

Supporting Information for

Amino acid conjugates of lithocholic acid as antagonists of the EphA2 receptor

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Molecular Modelling

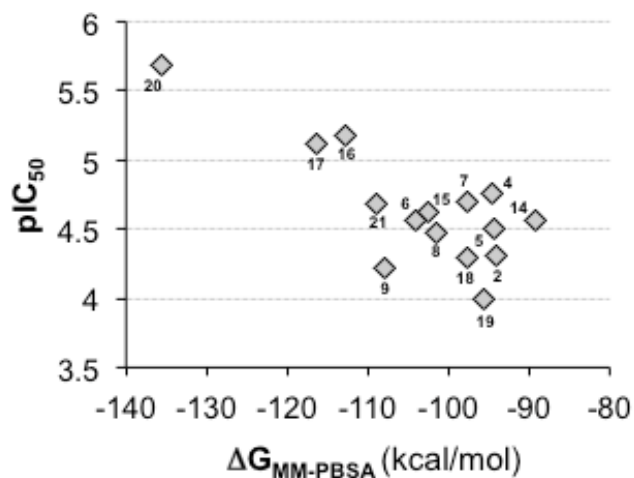


Figure S1. Plot of experimental pIC_{50} values versus calculated ΔG of binding from MM-PBSA calculations.

Pharmacology

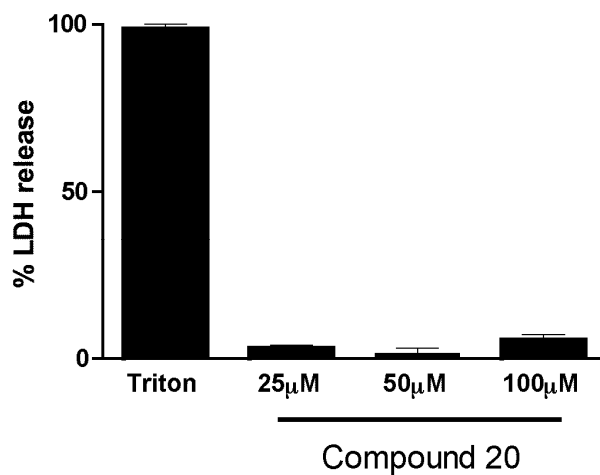


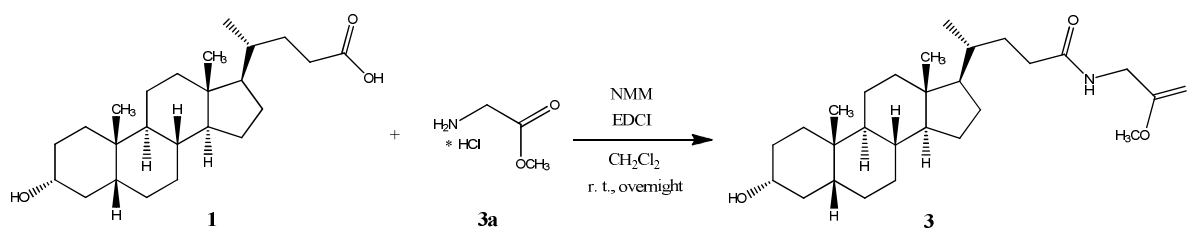
Figure S2. Compound **20** is not cytotoxic when incubated for 2 hours with PC3 cells and tested for the release of LDH enzymes. 0.5% DMSO (not shown) and 0.5% triton were considered as 0% and 100% toxicity, respectively.

Chemistry

General

Unless otherwise noted, reagents and solvents were purchased from commercial suppliers (Aldrich and Fluka) and were used without purification. The progress of the reactions was monitored by thin-layer chromatography with F₂₅₄ silica-gel precoated sheets (Merck Darmstadt, Germany). UV light, ninhydrin ethanolic solution (0.3% w/v) and potassium permanganate solution (10% w/v) were used for detection. Flash chromatography was performed using Merck silica-gel 60 (Si 60, 40-63 μ m, 230-400 mesh ASTM). Catalytic hydrogenation was performed using a Parr 3911 Hydrogenation apparatus. Dichloromethane (DCM) was dried by distillation over calcium hydride. All reactions were carried out using flame-dried glassware under atmosphere of nitrogen. Melting points were determined on a Gallenkamp melting point apparatus and were not corrected. The ¹H-NMR and ¹³C-NMR spectra were recorded on a Bruker Avance 400 spectrometer (400MHz); chemical shifts (δ scale) are reported in parts per million (ppm). ¹H-NMR spectra are reported in the following order: multiplicity, approximate coupling constants (*J* value) in Hertz (Hz) and number of protons; signals were characterized as s (singlet), d (doublet), t (triplet), quint. (quintuplet), m (multiplet), b (broad). Mass spectra were recorded on an Applied Biosystem API-150 EX system spectrometer with ESI interface. The final compounds were analyzed on a ThermoQuest (Italia) FlashEA 1112 Elemental Analyzer for C, H and N. The percentages found were within $\pm$ 0.4% of the theoretical values.

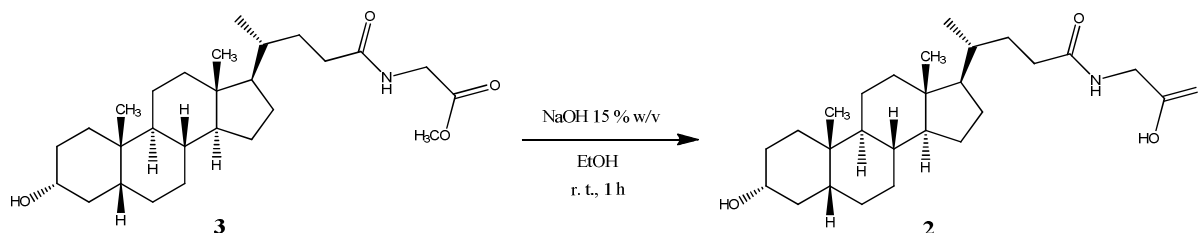
Methyl *N*-(3 α -hydroxy-5 β -cholan-24-oyl)glycinate (**3**)



Compound **3** was synthesized following a modification of a described procedure^[1] in which to a stirred solution of lithocholic acid **1** (0.8 mmol) O-methyl- L-glycinate hydrochloride (0.88 mmol) and N-methyl morpholine (NMM) (1.43 mmol) in dry CH₂Cl₂ (15 mL) under nitrogen was added N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDCI) (0.821 mmol). The reaction mixture was stirred at room temperature overnight and then was diluted with 30 mL of CH₂Cl₂, washed with HCl 2N, brine and dried over anhydrous Na₂SO₄. Evaporation of solvent under reduced pressure yielded a white solid that was purified by flash chromatography [SiO₂, CH₂Cl₂:EtOH 98:2]. The crude product was re-crystallized from ethanol-water to give **3**. Yield: 50%. Mp: 157-160°C. ¹H-NMR (400 MHz, DMSO-d₆) δ = 0.60 (s, 3H, CH₃), 0.86-0.87 (m, 6H), 0.91-1.20 (m, 11H), 1.33-1.35 (m, 7H), 1.47-1.68 (m, 5H), 1.74-1.77 (m, 2H), 1.90-1.93 (m, 1H), 1.98-2.05 (m, 1H), 2.10-2.17 (m, 1H), 3.31-3.35 (m, 1H), 3.60 (s, 3H, OCH₃), 3.78 (d, *J* = 6.0,

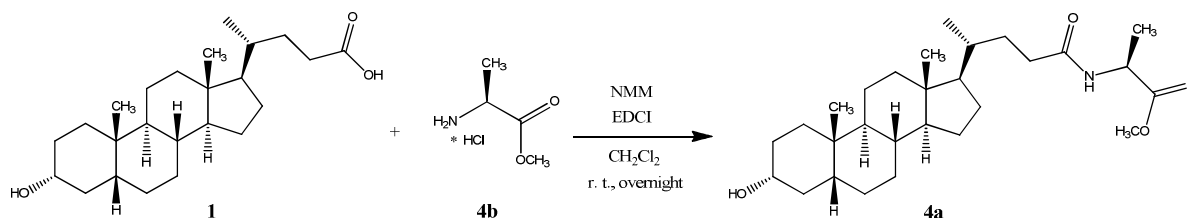
2H, CH₂), 4.42 (d, J = 4.4, 1H, OH), 8.20 (t, J = 6.0, 1H, NH). ¹³C-NMR (100 MHz, DMSO-d₆) δ = 12.06, 18.37, 20.83, 23.38, 24.21, 26.42, 27.20, 28.24, 30.55, 31.58, 33.25, 34.58, 35.35, 35.47, 35.85, 36.46, 40.18, 40.43, 41.21, 42.10, 42.75, 52.38, 53.37, 56.00, 56.49, 71.85, 170.64 (CONH), 173.72 (C=O). MS (ESI) calc for C₂₇H₄₅NO₄: 447.33; found: 446.3 [M-1].

N-(3 α -Hydroxy-5 β -cholan-24-oyl)-glycine (**2**)



Compound **2** was synthesized following a modification of a described procedure^[2] in which to a solution of compound **3** (0.32 mmol) in ethanol (15 mL) was added a solution of sodium hydroxide 15% w/v (10 mL) and the mixture was stirred at room temperature for 1 hour. Ethanol was removed under reduced pressure and the solution was acidified with concentrated hydrochloric acid until a precipitate was formed. The resulting suspension was filtered under vacuum and the white residue washed with water. The crude product was crystallized from ethanol-water to give the title compound **2** (95%) as a white solid. Mp: 178-181°C. ¹H-NMR (400 MHz, DMSO-d₆) δ = 0.59 (s, 3H, CH₃), 0.86-0.92 (m, 7H), 0.97-1.23 (m, 10H), 1.28-1.34 (m, 7H), 1.47-1.50 (m, 2H), 1.58-1.68 (m, 3H), 1.73-1.79 (m, 2H), 1.90-1.92 (m, 1H), 1.97-2.04 (m, 1H), 2.09-2.16 (m, 1H), 3.32-3.35 (m, 1H), 3.69 (d, J = 6.0, 2H, CH₂), 4.42 (bs, 1H, OH), 8.07 (t, J = 6.0, 1H, NH). ¹³C-NMR (100 MHz, DMSO-d₆) δ = 12.34, 18.75, 20.88, 23.74, 24.32, 26.63, 27.36, 28.18, 30.84, 31.89, 32.50, 34.67, 35.33, 35.62, 35.85, 36.76, 41.00, 41.99, 42.73, 56.09, 56.55, 70.32, 171.91 (CONH), 173.37 (C=O). MS (ESI) calc for C₂₆H₄₃NO₄: 433.32; found: 432.5 [M-1].

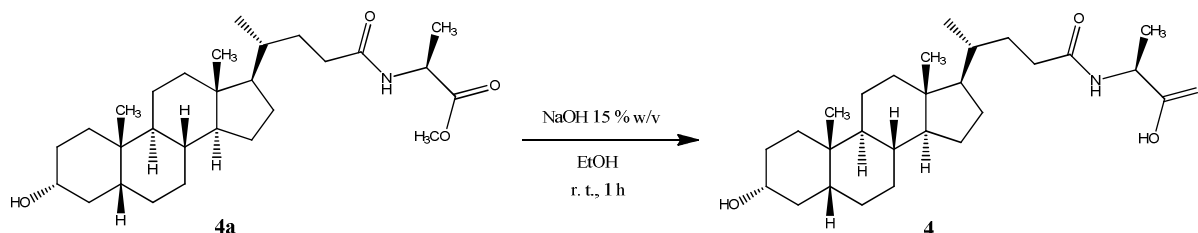
Methyl *N*-(3 α -hydroxy-5 β -cholan-24-oyl)-L-alaninate (**4a**).



Compound **4a** was synthesized following the procedure described for **3** using *O*-methyl-L-alaninate hydrochloride and was purified by flash chromatography [SiO₂, CH₂Cl₂:C₂H₅OH 98:2]. The crude product was re-crystallized from ethanol-water to give **4a**. Yield: 66%. Mp: 148-150°C. ¹H-NMR (400 MHz, CDCl₃) δ = 0.64 (s, 3H, CH₃), 0.91-0.93 (m, 6H), 0.96-1.31 (m, 10H), 1.34-1.41 (m, 9H), 1.50-1.64 (m, 4H), 1.71-

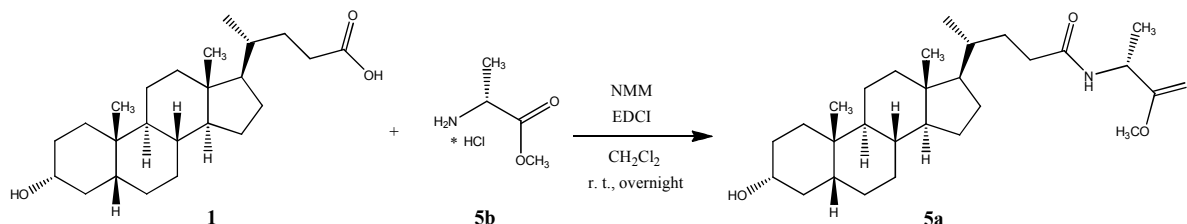
1.86 (m, 5H), 1.94-1.97 (m, 1H), 2.06-2.14 (m, 1H), 2.23-2.30 (m, 1H), 3.59-3.65 (m, 1H), 3.75 (s, 3H, OCH₃), 4.60 (quint, $J = 7.2$, 1H, CHCH₃), 6.00 (d, $J = 7.2$, 1H, NH). ¹³C-NMR (100 MHz, CDCl₃) δ = 12.03, 18.37, 18.54, 20.80, 23.38, 24.20, 26.41, 27.20, 28.24, 30.50, 31.57, 33.36, 34.56, 35.36, 35.44, 35.83, 36.41, 40.17, 40.40, 42.08, 42.40, 42.72, 47.84, 52.43, 56.00, 56.48, 71.74, 173.12 (CONH), 173.78 (C=O). MS (ESI) calc for C₂₈H₄₇NO₄: 461.35; found: 460.5 [M-1]⁻. Anal. calc for C₂₈H₄₇NO₄: C, 72.84; H, 10.26; N, 3.03; found: C, 72.78; H, 10.48; N, 2.90.

***N*-(3 α -Hydroxy-5 β -cholan-24-oyl)-L-alanine (4).**



Compound **4** was synthesized following the procedure described for **2** using compound **4a**. The crude product was re-crystallized from (EtOH/H₂O). Yield: 86%. Mp: 218-221°C. ¹H-NMR (400 MHz, DMSO-d₆) δ = 0.59 (s, 3H, CH₃), 0.87-0.91 (m, 7H), 1.00-1.08 (m, 4H), 1.11-1.21 (m, 9H), 1.28-1.34 (m, 7H), 1.47-1.55 (m, 2H), 1.58-1.68 (m, 3H), 1.73-1.79 (m, 2H), 1.90-2.01 (m, 2H), 2.07-2.12 (m, 1H), 3.34-3.36 (m, 1H), 4.10 (quint, $J = 7.2$, 1H, CHCH₃), 7.96 (d, $J = 7.2$, 1H, NH). ¹³C-NMR (100 MHz, DMSO-d₆) δ = 12.33, 17.97, 18.79, 20.87, 23.74, 24.32, 26.63, 27.36, 28.20, 30.83, 31.91, 32.55, 34.66, 35.37, 35.61, 35.84, 36.75, 41.99, 42.75, 48.13, 56.08, 56.56, 70.32, 172.64 (CONH), 174.91 (C=O). MS (ESI) calc for C₂₇H₄₅NO₄: 447.33; found: 446.4 [M-1]⁻. Anal. calc for C₂₇H₄₅NO₄: C, 72.44; H, 10.13; N, 3.13; found: C, 71.96; H, 10.08; N, 3.09.

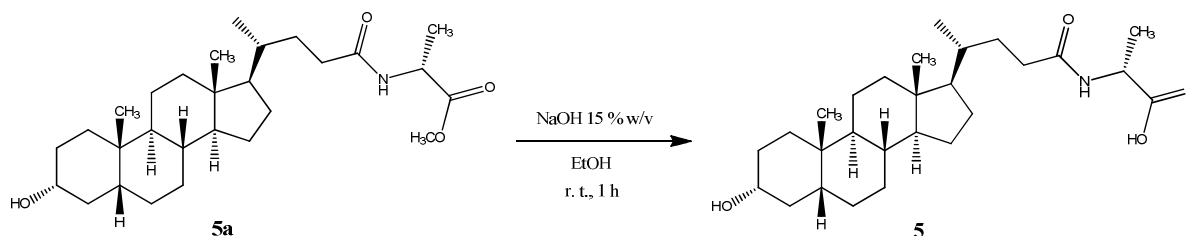
Methyl *N*-(3 α -hydroxy-5 β -cholan-24-oyl)-D-alaninate (5a).



Compound **5a** was synthesized following the procedure described for **3** using *O*-methyl-D-alaninate hydrochloride and was purified by flash chromatography [SiO₂, CH₂Cl₂:C₂H₅OH 98:2]. The crude product was re-crystallized from ethanol-water to give **5a** (EtOH/H₂O). Yield: 71%. Mp: 221-224°C. ¹H-NMR (400 MHz, DMSO-d₆) δ = 0.56 (s, 3H, OCH₃), 0.82-0.95 (m, 6H), 1.05-1.10 (m, 4H), 1.12-1.23 (m, 9H), 1.28-1.34 (m, 7H), 1.47-1.55 (m, 2H), 1.58-1.68 (m, 3H), 1.73-1.79 (m, 2H), 1.90-2.09 (m, 2H), 2.07-2.12 (m,

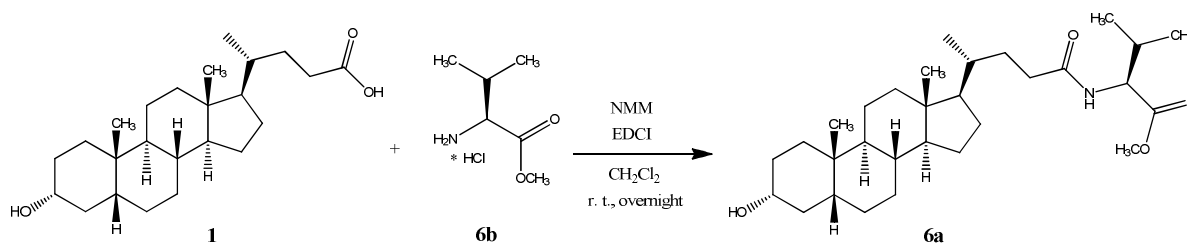
1H), 3.30-3.36 (m, 1H), 3.70 (s, 3H, OCH₃), 4.17 (quint, *J* = 7.2, 1H, CHCH₃), 7.95 (d, *J* = 7.2, 1H, NH). ¹³C-NMR (100 MHz, DMSO-d₆) δ = 12.25, 18.12, 18.81, 20.77, 23.55, 24.30, 26.52, 27.46, 28.40, 30.73, 32.41, 32.45, 34.67, 35.38, 35.51, 35.81, 36.76, 42.02, 42.43, 42.70, 48.20, 56.21, 56.76, 70.36, 172.84 (CONH), 174.31 (C=O). MS (ESI) calc for C₂₇H₄₇NO₄: 461.35; found: 460.4 [M-1]⁻. Anal. calc for C₂₇H₄₅NO₄: C, 72.44; H, 10.13; N, 3.13; found: C, 72.21; H, 10.05; N, 3.10.

***N*-(3 α -Hydroxy-5 β -cholan-24-oyl)-D-alanine (5).**



Compound **5** was synthesized following the procedure described for **2** using **5a**. The crude product was recrystallized from (EtOH/H₂O) to give **5**. Yield: 97%. Mp: 224-227°C. ¹H-NMR (400 MHz, DMSO-d₆) δ = 0.59 (s, 3H, CH₃), 0.85-0.91 (m, 7H), 1.01-1.06 (m, 4H), 1.09-1.22 (m, 9H), 1.32-1.34 (m, 7H), 1.47-1.50 (m, 2H), 1.58-1.68 (m, 3H), 1.73-1.80 (m, 2H), 1.90-1.92 (m, 1H), 1.95-2.03 (m, 1H), 2.07-2.14 (m, 1H), 3.32-3.38 (m, 1H), 4.15 (quint, *J* = 7.3, 1H, CHCH₃), 4.43 (bs, 1H, OH), 8.05 (d, *J* = 7.3, 1H, NH). ¹³C-NMR (100 MHz, DMSO-d₆) δ = 12.33, 17.66, 18.74, 20.87, 23.74, 24.32, 26.63, 27.36, 28.18, 30.84, 31.84, 32.43, 34.67, 35.36, 35.61, 35.84, 36.75, 41.99, 42.75, 47.72, 56.07, 56.54, 70.32, 172.79 (CONH), 174.77 (C=O). MS (ESI) calc for C₂₇H₄₅NO₄: 447.33; found: 446.4 [M-1]⁻. Anal. calc for C₂₇H₄₅NO₄: C, 72.44; H, 10.13; N, 3.13; found: C, 72.61; H, 10.29; N, 3.06.

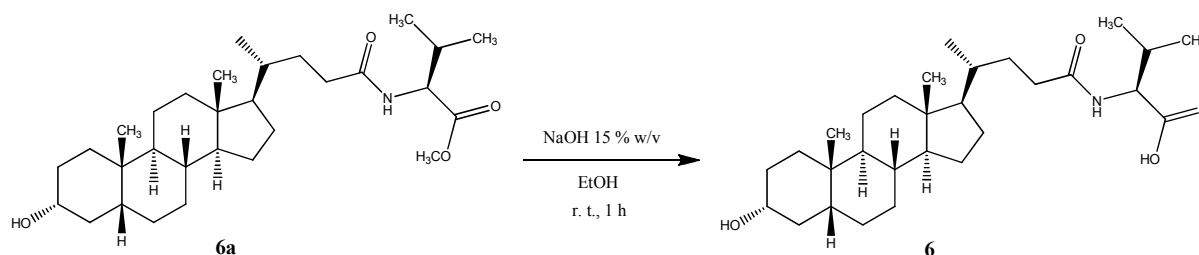
Methyl *N*-(3 α -hydroxy-5 β -cholan-24-oyl)-L-valinate (6a).



Compound **6a** was synthesized following the procedure described for **3** using *O*-methyl-L-valinate hydrochloride and was purified by flash chromatography [SiO₂, CH₂Cl₂:C₂H₅OH 98:2]. The crude product was re-crystallized from ethanol-water to give **6a** (EtOH/H₂O). Yield: 77%. Mp: 183-186°C. ¹H-NMR (400 MHz, CDCl₃) δ = 0.64 (s, 3H, CH₃), 0.89-0.94 (m, 12H), 0.96-1.16 (m, 5H), 1.24-1.40 (m, 11H), 1.52-1.64 (m, 4H), 1.71-1.86 (m, 5H), 1.94-1.97 (m, 1H), 2.09-2.17 (m, 2H), 2.26-2.34 (m, 1H), 3.62 (m, 1H), 3.74 (s,

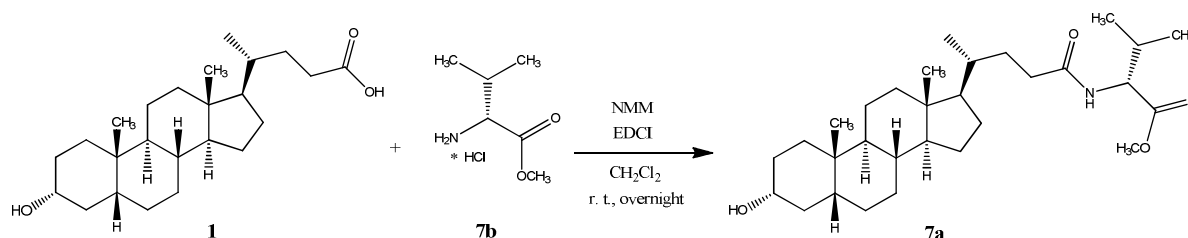
3H, OCH₃), 4.58 (dd, $J = 8.8, 4.8$, 1H, CHCH(CH₃)₂), 5.90 (d, $J = 8.8$, 1H, NH). ¹³C-NMR (100 MHz, CDCl₃) $\delta = 12.04, 17.86, 18.36, 18.93, 20.81, 23.37, 24.20, 26.42, 27.20, 28.24, 30.52, 31.35, 31.72, 33.55, 34.57, 35.35, 35.45, 35.84, 36.44, 40.18, 40.42, 42.09, 42.74, 52.11, 56.02, 56.48, 56.79, 71.80, 172.80$ (CONH), 173.47 (C=O). MS (ESI) calc for C₃₀H₅₁NO₄: 489.38; found: 488.5 [M-1]⁻. Anal. calc for C₃₀H₅₁NO₄: C, 73.57; H, 10.50; N, 2.86; found: C, 73.55; H, 10.47; N, 2.85.

***N*-(3 α -Hydroxy-5 β -cholan-24-oyl)-L-valine (6).**



Compound **6** was synthesized following the procedure described for **2** using **6a**. The crude product was re-crystallized from (EtOH/H₂O) to give **6**. Yield: 92%. Mp: 229-232°C. ¹H-NMR (400 MHz, DMSO-d₆) $\delta = 0.59$ (s, 3H, CH₃), 0.84-0.91 (m, 13H), 1.00-1.07 (m, 4H), 1.11-1.17 (m, 6H), 1.32 (m, 7H), 1.50 (m, 2H), 1.58-1.68 (m, 3H), 1.77 (m, 2H), 1.90-1.92 (m, 1H), 1.98-2.07 (m, 2H), 2.16-2.20 (m, 1H), 3.35 (m, 1H), 4.10 (dd, $J = 8.40, 6.00$, 1H, CHCH(CH₃)₂), 4.43 (bs, 1H, OH), 7.88 (d, $J = 8.40$, 1H, NH). ¹³C-NMR (100 MHz, DMSO-d₆) $\delta = 12.33, 18.54, 18.80, 19.63, 20.88, 23.74, 24.31, 26.64, 27.36, 28.17, 30.23, 30.85, 32.10, 32.44, 34.67, 35.41, 35.62, 35.85, 36.76, 41.99, 42.72, 56.04, 56.56, 57.48, 70.33, 173.38$ (CONH), 173.68 (C=O). MS (ESI) calc for C₂₉H₄₉NO₄: 475.37; found: 474.5 [M-1]⁻. Anal. calc for C₂₉H₄₉NO₄: C, 73.22; H, 10.38; N, 2.94; found: C, 73.26; H, 10.69; N, 2.96.

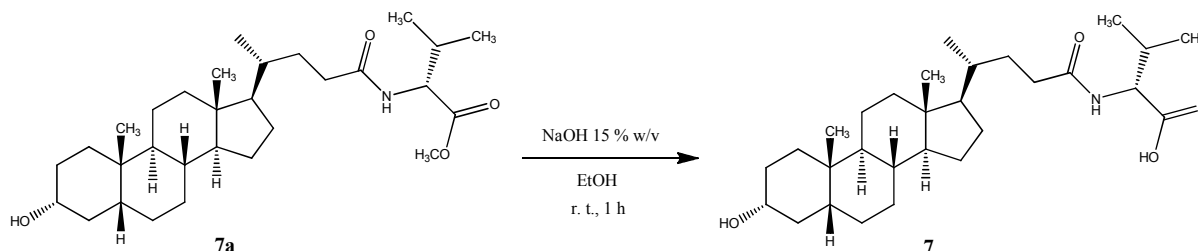
Methyl *N*-(3 α -hydroxy-5 β -cholan-24-oyl)-D-valinate (7a).



Compound **7a** was synthesized following the procedure described for **3** using *O*-methyl-D-valinate hydrochloride and was purified by flash chromatography [SiO₂, CH₂Cl₂:C₂H₅OH 98:2]. The crude product was re-crystallized from ethanol-water to give **7a** (EtOH/H₂O). Yield: 77%. Mp: 173-176°C. ¹H-NMR (400 MHz, CDCl₃) $\delta = 0.63$ (s, 3H, CH₃), 0.88-0.93 (m, 12H), 0.95-1.15 (m, 5H), 1.23-1.38 (m, 11H), 1.48-1.64 (m, 4H), 1.72-1.86 (m, 5H), 1.93-1.96 (m, 1H), 2.09-2.16 (m, 2H), 2.25-2.33 (m, 1H), 3.58-3.64 (m, 1H),

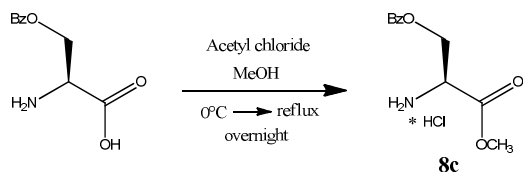
3.73 (s, 3H, OCH₃), 4.57 (dd, $J = 8.8, 5.2$, 1H, CHCH(CH₃)₂), 5.92 (d, $J = 8.8$, 1H, NH). ¹³C-NMR (100 MHz, CDCl₃) $\delta = 12.04, 17.85, 18.33, 18.93, 20.81, 23.37, 24.20, 26.41, 27.20, 28.21, 30.52, 31.33, 31.66, 33.58, 34.57, 35.39, 35.84, 36.44, 40.18, 40.41, 42.09, 42.74, 52.10, 56.03, 56.48, 56.81, 71.79, 172.79$ (CONH), 173.42 (C=O). MS (ESI) calc for C₃₀H₅₁NO₄: 489.38; found: 490.6 [M+H]⁺, 512.5 [M+Na]⁺. Anal. calc for C₃₀H₅₁NO₄: C, 73.57; H, 10.50; N, 2.86; found: C, 73.59; H, 10.66; N, 2.86.

***N*-(3 α -Hydroxy-5 β -cholan-24-oyl)-D-valine (7).**



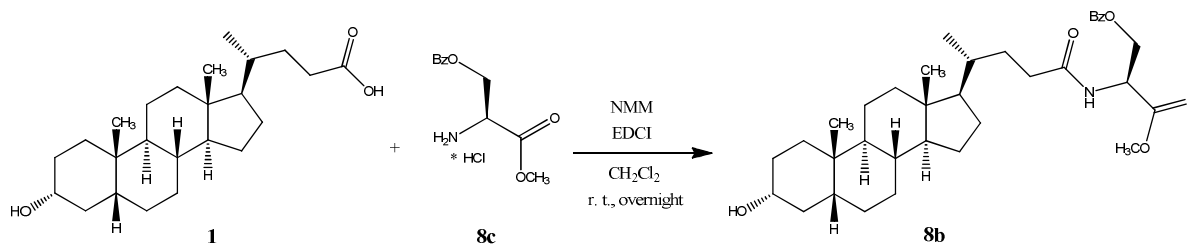
Compound **7** was synthesized following the procedure described for compound **2** starting from **7a**. The crude product was re-crystallized from (EtOH/H₂O) to give **7**. Yield: 92%. Mp: 215-218°C. ¹H-NMR (400 MHz, DMSO-d₆) $\delta = 0.61$ (s, 3H, CH₃), 0.87-0.94 (m, 13H), 1.02-1.27 (m, 10H), 1.29-1.38 (m, 7H), 1.50-1.61 (m, 3H), 1.64-1.71 (m, 2H), 1.76-1.83 (m, 2H), 1.91-1.94 (m, 1H), 2.00-2.07 (m, 2H), 2.12-2.17 (m, 1H), 3.37-3.39 (m, 1H), 4.15 (dd, $J = 8.4, 6.00$, 1H, CHCH(CH₃)₂), 7.62 (d, $J = 8.0$, 1H, NH). ¹³C-NMR (100 MHz, DMSO-d₆) $\delta = 12.35, 18.54, 18.78, 19.53, 20.94, 23.68, 24.29, 26.64, 27.43, 28.06, 30.31, 30.95, 32.03, 32.74, 34.75, 35.26, 35.74, 35.99, 36.92, 42.18, 42.85, 56.34, 56.67, 57.61, 70.43, 173.23$ (CONH), 173.39 (C=O). MS (ESI) calc for C₂₉H₄₉NO₄: 475.37; found: 474.5 [M-1]⁻. Anal. calc for C₂₉H₄₉NO₄ • 0.1H₂O: C, 72.94; H, 10.38; N, 2.93; found: C, 72.66; H, 10.47; N, 2.91.

***O*-Methyl *O*-benzyl-L-serinate hydrochloride (8c).**



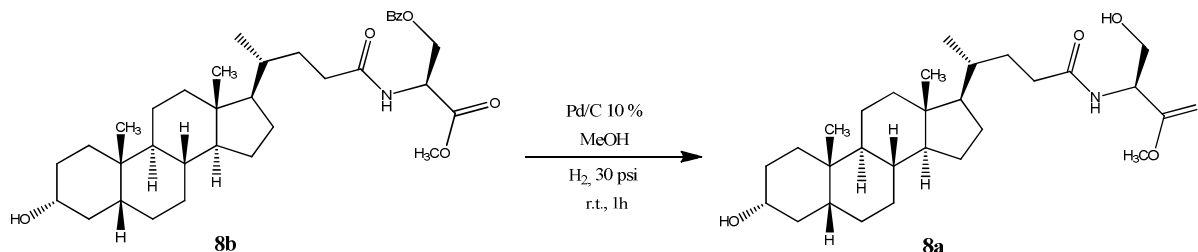
Compound **8c** was synthesized following a described procedure^[3] starting from *O*-Benzyl-L-Serine.

Methyl *O*-benzyl-*N*-(3 α -hydroxy-5 β -cholan-24-oyl)-L-serinate (8b).



Compound **8b** was synthesized following the procedure described for **3** starting from *O*-Methyl *O*-benzyl-L-serinate hydrochloride **8c** and was purified by flash chromatography [SiO_2 , CH_2Cl_2 : $\text{C}_2\text{H}_5\text{OH}$ 98:2]. The crude product was re-crystallized from ethanol-water to give **8b** ($\text{EtOH}/\text{H}_2\text{O}$). Yield: 79%. Mp: 145-148°C. ^1H -NMR (400 MHz, CDCl_3) δ = 0.63 (s, 3H, CH_3), 0.91-0.93 (m, 6H), 0.95-1.16 (m, 5H), 1.23-1.40 (m, 11H), 1.48-1.63 (m, 4H), 1.71-1.84 (m, 5H), 1.94-1.97 (m, 1H), 2.09-2.17 (m, 1H), 2.24-2.32 (m, 1H), 3.61-3.57 (m, 1H), 3.68 (dd, J = 9.44, 3.16 Hz, 1H, CHCHH), 3.74 (s, 3H, OCH_3), 3.89 (dd, J = 9.44, 3.08 Hz, 1H CHCHH), 4.48 (d, J = 12.2 Hz, 1H, CHHPh), 4.53 (d, J = 12.2 Hz, 1H, CHHPh), 4.76 (dt, J = 8.2, 3.1 Hz, 1H, CHCH_2), 6.30 (d, J = 7.9 Hz, 1H, NH), 7.25-7.36 (m, 5H, Ar). ^{13}C -NMR (100 MHz, CDCl_3) δ = 12.06, 18.38, 20.82, 23.38, 24.22, 26.42, 27.20, 28.24, 30.54, 31.53, 33.37, 34.58, 35.36, 35.45, 35.85, 36.45, 40.19, 40.42, 42.10, 42.75, 52.46, 52.52, 56.02, 56.50, 69.78, 71.83, 73.27, 127.66, 127.91, 128.46, 137.51, 170.92 (CONH), 173.39 (C=O). MS (ESI) calc for $\text{C}_{35}\text{H}_{53}\text{NO}_5$: 567.39; found: 568.6 $[\text{M}+\text{H}]^+$, 590.5 $[\text{M}+\text{Na}]^+$, 606.4 $[\text{M}+\text{K}]^+$. Anal. calc for $\text{C}_{35}\text{H}_{53}\text{NO}_5$: C, 74.04; H, 9.41; N, 2.47; found: C, 74.13; H, 9.63; N, 2.43.

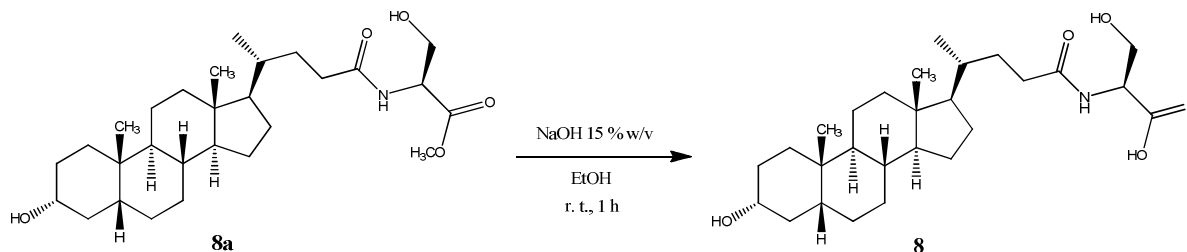
Methyl *N*-(3 α -hydroxy-5 β -cholan-24-oyl)-L-serinate (**8a**).



Compound **8b** (0.405 mmol) was dissolved in 25 mL of MeOH, Pd/C 10% (0.01032 mmol) was added to the solution and the mixture was shaken under H_2 atmosphere (30 psi) for 3h on a Parr apparatus. The reaction mixture was filtered over a pad of Celite and the solvent was removed under reduced pressure. The residue was then purified by flash chromatography (CH_2Cl_2 / HCOOH / EtOH : 89.5/0.5/10), giving the title compound as a white solid **8a**. Yield: 98%. Mp: 165-170°C. ^1H -NMR (400 MHz, CD_3OD) δ = 0.69 (s, 3H, CH_3), 0.94-0.98 (m, 6H), 1.06-1.19 (m, 5H), 1.25-1.49 (m, 13H), 1.61-1.63 (m, 2H), 1.72-1.82 (m, 3H), 1.86-1.90 (m, 2H), 2.01-2.04 (m, 1H), 2.16-2.24 (m, 1H), 2.28-2.36 (m, 1H), 3.49-3.56 (m, 1H), 3.73 (s, 3H, OCH_3), 3.78 (dd, J = 11.2, 4.3 Hz, 1H, CHCHH), 3.87 (dd, J = 11.2, 5.0 Hz, 1H, CHCHH), 4.52-4.97 (m, H, CHCH_2). ^{13}C -NMR (100 MHz, CD_3OD) δ = 11.12, 17.49, 20.56, 22.55, 23.88, 26.26, 26.97, 27.85, 29.79, 31.63, 32.34, 34.28, 35.09, 35.39, 35.78, 35.84, 40.15, 40.49, 42.14, 42.52, 51.36, 54.78, 56.11, 56.54, 61.43, 71.01,

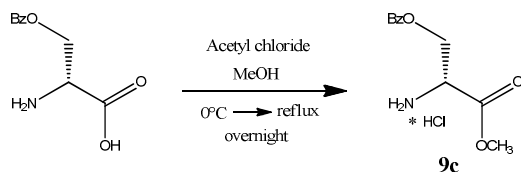
171.01 (CONH), 175.44 (C=O). MS (ESI) calc for $C_{28}H_{47}NO_5$: 477.35; found: 478.5 $[M+H]^+$, 500.4 $[M+Na]^+$. Anal. calc for $C_{28}H_{47}NO_5 \cdot 1H_2O$: C, 67.84; H, 9.96; N, 2.82; found: C, 68.12; H, 10.08; N, 2.70.

***N*-(3 α -Hydroxy-5 β -cholan-24-oyl)-L-serine (**8**).**



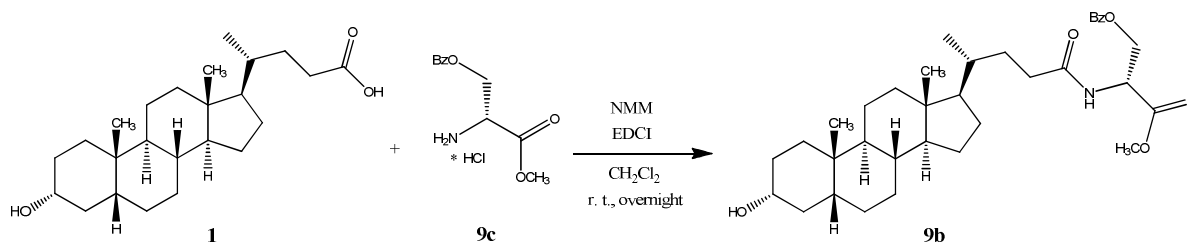
Compound **8** was synthesized following the procedure described for compound **2** starting from **8a**. The crude product was re-crystallized from (EtOH/H₂O) to give **8** Yield: 87%. Mp: 180-183°C. ¹H-NMR (400 MHz, DMSO-*d*₆) δ = 0.44 (s, 3H, CH₃), 0.87-0.91 (m, 6H), 1.02-1.06 (m, 7H), 1.11-1.17 (m, 6H), 1.33-1.43 (m, 5H), 1.50 (m, 2H), 1.58-1.68 (m, 3H), 1.77-1.85 (m, 2H), 1.90-1.92 (m, 1H), 2.00-2.06 (m, 1H), 2.14-2.16 (m, 1H), 3.57-3.66 (m, 2H, CHCHH), 4.24-4.29 (m, 1H, CHCH₂), 4.41 (bs, 1H, OH), 7.88 (d, J = 7.60, 1H, NH). ¹³C-NMR (100 MHz, DMSO-*d*₆) δ = 12.33, 18.80, 20.87, 23.73, 24.32, 26.63, 27.36, 28.19, 30.83, 31.90, 32.51, 34.66, 35.38, 35.61, 35.85, 36.75, 41.99, 42.72, 55.01, 56.11, 56.56, 61.90, 70.32, 172.63 (CONH), 173.15 (C=O). MS (ESI) calc for $C_{27}H_{45}NO_5$: 463.33; found: 462.5 $[M-H]^-$. Anal. calc for $C_{27}H_{45}NO_5$: C, 69.94; H, 9.78; N, 3.02; found: C, 69.77; H, 10.10; N, 2.89.

***O*-Methyl *O*-benzyl-D-serinate hydrochloride (**9c**).**



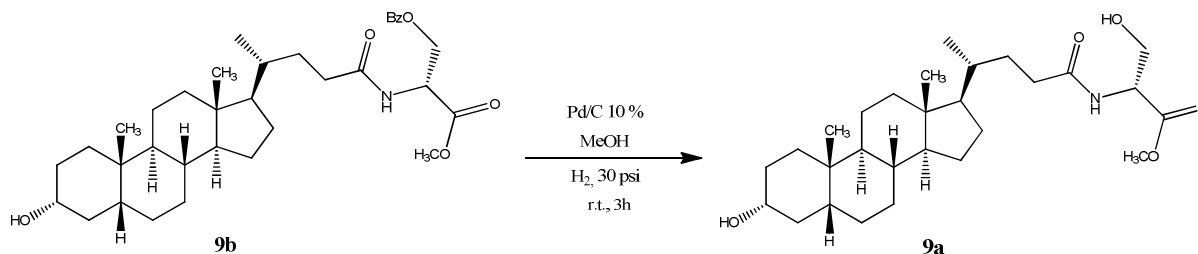
Compound **9c** was synthesized following a modification of a described procedure^[4] in which acetyl chloride (3.84 mmol) was added dropwise to-methanol (10 mL) at 0°C. The mixture was stirred for 15 min and *O*-benzyl-D-serine (0.512 mmol) was then added portionwise to the solution. The resulting mixture was heated to reflux overnight. The solvent evaporated under reduced pressure afforded **9c** as a white solid that was immediately used in the next step without further purification. Yield (99%). ¹H-NMR (400 MHz, D₂O) δ = 3.84 (s, 3H, OCH₃), 3.95 (dd, J = 14.8, 4.4 Hz, 1H, CHCHH), 4.02 (dd, J = 14.8, 5.8 Hz, 1H, CHCHH), 4.39 (dd, J = 6.0, 4.0 Hz, 1H, CHCH₂), 4.59 (d, J = 15.6 Hz, 1H, CHHPh), 4.68 (d, J = 16.0 Hz, 1H, CHHPh), 7.40-7.48 (m, 5H, Ar). (ESI) calc for $C_{11}H_{15}NO_3$: 209.24 found: 205.23 $[M+H]^+$.

Methyl *O*-benzyl-*N*-(3 α -hydroxy-5 β -cholan-24-oyl)-D-serinate (9b**).**



Compound **9b** was synthesized following the procedure described for **3** starting from *O*-Methyl *O*-benzyl-D-serinate hydrochloride **9c** and was purified by flash chromatography [SiO_2 , CH_2Cl_2 : $\text{C}_2\text{H}_5\text{OH}$ 98:2]. The crude product was re-crystallized from ethanol-water to give **9b** ($\text{EtOH}/\text{H}_2\text{O}$). Yield: 79%. Mp: 187-190°C. ^1H -NMR (400 MHz, CDCl_3) δ = 0.64 (s, 3H, CH_3), 0.92-0.93 (m, 6H), 0.96-1.00 (m, 1H), 1.08-1.16 (m, 5H), 1.24-1.40 (m, 11H), 1.48-1.63 (m, 5H), 1.75-1.84 (m, 4H), 1.94-1.97 (m, 1H), 2.08-2.16 (m, 1H), 2.26-2.32 (m, 1H), 3.59-3.65 (m, 1H), 3.68 (dd, J = 9.2, 3.2 Hz, 1H, CHCHH), 3.74 (s, 3H, OCH_3), 3.89 (dd, J = 9.2, 3.2 Hz, 1H, CHCHH), 4.48 (d, J = 12.4 Hz, 1H, CHHPh), 4.53 (d, J = 12.0 Hz, 1H, CHHPh), 4.76 (dt, J = 8.4, 3.2 Hz, 1H, CHCH_2), 6.28 (d, J = 8.0 Hz, 1H, 1H), 7.26-7.36 (m, 5H, Ar). ^{13}C -NMR (100 MHz, CDCl_3) δ = 12.06, 18.37, 20.83, 23.39, 24.22, 26.43, 27.21, 28.23, 30.54, 31.52, 33.38, 34.58, 35.37, 35.46, 35.86, 36.46, 40.19, 40.42, 42.10, 42.75, 52.47, 52.52, 56.02, 56.48, 69.81, 71.81, 73.28, 127.65, 127.91, 128.45, 137.52, 170.91 (CONH), 173.39 (C=O). MS (ESI) calc for $\text{C}_{35}\text{H}_{53}\text{NO}_5$: 567.39; found: 568.6 $[\text{M}+\text{H}]^+$, 590.5 $[\text{M}+\text{Na}]^+$, 606.4 $[\text{M}+\text{K}]^+$. Anal. calc for $\text{C}_{35}\text{H}_{53}\text{NO}_5$: C, 74.04; H, 9.41; N, 2.47; found: C, 74.20; H, 9.63; N, 2.42.

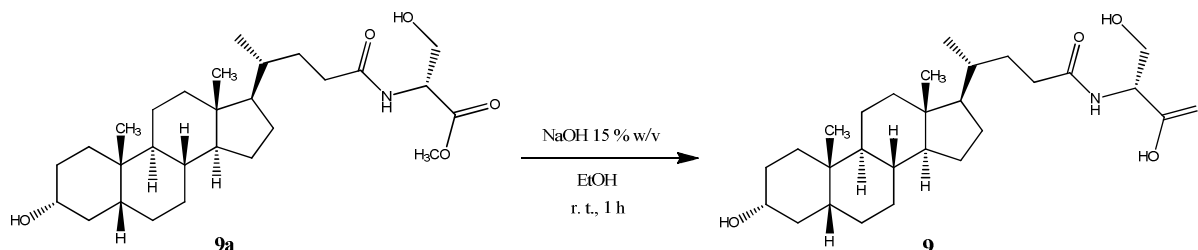
Methyl *N*-(3 α -hydroxy-5 β -cholan-24-oyl)-D-serinate (**9a**).



Compound **9a** was synthesized following the procedure described for **8a** starting from compound **9b**. Yield: 98 %. Mp: 173-176°C. ^1H -NMR (400 MHz, CDCl_3) δ = 0.64 (s, 3H, CH_3), 0.92-0.93 (m, 6H), 0.96-1.00 (m, 1H), 1.08-1.16 (m, 5H), 1.24-1.40 (m, 11H), 1.48-1.57 (m, 3H), 1.63-1.84 (m, 6H), 1.94-1.97 (m, 1H), 2.08-2.16 (m, 1H), 2.26-2.32 (m, 1H), 3.59-3.65 (m, 1H), 3.68 (dd, J = 9.2, 3.2 Hz, 1H, CHCHH), 3.74 (s, 3H, OCH_3), 3.89 (dd, J = 9.2, 3.2 Hz, 1H, CHCHH), 4.48 (d, J = 12.4 Hz, 1H, CHHPh), 4.53 (d, J = 12.0 Hz, 1H, CHHPh), 4.76 (dt, J = 8.4, 3.2 Hz, 1H, CHCH_2), 6.28 (d, J = 8.0 Hz, 1H, NH), 7.26-7.36 (m, 5H, Ar). ^{13}C -NMR (100 MHz, CDCl_3) δ = 12.10, 18.35, 20.78, 23.40, 24.29, 26.48, 27.31, 28.21, 30.52, 31.48, 33.35, 34.54, 35.30, 35.31, 36.58, 36.37, 40.31, 40.56, 42.20, 42.65, 52.48, 52.53, 56.07, 56.45, 69.71, 71.78, 73.24,

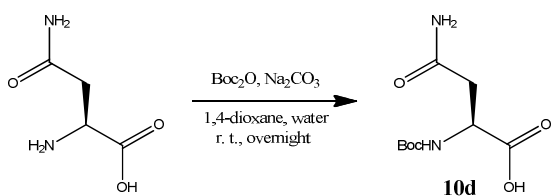
127.23, 127.80, 128.78, 137.45, 170.51 (CONH), 173.68 (C=O). MS (ESI) calc for $C_{28}H_{47}NO_5$: 477.35; found: 500.4 $[M+Na]^+$. Anal. calc for $C_{35}H_{53}NO_5$: C, 74.04; H, 9.41; N, 2.47; found: C, 74.30; H, 9.55; N, 2.58

***N*-(3 α -Hydroxy-5 β -cholan-24-oyl)-D-serine (9).**



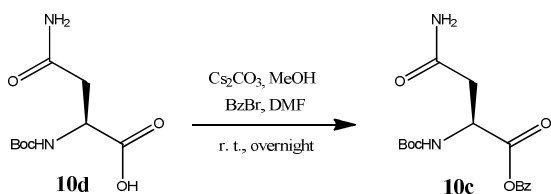
Compound **9** was synthesized following the procedure described for compound **2** starting from compound **9a**. The crude product was re-crystallized from (EtOH/H₂O) to give **9** Yield: 88%. Mp: 165-167°C. ¹H-NMR (400 MHz, DMSO-*d*₆) δ = 0.59 (s, 3H, CH₃), 0.86-0.91 (m, 7H), 1.01-1.07 (m, 4H), 1.14-1.17 (m, 6H), 1.33-1.46 (m, 7H), 1.50-1.58 (m, 3H), 1.61-1.68 (m, 2H), 1.77-1.84 (m, 2H), 1.90-1.92 (m, 1H), 2.00-2.07 (m, 1H), 2.14-2.16 (m, 1H), 3.56-3.63 (m, 2H, CHCHH), 4.23 (m, 1H, CHCH₂), 4.41 (bs, 1H, OH), 7.89 (d, *J* = 7.6, 1H, NH). ¹³C-NMR (100 MHz, DMSO-*d*₆) δ = 12.34, 18.77, 20.88, 23.74, 24.32, 26.63, 27.36, 28.19, 30.84, 31.86, 32.49, 34.67, 35.40, 35.61, 35.85, 36.75, 41.99, 42.73, 54.99, 56.09, 56.55, 61.91, 70.33, 172.62 (CONH), 173.13 (C=O). MS (ESI) calc for $C_{27}H_{45}NO_5$: 463.33; found: 461.9 $[M-H]^-$. Anal. calc for $C_{27}H_{45}NO_5 \cdot 0.1H_2O$: C, 69.67; H, 9.79; N, 3.00; found: C, 69.32; H, 10.09; N, 2.89.

***N*-(tert-Butoxycarbonyl)-L-asparagine (10d).**



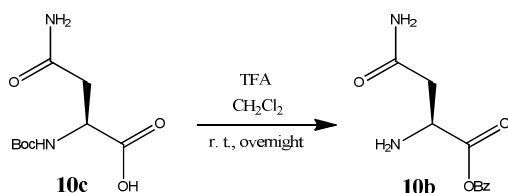
Compound **10d** was synthesized according to a described procedure^[5] starting from L-asparagine.

***O*-Benzyl *N*-(tert-butoxycarbonyl)-L-asparaginate (10c).**



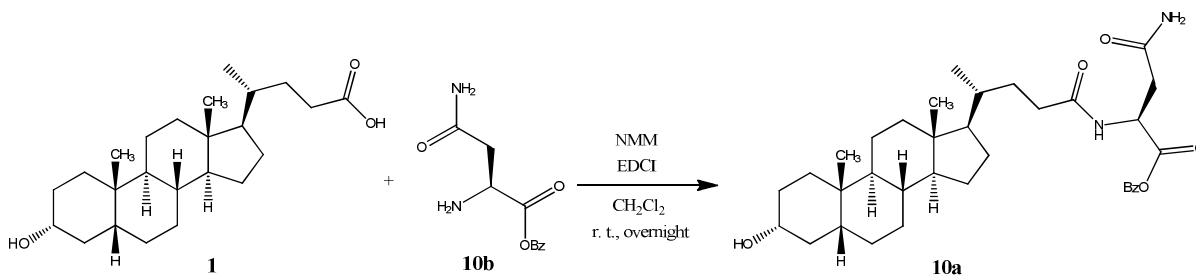
Compound **10c** was synthesized according to a described procedure^[5] starting from **10d**.

***O*-Benzyl-L-asparaginate (**10b**).**



Compound **10b** was synthesized following a modification procedure^[6] in which to a solution of **10c** (1.148 mmol) in CH_2Cl_2 (10 mL) was added trifluoroacetic acid (1 mL); the resulting solution was stirred at room temperature overnight. Evaporation of solvent under reduced pressure afforded **10b** as a white solid that was immediately used in the next step without further purification. Yield 98%. ^1H -NMR (400 MHz, CDCl_3) δ = 2.52 (dd, J = 16.0, 8.8 Hz, 1H, CHCHH), 2.70 (dd, J = 16.0, 3.6 Hz, 1H, CHCHH), 3.84 (dd, J = 8.8, 3.6 Hz, 1H, CHCH₂), 5.14 (d, J = 12.4 Hz, 1H, CHHPh), 5.18 (d, J = 12.4 Hz, 1H, CHHPh), 5.53 (bs, 1H, CONHH), 6.85 (bs, 1H, CONHH), 7.35-7.38 (m, 5H, Ar). ^{13}C -NMR (100 MHz, CDCl_3) δ = 39.35, 51.46, 67.23, 128.33, 128.51, 128.65, 135.36, 172.60 (CONH₂), 174.17 (C=O).

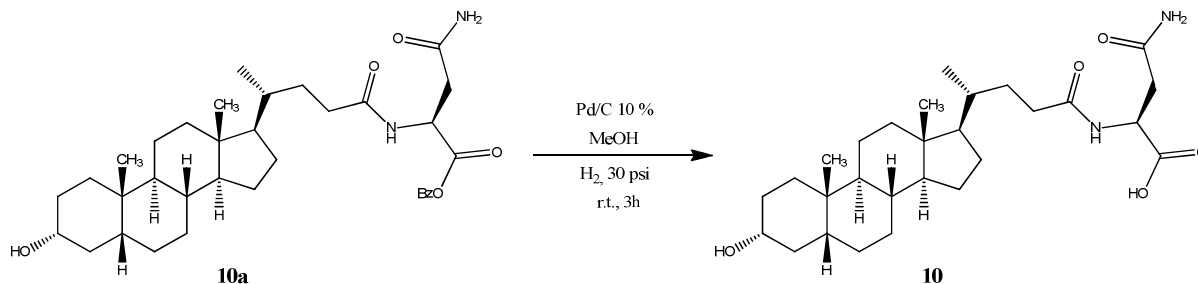
Benzyl *N*-(3 α -hydroxy-5 β -cholan-24-oyl)-L-asparaginate (10a**).**



Compound **10a** was synthesized following the procedure described for **3** starting from *O*-benzyl-L-asparaginate **10b** and was purified by flash chromatography [SiO_2 , CH_2Cl_2 : $\text{C}_2\text{H}_5\text{OH}$ from 98:2 to 95:5]. The crude product was re-crystallized from ethanol-water to give **10a** (EtOH/ H_2O). Yield: 72%. Mp: 65-68°C. ^1H -NMR (400 MHz, CDCl_3) δ = 0.60 (s, 3H, CH₃), 0.87-0.90 (m, 6H), 0.94-1.13 (m, 7H), 1.29-1.38 (m, 8H), 1.50 (m, 2H), 1.62 (m, 1H), 1.72-1.78 (m, 5H), 1.91-1.94 (m, 1H), 2.06-2.14 (m, 3H), 2.22-2.28 (m, 1H), 2.75 (dd, J = 16.2, 4.4 Hz, 1H, CHCHH), 2.93 (dd, J = 16.2, 4.8 Hz, 1H, CHCHH), 3.60 (m, 1H), 4.80 (dt, J = 8.0, 4.6 Hz, 1H, CHCH₂), 5.14 (d, J = 12.4 Hz, 1H, CHHPh), 5.18 (d, J = 12.4 Hz, 1H, CHHPh), 5.69 (bs, 1H, CONHH), 6.18 (bs, 1H, CONHH), 6.89 (d, J = 8.0 Hz, 1H, NH), 7.26-7.35 (m, 5H, Ar). ^{13}C -NMR (100 MHz, CDCl_3) δ = 12.02, 18.36, 20.80, 23.37, 24.19, 26.42, 27.19, 28.19, 30.48, 31.55, 33.32, 34.56, 35.35, 35.44, 35.83, 36.40, 36.91, 40.16, 40.42, 42.09, 42.72, 48.92, 55.98, 56.47, 58.28, 67.46, 71.78, 128.18, 128.37, 128.56, 135.32, 137.32, 171.03 (CONH₂), 172.54 (CONH), 173.93 (C=O). MS (ESI) calc for

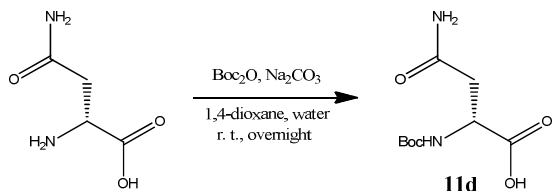
C₃₅H₅₂N₂O₅: 580.39; found: 581.5 [M+H]⁺, 603.4 [M+Na]⁺, 619.5 [M+K]⁺. Anal. calc for C₃₅H₅₂N₂O₅: C, 72.38; H, 9.02; N, 4.82; found: C, 72.42; H, 9.12; N, 4.93.

***N*-(3 α -Hydroxy-5 β -cholan-24-oyl)-L-asparagine (10).**



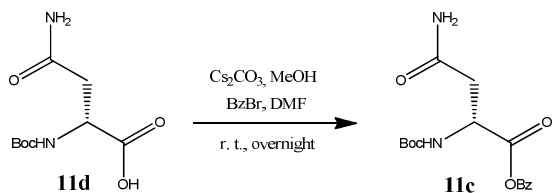
Compound **10a** (0.516 mmol) was dissolved in 20 mL of MeOH, Pd/C 10% (0.01032 mmol) was added to the solution and the mixture was shaken under H₂ atmosphere (30 psi) for 3h on a Parr apparatus. The reaction mixture was filtered over a pad of Celite and the solvent was removed under reduced pressure. The residue was then purified by flash chromatography (CH₂Cl₂/HCOOH/EtOH 89.5/0.5/10), giving the title compound as a white solid **10**. The crude product was re-crystallized from EtOH/H₂O. Yield: 98%. Mp: 164-167°C. ¹H-NMR (400 MHz, DMSO-d₆) δ = 0.59 (s, 3H, CH₃), 0.85-0.87 (m, 7H), 1.01-1.08 (m, 4H), 1.12-1.22 (m, 6H), 1.28-1.35 (m, 7H), 1.47-1.55 (m, 2H), 1.55-1.68 (m, 3H), 1.74-1.77 (m, 2H), 1.90-1.92 (m, 1H), 1.95-2.01 (m, 1H), 2.07-2.14 (m, 1H), 2.41 (dd, *J* = 15.5, 7.2 Hz, 1H, CHCHH), 2.49-2.54 (m, 1H, CHCHH), 3.35 (m, 1H), 4.45 (dd, *J* = 7.2, *J* = 7.0 Hz, 1H, CHCH₂), 6.84 (s, 1H, CONHH), 7.30 (s, 1H, CONHH), 7.93 (d, *J* = 7.9 Hz, 1H, NH). ¹³C-NMR (100 MHz, DMSO-d₆) δ = 12.34, 18.78, 20.88, 23.73, 24.31, 26.63, 27.36, 28.17, 30.85, 31.88, 32.59, 34.67, 35.33, 35.63, 35.86, 36.77, 37.27, 42.01, 42.74, 49.19, 56.11, 56.56, 70.34, 163.51, 171.73 (CONH₂), 172.85 (CONH), 173.45 (C=O). MS (ESI) calc for C₂₈H₄₆N₂O₅: 490.34; found: 489.2 [M-H]⁻. Anal. calc for C₂₈H₄₆N₂O₅: C, 68.54; H, 9.45; N, 5.71; found: C, 68.28; H, 9.82; N, 5.54.

***N*-(tert-Butoxycarbonyl)-D-asparagine (11d).**



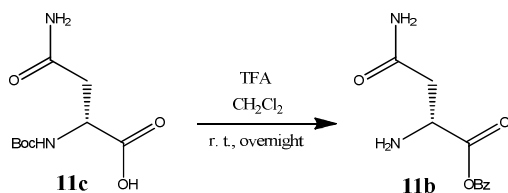
Compound **11d** was synthesized according to a described procedure^[7] starting from D-asparagine.

***O*-Benzyl *N*-(tert-butoxycarbonyl)-D-asparaginate (11c).**



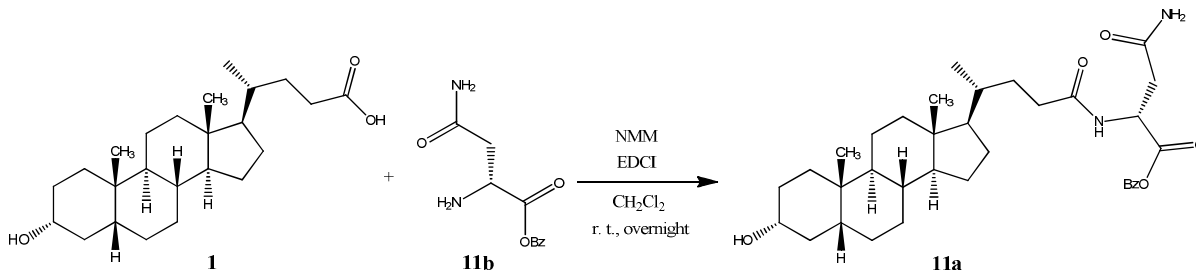
Compound **11c** was synthesized according to a described procedure^[8] starting from **11d**.

***O*-Benzyl-D-asparagine (**11b**).**



Compound **11b** was synthesized according to a described procedure^[8] starting from **11c**.

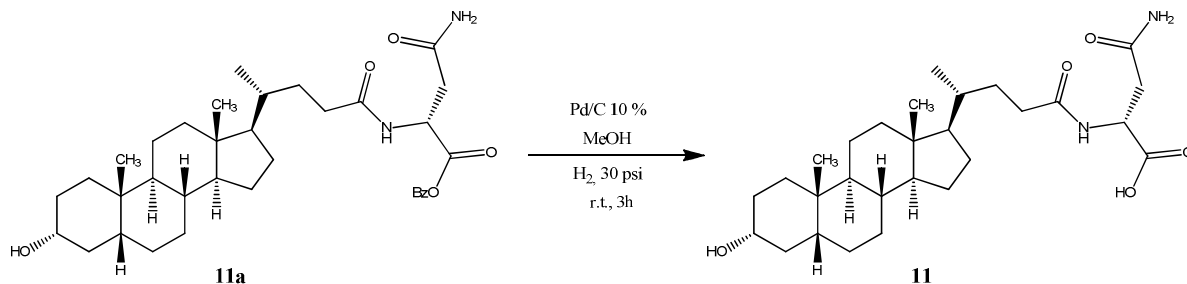
Benzyl *N*-(3 α -hydroxy-5 β -cholan-24-oyl)-D-asparagine (11a**).**



Compound **11a** was synthesized following the procedure described for **3** starting from *O*-benzyl-D-asparagine **11b** and was purified by flash chromatography [SiO_2 , CH_2Cl_2 : $\text{C}_2\text{H}_5\text{OH}$ from 98:2 to 95:5]. The crude product was re-crystallized from ethanol-water to give **11a** (EtOH/ H_2O). Yield: 65%. Mp: 169-173°C. ^1H -NMR (400 MHz, DMSO- d_6) δ = 0.58 (s, 3H, CH_3), 0.84-0.91 (m, 7H), 1.00-1.07 (m, 4H), 1.15-1.18 (m, 6H), 1.32-1.35 (m, 7H), 1.50-1.55 (m, 2H), 1.58-1.68 (m, 3H), 1.74-1.77 (m, 2H), 1.89-1.92 (m, 1H), 1.94-2.01 (m, 1H), 2.07-2.16 (m, 1H), 2.46-2.50 (m, 1H, CHCHH), 2.57 (dd, J = 15.6, 6.0 Hz, 1H, CHCHH), 3.32-3.35 (m, 1H), 4.43 (d, J = 4.4 Hz, 1H, OH), 4.56-4.62 (m, 1H, CHCH $_2$), 5.05-5.07 (m, 2H, CHHPh), 6.91 (s, 1H, CONHH), 7.30-7.36 (m, 6H, 5Ar, CONHH), 8.17 (d, J = 8.00 Hz, 1H, NH). ^{13}C -NMR (100 MHz, DMSO- d_6) δ = 12.35, 18.73, 20.87, 23.74, 24.31, 26.64, 27.36, 28.17, 30.85, 31.87, 32.47, 34.67, 35.40, 35.62, 35.85, 36.77, 37.12, 41.99, 42.72, 49.33, 56.04, 56.54, 66.28, 70.33, 128.02, 128.34, 128.78, 136.51, 171.33 (CONH $_2$), 171.90 (CONH), 173.08 (C=O). MS (ESI) calc for $\text{C}_{35}\text{H}_{52}\text{N}_2\text{O}_5$: 580.39; found:

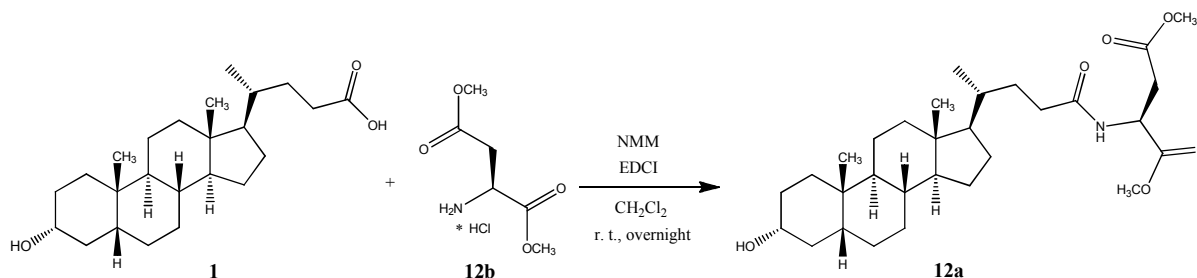
581.3 $[M+H]^+$, 603.4 $[M+Na]^+$, 619.4 $[M+K]^+$. Anal. calc for $C_{35}H_{52}N_2O_5$: C, 72.38; H, 9.02; N, 4.82; found: C, 72.57; H, 9.22; N, 4.97.

***N*-(3 α -Hydroxy-5 β -cholan-24-oyl)-D-asparagine (11).**



Compound **11** was synthesized following the procedure described for **10** starting from **11a** and was purified by flash chromatography ($CH_2Cl_2/HCOOH/EtOH$ 89.5/0.5/10). The crude product was re-crystallized from ($EtOH/H_2O$) to give **11**. Yield: 99%. Mp: 167-170°C. 1H -NMR (400 MHz, $DMSO-d_6$) δ = 0.59 (s, 3H, CH_3), 0.85-0.91 (m, 7H), 1.01-1.08 (m, 4H), 1.11-1.21 (m, 6H), 1.32-1.34 (m, 7H), 1.50-1.55 (m, 2H), 1.58-1.68 (m, 3H), 1.68-1.77 (m, 2H), 1.90-1.94 (m, 1H), 1.96-2.02 (m, 1H), 2.07-2.13 (m, 1H), 2.40 (dd, J = 15.5, 7.1 Hz, 1H, $CHCHH$), 2.48-2.54 (m, 1H, $CHCHH$), 3.31-3.38 (m, 1H), 4.45 (dd, J = 7.2, J = 7.0 Hz, 1H, $CHCH_2$), 6.86 (s, 1H, $CONHH$), 7.31 (s, 1H, $CONHH$), 7.96 (d, J = 7.92 Hz, 1H, NH). ^{13}C -NMR (100 MHz, $DMSO-d_6$) δ = 12.35, 18.74, 20.88, 23.73, 24.32, 26.63, 27.36, 28.18, 30.85, 31.87, 32.56, 34.67, 35.34, 35.62, 35.86, 36.77, 37.28, 42.00, 42.74, 49.17, 56.10, 56.55, 70.33, 163.53, 171.72 ($CONH_2$), 172.82 ($CONH$), 173.46 ($C=O$). MS (ESI) calc for $C_{28}H_{46}N_2O_5$: 490.34; found: 489.2 $[M-H]^-$. Anal. calc for $C_{28}H_{46}N_2O_5 \cdot 0.1H_2O$: C, 68.29; H, 9.45; N, 5.69; found: C, 67.96; H, 9.71; N, 5.63.

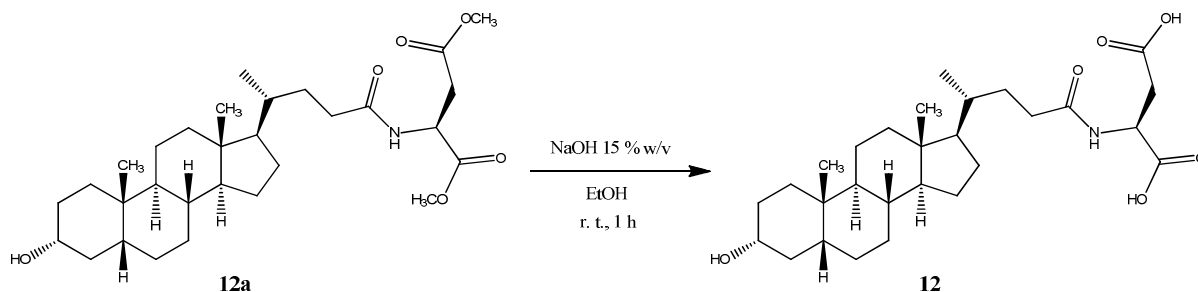
Dimethyl *N*-(3 α -hydroxy-5 β -cholan-24-oyl)-L-aspartate (12a).



Compound **12a** was synthesized following the procedure described for **3** starting from dimethyl L-aspartate hydrochloride and purified by flash chromatography [SiO_2 , $CH_2Cl_2:C_2H_5OH$ 98:2]. The crude product was re-crystallized from ethanol-water to give **12a** ($EtOH/H_2O$). Yield: 67%. Mp: 164-167°C. 1H -NMR (400 MHz, $CDCl_3$) δ = 0.64 (s, 3H, CH_3), 0.91-0.93 (m, 6H), 0.96-1.16 (m, 7H), 1.24-1.27 (m, 4H), 1.31-1.43 (m, 7H), 1.63-1.67 (m, 2H), 1.71-1.87 (m, 5H), 1.94-1.97 (m, 1H), 2.09-2.17 (m, 1H), 2.24-2.32 (m, 1H), 2.84

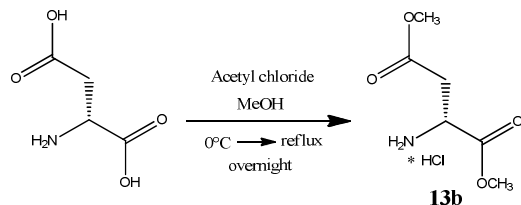
(dd, $J = 17.2, 4.4$ Hz, 1H, CHCHH), 3.03 (dd, $J = 17.2, 4.4$ Hz, 1H, CHCHH), 3.59-3.66 (m, 1H), 3.69 (s, 3H, OCH₃), 3.76 (s, 3H, OCH₃), 4.87 (dt, $J = 8.0, 4.4$ Hz, 1H, CHCH₂), 6.44 (d, $J = 8.0$ Hz, 1H, NH). ¹³C-NMR (100 MHz, CDCl₃) $\delta = 12.03, 18.33, 20.81, 23.37, 24.20, 26.41, 27.19, 28.22, 30.53, 31.49, 33.35, 34.57, 35.35, 35.41, 35.84, 36.11, 36.45, 40.18, 40.42, 42.09, 42.74, 48.30, 52.01, 52.78, 56.02, 56.48, 71.80, 171.32$ (CONH), 171.66 (C=O), 173.32 (C=O). MS (ESI) calc for C₃₀H₄₉NO₆: 519.36; found: 520.3 [M+H]⁺, 542.5 [M+Na]⁺, 558.3 [M+K]⁺. Anal. calc for C₃₀H₄₉NO₆: C, 69.33; H, 9.50; N, 2.70; found: C, 69.13; H, 9.69; N, 2.65.

***N*-(3 α -Hydroxy-5 β -cholan-24-oyl)-L-aspartic acid (**12**).**



Compound **12** was synthesized following the procedure described for **2** starting from compound **12a**. The crude product was re-crystallized from (EtOH/H₂O) to give **12**. Yield: 92%. Mp: 207-210°C. ¹H-NMR (400 MHz, DMSO-d₆) $\delta = 0.59$ (s, 3H, CH₃), 0.86-0.91 (m, 7H), 1.01-1.08 (m, 4H), 1.11-1.17 (m, 6H), 1.32 (m, 7H), 1.50 (m, 2H), 1.58-1.68 (m, 3H), 1.77 (m, 2H), 1.90-1.92 (m, 1H), 1.95-2.03 (m, 1H), 2.08-2.11 (m, 1H), 2.52 (dd, $J = 16.4, 7.2$ Hz, 1H, CHCHH), 2.65 (dd, $J = 16.4, 5.6$ Hz, 1H, CHCHH), 4.42-4.50 (m, 2H, OH+ CHCH₂), 8.09 (d, $J = 8.0$ Hz, 1H, NH). ¹³C-NMR (100 MHz, DMSO-d₆) $\delta = 12.33, 18.76, 20.88, 23.74, 24.32, 26.63, 27.36, 28.18, 30.85, 31.88, 32.53, 34.67, 35.32, 35.62, 35.85, 36.52, 36.76, 41.99, 42.73, 48.98, 56.09, 56.56, 70.33, 172.14$ (CONH), 172.93 (C=O), 173.03 (C=O). MS (ESI) calc for C₂₈H₄₅NO₆: 491.32; found: 490.5 [M-H]⁻. Anal. calc for C₂₈H₄₅NO₆: C, 68.40; H, 9.22; N, 2.85; found: C, 68.33; H, 9.40; N, 2.79.

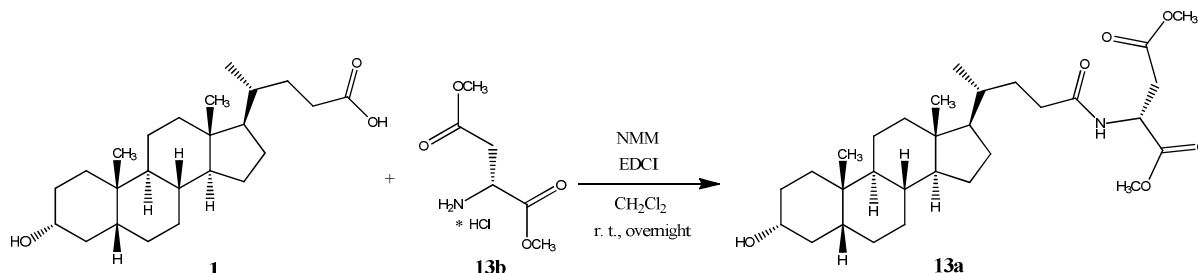
Dimethyl D-aspartate hydrochloride (13b**).**



Compound **13b** was synthesized following a modification of a described procedure^[9] in which acetyl chloride (15.40 mmol) was added dropwise to methanol (10 mL) at 0°C. The mixture was stirred for 15 min

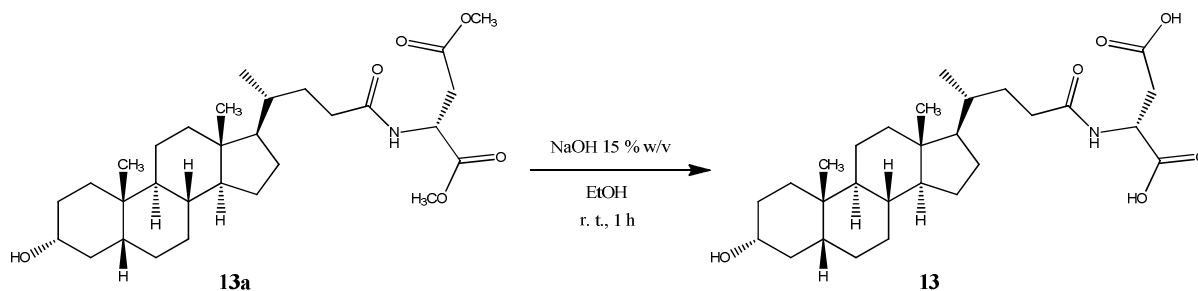
and D-aspartic acid (4.8 mmol) was then added portionwise to the solution. The resulting mixture was heated to reflux overnight. The solvent evaporated under reduced pressure afforded dimethyl D-aspartate hydrochloride **13b** as a white solid that was immediately used in the next step without further purification. Yield (73%). ¹H-NMR (400 MHz, D₂O) δ = 3.04 (dd, J = 18.0, 4.8 Hz, 1H, CHCHH), 3.12 (dd, J = 18.0, 6.0 Hz, 1H, CHCHH), 3.66 (s, 3H, OCH₃), 3.75 (s, 3H, OCH₃), 4.40-4.42 (m, 1H, CHCH₂). (ESI) calc for C₆H₁₂NO₄: 162.16 found: 163.14 [M+H]⁺.

Dimethyl *N*-(3 α -hydroxy-5 β -cholan-24-oyl)-D-aspartate (13a**).**



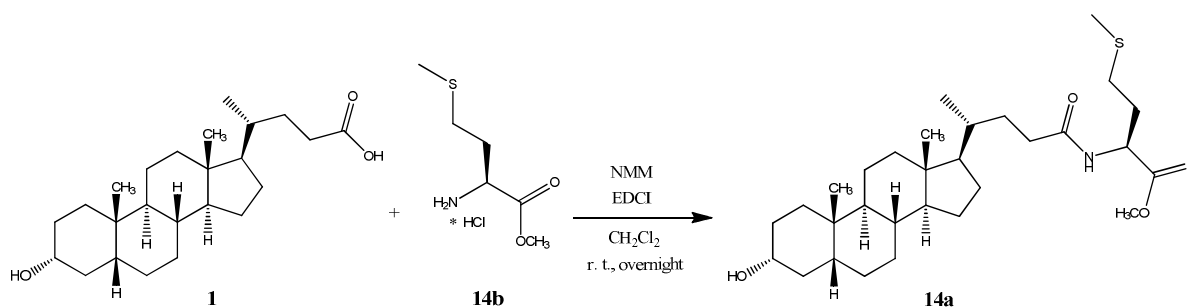
Compound **13a** was synthesized following the procedure described for **3** starting from dimethyl D-aspartate hydrochloride **13b** and was purified by flash chromatography [SiO₂, CH₂Cl₂:C₂H₅OH 98:2]. The crude product was re-crystallized from ethanol-water to give **13a** (EtOH/H₂O). Yield: 65%. Mp: 179-182°C. ¹H-NMR (400 MHz, CDCl₃) δ = 0.62 (s, 3H, CH₃), 0.89-0.90 (m, 6H), 0.94-1.11 (m, 7H), 1.14-1.38 (m, 11H), 1.63-1.65 (m, 1H), 1.70-1.86 (m, 6H), 1.92-1.95 (m, 1H), 2.06-2.14 (m, 1H), 2.24-2.32 (m, 1H), 2.83 (dd, J = 17.20, 4.40 Hz, 1H, CHCHH), 3.01 (dd, J = 17.20, 4.40 Hz, 1H, CHCHH), 3.57-3.64 (m, 1H), 3.68 (s, 3H, OCH₃), 3.74 (s, 3H, CH₃), 4.84 (dt, J = 8.00, 4.40 Hz, 1H, CHCH₂), 6.49 (d, J = 7.60 Hz, 1H, NH). ¹³C-NMR (100 MHz, CDCl₃) δ = 12.02, 18.32, 20.81, 23.37, 24.20, 26.41, 27.19, 28.21, 30.52, 31.49, 33.34, 34.57, 35.35, 35.42, 35.84, 36.13, 36.44, 40.17, 40.41, 42.09, 42.73, 48.33, 52.01, 52.78, 56.00, 56.47, 71.79, 171.31 (CONH), 171.64 (C=O), 173.36 (C=O). MS (ESI) calc for C₃₀H₄₉NO₆: 519.36; found: 518.3 [M-H]⁻. Anal. calc for C₃₀H₄₉NO₆: C, 69.33; H, 9.50; N, 2.70; found: C, 69.20; H, 9.67; N, 2.63.

***N*-(3 α -Hydroxy-5 β -cholan-24-oyl)-D-aspartic acid (**13**).**



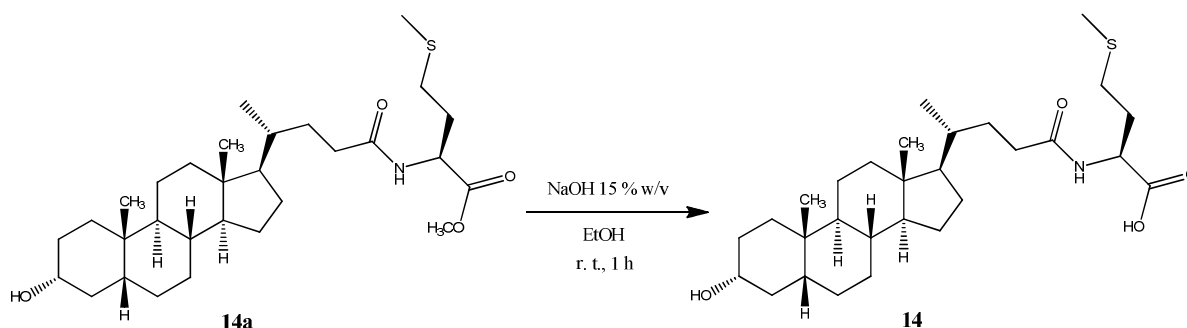
Compound **13** was synthesized following the procedure described for **2** using **13a**. The crude product was re-crystallized from (EtOH/H₂O) to give **13**. Yield: 96%. Mp: 200-204°C. ¹H-NMR (400 MHz, DMSO-d₆) δ = 0.59 (s, 3H, CH₃), 0.86-0.91 (m, 7H), 1.01-1.08 (m, 4H), 1.11-1.17 (m, 6H), 1.32-1.50 (m, 9H), 1.58-1.68 (m, 3H), 1.77 (m, 2H), 1.90-1.92 (m, 1H), 1.96-2.03 (m, 1H), 2.08-2.13 (m, 1H), 2.45-2.49 (m, 1H, CHCHH), 2.60 (dd, J = 16.4, 7.2 Hz, 1H, CHCHH), 4.40 (m, 1H, CHCH₂), 7.98 (d, J = 7.6 Hz, 1H, NH). ¹³C-NMR (100 MHz, DMSO-d₆) δ = 12.33, 18.74, 20.87, 23.74, 24.32, 26.63, 27.36, 28.18, 30.85, 31.88, 32.55, 34.67, 35.32, 35.62, 35.85, 36.76, 37.52, 41.99, 42.73, 48.96, 56.07, 56.55, 70.33, 172.36 (CONH), 172.72 (C=O), 173.23 (C=O). MS (ESI) calc for C₂₈H₄₅NO₆: 491.32; found: 490.6 [M-H]⁻. Anal. calc for C₂₈H₄₅NO₆ • 0.25H₂O: C, 67.78; H, 9.24; N, 2.82; found: C, 67.97; H, 9.36; N, 2.88.

Methyl *N*-(3 α -hydroxy-5 β -cholan-24-oyl)-L-methioninate (14a**).**



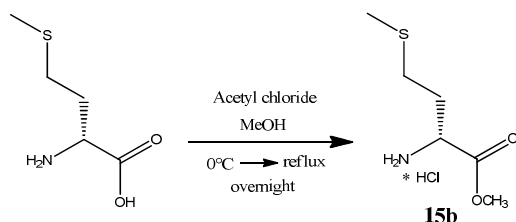
Compound **14a** was synthesized following the procedure described for **3** using methyl L-methioninate hydrochloride and purified by flash chromatography [SiO₂, CH₂Cl₂:C₂H₅OH 98:2]. The crude product was re-crystallized from ethanol-water to give **14a** (EtOH/H₂O). Yield: 67%. Mp: 149-152°C.^[10] ¹H-NMR (400 MHz, CDCl₃) δ = 0.64 (s, 3H, CH₃), 0.91-0.93 (m, 6H), 0.96-1.13 (m, 5H), 1.26 (m, 4H), 1.38 (m, 7H), 1.64 (m, 5H), 1.77-1.84 (m, 4H), 1.97 (m, 2H), 2.09 (s, 3H, SCH₃), 2.14 (m, 2H), 2.29 (m, 1H), 2.51 (m, 2H, CHCHH), 3.59-3.62 (m, 1H), 3.76 (s, 3H, OCH₃), 4.70-4.75 (m, 1H, CHCH₂), 6.12 (d, J = 7.7) Hz, 1H, NH). ¹³C-NMR (100 MHz, CDCl₃) δ = 12.04, 15.50, 18.37, 20.81, 23.37, 24.20, 26.41, 27.19, 28.26, 29.99, 30.53, 31.60, 31.77, 33.43, 34.57, 35.35, 35.44, 35.84, 36.45, 40.18, 40.42, 42.09, 42.74, 51.41, 52.51, 56.00, 56.48, 71.81, 172.67 (CONH), 173.40 (C=O). MS (ESI) calc for C₃₀H₅₁NO₄S: 521.35; found: 520.3 [M-H]⁻.

***N*-(3 α -Hydroxy-5 β -cholan-24-oyl)-L-methionine (**14**).**



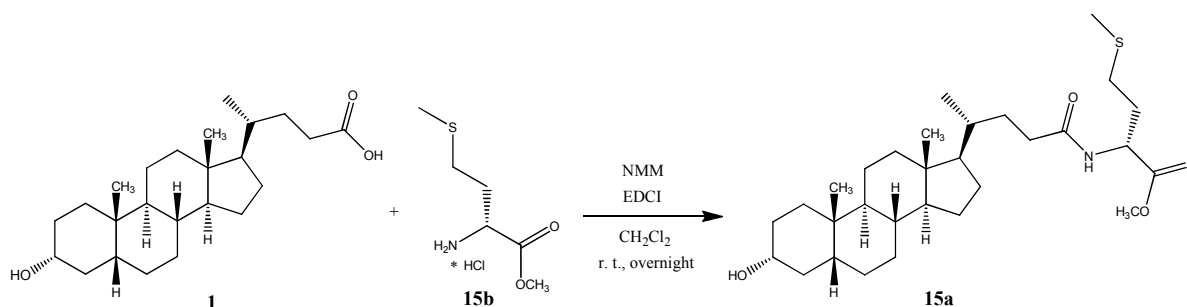
Compound **14** was synthesized following the procedure described for compound **2** using **14a**. The crude product was re-crystallized from (EtOH/H₂O) to give **14**. Yield: 100%. Mp: 178-182°C. ¹H-NMR (400 MHz, DMSO-d₆) δ = 0.59 (s, 3H, CH₃), 0.86-0.91 (m, 6H), 1.01-1.06 (m, 4H), 1.11-1.18 (m, 6H), 1.33-1.34 (m, 7H), 1.47-1.50 (m, 2H), 1.58-1.68 (m, 3H), 1.76-1.83 (m, 3H), 1.90-1.98 (m, 2H), 2.02-1.96 (m, 4H, -SCH₃ +CH), 2.07-2.14 (m, 2H, CHCHH +CH), 2.39-2.46 (m, 1H, CHCHH), 4.23-4.29 (m, 1H, CHCH₂), 4.42 (bs, 1H, OH), 8.04 (d, J = 7.60 Hz, 1H, NH). ¹³C-NMR (100 MHz, DMSO-d₆) δ = 12.32, 15.02, 18.77, 20.88, 23.74, 24.32, 26.63, 27.36, 28.21, 30.23, 30.84, 31.14, 31.23, 31.92, 32.50, 34.67, 35.31, 35.61, 35.85, 36.76, 41.99, 42.73, 51.24, 56.02, 56.55, 70.32, 173.27 (CONH), 173.96 (C=O). MS (ESI) calc for C₂₉H₄₉NO₄S: 507.34; found: 506.5 [M-H]⁻. Anal. calc for C₂₉H₄₉NO₄S: C, 68.60; H, 9.73; N, 2.76; found: C, 68.41; H, 9.99; N, 2.74.

Methyl D-methioninate hydrochloride (**15b**).



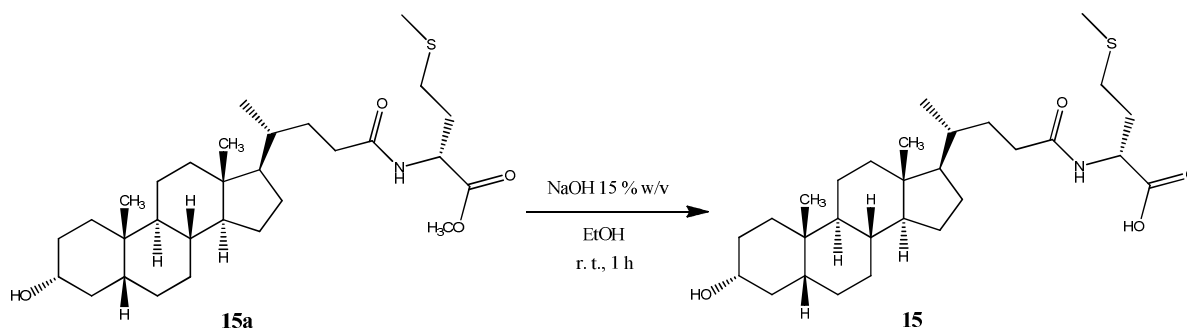
Compound **15b** was synthesized following a modification of a described procedure^[11] in which acetyl chloride (5.03 mmol) was added dropwise to a methanol (15 mL) at 0 °C. The mixture was stirred for 15 min and D-methionine (1.67 mmol) was then added portionwise to the solution. The resulting mixture was heated to reflux overnight. The solvent evaporated under reduced pressure afforded methyl D-methioninate hydrochloride **15b** as a white solid that was immediately used in the next step without further purification. Yield 86%. ¹H-NMR (400 MHz, D₂O) δ = 2.02 (s, 3H, SCH₃), 2.56-2.62 (m, 2H, CHCHH), 3.76 (s, 1H, OCH₃), 4.20-4.23 (m, 1H, CHCH₂). MS (ESI) calc for C₅H₁₁NO₂S: 149.21 found: 150.12 [M+H]⁺.

Methyl N-(3 α -hydroxy-5 β -cholan-24-oyl)-L-methioninate (**15a**).



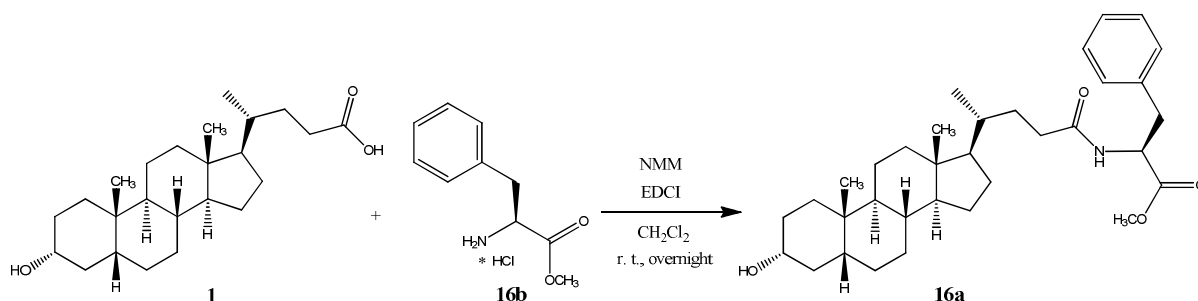
Compound **15a** was synthesized following the procedure described for **3** using methyl D-methioninate hydrochloride **15b** and was purified using flash chromatography (CH₂Cl₂/EtOH: 98/2) and re-crystallized from (EtOH/H₂O). Yield: 65%. Mp: 143-146°C. ¹H-NMR (400 MHz, CDCl₃) δ = 0.64 (s, 3H, CH₃), 0.91-0, 1.01-1.16 (m, 4H), 1.21-1.45 (m, 10H), 1.49-1.59 (m, 3H), 1.65 (m, 2H), 1.71-1.90 (m, 5H), 1.92-2.02 (m, 2H), 2.09 (s, 3H, SCH₃), 2.11-2.21 (m, 2H), 2.25-2.33 (m, 1H), 2.46-2.54 (m, 2H, CHCHH). 3.57-3.66 (m, 1H), 3.76 (s, 3H, OCH₃), 4.72 (ddd, J = 7.4, 5.5, 2.2 Hz, 1H, CHCH₂), 6.13 (d, J = 7.8 Hz, 1H, NH). ¹³C-NMR (100 MHz, CDCl₃) δ = 12.05, 15.49, 18.36, 20.81, 23.37, 24.20, 26.41, 27.19, 28.23, 29.99, 30.52, 31.57, 31.77, 33.43, 34.56, 35.35, 35.43, 35.83, 36.44, 40.18, 40.41, 42.09, 42.74, 51.40, 52.51, 56.00, 56.48, 71.79, 172.67 (CONH), 173.40 (C=O). MS (ESI) calc for C₃₀H₅₁NO₄S: 521.35; found: 520.3 [M-H]⁻. Anal. calc for C₃₀H₅₁NO₄S: C, 69.05; H, 9.85; N, 2.68; found: C, 68.56; H, 9.99; N, 2.50.

N-(3 α -Hydroxy-5 β -cholan-24-oyl)-D-methionine (**15**).



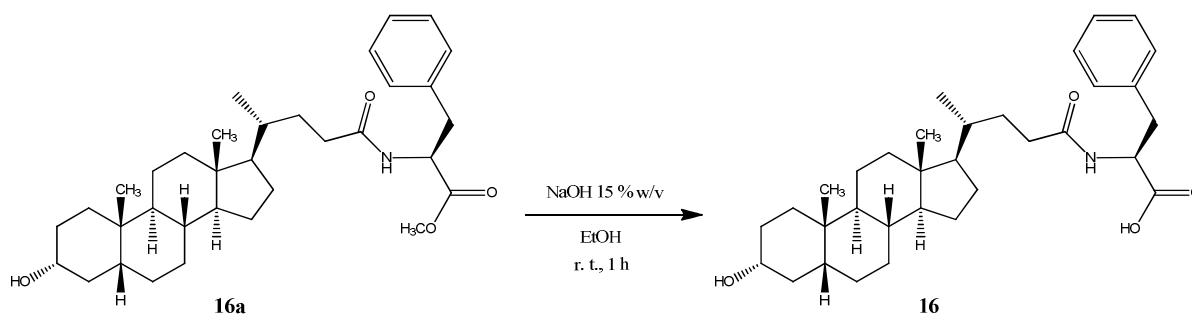
Compound **15** was synthesized following the procedure described for compound **2** starting from **15a**. The crude product was re-crystallized from (EtOH/H₂O) to give **15**. Yield: 96%. Mp: 169-172°C. ¹H-NMR (400 MHz, DMSO-d₆) δ = 0.59 (s, 3H, CH₃), 0.86-0.88 (m, 6H), 0.91-1.22 (m, 11H), 1.32-1.34 (m, 7H), 1.50-1.54 (m, 2H), 1.58-1.68 (m, 3H), 1.77-1.84 (m, 3H), 1.90-1.92 (m, 2H), 2.01 (s, 3H, SCH₃), 2.01-2.11 (m, 3H), 2.42-2.49 (m, 2H, CHCHH), 3.35 (m, 1H), 4.25-4.31 (m, 1H, CHCH₂), 4.42 (bs, 1H, OH), 8.05 (d, J = 7.6 Hz, 1H, NH). ¹³C-NMR (100 MHz, DMSO-d₆) δ = 12.33, 15.02, 18.70, 20.87, 23.74, 24.32, 26.64, 27.36, 28.20, 30.21, 30.84, 31.18, 31.90, 32.55, 34.67, 35.28, 35.61, 35.84, 36.76, 41.99, 42.73, 51.17, 56.11, 56.56, 70.32, 173.17 (CONH), 173.97 (C=O). MS (ESI) calc for C₂₉H₄₉NO₄S: 507.34; found: 506.4 [M-H]⁻. Anal. calc for C₂₉H₄₉NO₄S • 0.2H₂O: C, 68.11; H, 9.74; N, 2.74; found: C, 67.94; H, 9.72; N, 2.69.

Methyl *N*-(3 α -hydroxy-5 β -cholan-24-oyl)-L-phenylalaninate (16a).



Compound **16a** was synthesized following procedure described for compound **3** starting from methyl L-phenylalaninate hydrochloride and purified by flash chromatography [SiO₂, CH₂Cl₂:C₂H₅OH 98:2]. The crude product was re-crystallized from (EtOH/H₂O) to give **16a**. Yield: 80%. Mp: 185-187°C. ¹H-NMR (400 MHz, CDCl₃) δ = 0.63 (s, 3H, CH₃), 0.88-0.97 (m, 7H), 1.00-1.15 (m, 5H), 1.21-1.30 (m, 5H), 1.38-1.40 (m, 7H), 1.63-1.67 (m, 2H), 1.71-1.88 (m, 5H), 1.93-1.96 (m, 1H), 2.03-2.11 (m, 1H), 2.19-2.26 (m, 1H), 3.09 (dd, J = 13.6, 5.6 Hz, 1H, CHCHH), 3.15 (dd, J = 13.6, 5.6 Hz, 1H, CHCHH), 3.62 (m, 1H), 3.73 (s, 3H, OCH₃), 4.89 (dt, J = 8.0, 5.6 Hz, 1H, CHCH₂), 5.87 (d, J = 7.6 Hz, 1H, NH), 7.09 (d, J = 6.4 Hz, 2H, Ar), 7.25-7.31 (m, 3H, Ar). ¹³C-NMR (100 MHz, CDCl₃) δ = 12.06, 18.35, 20.82, 23.38, 24.21, 26.43, 27.20, 28.20, 30.53, 31.58, 33.37, 34.57, 35.36, 35.40, 35.85, 36.45, 37.89, 40.17, 40.43, 42.10, 42.73, 52.30, 52.92, 55.98, 56.48, 71.80, 127.10, 128.54, 129.27, 135.91, 172.22 (CONH), 173.14 (C=O). MS (ESI) calc for C₃₄H₅₁NO₄: 537.38; found: 536.4 [M-H]⁻. Anal. calc for C₃₄H₅₁NO₄: C, 75.94; H, 9.56; N, 2.60; found: C, 75.85; H, 9.85; N, 2.52.

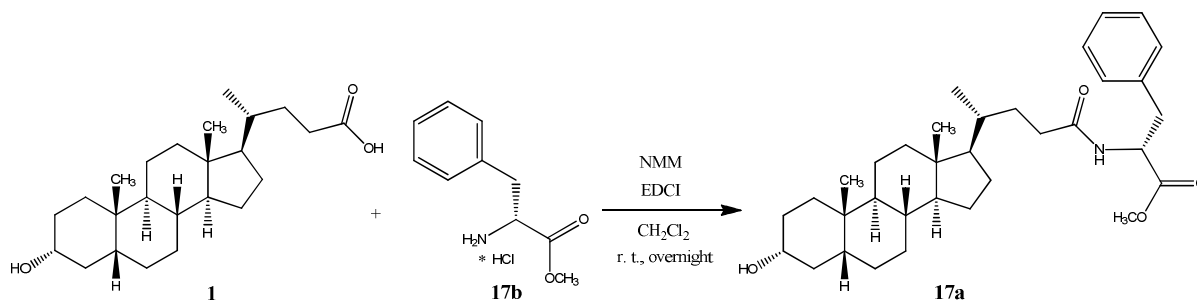
***N*-(3 α -Hydroxy-5 β -cholan-24-oyl)-L-phenylalanine (16).**



Compound **16** was synthesized following the procedure described for compound **2** starting from compound **16a**. The crude product was re-crystallized from (EtOH/H₂O) to give **16**. Yield: 95%. Mp: 225-228°C. ¹H-NMR (400 MHz, DMSO-d₆) δ = 0.57 (s, 3H, CH₃), 0.81-0.92 (m, 7H), 1.00-1.17 (m, 10H), 1.32-1.41 (m, 7H), 1.50-1.77 (m, 7H), 1.88-1.98 (m, 2H), 2.01-2.07 (m, 1H), 2.82 (dd, J = 13.6, 10.0 Hz, 1H, CHCHH), 3.02 (dd, J = 13.6, 4.4 Hz, 1H, CHCHH), 4.37-4.43 (m, 1H, CHCH₂), 7.22 (m, 5H, Ar), 8.06 (d, J = 8.0 Hz, 1H, NH). ¹³C-NMR (100 MHz, DMSO-d₆) δ = 12.33, 18.73, 20.87, 23.74, 24.31, 26.63, 27.36, 28.12, 30.85,

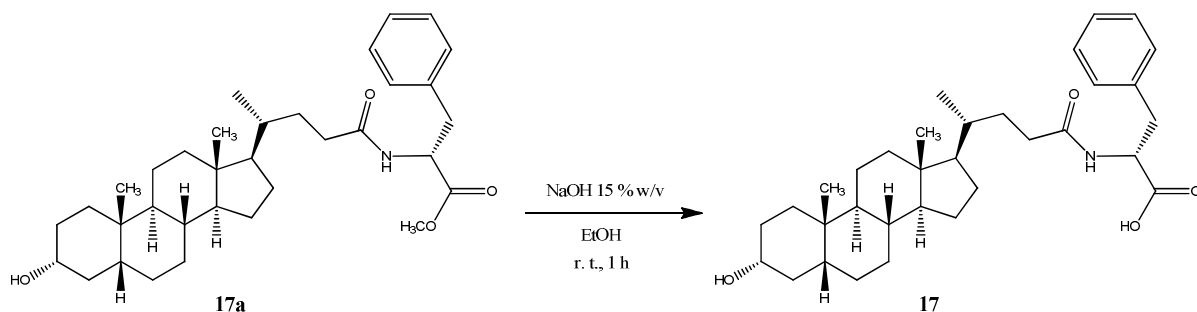
31.88, 32.49, 34.67, 35.29, 35.62, 35.84, 36.76, 37.20, 41.99, 42.70, 53.81, 56.01, 56.54, 70.32, 126.77, 128.54, 129.52, 138.27, 173.05 (CONH), 173.68 (C=O). MS (ESI) calc for $C_{33}H_{49}NO_4$: 523.37; found: 522.4 $[M-H]^-$. Anal. calc for $C_{33}H_{49}NO_4$: C, 75.68; H, 9.43; N, 2.67; found: C, 75.68; H, 9.64; N, 2.72.

Methyl *N*-(3 α -hydroxy-5 β -cholan-24-oyl)-*D*-phenylalaninate (17a).



Compound **17a** was synthesized following the procedure described for compound **3** starting from methyl *D*-phenylalaninate hydrochloride and purified by flash chromatography [SiO_2 , CH_2Cl_2 : C_2H_5OH 98:2]. The crude product was re-crystallized from ethanol-water to give **17a** ($EtOH/H_2O$. Yield: 40%. Mp: 228-231°C. 1H -NMR (400 MHz, $CDCl_3$) δ = 0.65 (s, 3H, CH_3), 0.88-0.95 (m, 7H), 1.05-1.17 (m, 5H), 1.22-1.35 (m, 5H), 1.37-1.41 (m, 7H), 1.60-1.66 (m, 2H), 1.69-1.85 (m, 5H), 1.92-1.98 (m, 1H), 2.07-2.15 (m, 1H), 2.20-2.28 (m, 1H), 3.23(dd, J = 13.6, 5.6 Hz, 1H, $CHCHH$), 3.28 (dd, J = 13.6, 5.6 Hz, 1H, $CHCHH$), 3.61 (m, 1H), 3.73 (s, 3H, OCH_3), 4.78 (dt, J = 8.0, 5.6 Hz, 1H, $CHCH_2$), 5.82 (d, J = 7.6 Hz, 1H, NH), 7.10 (d, J = 6.4 Hz, 2H, Ar), 7.20-7.29 (m, 3H, Ar). ^{13}C -NMR (100 MHz, $CDCl_3$) δ = 12.10, 18.25, 20.80, 23.34, 24.27, 26.53, 27.10, 28.30, 30.58, 31.57, 33.33, 34.54, 35.37, 35.48, 35.85, 36.47, 37.93, 40.21, 40.48, 42.20, 42.83, 52.28, 52.88, 52.97, 55.88, 56.51, 71.84, 127.15, 128.55, 129.25, 135.96, 172.31 (CONH), 173.17 (C=O). MS (ESI) calc for $C_{34}H_{51}NO_4$: 537.38; found: 536.4 $[M-H]^-$. Anal. calc for $C_{34}H_{51}NO_4 \cdot 3H_2O$: C, 69.00; H, 9.71; N, 2.34; found: C, 69.20; H, 9.67; N, 2.63.

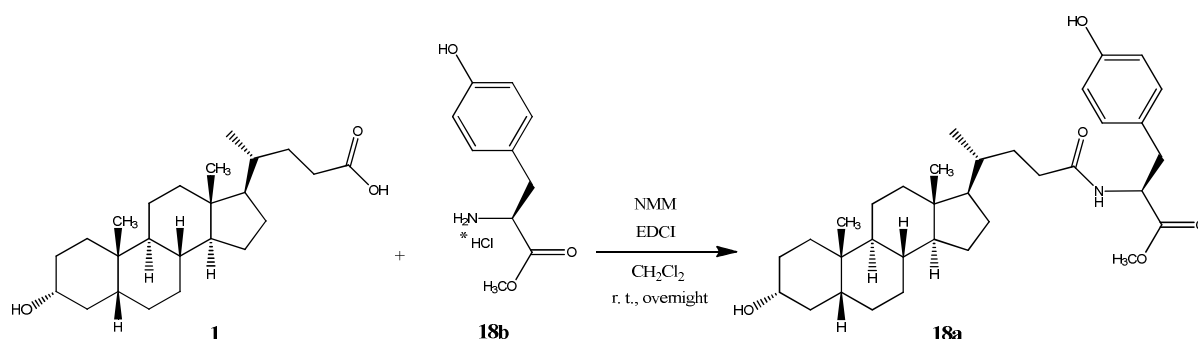
***N*-(3 α -Hydroxy-5 β -cholan-24-oyl)-*D*-phenylalanine (17).**



Compound **17** was synthesized following the procedure described for **2** using **17a**. The crude product was re-crystallized from ($EtOH/H_2O$) to give **17**. Yield: 93%. Mp: 155-158°C. 1H -NMR (400 MHz, $DMSO-d_6$) δ =

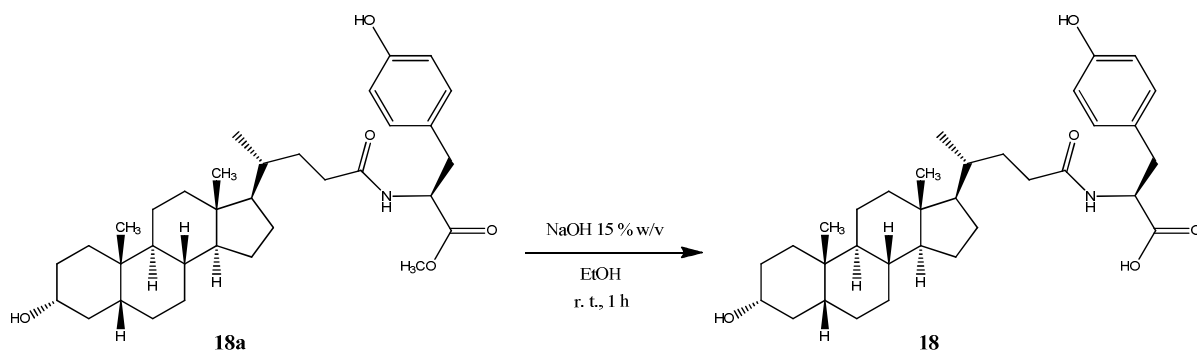
0.56 (s, 3H, CH₃), 0.79-0.92 (m, 7H), 0.99-1.22 (m, 10H), 1.27-1.34 (m, 7H), 1.47-1.77 (m, 7H), 1.88-1.99 (m, 2H), 2.03-2.12 (m, 1H), 2.82 (dd, $J = 13.6, 10.0$ Hz, 1H, CHCHH), 3.03 (dd, $J = 13.6, 4.4$ Hz, 1H, CHCHH), 4.32-4.37 (m, 2H, CHCH₂+ OH), 7.17-7.26 (m, 5H, Ar), 8.06 (d, $J = 8.0$ Hz, 1H, NH). ¹³C-NMR (100 MHz, DMSO-d₆) $\delta = 12.34, 18.64, 20.87, 23.74, 24.30, 26.62, 27.36, 28.13, 30.85, 31.83, 32.54, 34.67, 35.24, 35.62, 35.85, 36.77, 37.22, 42.00, 42.71, 53.72, 56.17, 56.54, 70.33, 126.77, 128.53, 129.51, 138.24, 172.93$ (CONH), 173.66 (C=O). MS (ESI) calc for C₃₃H₄₉NO₄: 523.37; found: 522.1 [M-H]⁻. Anal. calc for C₃₃H₄₉NO₄ • 0.5H₂O: C, 74.40; H, 9.46; N, 2.63; found: C, 74.43; H, 9.55; N, 2.59.

Methyl *N*-(3 α -hydroxy-5 β -cholan-24-oyl)-L-tyrosinate (18a).



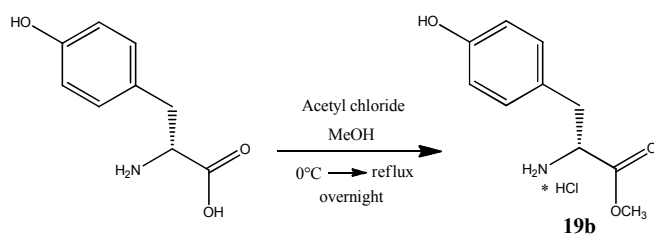
Compound **18a** was synthesized following the procedure described for compound **3** starting from methyl L-tyrosinate hydrochloride and was purified by flash chromatography [SiO₂, CH₂Cl₂:C₂H₅OH from 98:2 to 95:5]. The crude product was re-crystallized from ethanol-water to give **18a** (EtOH/H₂O. Yield: 87%. Mp: 203-206°C. ¹H-NMR (400 MHz, CD₃OD) $\delta = 0.66$ (s, 3H, CH₃), 0.91-1.01 (m, 7H), 1.08-1.45 (m, 17H), 1.61-1.67 (m, 3H), 1.72-1.90 (m, 4H), 1.98-2.11 (m, 2H), 2.17-2.23 (m, 1H), 2.16-2.83 (dd, $J = 14.0, 9.2$ Hz, 1H, CHCHH), 3.04 (dd, $J = 14.0, 5.6$ Hz, 1H, CHCHH), 3.51-3.53 (m, 1H), 3.68 (s, 3H, OCH₃), 4.58 (dd, $J = 9.2, 5.6$ Hz, 1H, CHCH₂), 6.69 (d, $J = 8.4$ Hz, 2H, Ar), 7.01 (d, $J = 8.8$ Hz, 2H, Ar). ¹³C-NMR (100 MHz, CD₃OD) $\delta = 11.12, 17.43, 20.55, 22.55, 23.87, 26.26, 26.97, 27.83, 29.79, 31.78, 32.35, 34.28, 35.09, 35.35, 35.77, 35.83, 36.22, 40.13, 40.48, 42.14, 42.49, 51.20, 54.01, 56.07, 56.50, 71.02, 114.81, 127.42, 129.76, 156.00, 172.41$ (CONH), 175.29 (C=O). MS (ESI) calc for C₃₄H₅₁NO₅: 553.38; found: 552.3 [M-H]⁻. Anal. calc for C₃₄H₅₁NO₅: C, 73.74; H, 9.28; N, 2.53; found: C, 73.08; H, 9.10; N, 2.46.

***N*-(3 α -Hydroxy-5 β -cholan-24-oyl)-L-tyrosine (18).**



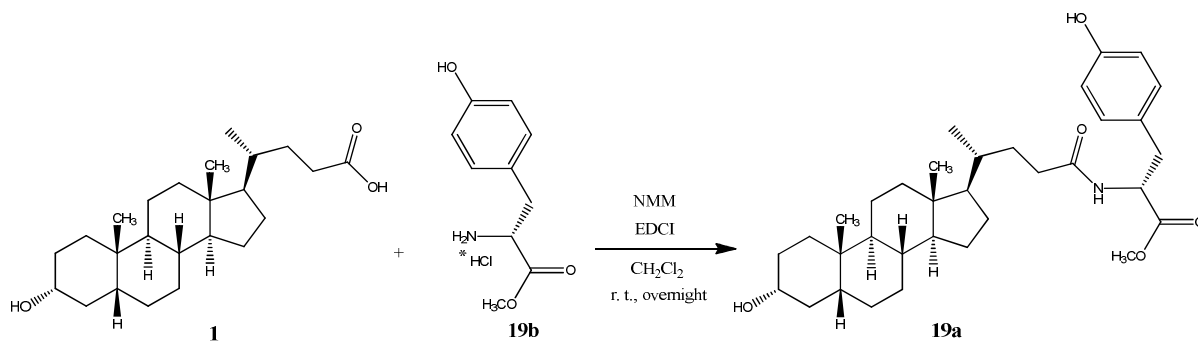
Compound **18** was synthesized following the procedure described for **2** using **18a**. The crude product was recrystallized from (EtOH/H₂O) to give **18**. Yield: 93%. Mp: 219–223°C. ¹H-NMR (400 MHz, DMSO-d₆) δ = 0.58 (s, 3H, CH₃), 0.82–0.94 (m, 7H), 1.01–1.32 (m, 17H), 1.47–1.77 (m, 7H), 1.89–1.98 (m, 2H), 2.02–2.08 (m, 1H), 2.70 (dd, J = 13.6, 9.6 Hz, 1H, CHCHH), 2.89 (dd, J = 13.6, 4.0 Hz, 1H, CHCHH), 4.28–4.32 (m, 1H, CHCH₂), 4.43 (bs, 1H, CH₂OH), 6.61 (d, J = 8.0 Hz, 2H, Ar), 6.98 (d, J = 8.0 Hz, 2H, Ar), 8.0 (d, J = 8.0 Hz, 1H, NH), 9.20 (s, 1H, ArOH). ¹³C-NMR (100 MHz, DMSO-d₆) δ = 12.33, 18.75, 20.88, 23.74, 24.32, 26.64, 27.36, 28.13, 30.84, 31.92, 32.51, 34.67, 35.31, 35.62, 35.85, 36.45, 36.76, 41.99, 42.71, 54.17, 56.04, 56.55, 70.33, 115.35, 128.19, 130.41, 156.35, 173.05 (CONH), 173.78 (C=O). MS (ESI) calc for C₃₃H₄₉NO₅: 539.36; found: 538.4 [M-H][–]. Anal. calc for C₃₃H₄₉NO₅ • 1.25H₂O: C, 70.49; H, 9.23; N, 2.49; found: C, 70.34; H, 9.10; N, 2.48.

Methyl D-tyrosinate hydrochloride (**19b**).



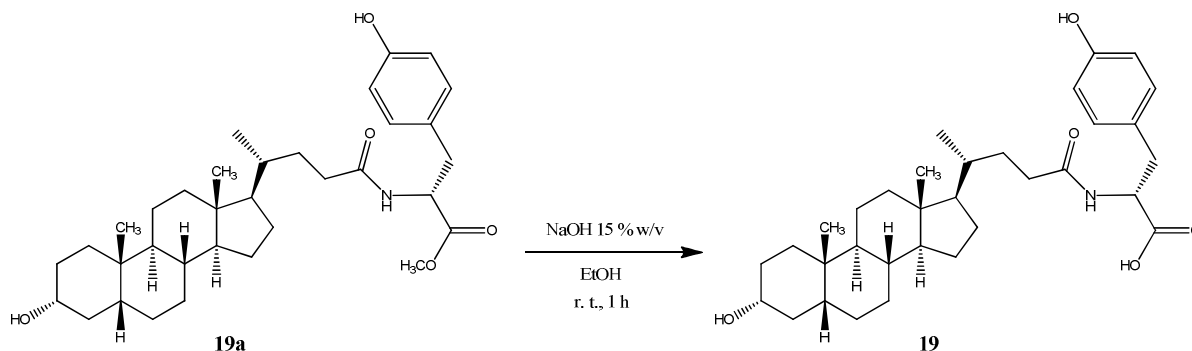
Compound **19b** was synthesized following the procedure described in literature^[12] using D-tyrosine.

Methyl N-(3 α -hydroxy-5 β -cholan-24-oyl)-D-tyrosinate (**19a**).



Compound **19a** was synthesized following the procedure described for compound **3** starting from methyl D-tyrosinate hydrochloride **19b** and purified by flash chromatography [SiO_2 , CH_2Cl_2 : $\text{C}_2\text{H}_5\text{OH}$ from 98:2]. The crude product was re-crystallized from ethanol-water to give **19a** (EtOH/ H_2O . Yield: 68%. Mp: 228-232°C. ^1H -NMR (400 MHz, DMSO-d_6) δ = 0.57 (s, 3H, CH_3), 0.81-0.91 (m, 7H), 1.00-1.32 (m, 17H), 1.50-1.77 (m, 7H), 1.89-2.07 (m, 3H), 2.73 (dd, J = 13.6, 9.6 Hz, 1H, CHCHH), 2.83 (dd, J = 13.6, 5.2 Hz, 1H, CHCHH), 3.41-3.44 (m, 1H), 3.56 (s, 3H, OCH_3), 4.31-4.36 (m, 1H, CHCH_2), 4.43 (d, J = 4.0 Hz, 1H, CH_2OH), 6.62 (d, J = 8.4 Hz, 2H, Ar), 6.96 (d, J = 8.0 Hz, 2H, Ar), 8.16 (d, J = 7.6 Hz, 1H, NH), 9.19 (s, 1H, ArOH). ^{13}C -NMR (100 MHz, DMSO-d_6) δ = 12.31, 18.65, 19.02, 20.87, 23.74, 24.31, 26.63, 27.35, 28.15, 30.85, 31.79, 32.44, 34.67, 35.28, 35.61, 35.84, 36.45, 36.76, 41.99, 42.71, 52.16, 54.23, 56.12, 56.49, 56.54, 70.33, 115.41, 127.72, 130.38, 156.42, 172.83 (CONH), 173.06 (C=O). MS (ESI) calc for $\text{C}_{34}\text{H}_{51}\text{NO}_5$: 553.38; found: 552.3 $[\text{M-H}]^-$. Anal. calc for $\text{C}_{34}\text{H}_{51}\text{NO}_5 \cdot 0.25\text{H}_2\text{O}$: C, 73.15; H, 9.29; N, 2.51; found: C, 73.12; H, 9.58; N, 2.44.

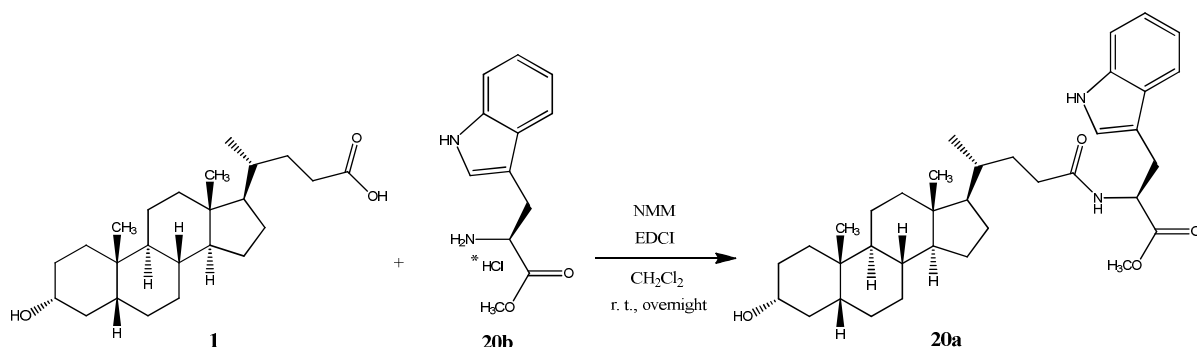
N-(3 α -Hydroxy-5 β -cholan-24-oyl)-D-tyrosine (**19**).



Compound **19** was synthesized following the procedure described for **2** using compound **19a**. The crude product was re-crystallized from (EtOH/ H_2O) to give **19**. Yield: 75%. Mp: 211-214°C. ^1H -NMR (400 MHz, DMSO-d_6) δ = 0.56 (s, 3H, CH_3), 0.82 (d, J = 6.40 Hz, 3H), 0.86 (s, 3H, CH_3), 0.89-0.91 (m, 6H), 1.00-1.09 (m, 5H), 1.12-1.17 (m, 5H), 1.27-1.34 (m, 7H), 1.50-1.54 (m, 2H), 1.58-1.68 (m, 3H), 1.74-1.77 (m, 2H), 1.88-1.91 (m, 1H), 1.96-2.02 (m, 1H), 2.04-2.06 (m, 1H), 2.70 (dd, J = 13.6, 9.6 Hz, 1H, CHCHH), 2.89 (dd, J = 13.6, 4.4 Hz, 1H, CHCHH), 3.34-3.39 (m, 1H), 4.26-4.32 (m, 1H, CHCH_2), 6.61 (d, J = 8.5 Hz, 2H, Ar),

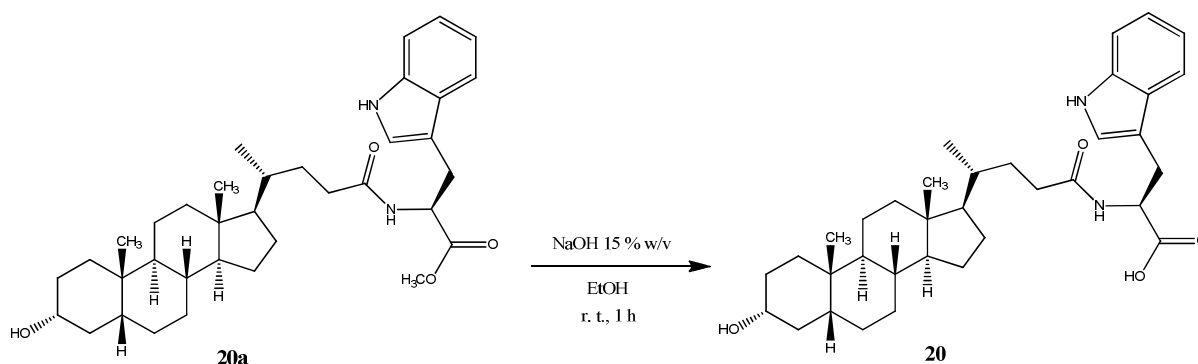
6.98 (d, $J = 8.5$ Hz, 2H, Ar), 7.98 (d, $J = 8.1$ Hz, 1H, NH). ^{13}C -NMR (100 MHz, DMSO- d_6) $\delta = 12.31$, 18.66, 20.88, 23.75, 24.31, 26.63, 27.36, 28.14, 30.84, 31.87, 32.57, 34.67, 35.28, 35.62, 35.84, 36.48, 36.76, 41.99, 42.71, 54.12, 56.18, 56.55, 70.33, 115.32, 128.20, 130.41, 156.31, 172.91 (CONH), 173.82 (C=O). MS (ESI) calc for $\text{C}_{33}\text{H}_{49}\text{NO}_5$: 539.36; found: 538.4 $[\text{M}-\text{H}]^-$. Anal. calc for $\text{C}_{33}\text{H}_{49}\text{NO}_5$: C, 73.43; H, 9.15; N, 2.59; found: C, 73.22; H, 8.81; N, 2.54.

Methyl *N*-(3 α -hydroxy-5 β -cholan-24-oyl)-L-tryptophanate (20a).



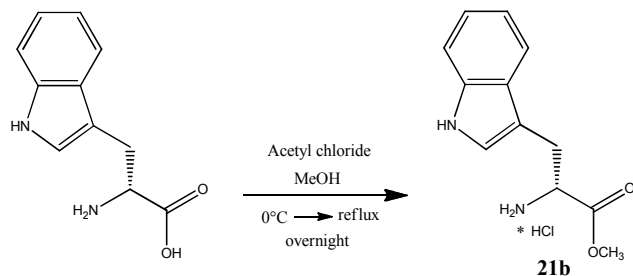
Compound **20a** was synthesized following the procedure described for compound **3** starting from methyl L-tryptophanate hydrochloride and was purified by flash chromatography [SiO_2 , CH_2Cl_2 : $\text{C}_2\text{H}_5\text{OH}$ from 99:1]. The crude product was re-crystallized from ethanol-water to give **20a** ($\text{EtOH}/\text{H}_2\text{O}$). Yield: 67%. Mp: 156–159°C. ^1H -NMR (400 MHz, DMSO- d_6) $\delta = 0.58$ (s, 3H, CH_3), 0.83–0.86 (m, 6H), 1.01–1.17 (m, 11H), 1.32 (bs, 7H), 1.50–1.77 (m, 7H), 1.89–1.91 (m, 1H), 2.00 (m, 1H), 2.08 (m, 1H), 3.00 (dd, $J = 14.4$, 8.4 Hz, 1H, CHCHH), 3.11 (dd, $J = 14.0$, 5.2 Hz, 1H, CHCHH), 3.32–3.36 (m, 1H), 3.55 (s, 3H, OCH_3), 4.43 (d, $J = 4.4$ Hz, 1H, CH_2OH), 4.47 (m, 1H, CHCH_2), 6.96 (t, $J = 7.2$ Hz, 1H, Ar), 7.05 (t, $J = 7.2$ Hz, 1H, Ar), 7.11 (s, 1H, Ar), 7.32 (d, $J = 8.0$ Hz, 1H, Ar), 7.47 (d, $J = 7.6$ Hz, 1H, Ar), 8.19 (d, $J = 7.2$ Hz, 1H, NH). ^{13}C -NMR (100 MHz, DMSO- d_6) $\delta = 12.32$, 18.74, 20.87, 23.74, 24.32, 26.64, 27.36, 27.53, 28.13, 30.85, 31.81, 32.43, 34.67, 35.32, 35.62, 35.85, 36.77, 41.99, 42.71, 52.18, 53.32, 56.03, 56.54, 70.33, 110.07, 111.87, 118.46, 118.83, 121.39, 124.04, 127.54, 136.56, 173.04 (CONH), 173.17 (C=O). MS (ESI) calc for $\text{C}_{36}\text{H}_{52}\text{NO}_4$: 576.39; found: 575.3 $[\text{M}-\text{H}]^-$. Anal. calc for $\text{C}_{36}\text{H}_{52}\text{N}_2\text{O}_4 \cdot 0.5\text{H}_2\text{O}$: C, 73.80; H, 9.12; N, 4.78; found: C, 73.73; H, 9.41; N, 4.80.

***N*-(3 α -Hydroxy-5 β -cholan-24-oyl)-L-tryptophan (20).**



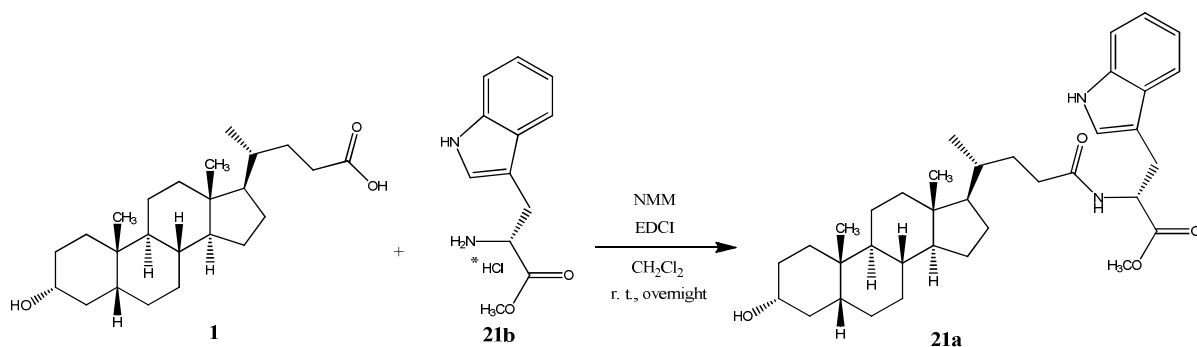
Compound **20** was synthesized following the procedure described for **2** using **20a**. The crude product was recrystallized from (EtOH/H₂O) to give **20**. Yield: 95%. Mp: 194-197°C^[13]. ¹H-NMR (400 MHz, DMSO-d₆) δ = 0.57 (s, 3H, CH₃), 0.82-0.91 (m, 6H), 1.00-1.17 (m, 10H), 1.32 (m, 7H), 1.50-1.68 (m, 5H), 1.77 (m, 2H), 1.88-1.91 (m, 1H), 1.99 (m, 1H), 2.08 (m, 1H), 2.97 (dd, J = 14.6, 8.7 Hz, 1H, CHCHH), 3.14 (dd, J = 14.6, 4.9 Hz, 1H, CHCHH), 4.43 (m, 2H, CHCH₂ + CH₂OH), 6.95 (t, J = 7.2 Hz, 1H, Ar), 7.04 (t, J = 7.2 Hz, 1H, Ar), 7.11 (m, 1H, Ar), 7.31 (d, J = 8.08 Hz, 1H, Ar), 7.51 (d, J = 7.8 Hz, 1H, Ar), 8.04 (d, J = 7.8 Hz, 1H, NH). ¹³C-NMR (100 MHz, DMSO-d₆) δ = 12.32, 18.77, 20.87, 23.74, 24.32, 26.63, 27.36, 27.57, 28.13, 30.84, 31.84, 32.53, 34.67, 35.34, 35.62, 35.84, 36.76, 41.99, 42.70, 53.37, 56.04, 56.54, 70.33, 110.50, 111.81, 118.62, 118.74, 121.30, 123.94, 127.68, 136.53, 173.05, 174.04. MS (ESI) calc for C₃₅H₅₀N₂O₄: 562.38; found: 561.4 [M-H]⁺.

Methyl-D-tryptophanate hydrochloride (**21b**).



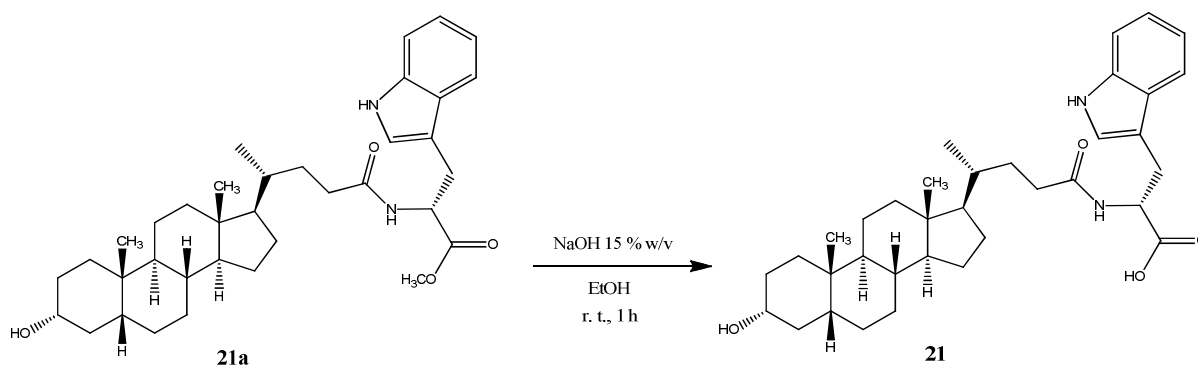
Compound **21b** was synthesized following a described procedure^[14] from D-tryptophan.

Methyl *N*-(3 α -hydroxy-5 β -cholan-24-oyl)-D-tryptophanate (**21a**).



Compound **21a** was synthesized following the procedure described for compound **3** starting from methyl D-tryptophanate hydrochloride **21b** and purified by flash chromatography [SiO_2 , CH_2Cl_2 : $\text{C}_2\text{H}_5\text{OH}$ from 98:2]. The crude product was re-crystallized from ethanol-water to give **21a** ($\text{EtOH}/\text{H}_2\text{O}$. Yield: 74%. Mp: 221-224°C. ^1H -NMR (400 MHz, $\text{DMSO}-d_6$) δ = 0.55 (s, 3H, CH_3), 0.83 (d, J = 6.44 Hz, 3H), 0.86 (s, 3H, CH_3), 1.01-1.14 (m, 11H), 1.32 (m, 7H), 1.50-1.77 (m, 7H), 1.88-1.90 (m, 1H), 2.00 (m, 1H), 2.10 (m, 1H), 3.00 (dd, J = 14.56, 8.52 Hz, 1H, CHCHH), 3.12 (dd, J = 14.52, 5.68 Hz, 1H, CHCHH), 3.55 (s, 3H, OCH_3), 4.43 (d, J = 4.56 Hz, 1H, CH_2OH), 4.48 (m, 1H, CHCH_2), 6.96 (t, J = 7.0 Hz, 1H, Ar), 7.0 (t, J = 6.9 Hz, 1H, Ar), 7.11 (d, J = 2.4, 1H, Ar), 7.31 (d, J = 8.0 Hz, 1H, Ar), 7.47 (d, J = 7.8 Hz, 1H, Ar), 8.19 (d, J = 7.6 Hz, 1H, NH). ^{13}C -NMR (100 MHz, $\text{DMSO}-d_6$) δ = 12.28, 18.68, 20.87, 23.74, 24.31, 26.63, 27.36, 27.55, 28.14, 30.85, 31.75, 32.44, 34.67, 35.30, 35.62, 35.84, 36.76, 41.99, 42.70, 52.18, 53.49, 56.07, 56.53, 70.33, 110.04, 111.87, 118.43, 118.82, 121.38, 124.04, 127.53, 136.55, 173.04 (CONH), 173.11 (C=O). MS (ESI) calc for $\text{C}_{36}\text{H}_{52}\text{NO}_4$: 576.39; found: 575.3 $[\text{M}-\text{H}]^-$. Anal. calc for $\text{C}_{36}\text{H}_{52}\text{N}_2\text{O}_4$: C, 74.96; H, 9.09; N, 4.86; found: C, 74.94; H, 9.48; N, 4.87.

N-(3 α -Hydroxy-5 β -cholan-24-oyl)-D-tryptophan (**21**).



Compound **21** was synthesized following the procedure described for **2** using compound **21a**. The crude product was re-crystallized from ($\text{EtOH}/\text{H}_2\text{O}$) to give **21**. Yield: 98%. Mp: 175-178°C. ^1H -NMR (400 MHz, $\text{DMSO}-d_6$) δ = 0.54 (s, 3H, CH_3), 0.81-0.88 (m, 6H), 0.98-1.06 (m, 5H), 1.09-1.14 (m, 6H), 1.31-1.34 (m, 7H), 1.50-1.68 (m, 5H), 1.77-1.82 (m, 2H), 1.88-1.90 (m, 1H), 1.97-1.99 (m, 1H), 2.07-2.09 (m, 1H), 2.97 (dd, J = 14.56, 8.72 Hz, 1H, CHCHH), 3.13 (dd, J = 14.5, 4.8 Hz, 1H, CHCHH), 4.43-4.50 (m, 2H, CHCH_2

+ CH₂OH), 6.95 (t, J = 7.0 Hz, 1H, Ar), 7.04 (t, J = 7.0 Hz, 1H, Ar), 7.10 (d, 1H, J = 2.5, Ar), 7.31 (d, J = 8.0 Hz, 1H, Ar), 7.51 (d, J = 7.8 Hz, 1H, Ar), 8.01 (d, J = 7.8 Hz, 1H, NH). ¹³C-NMR (100 MHz, DMSO-d₆) δ = 12.28, 18.69, 20.87, 23.74, 24.31, 26.63, 27.36, 27.59, 28.12, 30.85, 31.82, 32.55, 34.67, 35.29, 35.62, 35.84, 36.76, 41.99, 42.70, 53.32, 56.12, 56.53, 70.34, 110.47, 111.81, 118.60, 118.76, 121.30, 123.92, 127.66, 136.53, 172.99 (CONH), 174.06 (C=O). MS (ESI) calc for C₃₅H₅₀N₂O₄: 562.38; found: 561.5 [M-H]⁻. Anal. calc for C₃₅H₅₀N₂O₄ • 0.5H₂O: C, 73.52; H, 8.99; N, 4.90; found: C, 73.81; H, 9.21; N, 4.63.

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