Supporting Information

Title: Avicholic Acid: A Lead Compound from Birds on the Route to Potent TGR5 Modulators

Authors' Names: Roberto Pellicciari,*[,†] Antimo Gioiello,[†] Paola Sabbatini,[†] Francesco Venturoni,[†] Roberto Nuti,[†] Carolina Colliva,[‡] Giovanni Rizzo,[¥] Luciano Adorini,[¥] Mark Pruzanski,[¥] Aldo Roda,[‡] Antonio Macchiarulo[†]

Address:

[†]Dipartimento di Chimica e Tecnologia del Farmaco, Università di Perugia, Via del Liceo 1, 06123 Perugia, Italy.

[‡]Dipartimento di Scienze Farmaceutiche, Alma Mater Studiorum, Università di Bologna, Via Belmeloro 6, 40126 Bologna, Italy.

[¥]Intercept Pharmaceuticals, New York, NY 10013, USA.

*To whom correspondence should be addressed. Tel: +39 075 5855120. Fax: +39 075 5855124. Email: rp@unipg.it

Table of Contents:

I. Chemistry

IA. Synthesis and characterization (elemental analysis, mp, NMR) of avicholic acid (4), 16-*epi*-avicholic acid (5), 6α-ethyl-avicholic acid (6) and 6α-ethyl-16-*epi*-avicholic acid (7) sodium salt.

General Methods

Melting points were determined with a Buchi 535 electrothermal apparatus and are uncorrected. NMR spectra were obtained with a Bruker AC 200 MHz or 400 MHZ spectrometer and the chemical shifts are reported in parts per million (ppm). The abbreviations used are as follows: s, singlet; bs, broad singlet; d, doublet; dd, double doublet; m, multiplet. Flash and medium pressure liquid chromatography was performed using Merck silica gel 60 (0.040-0.063 mm) and a LOBAR Lichroprep RP-18 (40-63 μm), respectively. TLC were carried out on pre-coated TLC plates with silica gel 60 F-254 (Merck). Spots were visualized by staining and warming with phosphomolybdate reagent (5% solution in EtOH). All reaction were carried out under a nitrogen atmosphere. Purity of the new compounds was >95% according to the elemental analysis and HPLC traces.

*Methyl 3α-acetoxy-7α-hydroxy-5β-cholan-24-oate (**9a**)*

p-Toluenesulfonic acid monohydrate (0.49 g, 2.6 mmol) was added to a solution of CDCA (**2**) (10.0 g, 25.5 mmol) in methanol (200 mL). The mixture was sonicated at 30 °C for 2 h. The solvent was evaporated under reduce pressure, the residue was dissolved in $CHCl_3$ (300 mL), washed with aqueous $NaHCO_3$ saturated solution (2 x 100 mL), H_2O (100 mL) and brine (100 mL). The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure to afford the corresponding methyl ester (10.3 g, 25.3 mmol) as pure white solid.

The ester obtained (9.0 g, 22.16 mmol) was dissolved in freshly distilled THF (220 mL) and refluxed in the presence of acetic anhydride (40.7 g, 398.8 mmol) and $NaHCO_3$ (37.2 g, 443.0 mmol) overnight. The mixture was cooled to room temperature, slowly diluted with H_2O (220 mL) and extracted with EtOAc (3 x 150 mL). The combined organic layers were washed with

H_2O (2 x 150 mL), brine (150 mL), dried over Na_2SO_4 and evaporated under reduced pressure to get the desired monoacetylated compound **9a** (9.18 g, 20.5 mmol, 93%) as white solid (mp: 77-79 °C), that was used for the next step without further purification. ^{1}H-NMR ($CDCl_3$) δ: 0.64 (3H, s, 18-CH_3), 0.91-0.93 (6H, m, 19-CH_3 + 21-CH_3), 1.08-1.95 (25H, m), 2.01 (3H, s, 3-CHOC(O)CH_3), 2.18-2.26 (2H, m, 23-CH_2), 3.66 (3H, s, COOCH_3), 3.85 (1H, bs, 7-C*H*), 4.55-4.68 (1H, m, 3-C*H*).

*Methyl 3α-acetoxy-7α-hydroxy-6α-ethyl-5β-cholan-24-oate (**9b**)*

Prepared according to the same procedure described for **9a** starting from INT-747 (**8**). Yield: 90%. **9b** was obtained as white solid (mp: 69-71 °C). ^{1}H-NMR ($CDCl_3$) δ: 0.65 (3H, s, 18-CH_3), 0.84-0.93 (9H, m, 19-CH_3 + 21-CH_3, 26-CH_3), 1.11-1.91 (26H, m), 2.00 (3H, s, 3-CHOC(O)CH_3), 2.28-2.36 (2H, m), 3.65 (3H, s, COOCH_3), 3.70 (1H, bs, 7-C*H*), 4.48-4.59 (1H, m, 3-C*H*). ^{13}C-NMR ($CDCl_3$) δ: 11.61, 11.73, 18.21, 20.69, 21.46, 22.14, 23.17, 23.65, 26.60, 28.13, 29.58, 2x 30.96, 33.12, 35.13, 35.32, 35.49, 39.51, 39.95, 41.10, 42.70, 45.01, 50.43, 51.46, 55.73, 70.71, 74.67, 170.76, 174.72.

*Methyl 3α-acetoxy-7α-(3'-iodobenzoyl)oxy-5b-cholan-24-oate (**10a**)*

3-Iodobenzoic acid (1.5 g, 6.0 mmol) was suspended in CH_2Cl_2 (10 mL) and treated with oxalyl chloride (1.71 g, 13.6 mmol) in the presence of 2 drops of DMF at room temperature until the mixture become dissolved (about 1 h). Volatile solvents were removed under reduced pressure and the acylic chloride thus obtained was dissolved in 5 mL of toluene and added to a stirred solution of **9a** (1.5 g, 3.34 mmol) in toluene (5 mL). To the above solution, CaH_2 (1.0 g, 23.7 mmol) and $BnEt_3N^+Cl^-$ (0.19 g, 0.83 mmol) were added, and the mixture was refluxed for 48 h. The reaction mixture was then cooled to room temperature, the solvent was evaporated under reduced pressure, the residue was suspended in $CHCl_3$ (70 mL) and filtered. The organic filtrate was washed with aqueous $NaHCO_3$ saturated solution (2 x 30 mL), H_2O (30 mL), brine (30 mL), dried over Na_2SO_4 and evaporated under reduced pressure. The residue was purified by flash chromatography using from 5 to 10% of EtOAc in petroleum ether to yield 1.68 g (2.48 mmol, 74%) of **10a** as a white pure solid (mp: 60-62 °C). ^{1}H-NMR ($CDCl_3$) δ: 0.66 (3H, s, 18-CH_3), 0.92 (3H, d, *J*= 6.4 Hz, 21-CH_3), 0.98 (3H, s, 19-CH_3), 1.12-1.82 (25H, m), 2.02 (3H, s, 3-CHOC(O)CH_3), 2.18-2.30 (2H, m), 3.68 (3H, s, COOCH_3), 4.60-4.62 (1H, m, 3-C*H*), 5.21 (1H,

bs, 7-C*H*), 7.23 (1H, t, *J* = 6.5Hz, 5'-*H*), 7.90 (1H, d, *J*= 6.6 Hz, 4'-*H*), 7.98 (1H, d, *J*= 6.5 Hz, 6'-*H*), 8.34 (1H, s, 2'-*H*).

*Methyl 3α-acetoxy-6α-ethyl-7α-(3'-iodobenzoyl)oxy-5b-cholan-24-oate (**10b**)*

Prepared as previously described for **10a**. Conversion yield: 80%. **10b** was obtained as white solid (mp: 55-57 °C). ^{1}H-NMR ($CDCl_3$) δ: 0.67 (3H, s, 18-CH_3), 0.88-0.92 (6H, m, 21-CH_3, 26-CH_3), 0.99 (3H, s, 19-CH_3), 1.13-1.32 (15H, m), 1.7-1.74 (5H, m), 1.89-1.91 (5H, m), 2.05 (3H, s, 3-CHOC(O)CH_3), 2.18-2.30 (2H, m), 3.63 (3H, s, COOCH_3), 4.60-4.62 (1H, m, 3-C*H*), 5.41 (1H, bs, 7-C*H*), 7.23 (1H, dd, J_1 = 6.5 Hz, J_2= 6.7 Hz, 5'-*H*), 7.94 (1H, d, *J*= 6.7 Hz, 4'-*H*), 8.04 (1H, d, *J*= 6.5 Hz, 6'-*H*); 8.37 (1H, s, 2'-*H*). ^{13}C-NMR ($CDCl_3$) δ: 11.6, 11.7, 18.2, 20.7, 21.7, 22.2, 23.1, 23.9, 26.8, 27.9, 29.6, 2x 30.8, 34.4, 2x 35.1, 35.5, 39.2, 39.3, 41.3, 42.9, 44.6, 50.6, 51.4, 55.3, 74.2, 74.5, 93.9, 128.9, 130.1, 132.4, 138.6, 141.6, 164.5, 170.3, 174.6.

*Methyl 3α-acetoxy-7α-(3'-iodobenzoyl)oxy-17α-chloro-5β-cholan-24-oate (**11a**)*

Dichloroiodobenzene (3.11 g, 11.43 mmol) was added to a solution of **10a** (3.1 g, 4.57 mmol) in CH_2Cl_2 (250 mL) in the presence of 7.06 mL of a 0.3 M solution of ^{t}BuOH. The mixture was deoxygenated for 3 min by bubbling dry N_2, photolyzed at 0 °C using a tungsten lamps (200 W) for 1 h. The solvent was then evaporated under reduced pressure and the residue was purified by flash chromatography eluting with petroleum ether/EtOAc 8:2 (v/v) to yield 3.15 g (4.52 mmol, 96%) of the 17-chloro derivative **11a** as white solid (mp: 80-82 °C). ^{1}H-NMR ($CDCl_3$) δ: 0.85 (3H, s, 18-CH_3), 1.00 (3H, s, 19-CH_3), 1.02 (3H, d, *J*= 6.4 Hz, 21-CH_3), 1.11-1.90 (19H, m), 1.97 (3H, s, 3-CHOC(O)CH_3), 2.21-2.42 (4H, m), 3.67 (3H, s, COOCH_3), 4.54-4.62 (1H, m, 3-C*H*), 5.13-5.14 (1H, m, 7-C*H*), 7.24 (1H, t, *J* = 7.55 Hz, 5'-*H*), 7.92 (1H, dt, J_1 = 7.9 Hz, J_2 = 1.0 Hz, 4'-*H*), 8.04 (1H, dt, J_1 = 7.8 Hz, J_2 = 1.1 Hz, 6'-*H*), 8.40 (1H, t, *J*= 1.7 Hz, 2'-*H*). ^{13}C-NMR ($CDCl_3$) δ: 15.19, 15.30, 21.41, 22.23, 2x 23.43, 27.43, 29.42, 32.13, 32.48, 34.95, 35.07, 2x 35.44, 35.67, 39.45, 41.11, 41.47, 41.85, 46.07, 50.56, 52.27, 73.20, 74.53, 93.90, 94.71, 129.40, 130.92, 133.43, 139.28, 142.50, 165.34, 171.40, 174.87.

*Methyl 3α-acetoxy-6α-ethyl-7α-(3'-iodobenzoyl)oxy-17α-chloro-5β-cholan-24-oate (**11b**)*

Prepared as previously described for **11a**. Yield: 92%. **11b** was obtained as white solid (mp: 88-90 °C). ^{1}H-NMR ($CDCl_3$) δ: 0.81 (3H, s, 18-CH_3), 0.91 (3H, d, *J*= 7.3 Hz, 21-CH_3), 1.0 (6H, m,

19-CH_3 + 26-CH_3), 1.12-1.93 (24H,m), 2.03 (3H, s, 3-CHOC(O)CH_3), 2.18-2.25 (2H, m), 3.65 (3H, s, COOCH_3), 4.57-4.62 (1H, m, 3-C*H*), 5.40 (1H, bs, 7-C*H*), 7.22 (1H, t, *J* = 7.79 Hz, 5'-*H*), 7.91 (1H, d, *J* = 7.76 Hz, 4'-*H*), 8.02 (1H, d, *J* = 7.7 Hz, 6'-*H*), 8.38 (1H, s, 2'-*H*). ^{13}C-NMR ($CDCl_3$) δ: 11.6, 14.4, 14.5, 20.7, 21.6, 22.2, 2x 23.1, 26.7, 28.6, 29.4, 31.7, 2x 34.2, 35.1, 35.4, 39.6, 40.4, 41.1, 41.3, 44.6, 45.2, 49.9, 51.5, 74.1, 74.4, 92.9, 93.9, 128.8, 130.1, 132.3, 138.6, 141.7, 164.9, 170.7, 174.1.

*Methyl Δ^{16} 3α-acetoxy-7α-(3'-iodobenzoyl)oxy-5β-cholan-24-oate (**12a**)*

The 17-chloro derivative **11a** (2.65 g, 3.72 mmol) was dissolved in dry pyridine (120 mL) and refluxed overnight. The solvent was evaporated under reduced pressure and the residue was purified by flash chromatography eluting with petroleum ether/EtOAc 8:2 (v/v) to yield 2.24 g (2.15 g, 3.18 mmol, 81%) of the desired olefin **12a** as white solid (mp: 68-70 °C). ^{1}H-NMR ($CDCl_3$) δ: 0.77 (3H, s, 18-CH_3), 1.00-1.03 (6H, m, 19-CH_3 + 21-CH_3), 1.41-1.90 (22H,m), 1.99 (3H, s, 3-CHOC(O)CH_3), 2.22-2.34 (2H, m), 3.66 (3H, s, COOCH_3), 4.58-4.62 (1H, m, 3-C*H*), 5.23 (1H, s, 7-C*H*), 5.30 (1H, bs, 16-C*H*), 7.23 (1H, t, *J* = 7.8 Hz, 5'-*H*), 7.92 (1H, d, *J* = 7.9 Hz, 4'-*H*), 8.03 (1H, d, *J* = 7.9 Hz, 6'-*H*), 8.37 (1H, s, 2'-*H*).

*Methyl Δ^{16} 3α-acetoxy-6α-ethyl-7α-(3'-iodobenzoyl)oxy-5β-cholan-24-oate (**12b**)*

Prepared as previously described for **12a**. Yield: 72%. **12b** was obtained as white solid (mp: 77-79 °C). ^{1}H-NMR ($CDCl_3$) δ: 0.75 (3H, s, 18-CH_3), 0.89 (3H, t, *J* = 7.4 Hz, 21-CH_3), 0.98 (3H, t, *J* = 6.5 Hz, 26-CH_3), 1.03 (3H, s, 19-CH_3), 1.11-2.02 (22H, m), 2.05 (3H, s, 3-CHOC(O)CH_3), 2.22-2.30 (2H, m), 3.65 (3H, s, COOCH_3), 4.58-4.62 (1H, m, 3-C*H*), 5.20 (1H, bs, 7-C*H*), 5.51 (1H, s, 16-C*H*), 7.21 (1H, t, *J* = 7.9 Hz, 5'-*H*), 7.90 (1H, dt, J_1 = 7.9 Hz, J_2 = 1.1 Hz, 4'-*H*), 8.02 (1H, dt, J_1 = 7.9 Hz, J_2 = 1.1 Hz, 6'-*H*), 8.37 (1H, t, *J* = 1.3 Hz, 2'-*H*). ^{13}C-NMR ($CDCl_3$) δ: 11.6, 15.9, 20.6, 21.7, 21.8, 22.1, 23.1, 26.7, 29.6, 30.9, 31.1, 31.7, 32.2, 34.5, 34.9, 35.0, 35.7, 37.9, 41.3, 44.8, 47.5, 51.4, 51.7, 74.1, 74.7, 93.9, 121.4, 128.9, 130.1, 132.3, 138.6, 141.7, 158.5, 164.6, 170.7, 174.4.

*3α,7α,16α,24-tetrahydroxy-5β-cholane (**13a**)*

The olefin **12a** (0.4 g, 0.60 mmol) was dissolved in BH_3·THF (15 mL, 1M in THF) at 0 °C and then stirred at room temperature for 2 h. The reaction was then cooled at 0 °C and a mixture of

4M aqueous NaOH (25 mL) and H_2O_2 (25 mL) was added dropwise to the solution. The resulting reaction mixture was stirred at this temperature for 3 h. The reaction was acidified with 1N HCl (70 mL) and extracted with CH_2Cl_2 (3 x 60 mL). The combined organic layers were dried over Na_2SO_4 and evaporated under reduced pressure. The oily residue was dissolved in toluene (52 mL), a solution of 5% KOH in MeOH (7 mL) was added, and the resulting mixture was refluxed overnight. The solvent was removed under reduce pressure, the residue was dissolved in H_2O (25 mL) and extracted with EtOAc (3 x 30 mL). The combined organic layers were dried over Na_2SO_4 and evaporated under reduced pressure. The residue was purified by flash chromatography eluting with EtOAc/EtOH 95:5 (v/v) to afford 0.14 g (0.35 mmol, 58%) of the desired tetrol **13a** as white solid (mp: 190-192 °C). ^{1}H-NMR ($CDCl_3$) δ: 0.61 (3H, s, 18-CH_3), 0.83 (3H, s, 19-CH_3), 0.88 (3H, d, J = 6.4 Hz, 21-CH_3), 1.16-2.21 (21H, m), 3.31-3.35 (1H, m, 3-C*H*), 3.58-3.62 (2H, m, 24-CH_2), 3.74-3.76 (1H, bs, 7-*CH*), 3.85-3.90 (1H, bs, 16-*CH*).

*3α,7α,16α,24-tetrahydroxy-6α-ethyl-5β-cholane (**13b**)*

Prepared as previously described for **13a**. Yield: 47%. **13b** was obtained as white solid (mp: 124-126 °C). ^{1}H-NMR ($CDCl_3$) δ: 0.64 (3H, s, 18-CH_3), 0.85-0.91 (6H, m, 19-CH_3 + 26-CH_3), 0.92 (3H, d, J = 6.4 Hz, 21-CH_3), 1.21-1.91 (21H, m), 3.33-3.35 (1H, m, 3-C*H*), 3.54-3.61 (3H, m, 7-C*H* + 24-CH_2), 3.94 (1H, bs, 16-*CH*). ^{13}C-NMR ($CDCl_3$) δ: 11.7, 13.1, 18.8, 20.4, 22.4, 23.1, 28.7, 30.4, 31.8, 33.1, 33.5, 34.0, 2x 35.4, 35.5, 35.8, 39.5, 39.9, 41.3, 43.9, 45.3, 47.4, 62.6, 66.1, 70.6, 72.0.

*3α,7α-dihydroxy-5β-cholane O-24,16α-lactone (**14a**)*

A mixture of TEMPO (1.5 mg, 9.5 μmol) and Aliquat 336 (12 mg, 0.03 mmol) in CH_2Cl_2 (1 mL) was added to a stirred mixture of the tetrol **13a** (75 mg , 0.19 mmol) in CH_2Cl_2 (2 mL). A carbonate-bicarbonate buffer (0.5 M $NaHCO_3$/0.05 M K_2CO_3, 3 mL, pH 8.6) and solid *N*-chlorosuccinimide (125 mg, 0.94 mmol) were added, and the ensuing biphasic mixture was stirred vigorously for 2 h. The mixture was dilute with H_2O (6 mL), acidified with 3N HCl (3 mL) to pH 4 and extracted with CH_2Cl_2 (3 x 20 mL). The combined organic layers were dried over Na_2SO_4 and evaporated under reduced pressure. The oily residue was purified by flash chromatography eluting with EtOAc to obtain the desired lactone **14a** as white amorphous solid

(23 mg, 0.057 mmol, 30%). ^{1}H-NMR ($CDCl_3$) δ: 0.76 (3H, s, 18-CH_3), 0.91 (3H, s, 19-CH_3), 1.03 (3H, d, J = 6.4 Hz, 21-CH_3), 1.20-2.27 (21H, m), 2.60-2.77 (2H, m, 23-CH_2), 3.45-3.50 (1H, m, 3-C*H*), 3.85 (1H, bs, 7-CH), 4.68 (1H, t, J= 7.0 Hz, 16-*CH*). ^{13}C-NMR ($CDCl_3$) δ: 12.80, 20.00, 20.71, 22.64, 30.59, 32.13, 32.60, 33.18, 34.03, 34.47, 34.98, 35.22, 35.97, 38.90, 39.07, 39.63, 41.44, 45.01, 47.45, 64.10, 68.04, 71.82, 83.80, 175.81.

*3α,7α-dihydroxy-6α-ethyl-5β-cholane O-24,16α-lactone (**14b**)*

Prepared as previously described for **14a**. Yield: 37%. **14b** was obtained as white amorphous solid. ^{1}H-NMR ($CDCl_3$) δ: 0.73 (3H, s, 18-CH_3), 0.77-0.92 (6H, m, 19-CH_3 + 26-*CH*$_3$), 1.01 (3H, d, J= 6.8 Hz, 21-CH_3), 1.20-1.95 (23H, m), 2.61-2.75 (2H, m, 23-CH_2), 3.36-3.42 (1H, m, 3-C*H*), 3.66 (1H, bs, 7-CH), 4.63 (1H, t, J= 8.5 Hz, 16-*CH*).

*3α,7α,16α-trihydroxy-5β-cholan-24-oic acid sodium salt (**4**)*

The lactone derivative **14a** (60 mg, 0.15 mmol) was dissolved in 8 mL of 5% NaOH in MeOH and the resulting mixture was stirred at room temperature overnight. The solvent was then evaporated under reduced pressure and the resulting solid was dissolved into a mixture of H_2O/CH_3OH (1:1, 3mL) and purified by reverse phase chromatography (column RP-18, Lobar A) using a mixture of CH_3OH/H_2O as mobile phase to afford the desired sodium salt **4** (56 mg, 0.13 mmol, 88%) as pure white solid (mp: >250 °C). ^{1}H-NMR (CD_3OD) δ: 0.70 (3H, s, 18-CH_3), 0.91 (3H, s, 19-CH_3), 0.98 (3H, d, J = 6.6 Hz, 21-CH_3), 1.13-2.47 (27H, m), 3.30-3.37 (1H, m, 3-C*H*), 3.79 (1H, bs, 7-C*H*), 4.01 (1H, t, J= 6.7 Hz, 16-C*H*). ^{13}C-NMR (CD_3OD) δ: 13.52, 19.17, 21.00, 23.20, 31.19, 31.80, 31.52, 33.49, 34.47, 35.71, 35.78, 36.20, 37.25, 39.83, 40.63, 40.71, 42.72, 44.51, 48.22, 66.25, 68.03, 71.96, 76.89, 183.70. HPLC 100 % purity. Elemental Analysis: Calcd for $C_{24}H_{39}NaO_5$; C: calcd, 66.95; found, 66.97, H: calcd, 9.13; found, 9.12.

*3α,7α,16α-trihydroxy-6α-ethyl-5β-cholan-24-oic acid sodium salt (**6**)*

Prepared as previously described for **4**. Yield: 80%. **6** was obtained as white solid (mp: >250°C). ^{1}H-NMR (CD_3OD) δ: 0.70 (3H, s, 18-CH_3), 0.86-0.92 (6H, m, 19-CH_3 + 26-*CH*$_3$), 0.97 (3H, d, J = 6.4 Hz, 21-CH_3), 1.18-2.47 (29H, m), 3.26-3.34 (1H, m, 3-C*H*), 3.69 (1H, bs, 7-C*H*), 4.02 (1H, t, J= 6.4 Hz, 16-C*H*). ^{13}C-NMR (CD_3OD) δ: 11.89, 13.55, 19.14, 21.12, 23.04, 23.53, 31.19, 2x 31.35, 33.82, 34.43, 34.50, 36.18, 36.39, 37.18, 40.56, 40.65, 42.40, 44.57, 46.51, 48.26, 66.38,

70.29, 72.25, 76.90, 183.64. HPLC 100 % purity. Elemental Analysis: Calcd for $C_{26}H_{43}NaO_5$; C: calcd, 68.09; found, 68.10, H: calcd, 9.45; found, 9.48.

*3α,16α,24-trihydroxy-7α-(3'-iodobenzoyl)oxy-5β-cholane (**15a**)*

The olefin **12a** (0.5 g, 0.75 mmol) was dissolved in BH_3·THF (19 mL, 1M in THF) at 0 °C and then stirred at room temperature for 2 h. The reaction was then cooled at 0 °C and a mixture of 4M aqueous NaOH (30 mL) and 30% solution of H_2O_2 (30 mL) was added dropwise to the solution. The resulting reaction mixture was stirred at this temperature for 3 h. The reaction was acidified with 1N HCl (70 mL) and extracted with CH_2Cl_2 (3 x 60 mL). The combined organic layers were dried over Na_2SO_4 and evaporated under reduced pressure. The oily residue was dissolved in methanol (15 mL), and solid K_2CO_3 (0.15 g, 1.12 mmol) was added. The resulting mixture was stirred at room temperature for 2 h, then the solvent was removed under reduce pressure. The residue was dissolved in H_2O (25 mL) and extracted with EtOAc (3 x 30 mL). The combined organic layers were dried over Na_2SO_4 and evaporated under reduced pressure to afford 0.26 g (0.42 mmol, 56%) of compound **15a** as amorphous white solid. ^{1}H-NMR ($CDCl_3$) δ: 0.70 (3H, s, 18-C*H*$_3$), 0.96 (3H, d, *J* = 6.9 Hz, 21-C*H*$_3$), 0.98 (3H, s, 19-C*H*$_3$), 1.26-2.06 (22H, m), 3.45-3.53 (2H, m, 3-C*H* +24-C*H*$_2$), 3.53-3.61 (1H, m, 24-C*H*$_2$), 3.95 (1H, t, *J* = 6.1 Hz, 16-*CH*), 5.12 (1H, bs, 7-*CH*), 7.20 (1H, t, *J* = 7.9 Hz, 5'-*H*), 7.86 (1H, d, *J* = 7.9 Hz, 4'-*H*), 7.98 (1H, d, *J* = 7.9 Hz, 6'-*H*), 8.35 (1H, s, 2'-*H*). ^{13}C-NMR ($CDCl_3$) δ: 13.22, 18.79, 20.37, 22.75, 28.33, 29.67, 30.46, 31.07, 33.52, 34.39, 34.77, 35.04, 36.69, 37.65, 39.40, 39.64, 40.89, 44.34, 47.85, 62.87, 64.88, 71.91, 72.55, 76.80, 94.00, 128.45, 130.34, 132.57, 138.59, 141.63, 164.63.

*3α,16α,24-trihydroxy-6α-ethyl-7α-(3'-iodobenzoyl)oxy-5β-cholane (**15b**)*

Prepared as previously described for **15a**. Yield: 50%. **15b** was obtained as white amorphous solid. ^{1}H-NMR ($CDCl_3$) δ: 0.62 (3H, s, 18-C*H*$_3$), 0.82-0.92 (9H, m, 19-C*H*$_3$ + 21-C*H*$_3$ + 26-*CH*$_3$), 1.10-1.95 (23H, m), 3.40-3.50 (2H, m, 3-C*H* +24-C*H*$_2$), 3.51-3.56 (1H, m, 24-C*H*$_2$), 3.92 (1H, bs, 16-*CH*), 5.19 (1H, bs, 7-*CH*), 7.18 (1H, t, *J* = 7.9 Hz, 5'-*H*), 7.83 (1H, d, *J* = 7.9 Hz, 4'-*H*), 7.96 (1H, d, *J* = 7.9 Hz, 6'-*H*), 8.31 (1H, s, 2'-*H*). ^{13}C-NMR ($CDCl_3$) δ: 11.63, 13.25, 14.12, 18.79, 20.31, 22.13, 23.09, 28.53, 30.37, 31.16, 33.60, 34.26, 2x35.43, 37.02, 38.53, 39.35, 41.23, 44.26, 44.63, 47.51, 62.49, 64.40, 71.55, 74.55, 76.41, 93.95, 128.55, 130.32, 132.15, 138.63, 141.42, 164.93.

*Methyl 7α-(3'-iodobenzoyl)oxy-3,16-dioxo-5β-cholan-24-oate (**16a**)*

Jones reagent (2 mL) was added dropwise to a stirred solution of the triol **15a** (0.25 g, 0.40 mmol) in acetone (10 mL) at 0 °C and the mixture was stirred at room temperature for 2 h. Methanol (8 mL) was then added and the oxidized product was extracted with EtOAc (2 x 30 mL). The combined organic layers were dried over Na_2SO_4 and evaporated under reduced pressure. The brown residue was dissolved in MeOH (10 mL), *p*-toluensulfonic acid (0.01 g, 0.04 mmol) was added and the mixture was stirred at room temperature for 2 h. The solvent was evaporated under reduce pressure, the residue was dissolved in $CHCl_3$ (20 mL), washed with aqueous $NaHCO_3$ saturated solution (2 x 20 mL), H_2O (20 mL) and brine (20 mL). The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure. The residue was purified by silica gel flash chromatography using from 10 to 30% EtOAc in petroleum ether to afford **16a** (0.26 g, 0.39 mmol, 98%) as whitish amorphous solid. ^{1}H-NMR ($CDCl_3$) δ: 0.73 (3H, s, 18-C*H*$_3$), 0.98 (3H, d, *J* = 6.9 Hz, 21-C*H*$_3$), 1.01 (3H, s, 19-C*H*$_3$), 1.24-2.32 (18H, m), 2.56-2.63 (1H, m, 24-C*H*$_2$), 2.85-2.91 (1H, m, 23-C*H*$_2$), 3.57 (3H, s, COO*CH*$_3$), 5.12 (1H, bs, 7-*CH*), 7.23 (1H, t, *J* = 7.9 Hz, 5'-*H*), 7.92 (1H, d, *J* = 7.9 Hz, 4'-*H*), 7.97 (1H, d, *J* = 7.9 Hz, 6'-*H*), 8.34 (1H, s, 2'-*H*).

*Methyl 6α-ethyl-7α-(3'-iodobenzoyl)oxy-3,16-dioxo-5β-cholan-24-oate (**16b**)*

Prepared as previously described for **16a**. Yield: 92%. **16b** was obtained as white amorphous solid. ^{1}H-NMR ($CDCl_3$) δ: 0.77 (3H, s, 18-C*H*$_3$), 0.82-0.92 (6H, m, 21-C*H*$_3$ + 26-C*H*$_3$), 1.10 (3H, s, 19-C*H*$_3$), 1.22-2.28 (27H, m), 2.48-2.55 (1H, m, 23-C*H*$_2$), 2.73-2.88 (1H, m, 24-C*H*$_2$), 3.67 (3H, s, COO*CH*$_3$), 5.19 (1H, bs, 7-*CH*), 7.21 (1H, t, *J* = 7.9 Hz, 5'-*H*), 7.89 (1H, d, *J* = 7.9 Hz, 4'-*H*), 7.96 (1H, d, *J* = 7.9 Hz, 6'-*H*), 8.35 (1H, s, 2'-*H*)

*3α,7α,16β-trihydroxy-5β-cholan-24-oic acid sodium salt (**5**)*

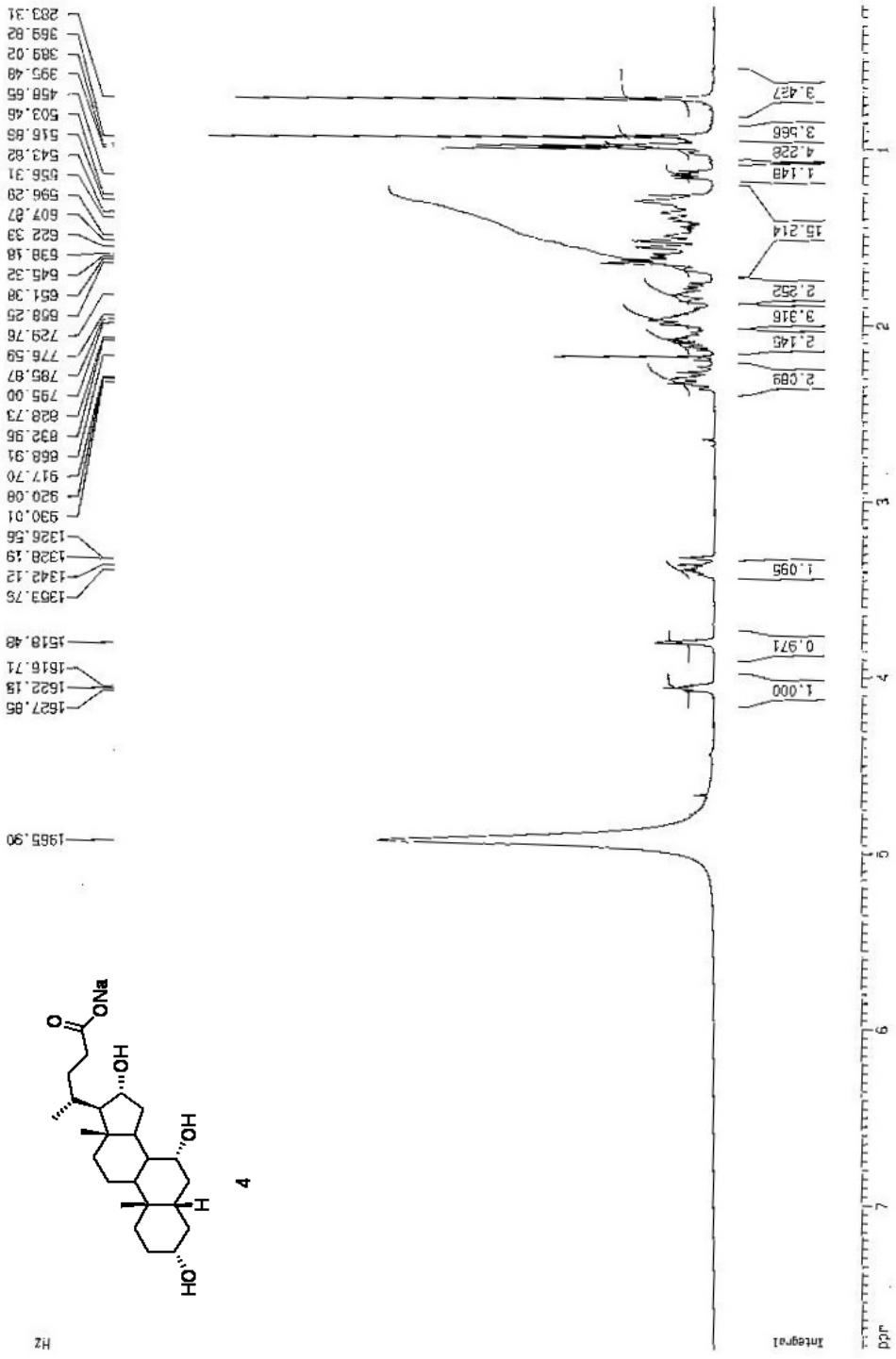
tert-Butylamine-borane complex (0.1 g, 1.12 mmol) was added to a stirred solution of **16a** (0.10 g, 0.24 mmol) in CH_2Cl_2 (8 mL). The reaction was refluxed overnight and then cooled to room temperature, acidified with 3N HCl (15 mL) and the resulting mixture stirred for additional 30 min. The organic layer was separated, washed with a saturated aqueous solution of $NaHCO_3$ (10 mL), H_2O (10 mL), dried over Na_2SO_4 and evaporated to dryness. The residue was dissolved in a

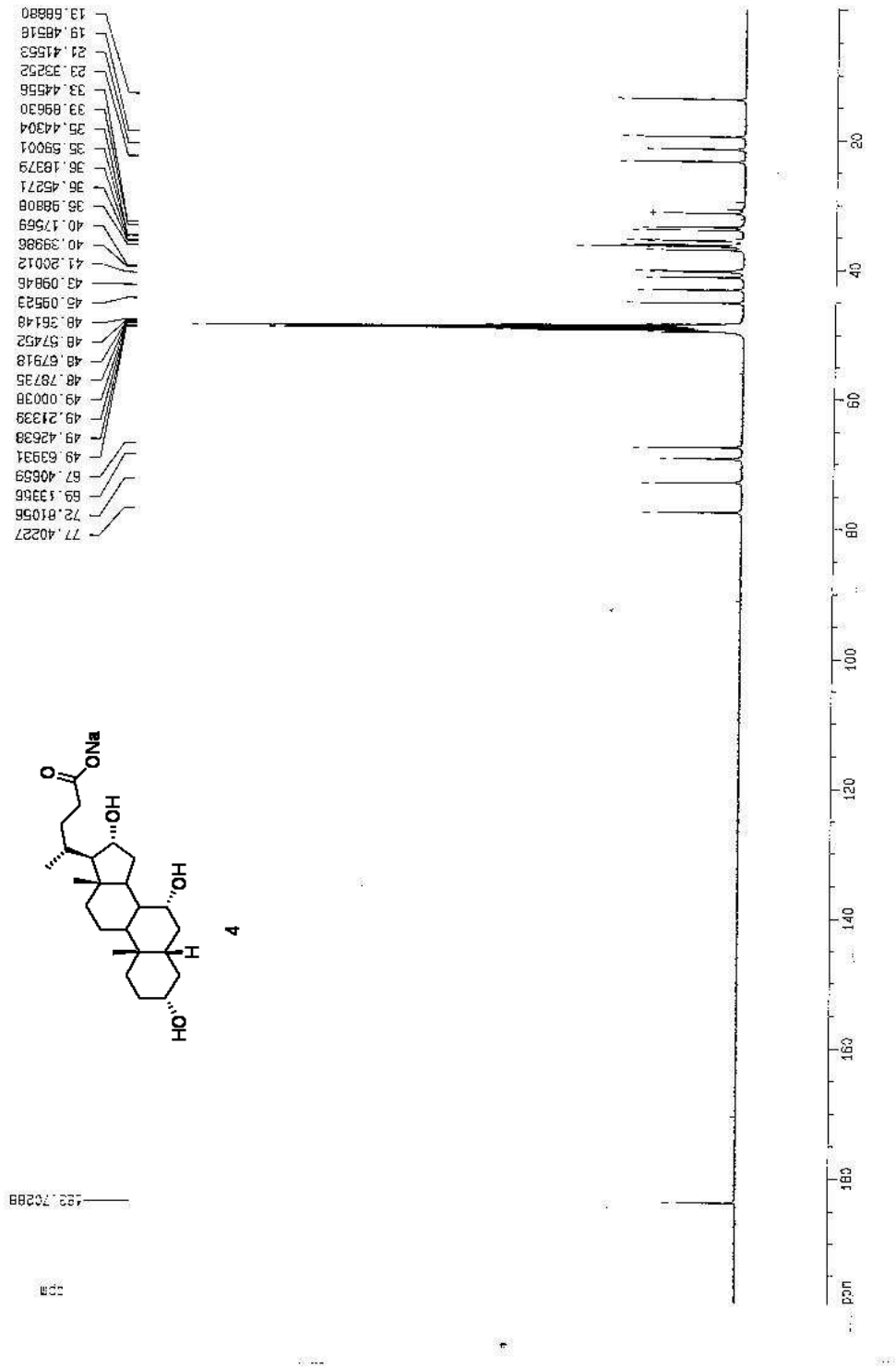
solution 1:4 (v/v) of H_2O/THF (5 mL) and $NaBH_4$ was added at 0 °C. The mixture was stirred at room temperature for 30 min, then H_2O (5 mL) and 3N HCl (5 mL) were added. The mixture was extracted with EtOAc (4 x 30 mL), the combined organic layers were dried over Na_2SO_4 and evaporated under reduced pressure. The residue was purified by silica gel flash chromatography using from 1 to 4 % MeOH in $CHCl_3$ to yield 0.042 g of methyl 3α,16β-dihydroxy-7α-(3'-iodobenzoyl)oxy-5β-cholan-24-oate, as an amorphous white solid. The ester thus obtained (40 mg, 0.09 mmol) was dissolved in 8 mL of 5% NaOH in MeOH and the resulting mixture was stirred at room temperature overnight. The solvent was evaporated under reduced pressure and the ensuing solid was purified by reverse phase chromatography (column RP-18 lobar A) using a mixture of CH_3OH/H_2O as mobile phase, to give the desired sodium salt **5** as white solid (35 mg, 0.08 mmol, 51%) (mp: >250 °C). ^{1}H-NMR (CD_3OD) δ: 0.89 (3H, s, 18-C*H*$_3$), 0.92 (3H, s, 19-C*H*$_3$), 1.00 (3H, d, *J*= 6.6 Hz, 21-C*H*$_3$), 1.15-1.99 (25H, m), 2.31-2.43 (2H, m, 23-*CH*$_2$), 3.30-3.38 (1H, m, 3-C*H*), 3.82 (1H, bs, 7-C*H*), 4.37-4.42 (1H, m, 16-C*H*). ^{13}C-NMR (CD_3OD) δ: 12.41, 17.42, 20.17, 22.43, 2x 28.52, 30.13, 30.41, 30.70, 32.74, 2x 34.97, 35.42, 39.24, 39.81, 39.97, 41.91, 42.08, 48.21, 61.99, 68.13, 72.12, 73.27, 183.03. HPLC 99% purity. Elemental Analysis: Calcd for $C_{24}H_{39}NaO_5$; C: calcd, 66.95; found, 66.98, H: calcd, 9.13; found, 9.11.

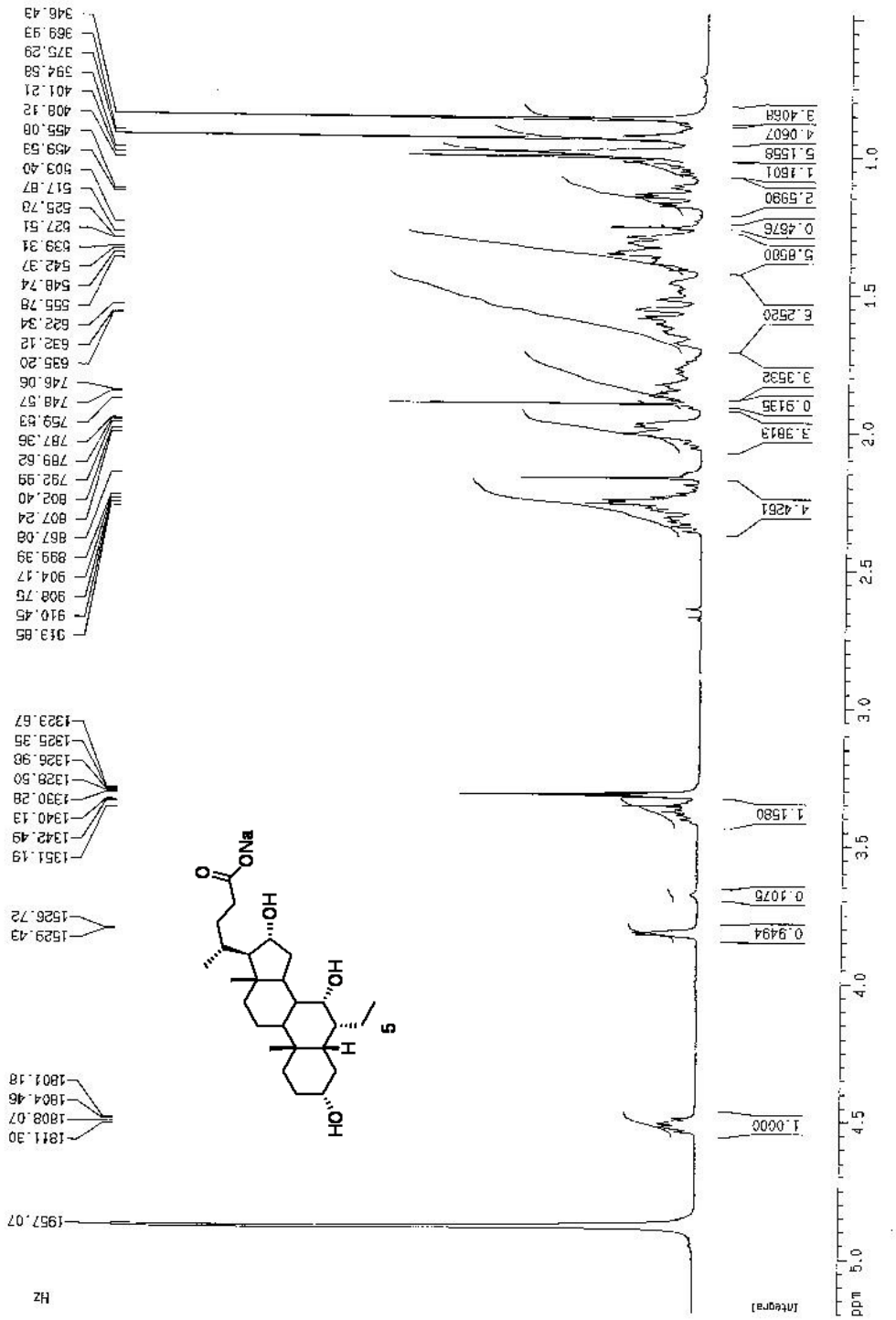
*6α-ethyl-3α,7α,16β-trihydroxy-5β-cholan-24-oic acid sodium salt (**7**)*

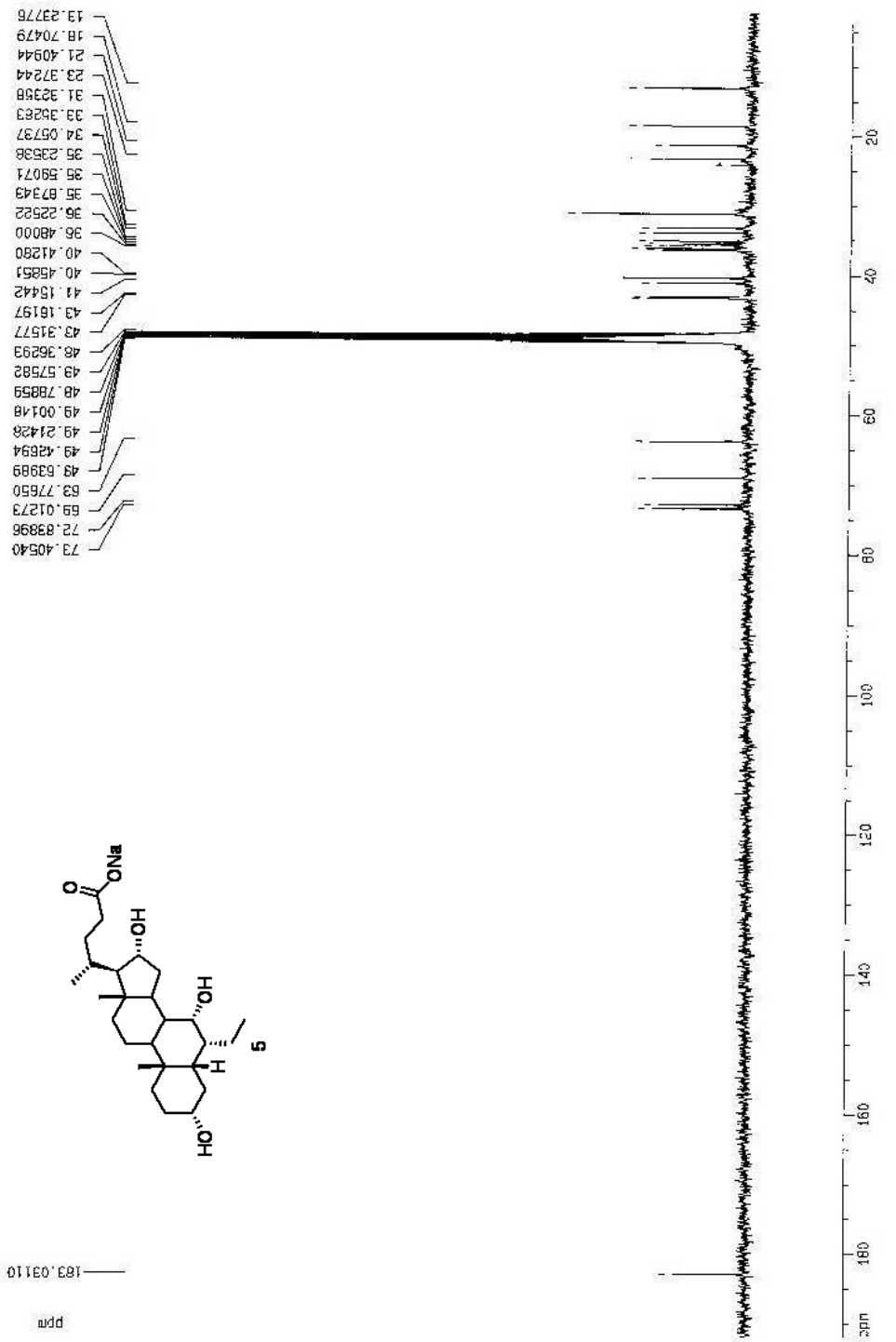
Prepared as previously described for **5**. Yield: 48%. The compound was obtained as white solid (mp: >250 °C). ^{1}H-NMR (CD_3OD) δ: 0.86 (3H, s, 18-C*H*$_3$), 0.88-0.93 (6H, m, 19-C*H*$_3$ + 26-C*H*$_3$), 0.99 (3H, d, *J* = 6.4 Hz, 21-C*H*$_3$), 1.06-1.41 (9H, m), 1.46-1.90 (11H, m), 1.97-2.04 (2H, m), 2.23-2.25 (2H, m), 2.30-2.35 (1H, m), 3.28-3.35 (1H, m, 3-C*H*), 3.67 (1H, bs, 7-C*H*), 4.49-4.52 (1H, m, 16-C*H*). ^{13}C-NMR (CD_3OD) δ: 12.0, 13.3, 18.7, 21.6, 23.5, 23.7, 31.2, 31.3, 33.4, 34.4, 34.5, 35.3, 35.5, 36.6, 36.7, 2x 41.2, 43.1, 43.4, 46.7, 49.5 (under solvent), 63.8, 71.1, 73.2, 73.4. 183.06. HPLC 99% purity. Elemental Analysis: Calcd for $C_{26}H_{43}NaO_5$; C: calcd, 68.09; found, 68.07, H: calcd, 9.45; found, 9.41.

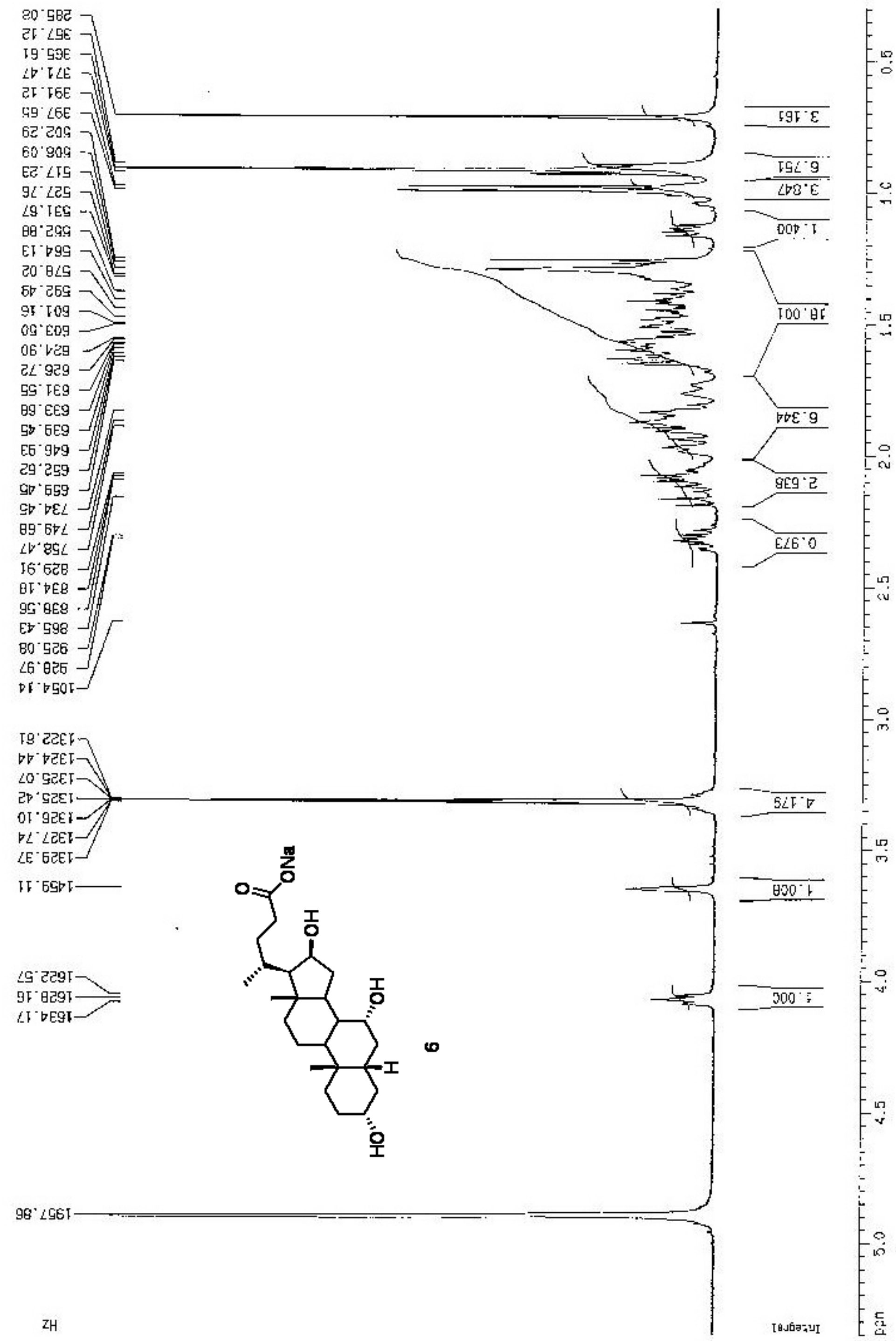
IB. Copy of ^{1}H- and ^{13}C-NMR for compounds 4-7

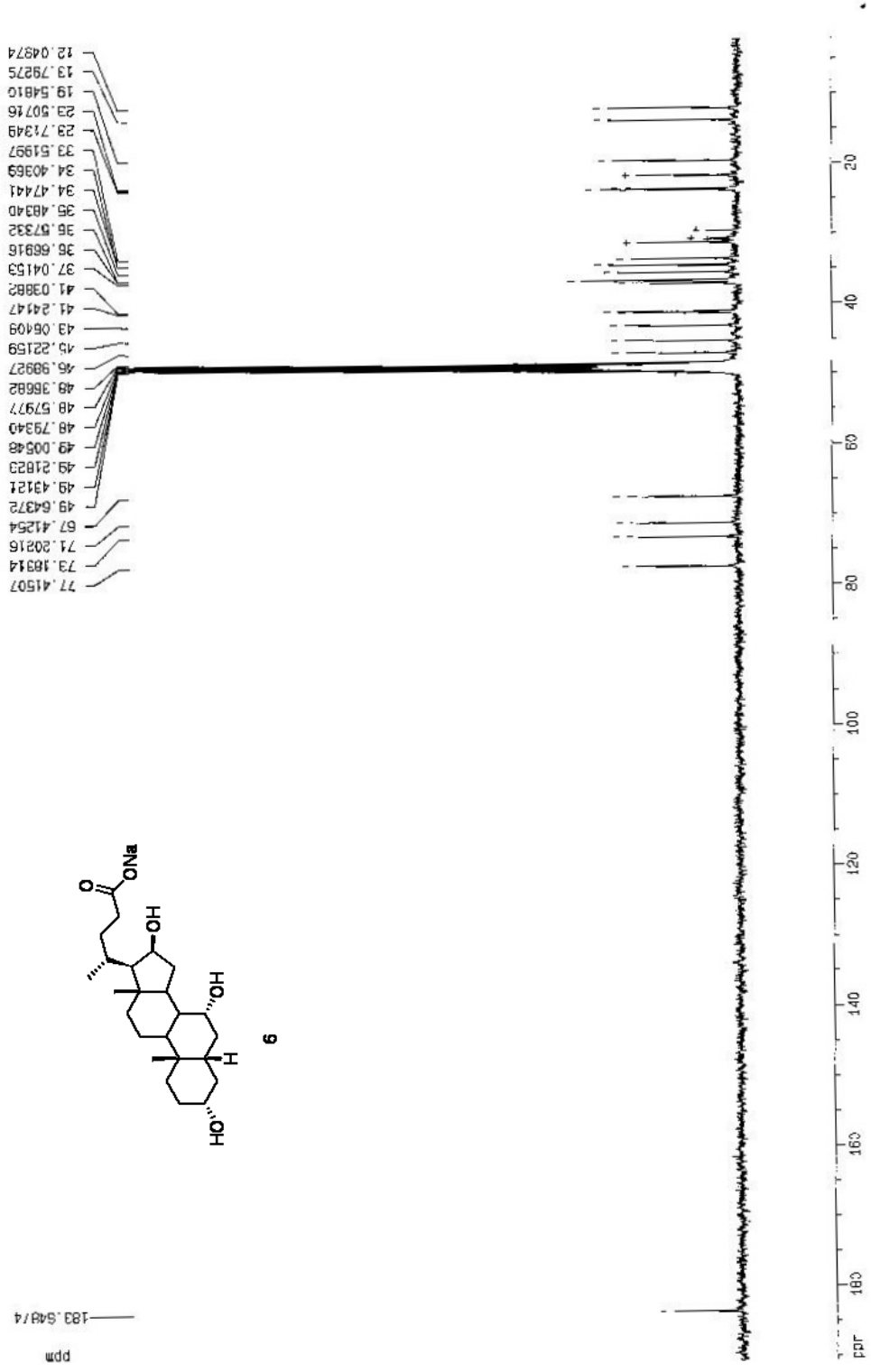


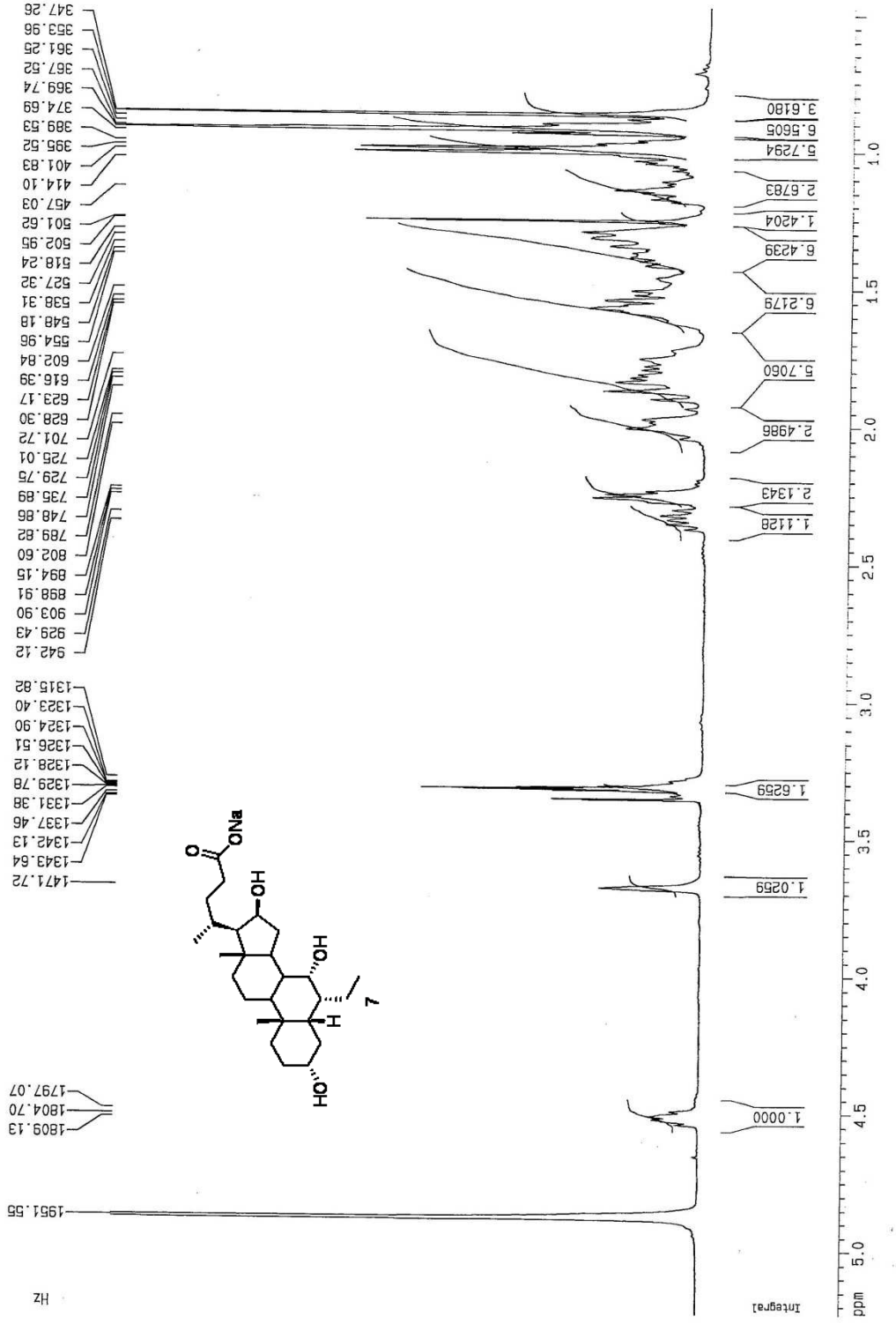

13.68880
19.48518
21.41563
23.33252
33.44556
33.89630
35.44304
35.59001
36.18379
36.45271
36.98808
40.17569
40.39986
41.20012
43.09846
45.09523
48.36148
48.57452
48.67918
48.78735
49.00038
49.21339
49.42638
49.63934
67.40659
69.13356
72.81056
77.40227
422.70288
ppm
20
40
60
80
100
120
140
160
180
O
ONa
OH
OH
H
HO
4

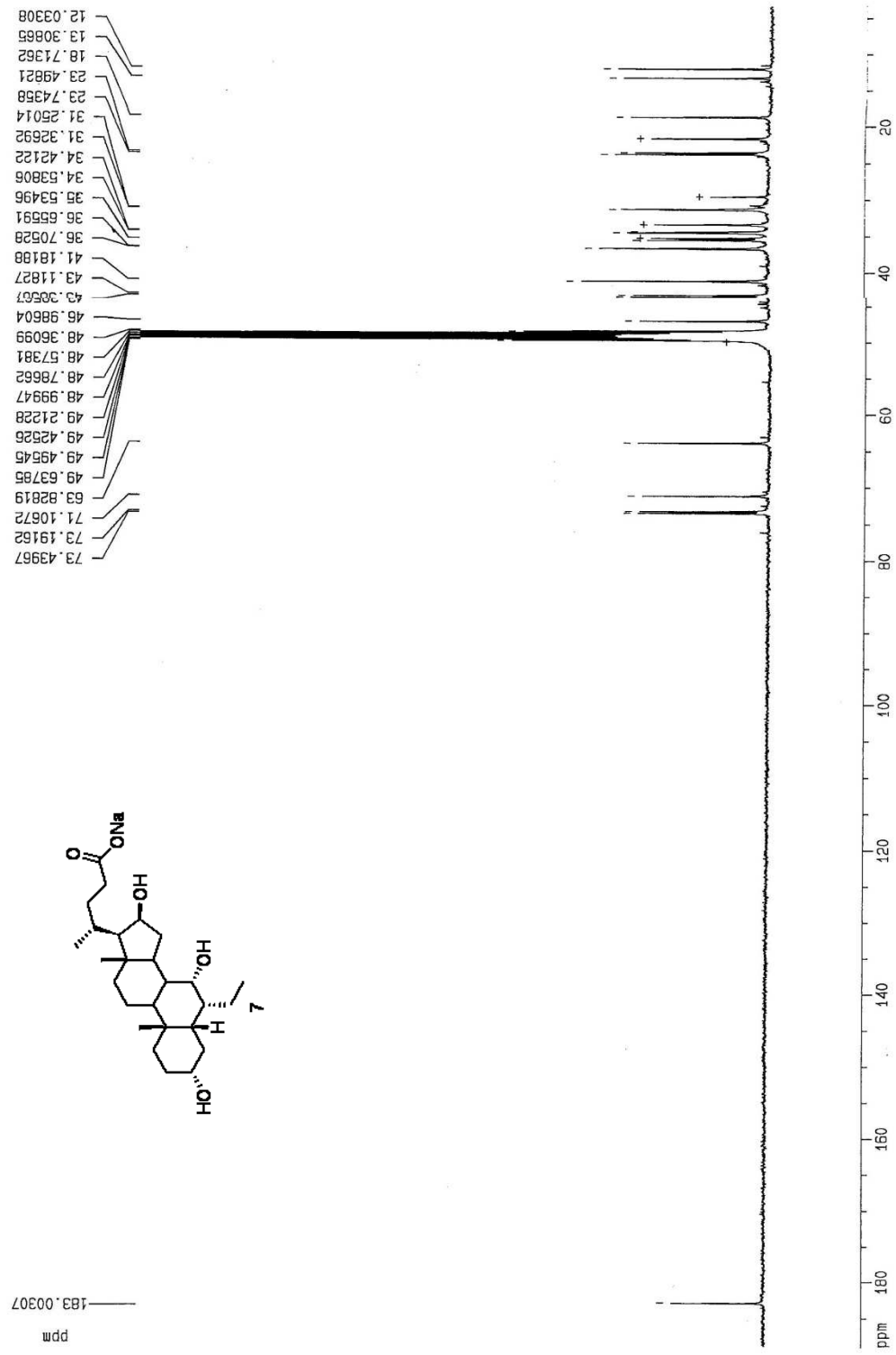

Hz
Integral
ppm
1957.07
1811.30
1808.07
1804.46
1801.18
1529.43
1526.72
1351.19
1342.49
1340.13
1330.28
1328.50
1326.90
1325.35
1323.67
913.85
910.45
908.75
904.17
899.39
867.08
807.24
802.40
792.99
789.62
787.36
759.63
748.57
748.06
635.20
632.12
622.34
555.78
548.74
542.37
539.34
527.51
525.73
517.87
503.40
459.53
455.08
408.12
401.21
394.58
375.29
369.93
346.43
1.0000
0.9494
0.1075
1.1580
4.4261
3.9813
0.9135
3.3532
6.2520
5.8500
0.4576
2.5990
1.1801
5.1558
4.0607
3.4068
ONa
O
OH
HO
H
5


13.23776
18.70479
21.40944
23.37244
31.32358
33.35263
34.05737
35.23538
35.59071
35.87343
36.22522
36.48000
40.41280
40.45851
41.15442
43.16197
43.31577
48.36293
49.57582
49.78859
49.00148
49.21428
49.42694
49.63989
63.77650
69.01273
72.83896
73.40540
183.03110
ppm
ONa
OH
OH
HO
H
5




12.04874
13.79275
19.54810
23.50716
23.71349
33.51997
34.40369
34.47441
35.48340
36.57332
36.66916
37.04153
41.03882
41.24147
43.06409
45.22159
46.98927
48.36682
48.57977
48.79340
49.00548
49.21823
49.43121
49.64372
67.41254
71.20216
73.18314
77.41507
183.5487/4
ppm
O
ONa
OH
HO
H
HO
6
20
40
60
80
100
120
140
160
180
ppm

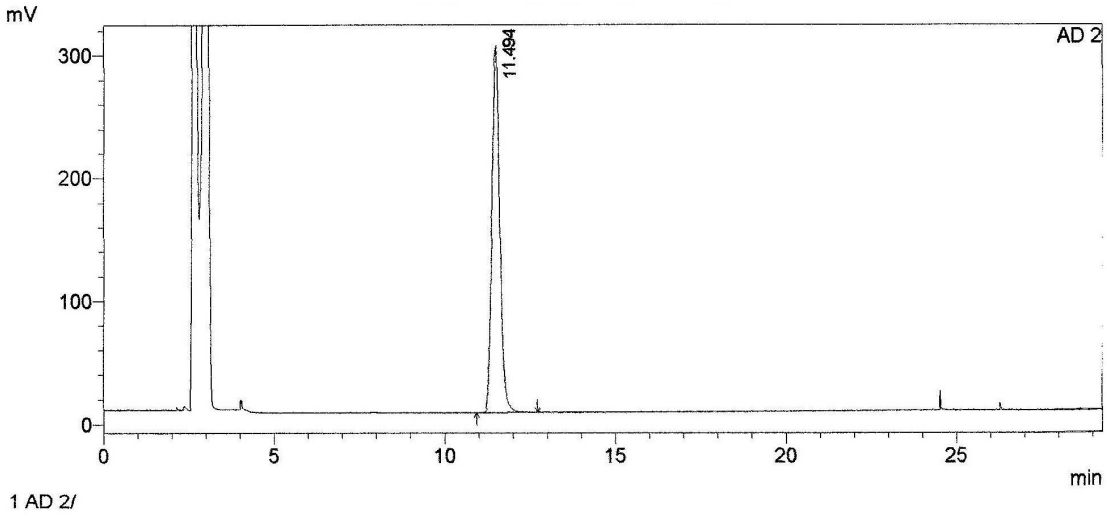

Hz
1951.55
1809.13
1804.70
1797.07
1471.72
1343.64
1342.13
1337.46
1331.38
1329.78
1328.12
1326.51
1324.90
1323.40
1315.82
942.12
929.43
903.90
898.91
894.15
802.60
789.82
748.86
735.89
729.75
725.01
701.72
628.30
623.17
616.39
602.84
554.96
548.18
538.31
527.32
518.24
502.95
501.62
457.03
414.10
401.83
395.52
389.53
374.69
369.74
367.52
361.25
353.96
347.26
Integral
1.0000
1.0259
1.6259
1.1128
2.1343
2.4986
5.7060
6.2179
6.4239
1.4204
2.6783
5.7294
6.5605
3.6180
ppm
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
OH
OH
H
OH
ONa
O


183.00307
73.43967
73.19162
71.10672
63.82819
49.63785
49.49545
49.42526
49.21228
48.99947
48.78662
48.57381
48.36099
46.98604
43.30507
43.11827
41.18188
36.70528
36.65591
35.53496
34.53806
34.42122
31.32692
31.25014
23.74358
23.49821
18.71362
13.30865
12.03308
ppm
20
40
60
80
100
120
140
160
180
ppm
ONa
O
OH
OH
H
HO
7

IC. HPLC Traces of Target Compounds (4-7)

The HPLC analyses were carried out on a Shimadzu (Kyoto, Japan) Class-VP equipped with a EZ Start chromatography data software, a LC-10 AT_{VP} pump, a SCL-$10A_{VP}$ system controller, a FCV-$10AL_{VP}$ low pressure gradient formation unit, a DGU-14A on-line degasser and a Rheodyne 7725i injector (Rheodyne, Cotati, CA, USA) with a 20 μL stainless steel loop. A PL-ELS 2100 Ice (Polymer Laboratories Varian, Inc., Amherst, MA, USA) was utilized as the evaporative light scattering detector (ELSD). A SS420X (Scientific Software, Inc., Pleasanton, CA, USA) interface allowed the analog-to-digital conversion of the output signal from the ELSD. An Ultra Aqueous C18 (Restek, Bellefonte, PA, USA) 250 x 4.6 mm i.d., 5 μm, 100Å polar end-capped analytical column was used after previous conditioning by passing through the column the selected mobile phase at a 1.0 mL/min flow rate for at least 30 min. The column temperature was controlled through a Grace (Sedriano, Italy) heather/chiller (Model 7956R) thermostat. The adopted chromatographic condition for the analysis of all compounds were: METHOD A) eluent composition, H_2O/MeCN (Sigma-Aldrich, Milano, Italy) - 50/50 (v,v) added with TFA 0.1%; eluent flow rate: 1.0 mL/min; column temperature, 25 °C; METOD B) eluent composition, H_2O/MeCN/MeOH (Sigma-Aldrich, Milano, Italy) - 50/40/10 (v,v,v) added with TFA 0.1%; eluent flow rate: 1.0 mL/min; column temperature, 25 °C The HPLC-grade water was obtained from a tandem Milli-Ro/Milli-Q apparatus (Millipore, Bedford, MA, USA). The mobile phase was always filtered through a 0.22 μm Millipore filter (Bedford, MA, USA) and then degassed with 20 min sonication. The adopted ELSD conditions for the analysis of all compounds were: nebulization temperature, 30 °C; evaporation temperature, 50 °C; gain factor, 2.0; gas flow rate (air), 1.5 L/min. The injected analyte were always solubilized in the filtered mobile phase.

HPLC trace of compound **4** (method A)

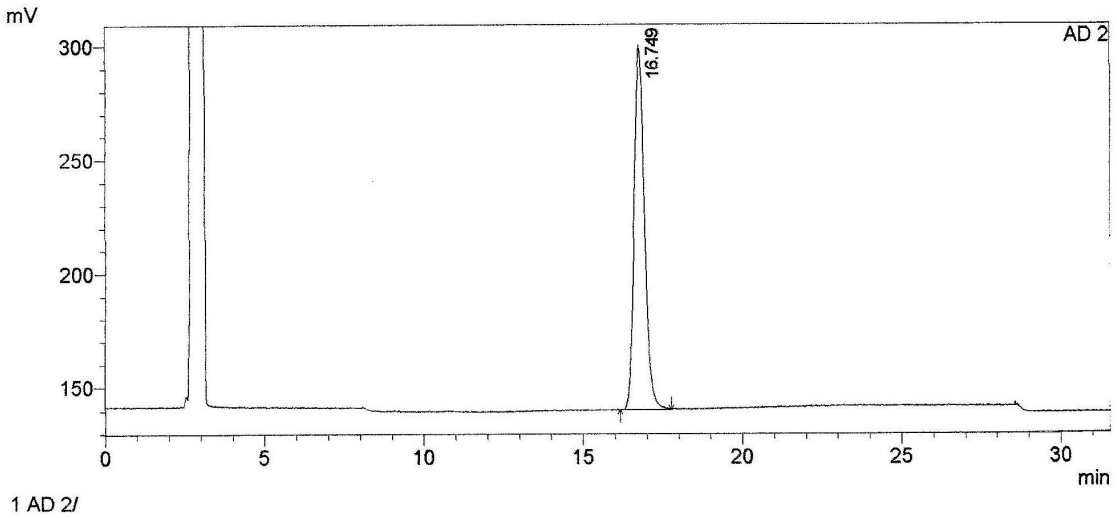


PeakTable

AD2 Ch1

Peak#	Ret. Time	Area	Height	Area %	Height %
1	11.494	4785253	298013	100.000	100.000
Total		4785253	298013	100.000	100.000

HPLC trace of compound **4** (method B)

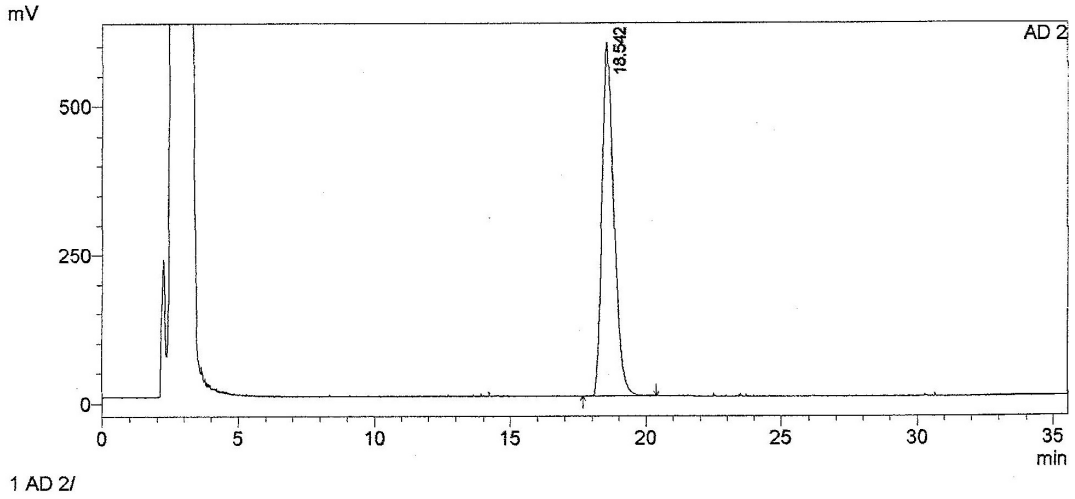


PeakTable

AD2 Ch1

Peak#	Ret. Time	Area	Height	Area %	Height %
1	16.749	3589338	159680	100.000	100.000
Total		3589338	159680	100.000	100.000

HPLC trace of compound **5** (method A)

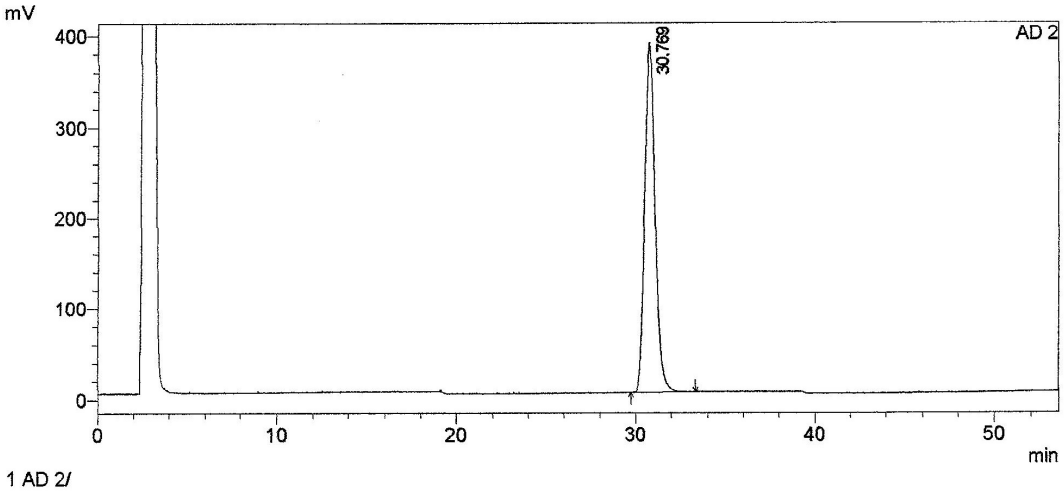


PeakTable

AD2 Ch1

Peak#	Ret. Time	Area	Height	Area %	Height %
1	18.542	18067969	592736	100.000	100.000
Total		18067969	592736	100.000	100.000

HPLC trace of compound **5** (method B)

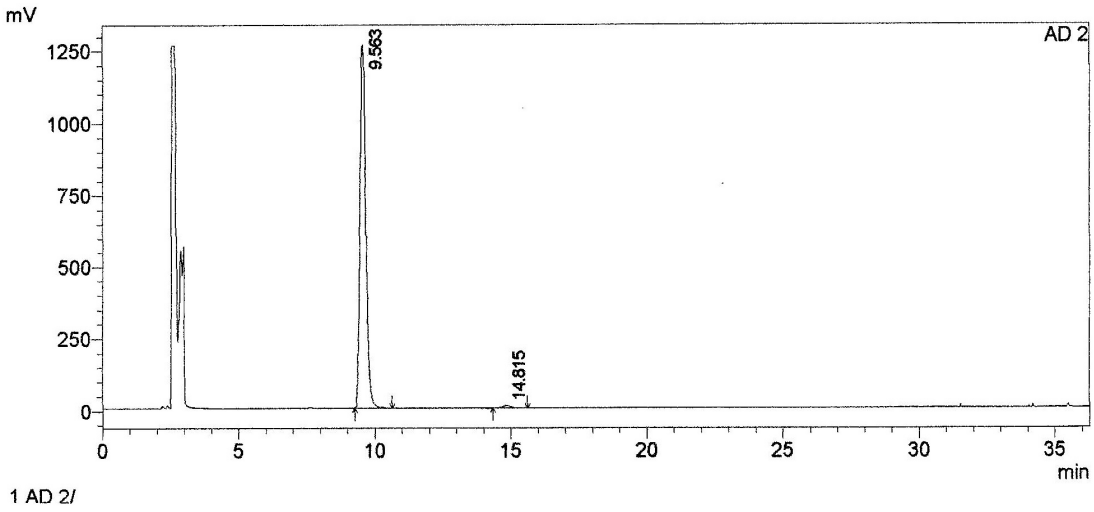


PeakTable

AD2 Ch1

Peak#	Ret. Time	Area	Height	Area %	Height %
1	30.769	15506562	384211	100.000	100.000
Total		15506562	384211	100.000	100.000

HPLC trace of compound **6** (method A)

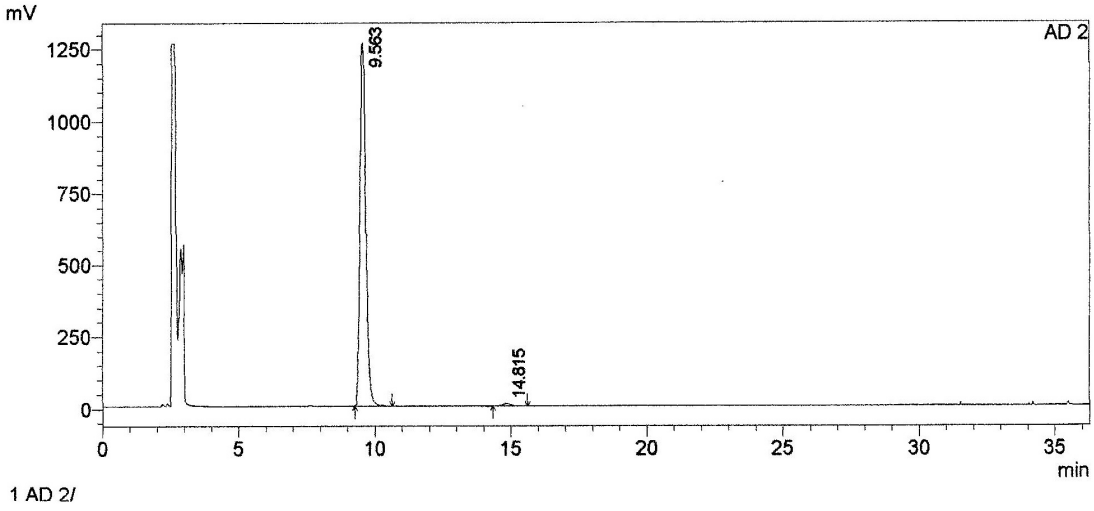


PeakTable

AD2 Ch1

Peak#	Ret. Time	Area	Height	Area %	Height %
1	9.563	18696619	1258375	98.932	99.222
2	14.815	201742	9868	1.068	0.778
Total		18898361	1268244	100.000	100.000

HPLC trace of compound **6** (method B)

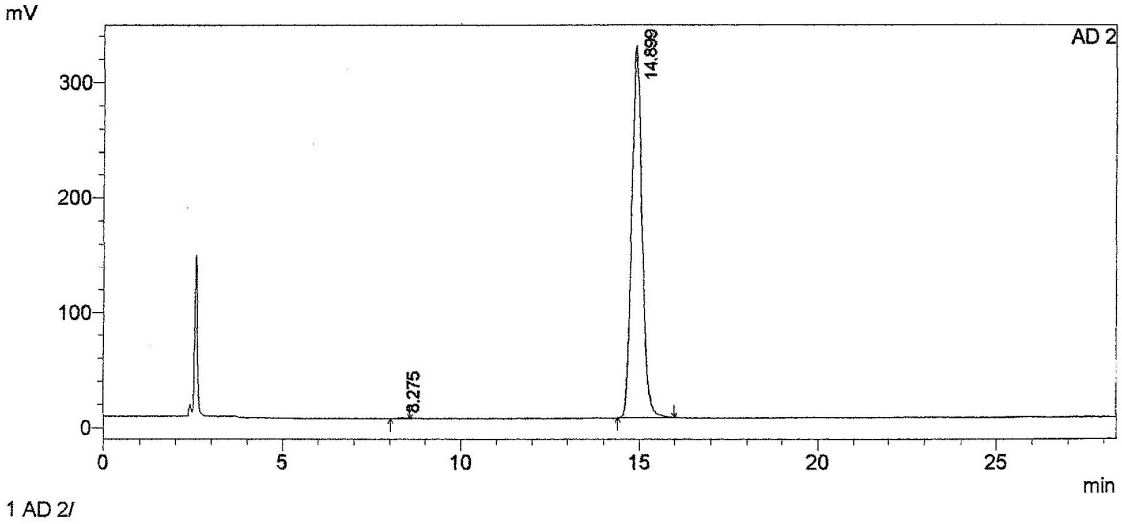


PeakTable

AD2 Ch1

Peak#	Ret. Time	Area	Height	Area %	Height %
1	9.563	18696619	1258375	98.932	99.222
2	14.815	201742	9868	1.068	0.778
Total		18898361	1268244	100.000	100.000

HPLC trace of compound **7** (method A)

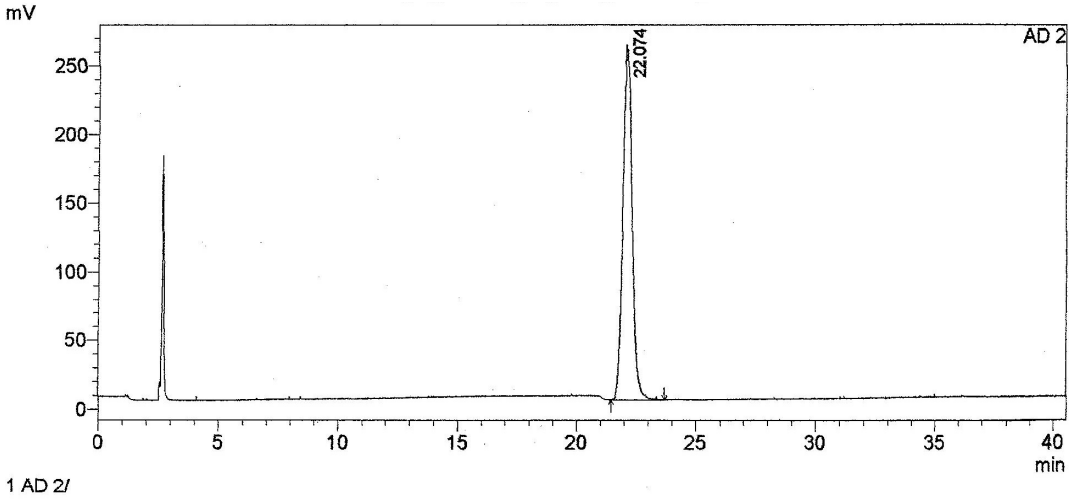


PeakTable

AD2 Ch1

Peak#	Ret. Time	Area	Height	Area %	Height %
1	8.275	14683	934	0.226	0.289
2	14.899	6487906	322420	99.774	99.711
Total		6502590	323354	100.000	100.000

HPLC trace of compound **7** (method B)



PeakTable

AD2 Ch1

Peak#	Ret. Time	Area	Height	Area %	Height %
1	22.074	7069647	257426	100.000	100.000
Total		7069647	257426	100.000	100.000

II. Biology

IIA. TGR5 FRET assay

The receptor activation was assessed by measuring the level of cyclic AMP (cAMP) using FRET assay. Human intestinal cell lines (NCI-H716) were plated in 96-well plates coated with 0.75 mg/ml Matrigel (BD Biosciences) according to manufacturer's instructions just prior to use, at a density of 12 x 10^3 cells/well in DMEM supplemented with 10% (v/v) FBS, 100 units/ml penicillin and 100 µg/mL streptomycin sulfate, and cultured for 24 h, which allowed cell adhesion to the bottom of the plate. The cells were washed twice with PBS and medium was exchanged for cAMP assay medium [OPTIMEM containing 0.1% (w/v) BSA and 1 mM 3-isobuty1-1-methylxanthine (IBMX)]. After incubation for 60 min. at 37 °C, the cells were treated with increasing concentrations of tested compound in stimulation Buffer (5 mM HEPES, 0,1% BSA in HBSS pH 7.4) containing the europium chelate – Streptavidin and the ALEXA Fluor 647-conjugated antibody anti-cAMP (PerkinElmer) for 1 h at room temperature. The level of intracellular cAMP was determined with Lance kit (PerkinElmer). Lithocholic acid was used as control ligand. Z' factor was used to validate assays. Non linear regression curves, without constraints, were performed by using four parameter equation and GraphPad Prism Software (GraphPad Inc.), to obtain the EC_{50} values.

IIB. FXR Alphascreen assay

Activity on FXR was assayed by using Alphascreen technology in a coactivator recruitment assay. Anti-GST-coated Acceptor beads were used to capture the GST-fusion FXR-LBD whereas the biotinylated-SRC-1 peptide was captured by the streptavidin Donor beads. Upon illumination at 680 nm chemical energy is transferred from Donor to Acceptor beads across the complex streptavidin-Donor/Src-1-Biotin/GSTFXR-LBD/Anti-GST-Acceptor and a signal is produced. The assay was performed in white, low-volume, 384-well Optiplates (PerkinElmer) using a final volume of 25 µL containing final concentrations of 10 nM of purified GST-tagged FXR-LBD protein, 30 nM biotinylated Src-1 peptide, 20 µg/ml anti-GST acceptor beads acceptor beads and 10 µg/ml of streptavidin donor bead (PerkinElmer). The assay buffer contained 50 mM Tris (pH 7.4), 50 mM KCl, 0.1% BSA, and 1 mM DTT. The stimulation times

with 1 μL of tested compound (solubilized in 100% DMSO) were fixed to 30 min. at room temperature. The concentration of DMSO in each well was maintained at a final concentration of 4%. After the addition of the detection mix (acceptor and donor beads) the plates were incubated in the dark for 4 h at room temperature and then were read in Envision microplate analyzer (PerkinElmer). Dose response curves were done in triplicate and Z' factor was used to validate the assays. Non linear regression curves, without constraints, were performed by using four parameter equation and GraphPad Prism Software (GraphPad Inc.), to obtain the EC_{50} values.

IIC. Physicochemical and biological properties

Avicholic acid analogues were admitted to a complete physico-chemical characterization following procedures as previously described elsewhere.[1] Water solubility of the protonated form was measured after equilibrium of a saturated water solution at 25°C keeping the pH below 3. After filtration the concentration of BA in the solution was measured by HPLC-ES-MS/MS.[2] The critical micellar concentration (CMC) was determined by surface tension (ST) measurements using a maximum bubble-pressure method. The surface tension values were plotted against the logarithm of the bile salt concentration; the regression lines corresponding to the two parts of the curve (monomeric and micellar phases) were calculated using the method of least squares, and the intersection of the lines was taken as the CMC value. The 1-Octanol/water partition coefficient ($logP_A$) was evaluated using a conventional shake-flask procedure.[3] The $logP_A$ values refer to the BA in the ionized form and the initial concentration of each BA was below its own CMC value. BA concentration in the water phase was measured by HPLC-ESI-MS/MS. The albumin binding was evaluated by equilibrium dialysis at a fixed BA-albumin ratio. BA concentrations of the dialyzed solution (corresponding to the free unbound fraction) and of the starting solution were determined with HPLC-ESI-MS/MS.

III. Molecular Modeling

All molecules were drawn with the sketch module of Cerius-2 (*Accelrys, Cerius-2*, version 4.6; Accelrys: San Diego, CA, 2001) and optimized using Universal force-field v.1.2 (Rappe, A. K.; Casewit, C. J.; Colwell, K. S.; Goddard, W. A.; Skiff, W. M. UFF, a full periodic table force field for molecular mechanics and molecular dynamics simulations. and the Smart Minimizer protocol of the Open Force Field module (OFF).[4] Acidic groups were considered in their protonated form and, as consequence, all molecules were endowed with a formal charge of 0. Atomic charges were calculated using the Gasteiger method,[5] as implemented in the polygraph version of Cerius-2. All the compounds of the study were aligned and analyzed according to the 3D-QSAR methodology reported in Macchiarulo et al. 2008.[6]

VI. References

1. a) Roda, A.; Pellicciari. R.; Cerrè. C.; Polimeni, C.; Sadeghpour, B. M.; Marinozzi, M.; Forti, G. C.; Sapigni, E. New 6-substituted bile acids: physico-chemical and biological properties of 6 alpha-methyl ursodeoxycholic acid and 6 alpha-methyl-7-epicholic acid. *J. Lipid Res*. **1994**, *35*, 2268-2279. b) Roda, A.; Hofmann, A. F.; Mysels, K. J. The influence of bile salt structure on self-association in aqueous solutions. *J. Biol Chem.* **1983**, *258*, 6362-6370.
2. Roda, A.; Gioacchini, A. M.; Cerrè, C.; Baraldini, M. High-performance liquid chromatographic-electrospray mass spectrometric analysis of bile acids in biological fluids. *J. Chromatogr. B Biomed. Appl.* **1995**, *665*, 281-294.
3. Roda, A.; Minutello, A.; Angellotti, M. A.; Fini, A. Bile acid structure-activity relationship: evaluation of bile acid lipophilicity using 1-octanol/water partition coefficient and reverse phase HPLC. *J. Lipid Res.* **1990**, *8*,1433-1443.
4. Kappe, A. K.; Casewit, C. J.; Colwell, K. S.; Goddard III, W. A.; Skiff, W. M. UFF, a full periodic table force field for molecular mechanics and molecular dynamics simulations. *J. Am. Chem. Soc.* **1992,** *114,* 10024-10035.
5. Gasteiger, J.; Marsili, M. Iterative partial equalization of orbital electronegativity - A rapid access to atomic charges. *Tetrahedron* **1980,** *36,* 3219-3228.
6. Macchiarulo, A.; Gioiello, A.; Thomas, C.; Massarotti, A.; Nuti, R.; Rosatelli, E.; Sabbatini, P.; Schoonjans, K.; Auwerx, J.; Pellicciari, R. Molecular field analysis and 3D-quantitative structure-activity relationship study (MFA 3D-QSAR) unveil novel features of bile acid recognition at TGR5. *J. Chem. Inf. Model* **2008**, *48*, 1792-1801.