Bile Acid Derivatives as Ligands of the Farnesoid X Receptor. Synthesis, Evaluation, and Structure−Activity Relationship of a Series of Body and Side Chain Modified Analogues of Chenodeoxycholic Acid

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Received February 3, 2004

The farnesoid X receptor (FXR) is activated by endogenous bile acids (BAs) and plays a variety of physiological roles related to modulation of gene transcription. In particular, FXR positively regulates the cholesterol catabolism while feedback inhibits the BA synthesis by repressing the expression of the CYP7A and CYP8B genes. We have previously shown that 6α-ethyl-CDCA (6ECDCA) is a potent and selective FXR agonist. In this paper we report an extensive structure−activity relationship for a series of synthetic bile acids. Our results indicate that the 6α position plays a fundamental role in determining affinity and that the side chain of BA is amenable to a variety of chemical modification. Although none of the new derivatives is more potent than 6ECDCA, we show here that a wide variability in efficacy, from full agonists to partial antagonists, can be obtained.

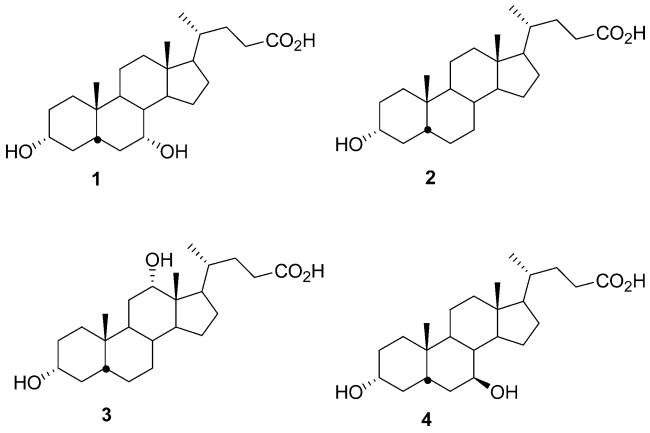
Introduction

The identification of the former orphan nuclear receptor farnesoid X receptor (FXR) as a bile acid (BA) receptor [1−3] has greatly increased the understanding of processes related to physiological control of BA homeostasis into the enterohepatic circulation. Indeed, FXR was shown to respond to physiological concentration of the primary BA chenodeoxycholic acid (CDCA, **1**, Chart 1), as well as to lithocholic acid (LCA, **2**) and deoxycholic acid (DCA, **3**), but not to ursodeoxycholic acid (UDCA, **4**) and to regulate the expression of several genes controlling the BA disposal through modulation of cholesterol metabolism and of the BA transport systems.[4,5]

Although many salient traits of the drawing still need to be painted, quite a clear picture has emerged for FXR as a BA sensor that feed-forward regulates the cholesterol catabolism and feedback inhibits the BA synthesis by repressing the expression of the CYP7A and CYP8B genes[6] through increase in the level of the nuclear receptor SHP-1[7,8] and by activating gene expression of the bile acid transporters I-BABP, BSEP, and MAOT.[9]

The intimate role of FXR in the transcriptional control of genes regulating cholesterol and BA biosynthesis and disposal makes it a particularly attractive target for the discovery of drugs for the treatment of cholestasis[10] or hyperlipedimia.[11] There is therefore pressure for the development of potent, specific, and pharmacokinetically suitable ligands to be employed in the validation of the viability of FXR as a therapeutic target.

Chart 1. Naturally Occurring BAs



A major breakthrough toward a more precise understanding of the FXR pathophysiological role was the discovery, facilitated by the availability of cell-free ligand sensing assays and reporter gene assays, of selective, highly potent agonists. Thus, exploitation of an isoxazole-based combinatorial library allowed GSK to discover GW4064 (**5**, Chart 2) as a nanomolar nonsteroidal activator of FXR.[12]

In a different approach, rational modification of 6α-methyl-CDCA (**6**), identified as an FXR ligand from the survey of a small libraries of synthetic BA, allowed us to develop 6α-ethyl-CDCA (6ECDCA, **7**), the most potent steroidal FXR agonist so far reported.[10] 6ECDCA (**7**) turned out to be very efficacious in recruiting the SRC-1 peptide in the cell-free assay, was significantly active in reverting cholestasis in an in vivo rat model, and was successfully employed in the determination of the first crystal structure of the holo conformation of FXR.[13]

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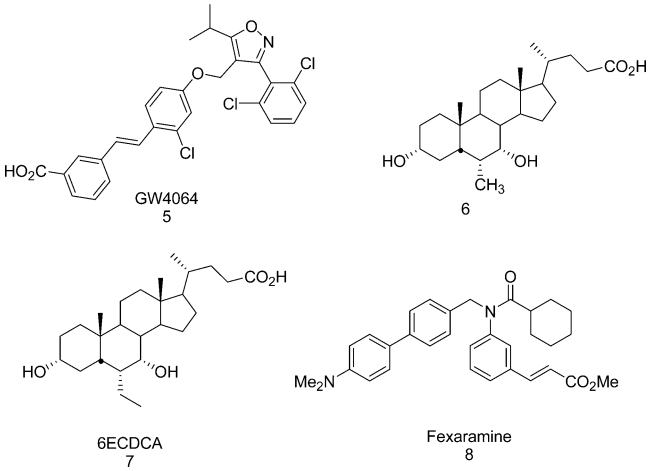
10.1021/jm049904b CCC: $27.50
Published on Web 07/23/2004

Chart 2. Synthetic FXR Ligands



Finally, optimization of a benzopyrane-based combinatorial derived libraries led to the identification of fexaramine (**8**) as a structurally diverse nonsteroidal agonist, also endowed with nanomolar potency.[14,15] Interestingly, the gene expression array induced by 6ECDCA (**7**), GW4064 (**5**), and fexaramine (**8**) is different,[15] thus pointing out a different genomic target for FXR when stimulated by structurally divergent agonists. Apparently consistent with this, the comparison of the high-resolution crystal structures of the two complexes fexaramine−FXR and 6ECDCA−FXR, indicated two similar but not overlapping binding sites and slightly different conformations of the two receptors, thus inferring that the two ligands may induce different degrees of receptor activation. Further evidence comes from the very recent observation that endogenous BAs may interact with FXR in unique ways leading to ligand and promoter selectivity for FXR-mediated gene transcription.[16]

Although combinatorial chemistry may offer the opportunity for the detection of structurally novel chemical entities able to interact with both the hormone binding site and possible modulatory sites, manipulation of the steroidal skeleton toward synthetic BA analogues is endowed with a number of potential advantages. In this regard, of particular interest is the possibility that a BA-based FXR ligand may efficiently be taken up into the enterohepathic circulation, thus preventing the depletion of the BA pool size secondary to the inhibition of BA synthesis induced by FXR activation. Furthermore, a BA-based ligand should inherently be targeted to hepatocytes and endowed with a pharmacokinetic profile suitable for in vivo administration. With this consideration in mind and as a continuation of our work in the field,[10,17,18a−l] we report here a thorough structure−activity relationship of a series of synthetic BA analogues as potential FXR modulators. Of these new derivatives, some were previously synthesized by us in attempts to optimize the physicochemical properties of BA; others were derive from a systematic modification over the CDCA (**1**) skeleton.

Design Strategy

Two major patterns of modification were elaborated around CDCA (**1**), namely, those relating to the B ring of the steroid body and those affecting the side chain

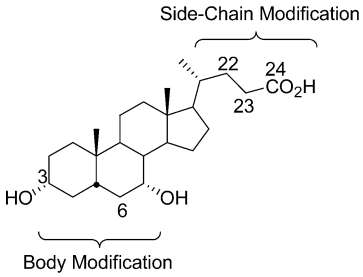


Figure 1. Selected modifications on CDCA skeleton.

(Figure 1). We have previously shown that 6α substitution of CDCA with small linear alkyl substituents increases the potency with respect to the parent compound, with 6α-ethyl (**7**) being the optimal one.[10] Now, we engaged ourselves in the synthesis and the evaluation of the effect of additional alkyl substituents, including 6α-allyl (**9**), 6α-(2′-hydroxyethyl) (**10**), and 6α-propargyl (**11**). Furthermore, more hydrophilic substituents were introduced, such as the 6α-hydroxy (**12**) and 6α-methoxy (**13**) ones. Fluorine was also considered as a potentially useful probe because of its small size, relative lipophilicity, and moderate ability to act as hydrogen acceptor. Thus, the 6α-F-CDCA (**14**) and 6*β*-F-CDCA (**15**) derivatives were synthesized. Finally, the effect of the hydroxyl group in the 3 position was evaluated by the synthesis of 3-deoxy-CDCA (**16**) (Chart 3).

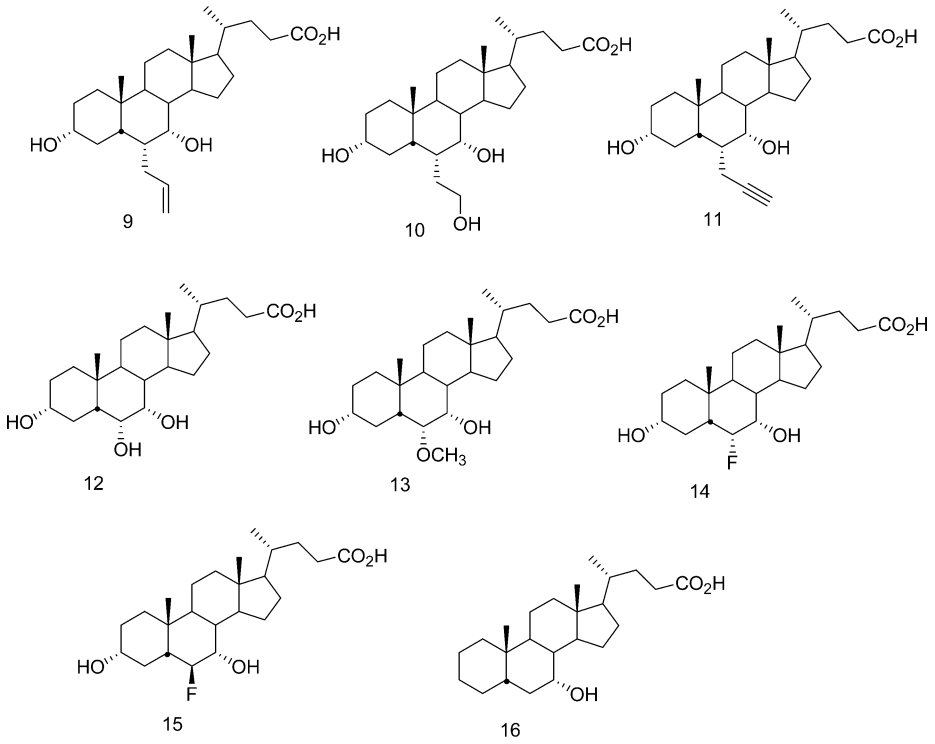
The side chain was also extensively studied (Chart 4). Positions 22 and 23 were explored by introducing a 22-hydroxy group (**17**), a 23-hydroxy group (**18**, **19**), and a 23-methyl group (**20**). Furthermore, the four diastereoisomers (**21**−**24**) resulting from the 22,23 cyclopropanation were analyzed in terms of the conformational requirement of the steroid side chain. The role of the side chain length was proved by degradation of CDCA (**1**) to bisnor-CDCA (**25**). Finally, but not less importantly, we decided to check the precise role of the acidic tail of BA. Thus, the distal 23 or 24 position was systematically scanned by the introduction of groups with different electrostatic, steric, hydrogen bonding, and pK_a profiles, such as amino (**26**), ethylcarbamate (**27**), oxazolidin-3,5-dione (**28**), sulfate (**29**), and sulfonate (**30**).

Chemistry

Compounds **1**−**4** and **12** were commercially available and purchased from Aldrich and Fluka (St. Louis, MO). All the other derivatives were synthesized as described below. Thus, compounds **14** (3α,7α-dihydroxy-6α-fluoro-5*β*-cholan-24-oic acid),[18a,j,19] **15** (3α,7α-dihydroxy-6*β*-fluoro-5*β*-cholan-24-oic acid),[18a,j,19] **16** (7α-hydroxy-5*β*-cholan-24-oic acid),[20] **17** (3α,7α,22α-trihydroxy-5*β*-cholan-24-oic acid),[18k,l] **18** (3α,7α,23*β*-trihydroxy-5*β*-cholan-24-oic acid),[18k,l] **19** (3α,7α,23α-trihydroxy-5*β*-cholan-24-oic acid,[18k,l] **25** (3α,7α-dihydroxy-22-bisnor-5*β*-cholanoic acid,[21] **29** (3α,7α,24-trihydroxy-5*β*-cholane-24-sulfate,[22] and **30** (3α,7α-dihydroxy-5*β*-cholan-24-ol-24-*O*-sulfonic acid)[23] were prepared as reported elsewhere.

The 6α-alkyl analogues of CDCA (**9**−**11**) were prepared according to the procedure previously described for the synthesis of 6αMeCDCA (**6**) and 6αECDCA (**7**).[10] In particular, 7-ketolithocholic acid **31** was protected at

Chart 3. Body Modified CDCA Analogues



the 3 position by treatment with 3,4-dihydro-2*H*-pyran in dioxane in the presence of a catalytic amount of *p*-toluenesulfonic acid (p-TSA) to give the corresponding 3-tetrahydropyranyloxy derivative of 7-ketolithocholic acid **32**. Reaction of **32** with allyl and propargyl bromide at −78 °C, using lithium diisopropylamide as a base and HMPTA/tetrahydrofuran (THF) as solvent followed by treatment with methanolic HCl, afforded the corresponding methyl 3α-hydroxy-7-keto-6α-allyl-5*β*-cholan-24-oate (**33**) and methyl 3α-hydroxy-7-keto-6α-propargyl-5*β*-cholan-24-oate (**34**) in 28% and 22% yield, respectively (Scheme 1). Selective reduction with sodium borohydride[24] and subsequent hydrolysis of the methyl esters **33** and **34** with alkali afforded the desired 3α,7α-dihydroxy-6*β*-allyl-5*β*-cholan-24-oic and 3α,7α-dihydroxy-6α-propargyl-5*β*-cholan-24-oic acids (**9** and **11**, respectively) in about quantitative yields.

Compound **10** (3α,7α-dihydroxy-6α-(2′-hydroxyethyl)-5*β*-cholan-24-oic) was obtained by submitting intermediate **33** of Scheme 1 to a reductive (sodium borohydride) ozonolysis to afford the corresponding hydroxymethyl derivative **37** in 31% yield (Scheme 2). Final basic saponification with alkali of **37** afforded the desired 3α,7α-hydroxy-6α-(2′-hydroxyethyl)-5*β*-cholan-24-oic acid (**10**) in 89% yield.

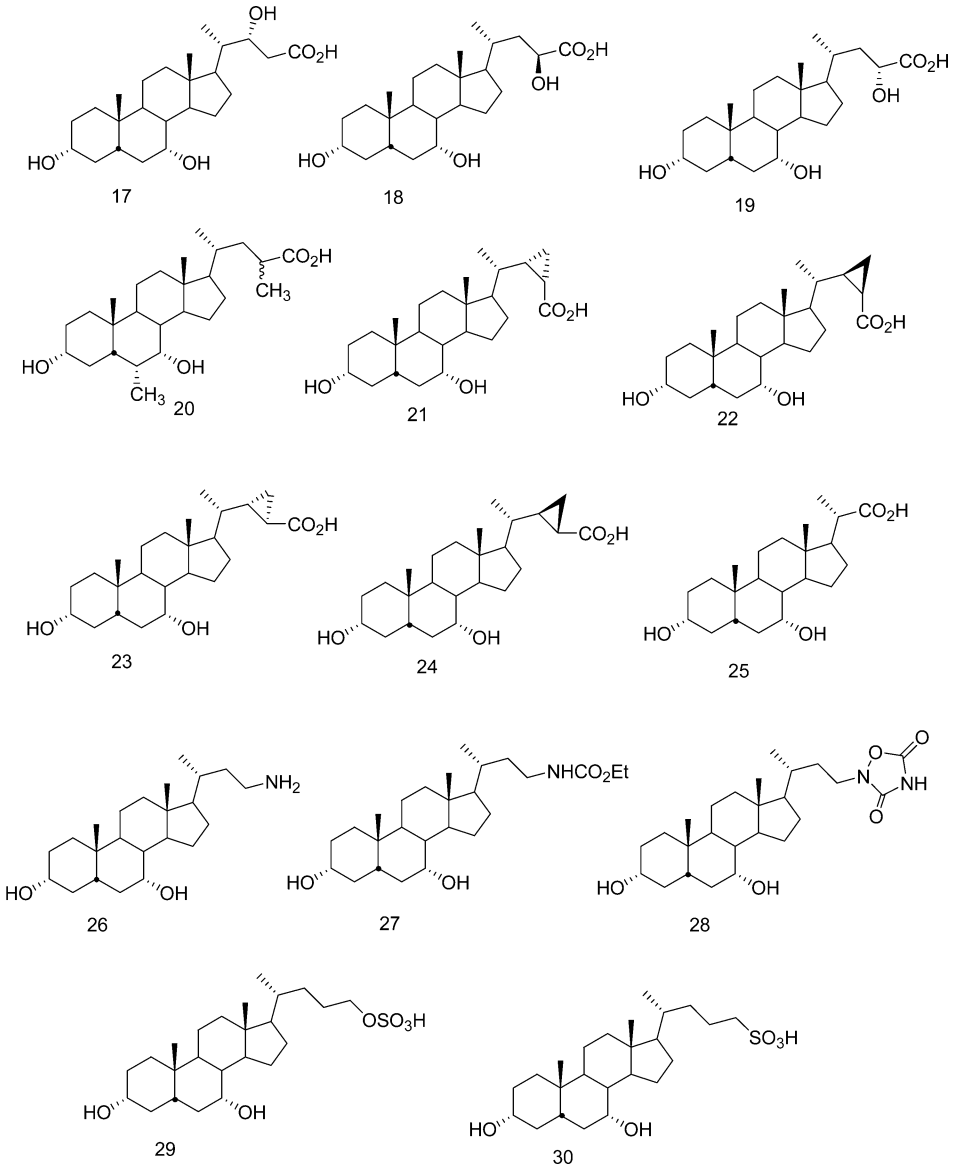
3α,7α-Dihydroxy-6α-methoxy-5*β*-cholan-24-oic acid (**13**) was synthesized as reported in Scheme 3.

Thus, methyl 3α,6α-dihydroxy-7-keto-5*β*-cholan-24-oate (**38**), obtained from commercially available 3α-hydroxy-7-keto-5*β*-cholan-24-oic acid (**31**) according to a known procedure,[25] was selectively protected at the 3 position with the *tert*-butyldiphenylsilyl group affording the corresponding derivative **39** in 43% yield. Alkylation of **39** with methyl iodide, 15-crown-5, and sodium hydride[26] and subsequent removal of the silyl protecting group with tetrabutylammonium fluoride gave methyl 3α,7α-hydroxy-6α-methoxy-5*β*-cholan-24-oate (**40**) in 45% yield. Selective reduction of **40** with sodium borohydride and basic saponification gave the final compound 3α,7α-dihydroxy-6α-methoxy-5*β*-cholan-24-oic acid (**13**) in 40% yield.

Compound **20** (3α,7α-dihydroxy-6α,23-dimethyl-5*β*-cholan-24-oic acid) was obtained as a byproduct in the synthesis of 6α-methyl-CDCA.[10] Briefly, alkylation of the 3-tetrahydropyranyloxy derivative of 7-ketolithocholic acid **32** with methyl iodide, followed by treatment with methanolic HCl, afforded the corresponding methyl 3α-hydroxy-7-keto-6α-methyl-5*β*-cholan-24-oate (**42**) and methyl 3α-hydroxy-7-keto-6α,23-dimethyl-5*β*-cholan-24-oate (**43**) in 21% and 4% yield, respectively (Scheme 4). Selective reduction with sodium borohydride and subsequent hydrolysis of the methyl ester **43** with alkali afforded the 3α,7α-dihydroxy-6α,23-dimethyl-5*β*-cholan-24-oic acid (**20**) in 52% yield.

3α,7α-Dihydroxy-22,23-methylene-5*β*-cholan-24-oic acid isomers **21**−**24** were prepared following the procedure reported elsewhere for the 3α,7*β*-dihydroxy-5*β*-cholan-24-oic analogues (UDCA derivatives).[18f−i] Protection with acetyl groups of CDCA and degradation of the side chain to afford the corresponding 21,22 unsaturated derivative **45** was performed according to a known procedure.[18k,28] As reported in Scheme 5, the derivative **45**[28] was submitted to a dirhodium(II) tetracetate catalyzed cyclopropanation with ethyldiazoacetate, thus obtaining a mixture of diacetyl 22,23-methylenecholanoic esters **46**. Alkaline hydrolysis followed by medium-pressure liquid chromatography afforded the (22*S*,23*S*)-3α,7α-dihydroxy-22,23-methylene-5*β*-cholan-24-oic acid (**21**), (22*R*,23*R*)-3α,7α-dihydroxy-22,23-methylene-5*β*-cholan-24-oic acid (**22**), (22*S*,23*R*)-3α,7α-dihydroxy-22,-23-methylene-5*β*-cholan-24-oic acid (**23**), and (22*R*, 23*S*)-3α,7α-dihydroxy-22,23-methylene-5*β*-cholan-24-oic acid (**24**) in 15%, 12%, 32%, and 27% yield, respectively. The assignment of absolute stereochemistry was performed

Chart 4. Side Chain Modified CDCA Analogues



by comparison with that previously reported for the ursoderivatives.[18f,i]

3α,7α-Dihydroxy-5β-23-norcholanylamine (**26**) and 23*N*-carbetoxy-3α,7α-dihydroxy-5β-23-norcholanylamine (**27**) were prepared as reported in Scheme 6. Thus, the diacetyl-protected CDCA **47** was converted into the corresponding acyl azides via the acyl chloride intermediate (thionyl chloride) followed by treatment with aqueous sodium azide. The crude acyl azide mixture was then refluxed overnight in EtOH to give the protected *N*-carbethoxyamino derivative **48** in 62% yield, which upon treatment with potassium hydroxide gave a mixture, which was separated by chromatography, of the corresponding *N*-carbethoxyamino **27** and norcholanilamino derivative **26** in 26% and 23% yield, respectively.

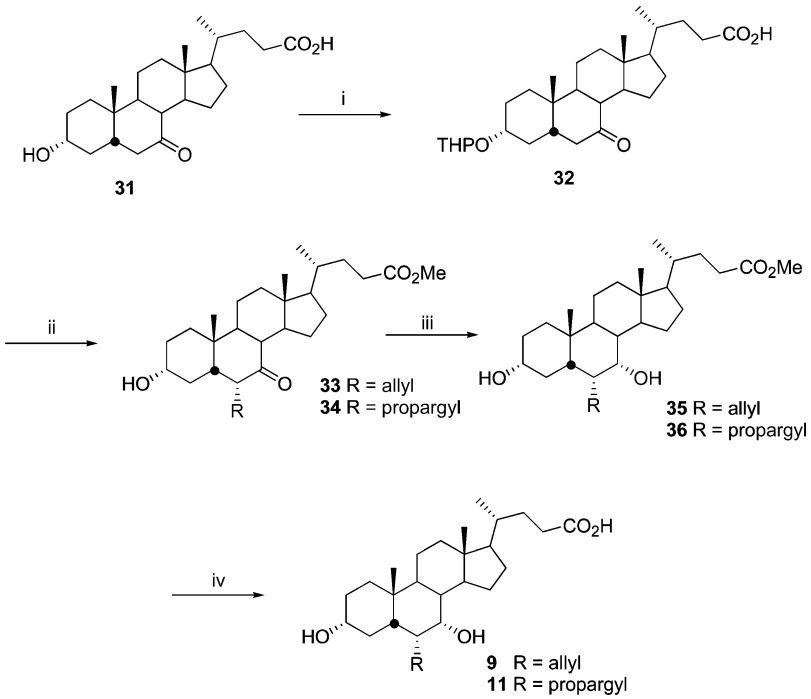
Compound **28** (3α,7α-dihydroxy-5β-23-norcholanyl)-3,5-dioxo-1,2,4-oxadiazolidine) was obtained in a multistep synthesis. Derivative **45**[28] was submitted to hydroboration and oxidation reactions[29] to yield the corresponding alcohol derivative **49** in 63% yield. Subsequent oxidation by utilizing pyridinium chlorocromate afforded 3α,7α-diacetoxy-5β-23-norcholanaldehyde (**50**) in 61% yield. The aldehyde group was then transformed to the 3,5-dioxo-1,2,4-oxadiazolidine moiety by following a known procedure.[30] Thus, condensation of **50** with *O*-benzylhydroxylamine in the presence of sodium cyanoborohydrate gave the corresponding *N*-benzyloxyamino derivative **51** in 42% yield. The intermediate **51** was then reacted with ethoxycarbonyl isocyanide to afford the derivative **52** in 72% yield. Removal of the benzyl protecting group by hydrogenolysis gave the corresponding *N*-hydroxy ester derivative **53** in 64% yield, which was submitted to the final hydrolysis under basic conditions leading to the cyclization and the removal of the acetyl protecting groups, thus obtaining the final compound **28** in 46% yield.

Biological Results and Discussion

All the studied compounds were tested in a cell-free ligand sensing assay that measures the ligand-dependent recruitment of the SRC peptide to FXR by fluorescence resonance energy transfer, as previously described.[12] The results are listed in Table 1.

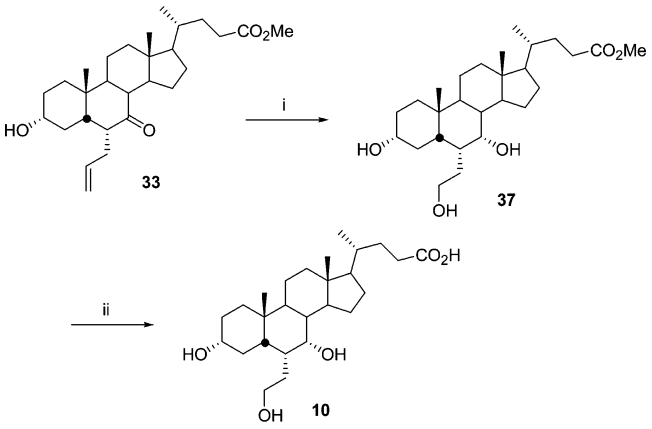
The 3α-hydroxy group (**16**) can be eliminated without significant loss of activity with respect to CDCA (**1**). This result could be interpreted on the basis of the crystal

Scheme 1[a]



[a] (i) DHP, pTSOH, dioxane, room temp; (ii) (a) LDA, THF, R−Br, −78 °C, (b) HCl, MeOH, reflux; (iii) $NaBH_4$, MeOH, room temp; (iv) NaOH, MeOH, reflux.

Scheme 2[a]



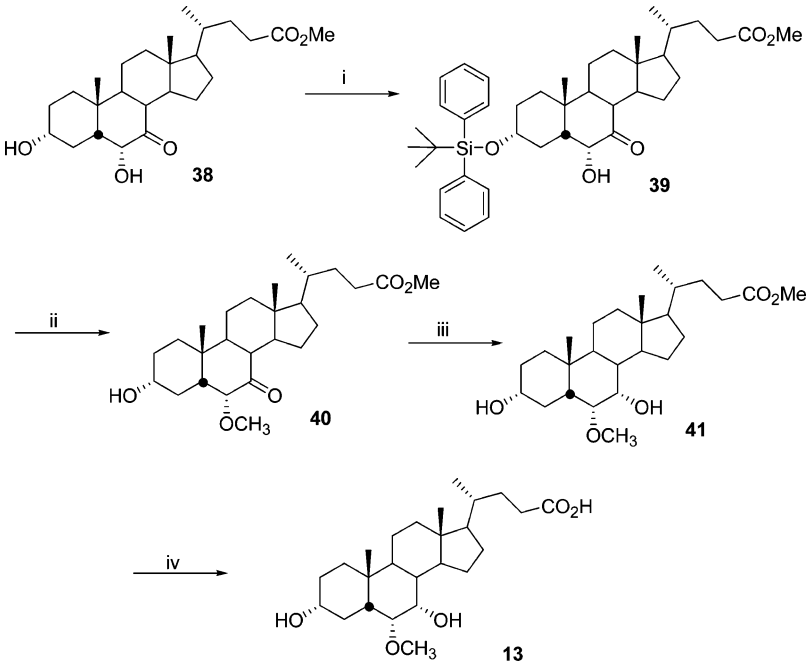
[a] (i) (a) O_3, CH_2Cl_2, MeOH, room temp, (b) $NaBH_4$, MeOH, room temp; (ii) NaOH, MeOH, reflux.

structure of the ligand binding domain of FXR.[13] Modification on ring B of the steroid body profoundly affected the ability of activating FXR. The key importance of the 6α position, figured out by the excellent potency of 6ECDCA (**7**),[10] is furthermore highlighted by the analysis of the novel 6-substituted CDCA analogues (compounds **9**−**15**). Indeed, while linear alkyl or alkyl-substituted groups (**9**−**11**) induced both high potency and efficacy consistent with the physicochemical nature of the pocket, more polar substituents such as the hydroxy (**12**) or methoxy (**13**) groups had a markedly reduced effect. This loss of potency is clearly not due to steric reasons and must be ascribed to the excessive desolvation cost that more hydrophilic molecules must pay to fit into the hydrophobic pocket. The effect of desolvation seems to be crucial for understanding the potency of BA analogues and will be thoroughly addressed in a following paper. Interestingly, insertion of the fluorine atom in the 6 position has an intermediate effect, according to its peculiar hydrophobic/hydrophilic balance. Thus, the 6α-fluoro derivative (**14**) is twice less potent than CDCA (**1**) but equally efficacious, while the 6*β* epimer (**15**) is about 8 times more potent but less efficacious (76%).

Modification of the side chain of CDCA (**1**) resulted in a complex pattern of results. Introduction of a hydroxyl group in the 22 or 23*R* or 23*S* position (**17** or **18** or **19**, respectively) had an overall unfavorable effect on EC_{50}, although it did not abolish the activity. In detail, functionalization of the 23 position is quite acceptable because compounds **18**−**20** showed only a minimal decrease in activity with respect to the parent CDCA (**1**) albeit with a marked reduction in the efficacy. Less prone to modification is the 22 position because compound **17** is 3 times less potent than CDCA (**1**). Remarkably, all the above side chain substituted CDCA analogues did not reach the maximum effect and behave as partial agonists. Cyclopropanation on the 22,23 position yielded very useful information. The two cis isomers (**21**, **22**) were devoid of any activity, whereas the two trans ones (**23**, **24**) were able to activate FXR. In particular, among the two trans derivatives, the 22*S*,-23*R* isomer (**23**) was about 4 times more potent than CDCA (**1**) although it was unable to reach the maximum efficacy. The 22*R*,23*S* isomer (**24**) was, in contrast, 5-fold less potent than CDCA (**1**). These data clearly indicate a conformational preference for an extended disposition of the side chain of BA when interacting with the FXR receptor. This is in agreement with the experimentally determined conformation of 6ECDCA (**7**) complexed with FXR and is indirectly confirmed by the lack of activity of bisnor-CDCA (**25**), where the carboxylic moiety cannot reach the position of that of a conformationally extended cholanoic acid.

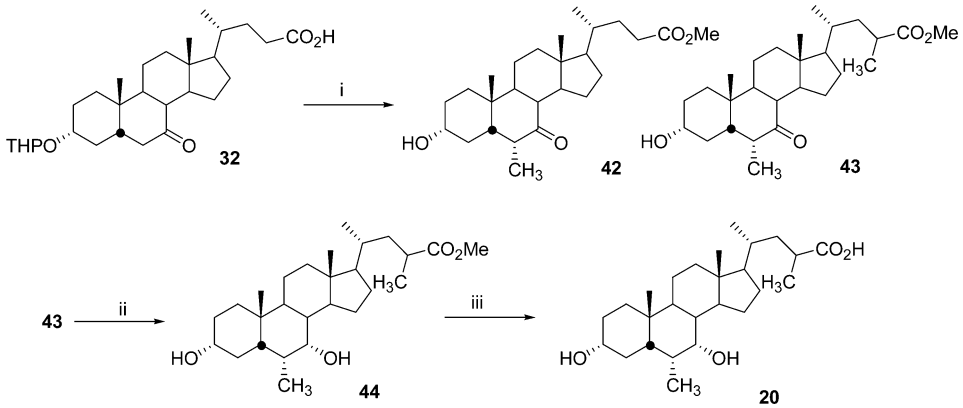
Of great interest is the analysis of the effect of the replacement of the 24-carboxylic acid group by structur-

Scheme 3[a]



[a] (i) TBDPSCl, imidazole, DMF, room temp; (ii) CH_3I, 15-crown-5, NaH, THF, room temp; (iii) $NaBH_4$, MeOH, room temp; (iv) NaOH, MeOH, reflux.

Scheme 4[a]



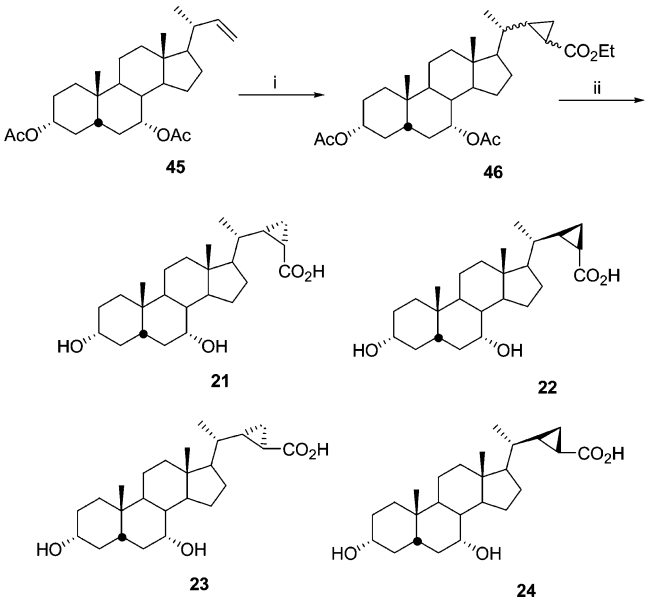
[a] (i) (a) LDA, THF, MeI, −78 °C, (b) HCl, MeOH, reflux; (ii) $NaBH_4$, MeOH, room temp; (iii) NaOH, MeOH, reflux.

ally diverse moieties. Introduction in the 23 position of a oxazolidin-3,5-dione moiety (**28**) turned out to represent a true bioisosteric substitution because the resulting derivative is endowed with both high potency and high efficacy. Other acidic groups had, however, a less straightforward behavior. Thus, while the 24-sulfonic acid derivative (**30**) kept both the potency and the efficacy comparable with that of CDCA (**1**), the 24-sulfate acid (**29**) had a slightly better potency than CDCA but only 27% of efficacy, thus demonstrating it to be endowed with marked antagonist properties. The substitution of the distal carboxylic acid group by an amino group yielded derivative **26**, which was surprisingly as equipotent and as efficacious as CDCA (**1**). Indeed, the substitution of the carboxylate by an amino group profoundly affects both the electrostatic profile and the hydrogen bond acceptor/donor characteristic of the molecule and the interpretation of the equiactivity between the two moieties requires extensive modeling approaches, whose results will be communicated in due course. Interestingly, the ethylcarbamate derivative (**27**) of compound **26** showed a potency 3 times higher than the parent derivative but only 47% of efficacy, being therefore a partial antagonist of FXR.

Conclusion

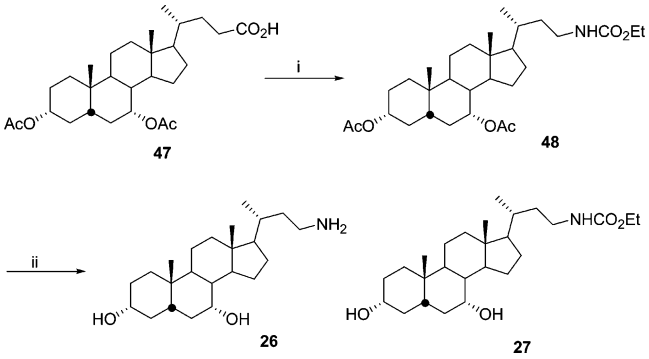
Taken together, these data offer quite a clear picture of the structural requirements needed for the modulation of the FXR receptor by steroid derivatives. While recent reports on the structure of ligand binding domain of FXR have allowed us to elucidate the main determinants of binding of steroids such as 6ECDCA (**7**)[13,17] and non-steroids such as fexaramine (**8**),[15] the present structure−activity relationship on a large series of CDCA analogues permits a closer examination of the possibility of fine-tuning the FXR response by selected chemical manipulations. The main findings can be grouped as follows. (i) Although none of the here-reported derivatives is more potent than 6ECDCA (**7**),[10] the beneficial effect of a 6α-alkyl substitution is confirmed. In particular, a group larger than an ethyl group can be fitted into the pocket, thus suggesting a certain

Scheme 5[a]



[a] (i) EDA, $Rh_2(OAc)_4$, CH_2Cl_2, room temp; (ii) (a) NaOH, EtOH, reflux, (b) MPLC.

Scheme 6[a]

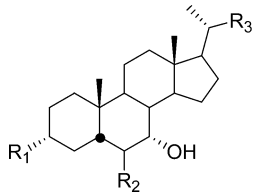


[a] (i) (a) $SOCl_2$, C_6G_6, reflux, (b) NaN_3, H_2O, room temp, (c) EtOH, reflux; (ii) NaOH, MeOH, reflux.

degree of receptor flexibility or ligand accommodation. Remarkably, some 6α-alkyl derivatives, such as **9**, have a very high efficacy. More polar and hydrogen-bonding groups have a negative impact on both potency and efficacy, consistent with the very hydrophobic nature of the pocket complementary to the 6α position. (ii) The side chain can be functionalized, and the resulting derivatives (**17**−**24**), despite a general, moderate loss of potency, display an interesting partial agonist character. The four cyclopropane isomers (**21**−**24**) provide stringent clues about the need of an extended disposition of the side chain of BA analogues because the two cis isomers **21** and **22**, reminiscent of a more folded conformation, are completely inactive. (iii) The carboxylic group can be substituted. Indeed, intuitive bioisosteric replacers such as oxazolidin-3,5-dione (**28**) kept the potency of the parent derivative, while others had an unpredictable effect. In particular, going from an amino (**26**), carbamate (**27**), and sulfate (**29**) to sulfonate (**30**), it is possible to achieve a variety of functional effects, from full agonism to partial antagonism, without significant loss of potency.

In conclusion, the present results complete and extend the structure−activity relationship for a class of semi-synthetic BA previously identified by us as potent and selective FXR agonist.[10]

Table 1. Binding Potency and Efficacy of Synthetic BAs to FXR



Comp.d	Trivial Name	R_1	R_2	R_3	EC_{50} (μM)	Efficacy (%)[a]
1	CDCA	OH	H	CO_2H	8.66	100
5	GW4064	OH	H	CO_2H	0.037	117
6	6αMeCDCA	OH	α-Me	CO_2H	0.75	148
7	6ECDCA	OH	α-Et	CO_2H	0.098	144
9		OH		CO_2H	0.48	170
10		OH		CO_2H	61.15	68
11		OH		CO_2H	0.54	105
12	HCA	OH	α-OH	CO_2H	>30	9
13		OH	α-OMe	CO_2H	14.73	113
14	6αFCDCA	OH	α-F	CO_2H	15.11	99
15	6βFCDCA	OH	β-F	CO_2H	1.21	76
16	3deoxyCDCA	H	H	CO_2H	1.30	87
17		OH	H	OH CO_2H	33.18	66
18		OH	H	CO_2H OH	13.20	58
19		OH	H	CO_2H OH	10.23	35
20		OH	H	CO_2H CH_3	15.62	60
21		OH	H	CO_2H	>30	50
22		OH	H	CO_2H	>30	0
23		OH	H	CO_2H	2.43	82
24		OH	H	CO_2H	42.70	42
25	bisnor-CDCA	OH	H	$-CO_2H$	>30	65
26		OH	H	NH_2	9.68	101
27		OH	H	$NHCO_2Et$	2.72	47
28		OH	H	O O NH O	1.22	111
29		OH	H	OSO_3H	5.99	27
30		OH	H	SO_3H	4.55	68

[a] Relative recruitment of the SRC1 peptide to FXR where CDCA (**1**) is 100%.

Experimental Section

Chemistry. Melting points were determined with a Buchi 535 electrothermal apparatus and are uncorrected. NMR

spectra were obtained with a Bruker AC 200 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. Specific rotations were recorded on a Jasco Dip-360 digital polarimeter. Flash column chromatography was performed using Merck silica gel 60 (0.040−0.063 mm). TLC was carried out on precoated TLC plates with silica gel 60 F-254 (Merck). Spots were visualized by staining and worming with phosphomolybdate reagent (5% solution in EtOH). All reactions were carried out under a nitrogen atmosphere.

6α-Alkylation of 7-Keto-5*β*-cholanoic Derivatives. Method A. *n*-Butyllithium (18.7 mL, 1.8 M solution in hexane) and then HMPA (5.8 mL. 33.7 mmol) were added dropwise at −78 °C to a solution of diisopropylamine (4.7 mL, 33.7 mmol) in dry THF (250 mL). The system was kept to −78 °C for an additional 30 min, and then 3α-tetrahydropyranyloxy-7-keto-5*β*-cholan-24-oic acid (**32**) (5 g, 10.5 mmol) dissolved in dry THF (50 mL) was added dropwise to the mixture. After 20 min, suitable alkyl bromide (105 mmol) dissolved in dry THF (20 mL) was slowly added and the mixture was allowed to warm overnight to room temperature. The solvents were removed under vacuum, acidified by 10% HCl, extracted with EtOAc (5 × 200 mL), washed with 5% $Na_2S_2O_3$ solution (2 × 300 mL), dried (over anhydrous Na_2SO_4), filtered, and evaporated under vacuum. The crude residue was then refluxed with a solution of 2 N HCl in MeOH (50 mL) for 12 h. The residue was evaporated under vacuum and taken up with EtOAc (300 mL), washed with a saturated $NaHCO_3$ solution (2 × 100 mL), dried (Na_2SO_4), and evaporated under vacuum. The residue was purified by silica gel flash chromatography.

Selective Reduction of 7-Keto-5*β*-cholanoic Derivatives.[24] Method B. Methyl 7-keto-5*β*-cholanoate derivative (0.85 mmol) was dissolved in MeOH (50 mL), and $NaBH_4$ (96 mg, 2.56 mmol) was added. The mixture was stirred at room temperature for 3 h. H_2O (10 mL) was then added, and the mixture was partially concentrated under vacuum and extracted with EtOAc (3 × 20 mL). The combined organic fractions were washed with a saturated NaCl solution (brine) (1 × 50 mL), dried (Na_2SO_4), and evaporated under vacuum to obtain the crude reduced derivative that was passed to the next step without further purification.

Saponification of Methyl 5*β*-Cholanoic Esters. Method C. Methyl 5*β*-cholanoate derivative (0.71 mmol) was dissolved in MeOH (25 mL), and 10% NaOH in MeOH (5.7 mL, 14.2.mmol) was added. The mixture was refluxed for 16 h. The mixture was acidified with 3 N HCl and extracted with EtOAc (3 × 20 mL). The combined organic fractions were washed with brine (1 × 50 mL), dried (Na_2SO_4), and evaporated under vacuum. The residue was purified by silica gel flash chromatography.

3α-Tetrahydropyranyloxy-7-keto-5*β*-cholan-24-oic Acid (32). *p*-Toluenesulfonic acid (0.12 g, 0.64 mmol) and 3,4-dihydro-2*H*-pyrane (1.9 mL, 20.4 mmol) were added to a solution of 7-ketolithocholic acid **31** (5.0 g, 12.8 mmol) in dioxane (50 mL). The reaction mixture was stirred at room temperature for 15 min. H_2O (50 mL) was then added, and the mixture was partially concentrated under vacuum and extracted with EtOAc (3 × 50 mL). The combined organic fractions were washed with brine (1 × 50 mL), dried (Na_2SO_4), and evaporated under vacuum. The residue was dissolved in MeOH (50 mL) and treated overnight with 10% NaOH (MeOH solution) at room temperature. The solvent was removed under vacuum, and the crude was dissolved in H_2O (100 mL), neutralized with 2 N HCl, and extracted with EtOAc (3 × 100 mL). The organic phases were dried (Na_2SO_4) and evaporated under vacuum. The residue was chromatographated on silica gel column. Elution with light petroleum/EtOAc 80/20 afforded 4.2 g of compound **32** (84% yield). ^{1}H NMR ($CDCl_3$) δ: 0.56 (s, 3H, 18-CH_3), 0.79 (d, 3H, J = 6.7 Hz21-CH_3), 1.03 (s, 3H, CH_3-19), 2.70 (m, 1H, CH-8), 3.37 (m, 4H), 3.79 (m, 1H, 3-CH).

Methyl 3α-Hydroxy-6α-allyl-7-keto-5*β*-cholan-24-oate (33). This compound was prepared using method A (allyl bromide 12.7 g, 105 mmol). Elution with light petroleum/EtOAc 70/30 afforded to 1.30 g of derivative **33** (27.8% yield). ^{1}H NMR ($CDCl_3$) δ: 0.58 (s, 3H, CH_3-18), 0.87 (d, J = 6.2 Hz, 3H, CH_3-21), 1.15 (s, 3H, CH_3-19), 3.30−3.55 (m, 1H, CH-3), 3.60 (s, 3H, CO_2CH_3), 4.90−5.10 (m, 2H, CH=CH_2), 5.50−5.70 (m, 1H, CH=CH_2).

Methyl 3α-Hydroxy-6α-propargyl-7-keto-5*β*-cholan-24-oate (34). This compound was prepared using method A (propargyl bromide 12.5 g, 105 mmol). Elution with light petroleum/EtOAc 70/30 afforded to 0.76 g of derivative **34** (22% yield). ^{1}H NMR ($CDCl_3$) δ: 0.58 (s, 3H, CH_3-18), 0.84 (d, J = 6.2 Hz, 3H, CH_3-21), 1.16 (s, 3H, CH_3-19), 3.40−3.59 (m, 1H, CH-3), 3.60 (s, 3H, CO_2CH_3).

Methyl 3α,7α-Dihydroxy-6α-allyl-5*β*-cholan-24-oate (35). Methyl 3α-hydroxy-6α-allyl-7-keto-5*β*-cholan-24-oate (**33**) (0.380 g, 0.85 mmol) was converted to 0.30 g of product **35** (81% yield) following method B. ^{1}H NMR ($CDCl_3$) δ: 0.58 (s, 3H, CH_3-18), 0.84−0.87 (d, J = 6.2 Hz, 3H, CH_3-21), 1.15 (s, 3H, CH_3-19), 3.30−3.47 (m, 1H, CH-3), 3.60 (m, 2H, CH-7 and CO_2CH_3), 4.90−5.10 (m, 2H, CH=CH_2), 5.60−5.80 (m, 1H, CH=CH_2).

Methyl 3α,7α-Dihydroxy-6α-propargyl-5*β*-cholan-24-oate (36). Methyl 3α-hydroxy-6α-propargyl-7-keto-5*β*-cholan-24-oate (**34**) (0.30 g, 0.68 mmol) was converted to 0.23 g of product **36** (76% yield) following method B. ^{1}H NMR ($CDCl_3$) δ: 0.64 (s, 3H, CH_3-18), 0.84 (s, 3H, CH_3-19), 0.89 (d, J = 6.2 Hz, 3H, CH_3-21), 3.40−3.59 (m, 1H, CH-3), 3.59 (s, 3H, CO_2CH_3), 3.74 (brs, 1H, CH-7).

Methyl 3α,7α-Dihydroxy-6α-(2-hydroxyethyl)-5*β*-cholan-24-oate (37). Methyl 3α-hydroxy-6α-allyl-7-keto-5*β*-cholan-24-oate (**33**) (0.380 g, 0.85 mmol) was dissolved in 50 mL of CH_2Cl_2/MeOH (1/1) and treated at 0 °C with O_3 until a blue solution was reached. The mixture was stirred at room temperature for 1 h, $NaBH_4$ (96 mg, 2.56 mmol) was added, and the mixture was stirred for an additional 3 h. The mixture was acidified with 3 N HCl and extracted with EtOAc (3 × 20 mL). The combined organic fractions were washed with brine (1 × 50 mL), dried with Na_2SO_4, and evaporated under vacuum. The residue was chromatographated on silica gel column. Elution with light petroleum/EtOAc 70/30 afforded 0.12 g of derivative **37** (31%yield). ^{1}H NMR (CD_3OD) δ: 0.58 (s, 3H, CH_3-18), 0.85 (m, 6H, CH_3-19 and CH_3-21), 3.24−3.30 (m, 1H, CH-3), 3.59 (s, 3H, CO_2CH_3), 3.64 (brs, 1H, CH-7).

3α,7α-Dihydroxy-6α-allyl-5*β*-cholan-24-oic Acid (9). Methyl 3α,7α-dihydroxy-6α-allyl-5*β*-cholan-24-oate (**35**) (0.1 g, 0.22 mmol) was treated according to method C. Elution with $CHCl_3$/MeOH 98/2 with 0.1% AcOH afforded 0.08 g of final compound **9** (86% yield) as a white solid (mp: 99−101 °C). ^{1}H NMR ($CDCl_3$) δ: 0.59 (s, 3H, CH_3-18), 0.83 (s, 3H, CH_3-19), 0.86 (d, J = 6.4 Hz, 3H, CH_3-21), 2.20−2.44 (2m, 2H, CH_2-23), 3.20−3.47 (m, 1H, CH-3), 3.60 (brs, 1H, CH-7), 4.90−5.10 (m, 2H, CH=CH_2), 5.50−5.80 (m, 1H, CH=CH_2). ^{13}C NMR ($CDCl_3$) δ: 11.79, 18.24, 20.69, 23.06, 23.67, 28.15, 30.39, 30.80, 30.99, 33.14, 33.85, 34.24, 35.39, 35.59, 39.43, 39.57, 40.03, 42.75, 45.42, 50.42, 55.79, 70.84, 72.25, 115.98, 137.32, 179.27.

3α,7α-Dihydroxy-6α-(2-hydroxyethyl)-5*β*-cholan-24-oic Acid (10). Methyl 3α,7α-dihydroxy-6α-(2-hydroxyethyl)-5*β*-cholan-24-oate (**37**) (0.1 g, 0.22 mmol) was treated according to method C. Elution with $CHCl_3$/MeOH 95/5 with 0.1% AcOH afforded of 0.07 g of compound **10** (73% yield) as a white solid (mp: 114−116 °C). ^{1}H NMR (CD_3OD) δ: 0.63 (s, 3H, CH_3-18), 0.87 (m, 6H, CH_3-19 and CH_3-21), 2.23−2.30 (m, 2H, CH_2-23), 3.22−3.28 (m, 1H, CH-3), 3.54 (3, 3H, CH_2−OH and CH-7). ^{13}C NMR (CD_3OD) δ: 11.50, 18.00, 20.53, 22.86, 23.35, 27.96, 29.48, 30.00, 30.92, 32.07, 32.92, 33.45, 35.24, 35.30, 35.43, 36.38, 39.42, 39.64, 42.48, 45.83, 49.01, 50.28, 55.69, 60.52, 70.46, 71.91, 177.04.

3α,7α-Dihydroxy-6α-propargyl-5*β*-cholan-24-oic Acid (11). Methyl 3α,7α-dihydroxy-6α-propargyl-5*β*-cholan-24-oate (**36**) (0.10 g, 0.22 mmol) was treated according to method C. Elution with $CHCl_3$/MeOH 98/2 with 0.1% AcOH afforded of 0.08 g of compound **11** (89% yield) as a white solid (mp: 108−110 °C). ^{1}H NMR (CD_3OD) δ: 0.64 (s, 3H, CH_3-18), 0.87 (s, 3H, CH_3-19), 0.90 (d, J = Hz, 3H, CH_3-19), 2.23−2.27 (m, 2H, CH_2-23), 3.24−3.27 (m, 1H, CH-3), 3.65 (brs, 1H, CH-7). ^{13}C NMR (CD_3OD) δ: 11.26, 17.81, 19.35, 22.60, 28.27, 30.16,

31.13, 31.40, 33.21, 33.30, 35.72, 40.01, 40.13, 40.56, 42.76, 45.38, 50.62, 56.36, 69.13, 69.92, 71.98, 78.48, 83.33, 177.48.

Methyl 3α-(*tert*-Buthyldiphenylsilyloxy)-6α-hydroxy-7-keto-5*β*-cholan-24-oate (39). Methyl 3α,6α-dihydroxy-7-keto-5*β*-cholan-24-oate (**38**) (4.0 g, mmol) was dissolved in DMF (80 mL), and *tert*-butyldiphenylsilyl chloride (5.8 mL, 14.2.mmol) and imidazole (1.5 g, mmol) were added. The reaction mixture was stirred at 60 °C for 5 h. Cold H_2O (250 mL) was then added, and the mixture was extracted with EtOAc (3 × 150 mL). The organic fraction was washed with brine (1 × 50 mL), dried (Na_2SO_4), and evaporated under vacuum. The residue was chromatographated on silica gel column. Elution with light petroleum/EtOAc 80/20 afforded 2.7 g of compound **39** (43% yield). ^{1}H NMR ($CDCl_3$) *δ*: 0.56 (s, 3H, CH_3-18), 0.81 (d, 3H J = 6.85 Hz, CH_3-21), 0.95 (s, 9H, *t*-Bu), 1.03 (s, 3H, CH_3-19), 3.40 (m, 1H, CH-3), 3.59 (s, 3H, CO_2CH_3), 3.69 (m, 1H, CH-6), 7.30 (m, 6H, Ph), 7.62 (m, 4H, Ph).

Methyl 3α-Hydroxy-6α-methoxy-7-keto-5*β*-cholan-24-oate (40). Methyl 3α-(*tert*-buthyldiphenylsilyloxy)-6α-hydroxy-7-keto-5*β*-cholan-24-oate (**39**) (0.5 g, 0.76 mmol) and 15-crown-5 (0.17 mL, 0.84 mmol) dissolved in dry THF (10 mL) were slowly added to a suspension of NaH (0.04 g, 1.06 mmol, 60% in oil) in dry ether (40 mL) at 0 °C. The reaction mixture was then treated dropwise with methyl iodide (0.12 g, 0.84 mmol) dissolved in THF (10 mL) and stirred at room temperature for an additional 4 h. Cold H_2O (50 mL) was added, and the mixture was extracted with EtOAc (3 × 20 mL). The organic fraction was washed with brine (1 × 50 mL), dried (Na_2SO_4), and evaporated under vacuum. The residue was taken up with THF (40 mL), tetrabutylammonium fluoride (0.26 g, 0.99 mmol) was added, and the mixture was stirred at room temperature for 16 h. H_2O (150 mL) was added, and the mixture was extracted with EtOAc (3 × 50 mL). The organic fraction was washed with brine (1 × 50 mL), dried (Na_2SO_4), and evaporated under vacuum. The residue was chromatographated on a silica gel column. Elution with light petroleum/EtOAc 50/50 afforded 0.15 g of the derivative **40** (45% yield). ^{1}H NMR ($CDCl_3$) *δ*: 0.56 (s, 3H, CH_3-18), 0.81 (d, 3H J = 7.06 Hz, CH_3-21), 1.04 (s, 3H, CH_3-19), 3.18 (s, 3H, OCH_3), 3.41 (m, 1H, CH-3), 3.59 (s, 3H, CO_2CH_3), 3.95 (m, 1H, CH-6).

Methyl 3α,7α-Dihydroxy-6α-methoxy-5*β*-cholan-24-oate (41). Methyl 3α-hydroxy-6α-methoxy-7-keto-5*β*-cholan-24-oate (**40**) (0.12 g, 0.28 mmol) was converted to 0.085 g of product **41** (71% yield) following method B. ^{1}H NMR ($CDCl_3$) *δ*: 0.55 (s, 3H, CH_3-18), 0.81 (m, 6H, CH_3-19 and CH_3-21), 3.27−3.37 (m and s, 4H, CH-3 and OCH_3), 3.56 (s, 3H, CO_2CH_3), 3.70 (brs, 1H, CH-7).

3α,7α-Dihydroxy-6α-methoxy-5*β*-cholan-24-oic Acid (13). Methyl 3α,7α-dihydroxy-6α-methoxy-5*β*-cholan-24-oate (**41**) (0.08 g, 0.18 mmol) was treated according to method C. Elution with $CHCl_3$/MeOH 90/10 and further purification on an RP8 column, eluting with CH_3CN/H_2O 80/20, afforded of 0.04 g of compound **13** (56% yield) as a white solid (mp: 105−107 °C). ^{1}H NMR ($CDCl_3$) *δ*: 0.59 (s, 3H, CH_3-18), 0.83 (s, 3H, CH_3-19), 0.87 (d, J = Hz, 3H, CH_3-19), 2.18−2.32 (2m, 2H, CH_2-23), 3.27−3.37 (m, 1H, CH-3), 3.30 (s, 3H, OCH_3), 3.88 (brs, 1H, CH-7). ^{13}C NMR ($CDCl_3$) *δ*: 11.67, 18.24, 20.64, 23.13, 23.55, 28.10, 30.51, 30.79, 30.90, 32.75, 32.85, 35.39, 35.68, 35.76, 38.24, 39.41, 42.66, 44.74, 50.06, 55.65, 55.91, 68.96, 71.90, 78.76, 179.12.

Methyl 3α-Hydroxy-6α,23-dimethyl-7-keto-5*β*-cholan-24-oate (43). This compound was prepared using method A (methyl iodide 15 g, 105 mmol). Elution with light petroleum/EtOAc 70/30 afforded 0.92 g of methyl 3α-hydroxy-6α-methyl-7-keto-5*β*-cholan-24-oate (**42**) (21% yield) and 0.18 g of product **43** (4% yield). ^{1}H NMR ($CDCl_3$) *δ*: 0.60 (s, 3H, CH_3-18), 0.87 (d, J = 6.3 Hz, 3H, CH_3-21), 0.88 (d, J = 6.6 Hz, 3H, CH_3-6), (m, 3H, CH_3-23), 1.19 (s, 3H, CH_3-19), 3.45−3.53 (m, 1H, CH−OH), 3.61 (s, 3H, CO_2CH_3).

Methyl 3α,7α-Dihydroxy-6α,23-dimethyl-5*β*-cholan-24-oate (44). Methyl 3α-hydroxy-6α,23-dimethyl-7-keto-5*β*-cholan-24-oate (**43**) (0.10 g, 0.24 mmol) was converted to 0.08 g of product **44** (80% yield) following method B. ^{1}H NMR ($CDCl_3$) *δ*: 0.62 (s, 3H, CH_3-18), 0.87 (s, 3H, CH_3-19), 0.92 (d, J = 6.0 Hz, 3H, CH_3-21), 0.97 (d, J = 7.1 Hz, 3H, CH_3-6), 3.27−3.40 (m, 1H, CH-3), 3.55 (brs, 1H, CH-3), 3.63 (s, 3H, CO_2CH_3).

3α,7α-Dihydroxy-6α,23-dimethyl-5*β*-cholan-24-oic Acid (20). Methyl 3α,7α-dihydroxy-6α,23-dimethyl-5*β*-cholan-24-oate (**44**) (0.08 g, 0.19 mmol) was treated according to method C. Elution with CH_2Cl_2/MeOH 95/5 with 0.1% AcOH afforded of 0.05 g of product **20** (65% yield) as a white solid (mp: 103−105 °C). ^{1}H NMR ($CDCl_3$) *δ*: 0.67 (s, 3H, CH_3-18), 0.92 (s, 3H, CH_3-19), 0.95 (m, complex, CH_3-21 and CH_3-23), 1.01 (d, J = 7.1 Hz, 3H, CH_3-6), 2.54 (m, 1H, CH-23), 3.39−3.48 (m, 1H, CH-3), 3.56 (brs, 1H, CH-7). ^{13}C NMR ($CDCl_3$) *δ*: 11.77, 15.72, 18.58, 18.88, 20.65, 23.12, 23.66, 25.63, 28.19, 30.51, 32.67, 33.84, 33.99, 34.62, 35.44, 37.03, 38.91, 39.62, 40.04, 40.73, 47.37, 50.46, 56.61, 72.35, 72.87, 179.10.

3α,7α-Dihydroxy-22,23-methylene-5*β*-cholan-24-oic Acids (21−24). Ethyl diazoacetate (0.478 g, 1.19 mmol) in dry CH_2Cl_2 (15 mL) was slowly added dropwise to a stirred suspension of 3α,7α-diacetoxy-5*β*-norcholan-22,23-ene (45) (0.6 g, 1.39 mmol) in the presence of dirhodium(II) tetraacetate (9 mg, 0.02 mmol) in dry CH_2Cl_2 (15 mL) under nitrogen at room temperature. The reaction mixture was filtered and washed with H_2O (20 mL), dried (Na_2SO_4), and evaporated under vacuum, thus affording a mixture of the four diastereoisomeric esters **46**. The esters **46** were successively dissolved in EtOH (15 mL) and treated with a solution of 10 N NaOH (10 mL) at reflux for 4 h, cooled, poured onto cold H_2O (50 mL), acidified with 2 N HCl, and extracted with EtOAc (3 × 15 mL). The organic phase was washed with brine (10 mL), dried (Na_2SO_4), and concentrated under vacuum. The residue was chromatographated on silica gel. Elution with CH_2Cl_2/MeOH 96/4 with AcOH 0.1% afforded 0.087 g (15% yield) of (22*S*,23*S*)-3α,7α-dihydroxy-22,23-methylene-5*β*-cholan-24-oic acid (**21**) and 0.065 g (11.5% yield) of (22*R*,23*R*)-3α,7α-dihydroxy-22,23-methylene-5*β*-cholan-24-oic acid (**22**). Elution with CH_2Cl_2/MeOH 95.5/4.5 with 0.1% AcOH afforded 0.18 g (32% yield) of (22*S*,23*R*)-3α,7α-dihydroxy-22,23-methylene-5*β*-cholan-24-oic acid (**23**) and 0.15 g (26.7% yield) of (22*R*, 23*S*)-3α,7α-dihydroxy-22,23-methylene-5*β*-cholan-24-oic acid (**24**) as white solids.

21. Mp: 148−150 °C. $[\alpha]^{20}_D$ +5.16 (*c* 1, EtOH). ^{1}H NMR (CD_3OD and $CDCl_3$) *δ*: 0.67 (s, 3H, 18-CH_3), 0.90 (s, 3H, 19-CH_3), 0.96 (d, J = 6.68 Hz, 3H, 21-CH_3), 3.40−3.50 (m, 1H, 3-CH), 3.85 (m, 1H, 7-CH). ^{13}C NMR ($CDCl_3$) *δ*: 12.20, 16.80, 17.08, 20.80, 21.00, 23.15, 24.10, 28.30, 30.80, 31.30, 33.30, 34.80, 34.90, 35.40, 35.70, 39.80, 39.90, 41.80, 43.40, 50.55, 58.20, 68.90, 72.30, 177.00.

22. Mp: >230 °C. $[\alpha]^{20}_D$ −38.19 (*c* 1.1, CH_3Cl/MeOH 1:1). ^{1}H NMR (CD_3OD and $CDCl_3$) *δ*: 0.50 (s, 3H, 18-CH_3), 0.86 (s, 3H, 19-CH_3), 0.96 (d, J = 6.40 Hz, 3H, 21-CH_3), 3.40−3.60 (m, 1H, 3-CH), 3.80 (m, 1H, 7-CH). ^{13}C NMR ($CDCl_3$) *δ*: 12.00, 12.50, 20.90, 21.00, 21.10, 23.00, 23.80, 27.10, 30.50, 31.00, 32.10, 33.10, 34.80, 35.30, 35.60, 39.50, 39.70, 39.85, 41.80, 43.00, 50.40, 58.50, 68.60, 72.00, 176.90.

23. Mp: 221−225 °C. $[\alpha]^{20}_D$ −40.22 (*c* 1, EtOH). ^{1}H NMR (CD_3OD and $CDCl_3$) *δ*: 0.56 (s, 3H, 18-CH_3), 0.86 (s, 3H, 19-CH_3), 1.16 (d, J = 6.60 Hz, 3H, 21-CH_3), 3.10−3.30 (m, 1H, 3-CH), 3.85 (m, 1H, 7-CH). ^{13}C NMR ($CDCl_3$) *δ*: 12.10, 18.30, 18.55, 20.00, 20.90, 23.10, 24.00, 28.20, 30.70, 31.70, 33.20, 34.80, 35.40, 35.70, 39.80, 40.10, 41.80, 43.15, 50.40, 57.80, 68.90, 72.20, 178.40.

24. Mp: 136−140 °C. $[\alpha]^{20}_D$ +13.66 (*c* 1, EtOH). ^{1}H NMR (CD_3OD and $CDCl_3$) *δ*: 0.56 (s, 3H, 18-CH_3), 0.86 (s, 3H, 19-CH_3), 0.96 (d, J = 6.66 Hz, 3H, 21-CH_3), 3.40−3.60 (m, 1H, 3-CH), 3.80 (m, 1H, 7-CH). ^{13}C NMR ($CDCl_3$) *δ*: 12.00, 13.50, 19.90, 20.90, 22.50, 23.10, 24.00, 28.00, 30.70, 31.60, 33.20, 34.90, 35.40, 35.65, 39.70, 39.73, 41.80, 43.10, 50.40, 58.00, 68.80, 72.20, 177.60.

23-*N*-Carbethoxy-3α,7α-diacetoxy-5*β*-norcholanilamine (48). 3α,7α-Diacetoxy-5*β*-cholan-24-oic acid (**47**) (0.3 g, 0.63 mmol) was suspended in dry benzene (30 mL) and treated with $SOCl_2$ (0.8 mL). The mixture was refluxed for 2 h. After the mixture was cooled, the solvents were removed under vacuum and THF (10 mL) was added and the mixture was

treated at 0 °C with a solution of NaN_3 (0.12 g, 1.9 mmol) in H_2O (5 mL). After 1 h, the reaction mixture was poured into cold H_2O (100 mL) and extracted with EtOAc (3 × 100 mL). The organic phase was dried (Na_2SO_4) and evaporated to obtain the acyl azide intermediate (IR: 2134 and 2267 cm^{-1}). The residue was then dissolved in absolute EtOH (50 mL) and refluxed overnight. The reaction mixture was concentrated under vacuum to give 0.21 g of the crude product **48** (65% yield). ^1H NMR ($CDCl_3$) δ: 0.63 (s, 3H, CH_3-18), 0.91 (s, 3H, CH_3-19), 0.91 (d, J = 6.07 Hz, 3H, CH_3-21), 1.23 (t, 3H J = 4.06 Hz, CH_2CH_3), 2.01 and 2.03 (2s, 6H, $2CH_3CO$), 4.90−4.53 (m, 1H, CH-3), 4.74 (bs, 1H, CH-7).

3α,7α-Dihydroxy-5β-norcholanilamine (26) and 23-*N*-Carbethoxy-3α,7α-dihydroxy-5β-norcholanilamine (27). 23-*N*-Carbethoxy-3α,7α-diacetoxy-5β-norcholanilamine (**48**) (0.2 g, 0.39 mmol) was treated according to method C. Elution with CH_2Cl_2/MeOH 90/10 afforded 0.035 g of the less polar product **27** (26% yield) and 0.028 g of the more polar product **26** (23% yield) as white solids.

26. Mp: 131−133 °C. ^1H NMR (CD_3OD) δ: 0.64 (s, 3H, CH_3-18), 0.87 (s, 3H, CH_3-19), 0.90 (d, J = 6.45 Hz, 3H, CH_3-21), 2.20−2.40 (m, 2H, CH_2-24), 3.27−3.33 (m, 1H, CH-3), 3.74 (bs, 1H, CH-7). ^{13}C NMR (CD_3OD) δ: 17.45, 20.33, 22.07, 23.18, 27.79, 29.88, 31.01, 32.56, 34.43, 34.77, 35.11, 35.31, 38.96, 39.26, 39.55, 41.64, 42.23, 48.14, 50.06, 55.83, 67.62, 71.40.

27. Mp: 90−92 °C. ^1H NMR (CD_3OD) δ: 0.61 (s, 3H, CH_3-18), 0.85 (s, 3H, CH_3-19), 0.90 (d, J = 6.52 Hz, 3H, CH_3-21), 2.95−3.24 (2m, 2H, CH_2-24), 3.36−3.44 (m, 1H, CH-3), 3.79 (bs, 1H, CH-7), 4.04 (bq, CH_2CH_3). ^{13}C NMR ($CDCl_3$) δ: 14.68, 18.61, 20.57, 22.77, 23.71, 28.33, 30.68, 32.84, 33.80, 34.61, 35.05, 35.32, 36.16, 38.56, 39.43, 39.63, 39.92, 41.48, 42.74, 50.47, 56.02, 60.64, 68.53, 72.01, 199.98.

3α,7α-Diacetoxy-5β-norcholan-23-ole (49). 3α,7α-Diacetoxy-5β-norcholan-22,23-ene (**45**) (6.4 g, 14.8 mmol) was dissolved in 150 mL of THF, and 1 M solution of the BH_3·THF complex (30 mL) was added at 0 °C. The mixture was then stirred at room temperature for 3 h. The reaction mixture was then cooled (ice bath) and treated with 3 N NaOH (23 mL) and 30% H_2O_2 (26 mL) and stirred for an additional 16 h at room temperature. The mixture was acidified with 3 N HCl and extracted with EtOAc (3 × 300 mL). The combined organic fractions were washed with brine (1 × 150 mL), dried (Na_2SO_4), and evaporated under vacuum. The residue was chromatographated on silica gel. Elution with CH_2Cl_2/MeOH 95/5 gave 4.2 g of product **49** (63% yield). ^1H NMR ($CDCl_3$) δ: 0.69 (s, 3H, CH_3-18), 0.97 (m, 6H, CH_3-19 and CH_3-21), 2.08 and 2.10 (2s, 6H, $2CH_3CO$), 3.65 (m, 2H, CH_2-23), 4.53 (m, 1H, CH-3), 4.92 (bs, 1H, CH-7).

3α,7α-Diacetoxy-5β-norcholan-23-aldehyde (50). PCC (2.41 g, 11.2 mmol) suspended in dry methylene chloride (70 mL) was added dropwise to 3α,7α-diacetoxy-5β-norcholan-23-ole (**49**) (4.1 g, 9.3 mmol) dissolved in dry dichloromethane (70 mL). The mixture was stirred at room temperature for 12 h. Et_2O (25 mL) was then added, and the black solid that precipitated was filtered and washed with diethyl ether several times. The filtrate was concentrated under vacuum and the residue was passed on fluorisil (CH_2Cl_2), affording 2.6 g of crude product **50** (61% yield) that was used for the next step without further purification. ^1H NMR ($CDCl_3$) δ: 0.67 (s, 3H, CH_3-18), 0.95 (m, 6H, CH_3-19 and CH_3-21), 2.05 and 2.08 (2s, 6H, $2CH_3CO$), 2.38 (m, 2H, CH_2-23), 4.51 (m, 1H, CH-3), 4.86 (bs, 1H, CH-7), 9.76 (s, 1H, CHO).

3α,7α-Diacetoxy-5β-norcholan-23-(*N*-benzyloxy)-amine (51). Benzylhydroxylamine (0.43 g, 2.68 mmol), 3α,7α-diacetoxy-5β-norcholan-23-aldehyde (**50**) (1.2 g, 2.68 mmol), and sodium cyanoborohydride (0.34 g, 5.4 mmol) were dissolved in distilled MeOH (50 mL). The pH of the solution was brought to 5 by addition of 2 N HCl in MeOH, and the reaction mixture was stirred for 48 h at room temperature. H_2O (50 mL) was added, and the solvents were removed under vacuum. The crude product was extracted with EtOAc (3 × 50 mL) and H_2O (50 mL). The organic layer was washed with brine (1 × 50 mL) and dried (Na_2SO_4). The residue was chromatographated on silica gel. Elution with $CHCl_3$ afforded 0.63 g of product **51** (42% yield). ^1H NMR ($CDCl_3$) δ: 0.63 (s, 3H, CH_3-18), 0.86 (s, 3H, CH_3-19), 0.89 (d, J = 6.1 Hz, 3H, CH_3-19), 2.05 and 2.08 (2s, 6H, $2CH_3CO$), 2.73 (m, 2H, CH_2-23), 4.24 (q, J = 6 Hz, 2 H, CH_2CH_3), 4.41 (m, 1H, CH-3), 4.56 (m, 1H, CH-7), 4.91 (s, 2 H, CH_2Ph), 5.77 (brs, 1 H, *H*NOBn), 7.35 (brs, 5 H, Ph).

3α,7α-Diacetoxy-5β-norcholan-23-(*N*-benzyloxy)-*N*-(ethoxycarbonylureido)amine (52). 3α,7α-Diacetoxy-5β-norcholan-23-(*N*-benzyloxy)amine (**51**) (0.6 g, 1.08 mmol) was dissolved in dry THF (5 mL), and 90% ethoxycarbonyl isocyanate (135 μL, 1.32 mmol) was added after which the reaction mixture was stirred for 30 min. The solvent was removed under vacuum, and the residue was chromatographated on silica gel. Elution with $CHCl_3$ gave 0.52 g of the derivative **52** (72% yield). ^1H NMR ($CDCl_3$) δ: 0.63 (s, 3H, CH_3-18), 0.86 (s, 3H, CH_3-19), 0.89 (d, J = 6.5 Hz, 3H, CH_3-19), 1.38 (m, 3H), 2.05 and 2.08 (2s, 6H, $2CH_3CO$), 2.73 (m, 2H, CH_2-23), 4.27 (m, 3H, CH-3 and CH_2CH_3), 4.58 (m, 1H, CH-7), 4.89 (s, 2H, CH_2Ph), 7.41 (br s, 5 H, Ph), 7.80 (br s, 1 H, CONHCO).

3α,7α-Diacetoxy-5β-norcholan-23-(*N*-hydroxy)-*N*-(ethoxycarbonylureido)amine (53). 3α,7α-Diacetoxy-5β-norcholan-23-(*N*-benzyloxy)-*N*-(ethoxycarbonylureido)amine (**52**) (0.47 g, 0.70 mmol) was dissolved in 20 mL of 96% EtOH, and this solution was carefully mixed with about 0.1 g of 10% Pd/C. The solution was hydrogenated at 30 psi for 3 h. The solution was then filtered through Celite and washed with EtOH, and the solvent was removed under vacuum. The residue was chromatographated on silica gel. Elution with $CHCl_3$/MeOH 95/5 gave 0.26 g of the derivative **53** (64% yield). ^1H NMR ($CDCl_3$) δ: 0.66 (s, 3H, CH_3-18), 0.95 (s, 3H, CH_3-19), 0.99 (d, J = 6.7 Hz, 3H, CH_3-19), 1.32 (m, 3 H, CH_3), 2.05 and 2.08 (2s, 6H, $2CH_3CO$), 3.61 (m, 1H), 3.72 (m, 1H), 4.27 (m, 2H, CH_2), 4.61 (m, 1H, CH-3), 4.90 (bs, 1H, CH-7), 7.29 (s, 1H, NHO), 8.06 (s, 1 H, CONHCO), 8.72 (s, 1 H, NOH).

1-(3α,7α-Dihydroxy-5β-23-norcholanyl)-3,5-dioxo-1,2,4-oxadiazolidine (28). 3α,7α-Diacetoxy-5β-norcholan-23-(*N*-hydroxy)-*N*-(ethoxycarbonylureido)amine (**53**) (0.17 g, 0.29 mmol) was dissolved in a mixture of THF and H_2O (20 mL, 1/1). Sodium hydroxide (0.12 g, 0.29 mmol) was added, and the reaction mixture was stirred at room temperature for 1 h. The reaction then was quenched with 10% citric acid solution. The residue was extracted with EtOAc (5 × 50 mL). The organic layer was dried (Na_2SO_4), and the solvent was then removed under vacuum. The residue was chromatographated on silica gel. Elution with $CHCl_3$/MeOH 90/10 afforded 0.06 g of product **28** (46% yield) as a white solid (mp: 96−98 °C). ^1H NMR (CD_3OD) δ: 0.63 (s, 3H, CH_3-18), 0.86 (s, 3H, CH_3-19), 0.89 (d, J = Hz, 3H, CH_3-19), 2.63−2.73 (m, 2H, CH_2-24), 3.26−3.41 (m, 1H, CH-3), 3.80 (m, 2H, CH2), 3.92 (brs, 1H, CH-7), 7.95 (s, 1H, NH). ^{13}C NMR (CD_3OD) δ: 10.57, 17.46, 20.26, 21.87, 23.09, 27.79, 29.85, 32.24, 32.55, 33.29, 34.38, 34.72, 35.05, 38.98, 39.26, 39.50, 41.68, 46.56, 50.03, 55.75, 67.53, 71.35, 77.97, 152.75, 157.99.

Biology. Compounds were assayed by fluorescence resonance energy transfer for recruitment of the SRC1 peptide to human FXR using a cell-free LiSA as described elsewere.[2,12]

Acknowledgment. A.E. gratefully acknowledges financial support from the "Secretaria de Estado de Universidades y Educacion" of Spain. The authors are grateful to Erregierre (Bergamo, Italy) for the starting material 7-ketolithocholic acid and to Intercept Pharmaceuticals (New York) for financial support.

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JM049904B