *N*-methylpyrrolidone), the same endproducts were obtained in similar yields: the less polar fraction was a mixture of elimination compounds non-separable by column chromatography. Then, the more polar material was eluted from the column, which appeared to be a non-separable mixture of sulfur-containing substances. From this mixture, disulfide **6** was isolated in a very poor yield after two recrystallizations (Scheme 2). In the mass spectrum taken from the mother liquor, three peaks were present, among which 1065 ($M^+ + Na$;

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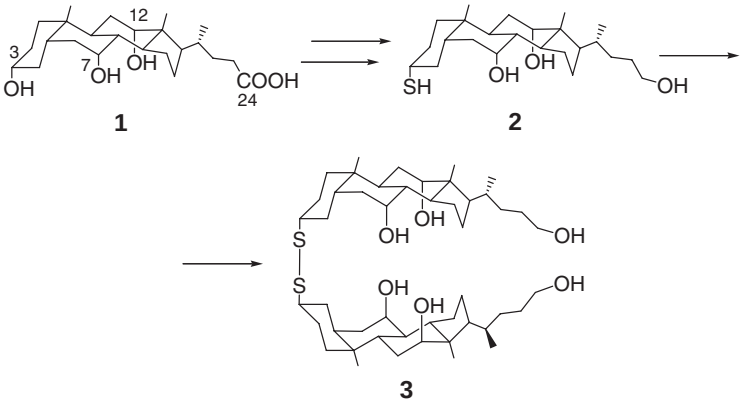
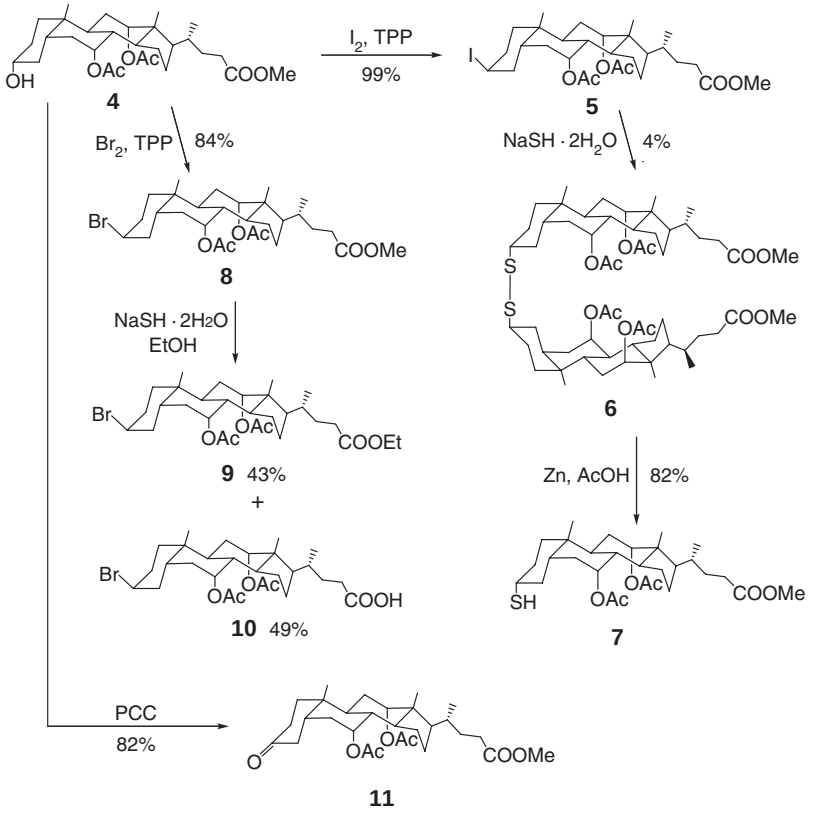


Scheme 1. Proposed synthesis of compounds **2** and **3**.

6) was the most intensive. The two other signals represented probably the molecular ions of dimers with oxidized spacers: $-S_2O_2-$ (1097; $M^+ + Na$) and $-S_2O_4-$ (1129; $M^+ + Na$). All these three compounds were inseparable by column chromatography. Reduction of **6** with zinc in acetic acid gave thiol **7** (Scheme 2) in a high yield. To confirm the structures of the two other components of the mother liquor, a small portion of this fraction was also subjected to the reduction under



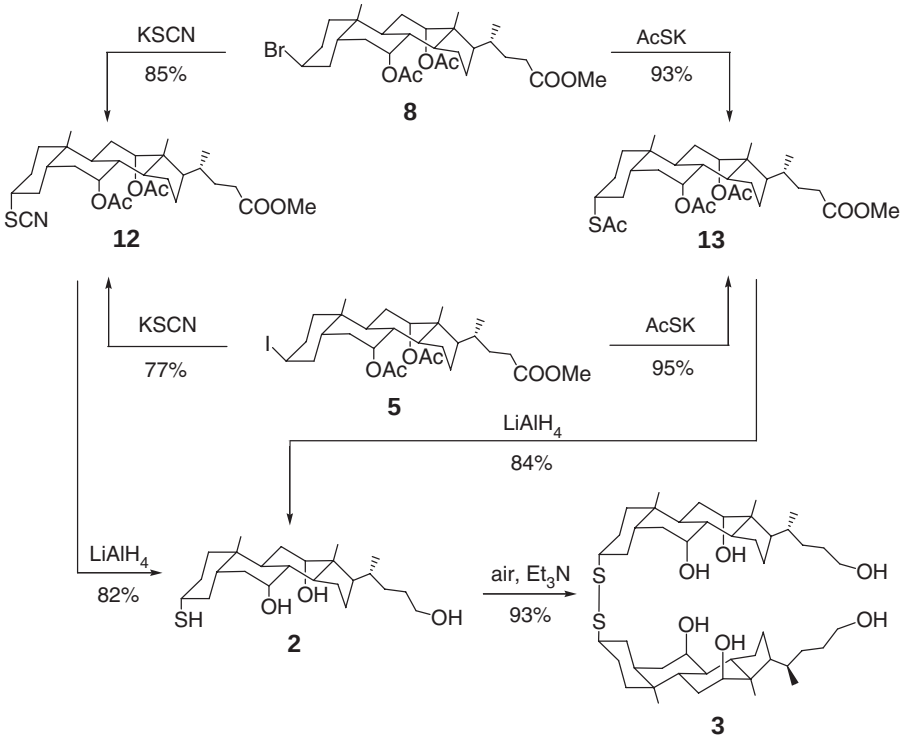
Scheme 2. Preparation of halides (**5** and **8**) and their reactions with sodium hydrogen sulfide.

the same conditions as disulfide **6**. In this case, thiol **7** was obtained as the only product of this reaction.

To avoid the formation of elimination products, bromide **8** was prepared and subjected to reaction with $NaSH{\cdot}2H_2O$ in absolute ethanol. But, in this case, a mixture of transesterification (**9**) and hydrolysis (**10**) products was only obtained (Scheme 2).

An attempt to substitute both iodide and bromide ions by SH^- via Bunte salts (*3*) was unsuccessful: the formation of elimination products was detected at the stage of the reaction of **5** and **8** with sodium thiosulfate. Analogous result was achieved when the conversion of halides **5** and **8** to thiol **7** with thiourea (through S-alkylthiuronium salts) (*4*) was attempted. Under the reaction conditions (THF, 40°C), both substrates underwent elimination to yield the mixture of olefins in different yields (10% from bromide **8** and 80% from iodide **5**). In the next attempt to obtain thiol **7**, alcohol **4** and ketone **11** were treated with the Lawesson's reagent (*5*) but no reaction was observed.

Thiol **2** was obtained in high yields in two ways (Scheme 3). In the first method, both substrates (bromide **8** and iodide **5**) were subjected to reaction with KSCN in a mixed acetone/DMF solution (*6*). Higher yield of thiocyanate **12** was obtained from bromide **8** (85%); in the case of iodide **5** as a substrate, some olefinic by-products were formed, so the crude reaction mixture needed careful purification by column chromatography. Then, the halides (**5** and **8**) were treated with potassium thioacetate (*7*) (Scheme 3) and the product (thioacetate **13**) was obtained in excellent yields with no detectable amounts of olefinic by-products. Both thiocyanate **12** and thioacetate **13** were subjected to reduction with $LiAlH_4$ in THF solution and the same thiol **2** was formed in high yields (82% from thiocyanate and 84% from thioacetate). The last step of the



Scheme 3. Synthetic route to products **2** and **3**.

synthesis – transformation of thiol **2** to disulfide **3** – was achieved by air oxidation of **2** with catalytic amounts of triethylamine in CH_2Cl_2 solution.

3. Conclusions

The steroidal thiol **2** and its C(3)–C(3′) disulfide dimer **3** were obtained from halides **5** and **8** in good overall yields. The best methods for the transformation of halides into the corresponding thiol appeared to be the two-step procedures via thiocyanate or thioacetate as intermediates followed by their reduction as demonstrated in Scheme 3. In all S_N2 transformations carried out at C(3) carbon atom in steroidal skeleton (alcohol **4** → iodide **5** or bromide **8**, iodide **5** → disulfide **6** or thiocyanate **12** or thioacetate **13**, bromide **8** → thiocyanate **12** or thioacetate **13**), all the products were diastereomerically pure. The desired disulfide dimer **3** was obtained by air oxidation of thiol **2** in the presence of triethylamine as a catalyst. This dimer is expected to be a good supramolecular host for ions or small molecules, and physicochemical studies to determine its complexation properties are in progress.

4. Experimental

Melting points were determined on a *Kofler* apparatus of the *Boëtius* type. NMR spectra were taken with a Bruker Ultrashield Plus 400 spectrometer with *TMS* as the internal standard. Infrared spectra were recorded on a Nicolet series II Magna-IR 550 FT-IR spectrometer. Mass spectra were obtained with the AMD-604 spectrometer. Optical rotations were measured on a Perkin-Elmer 141 polarimeter at the sodium D line (589 nm). The reaction products were isolated by column chromatography performed on 70–230 mesh silica gel (J.T. Baker). Thin-layer chromatograms were developed on aluminum TLC sheets precoated with silica gel F_{254} (Merck) and visualized with 50% H_2SO_4 after heating. All solvents were dried and freshly distilled prior to use. Cholic acid (**1**) was purchased from ABCR GmbH & Co. KG and it was used without further purification. 7α,12α-Diacetylmethyl cholate (**4**) was prepared according to the known procedure (*2*).

4.1. *Methyl 7α,12α-diacetoxy-3β-iodo-5β-cholan-24-ate (5) ($C_{29}H_{45}IO_6$)*

To a solution of 2.94 g of **4** (5.81 mmol) in 100 ml of benzene/acetonitrile 4:1 mixture, 8.40 g of triphenylphosphine (32.06 mmol) and 2.30 g of imidazole (33.82 mmol) were added. After a few minutes, 7.4 g of I_2 (29.13 mmol) was added portion-wise with vigorous stirring. The reaction was continued for 15 min and then the mixture was poured into H_2O containing a few drops of 30% H_2O_2 and extracted with benzene. The combined organic layers were washed with aqueous $Na_2S_2O_5$ and dried ($MgSO_4$). The crude product was subjected to column chromatography. Pure iodide **5** was eluted with *n*-hexane/ethyl acetate 8:2 (3.54 g; 99% yield). Colorless crystals, mp 133–135°C (*n*-hexane/CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 5.09$ (m, 1H, 12β-H), 4.94 (m, 1H, 3α-H), 4.91 (m, 1H, 7β-H), 3.67 (s, 3H, OCH_3), 2.11 (s, 3H, 12-CH_3CO), 2.06 (s, 3H, 7-CH_3CO), 1.01 (s, 3H, 19-CH_3), 0.81 (d, 3H, $J = 6.4$ Hz, 21-CH_3), 0.74 (s, 3H, 18-CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 174.5$ (C), 170.4 (C), 170.1 (C), 75.4 (CH), 70.7 (CH), 51.5 (CH_3), 47.3 (CH), 45.0 (C), 43.4 (CH), 38.9 (CH_2), 38.3 (CH), 38.2 (CH), 37.7 (CH), 35.2 (C), 34.6 (CH), 32.3 (CH_2), 30.92 (CH_2), 30.90 (CH_2), 30.89 (CH_2), 30.7 (CH_2), 29.9 (CH), 27.1 (CH_2), 25.5 (CH_2), 22.8 (CH_2), 22.7 (CH_3), 21.5 (CH_3), 21.3 (CH_3), 17.5 (CH_3), 12.2 (CH_3) ppm; IR ($CHCl_3$): $\tilde{\nu} = 1727$, 1252, 1022 cm^{-1}; $[\alpha]_D^{20} + 34.3$ ($c = 1$, $CHCl_3$); ESI MS: $m/z = 639.2$ ($M^+ + Na$; 100%).

4.2. *Di(7α,12α-diacetoxy-23-methoxycarbonyl-5β-cholan-3α-yl) disulfide (6) ($C_{58}H_{90}O_{12}S_2$)*

Three hundred milligrams (0.49 mmol) of **5** was dissolved in 10 ml of dry DMSO and then 460 mg (5 mmol) of powdered $NaSH{\cdot}2H_2O$ was added under argon. The reaction mixture was stirred at room temperature for 48 h. Then, the crude product was extracted twice with benzene/ethyl acetate 9:1 mixture, and combined organic layers were dried over $MgSO_4$ and evaporated to dryness. The residue was subjected to column chromatography. The less polar fraction (a mixture of elimination products) was eluted with hexane/ethyl acetate 85:15 mixture. The more polar fraction (154 mg) was crystallized twice from *n*-hexane/CH_2Cl_2 mixture. Pure disulfide **6** was obtained in 4% yield (11 mg). Colorless crystals, mp 97–99°C (*n*-hexane/CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 5.06$ (m, 2H, 2 12β-H), 4.87 (m, 2H, 2 7β-H), 3.65 (s, 6H, 2 OCH_3), 2.53 (m, 2H, 2 3β-H), 2.11 (s, 6H, 2 12-CH_3CO), 2.07 (s, 6H, 2 7-CH_3CO), 0.90 (s, 6H, 2 19-CH_3), 0.81 (d, 6H, $J = 5.9$ Hz, 2 21-CH_3), 0.71 (s, 6H, 2 18-CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 174.4$ (2 C), 170.5 (2 C), 170.4 (2 C), 75.3 (2 CH), 70.7 (2 CH), 51.4 (2 CH_3), 50.3 (2 CH), 47.3 (2 CH), 45.0 (2 C), 43.3 (2 CH), 42.9 (2 CH), 37.7 (2 CH), 36.8 (2 CH_2), 35.8 (2 CH_2), 34.5 (2 CH), 34.4 (2 C), 34.42 (2 C), 31.2 (2 CH_2), 30.8 (2 CH_2), 30.7 (2 CH_2), 28.8 (2 CH), 27.9 (2 CH_2), 27.1 (2 CH_2), 25.4 (2 CH_2), 22.8 (2 CH_3), 22.7 (2 CH_2), 21.6 (2 CH_3), 21.3 (2 CH_3), 17.4 (2 CH_3), 12.1 (2 CH_3) ppm; IR ($CHCl_3$): $\tilde{\nu} = 1725, 1258, 1021\ cm^{-1}$; $[\alpha]_D^{20} + 31.7$ ($c = 1$, $CHCl_3$); ESI MS: $m/z = 1065.5$ ($M^+ + Na$; 100%).

4.3. *Methyl 7α,12α-diacetoxy-3α-mercapto-5β-cholan-24-ate (7) ($C_{29}H_{46}O_6S$)*

Compound **6** (11 mg; 0.011 mmol) was dissolved in 5 ml of benzene/methanol 2:1 mixture. Then, one drop of glacial acetic acid and 5 mg (0.077 mmol) of zinc powder were added to the solution and the mixture was stirred at room temperature for 24 h. A few drops of aqueous $NaHCO_3$ were added to complete the reaction. The reaction mixture was dried ($MgSO_4$) and the solvents were evaporated. Purification with column chromatography gave pure **7** (9 mg) in 82% yield (elution with *n*-hexane/ethyl acetate 82:18). Colorless crystals, mp 72–75°C (*n*-hexane/CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 5.09$ (m, 1H, 12β-H), 4.90 (m, 1H, 7β-H), 3.67 (s, 3H, OCH_3), 2.68 (m, 1H, 3β-H), 2.15 (s, 3H, 12-CH_3CO), 2.11 (s, 3H, 7-CH_3CO), 0.91 (s, 3H, 19-CH_3), 0.82 (d, 3H, $J = 6.4$ Hz, 21-CH_3), 0.74 (s, 3H, 18-CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 174.4$ (C), 170.5 (C), 170.4 (C), 75.3 (CH), 70.7 (CH), 51.4 (CH_3), 47.3 (CH), 45.0 (C), 43.3 (CH), 43.1 (CH), 40.7 (CH_2), 38.9 (CH), 37.7 (CH), 37.1 (CH_2), 34.5 (CH), 34.0 (C), 32.8 (CH_2), 31.2 (CH_2), 30.8 (CH_2), 30.7 (CH_2), 28.9 (CH), 27.1 (CH_2), 25.5 (CH_2), 22.76 (CH_3), 22.73 (CH_2), 21.5 (CH_3), 21.3 (CH_3), 17.4 (CH_3), 12.1 (CH_3) ppm; IR ($CHCl_3$): $\tilde{\nu} = 1725, 1251, 1021\ cm^{-1}$; $[\alpha]_D^{20} + 28.4$ ($c = 0.25$, $CHCl_3$); ESI MS: $m/z = 545.4$ ($M^+ + Na$; 100%).

4.4. *Methyl 7α,12α-diacetoxy-3β-bromo-5β-cholan-24-ate (8) ($C_{29}H_{45}BrO_6$)*

To a solution of 1.5 g (5.73 mmol) of triphenylphosphine in 15 ml of benzene, 1 ml (12.4 mmol) of dry pyridine was added and the mixture was stirred at room temperature for 10 min. Then, 0.25 ml (4.84 mmol) of bromine was carefully added dropwise with vigorous stirring. After additional 15 min of stirring, 1.04 g (2.06 mmol) of compound **4** was added portion-wise and the content of the flask was stirred at room temperature for 24 h. Then, the reaction mixture was poured into a separating funnel containing aqueous $Na_2S_2O_3$, and the crude product was extracted with benzene. The organic layer was extracted again with acidified water and dried over $MgSO_4$. The solvent was evaporated and the residue was subjected to column chromatography. Pure bromide **8** was eluted with *n*-hexane/ethyl acetate 8:2 (0.98 g; 84% yield). Colorless crystals, mp 153–155°C; (*n*-hexane/CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 5.05$ (m, 1H, 12β-H), 4.88 (m,

1H, 7β-H), 4.70 (m, 1H, 3α-H), 3.62 (s, 3H, OCH_3), 2.07 (s, 3H, 12-CH_3CO), 2.02 (s, 3H, 7-CH_3CO), 0.96 (s, 3H, 19-CH_3), 0.77 (d, 3H, $J = 6.0$ Hz, 21-CH_3), 0.70 (s, 3H, 18-CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 174.4$ (C), 170.2 (C), 170.0 (C), 75.3 (CH), 70.7 (CH), 55.9 (CH), 51.4 (CH_3), 47.2 (CH), 44.9 (C), 43.3 (CH), 37.6 (CH), 37.2 (CH_2), 36.3 (CH), 34.9 (C), 34.5 (CH), 30.7 (CH_2), 30.63 (CH_2), 30.61 (CH_2), 30.3 (CH_2), 29.3 (CH_2), 29.0 (CH), 27.0 (CH_2), 25.5 (CH_2), 22.74 (CH_2), 22.72 (CH_3), 21.4 (CH_3), 21.2 (CH_3), 17.4 (CH_3), 12.1 (CH_3) ppm; IR ($CHCl_3$): $\tilde{\nu} = 1727$, 1252, 1023 cm^{-1}; $[\alpha]_D^{20} + 57.2$ ($c = 1$, $CHCl_3$); ESI MS: $m/z = 591.3$ ($M^+ + Na$; 86%), 593.3 ($M^+ + Na$; 100%).

4.5. *Reaction of bromide (8) with NaSH· 2H$_2$O. Ethyl 7α,12α-diacetoxy-3β-bromo-5β-cholan-24-ate (9) (C$_{30}$H$_{47}$BrO$_6$) and 7α,12α-diacetoxy-3β-bromo-5β-cholan-24-oic acid (10) (C$_{28}$H$_{43}$BrO$_6$)*

Compound **8** (50 mg; 0.09 mmol) was dissolved in 5 ml of absolute ethanol and argon was then passed through the solution for a few seconds. Then, 83 mg (0.90 mmol) of powdered $NaSH \cdot 2H_2O$ was added and the content of the flask was stirred at room temperature for 20 h. Then the reaction mixture was diluted with water and extracted twice with benzene/ethyl acetate 6:4 mixture. The organic layer was dried over $MgSO_4$ and the solvents were evaporated. The crude mixture was subjected to column chromatography. The less polar fraction (compound **9**) was eluted with *n*-hexane/ethyl acetate 85:15 (22 mg; 43% yield), the more polar (compound **10**) with *n*-hexane/ethyl acetate 2:8 (24 mg; 49% yield).

Compound **9**: colorless crystals, mp 151–153°C (*n*-hexane/CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 5.09$ (m, 1H, 12β-H), 4.92 (m, 1H, 7β-H), 4.74 (m, 1H, 3α-H), 4.13 (q, 2H, $J = 7.1$ Hz, $OC\underline{H}_2CH_3$), 2.19 (s, 3H, 12-CH_3CO), 2.11 (s, 3H, 7-CH_3CO), 1.25 (t, 3H, $J = 7.1$ Hz, $OCH_2C\underline{H}_3$), 0.99 (s, 3H, 19-CH_3), 0.81 (d, 3H, $J = 6.5$ Hz, 21-CH_3), 0.74 (s, 3H, 18-CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 174.1$ (C), 170.4 (C), 170.1 (C), 75.4 (CH), 70.8 (CH), 60.2 (CH_2), 56.0 (CH), 47.3 (CH), 45.0 (C), 43.4 (CH), 37.7 (CH), 37.3 (CH_2), 36.4 (CH), 34.9 (C), 34.6 (CH), 31.1 (CH_2), 30.75 (CH_2), 30.74 (CH_2), 30.4 (CH_2), 29.4 (CH_2), 29.1 (CH), 27.1 (CH_2), 25.6 (CH_2), 22.8 (CH_2), 22.7 (CH_3), 21.5 (CH_3), 21.3 (CH_3), 17.5 (CH_3), 14.2 (CH_3), 12.2 (CH_3) ppm; IR ($CHCl_3$): $\tilde{\nu} = 1725$, 1254, 1025 cm^{-1}; $[\alpha]_D^{20} + 58.1$ ($c = 1$, $CHCl_3$); ESI MS: $m/z = 605.3$ ($M^+ + Na$; 89%), 607.3 ($M^+ + Na$; 100%).

Compound **10**: colorless crystals, mp 136–138°C (*n*-hexane/CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 5.10$ (m, 1H, 12β-H), 4.92 (m, 1H, 7β-H), 4.74 (m, 1H, 3α-H), 2.11 (s, 3H, 12α-CH_3CO), 2.07 (s, 3H, 7α-CH_3CO), 1.00 (s, 3H, 19-CH_3), 0.83 (d, 3H, $J = 6.5$ Hz, 21-CH_3), 0.74 (s, 3H, 18-CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 179.3$ (C), 170.4 (C), 170.2 (C), 75.4 (CH), 70.8 (CH), 55.9 (CH), 47.3 (CH), 45.1 (C), 43.4 (CH), 37.7 (CH), 37.4 (CH_2), 36.4 (CH), 35.0 (C), 34.5 (CH), 30.8 (CH_2), 30.7 (CH_2), 30.5 (CH_2), 30.4 (CH_2), 29.4 (CH_2), 29.1 (CH), 27.1 (CH_2), 25.6 (CH_2), 22.8 (CH_2), 22.7 (CH_3), 21.5 (CH_3), 21.3 (CH_3), 17.5 (CH_3), 12.2 (CH_3) ppm; IR ($CHCl_3$): $\tilde{\nu} = 1724$, 12532, 1023 cm^{-1}; $[\alpha]_D^{20} + 33.3$ ($c = 1$, $CHCl_3$); ESI MS: $m/z = 577.3$ ($M^+ + Na$; 88%), 579.3 ($M^+ + Na$; 100%).

4.6. *Methyl 7α,12α-diacetoxy-3-oxo-5β-cholan-24-ate (11) (C$_{29}$H$_{44}$O$_7$)*

Compound **4** (200 mg; 0.40 mmol) was dissolved in 20 ml of CH_2Cl_2. Then 172 mg (0.80 mmol) of pyridinium chlorochromate was added to the solution and the mixture was stirred at room temperature for 3 h. The precipitate was filtered off and the crude product was subjected to column chromatography. Pure ketone **11** was eluted with *n*-hexane/ethyl acetate 7:3 (163 mg; 82% yield). Colorless crystals, mp 198–199°C; (*n*-hexane/CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 5.11$ (m, 1H, 12β-H), 4.98 (m, 1H, 7β-H), 3.64 (s, 3H, OCH_3), 2.97 (dd, 1H, $J_1 = 15.2$ Hz,

$J_2 = 13.7$ Hz, 4α-H), 2.09 (s, 3H, 12-CH_3CO), 2.05 (s, 3H, 7-CH_3CO), 1.00 (s, 3H, 19-CH_3), 0.80 (d, 3H, $J = 6.1$ Hz, 21-CH_3), 0.75 (s, 3H, 18-CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 212.0$ (C), 174.4 (C), 170.3 (C), 170.1 (C), 75.2 (CH), 70.5 (CH), 51.4 (CH_3), 47.3 (CH), 45.0 (C), 44.5 (CH_2), 43.2 (CH), 42.1 (CH), 37.7 (CH), 36.5 (CH_2), 36.1 (CH_2), 34.5 (CH), 34.3 (C), 30.8 (CH_2), 30.8 (CH_2), 30.7 (CH_2), 29.7 (CH), 27.1 (CH_2), 25.8 (CH_2), 22.7 (CH_2), 21.6 (CH_3), 21.4 (CH_3), 21.2 (CH_3), 17.4 (CH_3), 12.2 (CH_3) ppm; IR ($CHCl_3$): $\tilde{\nu} = 1724$, 1248, 1022 cm^{-1}; $[\alpha]_D^{20} + 22.8$ ($c = 1$, $CHCl_3$); ESI MS: $m/z = 527.3$ ($M^+ + Na$; 100%).

4.7. *Methyl 7α,12α-diacetoxy-3α-thiocyanate-5β-cholan-24-ate (12) ($C_{30}H_{45}NO_6S$)*

Bromide **8** (400 mg; 0.73 mmol) was dissolved in 30 ml of acetone/DMF (1:2 v/v) mixture and 200 mg (2.06 mmol) of powdered KSCN was added to the solution. The reaction mixture was then stirred at room temperature for 4 days. Then the crude product was extracted twice with benzene/ethyl acetate 9:1 mixture. The organic layer was dried over $MgSO_4$, the solvents were evaporated and the residue was subjected to column chromatography. Pure thiocyanate **12** was eluted with *n*-hexane/ethyl acetate 85:15 (328 mg; 85% yield). The same procedure was repeated using iodide **5** as a substrate. In this case, the yield of the endproduct achieved was 77%. Colorless crystals, mp 161–162°C; (*n*-hexane/CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 5.07$ (m, 1H, 12β-H), 4.91 (m, 1H, 7β-H), 3.65 (s, 3H, OCH_3), 2.87 (m, 1H, 3β-H), 2.143 (s, 3H, 12-CH_3CO), 2.140 (s, 3H, 7-CH_3CO), 0.92 (s, 3H, 19-CH_3), 0.82 (d, 3H, $J = 6.2$ Hz, 21-CH_3), 0.72 (s, 3H, 18-CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 174.5$ (C), 170.8 (C), 170.5 (C), 110.5 (C), 75.0 (CH), 70.3 (CH), 51.5 (CH_3), 47.3 (CH), 47.2 (CH), 44.9 (C), 43.1 (CH), 43.0 (CH), 37.7 (CH), 36.8 (CH_2), 36.5 (CH_2), 34.6 (CH), 34.0 (C), 31.0 (CH_2), 30.8 (CH_2), 30.7 (CH_2), 28.9 (CH_2), 28.6 (CH), 27.1 (CH_2), 25.1 (CH_2), 22.8 (CH_2), 22.4 (CH_3), 21.5 (CH_3), 21.2 (CH_3), 17.5 (CH_3), 12.1 (CH_3) ppm; IR ($CHCl_3$): $\tilde{\nu} = 2154$, 1727, 1249, 1021 cm^{-1}; $[\alpha]_D^{20} + 29.2$ ($c = 1$, $CHCl_3$); ESI MS: $m/z = 570.3$ ($M^+ + Na$; 100%).

4.8. *Methyl 7α,12α-diacetoxy-3α-acetylthio-5β-cholan-24-ate (13) ($C_{31}H_{48}O_7S$)*

To a solution of **5** (100 mg; 0.16 mmol) in 10 ml of DMF, powdered potassium thioacetate (37 mg; 0.32 mmol) was added and the reaction mixture was heated to 110°C with stirring for 18 h. The reaction mixture was extracted twice with CH_2Cl_2, the organic layer was dried over $MgSO_4$ and the solvent was evaporated. Column chromatography gave pure **13** (87 mg) as colorless oil (elution with *n*-hexane/ethyl acetate 85:15) in 95% yield. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 5.06$ (m, 1H, 12β-H), 4.87 (m, 1H, 7β-H), 3.65 (s, 3H, OCH_3), 3.24 (m, 1H, 3β-H), 2.28 (s, 3H, CH_3COS), 2.11 (s, 3H, 12-CH_3CO), 2.05 (s, 3H, 7-CH_3CO), 0.90 (s, 3H, 19-CH_3), 0.80 (d, 3H, $J = 6.1$ Hz, 21-CH_3), 0.71 (s, 3H, 18-CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 195.4$ (C), 174.4 (C), 170.4 (C), 170.2 (C), 75.3 (CH), 70.7 (CH), 51.4 (CH_3), 47.3 (CH), 45.0 (C), 43.3 (CH), 42.9 (CH), 42.8 (CH), 37.6 (CH), 36.8 (CH_2), 35.6 (CH_2), 34.5 (CH), 34.1 (C), 31.2 (CH_2), 30.8 (CH_2), 30.71 (CH_3), 30.69 (CH_2), 28.7 (CH), 27.8 (CH_2), 27.1 (CH_2), 25.4 (CH_2), 22.73 (CH_3), 22.72 (CH_2), 21.5 (CH_3), 21.3 (CH_3), 17.4 (CH_3), 12.1 (CH_3) ppm; IR ($CHCl_3$): $\tilde{\nu} = 1725$, 1685, 1247 cm^{-1}; $[\alpha]_D^{20} + 62.4$ ($c = 1$, $CHCl_3$); ESI MS: $m/z = 587.3$ ($M^+ + Na$; 100%).

4.9. *3α-Mercapto-5β-cholane-7α,12α,24-triol (2) ($C_{24}H_{42}O_3S$)*

To a solution of 60 mg (0.11 mmol) of **13** in 5 ml of anhydrous THF, 20 mg of $LiAlH_4$ (6.67 mmol) was added under argon, and the mixture was stirred at room temperature for 16 h. Then several drops of water were added and the precipitate was filtered off. Purification with column chromatography gave pure **2** (37 mg) in 84% yield (elution with ethyl acetate). Colorless crystals,

mp 193–195°C (*n*-heptane/ethyl acetate); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 3.97$ (m, 1H, 12β-H), 3.84 (m, 1H, 7β-H), 3.50 (t, 4H, $J = 5.7$ Hz, C$\underline{H}_2$OH), 2.53 (m, 1H, 3β-H), 0.98 (d, 3H, $J = 6.6$ Hz, 21-CH_3), 0.89 (s, 3H, 19-CH_3), 0.69 (s, 3H, 18-CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 73.7$ (2 CH), 68.8 (2 CH), 63.3 (2 CH_2), 51.2 (2 CH), 48.1 (2 CH), 47.0 (2 C), 44.5 (2 CH), 42.2 (2 CH), 40.3 (2 CH), 38.1 (2 CH_2), 37.7 (2 CH_2), 36.6 (2 CH), 35.7 (2 C), 35.3 (2 CH_2), 32.7 (2 CH_2), 30.2 (2 CH_2), 29.0 (2 CH_2), 28.3 (2 CH_2), 28.3 (2 CH_2), 27.2 (2 CH), 23.7 (2 CH_2), 23.2 (2 CH_3), 17.9 (2 CH_3), 12.9 (2 CH_3) ppm; IR ($CHCl_3$): $\tilde{\nu} = 3610$, 3414, 2458, 1075, 1039 cm^{-1}; $[\alpha]_D^{20} + 52.1$ ($c = 0.5$, $CHCl_3$); HRMS: calcd. mass for $C_{24}H_{42}NaO_3S$: 433.2752, found: 433.2751.

4.10. *Di(7α,12α,24-trihydroxy-5β-cholan-3α-yl) disulfide (3) ($C_{48}H_{82}O_6S_2$)*

Compound **2** (30 mg; 0.07 mmol) was dissolved in 4 ml of CH_2Cl_2 containing 5 μl of triethylamine and allowed to stay for 7 h at room temperature. Then the solvent was evaporated and the product was crystallized from *n*-heptane/ethyl acetate mixture giving 28 mg (93% yield) of **3**. Colorless crystals, mp 220–222°C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 3.94$ (m, 2H, 12β-H), 3.80 (m, 2H, 7β-H), 3.51 (t, 4H, $J = 5.7$ Hz, 24-C$\underline{H}_2$-OH), 2.52 (m, 2H, 3β-H), 0.99 (d, 6H, $J = 6.3$ Hz, 21-CH_3), 0.90 (s, 6H, 19-CH_3), 0.69 (s, 6H, 18-CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 73.8$ (2 CH), 68.7 (2 CH), 63.3 (2 CH_2), 51.0 (2 CH), 48.0 (2 CH), 47.0 (2 C), 44.4 (2 CH), 42.4 (2 CH), 40.3 (2 CH), 38.1 (2 CH_2), 37.6 (2 CH_2), 36.6 (2 CH), 35.8 (2 C), 35.3 (2 CH_2), 32.7 (2 CH_2), 30.0 (2 CH_2), 29.0 (2 CH_2), 28.4 (2 CH_2), 28.3 (2 CH_2), 27.3 (2 CH), 23.8 (2 CH_2), 23.2 (2 CH_3), 17.9 (2 CH_3), 12.9 (2 CH_3) ppm; IR (KBr): $\tilde{\nu} = 3422$, 1260, 1072, 1037 cm^{-1}; $[\alpha]_D^{20} + 104.1$ ($c = 0.5$, $CHCl_3/CH_3OH$ 1:1); HRMS: Calcd. mass for $C_{48}H_{82}NaO_6S_2$: 841.5451, found: 841.5450.

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