Synthesis of novel amide-linked dimers of lithocholic acid

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An efficient synthesis of lithocholic amides and amide-linked dimers under mild conditions is described. New *o*-, *m*- and *p*-phenylenediamine-derived dimers of lithocholic acid were synthesised by the activation of the carboxyl group of the bile acid as a mixed anhydride resulting from the reaction of the acid with ethyl chloroformate. The reduction of lithocholic amides afforded the corresponding 24-amino-5β-cholane derivatives. All compounds were characterised by IR, ^{1}H and ^{13}C NMR and mass spectra.

Keywords: steroids, bile acid, steroid dimers, lithocholic amides

Bile acid amides exhibit various interesting properties. Antibacterial[1,2], antifungal[1,3] and antiviral[4] activity of bile acid amides have been reported. Metal complexing properties of these compounds have also been well documented.[5-7] Among other compounds of natural origin like amino acids, peptides and carbohydrates, bile acid amides have been shown to act as gelators of aqueous fluids,[8] while alkyl derivatives of cholic acid amide[9,10] and bile acid amidoalcohols[11] have proved to be effective organogelators.[12]

The concept of linking two steroidal units to form a molecular cleft for molecular recognition was first described by McKenna *et al*.[13] In recent years, dimeric steroids – mainly derivatives of bile acids – have received growing attention.[14] The synthesis of head-to-head, head-to-tail and tail-to-tail dimers of bile acids has been reported.[14-16] Some of these dimers undergo macrocyclisation to cholaphanes,[14,16,17] which are potential hosts in supramolecular assemblies. For example, a piperazine linked dimer of lithocholic acid has been used as the substrate for preparation of a cholaphane showing complexing binding properties toward Ag^+.[7,18] Various steroid dimers are also active as cation-transporting *trans*-membrane channels.[19,20] The head-to-head amide-linked derivative of cholic acid has been reported as a host for the effective binding of carbohydrates.[21] Recently derivatives of cholic acid amide which behave as tripodal compounds have been shown to exhibit efficient gelation ability.[8] The synthesis of linear and cyclic dimeric cholapeptides derived from cholic acid and glycine,[22] ester-linked lithocholic acid dimers linking C-24 and C-3' carbon atoms and an oxalate diester linking position 3–3' have also been reported.[23] Whilst this work was in progress a paper by Hazra and co-workers was published reporting the synthesis of four steroidal amide-linked dimers showing antifungal and antiproliferative activity.[24] In the synthesis of these amides, the carboxyl group of cholic and deoxycholic acid was activated as *N*-succinimidyl esters.[3,24] Other activating agents for the carboxyl group utilised in the preparation of bile acid amides have also been reported. These include thionyl chloride,[18,25] dicyclohexylcarbodiimide,[8,26,27] diethylphosphoryl cyanide,[10,28] *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline,[4,29,30] *p*-nitrophenol[22,27,31,32] and *N,N*'-carbonyldiimidazole.[4]

The practical importance of bile acid amides and their possible use as substrates for the preparation of cholaphanes prompted us to examine the efficient synthesis of representative compounds. Our preliminary results showed that activation of the carboxyl group of lithocholic acid as a mixed anhydride formed upon reaction with ethyl chloroformate provided an effective method for synthesising bile acid amides. The chloroformate method has originally been used for the preparation of unsubstituted amides[33] and peptides.[34,35]

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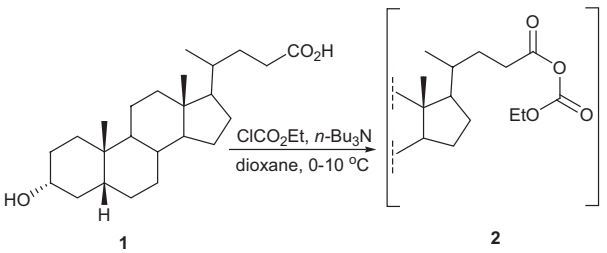
Amide and amine, derivatives of lithocholic acid have occasionally been reported.[1,2,36] However most were poorly characterised, mainly by melting point and microanalyses. Moreover, in some cases the bile acid amides were prepared via acid chlorides obtained under vigorous conditions by treating the acid with thionyl chloride.[37-40] We now present the synthesis of lithocholic acid amides and amide-linked dimers using activation of the carboxyl group with ethyl chloroformate under mild conditions. The method proved to be efficient, convenient and inexpensive.

Results and discussion

In preliminary experiments lithocholic and amides were prepared according to a procedure described by Chaplin *et al*.[40] Thus, 3α-acetoxy-5β-cholan-24-oic acid was treated with thionyl chloride and pyridine in anhydrous benzene to give the acid chloride which upon treatment with gaseous ammonia or an amine gave the respective amide in yields ranging from 50 to 70%. However, the preparation of the acid chloride was troublesome and isolation and purification of the crude amide required chromatography on a silica gel column. In the best experiment, 3α-acetoxy-5β-cholanamide, for example, was isolated in 78% yield. Therefore we turned our attention to the activation of the carboxyl group as a mixed anhydride under mild conditions.[1] Under these conditions protection of the 3-hydroxyl of lithocholic acid was not required. The reaction of lithocholic acid (**1**) with a two molar excess of ethyl chloroformate in dioxan in the presence of excess tri-*n*-butylamine gave mixed anhydride (**2**), which without isolation was treated with *n*-butylamine and benzylamine to give pure amides (**4**) and (**5**) in almost quantitative yield (Scheme 1). After crystallisation the amides were obtained in 80 and 75% yield, respectively.

The reaction of (**2**) with ammonium hydroxide (25%) in dioxan afforded amide (**3**)[42] in 90% yield. When the anhydride (**2**) was treated with 24-amino-5β-cholane-3α-ol (*vide infra*) a new type of dimeric steroid amide (**7**) was prepared. Under similar conditions the weakly nucleophilic aniline was effective in transforming (**2**) to the anilide (**6**),[37] which was isolated in 85% yield after crystallisation.

This result prompted us to synthesise a new type of symmetric dimers in which two lithocholic acid units were linked by an amide bond formed with participation of aromatic diamines. Thus, the reaction of the intermediate (**2**) with *o*-, *m*- and *p*-phenylenediamine afforded pure dimers (**9**), (**11**) and (**13**) in 95, 90 and 95% yield, respectively. The dimeric amides may exist in solution as *syn* and *anti* conformers, depending on the polarity of the solvent. The *syn* geometry of the synthesised dimers should allow for the preparation of macrocyclic cholaphanes characterised by cavities of variable size.



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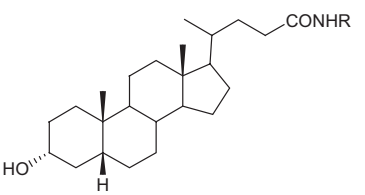


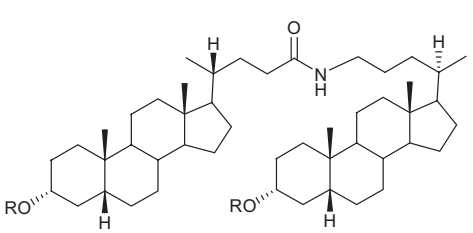
3 R = H,
4 R = *n*-Bu
5 R = $CH_2C_6H_5$
6 R = C_6H_5

Scheme 1

The effectiveness of the activation of the carboxyl group by the formation of the mixed anhydride under the conditions described above is illustrated in the synthesis of dimer (**17**), a dicholanoyl derivative of piperazine, which we prepared as a pure compound in 86% yield and which did not require chromatography for purification.[7] Under similar conditions, the acylation of ethylenediamine with anhydride (**2**) afforded an ethylene-linked amide dimer (**15**) in 84% yield. Previously, amide (**17**) had been prepared in 56% yield via the acid chloride in a reaction requiring heating with the amine at 90°C for 90 h[18] or in another preparation in 51% yield.[7]

3-Hydroxy-5β-cholan-24-amide dimers were high-melting solids, mostly insoluble in nonpolar solvents, such as chloroform or benzene. Moreover, dimers (**13**) and (**15**) were not soluble even in dimethylsulfoxide-d_6 and pyridine-d_5, which are usually effective for amide dissolution. In order to improve the solubility of the amides (**9**), (**11**), (**13**), (**15**) and (**17**), acetylation was carried out under standard conditions (acetic anhydride in pyridine at room temperature) which afforded the corresponding 3,3'-bis-acetates (**10**), (**12**), (**14**), (**16**) and (**18**).

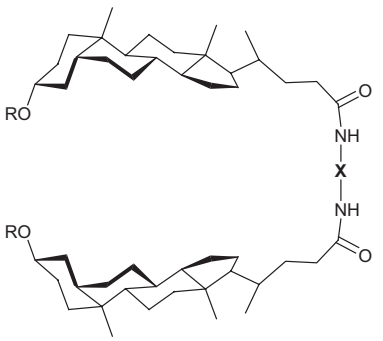
Reduction of the amides (**3**–**5**) to the respective amines with lithium aluminium hydride required tetrahydrofuran at reflux. Amines (**19**), (**20**) and (**21**) were isolated in 50, 60 and



7 R = H

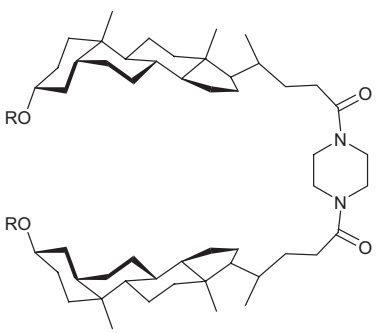
8 R = Ac

Scheme 2



9 R = H, X = *o*-phenylene
10 R = Ac, X = *o*-phenylene
11 R = H, X = *m*-phenylene
12 R = Ac, X = *m*-phenylene
13 R = H, X = *p*-phenylene
14 R = Ac, X = *p*-phenylene
15 R = H, X = ethylene
16 R = Ac, X = ethylene

Scheme 3

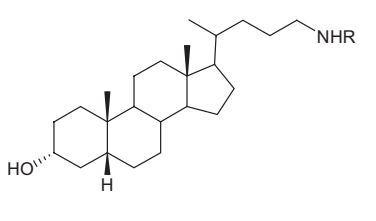


17 R = H
18 R = Ac

Scheme 4

96% yield. The reduction of anilide (**6**) was best performed with the aid of the diborane-dimethyl sulfide complex to give amine (**22**).

The synthesised amides and amines were characterised by IR, ^{1}H and ^{13}C NMR and mass spectra (see Experimental). The complete assignment of ^{13}C NMR spectra of amides and amines as well as amide-linked dimers is presented in Table 1 and Table 2, respectively. In a recent review, ^{13}C NMR spectra of bile acids and their derivatives have been analysed and summarised.[41] As for lithocholic acid, only the data for 3-*O*-substituted carboxylic acids and esters were presented. Therefore our spectral data for lithocholic amides and amide-linked dimers provide a supplement to that study.



19 R = H,
20 R = *n*-Bu
21 R = $CH_2C_6H_5$
22 R = C_6H_5

Scheme 5

In summary, our work demonstrates the effectiveness of the activation of the carboxylic group of lithocholic acid as a mixed anhydride with the inexpensive and readily available ethyl chloroformate allowing preparation of bile acid amides under mild conditions and with excellent yields. This method proved effective in the one-step synthesis of a new type of lithocholic amide-linked dimers, derived from isomeric phenylenediamines. This is a general method allowing the preparation of diamides, derivatives of aromatic and aliphatic primary and secondary diamines. The synthesised derivatives of lithocholic acid were fully characterised by spectroscopic methods. The lithocholic acid dimers are excellent substrates for the preparation of new lithiocholaphanes which is the subject of our further investigations.

Experimental

General

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. IR spectra were determined with an FT-IR Bruker FS 113V spectrophotometer for solutions in chloroform or as KBr pellets. ^{1}H and ^{13}C NMR spectra were recorded with a Varian Gemini 300 VT spectrometer (300 and 75.5 MHz, respectively) operating in the Fourier transform mode using solutions in $CDCl_3/CD_3OD$ (4:1) or pyridine-d_5. Chemical shifts (δ) are expressed in ppm relative to tetramethylsilane as the internal standard. The DEPT technique was used for the assignment of multiplicity of carbon signals in ^{13}C NMR spectra. Additivity rules and comparison with data[41] reported for compounds of similar structure were helpful for signal assignment. Electron impact (ionisation energy of 70 eV) and FAB mass spectra were recorded using an AMD 402 or AMD 604 spectrometer. Electrospray mass spectra (ESI MS) experiments were performed using a Waters/Micromass ZQ ES mass spectrometer with methanol solvent system. Solvents were dried and distilled according to the standard procedures. Reaction progress and purity of compounds was monitored by TLC using precoated aluminum-backed silica plates (E. Merck, no. 5554). Silica gel 60 (Merck 70-230 mesh, no. 7734) was used for flash chromatography.

Preparation of lithocholic amides and amide-linked dimers; general procedure

Freshly distilled tri-*n*-butylamine (1.2 ml, 5 mmole) was added to a solution of lithocholic acid (**1**) (188 mg, 0.5 mmole) in anhydrous dioxane (15 ml) and the solution was stirred at 10°C for 10 min. Ethyl chloroformate (48 µl, 0.5 mmole) was added with cooling and stirring continued for 10 min. at 10°C. Subsequently a solution of an amine was added dropwise and the mixture was stirred for 24 h at room temperature. Water (20 ml) was added and the product was extracted with ethyl acetate or chloroform. The organic layer was washed with water, dilute HCl, sodium hydrogen carbonate (5%) and water and dried over magnesium sulfate. Filtration and evaporation of the solvent under reduced pressure gave the crude amide which was crystallised.

3α-Hydroxy-5β-cholan-24-amide (**3**):[1] Solution of NH_4OH (25%) was used (0.65 mmole), 98% yield of the crude amide, 90% yield after crystallisation. M.p. 214–216°C ($CHCl_3$), lit.[1] m.p. 208–210°C ($MeOH/H_2O$). ^{1}H NMR ($CDCl_3$): δ = 5.36 (bs, 2 H, NH_2), 3.62 (m, 1 H, 3β-H), 0.92 (d, J = 6.3 Hz, 3 H, 21-CH_3), 0.91 (s, 3 H, 19-CH_3), 0.64 (s, 3 H, 18-CH_3). IR (KBr pellets) ν_{max}: 3456, 3390, 3195, 1669, 1625 cm^{-1}. MS (EI) *m/z*: 375 $[M]^+$, 357 $[M\text{-}H_2O]^+$. MS (ESI) *m/z*: 773 $[2M + Na]^+$, 751 $[2M + H]^+$, 398 $[M + Na]^+$, 376 $[M + H]^+$, 358 $[M + H\text{-}H_2O]^+$.

N-n-Butyl-3α-hydroxy-5β-cholan-24-amide (**4**): Solution of *n*-butylamine (1M) was used (0.65 mmole), 98% yield of the crude amide (pure on TLC), 80% yield after crystallisation. M.p. 200–202°C (AcOEt). ^{1}H NMR ($CDCl_3$): δ = 5.38 (bs, 1 H, NH), 3.62 (m, 1 H, 3β-H), 3.25 (dd, J_1 = 12.9 Hz, J_2 = 7.1 Hz, 2 H, $NHCH_2$), 2.41 (t, J = 7.1 Hz, 2 H, 23-CH_2), 0.93 (d, J = 7.1 Hz, 3 H, 21-CH_3), 0.91 (s, 3 H, 19-CH_3), 0.63 (s, 3H, 18-CH_3). IR ($CHCl_3$) ν_{max}: 3607, 3450, 1660, 1518 cm^{-1}. MS (EI) *m/z*: 431 $[M]^+$, 374 $[M\text{-}C_4H_9]^+$. *Anal*. Calcd. for $C_{28}H_{49}NO_2$: C, 77.9; H, 11.4; N, 3.2. Found: C, 77.25; H, 11.5; N, 3.1%.

N-Benzyl-3α-hydroxy-5β-cholan-24-amide (**5**):[1] Benzylamine (0.65 mmole)/water (1 : 10) was used, 98% yield of the crude amide (pure on TLC), 75% yield after crystallisation. M.p. 197–198°C (AcOEt); lit.[1] m.p. 183–185°C ($AcOEt/Et_2O$). ^{1}H NMR ($CDCl_3$): δ = 7.33–7.27 (m, 5 H, ArH), 5.74 (s, 1 H, NH), 4.44 (d, J = 5.8 Hz, 2 H, $NHCH_2$), 3.62 (m, 1 H, 3β-H), 2.33–2.23 (m, 1 H, 23-CH_2), 2.15–2.05 (m, 1 H, 23-CH_2), 0.91 (d, J = 6.0 Hz, 3 H, 21-CH_3), 0.91 (s, 3 H, 19-

Table 1 ^{13}C NMR data of lithocholic amides (**3–6**) and 3α-hydroxy-5β-cholanyl-24-amine derivatives (**19–22**)

C	3[a]	4[a]	5[a]	6[a]	19[a]	20[a]	21[a]	22[b]
1	35.1	35.2	35.1	35.1	34.9	35.1	35.2	35.4
2	29.9	30.2	30.0	30.0	27.9	29.9	28.2	28.4
3	71.2	71.5	71.4	71.3	70.9	71.3	71.4	71.9
4	35.8	36.1	35.9	35.9	35.4	35.8	36.1	36.5
5	41.9	42.0	41.9	41.9	41.7	41.9	41.9	42.2
6	27.0	27.1	27.0	27.0	26.7	30.0	26.3	26.5
7	26.2	26.3	26.2	26.2	26.0	26.2	25.8	26.3
8	35.6	35.7	35.7	35.7	35.4	35.6	35.7	35.9
9	40.3	40.3	40.3	40.3	40.1	40.3	40.3	40.5
10	34.3	34.5	34.4	34.4	34.1	34.4	34.4	34.6
11	20.6	20.7	20.6	20.6	20.3	20.6	20.7	20.9
12	40.0	40.1	40.0	40.0	39.8	40.0	40.0	40.3
13	42.5	42.6	42.6	42.6	42.2	42.5	42.5	42.8
14	56.3	56.4	56.3	56.3	56.1	56.3	56.4	56.5
15	24.0	24.1	24.0	24.0	23.7	24.0	24.1	24.3
16	27.9	28.1	28.0	28.0	27.8	28.1	27.1	27.3
17	55.8	55.9	55.8	55.8	55.7	56.0	56.0	56.2
18	11.7	11.9	11.8	11.8	11.4	11.8	11.9	12.2
19	23.1	23.3	23.2	23.2	22.8	23.2	23.3	23.5
20	35.3	35.4	35.3	35.4	35.2	35.5	35.5	35.7
21	18.0	18.2	18.1	18.1	17.9	18.3	18.5	18.7
22	31.5	31.5[c]	31.7	31.6	29.5	30.4	30.2	30.6
23	32.4	33.5	33.3	34.1	32.5	33.1	33.3	33.4
24	177.6	174.0	174.2	172.9	41.2	49.3	49.3	44.6
1'		13.6	43.3			13.6	53.4	
2'		20.0				20.1		
3'		31.8[c]				29.5		
4'		39.0				48.5		
ipso			138.1	138.1			138.8	148.4
ortho			128.4[c]	128.7			128.4[c]	129.1
meta			127.5[c]	119.8			128.3[c]	112.6
para			127.2[c]	123.8			127.1[c]	117.0

[a]For solution in $CDCl_3/CD_3OD$ (4:1); [b]for solution in $CDCl_3$; [c]these signals may be interchanged.

Table 2 ^{13}C NMR data of amide-linked lithocholic acid dimers (**8**), (**9**), (**11**), (**14**) and (**16**)

C	**8**[a]	**9**[b]	**11**[b]	**14**[a]	**16**[a]
1	34.6	35.1	35.1	34.5	34.6
2	28.3 + 28.2[d]	29.9	30.0	28.3	28.2
3	74.4	71.3	71.3	74.4	74.4
4	35.0	35.8	35.9	35.0	35.1
5	41.9	41.8	41.9	41.8	42.0
6	26.6	27.0	27.0	26.6	26.7
7	26.3	26.3	26.2	26.3	26.3
8	35.8	35.6	35.6	35.8	35.9
9	40.4	40.2	40.3	40.4	40.5
10	34.6	34.4	34.4	34.5	35.6
11	20.8	20.6	20.6	20.8	20.8
12	40.2	40.0	40.0	40.1	40.2
13	42.73 + 42.69[d]	42.6	42.5	42.7	42.8
14	56.5	56.3	56.3	56.5	56.6
15	24.2	24.0	24.0	24.2	24.2
16	27.0	28.1	28.0	27.0	27.0
17	56.14 + 56.09[d]	55.8	55.8	56.1	56.1
18	12.0	11.8	11.8	12.1	12.1
19	23.3	23.2	23.2	23.3	23.3
20	35.49 + 35.44[d]	35.4	35.4	35.5	35.5
21	18.59 + 18.37[d]	18.1	18.1	18.4	18.4
22	31.9 + 32.2[d]	31.7	31.6	31.6	31.8
23	33.0 + 33.8[d]	33.8	34.0	32.2	32.3
24	173.4	173.6	173.1	171.8[c]	174.9
24'	39.9				
1'					40.2
ipso		130.4	138.6	134.2	
ortho		125.9[c]	111.0	120.5	
meta		125.2[c]	115.3		
ortho'			129.0		
$\underline{C}H_3COO$	21.4			21.5	21.4
CH_3COO	170.6			170.7[c]	170.5

[a]For solution in $CDCl_3$; [b]for solution in $CDCl_3/CD_3OD$ (4:1); [c]these signals may be interchanged; [d]for unsymmetrical dimer two signal were observed.

H_3), 0.63 (s, 3 H, 18-CH_3). IR (KBr pellets) ν_{max}: 3413, 3334, 3060, 3038, 1652 cm^{-1}. MS (ESI) *m/z*: 953 $[2M + Na]^+$, 931 $[2M + H]^+$, 488 $[M + Na]^+$, 466 $[M + H]^+$. *Anal*. Calcd. for $C_{31}H_{47}NO_2 \times H_2O$: C, 77.0; H, 10.2; N, 2.9. Found: C, 77.7; H, 10.5; N, 2.4%.

N-Phenyl-3α-hydroxy-5β-cholan-24-amide (6): Aniline (0.5 mmole) was used, 85% yield of the crude amide (pure on TLC). M.p. 225–227°C (AcOEt), 216–219°C ($CHCl_3$). ^1H NMR ($CDCl_3$): δ = 7.56–7.06 (m, 6 H, NH and ArH), 3.63 (m, 1 H, 3β-H), 2.47–2.29 (m, 1 H, 23-CH_2), 2.24–2.19 (m, 1 H, 23-CH_2), 0.96 (d, *J* = 6.3 Hz, 3 H, 21-CH_3), 0.92 (s, 3 H, 19-CH_3), 0.65 (s, 3 H, 18-CH_3). IR (KBr pellets) ν_{max}: 3433, 3307, 3198, 3137, 3059, 3021, 1664, 1600, 754, 693 cm^{-1}. MS (FAB) *m/z*: 962 $[2M + 2H_2O + Na]^+$, 939 $[2M + H + 2H_2O]^+$, 903 $[2M + Na]^+$, 488 $[M + H + 2H_2O]^+$, 452 $[M + H]^+$, 434 $[M + H\text{-}H_2O]^+$. HR MS: calcd. for $[M + H]^+$ $C_{30}H_{46}NO_2$: 452.35284; found 452.35219.

N-(3α-Hydroxy-5β-cholan-24-yl)-3α-hydroxy-5β-cholan-24-amide (**7**): 24-Amino-5β-cholan-3α-ol (0.62 mmole) was used, 98% yield of the crude amide (pure on TLC), which was purified by washing the precipitate with ethyl acetate to give product (7) in 68% yield. ^1H NMR (pyridine-d_5): δ = 8.49 (t, *J* = 5.5 Hz, 1 H, NH), 3.91 (m, 2 H, 3β- and 3'β-H), 3.56 (dd, J_1 = 12.8 Hz, J_2 = 6.0 Hz, 2 H, 24'-CH_2), 2.57–2.43 (m, 2 H, 23-CH_2), 0.95 and 0.98 (two d, *J* = 6.6 Hz, 6 H, 21- and 21'-CH_3), 0.92 and 0.91 (two s, 6 H, 19- and 19'-CH_3), 0.612 and 0.619 (two s, 6 H, 18- and 18'-CH_3). IR (KBr pellets) ν_{max}: 3298, 1645, 1553 cm^{-1}. MS (ESI) *m/z*: 742 $[M + Na]^+$, 720 $[M + H]^+$. HR MS (FAB): calcd. for $[M\text{-}OH]^+$ $C_{48}H_{80}NO_2$: 702.61890; found 702.62125.

N,N'-Bis(3α-hydroxy-5β-cholan-24-oyl)-o-phenylenediamine (**9**): *o*-Phenylenediamine (0.25 mmole)/dioxane (1:10) was used, 95% yield of the crude amide (pure on TLC). M.p. > 250°C (AcOEt). ^1H NMR ($CDCl_3$): δ = 7.98 (s, 2 H, NH), 7.41–7.34 and 7.28–7.25 (two m, 4 H, ArH), 3.62 (m, 2 H, 3β- and 3'β-H), 2.45–2.23 (m, 4 H, 23- and 23'-CH_2), 0.97 (d, *J* = 6.0 Hz, 6 H, 21- and 21'-CH_3), 0.92 (s, 6 H, 19- and 19'-CH_3), 0.66 (s, 6 H, 18- and 18'-CH_3). IR (KBr pellets) ν_{max}: 3291, 3129, 3064, 1668, 751 cm^{-1}. MS (ESI) *m/z*: 847 $[M + Na]^+$, 848 [M + Na + H], 186, 151. HR MS: calcd. for $[M + H]^+$ $C_{54}H_{85}N_2O_4$: 825.65094; found 825.65017.

N,N'-Bis(3α-hydroxy-5β-cholan-24-oyl)-m-phenylenediamine (**11**): *m*-Phenylenediamine (0.25 mmole)/dioxane (1:10) was used, 90% yield of the crude amide (pure on TLC). M.p. 152–153°C (AcOEt). ^1H NMR ($CDCl_3$): δ = 7.25–7.17 (6 H, NH and ArH), 3.62 (m, 2 H, 3β- and 3'β-H), 2.41–2.36 (m, 2 H, 23- and 23'-CH_2), 2.54–2.20 (m, 2 H, 23- and 23'-CH_2), 0.97 (d, *J* = 5.9 Hz, 6 H, 21- and 21'-CH_3), 0.91 (s, 6 H, 19- and 19'-CH_3), 0.65 (s, 6 H, 18- and 18'-CH_3). IR (KBr pellets) ν_{max}: 3308, 3081, 1668, 1610, 784 cm^{-1}. MS (FAB) *m/z*: 847 $[M + Na]^+$, 575, 488, 186, 154, 128. HR MS: calcd. for $[M + H]^+$ $C_{54}H_{85}N_2O_4$: 825.65094; found 825.65000.

N,N'-Bis(3α-hydroxy-5β-cholan-24-oyl)-p-phenylenediamine (**13**): *p*-Phenylenediamine (0.25 mmole)/dioxane (1:10) was used, 95% yield of the crude amide (pure on TLC). M.p. 185–188°C (CH_2Cl_2). ^1H NMR (pyridine-d_5): δ = 10.81 (s, 2 H, NH), 8.41 (s, 4 H, ArH), 3.90 (m, 2 H, 3β- and 3'β-H), 2.67–2.43 (m, 4 H, 23- and 23'-CH_2), 0.91 (d, *J* = 6.3 Hz, 6 H, 21- and 21'-CH_3), 0.90 (s, 6 H, 19- and 19'-CH_3), 0.56 (s, 6 H, 18- and 18'-CH_3). IR (KBr pellets) ν_{max}: 3447, 3305, 3073, 1668, 1610, 838 cm^{-1}. MS (ESI) *m/z*: 847 $[M + Na]^+$, 186, 182, 151. HR MS (FAB): calcd. for $[M + H]^+$ $C_{54}H_{85}N_2O_4$: 825.65094; found 825.64710.

N,N'-Bis(3α-hydroxy-5β-cholan-24-oyl)-ethylenediamine (**15**): Ethylenediamine (0.25 mmole) was used, 84% yield of the crude amide (pure on TLC). M.p. 202–205°C. IR (KBr pellets) ν_{max}: 3442, 1660, 1558 cm^{-1}. MS (FAB) *m/z*: 831 $[M + H + 3H_2O]^+$, 813 $[M + H + 2H_2O]^+$, 777 $[M + H]^+$, 740 $[M\text{–}2H_2O]^+$. Compound (15) was not soluble in $CDCl_3$, DMSO-d_6 and pyridine-d_5. HR MS: calcd. for $[M + H]^+$ $C_{50}H_{85}N_2O_4$: 777.65094; found 777.64824.

N,N'-Bis(3α-hydroxy-5β-cholan-24-oyl)-piperazine (**17**):[7,18] Piperazine (0.25 mmole)/dioxane (1:5) was used, 86% yield of the crude amide (pure on TLC). M.p. 120–123°C (AcOEt). ^1H NMR ($CDCl_3$): δ = 3.63–3.46 (m, 10 H, 3β- and 3'β-H and $N(CH_2)_2(CH_2)_2N$), 2.44–2.18 (m, 4 H, 23- and 23'-CH_2), 0.94 (d, *J* = 6.5 Hz, 6 H, 21- and 21'-CH_3), 0.91 (s, 6 H, 19- and 19'-CH_3), 0.64 (s, 6 H, 18- and 18'-CH_3). IR (KBr pellets) ν_{max}: 3385, 1634, 1601 cm^{-1}. MS (FAB) *m/z*: 826 $[M + Na]^+$, 803 $[M + H]^+$, 767 $[M\text{–}2H_2O]^+$. HR MS: calcd. for $[M + H]^+$ $C_{52}H_{87}N_2O_4$: 803.66656; found 803.66692.

Acetylation of lithocholic amides and amide dimers; general procedure

The lithocholic amides were acetylated under standard conditions in pyridine/acetic anhydride (10:1) mixture at room temperature for 24 h. The usual workup (extraction with benzene or addition of water and filtration of the precipitate) gave a crude product.

N-(3α-Acetoxy-5β-cholan-24-yl)-3α-acetoxy-5β-cholan-24-amide (8): Yield 72% of the crude compound (pure on TLC), oil. ^1H NMR ($CDCl_3$): δ = 5.35 (t, *J* = 5.5 Hz, 1 H, NH), 4.71 (m, 2 H, 3β- and

3'β-H), 3.21 (m, 2 H, 24'-CH_2), 2.03 (s, 6 H, two CH_3CO_2), 0.90 (d, J = 6.6 Hz, 6 H, 21- and 21'-CH_3), 0.92 (s, 6 H, 19- and 19'-CH_3), 0.64 and 0.63 (two s, 6 H, 18- and 18'-CH_3). IR (KBr pellets) ν_{max}: 3307, 1737, 1647, 1544 cm^{-1}. MS (ESI) *m/z*: 826 [M + Na]$^+$, 744 [M–CH_3COO^-]$^+$. HR MS (FAB): calcd. for [M + H]$^+$ $C_{52}H_{86}NO_5$: 804.65057; found 804.64244.

N,N'-Bis(3α-acetoxy-5β-cholan-24-oyl)-o-phenylenediamine (**10**): Yield 82% of the crude compound (pure on TLC). M.p. 156–158°C (AcOEt). ^{1}H NMR ($CDCl_3$): δ = 8.12 (s, 2 H, two NH), 7.41–7.35 and 7.24–7.15 (two m, 4 H, ArH), 4.72 (m, 2 H, 3β- and 3'β-H), 2.03 (s, 6 H, two CH_3CO_2), 0.98 (d, J = 6.0 Hz, 6 H, 21- and 21'-CH_3), 0.93 (s, 6 H, 19- and 19'-CH_3), 0.66 (s, 6 H, 18- and 18'-CH_3). IR (KBr pellets) ν_{max}: 3291, 3131, 3064, 1737, 1667, 753 cm^{-1}. MS (ESI) *m/z*: 931 [M + Na]$^+$, 573, 552, 496. Anal. Calcd. for $C_{58}H_{88}N_2O_6 \times 1.5$ H_2O: C, 74.4; H, 9.8; N, 3.0. Found: C, 74.3; H, 10.1; N, 2.8%.

N,N'-Bis(3α-acetoxy-5β-cholan-24-oyl)-m-phenylenediamine (**12**): Yield 64% of the crude compound (pure on TLC), oil. ^{1}H NMR ($CDCl_3$): δ = 7.84–7.18 (m, 6 H, two NH and ArH), 4.72 (m, 2 H, 3β- and 3'β-H), 2.03 (s, 6 H, two CH_3CO_2), 0.96 (d, J = 6.0 Hz, 6 H, 21- and 21'-CH_3), 0.92 (s, 6 H, 19- and 19'-CH_3), 0.64 (s, 6 H, 18- and 18'-CH_3). IR (KBr pellets) ν_{max}: 3317, 3152, 3086, 1736, 1667, 1609, 732 cm^{-1}. MS (ESI) *m/z*: 931 [M + Na]$^+$, 573, 552, 496. HR MS (FAB): calcd. for [M + H]$^+$ $C_{58}H_{89}N_2O_6$: 909.67206; found 909.67454.

N,N'-Bis(3α-acetoxy-5β-cholan-24-oyl)-p-phenylenediamine (**14**): Yield 78% of the crude compound (pure on TLC). M.p. 135–136°C (AcOEt). ^{1}H NMR ($CDCl_3$): δ = 7.46 (s, 4 H, ArH), 7.16 (s, 2 H, two NH), 4.72 (m, 2 H, 3β- and 3'β-H), 2.03 (s, 6 H, two CH_3CO_2), 0.96 (d, J = 6.0 Hz, 6 H, 21- and 21'-CH_3), 0.92 (s, 6 H, 19- and 19'-CH_3), 0.65 (s, 6 H, 18- and 18'-CH_3). IR (KBr pellets) ν_{max}: 3355, 3161, 3062, 1736, 1718, 1689, 1667, 1609, 838 cm^{-1}. MS (ESI) *m/z*: 931 [M + Na]$^+$, 573, 552, 496. HR MS (FAB): calcd. for [M + H]$^+$ $C_{58}H_{89}N_2O_6$: 909.67206; found 909.67475.

N,N'-Bis(3α-acetoxy-5β-cholan-24-oyl)-ethylenediamine (**16**): Yield 92% of the crude compound, chromatography on silica gel column, 76% yield. M.p. 185–186°C (AcOEt). ^{1}H NMR ($CDCl_3$): δ = 6.16 (s, 2 H, two NH), 4.71 (m, 2 H, 3β- and 3'β-H), 3.38 (s, 4 H, NCH_2CH_2N), 2.03 (s, 6 H, two CH_3CO_2), 0.92 (s, 6 H, 19- and 19'-CH_3), 0.64 (s, 6 H, 18- and 18'-CH_3). IR (KBr pellets) ν_{max}: 3323, 3275, 1739, 1652, 1640, 1550 cm^{-1}. MS (FAB) *m/z*: 883 [M + Na]$^+$, 861 [M + H]$^+$, 801 [M–CH_3COO-]$^+$, 741 [M–2CH_3COOH]$^+$. HR MS: calcd. for [M + H]$^+$ $C_{54}H_{89}N_2O_6$: 861.67206; found 861.67422.

N,N'-Bis(3α-acetoxy-5β-cholan-24-oyl)-piperazine (**18**): Yield 91% of the crude compound (pure on TLC). M.p. 203–205°C (AcOEt). ^{1}H NMR ($CDCl_3$): δ = 4.72 (m, 2 H, 3β- and 3'β-H), 3.70–3.46 (two bs, 8 H, N(CH_2)$_2$(CH_2)$_2$N), 2.44–2.18 (m, 4 H, 23- and 23'-CH_3), 2.03 (s, 6 H, two CH_3CO_2), 0.96 (d, J = 5.8 Hz, 6 H, 21- and 21'-CH_3), 0.92 (s, 6 H, 19- and 19'-CH_3), 0.64 (s, 6 H, 18- and 18'-CH_3). IR (KBr pellets) ν_{max}: 3446, 1734, 1650 cm^{-1}. MS (ESI) *m/z*: 909 [M + Na]$^+$, 887 [M + H]$^+$, 826 [M-CH_3COOH]$^+$. Anal. Calcd. for $C_{56}H_{90}N_2O_6 \times 1.5$ H_2O: C, 73.6; H, 10.25; N, 3.1. Found: C, 73.3; H, 10.6; N, 2.7%.

Preparation of amines (**19–21**); *general procedure*

Lithium aluminohydride (100 mg, 2.6 mmole) was added to a solution of an amide (0.3 mmole) in anhydrous tetrahydrofuran and the mixture was refluxed for 24 h. The excess reducing agent was decomposed by careful addition of ethyl acetate and a saturated solution of magnesium sulfate. The solution was dried over anhydrous magnesium sulfate and the solvent evaporated to give a crude product.

24-Amino-5β-cholan-3α-ol (**19**):[1] Yield 69% of the crude amine, chromatography on a silica gel column gave the amine in 50% yield. M.p. 145–148°C (EtOH), lit. [1] m.p. 148–150°C (MeOH). ^{1}H NMR ($CDCl_3$): δ = 3.57 (m, 1 H, 3β-H), 2.63 (m, 2 H, 24-CH_2), 0.91 (d, J = 6.6 Hz, 3 H, 21-CH_3), 0.91 (s, 3 H, 19-CH_3), 0.64 (s, 3 H, 18-CH_3). IR (KBr pellets) ν_{max}: 3362, 1561 cm^{-1}. MS (ESI) *m/z*: 362 [M + H]$^+$, 343 [M-H_2O]$^+$, 242, 133.

24-n-Butylamino-5β-cholan-3α-ol (**20**): Yield 98% of the crude amine, purified as the hydrochloride, 60% yield. M.p. 89–91°C (EtOH). ^{1}H NMR ($CDCl_3$): δ = 3.61 (m, 1 H, 3β-H), 2.51–2.62 (m, 4H, CH_2NHCH_2), 0.94 (d, J = 6.6 Hz, 3 H, 21-CH_3), 0.91 (s, 3 H, 19-CH_3), 0.63 (s, 3 H, 18-CH_3). IR (KBr pellets) ν_{max}: 3405, 1645 cm^{-1}. MS (ESI) *m/z*: 419[M + 2H], 418 [M + H]$^+$. Anal. Calcd. for $C_{28}H_{51}NO \times 1.5$ H_2O: C, 75.6; H, 12.2; N, 3.1. Found: C, 75.9; H, 12.4; N, 3.0%.

24-Benzylamino-5β-cholan-3α-ol (**21**):[1] Yield 98% of the crude compound (pure on TLC). M.p. 74–75°C (EtOH), lit.[1] m.p. 193–195°C. ^{1}H NMR ($CDCl_3$): δ = 7.41–7.23 (m, 5 H, ArH), 3.77 (s, 2H, NHCH_2ArH), 3.59 (m, 1 H, 3β-H), 2.61–2.53 (m, 2 H, 24-CH_2), 0.94 (d, J = 6.6 Hz, 3 H, 21-CH_3), 0.90 (s, 3 H, 19-CH_3), 0.62 (s, 3 H, 18-CH_3). IR (KBr pellets) ν_{max}: 3319, 3085, 3062, 3027, 1645, 754, 698 cm^{-1}. MS (ESI) *m/z*: 474 [M + Na]$^+$, 452 [M + H]$^+$.

24-Phenylamino-5β-cholan-3α-ol (**22**): A solution of diborane–dimethylsulfide complex (10M, 100 μl, 0.649 mmole) was added dropwise to a boiling solution of amide (6) (100 mg, 0.203 mmole) under argon in anhydrous tetrahydrofuran (3 ml) and the solution was refluxed for 1.5 h. The solution was acidified by the addition of methanolic hydrogen chloride and the solvent was evaporated. The residue was dissolved in benzene/methanol (1:1), the solution was washed with sodium carbonate (10%), brine and the solvent evaporated to give yellow oil (82 mg) which was chromatographed on a silica gel column to give (22) (56 mg, 63% yield).

^{1}H NMR ($CDCl_3$): δ = 7.16 (t, J = 7.4 Hz, 2 H, ArH), 6.67 (t, J = 7.3 Hz, 1 H, ArH), 6.59 (d, J = 7.7 Hz, 2 H, ArH), 3.66 (m, 1 H, 3β-H), 3.05 (m, 2 H, 24-CH_2), 0.92 (d, J = 6.8 Hz, 3 H, 21-CH_3), 0.91 (s, 3 H, 19-CH_3), 0.64 (s, 3 H, 18-CH_3). IR (KBr pellets) ν_{max}: 3315, 3083, 3050, 3019, 2932, 2862, 1603, 1506, 1465, 1447, 1373, 1365, 1321, 1260, 1176, 1069, 1040, 746, 691 cm^{-1}. MS (ESI) *m/z*: 439 [M + H]$^+$. Anal. Calcd. for $C_{30}H_{47}NO \times 0.5$ H_2O: C, 80.7; H, 10.8; N, 3.1. Found: C, 80.3; H, 10.6; N, 3.1%.

Financial support by the Ministry of Science and Higher Education (project No. 3 T09A 085 28) is gratefully acknowledged.

Received 23 February 2008; accepted 27 April 2008
Paper 08/5231 *doi: 10.3184/030823408X318325*

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