STEROIDS 72 (2007) 756–764

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/steroids

Preparation and characterization of some keto-bile acid azines

Valerio Bertolasi[a], *Olga Bortolini*[b], *Giancarlo Fantin*[a], *Marco Fogagnolo*[a,*], *Daniela Perrone*[c]

[a] *Dipartimento di Chimica, Università di Ferrara, Via Borsari 46, 44100 Ferrara, Italy*
[b] *Dipartimento di Chimica, Università della Calabria, Via Bucci 12 C, 87036 Rende CS, Italy*
[c] *Dipartimento di Biologia ed Evoluzione, Università di Ferrara, C.so Ercole I d'Este, 32, 44100 Ferrara, Italy*

ARTICLE INFO

Article history:
Received 26 February 2007
Received in revised form
22 June 2007
Accepted 26 June 2007
Published on line 6 July 2007

Keywords:
Steroids
Dimeric bile acids
Homo- and heteroazines
Synthesis

ABSTRACT

New acyclic dimers of ketocholanic acids with hydrazine were obtained. Crystal structure was determined for the 3,7-dihydroxy-12-ketocholanic acid azine. Some distinctive ^{1}H NMR signals are assigned for the entire set of azines.

1. Introduction

Bile acids are versatile building blocks that have become increasingly important in a number of fields, such as pharmacology, biomimetic and supramolecular chemistry [1]. A vast amount of interesting medical applications based on these derivatives have been reported as well [2]. The synthesis of specific steroidal dimers and oligomers has become of interest because of their possible application as catalysts for some types of reactions and as pharmacologically active derivatives, in particular in fungal infections [3]. A pointed out by Li and Dias [4], initially the synthesis of dimeric derivatives with various inter-steroid linkage occurred as by-product formation. Only in recent years these compounds were produced by design [5]. Of the dimeric bile acids those derived by ring A–ring A connection are the most abundant, whereas ring C–ring C connections are no so frequent [4,6]. For connection and numbering see Fig. 1.

In 1996 we reported the preparation of a ring C–ring C connected 12-azine, starting from dehydrocholic acid (3,7,12-triketo-5β-cholan-24-oic acid), upon reduction and treatment with NH_2NH_2 [7]. In the present paper we have modified and extended our synthetic strategy to the preparation of new acyclic homo and hetero dimers with various ring connections utilizing the bile acids 3α-hydroxy-7-keto-5β-cholan-24-oic acid **1**, 3α,12α-dihydroxy-7-keto-5β-cholan-24-oic acid **2**, 3α-hydroxy-12-keto-5β-cholan-24-oic acid **3** and 3α,7α-dihydroxy-12-keto-5β-cholan-24-oic acid **4** as building blocks. The X-ray structure of the homo ring C–ring C connected azine, derived from bile acid **4**, is also reported and discussed.

* *Corresponding author*. Fax: +39 0532 240709.
E-mail address: fgr@unife.it (M. Fogagnolo).
0039-128X/$ – see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.steroids.2007.06.005

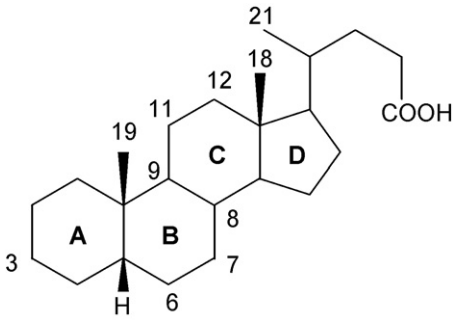
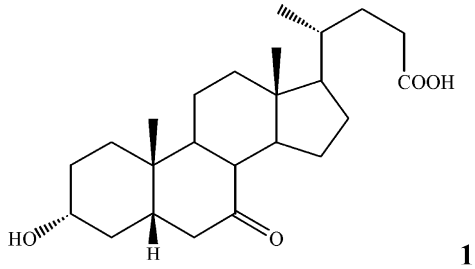
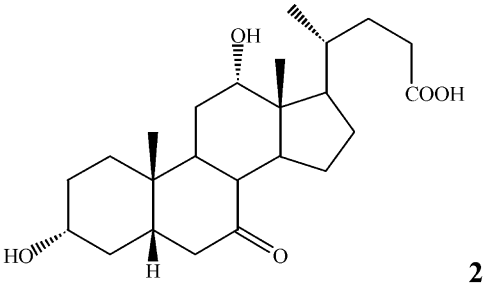


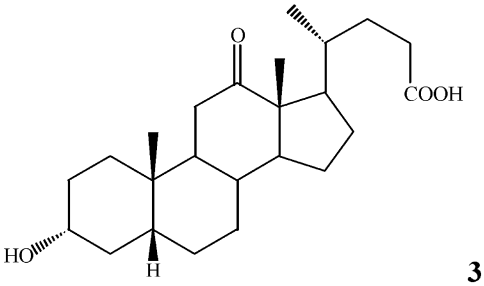
Fig. 1 – Bile acid steroid skeleton.



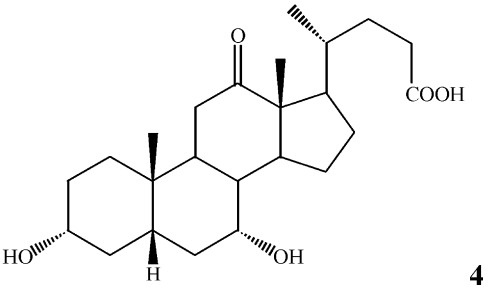
1



2



3



4

2. Experimental

Melting points are uncorrected and were determined on a 510 Bücki Meltig Point instrument. TLC were performed on precoated Silica Gel plates (thickness 0.25 mm, Merck) and Silica Gel (Fluka, Kiesegel 60, 70–230 mesh) was used for preparative column chromatography. The ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ or CD_3OD solutions, in 5 mm tubes, at room temperature, with a Varian Gemini 300 spectrometer operating at 300 MHz (^{1}H) and 75 MHz (^{13}C), with TMS as external standard. Mass spectra were run on MICROMASS ZMD2000 instrument operating in electrospray ionization. X-ray structure were collected at room temperature, 295 K, on a Nonius Kappa CCD diffractometer with graphite monochromated Mo Kα radiation (λ = 0.7107 Å).

2.1. *Preparation of bile acids 1–4*

2.1.1. *3α-Hydroxy-7-keto-5β-cholan-24-oic acid* **1**

The compound was prepared according to a literature procedure [8]. M.p. 208–211 °C; ^{1}H NMR (CD_3OD): δ 0.73 (s, 3H, H-18); 0.98 (d, *J* = 6.2 Hz, 3H, H-21); 1.28 (s, 3H, H-19); 2.60 (dd, *J* = 10.9 Hz, 1H, H-8β); 3.03 (dd, *J* = 12.1; 4.8 Hz, 1H, H-6β); 3.58 (br, 1H, H-3β).

2.1.2. *3α,12α-Dihydroxy-7-keto-5β-cholan-24-oic acid* **2**

The compound was prepared according to a literature procedure [9]. The product was purified by column chromatography on silica gel (ethyl acetate/ acetic acid 50:1): m.p. 195–198 °C; ^{1}H NMR (CD_3OD): δ 0.77 (s, 3H, H-18); 1.06 (d, *J* = 6.2 Hz, 3H, H-21); 1.26 (s, 3H, H-19); 2.60 (dd, *J* = 12 Hz, 1H, H-8β); 3.02 (dd, *J* = 13; 6.6 Hz, 1H, H-6β); 3.58 (br, 1H, H-3β); 4.04 (s, 1H, H-12β).

2.1.3. *3α-Hydroxy-12-keto-5β-cholan-24-oic acid* **3**

To a r.t. stirred solution of methyl deoxycholate (2.5 g, 6.1 mmol) and triethylamine (1.28 mL, 9.2 mmol) in CH_2Cl_2 (40 mL) was added drop by drop a solution of pivaloyl chloride (1.13 mL, 9.2 mmol) in CH_2Cl_2 (20 mL). After 24 h the reaction mixture was treated with aqueous solution of $NaHCO_3$ (30 mL). The organic phase was separated, dried on anhydrous Na_2SO_4, concentrated and the expected derivative (**6**), purified by column chromatography (SiO_2, cyclohexane/ ethyl ether 75/25), was obtained as sirup in 73% yield (2.2 g). ^{1}H NMR ($CDCl_3$): δ 0.71 (s, 3H, 18-H3); 0.94 (s, 3H, 19-H3); 1.0 (d, *J* = 6.1 Hz, 3H, 21-H3), 1.2 (s, 9H, COOt-Bu); 3.67 (s, 3H, COOMe); 4.0 (s, 1H, H-12β); 4.70 (br, 1H, H-3β).

A stirred solution of pivaloyl derivative (2 g, 4.1 mmol) in acetone at 0 °C (40 mL) was treated with the Jones' reagent until a slight permanent orange color was obtained. After 10 min at r.t. the reaction mixture was quenched with 2-propanol (0.5 mL), filtered over celite, concentrated, treated with an aqueous solution of $NaHCO_3$ and extracted with ethyl acetate. After removal of the solvent in a vacuum, the residue was dissolved in dioxane (20 mL), treated with aqueous NaOH 15% (10 mL) and refluxed for 6 h. Most of the organic solvent was evaporated, the residue was cooled in an ice-bath and acidified with HCl 30%. The expected product **3** was obtained as white solid (1.3 g, yield 82%); m.p. 165–167 °C; ^{1}H NMR (CD_3OD): δ 0.8 (d, *J* = 6.2 Hz, 3H, H-21); 1.10 (s, 3H, H-18); 1.12 (s, 3H, H-19); 2.62 (dd, *J* = 12.2 Hz, 1H, H-11β); 3.59 (br, 1H, H-3β).

2.1.4. *3α,7α-Dihydroxy-12-keto-5β-cholan-24-oic acid* **4**

The compound was prepared from methyl 3α,7α-diacethoxy-12-hydroxycholanate via oxidation with the Jones' reagent

according to a literature procedure [10] followed by saponification as described for compound **3**. The expected product was obtained as white solid (1.1 g, 87%); m.p. 219–221 °C; ^{1}H NMR (CD_3OD): δ 0.90 (d, J = 6.3 Hz, 3H, H-21); 1.10 (s, 3H, H-18); 1.13 (s, 3H, H-19); 2.62 (dd, J = 13.3, 1H, H-11β); 3.42 (br, 1H, H-3β); 3.94 (brs, 1H, H-7β).

2.2. *General procedure for the synthesis of the azines*

2.6 mmol of the appropriate ketocholanic acid **1–4** (or 1.3 mmol of each one of the two different bile acids for the preparation of heteroazines) were dissolved in 5 mL of glacial acetic acid, under gentile heating. Hydrazine hydrate (13 mmols, 80%) was slowly added to the mixture. The reaction was left under stirring at room temperature for 24 h, poured in a flask containing 30 g of water and ice crushed. The resulting crude white solid was filtered, washed with distilled water and dried in air. The pure azines were obtained by recrystallization or by column chromatography.

2.2.1. *Homoazine (connection 7-7)* **7**

Ninety-one percent yield, crystallized from ethanol, m.p. 163–170 °C with dec. ^{1}H NMR ($CDCl_3$): δ 0.77 (s, 6H, H-18); 1.01 (d, J = 6.3 Hz, 6H, H-21); 1.14 (s, 6H, H-19); 3.04 (d, J = 12.6 Hz, 2H, H-6α); 3.50 (br, 2H, H-3β). Selected ^{13}C NMR resonances (CD_3OD): δ 178.20 (24-C); 167.89 (7-C).

2.2.2. *Homoazine (connection 7-7)* **8**

Ninety-three percent yield, crystallized from ethanol, m.p. 223–230 °C with dec; ^{1}H NMR (CD_3OD): δ 0.79 (s, 6H, H-18); 1.05 (d, J = 6.6 Hz, 6H, H-21); 1.13 (s, 6H, H-19); 2.98 (dd, J = 12.6; 1.4 Hz, 2H, H-6α); 3.50 (br, 2H, H-3β); 4.02 (brs, 2H, H-12β). Selected ^{13}C NMR resonances (CD_3OD): δ 178.32 (24-C); 166.91 (7-C).

2.2.3. *Heteroazine (connection 7-7)* **9**

Forty-one percent yield, column chromatography (SiO_2, ethyl acetate/cyclohexane/acetic acid 30:20:1); m.p. 228–233 °C dec. ^{1}H NMR (CD_3OD): δ 0.76 (s, 3H, H-18); 0.78 (s, 3H, H-18); 1.0 (d, J = 6.4 Hz, 3H, H-21); 1.06 (d, J = 6.3 Hz, 3H, H-21); 1.12 (s, 3H, H-19); 1.14 (s, 3H, H-19); 3,02 (d, J = 12.8 Hz, 2H, H-6α); 3.50 (br, 2H, H-3β); 4.03 (brs, 1H, H-12β). Selected ^{13}C NMR resonances (CD_3OD): δ 178.48 (24-C); 178.41 (24-C); 167.73 (7-C); 167.41 (7-C).

2.2.4. *Homoazine (connection 12-12)* **10**

Eighty-six percent yield, column chromatography (SiO_2, ethyl acetate/cyclohexane/acetic acid 20:30:1), m.p. 135–140 °C dec. ^{1}H NMR (CD_3OD): δ 1.02 (m, 12H); 1.06 (s, 6H); 3.56 (br, 2H, H-3β); 3.69 (dd, J = 12; 3,5 Hz, 2H, H-11α). Selected ^{13}C NMR resonances (CD_3OD): δ 178,21 (24-C); 175,43 (12-C). ESI-MS: $[M+H]^+$ m/z 777.6.

2.2.5. *Homoazine (connection 12-12)* **11**

Ninety-six percent yield, crystallized from methanol, m.p. 240–245 °C dec. ^{1}H NMR (CD_3OD): δ 0.97–1.03 (m, 18H); 3.34 (br, 2H, H-3β); 3.63 (dd, J = 9.2; 3,6 Hz, 2H, H-11α); 3.83 (brs, 2H, H-7β). Selected ^{13}C NMR resonances (CD_3OD): δ 178.34 (24-C); 174.35 (12-C). ESI-MS: $[M+H]^+$ m/z 809.6.

2.2.6. *Heteroazine (connection 12-12)* **12**

Thirty-seven percent yield, column chromatography (SiO_2, ethyl acetate/cyclohexane/acetic acid 30:20:1), m.p. 195–202 °C with dec ^{1}H NMR (CD_3OD): δ 0.99–1.07 (m, 18H); 3.4 (br, 1H, H-3β); 3.51 (br, 1H, H-3β); 3.71 (dd, J = 12 3.5 Hz, 1H, H-11α); 3.80 (dd, J = 12.2 3.5 Hz, 1H, H-11α), 3.88 (brs, 1H, H-7β). Selected ^{13}C NMR resonances (CD_3OD): δ 178.33 (24-C); 175.31 (12-C); 175.83 (12-C).

2.2.7. *Heteroazine (connection 7-12)* **13**

Forty-five percent yield, column chromatography (SiO_2, ethyl acetate/cyclohexane/acetic acid 40:10:1), m.p. 228–235 °C dec.; ^{1}H NMR (CD_3OD): δ 0.74 (s, 3H, H-18); 1.0 (m, 9H, H-18, H-19, H-21); 1.08 (d, J = 6.3 Hz, 3H, H-21); 1.15 (s, 3H, H-19); 2.85 (d, J = 12.7 Hz, 1H, H-6α); 2.92 (dd, J = 12.3; 3.5 Hz, 1H, H-11α); 3.39 (br, 1H, H-3β); 3.55 (br, 1H, H-3β); 3.88 (brs, 1H, H-7β). Selected ^{13}C NMR resonances (CD_3OD): δ 178.54; 178.46 (24-C); 170.53 (12-C); 165.99 (7-C). ESI-MS: $[M+H]^+$ m/z 793.7.

2.2.8. *Heteroazine (connection 7-12)* **14**

Forty-seven percent yield, column chromatography (SiO_2, ethyl acetate/cyclohexane/acetic acid 30:20:1), m.p. 205–210 °C dec. ^{1}H NMR (CD_3OD): δ 0.75 (s, 3H); 0.97–1.07 (m, 12H); 1.1 (s, 3H); 2.79 (d, J = 10.1 Hz, 1H, H-6α or 11α); 2.82 (dd, J = 9.6; 2.6 Hz, 1H, H-11α or 6α); 2.52 (br, 2H, H-3β); 4,01 (brs, 1H, H-12β). Selected ^{13}C NMR resonances (CD_3OD): δ 178.44 (24-C); 170.53 (12-C); 165.38 (7-C).

2.2.9. *Heteroazine (connection 7-12)* **15**

Fifty-seven percent yield, column chromatography (SiO_2, ethyl acetate/cyclohexane/acetic acid 20:30:1), mp. 176–180 °C with dec. ^{1}H NMR (CD_3OD): δ 0.76 (s, 3H, H-18); 0.96–1.06 (m, 15H); 2.81 (m, 2H, H-6α, H-11α); 3.54 (br, 2H, H-3β). Selected ^{13}C NMR resonances (CD_3OD): δ 178.30 (24-C); 178.35 (24-C); 170.60 (12-C); 165.76 (7-C).

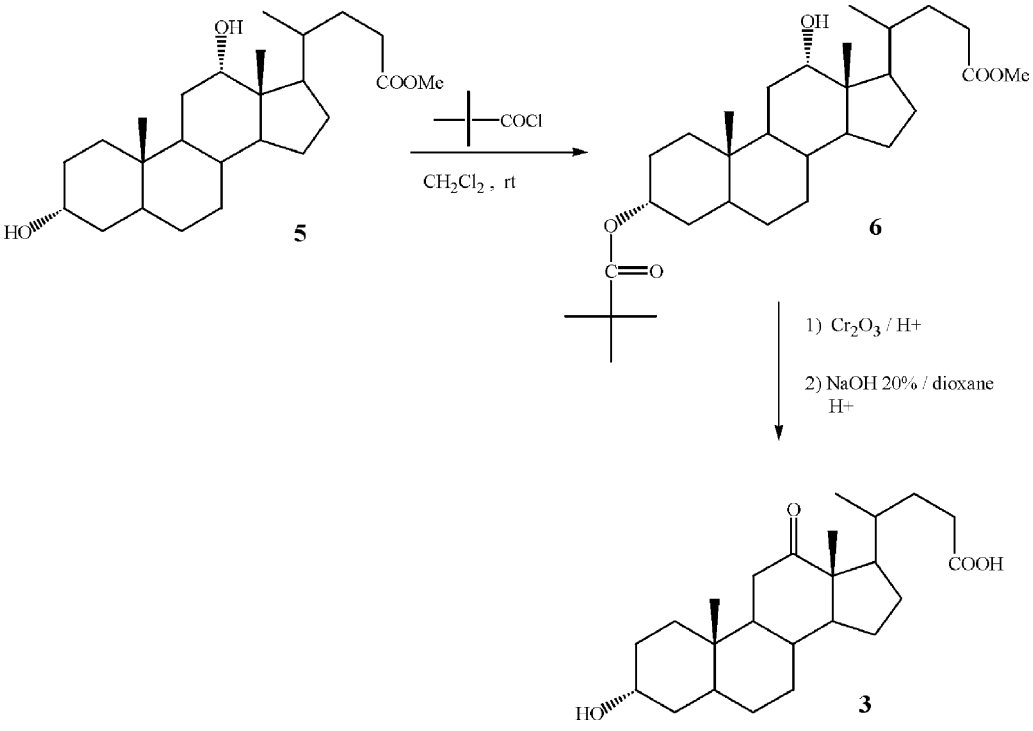
2.2.10. *Heteroazine (connection 7-12)* **16**

Thirty-eight percent yield, column chromatography (SiO_2, ethyl acetate/cyclohexane/acetic acid 25:25:1), m.p. 201–206 °C with dec. ^{1}H NMR (CD_3OD):δ 0.78 (s, 3H, H-18); 0.97–1.1 (m, 12H); 1.15 (s, 3H, H-19); 2.88 (dd, J = 13; 1.5 Hz, 1H, H-6α or 11α); 2.94 (dd, J = 13.5; 3.6 Hz, 1H, H-11α or 6α); 3.39 (br, 1H, H-3β); 3.55 (br, 1H, H-3β); 3.87 (brs, 1H, H-7β); 4.03 (brs, 1H, H-12β). Selected ^{13}C NMR resonances (CD_3OD): δ 178.50 (24-C); 170.48 (12-C); 165.63 (7-C).

3. Results and discussion

3.1. *Chemistry*

The keto-bile acid precursors **1** and **2** were easily prepared from the corresponding commercial available chenodeoxycholic and cholic acids by selective oxidation of the hydroxyl group at position C(7) with *N*-bromosuccinimide [8,9]. The keto derivative **3** was prepared in good yield from methyl deoxycholate **5** following an unprecedented reported procedure, consisting in the selective protection of the hydroxyl group at C(3) with pivaloyl chloride, followed by Jones' oxidation and saponification, according to Scheme 1.



Scheme 1

The keto-bile acid **4** was prepared from methyl 3α,7α-diacethoxy-12-hydroxycholanate via oxidation with the Jones' reagent, followed by saponification [10].

With the four precursors in our hands we have undertaken the synthesis of the different azines shown in Fig. 2. The term homo refers to the azines obtained starting from the same keto-bile acids, while the term hetero is used for azines derived from two different bile acid precursors.

The synthetic procedure is general, simple and straightforward. The appropriate ketocholanic acid **1**–**4** was dissolved in glacial acetic acid and the hydrazine hydrate was slowly added to the mixture. The reaction mixture was left under stirring at room temperature for 24 h, followed by the usual work-up, Eq. (1).

$$\mathbf{1-4} + NH_2\text{–}NH_2 \xrightarrow[\text{r.t.,24 h}]{\text{AcOH}} \text{Azine} \quad (1)$$

For the preparation of heteroazines equimolar amounts of two different bile acid building blocks are used. Heteroazine **9**, for example, is obtained by reacting a 1:1 mixture of bile acids **1** and **2** with hydrazine hydrate in AcOH, Eq. (2), **12** was prepared from a mixture of **3** and **4**, **13** by reacting **1** and **4**, **14** from a mixture of **2** and **3**, **15** from **1** and **3** and **16** was prepared from a mixture of **2** and **4**. The obvious consequence is that the homoazines **7**, **8**, **10**, **11**, derived from an unique ketocholanic acid precursor, are obtained in nearly quantitative yield (86–96%), Eq. (1), whereas for heterozines **9**, **12**, **13**–**16**, derived from two different ketocholanic acid precursors, the yield is lower (37–57%), since the concomitant formation of the related homoazines are observed (TLC) as by-products, but not quantified, Eq. (2). After work-up, only the heteroazine of interest was isolated.

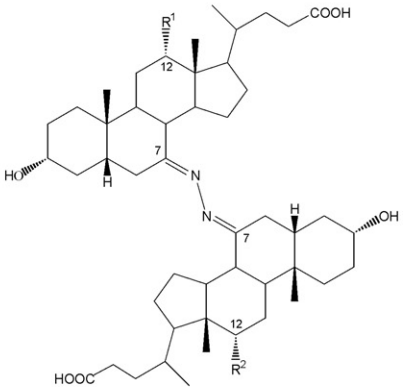
$$\underbrace{\mathbf{1+2}}_{\text{equimolar amounts}} + NH_2\text{–}NH_2 \xrightarrow[\text{r.t.,24 h}]{\text{AcOH}} \mathbf{9} + \underbrace{\mathbf{7+8}}_{\text{by product homoazines}} \quad (2)$$

3.2. *NMR spectra*

As pointed out in several publications the ^{1}H NMR spectra of bile acids are typified in two major zones, e.g. the methylene-methine envelope centred in the 0.5–2.5 ppm region and the portion of the spectra between 3 and 5 ppm [11]. Oxo-hydroxy bile acids exhibited additional and specific signals in the range 2.5–3.6 ppm, likely due to the presence of the carbonyl groups on selected adjacent protons [12]. A similar deshielding effect induced by the C=N groups of the azine may be found in the ^{1}H NMR spectra of compounds **7**–**16**, on the protons closest to this group [6]. Table 1 reports the related resonances multiplicities and coupling constants.

For the azines **10**–**12**, C(12)-C(12) connected, the specific resonances have to be assigned to one of the two protons at C(11), either equatorial (α) or axial (β). The multiplicity of the signal, a doublet of doublet, is characterized by two *J* values: the first one in the range of 9.2–12 Hz (geminal coupling) and the second one of about 3.5 Hz, attributable to a vicinal coupling to the axial proton at C(9) position. This considerations unequivocally attribute the specific resonances to the equatorial C(11) protons. Similar arguments may be used to assign to the equatorial C(11) protons the specific resonances, collected in Table 1, for compounds **13**–**16**. On the other hand, the distinctive resonances found for the azines **7**–**9** and **13**–**16** are assigned to the equatorial C(6) protons. Further support to this attribution may be found in independent experiments carried

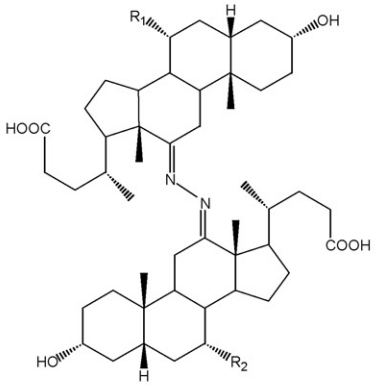
Azines via ring B-ring B connection



Homo azine — **7** $R^1 = R^2 = H$; **8** $R^1 = R^2 = OH$

Hetero azine — **9** $R^1 = OH$; $R^2 = H$

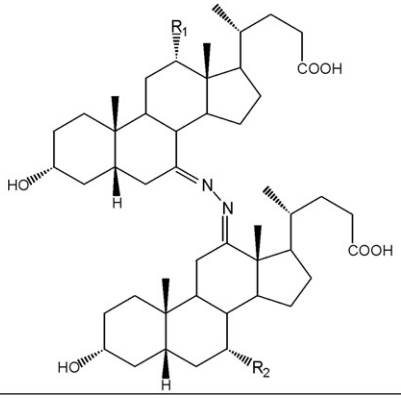
Azines via ring C-ring C connection



Homo azine — **10** $R^1 = R^2 = H$; **11** $R^1 = R^2 = OH$

Hetero azine — **12** $R^1 = OH$; $R^2 = H$

Azines via ring B-ring C connection



Hetero azine — **13** $R^1 = H$; $R^2 = OH$; **14** $R^1 = OH$; $R^2 = H$; **15** $R^1 = H$; $R^2 = H$; **16** $R^1 = R^2 = OH$

Fig. 2 – Structure of the homo- and heteroazines of this study.

out on cholanoic acid **2** and on the corresponding azine **8**. As shown in Scheme 2 and described in Section 2, bile acid **2** is characterized, among others, by two NMR resonances at δ 2.60 and 3.02, respectively. Treatment of this compound with basic deuterated water leads to the disappearance of the signal at 3.02 ppm, attributable to axial C(6)-hydrogen [12]. Exchange of the conformationally forced hydrogen at carbon C(8), in fact, is prevented. The deuterated azine **8bis**, prepared starting from the deuterated cholanoic acid **2bis**, showing the absence of the resonance at 2.98 ppm, clearly assigned this resonance to C(6)-hydrogens.

3.3. *X-ray structure determination*

For the azine **11** the formation of crystals has permitted the X-ray structure of this compound. X-ray diffraction data for compound **11** were collected at room temperature, 295 K, on a Nonius Kappa CCD diffractometer with

Table 1 – ^{1}H NMR chemical shifts, multiplicities and coupling constants of α-protons adjacent to the C=N groups of 7–16

	Diazine									
	7-7 connection			12-12 connection			7-12 connection			
	7	8	9	10	11	12	13	14	15	16
Proton	6[a]	6[a]	6[a]	11[b]	11[b]	11β	6β	6β[c]	6.11	6β[c]
δ (ppm)	3.04	2.98	3.02	3.69	3.63	3.71	2.85	2.79	2.81	2.88
Multip.	d	dd	d	dd	dd	dd	d	d	m	dd
J (Hz)	12.6	12.6	12.8	12.0	9.2	12.0	12.7	10.1	–	13.0
J (Hz)		1.4		3.5	3.6	3.5				1.5
Proton						11α	11β	11β[c]		11β[c]
δ (ppm)						3.80	2.92	2.82		2.94
Multip.						dd	dd	dd		dd
J (Hz)						12.2	12.3	9.6		13.5
J (Hz)						4.4	3.5	2.6		3.6

For C(6) and C(11) positions α-proton is equivalent to equatorial proton while β-proton is equivalent to axial proton.

[a] Assigned to both equatorial protons at carbons C(6) of the first and second cholanic framework forming the azine.

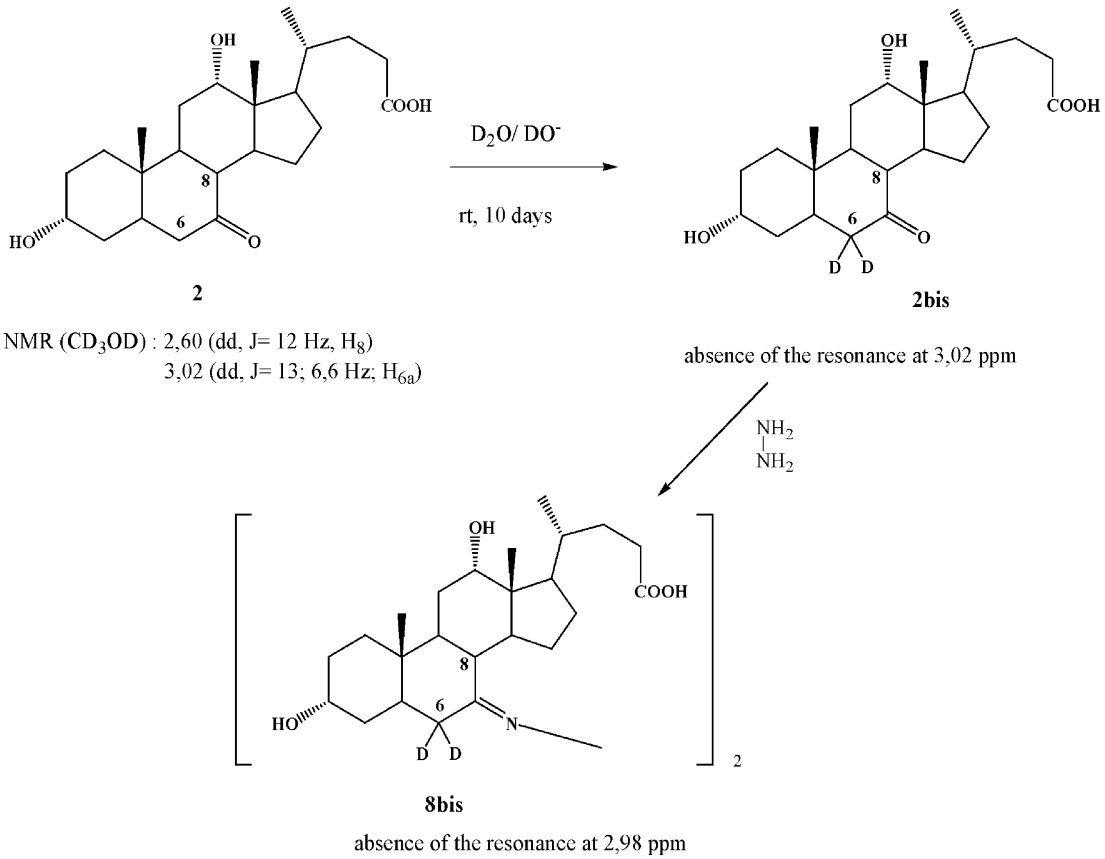
[b] Assigned to both equatorial protons at carbons C(11) of the first and second cholanic framework forming the azine.

[c] The assignment along the column could be reversed.

graphite monochromated Mo Kα radiation (λ = 0.7107 Å). The structure was solved by direct methods (SIR97) [13] and refined (SHELXL-97) [14] by full matrix least squares with anisoptropic non-H atoms and hydrogen atoms included on calculated positions riding on their carrier atoms, except for some OH hydrogens which were refined isotropically. The hydrogen positions of the water molecules could not be determined. All other calculations have been performed using PARST [15] and PLATON [16] systems of programs.

3.3.1. *Crystal data*

11, $C_{48}H_{76}N_2O_8{\cdot}2H_2O$; monoclinic, space group $P2_1$, $a = 12.9669(2)$, $b = 18.6740(3)$, $c = 12.2846(3)$ Å, $\beta = 118.8423(7)^\circ$, $V = 2404.69(8)$ Å^3, $Z = 2$, $Dc = 1.167$ g cm^{-3}. Intensity data collected with $\theta \leq 27.5^\circ$; 10,508 independent reflections mea-



Scheme 2

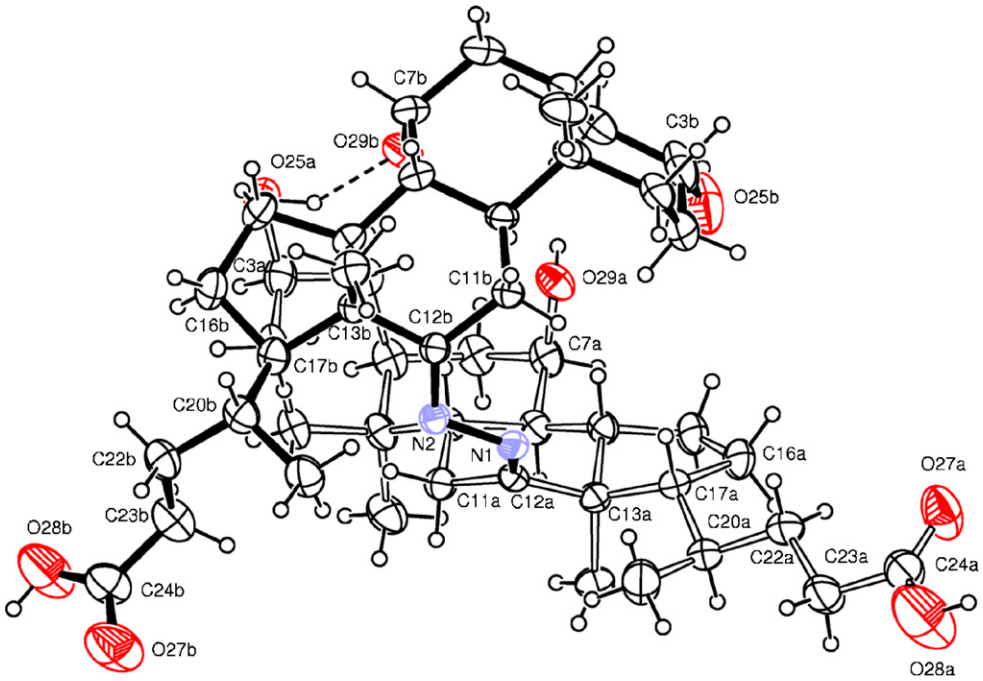


Fig. 3 – ORTEP view and atom numbering of compound 11 displaying the thermal ellipsoids at 30% probability.

sured; 8681 observed [$I > 2\sigma(I)$]. Final R index = 0.0653 (observed reflections), Rw (all reflections) = 0.1924 and S = 1.057.

Complete crystallographic data (excluding structural factors) have been deposited with the Cambridge Crystallographic Data Centre and allocated the deposition number CCDC 637989. These data can be obtained, free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html or on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk].

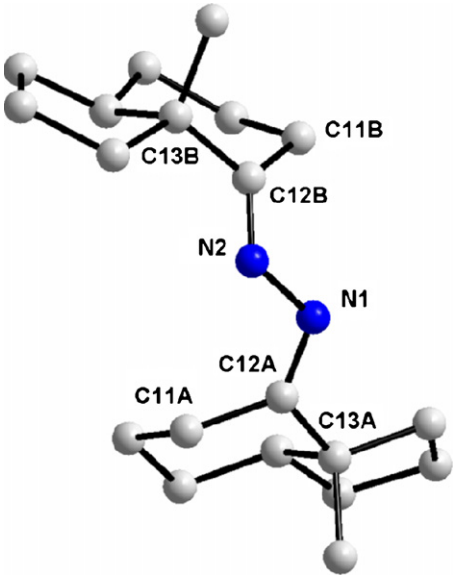


Fig. 4 – Molecular fragment of compound 11 showing the *E* configuration of C12A=N1 and C12B=N2 double bonds, and the *trans* conformation around the N1–N2 single bond.

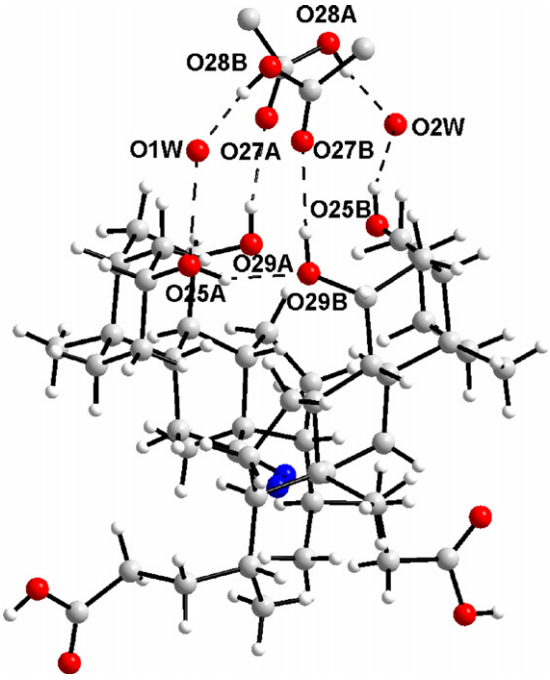


Fig. 5 – The hydrogen bond system involved in the formation of the hydrophilic channels.

ORTEP [17] view of compound **11** is given in Fig. 3. The molecular fragment in Fig. 4 shows the configurations and the conformation determined by the azino linkage. The cholic acid moieties, A and B, forming the molecular dimer, exhibit two different carboxylic side-chain conformations: full extended, *t t t t*, for A and folded *t g t g* for B, Table 2 [18].

Table 2 – Selected torsion angles (°) for compound 11

C13A-C17A-C20A-C22A: ψ_1	179.2(3)	*t*	C13B-C17B-C20B-C22B: ψ_1	−179.1(3)	*t*
C17A-C20A-C22A-C23A: ψ_2	−176.8(4)	*t*	C17B-C20B-C22B-C23B: ψ_2	64.7(4)	*g*
C20A-C22A-C23A-C24A: ψ_3	174.5(4)	*t*	C20B-C22B-C23B-C24B: ψ_3	155.5(4)	*t*
C22A-C23A-C24A-O28A: ψ_4	160.5(5)	*t*	C22B-C23B-C24B-O28B: ψ_4	95.8(5)	*g*
C12A-N1-N2-C12B	−137.1(3)	*t*			
C11A-C12A-N1-N2	3.2(4)		C11B-C12B-N2-N1	3.2(4)	
C13A-C12A-N1-N2	170.8(3)	*E*	C13B-C12B-N2-N1	170.9(3)	*E*

Carboxylic side-chain conformations; configurations and conformation around the azino linkage.

Table 3 – Hydrogen bond parameters (Å, °) for compound 11

D–H...A	Symm. Op.	D–H	H...A	D...A	D–H...A
O25A-H25A...O29B	x, y, z	1.01(5)	1.90(5)	2.822(5)	151(4)
O29A-H29A...O27A	−x, 1/2 + y, 1 − z	0.81(3)	2.08(4)	2.812(4)	150(4)
O28A-H28A...O2w	1 − x, y − 1/2, 1 − z	0.82[a]	1.81	2.621(8)	172
O25B-H25B...O2w	x − 1, y, z	0.82[a]	2.07	2.589	120
O29B-H29B...O27B	x − 1, y, z − 1	0.89(4)	1.97(4)	2.860(5)	173(4)
O28B-H28B...O1w	2 − x, 1/2 + y, 2 − z	0.82[a]	1.83	2.614(3)	160
O1w-H...O25A	1 − x, y − 1/2, 1 − z			2.699(5)	

[a] Calculated hydrogens.

Both C12A=N1 and C12B=N2 double bonds display *E* configurations, while the conformation around the N1–N2 single bond can be classified *trans* owing to the C12A=N1–N2=C12B torsion angle value of −137.1(3)°. The overall structure of the dimer is stabilized by an intramolecular O25A-H...O29B hydrogen bond.

X-ray crystallographic studies have revealed that cholic acid frequently forms antiparallel layer structures consisting of alternate stacking of lipophilic and hydrophilic layers and that host cavities are formed in the lipophilic layers which include a large variety of guest molecules [19–23]. Conversely, in this dimeric cholic acid derivative, the hydrogen bonded

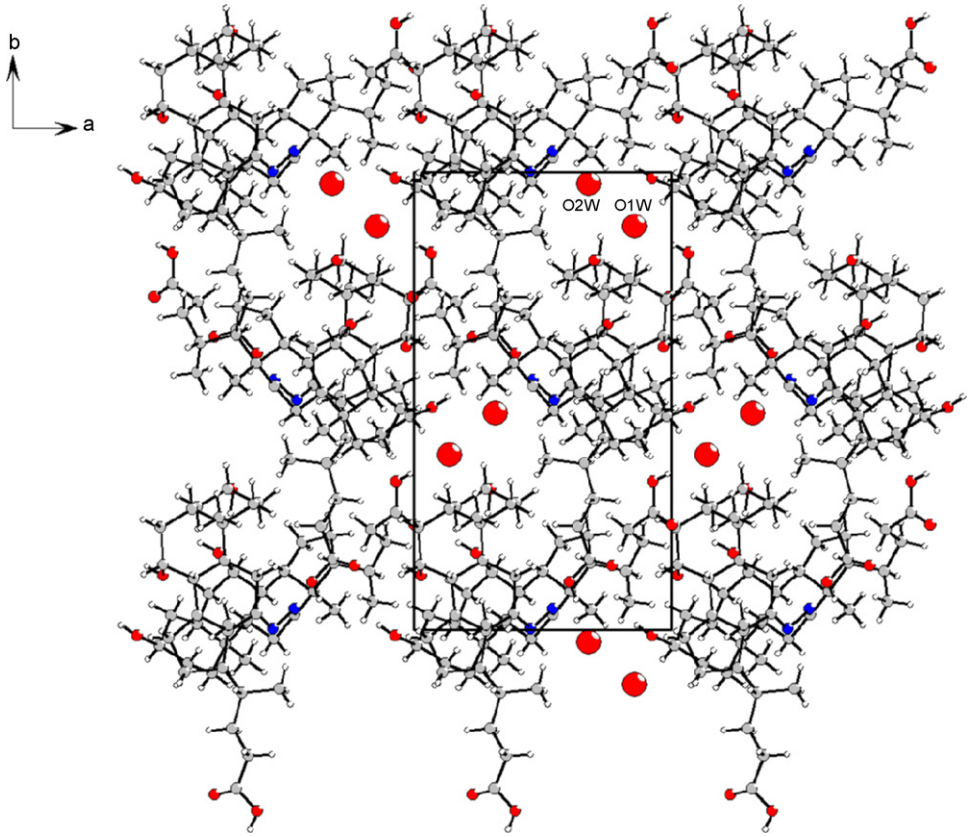


Fig. 6 – The unit cell of compound 11 as viewed down the crystallographic *c*-axis showing the channels containing water molecules.

multilayer arrangement of cholic acid host is absent. Instead of lipophilic layers, the structure exhibits hydrophilic channels built up by the hydroxyl groups, except O25A-H, of a cholic acid dimer and the carboxylic groups of other dimers, forming with water molecules a three-dimensional array of hydrogen bonds which stabilize the crystal lattice (Table 3; Fig. 5). In the crystal packing the hydrophilic channels, where the water molecules O1w and O2w are trapped, run along the crystallographic *c*-axis, as shown in Fig. 6. The formation of hydrophilic channels in crystal lattices of cholic acid (or cholic acid derivatives) inclusion complexes is uncommon. Only few examples of these crystals containing water and/or alcohol molecules are reported, for instance: deoxycholic acid with ethanol and water [24], cholic acid with water [25], cholic acid with methanol or ethanol or 1-propanol [26].

The observation that water molecules are trapped within the channels of crystals of compound **11** opens on the possibility to test azines in host–guest inclusion processes. Inclusion of molecules within others is a well known phenomenon observed both with achiral and chiral compounds, having important application in the separation of isomers and enantiomers [27]. In this frame we have carried out preliminary tests using azines as hosts, experiments that give evidence that derivative **8** is able to accommodate 2-methyl cyclohexanone within its channels, with a host to guest ratio of 1:1.

4. Conclusions

Novel bile acid moieties containing the structures presented in this paper have a great deal of potential in pharmacology, biomimetic and supramolecular chemistry. In addition bile acids are versatile building blocks for the design of frameworks capable of ionic and molecular recognition. Preliminary results on host–guest enclathration confirmed the capability of some azines to include organic molecules. Work is in progress in this field.

REFERENCES

[1] Virtanen E, Kolehmainen E. Use of bile acids in pharmacological and supramolecular applications. Eur J Org Chem 2004:3385–99, and references.

[2] Salunke DB, Hazra BG, Pore VS. Steroidal conjugates and their pharmacological applications. Curr Med Chem 2006;13:813–47.

[3] Salunke DB, Hazra BG, Pore VS, Manoj KB, Pallavi BN, Mukund VD. New steroidal dimers with antifungal and antiproliferative activity. J Med Chem 2004;47:1591–4.

[4] Li Y, Dias JR. Dimeric and oligomeric steroids. Chem Rev 1997;97:283–304.

[5] Lotowski Z, Guzmanski D. Ne acyclic dimmers of cholic acid with oxamide and hydrazide spacers. Monatsh Chem 2006;137:117–24.

[6] Gao H, Dias JR. Cyclocholates with 12-oxo and 7,12-oxo groups. Eur J Org Chem 1998:719–24.

[7] Bortolini O, Cova U, Fantin G, Medici A. A new non-enzymatic route to chenodeoxycholic acid. Chem Lett 1996:335.

[8] Hofmann AF. The preparation of chenodeoxycholic acid and its glycine and taurine conjugates. Acta Chem Scand 1963;17:173–86.

[9] Fieser LF, Rajagopalan S. Selective oxidation with *N*-bromosuccinimide. I. Cholic acid. J Am Chem Soc 1949;71:3935–8.

[10] Fieser LF, Rajagopalan S. Oxidation of steroids. III. Selective oxidations and acylations in the bile acid series. J Am Chem Soc 1950;72:5530–6.

[11] Brane S, Kirk DN. The bile acids, chemistry, physiology and metabolism, vol. 4. New York: Plenum; 1988.

[12] Bettarello L, Bortolini O, Fantin G, Guerrini A. Mixed oxo-hydroxo bile acids as actual or potential impurities in ursodeoxycholic acid preparation: a ^{1}H and ^{13}C NMR study. Il Farmaco 2000;55:51–5.

[13] Altomare A, Burla MC, Camalli M, Cascarano MG, Giacovazzo C, Guagliardi A, et al. *SIR97*: a new tool for crystal structure determination and refinement. J Appl Crystallogr 1999;32:115–6.

[14] Sheldrick GM. Program for refinement of crystal structures, SHELXL-97. Germany: University of Göttingen; 1996.

[15] Nardelli M. *PARST95*—an update to *PARST*: a system of Fortran routines for calculating molecular structure parameters from the results of crystal structure analyses. J Appl Crystallogr 1995;28:659.

[16] Spek AL. Single-crystal structure validation with the program *PLATON*. J Appl Crystallogr 2003;36:7–13.

[17] Burnett MN, Johnson CK. ORTEP-III, Report ORNL-6895. Oak Ridge National Laboratory, Oak Ridge, TN; 1996.

[18] Bertolasi V, Ferretti V, Pretto L, Fantin G, Fogagnolo M, Bortolini O. Hydrogen-bonded aggregations of oxo-cholic acids. Acta Crystallogr Sect B 2005;61:346–56.

[19] Nakano K, Sada K, Kurozumi Y, Miyata M. Importance of packing coefficient of host cavities in the isomerization of open host frameworks: guest-size-dependent isomerization in cholic acid inclusion crystals with monosubstituted benzenes. Chem Eur J 2001;7:209–20.

[20] Yoswathananont N, Sada K, Miyata M, Akita S, Nakano K. Dependence of selective enclathration on types of cholic acid crystals. Org Biomol Chem 2003;1:210–4.

[21] Nakano K, Mochizuki E, Yasui N, Morioka K, Yamauchi Y, Kanehisa N, et al. Mechanism of selective and unselective enclathration by a host compound possessing open, flexible host frameworks. Eur J Org Chem 2003:2428–36.

[22] Bertolasi V, Bortolini O, Fogagnolo M, Fantin G, Pedrini P. Enantioselective inclusion in bile acids: resolution of cyclic ketones. Tetrahedron: Asymm 2001;12:1479–83.

[23] Bertolasi V, Bortolini O, Fantin G, Fogagnolo M, Pretto L. Inclusion of cyclic carbonates by a cholic acid host: structure and enantioselection. Tetrahedron: Asymm 2006;17:308–12.

[24] Candeloro De Sanctis S, Coiro VM, Giglio E, Pagliuca S, Pavel NV, Quagliata C. The hexagonal phase of the 3:2:1 canal complex between Deoxycholic acid, ethanol and water: an inclusion compound with hydrophilic channels. Acta Crystallogr Sect B 1978;34:1928–33.

[25] Shibakami M, Tamura M, Sekiya A. Topological and nontopological rearrangement in crystal lattice of cholic acid inclusion complex induced by guest exchange. J Am Chem Soc 1995;117:4499–505.

[26] Jones EL, Nassimbeni LR. Crystal and molecular structures of the inclusion compounds of cholic acid with methanol, ethanol and 1-propanol. Acta Crystallogr Sect B 1990;46:399–405.

[27] Toda F, Bishpo R, editors. Separation and reactions in organic supramolecular chemistry: perspectives in supramolecular chemistry, vol. 8. Wiley; 2004. p. 123.