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Research paper

The discovery of 12β-methyl-17-*epi*-18-*nor*-bile acids as potent and selective TGR5 agonists

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ABSTRACT

Recent discoveries have demonstrated that the physiological function of bile acids extends to the regulation of diverse signaling processes through interactions with nuclear and G protein-coupled receptors, most notably the Farnesoid-X nuclear receptor (FXR) and the G protein-coupled bile acid receptor 1 (GPBAR1, also known as TGR5). Targeting such signaling pathways pharmacologically, *i.e.* with bile acid-derived therapeutics, presents great potential for the treatment of various metabolic, inflammatory immune, liver, and neurodegenerative diseases.

Here we report the discovery of two potent and selective TGR5 agonists (NZP196 and 917). These compounds are the taurine conjugates of 6α-ethyl-substituted 12β-methyl-18-*nor*-bile acids with the side chain being located on the α-face of the steroid scaffold. The compounds emerged from a screening effort of a diverse library of 12β-methyl-18-*nor*-bile acids that were synthesized from 12β-methyl-18-*nor*-chenodeoxycholic acid and its C17-epimer. Upon testing for FXR activity, both compounds were found to be inactive, thus revealing selectivity for TGR5.

1. Introduction

Research over the past two decades has demonstrated that bile acids modulate various cellular processes through interaction with nuclear and G protein-coupled receptors (GPCRs) [1,2]. Most prominent amongst these receptors are the Farnesoid X nuclear receptor (FXR) [3] and the Takeda G protein-coupled receptor 5 (TGR5) [4], also known as G-protein-coupled bile acid receptor 1 (GPBAR1). FXR is an important transcriptional regulator of bile acid (BA) homeostasis and genes that are involved in lipid and glucose metabolism [1,2,5–7]. TGR5 is a cell-surface transmembrane receptor that is widely expressed in human tissues including brown adipose tissue, muscle, intestine, gallbladder, liver, immune cells and the brain [1,2]. TGR5 is activated by the majority of endogenous bile acids at varying concentrations, however the secondary bile acids, lithocholic acid (LCA, **3**) and deoxycholic acid (DCA, **5**) and their taurine conjugates (**4** and **6**), are ligands with the greatest potency. The primary bile acid chenodeoxycholic acid (CDCA, **2**) is the most potent natural ligand of FXR (Fig. 1).

Extracellular ligand-binding to TGR5 promotes the upregulation of secondary messenger cyclic AMP (cAMP) and subsequent activation of downstream effectors in target cells [*e.g.* protein kinase A (PKA) and B (PKB/AKT)] [1,2,4,9]. In muscle and brown adipose tissue, TGR5 agonism leads to enhanced energy expenditure and oxygen consumption *via* increased activity of the cAMP-dependent thyroid hormone-activating enzyme, iodothyronine deiodinase 2 (D2), and uncoupling protein (UCP)-1 [10,11]. In enteroendocrine cells, stimulation of TGR5 induces the secretion of the glucagon-like peptide (GLP)-1. This leads to overall positive outcomes on pancreas function, insulin secretion and sensitivity as well as glucose homeostasis [6b,12,13]. Furthermore, activation of TGR5 exerts potent anti-inflammatory effects by inhibiting NF-κB signaling and NLRP3-ASC inflammasome assembly, thus, decreasing the release of proinflammatory cytokines and mediators [4b,15-16]. Whereas, in endothelial cells, activation of TGR5 stimulates release of

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Abbreviations

ASC	apoptosis-associated speck-like protein containing a CARD
BAIB	bis(acetoxy)iodobenzene
DMEM	Dulbecco's modified Eagle's medium
CCL	C–C motif ligand (*e.g.* chemokine CCL2 also known as monocyte chemoattractant protein-1 MCP-1)
CRE	cAMP response element
DMF	*N*,*N*-dimethylformamide
DMSO	dimethyl sulfoxide
DCA	deoxycholic acid
DPPA	diphenylphosphoryl azide;
DSC	differential scanning calorimetry
EEDQ	2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline
EGFR	epidermal growth factor receptor
eNOS	endothelial nitric oxide (NO) synthase
ESI	electrospray ionization
FBS	fetal bovine serum
FDA	US Food and Drug Administration
FXR	Farnesoid X nuclear receptor
GPBAR1	G protein-coupled bile acid receptor 1
GPCR	G protein-coupled receptor
HEK	human embryonic kidney cells
HRMS	high resolution electrospray mass spectroscopy
IBCF	isobutyl chloroformate
LBD	ligand-binding domain
LCA	lithocholic acid
LDA	lithium diisopropylamide
NASH	non-alcoholic steatohepatitis
NF-κB	kappa-light-chain-enhancer of activated B-cells
NLRP3	NLR Family Pyrin Domain Containing 3
NMO	*N*-methylmorpholine *N*-oxide
o/n	overnight
PKA	protein kinase A
PKB (or AKT)	protein kinase B
RLU	relative luminescent units
ROS	reactive oxygen species
rt	room temperature
SD	standard deviation
SEAP	secreted alkaline phosphatase
T	taurine
TBAB	tetra-*n*-butylammonium bromide
TEA	triethylamine
TEMPO	2,2,6,6-Tetramethylpiperidine 1-oxyl free radical
TFA	trifluoroacetic acid
TFAA	trifluoroacetic acid anhydride
TGR5	Takeda G-coupled protein receptor 5
THF	tetrahydrofuran
TLCA	sodium taurolithocholate
TMSCl	trimethylsilyl chloride
TNF-α	tumor necrosis factor alpha

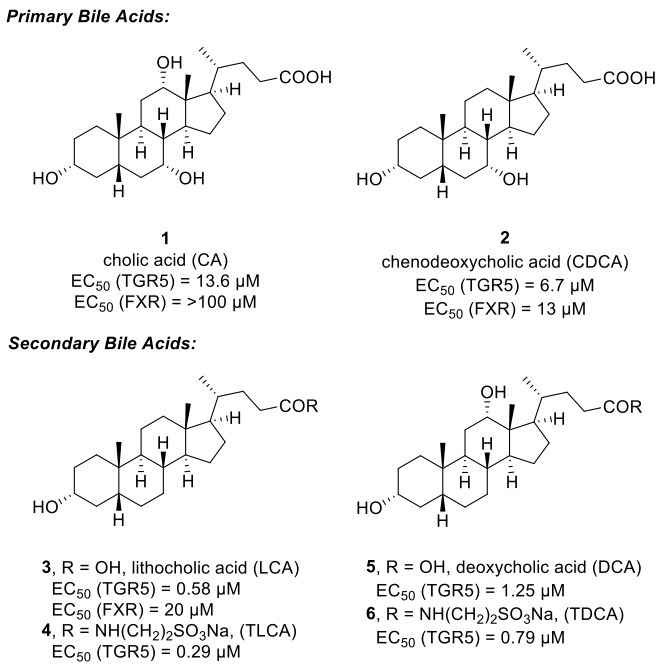


Fig. 1. Primary and secondary bile acids and their EC_{50} values at TGR5 and FXR [8].

nitric oxide and hydrogen sulfide, through activation of endothelial nitric oxide synthase (eNOS) and cystathionine-γ-lyase (CSE). These actions improve vasodilation in the systemic and portal circulation, reduce the expression of adhesion molecules and of the vasoconstrictor endothelin-1 (ET-1) [17,18]. In cholangiocytes and gallbladder epithelial cells, TGR5 activation promotes cytoprotective regulations. These effects include the secretion of bicarbonate, which prevents excessive cell entry of hydrophobic BAs, reduction of bile salt- and CD95-mediated apoptosis, and increased proliferation of cholangiocytes *via* ROS/EGFR to maintain the integrity of the biliary tree [9,19]. TGR5 is also expressed in neurons, microglia and astrocytes and here, TGR5 activation exerts anti-inflammatory effects by downregulating the production of proinflammatory cytokines and decreasing CCL2 chemokine discharge and the associated microglia activation and proliferation [20,21]. Direct modulation of neuronal TGR5 mediates anorexigenic effects and additionally, in a murine model of Parkinson's disease, TGR5 activation decreased the release of TNF-α and other secondary chemokines (CCL3 and 6), preventing microglia polarization and neurodegeneration [22,23]. Considering all these factors, TGR5 agonists offer intriguing therapeutic potential for the treatment of metabolic, inflammatory immune, liver and neurological diseases including diabetes, obesity, metabolic syndrome, atherosclerosis, colitis, non-alcoholic steatohepatitis (NASH) and associated comorbidities [9,14,20–29]. However, systemic stimulation of TGR5 can produce adverse side effects including excessive filling and inadequate emptying of the gallbladder with an elevated risk for gallstone formation, pruritus and changes to heart rate and blood pressure [30–32]. Additionally, to avoid co-activation of FXR and other bile acid sensitive receptors, the development of TGR5 selective agonists is of great importance.

A selection of BA-based TGR5 agonists is shown in Fig. 2. Obeticholic acid (**7**; OCA, INT-747) and the 24-*nor*-23-sulfate analog, INT-767 (**8**), are dual activators of FXR and TGR5 [33,34]. OCA is FDA-approved for the treatment of primary biliary cholangitis (PBC) and is also pursued in phase III clinical trials for the treatment of liver fibrosis due to NASH [40,41]. INT-767 (**8**) has so far been evaluated in phase I clinical trials as a potential candidate for the treatment of liver and metabolic diseases [42,43]. INT-777 (**9**) is a potent and selective TGR5 agonist that has shown preclinical potential for the treatment of metabolic diseases and neuroinflammation [21–23,35,44]. Bile alcohol BAR501 (**11**), with β-oriented 6-ethyl and 7-hydroxy groups, is another example of a potent, BA-derived TGR5 agonist with no associated FXR activity [37]. Recent studies have demonstrated the anti-inflammatory and portal

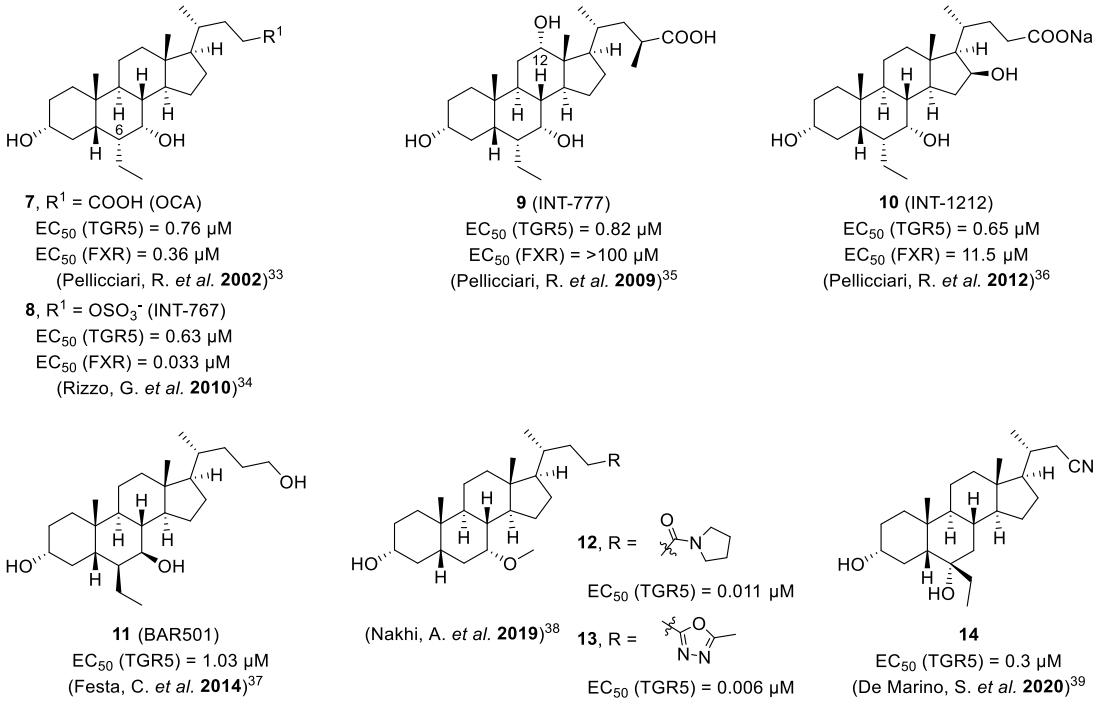


Fig. 2. A selection of BA-derived TGR5 agonists and their EC_{50} values [33–39].

pressure-lowering effects of **11** in rodent models of colitis and portal hypertension, with the possible application for the treatment of inflammatory bowel diseases and portal hypertension in liver disorders [45,18b]. BAR501 also reversed liver steatosis and fibrosis in mice [46].

More recently, Nakhi, A. *et al.* found that substituting the carboxylic acid function in CDCA (**2**) with a pyrrolidine amide or a 1,3,4-oxadiazole, can enhance potency towards TGR5 up to 10-70-fold and that additional methylation of the 7-hydroxy group gave rise to TGR5 agonists with activities in the low nanomolar range (**12** and **13**) [38]. In 2020, **14** was reported to selectively activate TGR5 [39]. In cellular assays, this compound was found to reduce the expression of pro-inflammatory cytokines and also to reverse inflammation in a murine model of colitis.

Our approach is to explore 12β-methyl-18-*nor*-BA scaffolds for the development of new BA-based drugs (Fig. 3) [47–49]. Following our initial work on the synthesis of 12β-methyl-18-*nor*-BAs, we also recently published results from an investigation into the applicability of 12β-methyl-18-*nor*-avicholic acids as potential TGR5 agonists [47,48]. Our research revealed the 23(*R*)-methylated 12β-methyl-18-*nor*-chenodeoxycholic acid (**16**) and the 17-*epi*-bile acid **17** as weak TGR5 activators [48]. Herein, we have used 12β-methyl-18-*nor*-chenodeoxycholic acid (**15**) and its C17-epimer (**17**) as starting materials to build a wider library of 12β-methyl-18-*nor*-BA analogues, with varying side chain lengths, 6α-ethyl substitution and taurine conjugation. These compounds were then examined for their ability to activate TGR5. As a result, we identified two compounds, NZP196 (**18**) and NZP917 (**19**), as potent TGR5 agonists with EC_{50} values of 0.31 and 0.27 μM. Both compounds are the taurine conjugates of the 6α-ethyl-substituted 12β-methyl-17-*epi*-18-*nor*-chenodeoxycholic acid (**17**) and are unique due to the configuration at the C17 position, exhibiting the amidosulfonate-containing side chain at the α-face of the steroid scaffold. A follow-up screen to assess FXR activity uncovered no response for both compounds, thus rendering them selective for TGR5.

2. Results and discussion

Chemistry. Our synthetic approaches to the compounds evaluated in this study are summarized in Schemes 1–5. Accordingly, previously reported 12β-methyl-18-*nor*-BAs [47] (**15** and its 24-*nor*-analogue **21**, as well as **17** and its 25-*homo*-analogue **20**) were conjugated with the amino sulfonic acid **22** (taurine) using either 2-ethoxy-1-ethoxycarbonyl-1, 2-dihydroquinoline (EEDQ), diphenylphosphoryl azide (DPPA) or isobutyl chloroformate (IBCF; for **21**) as coupling reagents (Scheme 1) [50,51].

The 6α-ethyl-modified 12β-methyl-18-*nor*-BAs **34** and **43** as well as their 25-*homo*-analogues **52** and **61** were prepared in seven steps from

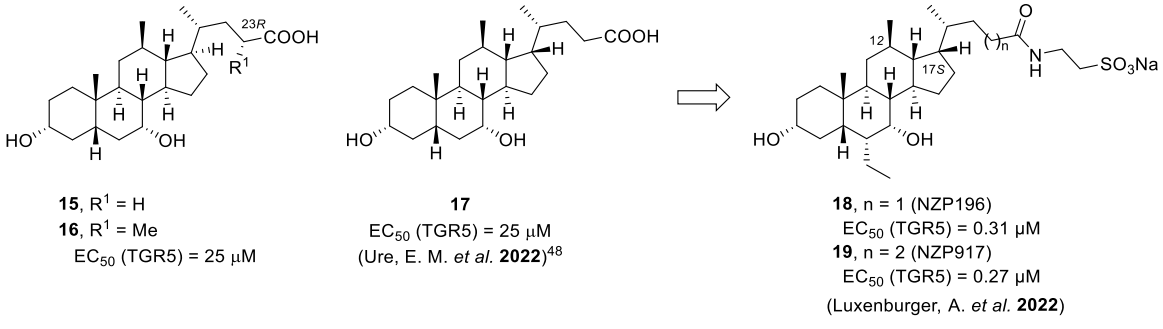
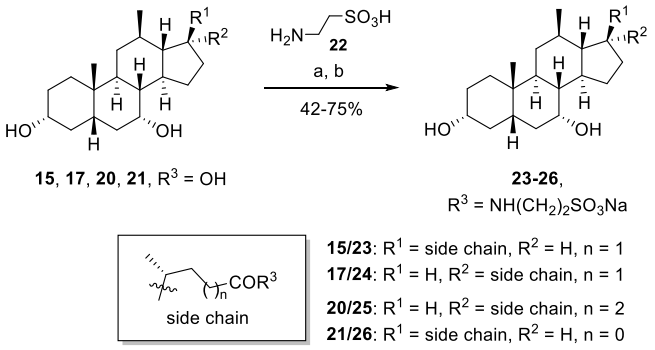
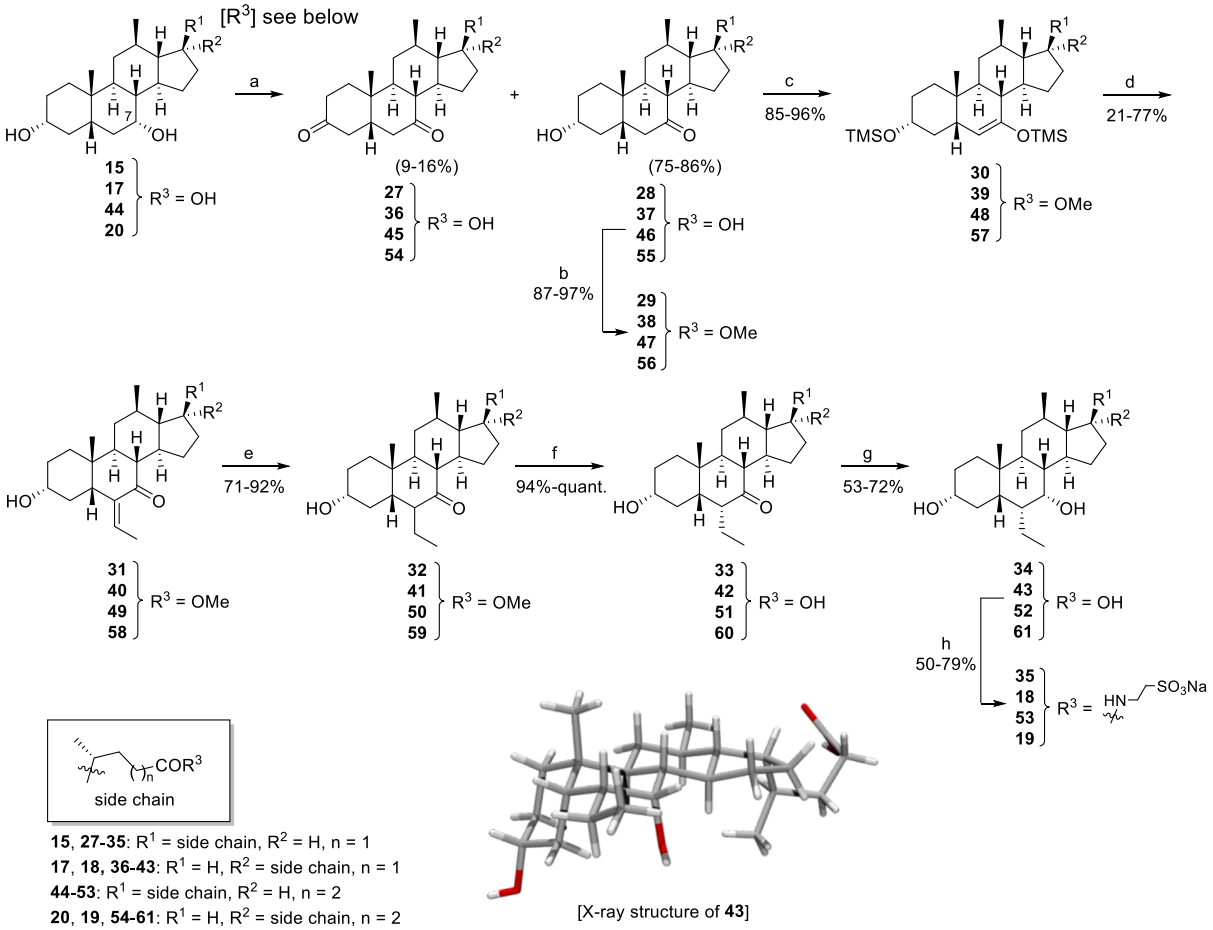


Fig. 3. 12β-Methyl-18-*nor*-bile acid TGR5 agonists (**18** and **19**) as described in this paper.

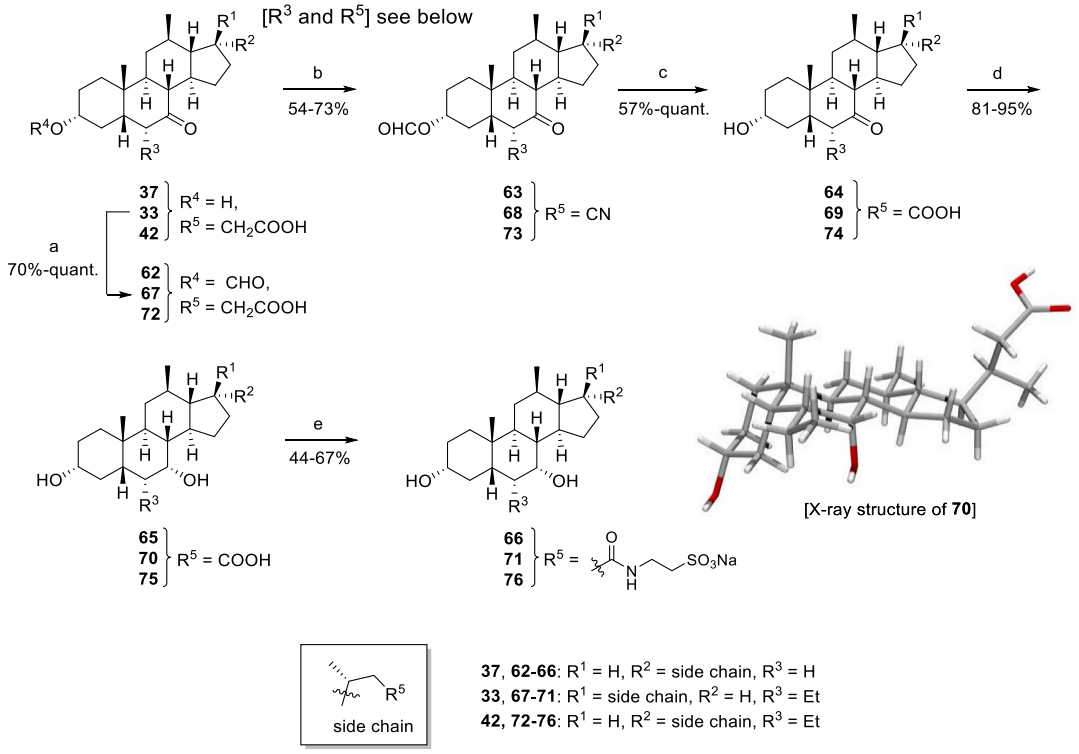


Scheme 1. Synthesis of taurine-conjugated 12β-methyl-18-*nor*-BAs **23**–**26**. Reagents and conditions: (a) EEDQ, NEt_3, DMF, 90 °C or DPPA, NEt_3, DMF, 0 °C→rt; or for **21**: IBCF, NEt_3, THF, −10 °C→rt; (b) 2 M $NaOH_{(aq)}$, MeOH.

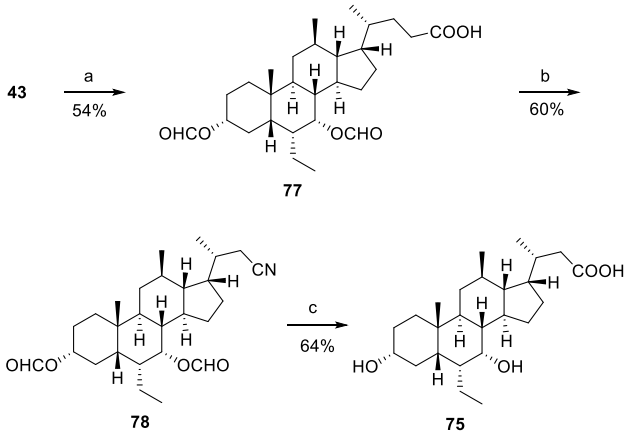
15, **17**, **44** [47] and **20**, respectively, using established methods (Scheme 2) [35,52]. The synthetic sequence started with the selective oxidation of the 7-hydroxy group by treating the BA starting materials (**15**, **17**, **44** and **20**) with a 2 M aqueous sodium hypochlorite solution in the presence tetra-*n*-butylammonium bromide (TBAB) and catalytic amounts of sodium bromide or potassium bromide only. While the desired 7-oxo-BAs **28**, **37**, **46** and **55** were produced in good yields, we also isolated minor amounts of the over-oxidized 3,7-di-keto BAs **27**, **36**, **45** and **54** from the reaction. The formation of 3-oxo-BA side products was also observed, but these compounds were not isolated consistently, nor characterized. Following methyl esterification of the 7-oxo-BAs (**28**/**37**/**46**/**55**), the silyl enol ethers **30**, **39**, **48** and **57** were generated through reaction with lithium diisopropylamide (LDA) and trimethylsilyl chloride (TMSCl) at low temperatures. The silyl ethers were then subjected to a boron trifluoride-catalyzed Mukaiyama aldol reaction with acetaldehyde and subsequent acidic condensation to yield the 6-ethylidene-substituted ketones **31**, **40**, **49** and **58**. Following hydrogenation of the double bond over 10% palladium on charcoal, and basic hydrolysis of the methyl ester, the 6α-ethyl-7-oxo-BAs **33**, **42**, **51** and **60** were obtained in good yields. Finally, reduction of the 7-oxo-group of **33**, **42**, **51** and **60** with sodium borohydride at 0 °C gave rise to the 12β-methyl-18-*nor*-OCA derivatives **34** and **43** and their 25-*homo*-analogues **52** and **61**. Conjugations with taurine (**22**) were accomplished by using IBCF- (for **35** and **18**), DPPA- (for **53**) or EEDQ-mediated (for **19**)



Scheme 2. Synthesis of 6α-ethyl-modified 12β-methyl-18-*nor*-BAs **34**, **43**, **52** and **61** and their respective taurine conjugates **18**, **19**, **35** and **53**. Reagents and conditions: (a) 2 M $NaClO_{(aq)}$, TBAB/NaBr or KBr, MeOH/HOAc/EtOAc/H_2O, 0 °C or Br_2, $NaHCO_3$, MeOH, 0 °C; (b) *p*-TsOH, MeOH, Δ, 3–4 h; pyridine; (c) LDA, TMSCl, THF, −78 °C; NEt3; (d) (i) CH_3CHO, $BF_3{\cdot}OEt_2$, DCM, −60 °C; (ii) 3 M $HCl_{(aq)}$, DCM, 0 °C; (e) 10% Pd/C, H_2, EtOH (for 32 and 50) or THF/MeOH, (1:1; for 41 and 59), o/n; (f) 8 M $KOH_{(aq)}$, MeOH, rt, o/n or for 33: 2 M $NaOH_{(aq)}$, MeOH, 90 °C; (g) $NaBH_4$, THF/water (4:1) 0 °C, 1 h; (h) (i) 22, for 35 and 18: IBCF, NEt_3, THF, 0 °C→rt; for 53: DPPA, NEt_3, DMF, 0 °C→rt; for 19: EEDQ, NEt_3, DMF, 90 °C; (ii) 2 M $NaOH_{(aq)}$, MeOH.



Scheme 3. Preparation of the 12β-methyl-17-*epi*-18,24-*bisnor*-BA **65** and the 6α-ethyl-substituted 12β-methyl-18,24-bis*nor*-BAs **70** and **75** and their respective taurine conjugates **66**, **71** and **76**. Reagents and conditions: (a) HCOOH, $HClO_4$, 60 °C, 2.5–3 h; then Ac_2O; 40 °C, 1 h; (b) TFA, TFAA, $NaNO_2$, −10 °C→40 °C; (c) $KOH_{(aq)}$, EtOH, 90 °C; (d) $NaBH_4$, THF/H_2O (4:1), 0 °C; (e) (i) IBCF, NEt_3, THF, 0 °C→rt; (ii) 2 M $NaOH_{(aq)}$, MeOH.



Scheme 4. Alternative route to the 6α-ethyl-12β-methyl-18,24-bis*nor*-BA **75**. Reagents and conditions: (a) HCOOH, $HClO_4$, 60 °C, 4 h; then Ac_2O, 40 °C, 1 h; (b) TFA, TFAA, $NaNO_2$, 0 °C→40 °C; (c) 7 M $KOH_{(aq)}$, EtOH, 90 °C.

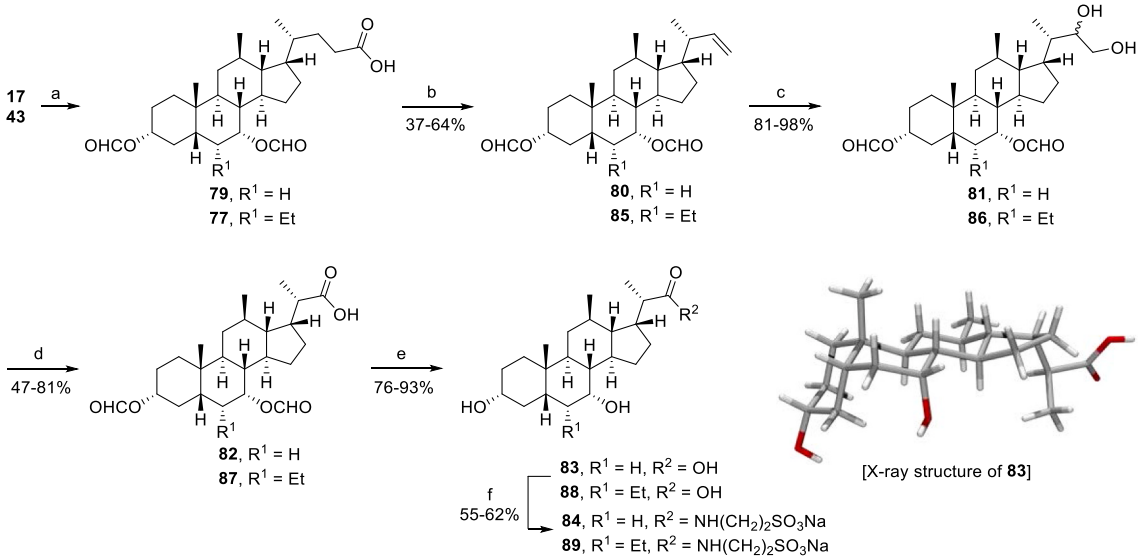
coupling strategies [50,51].

The 12β-methyl-18-*nor*-BAs with the side chain one methylene shorter, 12β-methyl-17-*epi*-18,24-*bisnor*-BA **65** and the 6α-ethyl-substituted 12β-methyl-18,24-*bisnor*-BAs **70** and **75**, were synthesized *via* application of a second-order Beckmann rearrangement (Scheme 3) [53]. For this purpose we started by formyl protecting the 3-hydroxy group of the 7-keto BAs **37**, **33** and **42**. These were then reacted with sodium nitrite in a mixture of trifluoroacetic anhydride and trifluoroacetic acid, which enabled the formation of the 23-nitriles **63**, **68** and **73**. Subsequent basic hydrolysis of the nitrile function gave the corresponding 7-oxo-24-*nor*-BAs **64**, **69** and **74** which, upon sodium borohydride-mediated reduction of the 7-keto group, were converted into the desired 24-*nor*-BA derivatives **65**, **70** and **75**. This approach proved to be superior compared to our initial attempt to access **75** *via* formyl protection of the two hydroxy groups in position 3 and 7 (**77**) followed by TFA/TFAA/$NaNO_2$-mediated rearrangement and final hydrolysis of the so formed nitrile **78** (Scheme 4). In this case we observed an increase in various elimination products, which accounted for the reduced yields of the sequence. Finally, coupling of these bile acids with taurine was achieved *via* IBCF-mediated coupling reaction.

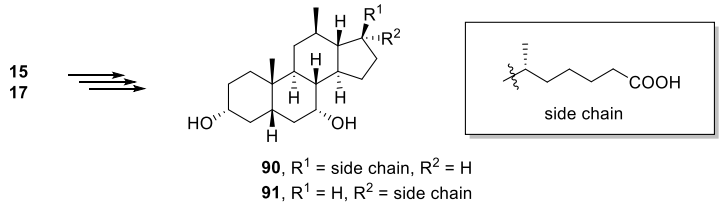
The 12β-methyl-17-*epi*-18,23,24-*trisnor*-BAs **83** and **88** and their respective taurine conjugates **84** and **89** were synthesized from **17** and **43** as depicted in Scheme 5. Upon formyl protection of the two hydroxy groups of **17** [47] and **43**, the side chain was first subjected to a bis(acetoxy)iodobenzene (BAIB)/copper(II) acetate-mediated oxidative decarboxylation to yield the olefins **80** and **85** [54]. These were then dihydroxylated by means of osmium tetroxide and *N*-methylmorpholine *N*-oxide (NMO) and the resulting vicinal diols **81** and **86** underwent oxidative cleavage to the desired 17-*epi*-18,23,24-*trisnor*-BAs **83** and **88** when treated with BAIB and catalytic amounts of TEMPO [55,56]. Final conjugation of **83** and **88** with taurine (**22**) was accomplished by using DPPA as the coupling reagent [51].

For the synthesis of the 25,26-*bishomo*-BAs **90** and **91** we employed methods that were designed previously by D'Amore *et al.* for the natural CDCA system (Scheme 6) [57]. Accordingly, the compounds were prepared in eight steps by employing a Wittig reaction as a key transformation with subsequent hydrogenation of the formed double bond. The aldehyde precursor was obtained by reducing the carboxylate function of **15** and **17** with $LiBH_4$ followed by a Swern-oxidation [58].

In vitro TGR5 efficacy. Following the synthesis of the 12β-methyl-18-*nor*-CDCA and OCA analogues as illustrated in Schemes 1–6, we evaluated their activity towards TGR5 at a ligand concentration of 10 μM. A commercially available secreted alkaline phosphatase (SEAP) reporter assay was employed. This assay measures the ability of compounds to stimulate TGR5 in transfected HEK293 cells and activate the SEAP reporter system HEK. This occurs *via* the Gαs subunit and the



Scheme 5. Synthesis of the 12β-methyl-17-*epi*-18,23,24-*trisnor*-BAs **83** and **88** and their taurine conjugates **84** and **89**. Reagents and conditions: (a) HCOOH, $HClO_4$, 60 °C, 2.5–3 h then 40 °C, Ac_2O, 1 h [47]; (b) $Cu(OAc)_2$, BAIB, pyridine, PhMe, 2–4 h, Δ; (c) OsO_4, NMO, CH_3CN, H_2O, rt, 4 h-o/n; (d) TEMPO, BAIB, CH_3CN, H_2O, rt; (e) 2 M $NaOH_{(aq)}$, MeOH, 90 °C, o/n; (f) (i) taurine (**22**), DPPA, NEt_3, DMF, 0 °C→rt; (ii) 2 M $NaOH_{(aq)}$, MeOH.



Scheme 6. Preparation of the 12β-methyl-24,25-*bishomo*-18-*nor*-BAs **90** and **91** [58].

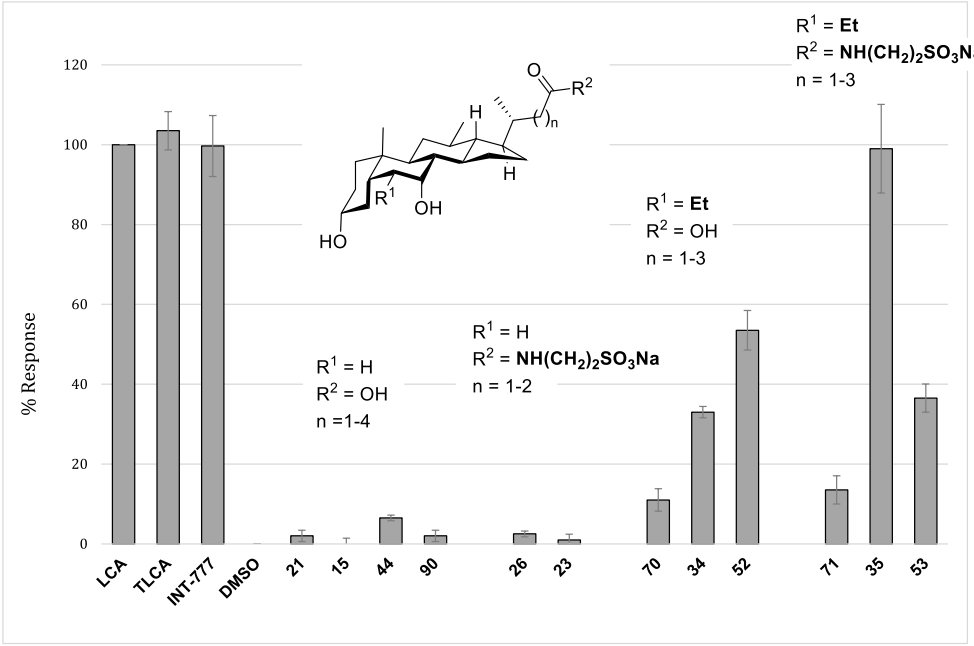


Fig. 4. Efficacies of 12β-methyl-18-*nor*-BAs with a β-facing side chain (natural orientation) at TGR5 at a ligand concentration of 10 μM. Data for TLCA (**4**), INT-777 (**9**) and **35** are expressed as mean values ± SD of five independent experiments. Efficacies for the remaining compounds are reported as the mean values ± SD of at least two independent experiments with three technical replicates each. SD = standard deviation.

adenylate cyclase pathway. Thus, intracellular cAMP (cyclic adenosine monophosphate) levels are increased followed by protein kinase A (PKA) activation and subsequent phosphorylation of the cAMP response element (CRE) binding protein (CREB). Within the nucleus this activated transcription factor then binds to the CRE promoter region of the reporter gene responsible for the upregulation of SEAP expression. The activities of the bile acids examined, as detected by a luminescence output, were normalized to the response produced by lithocholic acid (**3**, LCA) at the same concentration.

Evaluation of the individual test results of the 12β-methyl-18-*nor*-BAs with the naturally oriented, β-facing side chain revealed that compounds lacking the 6α-ethyl group (**21**, **15**, **44**, **90**) were either inactive or exhibited only negligible responses, whereas the presence of a 6α-ethyl substituent produced an apparent positive effect on efficacy (**70**, **34**, **52**). Accordingly, we observed a steady gain in activity for the suite of 6α-ethyl-substituted 12β-methyl-18-*nor*-BAs **70**, **34** and **52** concurrent with their side chain length increasing. While the 24-*nor*-analogue **70**, with the shortest side chain generated only a minor response of 11%, the compound with the longest side chain in this set, the 25-*homo*-analogue **52**, displayed an efficacy of 54%. In this context it is also noteworthy that the overall performance of compound **34**, the 12β-methyl-18-*nor*-analogue of obeticholic acid (**7**), remained behind expectations with only a 33% efficacy at TGR5. This outcome demonstrated that the shift of the angular 18-methyl group into the 12β-position was not tolerated particularly well, presumably as a result of steric interference within the TGR5 ligand-binding domain (LBD). However, when the side chain of **34** was extended with taurine (**35**), we recorded a significant enhancement in activity (efficacy: 99%) which matched that of LCA (**3**), TLCA (**4**) and INT-777 (**9**). On the other hand, the taurine conjugates with the side chain one methylene shorter or longer (**71** and **53**) were significantly less active and **53** was even less active than its unconjugated counterpart **52**. From these results we concluded that the taurine conjugation of **34** led to a side chain length that was well suited to engage in favorable ligand-receptor interactions, whereas a shorter or longer side chain proved disadvantageous.

When reviewing the assay results for the 12β-methyl-18-*nor*-BAs exhibiting the α-facing side chain we were surprised to see that these performed generally better (Fig. 5) than the series of 12β-methyl-18-*nor*-BAs with the side chain located on the convex side (Fig. 4). Here we found that the activity of 12β-methyl-17-*epi*-18-*nor*-BAs without a 6α-ethyl residue, already increased noticeably as the side chain became longer. While those compounds with a shorter side chain **83** (23,24-*bisnor*, n = 0) and **65** (24-*nor*, n = 1) displayed only a 4% response towards TGR5, the efficacy rose to 26 and 43% for the 25-*homo*- and 25,26-*bishomo*-analogues **20** and **91**, respectively. When the side chains of these compounds were extended with taurine, the activity of the compounds improved even further to 56–62%. Only the 24-*nor*-analogue **66** did not benefit from this side chain modification suggesting that this compound could not engage in beneficial interactions with TGR5. For the 17-*epi*-bile acids, that were equipped with a 6α-ethyl group, the same trends as already discussed earlier became apparent: in comparison to the 17-*epi*-bile acids without C6 substitution (**83**/**65**/**17**/**20**/**91**) the presence of the extra 6α-ethyl group significantly enhanced the activity towards TGR5 and the compounds' activities rose with the length of the side chain. Amongst these, the 6α-ethyl-17-*epi*-bile acids **43** and the 25-*homo*-analogue **61** produced the best responses at 62 and 69%. Moreover, these results lay in the same range as those of the taurine conjugates **84** (56%), **24** (61%) and **25** (62%). Among these compounds, the 6α-ethyl-17-*epi*-25-*homo*-bile acid **61** was the most active with an efficacy of 69%. It is also of note that 6α-ethyl-modification of 17-*epi*-24-*nor*-BA **65** improved the compound's activity towards TGR5 significantly from 4% (**65**) to 42% (**75**), while conjugation with taurine alone induced only a marginal effect (**66**: 7%). Eventually, the combination of taurine conjugation and 6α-ethyl-substitution revealed **18** and the 25-*homo*-analogue **19** as the best-performing compounds in this set of 17-*epi*-BAs, with maximum responses of 99–100%. For the 23,24-*bisnor*-analogue **89** we also observed a significant increase in activity to 61% when compared to its unconjugated counterpart **88** (10%). However, with respect to the taurine conjugate **84** (efficacy: 56%), which did not carry a 6α-ethyl group, the gain in activity was only minor. For the 17-*epi*-24-*nor*-BA **75** additional conjugation with taurine did not bring any improvements as **75** and **76** displayed similar efficacies at 42–43%.

After **35**, **18** and **19** emerged as the three best-performing compounds from our screening effort, we next sought to acquire dose response data for these, to determine their corresponding EC_{50} values

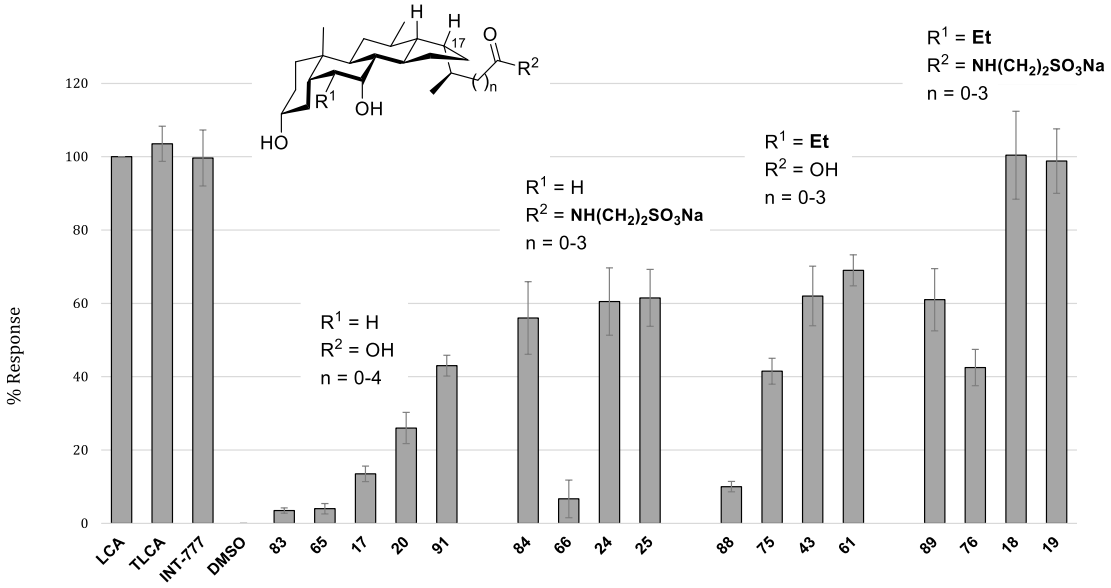


Fig. 5. Efficacies of 12β-methyl-18-*nor*-BAs with an α-oriented side chain (17-*epi*) at TGR5 at a ligand concentration of 10 μM. Data for TLCA (**4**), INT-777 (**9**), **18** and **19** are expressed as mean values ± SD of five independent experiments. Efficacies for the remaining compounds are reported as the mean values ± SD of at least two independent experiments with three technical replicates each. SD = standard deviation.

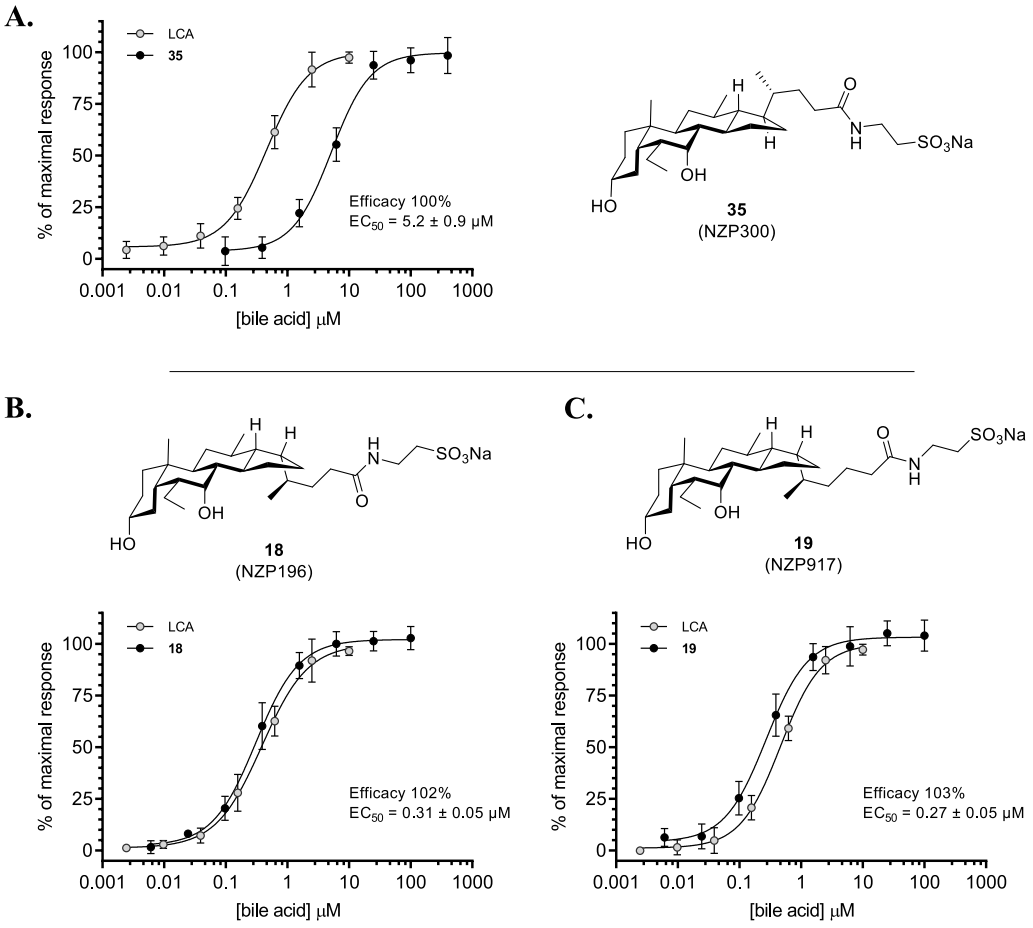


Fig. 6. Dose response data for **35** (A), **18** (B) and **19** (C). The average potency is calculated as the mean EC_{50} value ± SD of three independent experiments. SD = standard deviation.

(Fig. 6). The two 17-*epi*-compounds **18** and **19** were found to be the most potent compounds with EC_{50} values of 0.31 ± 0.05 (**18**) and 0.27 ± 0.05 μM (**19**), respectively. For **35**, bearing the side chain in a β-facing orientation, we recorded an EC_{50} value of 5.2 ± 0.9 μM. With respect to its C17-epimer **18**, the compound was ~17 fold less active. The compounds did not display any cytotoxic effects at the concentrations tested.

To assess the potential of the 12β-methyl-18-*nor*-BAs to transactivate FXR, we also screened the compounds for efficacy at the FXR receptor at a drug concentration of 10 μM using a nuclear hormone receptor (NHR) assay. This screening effort, which was carried out at the drug discovery company Eurofins DiscoverX, showed that none of the 12β-methyl-18-*nor*-BAs tested presented as a viable FXR agonist (Table S1-2 and Figure S1). Only two compounds generated a weak response at FXR. 6α-Ethyl-12β-methyl-17-*epi*-18-*nor*-CDCA (**43**) produced the highest measured response at 31% and 6α-ethyl-12β-methyl-18-*nor*-17-*epi*-25-*homo*-CDCA (**61**) imparted a response of 23%. Both compounds triggered an efficacy of 62 and 69% at the TGR5 receptor at the same concentration. Interestingly, the most efficacious compounds are yet again those with an unnatural, C17-epimerized configuration. Most importantly, the top three BAs from the TGR5 screen (**35**, **18** and **19**) all produced baseline responses at FXR, thus rendering them selective agonists for TGR5.

Modeling of BA analogues to human TGR5. The series of 12β-methyl-18-*nor*-BA analogues were modelled into the orthosteric site of human TGR5 to gain insight into the possible binding modes of the synthetic analogues and to inform future design. The recently published cryo-electron microscopy (cryo-EM) structure of the GPBAR–Gs in

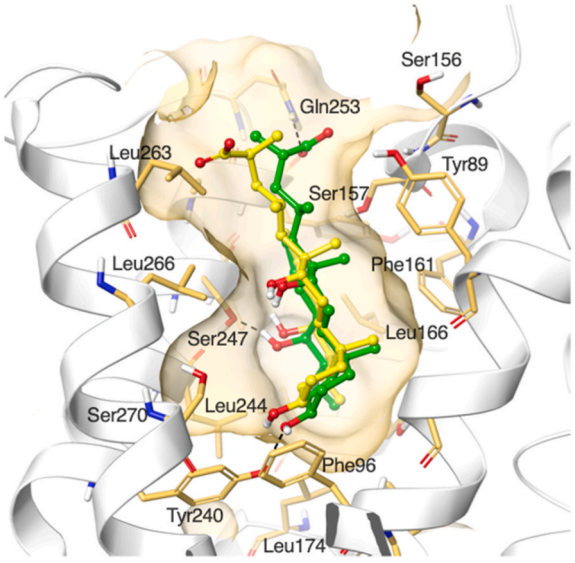


Fig. 7. Crystallographic (yellow carbon atoms) and modeling predicted (green carbon atoms) binding conformations for INT-777 (**9**). The residues on TGR5 are displayed with golden carbon atoms. The orthosteric pocket of TGR5 (PDB 7CFN) is displayed as golden surface. Residues 68–81 were omitted in the figure for clarity.

complex with INT-777 (**9**; PDB 7CFN) was used for our modeling studies [59]. To begin with, we docked INT-777 back into the receptor and found that the predicted conformation of the steroid nucleus of INT-777 superimposed well with its crystallographic binding pose in the orthosteric site (Fig. 7), validating the *in silico* calculations for predicting the binding conformations of the steroid scaffold. However, a small deviation from the orientation of the terminal end of the side chain of INT-777 can be observed between the predicted and crystal conformations. While the carboxylate group of INT-777 resided close to the hydrophobic Leu263 in the crystallographic pose, modeling predicted the formation of a hydrogen bond between the side chain carboxylate group of INT-777 and Gln253. This small difference between modeling and the crystal structure implies a likely degree of flexibility at the more spacious top of the TGR5 binding pocket.

Overall, all 12β-methyl-18-*nor*-BA analogues were found to bind in the same orientation as INT-777, with the steroid scaffold buried in the TGR5 pocket and the side chain interacting with the top of the TGR5 pocket (Figs. S2–S6). Furthermore, the great majority of BA analogues were predicted to establish polar interactions between their 3- and 7-hydroxy groups and the amino acid residues of Tyr240 and Ser247, respectively, which was consistent with those observed for INT-777. The exceptions were **44**, which was missing the interaction with its 7-hydroxy group, as well as **26** and **89** whose 3-hydroxy group was not engaged in any binding (Figs. S2 and 6).

Our test results of the 12β-methyl-18-*nor*-BA analogues displayed increased efficacy with the incorporation of a 6α-ethyl group. Comparison of the predicted binding conformations for compounds with and without the 6α-ethyl group revealed that the extra ethyl group binds in a small pocket surrounded largely by hydrophobic residues. Extra hydrophobic interactions could be established with Leu166, Leu174, Leu244, and Leu97 (Fig. 8), which likely contributed to the observed increased efficacies in the BA analogues incorporated with a 6α-ethyl group.

For bile acids with a terminal carboxylate side chain, the charged carboxylate group was able to reach further to the top of the TGR5 binding pocket as the side chain lengths increased (Fig. 9A and B). Favorable polar interactions could be established with residues such as Gln158 and Gln253 at the top of the binding pocket and likely contributed to the observed benefit on efficacies with increasing side chain lengths. Similarly, taurine conjugation to short carboxylate side chains (n = 0 or 1) effectively increased the length of the side chain and brought the polar end groups to the top of the binding pocket (**70**/**71**,

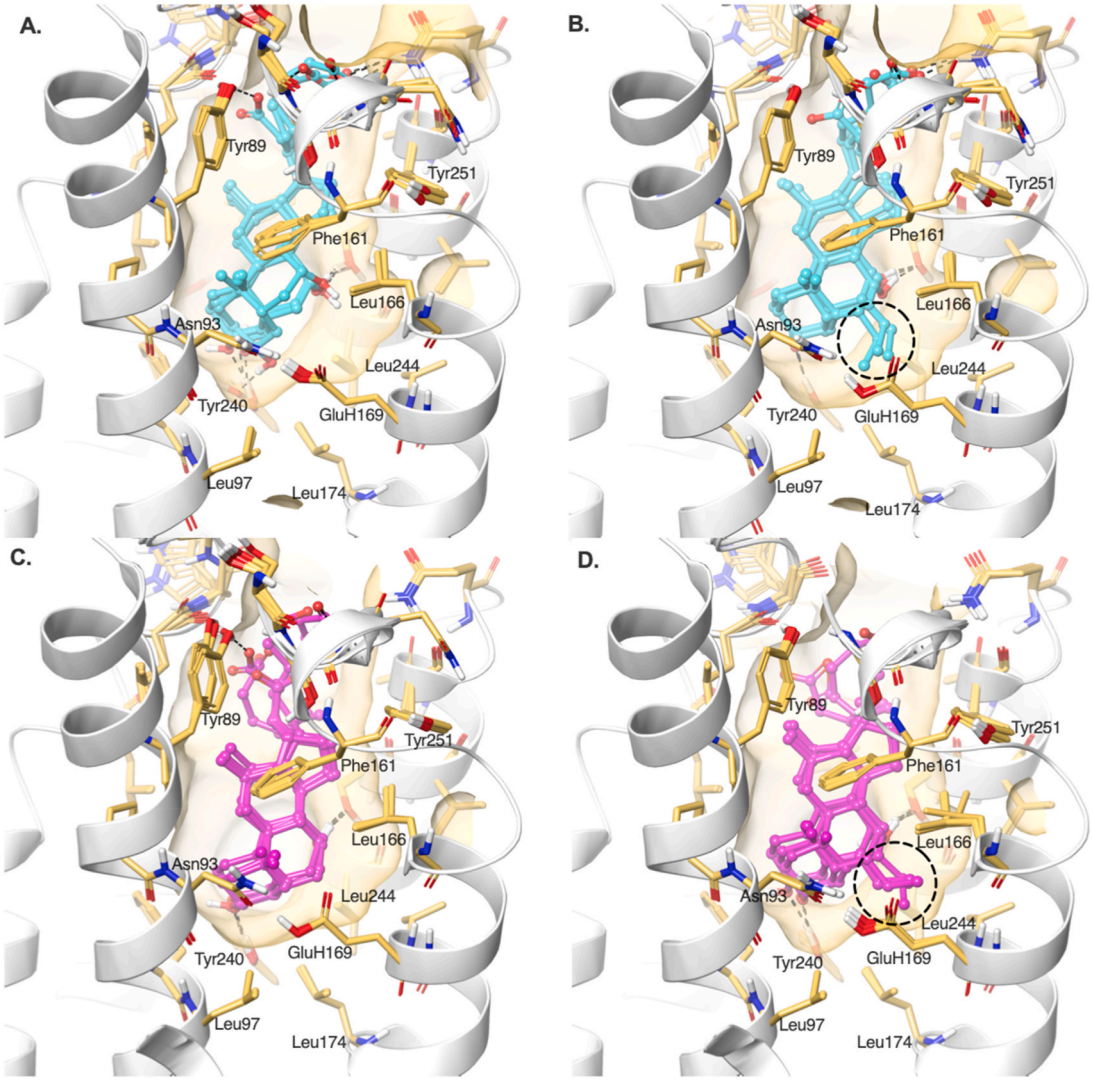


Fig. 8. Comparison of predicted binding conformations for compounds with and without 6α-ethyl group within the orthosteric pocket of TGR5 (PDB 7CFN). (A) Superimposition of compounds **15**, **21**, and **90**, with β-facing side chain and no 6α-ethyl group. (B) Superimposition of compounds **70**, **34**, and **52**, with β-facing side chain and additional 6α-ethyl group (highlighted in the dashed circle). (C) Superimposition of compounds **83**, **65**, **17**, **20**, and **91**, with α-facing side chain and no 6α-ethyl group. (D) Superimposition of compounds **88**, **75**, **43**, and **61**, with α-facing side chain and additional 6α-ethyl group (highlighted in the dashed circle).

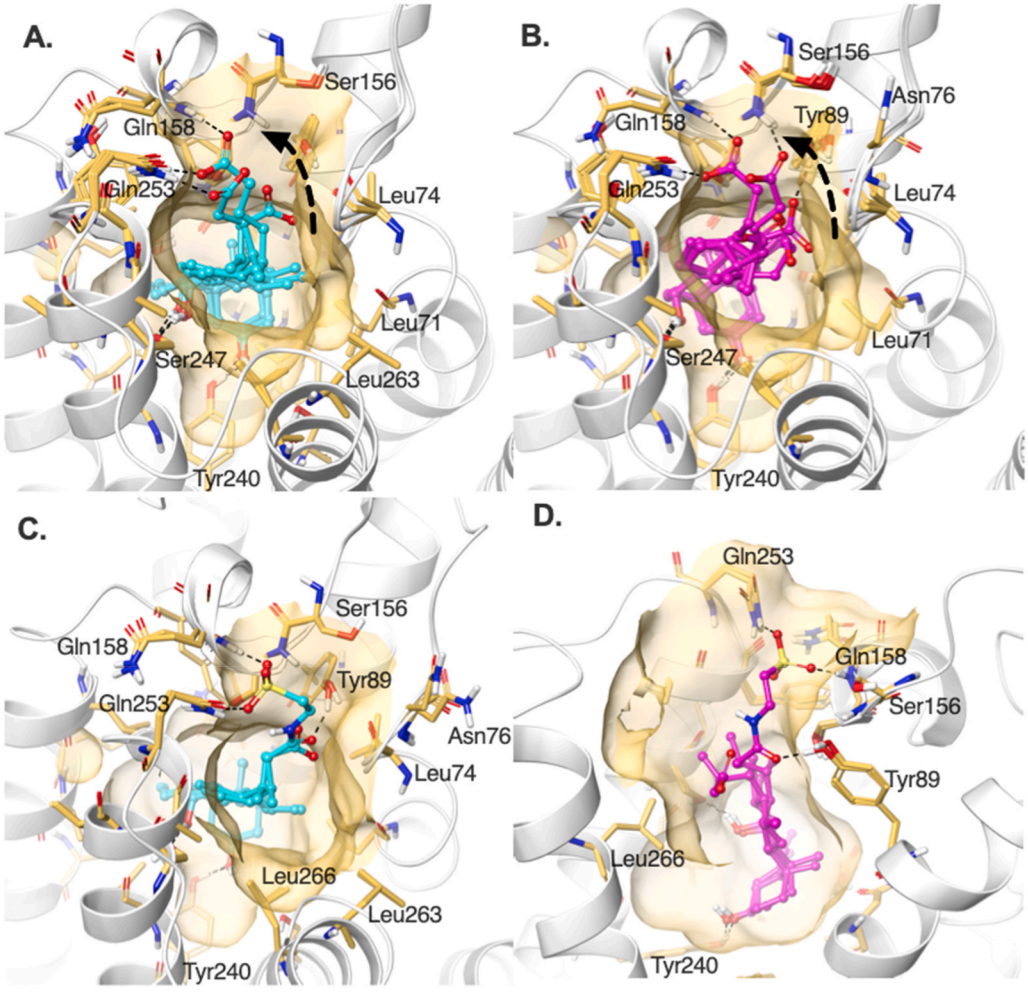


Fig. 9. (A) Predicted binding conformation for compounds **70**, **34**, and **52**, with increasing length of the β-facing side chain within the orthosteric pocket of TGR5 (PDB 7CFN). (B) Predicted binding conformation for compounds **83**, **65**, **17**, **20**, and **91**, with increasing length of the α-facing side chain. For both (A) and (B) the direction of increasing side chain length is indicated by the black dashed arrow. (C) Comparison of the predicted conformation for **70** (β-facing side chain) with its corresponding taurine conjugated compound **71**. (D) Comparison of the predicted conformation for **75** (α-facing side chain) with its corresponding taurine conjugated compound **76**.

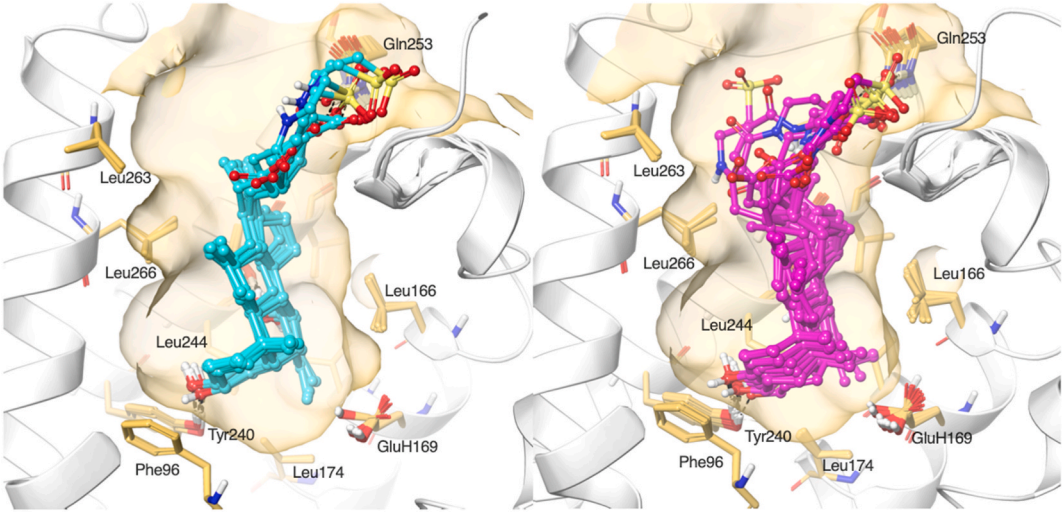


Fig. 10. Superimposition of the predicted binding pose for compounds with β-facing side chains (cyan carbon atoms, compounds **15**, **21**, **90**, **23**, **70**, **34**, **52**, **71**, **35**, and **53**) and with α-facing side chains (magenta carbon atoms, compounds **17**, **83**, **65**, **20**, **91**, **84**, **66**, **24**, **25**, **88**, **75**, **43**, **61**, **76**, **18**, and **19**) within the orthosteric pocket of TGR5 (PDB 7CFN).

Fig. 9C), which likely accounted for the positive impact on efficacies. On the other hand, it remained elusive as to why taurine conjugation of **75** did not result in similar increase in efficacy even though the same extension and interactions were observed in the modelled conformation for **76** (Fig. 9D).

In contrast to BAs with β-facing side chains, 12β-methyl-18-*nor*-BA analogues exhibiting α-facing side chains (epimerized at C17) were found to bind deeper, thus placing the BA A/B ring system closer to the

amino acids lining the bottom of the TGR5 binding pocket (Fig. 10), including Phe96, Glu169 (protonated), Leu174, Tyr240 and Leu244. Some of these residues have been identified as critical to BA recognition and in promoting the agonistic response (Fig. 10) [59]. For example, Tyr240 and Phe96 are important for sensing the agonistic signal; Leu244 is important for interaction with the 7-hydroxy group of BAs, and Glu169 contributes to the small binding pocket accommodating the 6α-ethyl substituent. As a result of the deeper binding, the 17-*epi*-BAs exhibit, in general, more favorable interaction energies with these critical residues (Table S3), thus providing a rationale for the enhanced efficacies of 17-*epi*-BAs towards TGR5.

3. Conclusion

In summary, we evaluated twenty-nine 12β-methyl-18-*nor*-bile acids for their ability to agonize TGR5. These compounds varied in the orientation of the side chain (α- *vs* β-side), the length of the side chain (ranging from *bisnor* (n = 0) to *bishomo* (n = 4)), taurine conjugation and the presence or absence of a 6α-ethyl group. Our results revealed that 12β-methyl-18-*nor*-bile acids with epimerization at the C17 position (α-oriented side chain) performed better as TGR5 agonists than those with a β-facing side chain (natural orientation). The introduction of a 6α-ethyl group as well as an extension of the side chain generally enhanced the ability of compounds to stimulate TGR5, although, for the series of 12β-methyl-18-*nor*-bile acids with a β-oriented side chain, this effect was only observed for the unconjugated, 6α-ethyl-modified analogues. Moreover, conjugation with taurine was able to increase the activities of 17-*epi*-bile acids without 6α-ethyl-substitution to levels similar to the best-performing, 6α-ethyl-modified and unconjugated analogues. The combination of 6α-ethyl-substitution and taurine extension of the side chain then led to the discovery of compounds **35**, **18** and **19** that exerted maximum responses at 99–100% in a commercially available secreted alkaline phosphatase (SEAP) reporter assay, matching those produced by LCA (**3**), TLCA (**4**) and INT-777 (**9**). Among these three compounds the 17-*epi*-BA analogues **18** and **19** emerged as the most potent agonists with EC_{50} values of 0.31 ± 0.05 (**18**) and 0.27 ± 0.05 μM (**19**) while **35** with a natural β-oriented side chain was less active with an EC_{50} value of 5.2 ± 0.9 μM. To assess the selectivity of our collection of 12β-methyl-18-*nor*-bile acids we also screened these for activity at FXR at a concentration of 10 μM. All compounds were found to be inactive; except the unconjugated 6α-ethyl-17-*epi*-bile acids **43** and **61** displayed activities of 23 and 31%, respectively. Therefore, our most active agonists **35**, **18** and **19** represent selective TGR5 agonists. We also conducted an *in silico* study into the likely binding mode of the tested 12β-methyl-18-*nor*-bile acids. Our results showed that 17-*epi*-bile acids were able to form stronger interactions with those amino acid residues in the TGR5 binding pocket that were found to be critical for recognition and response. A 6α-ethyl substituent had the additional benefit of occupying a small hydrophobic pocket. Moreover, increasing side chain lengths could place the hydrophilic end group closer to the top of the TGR5 pocket and establish favorable polar interactions. Taken together, these effects likely contributed to the increased efficacies of BAs with longer side chains. Despite decades of BA research leading to potent and selective BA-based TGR5 agonists, to our knowledge, we report the first examples where unnatural 17-*epi*-bile acids are not only agonists of TGR5 but are more potent than their congeners with a naturally-configured side chain. This work lays the foundation for further evaluation of this new class of 17-*epi*-bile acids.

4. Experimental section

4.1. General methods

Melting points were determined by differential scanning calorimetry (DSC) on a Mettler Toledo DSC1 instrument at a heating rate of 10 K min^{-1}. Proton (^{1}H) and carbon (^{13}C) NMR-spectra were recorded on a Bruker Avance (III)-500 spectrometer. Chemical shifts are reported in ppm relative to Me_4Si (TMS, δ 0), or residual solvent peaks as an internal standard set to δ 7.26 and 77.00 ($CDCl_3$), or δ 3.31 and 49.05 (CD_3OD), or δ 2.50 and 39.50 (DMSO-d_6), or δ 4.79 (D_2O) or using the spectrometer's default referencing. NMR data is reported as follows: chemical shift in ppm, multiplicity (ap, apparent; s, singlet; d, doublet; t, triplet; q, quartet; sext., sextet; br, broad; dd, doublet of doublets; td, triplet of doublets; dt, doublet of triplets; m, multiplet), coupling constant in Hz, integration.

High resolution electrospray mass spectra (ESI-HRMS) were recorded on a Waters Xevo G2-XS Q-Tof mass spectrometer equipped with an electrospray ion source and controlled by MassLynx software (version 4.2). Sodium formate solution was used to calibrate the system over a mass range from 100 to 1200 u and leucine enkephalin was employed as lockmass for accurate mass measurements. The optimized conditions are as follows: capillary voltage 1–3 kv, sampling cone 30–50 v, source temperature 120 °C, desolvation gas flow 800 L/h and desolvation temperature 500 °C.

The purity of all relevant compounds including NZP196 (**18**) and NZP917 (**19**) was evaluated by liquid chromatography-mass spectrometry (LCMS) and determined to be ≥ 95% except bile acid **21** which was >91%. The analytical LCMS analyses were carried out on an Agilent 1260 Infinity II Series LC System with an Agilent 6120B Single Quadrupole LC/MS (ESI), equipped with an Agilent 1100 Multi Wavelength Detector (WD) and an Agilent Infinity II 1290 Evaporative Light Scattering Detector (ELSD). Specific optical rotations were acquired on a Rudolph Autopol IV Automatic polarimeter at ambient temperature (20 °C), unless otherwise stated, λ = 589 nm and concentration (g/100 mL) in the solvent indicated, using a cell of path length 100 mm. All reactions were monitored by thin layer chromatography (TLC) using 0.2 μm silica gel (Merck Kieselgel 60 F_{254}) precoated aluminium plates, staining with ammonium molybdate staining solution to visualize. Flash column chromatography was performed on Davisil silica gel (60, particle size 0.040–0.063 mm), or using pre-packed silica cartridges from Reveleris or SiliCycle on a Grace Reveleris automated flash system with continuous gradient facility. Solvents for reactions and chromatography were analytical grade and were used as supplied unless stated otherwise. The 12β-methyl-18-*nor*-bile acids **15**, **17**, **20**, **21** and **44** were prepared as described in [47]. LCA (**3**, Lot A0323861) was purchased from Acros Organics and TLCA (**4**, Lot # SLBQ8462V) from Sigma Aldrich. INT-777 (**9**) was used as supplied by Cayman Chemical.

4.2. Experimental procedures

***N*-(3α,7α-Dihydroxy-12β-methyl-18-*nor*-5β-cholan-24-oyl)taurine sodium salt (23). Method A**: To a solution of **15** [47] (201 mg, 0.512 mmol) in dry *N*,*N*-dimethylformamide (DMF; 6 mL) were added 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ; 164 mg, 0.663 mmol), taurine (**22**; 70.1 mg, 0.560 mmol) and triethylamine (0.09 mL, 0.646 mmol) and the whole was heated at 90 °C for 70 min. The solvent was evaporated, and the residue purified on silica gel eluting with 0–45% methanol/dichloromethane (+1% acetic acid). The semi-purified product was then dissolved in methanol and a 2 M aqueous solution of sodium hydroxide was added until the pH was adjusted to 10. This was adsorbed onto on C18-silica gel and subsequently purified by automated reversed-phase column chromatography (C18-silica gel, 0–60% methanol/water) to yield 112 mg (42%) of the corresponding taurine conjugate **23** as a colorless resin.

Method B: To a solution of **15** (29 mg, 73.9 μmol) in dry *N*,*N*-dimethylformamide (DMF; 42 mL/g, 1.2 mL) at 0 °C were added triethylamine (TEA; 51 μL, 0.367 mmol, 5 equiv.) and diphenylphosphoryl azide (DPPA; 31 mg, 0.113 mmol, 1.5 equiv.). The solution was stirred at 0 °C for 20 min, at which point taurine (**22**; 46 mg, 0.370 mmol, 5 equiv.) was added. The reaction was allowed to warm to room temperature overnight before the DMF was removed under reduced pressure. The crude reaction mixture was re-dissolved in methanol (35 mL/g, 1 mL) and a 2 M aqueous solution of sodium hydroxide was added

dropwise until the pH was adjusted to 10. The resultant solution was adsorbed onto C18 silica gel and purified by reversed-phase flash chromatography (C-18 silica gel, 20–80% methanol in water), giving rise to 22 mg (57%) of conjugate **23** as a white powder.

$[\alpha]_D^{20}$ = +15.0 (*c* 0.25, water); ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.67 (t, J = 5.3 Hz, 1H, NH), 4.28 (d, J = 4.8 Hz, 1H, OH), 4.09 (d, J = 3.4 Hz, 1H, OH), 3.76-3.72 (m, 1H), 3.32-3.24 (m, 2H), 3.23-3.14 (m, 1H), 2.55-2.49 (m, 2H), 2.19 (dt, J = 12.0, 13.1 Hz, 1H), 2.09 (ddd, J = 14.1, 9.1, 5.1 Hz, 1H), 1.95-1.05 (m, 20H), 0.90 (d, J = 6.4 Hz, 3H), 0.89-0.67 (overlapping signals: m, 4H; 0.85, d, J = 6.6 Hz, 3H and 0.81, s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 171.79, 70.30, 65.98, 52.33, 50.55, 48.61, 47.27, 45.16, 41.35, 39.48, 38.03, 35.49, 35.41, 35.20, 34.54, 34.50, 34.16, 32.46, 30.57, 28.26, 25.23, 23.83, 22.84, 21.14, 19.56; HRMS (ESI) *m/z* calcd for $C_{26}H_{44}NO_6SNa_2^+$ 544.2679, found 544.2680. ^{1}H NMR (500 MHz, CD_3OD) δ 3.93-3.89 (m, 1H), 3.58 (t, J = 6.9 Hz, 2H), 3.38 (tt, J = 11.1, 4.4 Hz, 1H), 2.96 (t, J = 7.0 Hz, 2H), 2.31-2.19 (m, 2H), 2.07 (ddd, J = 14.0, 9.2, 7.3 Hz, 1H), 2.02-1.94 (m, 2H), 1.91-1.79 (m, 3H), 1.78-1.69 (m, 2H), 1.69-1.48 (m, 6H), 1.45-1.21 (m, 6H), 1.03-0.91 overlapping signals (m, 2H; 0.97, d, J = 6.6 Hz, 3H and 0.94, d, J = 6.7 Hz, 3H), 0.89 (s, 3H), 0.88-0.79 (m, 2H); ^{13}C NMR (125 MHz, CD_3OD) δ 176.31, 72.93, 68.98, 54.40, 51.51, 50.45, 49.00, 46.82, 43.18, 40.43, 39.97, 37.12, 36.63, 36.57 (2C), 36.11, 35.90, 35.66, 34.27, 31.40, 29.91, 27.20, 25.28, 23.56, 21.82, 20.03; HRMS (ESI) *m/z* calcd for $C_{26}H_{44}NO_6S^-$ 498.2895, found 498.2896.

***N*-(3α,7α-Dihydroxy-12β-methyl-17-*epi*-18-*nor*-5β-cholan-24-oyl)taurine sodium Salt (24). Method A**: Using the same procedure as described for the preparation of taurine conjugate **23** a mixture of **17** [47] (502 mg, 1.28 mmol), 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ; 413 mg, 1.67 mmol), taurine (**22**; 179 mg, 1.43 mmol) and triethylamine (0.23 mL, 1.65 mmol) in dry *N*,*N*-dimethylformamide (6 mL) was heated at 90 °C for 70 min to afford 452 mg (68%) of **24** as a colorless resin.

Method B: Conjugation of **17** (48 mg, 0.122 mmol) with taurine (**22**; 0.639 mmol, 80 mg) was carried out in dry *N*,*N*-dimethylformamide (2 mL) and employing DPPA (0.181 mmol, 39 μL) in the presence of triethylamine (TEA; 0.617 mmol, 86 μL) according to Method B described for the preparation of **23**. The crude product was purified by automated reversed-phase chromatography on C-18 silica gel (20–80% methanol in water) to afford 31 mg (49%) of **24** as a white powder.

$[\alpha]_D^{20}$ = −21.6 (*c* 1.0, H_2O); ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.66 (t, J = 5.5 Hz, 1H, NH), 4.28 (d, J = 5.1 Hz, 1H, OH), 4.10 (d, J = 4.7 Hz, 1H, OH), 3.73-3.69 (m, 1H), 3.31-3.25 (m, 2H), 3.21-3.13 (m, 1H), 2.52 (dd, J = 7.9, 7.1 Hz, 2H), 2.19 (dt, J = 11.9, 13.2 Hz, 1H), 2.15-2.09 (m, 1H), 2.07-1.97 (m, 2H), 1.90-1.15 (m, 16H), 0.98-0.81 overlapping signals (m, 4H and 0.91, d, J = 6.3 Hz, 3H), 0.80-0.75 (overlapping signals: d, 3H and 0.77, s, 3H), 0.58 (dt, J = 11.7, 12.3 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 171.72, 70.35, 65.84, 55.57, 50.52, 46.66, 42.71, 41.47, 40.89, 39.42, 35.38, 35.25 (2C), 34.50, 34.44, 33.69, 32.56, 32.33, 31.59, 31.12, 30.60, 28.79, 22.92, 22.16, 21.13, 16.70; HRMS (ESI) *m/z* calcd for $C_{26}H_{44}NO_6SNa_2^+$ 544.2679, found 544.2688. ^{1}H NMR (500 MHz, CD_3OD) δ 3.90-3.86 (m, 1H), 3.63-3.53 (m, 2H), 3.37 (tt, J = 11.2, 4.4 Hz, 1H), 2.96 (t, J = 6.9 Hz, 2H), 2.30-2.14 (m, 4H), 1.99-1.86 (m, 4H), 1.86-1.78 (m, 1H), 1.78-1.59 (m, 5H), 1.58-1.31 (m, 7H), 1.10-0.90 overlapping signals (m, 4H and 0.97, d, J = 6.3 Hz, 3H), 0.89 (d, J = 6.8 Hz, 3H), 0.86 (s, 3H), 0.68 (dt, J = 11.6, 12.4 Hz, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 176.24, 72.91, 68.69, 57.61, 51.48, 48.49, 44.50, 43.26, 42.89, 40.32, 36.99, 36.58, 36.58, 36.02, 35.63, 35.43, 34.24, 34.15, 33.29, 33.14, 31.43, 30.35, 23.65, 23.60, 21.80, 17.21; HRMS (ESI) *m/z* calcd for $C_{26}H_{44}NO_6S^-$ 498.2895, found 498.2894.

***N*-(3α,7α-Dihydroxy-12β-methyl-17-*epi*-25-*homo*-18-*nor*-5β-cholan-24-oyl)taurine sodium salt (25).** Employing Method A as outlined for the preparation of taurine conjugate **23**, bile acid **20**][47] (39 mg, 95.9 μmol) dissolved in *N*,*N*-dimethylformamide (1 mL) was coupled with taurine (**22**; 26 mg, 0.199 mmol; 2 equiv.) by treatment with EEDQ (39 mg, 0.149 mmol; 1.5 equiv.) in the presence of triethylamine (28 μL, 0.199 mmol; 2 equiv.) for 2 h at 90 °C to give 30 mg (58%) of **25** as a colorless powder. The compound was first purified by automated column chromatography on normal-phase silica gel eluting with methanol/dichloromethane (+1% acetic acid) 5–40% followed by adjusting the pH of the product to pH 10 with 2 M aqueous sodium hydroxide solution before additional purification on reversed-phase silica gel eluting with water/methanol 20–80%. $[\alpha]_D^{20}$ = −22.7 (*c* 0.525, MeOH); ^{1}H NMR (500 MHz, CD_3OD) δ 3.90-3.86 (m, 1H), 3.59 (t, J = 6.9 Hz, 2H), 3.37 (tt, J = 11.2, 4.4 Hz, 1H), 2.96 (t, J = 6.9 Hz, 2H), 2.30-2.19 (m, 2H), 2.16 (t, J = 7.6 Hz, 2H), 1.99-1.86 (m, 4H), 1.86-1.78 (m, 1H), 1.78-1.69 (m, 2H), 1.68-1.57 (m, 5H), 1.57-1.47 (m, 3H), 1.43-1.31 (m, 2H), 1.25-1.09 (m, 2H), 1.09-1.00 (m, 2H), 1.00-0.89 overlapping signals (m, 2H and 0.98, d, J = 6.3 Hz, 3H), 0.88 (d, J = 6.8 Hz, 3H), 0.86 (s, 3H), 0.69 (dt, J = 11.6, 12.4 Hz, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 176.02, 72.94, 68.73, 57.67, 51.49, 48.49, 44.49, 43.29, 43.07, 40.35, 38.04, 37.56, 37.00, 36.58 (2C), 36.03, 35.63, 34.17, 33.33, 33.25, 31.45, 30.34, 24.98, 23.68, 23.66, 21.83, 17.42; HRMS (ESI) *m/z* calcd for $C_{27}H_{46}NO_6S^-$ 512.3051, found 512.3047.

***N*-(3α,7α-Dihydroxy-12β-methyl-18,24-*bisnor*-5β-cholan-23-oyl)taurine sodium salt (26).** To a solution of the 24-*nor*-bile acid **21** [47] (302 mg, 0.80 mmol) and triethylamine (0.13 mL, 0.933 mmol) in dry tetrahydrofuran (16 mL) was added dropwise isobutyl chloroformate (IBCF; 0.10 mL, 0.771 mmol) at −10 °C. The reaction was stirred for 1 h while maintaining the reaction temperature between −10 and −2 °C. Then a second portion of triethylamine (0.14 mL, 1.01 mmol) was introduced at −10 °C followed by addition of an aqueous solution of taurine (**22**; 126 mg, 0.99 mmol in 1.6 mL of water). Subsequently, the reaction was allowed to warm to room temperature and stirred overnight. Then the reaction was concentrated, re-dissolved/suspended in methanol and treated with a 2 M aqueous solution of sodium hydroxide (1.6 mL), adsorbed onto normal phase silica gel and purified by automated column chromatography (silica gel, methanol/chloroform 0–50%) before additional purification on reversed-phase silica gel eluting with water/methanol 20–80% to yield 304 mg of **26** (75%) as a colorless powder. $[\alpha]_D^{20}$ +10.7 (*c* 0.998, water); ^{1}H NMR (500 MHz, D_2O) δ 4.04 (s_{br}, 1H), 3.72-3.60 (m, 2H), 3.60-3.52 (m, 1H), 3.16 (t, J = 7.1 Hz, 2H), 2.44-2.34 (m, 2H), 2.17 (ap q, J = 12.1 Hz, 1H), 2.07-1.86 (m, 6H), 1.82-1.63 (m, 5H), 1.59-1.39 (m, 5H), 1.31 (t, J = 10.2 Hz, 1H), 1.15-1.03 (overlapping signals: 1.12, d, J = 5.9 Hz, 3H and m, 1H), 1.03-0.91 (overlapping signals: m, 4H and 0.98, s, 3H), 0.90-0.80 (m, 1H), 0.74 (ap q, J = 9.6 Hz, 1H); ^{13}C NMR (125 MHz, D_2O) δ 175.84, 71.67, 67.93, 53.49, 50.10, 48.04, 47.44, 45.49, 41.36, 38.43, 38.05, 37.27, 35.98, 35.39, 35.17, 34.85, 34.02, 32.91, 32.84, 29.90, 28.58, 24.20, 23.35, 21.32, 19.04; HRMS (ESI) *m/z* calcd for $C_{25}H_{42}NO_6SNaH^+$ 508.2703, found 508.2708 and *m/z* calcd for $C_{25}H_{42}NO_6SNa_2^+$ 530.2523, found 530.2527.

3α-Hydroxy-12β-methyl-7-oxo-18-*nor*-5β-cholan-24-oic acid (28). Method A: To a solution of compound **15** [47] (3.94 g, 10.0 mmol) in a 3:1:6.4:0.25 mixture of methanol (48 mL), acetic acid (16 mL), ethyl acetate (102 mL) and water (4 mL) were added sodium bromide (51.0 mg, 0.496 mmol) and tetra-*n*-butylammonium bromide (TBAB; 10.8 g, 33.5 mmol). This mixture was cooled to 0 °C and bleach (2 M; 5.5 mL, 11.0 mmol) was introduced dropwise. The ice bath was removed, and the reaction stirred for a further 4.5 h. After complete oxidation (TLC analysis; if required more bleach was added to drive the reaction to completion) the reaction was quenched with sodium sulfite solution (3.3%) and subsequently extracted with ethyl acetate (3 ×). The combined organic phases were washed with brine, dried over $MgSO_4$ and concentrated. The residue was purified by automated flash column chromatography [silica gel, acetone (+1% acetic acid)/dichloromethane (+1% acetic acid) 2–25%] to give 3.14 g (80%) of **28** as a colorless foam. In addition, 634 mg (16%) of the 3,7-dioxo by-product **27** and 137 mg (4%) of the 3-oxo isomer were recovered. An analytical sample of the two side products was obtained by recrystallization

from ethyl acetate/petroleum ether.

Method B: To a solution of compound **15** (6.70 g, 17.1 mmol) in methanol (40 mL/g; 270 mL) was added water (1.3 mL/g; 9 mL) and sodium bicarbonate (3 equiv., 51 mmol). The suspension was cooled to 0 °C before bromine (1.3 equiv.; 1.11 mL, 21.5 mmol) was introduced dropwise. After 1 h water (3 mL) was added and the mixture stirred at 0 °C overnight. Then *iso*-propanol (5 mL) was added and the mixture allowed to warm to room temperature before 2 M aqueous sodium hydroxide solution (20 mL) was added followed by 1 M aqueous thiosulfate solution (5 mL). The mixture was concentrated to remove methanol before ethyl acetate 100 mL and 1 M hydrochloric acid solution (100 mL) were added. The aqueous phase was extracted with ethyl acetate (3 ×) and the combined organics were washed with brine, dried over $MgSO_4$ and concentrated. The crude product was purified by automated flash column chromatography [silica gel, acetone (+1% acetic acid)/ dichloromethane (+1% acetic acid) 0–40%] to yield 4.1 g (62%) of **28** as an off-white solid.

3α-Hydroxy-12β-methyl-7-oxo-18-nor-5β-cholan-24-oic acid (**28**). $[\alpha]_D^{20} = -25.2$ (*c* 1.00, $CHCl_3$); $[\alpha]_D^{20} = -15.4$ (*c* 0.39, MeOH); ^{1}H NMR (500 MHz, CD_3OD) δ 3.53 (tt, $J = 10.9$, 4.7 Hz, 1H), 2.98 (dd, $J = 13.0$, 6.2 Hz, 1H), 2.37 (ddd, $J = 15.4$, 8.7, 5.5 Hz, 1H), 2.31 (ap t, $J = 11.0$ Hz, 1H), 2.22-2.14 (m, 2H), 1.98-1.45 (m, 12H), 1.40-1.13 (m, 6H), 1.19 (s, 3H), 1.05 (ap q, $J = 12.1$ Hz, 1H), 0.99 (d, $J = 6.6$ Hz, 3H), 0.93 (d, $J = 6.7$ Hz, 3H), 0.91-0.78 (m, 2H); ^{13}C NMR (125 MHz, CD_3OD) δ 215.03, 177.91, 71.58, 55.67, 53.72, 49.26, 47.71, 47.53, 46.17, 44.31, 39.42, 38.30, 38.16, 36.27, 36.20, 35.25, 33.56, 31.07, 30.75, 26.23, 25.29, 23.58, 21.52, 19.89; HRMS (ESI) *m/z* calcd for $C_{24}H_{38}O_4Na^+$ 413.2662, found 413.2660.

12β-Methyl-3,7-dioxo-18-nor-5β-cholan-24-oic acid (**27**). Mp 130–134 °C (DSC); $[\alpha]_D^{20} = -22.9$ (*c* 0.615, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.87 (dd, $J = 13.0$, 5.6 Hz, 1H), 2.46 (ddd, $J = 15.7$, 9.7, 5.4 Hz, 1H), 2.34-2.17 (m, 8H), 2.12 (ddd, $J = 14.4$, 5.3, 2.9 Hz, 1H), 2.04 (dt, $J = 11.9$, 3.6 Hz, 1H), 1.98 (dd, $J = 13.0$, 2.2 Hz, 1H), 1.85-1.69 (m, 3H), 1.68-1.59 (m, 2H), 1.57 (td, $J = 14.4$, 4.7 Hz, 1H), 1.51-1.39 (m, 2H), 1.37-1.24 (m, 2H), 1.27 (s, 3H), 1.12 (ap q, $J = 12.1$ Hz, 1H), 0.99 (d, $J = 6.6$ Hz, 3H), 0.93 (d, $J = 6.7$ Hz, 3H), 0.89-0.77 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 211.00, 210.26, 179.33, 54.55, 52.32, 47.77, 47.69, 46.07, 44.72, 42.93, 42.64, 37.84, 37.15, 36.81, 35.44, 35.31, 34.87, 32.41, 29.90, 24.64, 24.20, 22.46, 20.96, 19.40; HRMS (ESI) *m/z* calcd for $C_{24}H_{36}O_4Na^+$ 411.2506, found 411.2521.

7α-Hydroxy-12β-methyl-3-oxo-18-nor-5β-cholan-24-oic acid. $[\alpha]_D^{20} = +36.4$ (*c* 0.585, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.05-4.01 (m, 1H), 3.37 (dd, $J = 15.2$, 13.8 Hz, 1H), 2.50-2.37 (m, 2H), 2.26 (ddd, $J = 16.0$, 9.3, 6.8 Hz, 1H), 2.23-2.04 (m, 4H), 1.95 (ddd, $J = 15.0$, 5.3, 3.6 Hz, 1H), 1.87-1.72 (m, 5H), 1.66 (dt, $J = 13.1$, 3.6 Hz, 1H), 1.63-1.48 (m, 3H), 1.46-1.24 (m, 5H), 1.06-0.96 (overlapping signals: m, 1H; 0.98, d, $J = 6.8$ Hz, 3H and 0.97, s, 3H), 0.95-0.84 (overlapping signals: m, 2H and 0.93, d, $J = 6.7$ Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 213.23, 179.07, 68.36, 53.00, 48.91, 47.60, 45.58, 45.36, 43.10, 38.43, 37.00, 36.82, 36.06, 35.25, 35.02, 33.69, 33.43, 32.45, 28.68, 24.81, 24.25, 22.04, 21.18, 19.45; HRMS (ESI) *m/z* calcd for $C_{24}H_{38}O_4Na^+$ 413.2662, found 413.2675.

Methyl 3α-hydroxy-12β-methyl-7-oxo-18-*nor*-5β-cholan-24-oate (29). To a solution of **28** (2.84 g, 7.27 mmol) in dry methanol (120 mL) was added a catalytic amount of *p*-toluenesulfonic acid monohydrate (100 mg, 0.526 mmol) and the whole was refluxed for 2.5-4 h. After complete methyl ester formation, the reaction was allowed to cool to room temperature before being quenched with pyridine (3 mL, 37.1 mmol) and concentrated. The crude methyl ester was purified by automated flash column chromatography (silica gel, ethyl acetate/petroleum ether 2–50%) to yield 2.59 g (88%) of the corresponding methyl ester **29** as a colorless foam. ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.66 (s, 3H), 3.58 (tt, $J = 11.0$, 4.7 Hz, 1H), 2.84 (dd, $J = 12.6$, 6.0 Hz, 1H), 2.40 (ddd, $J = 15.3$, 9.7, 5.4 Hz, 1H), 2.25-2.12 (m, 3H), 1.99-1.92 (m, 2H), 1.91-1.83 (m, 2H), 1.82-1.65 (m, 5H), 1.63-1.53 (m, 2H), 1.48-1.40 (m, 1H), 1.40-1.12 (m, 6H), 1.16 (s, 3H), 1.03-0.93 (overlapping signals: m, 1H and 0.96, d, $J = 6.6$ Hz, 3H), 0.90 (d, $J = 6.7$ Hz, 3H), 0.85-0.73 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 212.18, 174.47, 70.79, 54.45, 52.26, 51.38, 47.79, 46.13 (2C), 45.07, 42.59, 37.77, 37.39, 36.85, 34.96, 34.77, 34.14, 32.56, 29.92, 29.81, 24.81, 24.10, 23.02, 20.89, 19.36; HRMS (ESI) *m/z* calcd for $C_{25}H_{40}O_4Na^+$ 427.2819, found 427.2817.

Methyl 12β-methyl-3α,7-*bis*(trimethylsilyloxy)-18-*nor*-5β-cholan-6(7)-en-24-oate (30). To a solution of lithium diisopropylamide (2 M in THF/heptane/ethyl benzene; 21.5 mL, 43.0 mmol) in dry tetrahydrofuran (26 mL) was added slowly trimethylsilyl chloride (6.7 mL, 52.8 mmol) at −78 °C. After 10 min a solution of **29** (2.45 g, 6.06 mmol) in dry tetrahydrofuran (10 mL) was introduced dropwise at −78 °C *via* cannula and the reaction was stirred for another 30–40 min. Then triethylamine (1.33 mL, 9.54 mmol) was added at the same temperature. The reaction was stirred for another hour at −78 °C before being allowed to warm to −20 °C at which point saturated bicarbonate solution was added to quench. At room temperature the mixture was diluted with water and the aqueous phase was extracted with ethyl acetate (3 ×). The organic phases were combined, washed with brine, dried over Na_2SO_4 and concentrated. The crude product was purified by automated flash column chromatography (silica gel, ethyl acetate/petroleum ether 2–10%) to afford 2.81 g (85%) of the silyl enol ether **30** as a colorless oil that solidified on standing. ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.72 (dd, $J = 5.9$, 1.7 Hz, 1H), 3.67 (s, 3H), 3.52 (tt, $J = 10.9$, 4.5 Hz, 1H), 2.40 (ddd, $J = 15.2$, 9.8, 5.2 Hz, 1H), 2.20 (ddd, $J = 15.5$, 9.1, 7.1 Hz, 1H), 2.15-2.08 (m, 1H), 1.86-1.58 (m, 8H), 1.58-1.42 (m, 4H), 1.38-1.23 (m, 4H), 1.22-1.06 (m, 2H), 1.05 (td, $J = 14.2$, 3.1 Hz, 1H), 0.95 (d, $J = 6.6$ Hz, 3H), 0.90 (d, $J = 6.8$ Hz, 3H), 0.88-0.74 (overlapping signals: m, 2H and 0.80, s, 3H), 0.17 (s, 9H), 0.11 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.63, 151.65, 108.44, 71.62, 55.01, 51.44, 51.28, 47.63, 47.11, 42.84, 40.88, 39.34, 38.87, 35.48, 35.21, 34.68, 33.08, 32.79, 32.39, 30.73, 25.00, 24.81, 22.47, 21.39, 19.45, 0.39 (3C), 0.24 (3C); HRMS (ESI) *m/z* calcd for $C_{28}H_{48}O_4SiNa^+$ ([M-Si$(CH_3)_3$+Na]$^+$) 499.3214, found 499.3219, and calcd for $C_{31}H_{56}O_4Si_2Na^+$ 571.3609, found 571.3622.

Methyl 6-ethyliden-3α-hydroxy-12β-methyl-7-oxo-18-*nor*-5β-cholan-24-oate (31). To a solution of **30** (2.80 g, 5.10 mmol) and acetaldehyde (0.55 mL, 9.80 mmol) in dry dichloromethane (11 mL) was added dropwise a solution of boron trifluoride diethyl etherate (2.33 mL, 18.9 mmol) in dry dichloromethane (7 mL) at -60 °C. After being stirred for 2 h at this temperature the reaction was allowed to warm to room temperature at which point it was quenched with saturated bicarbonate solution. The phases were separated, and the aqueous layer was extracted with ethyl acetate (3 ×). The combined organic phases were washed with brine, dried over Na_2SO_4 and concentrated. The crude reaction product was re-dissolved in dichloromethane (15 mL) and stirred vigorously with a 3 M aqueous solution of hydrochloric acid (7 mL) for 1 h. The reaction mixture was subsequently quenched with saturated bicarbonate solution and the phases were separated. The aqueous layer was extracted another three times with ethyl acetate and the combined organic phases were washed with brine, dried over Na_2SO_4 and concentrated. The crude product was purified by automated flash column chromatography (silica gel, ethyl acetate/petroleum ether 2–40%) to give 1.66 g (76%) of **31** as a colorless foam. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.24-6.19 (m, 1H), 3.71-3.62 (overlapping signals: m, 1H and 3.66, s, 3H), 2.58 (dd, $J = 13.1$, 4.2 Hz, 1H), 2.53-2.46 (m, 1H), 2.40 (ddd, $J = 15.3$, 9.8, 5.4 Hz, 1H), 2.19 (ddd, $J = 15.5$, 9.2, 7.1 Hz, 1H), 2.09 (td, $J = 11.8$, 3.6 Hz, 1H), 2.02 (dd, $J = 12.1$, 9.6 Hz, 1H), 1.97 (dt, $J = 14.4$, 3.3 Hz, 1H), 1.83-1.55 (m, 7H), 1.70 (d, $J = 7.1$ Hz, 3H), 1.54 (d, $J = 4.0$ Hz, 1H, OH), 1.50-1.43 (m, 1H), 1.40-1.23 (m, 5H), 1.17 (td, $J = 14.2$, 3.2 Hz, 1H), 1.04-0.93 (overlapping signals: m, 2H; 0.97, d, $J = 6.6$ Hz, 3H and 0.97, s, 3H), 0.91 (d, $J = 6.7$ Hz, 3H), 0.81 (ap q, $J = 10.2$ Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 204.06, 174.55, 143.16, 130.08, 70.62, 53.73, 53.52, 51.44, 47.83, 47.50, 45.60, 39.01, 37.87, 37.59, 36.42, 34.91, 34.55, 34.52, 32.69, 31.05, 29.85, 24.90, 24.36, 22.84, 21.07, 19.41, 12.71; HRMS (ESI) *m/z* calcd for $C_{27}H_{42}O_4Na^+$ 453.2975, found 453.2976.

Methyl 6-ethyl-3α-hydroxy-12β-methyl-7-oxo-18-*nor*-5β-cholan-24-oate (32). To a solution of the 6-ethylidene methyl ester **31** (1.30 g, 3.02 mmol) dissolved in ethanol (40 mL) was added 10% palladium on charcoal (200 mg) and the resulting mixture was stirred under an atmosphere of hydrogen at room temperature and atmospheric pressure overnight. After complete hydrogenation the reaction was filtered through a pad of celite and the filtrate was concentrated. The crude product was then purified by automated flash column chromatography (silica gel, ethyl acetate/petroleum ether 0–50%) to afford 921 mg (71%) of **32** as a colorless foam. ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.67 (s, 3H), 3.56 (tt, J = 10.5, 4.7 Hz, 1H), 2.41 (ddd, J = 15.4, 9.9. 5.3 Hz, 1H), 2.33 (dd, J = 11.9, 10.2 Hz, 1H), 2.24-2.15 (m, 2H), 1.99-1.86 (m, 3H), 1.86-1.53 (m, 11H), 1.48-1.37 (m, 2H), 1.32-1.13 (m, 5H), 1.18 (s, 3H), 1.02-0.93 (overlapping signals: m, 1H and 0.95, d, J = 6.6 Hz, 3H), 0.91 (d, J = 6.7 Hz, 3H), 0.85 (t, J = 7.3 Hz, 3H), 0.84-0.72 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 215.39, 174.59, 70.73, 61.91, 52.23, 51.45, 50.62, 49.65, 47.93, 46.04, 42.96, 39.93, 37.74, 36.75, 35.43, 35.41, 34.89, 32.65, 29.96, 29.69, 26.50, 26.06, 24.91, 24.09, 21.00, 19.42, 13.05; HRMS (ESI) *m/z* calcd for $C_{27}H_{44}O_4Na^+$ 455.3132, found 455.3143.

6α-Ethyl-3α-hydroxy-12β-methyl-7-oxo-18-*nor*-5β-cholan-24-oic acid (33). To a solution of compound **32** (1.20 g, 2.77 mmol) in methanol (14 mL) was added 2 M aqueous sodium hydroxide solution (14 mL, 28 mmol). The reaction was heated to 90 °C overnight before allowing to cool and evaporating off the methanol. Water (10 mL) was added and the solution was poured dropwise into cold 1 M hydrochloric acid solution. The precipitate was dissolved in ethyl acetate and the organic phase separated. The aqueous phase was extracted another three times with ethyl acetate and the combined organic fractions were concentrated to afford 1.18 g (quant.) of crude **33**. ^{1}H NMR (500 MHz, CD_3OD) δ 3.47 (tt, J = 10.8, 4.6 Hz, 1H), 2.85-2.79 (m, 1H), 2.37 (ddd, J = 15.3, 8.8, 5.5 Hz, 1H), 2.31-2.24 (m, 1H), 2.22-2.13 (m, 2H), 1.95 (td, J = 11.8, 3.2 Hz, 1H), 1.92-1.54 (m, 10H), 1.54-1.44 (m, 1H), 1.43-1.34 (m, 1H), 1.33-1.09 (m, 5H), 1.22 (s, 3H), 1.09-0.98 (overlapping signals: m, 1H and 1.00, d, J = 6.6 Hz, 3H), 0.94 (d, J = 6.7 Hz, 3H), 0.91-0.76 (overlapping signals: m, 3H and 0.83, t, J = 7.4 Hz, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 215.34, 177.86, 71.75, 56.22, 53.61, 53.00, 52.09, 49.30, 47.70, 45.11, 39.35, 38.22, 36.70, 36.21, 35.32, 33.64, 32.57, 30.85, 30.63, 26.19, 25.30, 24.10, 21.58, 19.99 (2C), 12.36; HRMS (ESI) *m/z* calcd for $C_{26}H_{42}O_4Na^+$ 441.2975, found 441.2985.

6α-Ethyl-3α,7α-dihydroxy-12β-methyl-18-*nor*-5β-cholan-24-oic acid (34). To a solution of **32** (921 mg, 2.13 mmol) dissolved in methanol (13.5 mL) was added an 8 M aqueous potassium hydroxide solution (1.35 mL) and the resulting reaction mixture was stirred at room temperature overnight. After complete reaction (TLC analysis) the solvent (methanol) was evaporated and the crude product re-dissolved in water and subsequently acidified by adding dropwise a 1 M hydrochloric acid solution. The formed precipitate was extracted with ethyl acetate (3 ×) and the combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated to give crude **33** (972 mg) which was used in the next reaction step without further purification.

The crude (**33**) was re-dissolved in a 4:1 mixture of tetrahydrofuran and water (50 mL) and sodium borohydride (450 mg, 11.9 mmol) was added in portions at 0 °C. After being stirred at this temperature for 2 h the solvents were evaporated, and the residue was taken up in water and acidified to pH 1 by dropwise addition of a 1 M aqueous solution of hydrochloric acid. The formed colorless precipitate was extracted with ethyl acetate (3 ×) and the combined organic phases were washed with brine, dried over $MgSO_4$ and concentrated. The crude product was purified by automated flash column chromatography [silica gel, acetone (+1% acetic acid)/dichloromethane (+1% acetic acid) 2–30%] to yield 639 mg (71%) of **34** over two steps as a colorless solid. Mp 148–150 °C (DSC); $[\alpha]_D^{20}$ = +11.4 (*c* 1.00, MeOH); ^{1}H NMR (500 MHz, CD_3OD) δ 3.79-3.75 (m, 1H), 3.32 (tt, J = 11.2, 4.4 Hz, 1H), 2.38 (ddd, J = 15.3, 8.9, 5.5 Hz, 1H), 2.22-2.14 (m, 1H), 1.98-1.80 (m, 5H), 1.80-1.70 (m, 3H), 1.67-1.48 (m, 6H), 1.48-1.34 (m, 3H), 1.33-1.20 (m, 4H), 1.05-0.96 (overlapping signals: m, 1H and 0.97, d, J = 6.6 Hz, 3H), 0.95-0.88 (overlapping signals: m, 1H; 0.93, d, J = 6.9 Hz, 3H and 0.91, t, J = 7.2 Hz, 3H), 0.88 (s, 3H), 0.87-0.77 (m, 2H); ^{13}C NMR (125 MHz, CD_3OD) δ 178.01, 73.30, 71.16, 54.51, 50.50, 49.07, 47.82, 46.99, 42.92, 39.97, 37.40, 36.86, 36.61, 36.47, 34.75, 34.46, 33.69, 31.35, 29.94, 26.43, 25.36, 23.97, 23.52, 21.84, 19.95, 12.09; HRMS (ESI) *m/z* calcd for $C_{26}H_{44}O_4Na^+$ 443.3132, found 443.3138.

***N*-(6α-Ethyl-3α,7α-dihydroxy-12β-methyl-18-*nor*-5β-cholan-24-oyl)taurine sodium salt (35).** Deploying the same method as described for the preparation of compound **26**, bile acid **34** (250 mg, 0.597 mmol) dissolved in dry tetrahydrofuran (12 mL) was activated with isobutyl chloroformate (IBCF; 0.11 mL, 0.848 mmol) in the presence of triethylamine (TEA; 0.1 mL, 0.718 mmol) and subsequently treated with taurine (**22**; 100 mg, 0.799 mmol) and a second addition of triethylamine (0.1 mL) to give 257 mg (79%) of **35** as a colorless powder. The compound was additionally purified by automated reversed-phase column chromatography (C18 silica gel, water/methanol 5–70%). $[\alpha]_D^{20}$ = +7.5 (*c* 1.0, water); ^{1}H NMR (500 MHz, CD_3OD) δ 3.79-3.76 (m, 1H), 3.59 (t, J = 6.9 Hz, 2H), 3.35-3.29 (m, 1H), 2.96 (t, J = 6.9 Hz, 2H), 2.28 (ddd, J = 14.4, 9.6, 5.1 Hz, 1H), 2.12-2.03 (m, 1H), 1.97-1.80 (m, 5H), 1.78-1.68 (m, 3H), 1.67-1.49 (m, 6H), 1.49-1.34 (m, 3H), 1.34-1.21 (m, 4H), 1.05-0.78 overlapping signals (m, 4H; 0.97, d, J = 6.7 Hz, 3H; 0.94, d, J = 6.6 Hz, 3H; 0.91, t, J = 7.1 Hz, 3H and 0.88, s, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 176.30, 73.25, 71.11, 54.42, 51.50, 50.47, 47.66, 46.94, 42.85, 39.92, 37.29, 36.79, 36.61, 36.56 (2C), 35.89, 34.69, 34.40, 31.30, 29.84, 27.19, 25.32, 23.93, 23.46, 21.84, 20.04, 12.03; HRMS (ESI) *m/z* calcd for $C_{28}H_{48}NO_6S^-$ 526.3208, found 526.3194.

3α-Hydroxy-12β-methyl-7-oxo-17-*epi*-18-*nor*-5β-cholan-24-oic acid (37). Method A: Following the same procedure as outlined in Method A for the preparation of **28**, 17-*epi*-bile acid **17** [47] (5.42 g, 13.8 mmol) was dissolved in a mixture of methanol (112 mL), acetic acid (37 mL), water (9 mL) and ethyl acetate (242 mL) followed by the addition of sodium bromide (71.0 mg, 0.690 mmol) and tetra-*n*-butylammonium bromide (TBAB; 14.8 g, 45.9 mmol). Subsequently, bleach (2 M, 7.5 mL, 14.0 mmol) was introduced dropwise at 0 °C to give 4.42 g (82%) of **37** as a colorless foam along with 477 mg (9%) of the corresponding diketone by-product **36** and 325 mg (6%) of the 3-oxo-isomer. A crystalline sample of each of the side products was obtained by crystallisation from ethyl acetate/petroleum ether.

Method B: Employing the same procedure as for the preparation of **28** according to Method B, a suspension of compound **17** (6.73 g, 17.1 mmol) in methanol (235 mL), water (13.5 mL) and sodium bicarbonate (6.0 g, 51.9 mmol) was treated with bromine (1.11 mL, 21.5 mmol) at 0 °C to yield 4.8 g (72%) of **37** as an off-white solid. The crude product was purified by automated flash column chromatography [silica gel, acetone (+1% acetic acid)/dichloromethane (+1% acetic acid) 0–40%]

3α-Hydroxy-12β-methyl-7-oxo-17-epi-18-nor-5β-cholan-24-oic acid (**37**). $[\alpha]_D^{20}$ = −65.4 (*c* 0.50, $CHCl_3$); ^{1}H NMR (500 MHz, CD_3OD) δ 3.53 (tt, J = 10.9, 4.8 Hz, 1H), 2.96 (dd, J = 13.1, 6.2 Hz, 1H), 2.35-2.15 (m, 4H), 2.06 (ap t, J = 10.7 Hz, 1H), 1.95-1.84 (m, 4H), 1.83 (sext d, J = 6.9, 2.7 Hz, 1H), 1.74 (dt, J = 12.9, 6.8 Hz, 1H), 1.71-1.58 (m, 4H), 1.57-1.40 (m, 4H), 1.34-1.25 (m, 1H), 1.23-1.11 (overlapping signals: m, 2H and 1.16, s, 3H), 0.99 (d, J = 6.3 Hz, 3H), 0.98-0.88 (m, 3H), 0.87 (d, J = 6.8 Hz, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 214.98, 177.86, 71.56, 57.10, 57.03, 47.43, 45.97, 43.93, 43.48, 41.84, 38.27, 37.87, 36.07, 35.23, 33.40, 33.17, 33.14, 32.89, 31.72, 30.81, 23.65, 23.60, 21.33, 17.16; HRMS (ESI) *m/z* calcd for $C_{24}H_{38}O_4Na^+$ 413.2662, found 413.2678.

12β-Methyl-3,7-dioxo-17-epi-18-nor-5β-cholan-24-oic acid (**36**). Mp 165–167 °C; $[\alpha]_D^{20}$ = −73.9 (*c* 0.62, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.85 (dd, J = 13.1, 5.4 Hz, 1H), 2.42-2.10 (m, 10H), 2.06-1.94 (m, 3H), 1.84-1.72 (overlapping signals: 1.80, sext d, J = 6.8, 1.9 Hz, 1H and 1.75, dt, J = 13.2, 3.2 Hz, 1H), 1.72-1.44 (m, 7H), 1.24 (s, 3H), 1.04-0.89 (overlapping signals: m, 3H and 0.99, d, J = 6.3 Hz, 3H), 0.87 (d, J = 6.8 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 210.81, 210.22,

179.15, 56.01, 55.67, 47.59, 44.54, 43.01, 42.14, 41.91, 40.56, 36.87 (2C), 35.46, 35.22, 32.02, 31.79 (2C), 31.66, 30.69, 22.79, 22.50, 20.87, 16.72; HRMS (ESI) *m/z* calcd for $C_{24}H_{36}O_4Na^+$ 411.2506, found 411.2514.

7α-Hydroxy-12β-methyl-3-oxo-17-epi-18-nor-5β-cholan-24-oic acid. Mp 195–197 °C (DSC); $[\alpha]_D^{20} = -26.0$ (*c* 0.65, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.01 (dd, $J = 5.7$, 2.9 Hz, 1H), 3.37 (dd, $J = 15.2$, 13.9 Hz, 1H), 2.43 (td, $J = 14.3$, 5.1 Hz, 1H), 2.39-2.30 (m, 2H), 2.26-2.13 (m, 3H), 2.08 (dt, $J = 14.0$, 4.5 Hz, 1H), 2.02 (td, $J = 11.8$, 3.4 Hz, 1H), 1.94 (ddd, $J = 15.0$, 5.3, 3.6 Hz, 1H), 1.88-1.81 (m, 2H), 1.81-1.74 (m, 2H), 1.74-1.61 (m, 2H), 1.61-1.45 (m, 5H), 1.42 (td, $J = 14.1$, 4.1 Hz, 1H), 1.17-1.08 (m, 2H), 1.08-0.99 (m, 1H), 0.98 (d, $J = 6.3$ Hz, 3H), 0.95 (s, 3H), 0.87 (d, $J = 6.8$ Hz, 3H), 0.81 (dt, $J = 11.7$, 12.5 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 213.43, 179.33, 68.19, 56.10, 46.81, 45.51, 43.14 (2C), 41.57, 36.98, 36.80, 35.80, 35.19, 33.56, 33.30, 32.16, 31.99, 31.91, 31.67, 29.09, 22.69, 22.12, 21.12, 16.79; HRMS (ESI) *m/z* calcd for $C_{24}H_{38}O_4Na^+$ 413.2662, found 413.2664.

Methyl 3α-hydroxy-12β-methyl-7-oxo-17-*epi*-18-*nor*-5β-cholan-24-oate (38). Using the same procedure as for the preparation of compound **29**, a solution of compound **37** (4.77 g, 12.2 mmol) and a catalytic amount of *p*-toluenesulfonic acid monohydrate in dry methanol (220 mL) was heated under reflux for 3 h. Subsequently the reaction was quenched with pyridine (5 mL, 61.8 mmol) at room temperature to yield 4.77 g (97%) of the corresponding 7-oxo-methyl ester **38** as a colorless oil. $[\alpha]_D^{20} = -66.8$ (*c* 0.37, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.66 (s, 3H), 3.61 (tt, $J = 10.9$, 4.8 Hz, 1H), 2.82 (dd, $J = 12.9$, 5.9 Hz, 1H), 2.37-2.26 (m, 3H), 2.14 (tt, $J = 8.6$, 3.2 Hz, 1H), 1.96 (dd, $J = 13.0$, 2.3 Hz, 1H), 1.93-1.82 (m, 4H), 1.80-1.39 (m, 11H), 1.35-1.20 (m, 2H), 1.20-1.11 (overlapping signals: m, 1H and 1.13, s, 3H), 0.95 (d, $J = 6.3$ Hz, 3H), 0.94-0.81 (overlapping signals: m, 3H and 0.85, d, $J = 6.8$ Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 211.44, 174.45, 71.03, 56.00, 55.78, 51.45, 45.96, 44.92, 42.13, 41.96, 40.47, 37.40, 36.67, 34.91, 34.15, 32.29, 32.12, 31.72 (2C), 30.79, 30.05, 23.13, 22.75, 20.89, 16.70; HRMS (ESI) *m/z* calcd for $C_{25}H_{40}O_4Na^+$ 427.2819, found 427.2822.

Methyl 12β-methyl-3α,7-*bis*(trimethylsilyloxy)-17-*epi*-18-*nor*-5β-cholan-6(7)-en-24-oate (39). Employing the same protocol as for the synthesis of compound **30**, trimethylsilyl chloride (12.1 mL, 95.3 mmol) was added slowly to a solution of a solution of lithium diisopropylamide (2 M in THF/heptane/ethyl benzene; 39.0 mL, 78.0 mmol) in dry tetrahydrofuran (48 mL) at −78 °C which was followed after 10 min by introducing dropwise a solution of 7-oxo-methyl ester **38** (4.77 g, 11.8 mmol) in dry tetrahydrofuran (18 mL). The reaction was stirred for a further 40 min before triethylamine (2.50 mL, 17.9 mmol) was added to give 6.23 g (96%) of the bis(silyl) ether **39** as a colorless oil. $[\alpha]_D^{20} = +13.2$ (*c* 0.63, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.72 (dd, $J = 5.8$, 1.0 Hz, 1H), 3.66 (s, 3H), 3.51 (tt, $J = 11.0$, 4.4 Hz, 1H), 2.36-2.25 (m, 2H), 2.21-2.14 (m, 1H), 2.14-2.08 (m, 1H), 1.84 (dt, $J = 14.4$, 3.2 Hz, 1H), 1.80-1.18 (m, 16H), 1.05 (td, $J = 14.3$, 3.1 Hz, 1H), 0.94-0.87 (overlapping signals: m, 1H and 0.93, d, $J = 6.3$ Hz, 3H), 0.79 (s, 3H), 0.78 (d, $J = 6.8$ Hz, 3H), 0.64 (dt, $J = 11.2$, 12.8 Hz, 1H), 0.17 (s, 9H), 0.11 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.51, 151.75, 108.52, 71.76, 57.66, 51.43, 48.47, 46.73, 42.84, 40.75, 39.72, 38.74, 34.78 (2C), 33.14, 32.83, 32.34, 32.14, 32.01, 31.63, 30.70, 22.65, 22.52, 21.17, 16.47, 0.40 (3C), 0.22 (3C); HRMS (ESI) *m/z* calcd for $C_{28}H_{48}O_4SiNa^+$ ([M-Si$(CH_3)_3$+Na]$^+$) 499.3214, found 499.3225, and calcd for $C_{31}H_{56}O_4Si_2Na^+$ 571.3609, found 571.3620.

Methyl 6-ethyliden-3α-hydroxy-12β-methyl-7-oxo-17-*epi*-18-*nor*-5β-cholan-24-oate (40). According to the same method as for the preparation of compound **31**, a solution of bis(silyl) ether **39** (6.20 g, 11.3 mmol) and acetaldehyde (1.20 mL, 21.4 mmol) in dry dichloromethane (25 mL) was treated with a solution of boron trifluoride diethyl etherate (5.16 mL, 41.8 mmol) in dry dichloromethane (15 mL) at -60 °C. The crude product from this reaction was subsequently re-dissolved in dichloromethane (52 mL) and stirred vigorously with a 3 M aqueous hydrochloric acid solution (23 mL) at 0 °C for 1 h to afford 3.72 g (77%) of **40** as a colorless foam. $[\alpha]_D^{20} = -70.7$ (*c* 0.785, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.23 (ap q, $J = 7.1$ Hz, 1H), 3.70-3.61 (overlapping signals: m, 1H and 3.64, s, 3H), 2.54 (dd, $J = 13.0$, 4.0 Hz, 1H), 2.52-2.45 (m, 1H), 2.35-2.24 (m, 2H), 2.14-2.07 (m, 1H), 2.04-1.94 (m, 2H), 1.80-1.68 (m, 5H), 1.68 (d, $J = 7.1$ Hz, 3H), 1.65-1.38 (m, 7H), 1.38-1.28 (m, 2H), 1.20-1.05 (m, 2H), 0.95 (d, $J = 6.3$ Hz, 3H), 0.92 (s, 3H), 0.91-0.84 (m, 1H), 0.84-0.74 (overlapping signals: 0.82, d, $J = 6.8$ Hz, 3H and 0.79, dt, $J = 11.8$, 12.2 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 203.77, 174.47, 142.79, 130.50, 70.67, 56.82, 55.15, 51.46, 45.46, 43.62, 40.10, 38.60, 37.54, 36.01, 34.53 (2C), 32.30, 32.10, 31.89, 31.68 (2C), 29.82, 22.76 (2C), 20.91, 16.70, 12.75; HRMS (ESI) *m/z* calcd for $C_{27}H_{42}O_4Na^+$ 453.2975, found 453.2991.

Methyl 6-ethyl-3α-hydroxy-12β-methyl-7-oxo-17-*epi*-18-*nor*-5β-cholan-24-oate (41). Employing the same procedure as for the preparation of **32**, a solution of **40** (3.72 g, 8.64 mmol) was dissolved in a 1:1 mixture of methanol (50 mL) and tetrahydrofuran (50 mL) and hydrogenated over 10% palladium on charcoal (192 mg) to give 3.42 g (92%) of **41** as a colorless foam. ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.66 (s, 3H), 3.62-3.53 (m, 1H), 2.37-2.21 (m, 3H), 2.18-2.11 (m, 1H), 2.07 (dd, $J = 11.8$, 9.8 Hz, 1H), 1.96 (ddd, $J = 9.8$, 5.7, 1.3 Hz, 1H), 1.92-1.81 (m, 2H), 1.81-1.57 (m, 9H), 1.56-1.40 (m, 4H), 1.38 (d, $J = 4.7$ Hz, 1H, OH), 1.28 (dt, $J = 11.2$, 13.1 Hz, 1H), 1.25-1.15 (m, 2H), 1.14 (s, 3H), 0.95 (d, $J = 6.3$ Hz, 3H), 0.94-0.88 (m, 2H), 0.88-0.80 (overlapping signals: 0.858, t, $J = 7.3$ Hz, 3H; 0.863, d, $J = 6.6$ Hz, 3H and m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 214.88, 174.46, 70.76, 61.44, 55.76, 52.31, 51.45, 49.29, 42.40, 41.97, 40.70, 39.96, 36.43, 35.36, 35.27, 32.30, 32.18, 31.70, 31.65, 30.85, 29.74, 26.45, 26.00, 22.76, 20.90, 16.75, 13.02; HRMS (ESI) *m/z* calcd for $C_{27}H_{44}O_4Na^+$ 455.3132, found 455.3136.

6α-Ethyl-3α-hydroxy-12β-methyl-7-oxo-17-*epi*-18-*nor*-5β-cholan-24-oic acid (42). Following the same method used for the preparation of **33**, compound **41** (3.40 g, 7.86 mmol) was dissolved in methanol (50 mL) and saponified in the presence of an 8 M aqueous potassium hydroxide solution (5 mL) at room temperature overnight to give rise to 3.14 g (96%) of crude **42** as a colorless foam which was used in the next reaction step without further purification. $[\alpha]_D^{25} = -79.6$ (*c* 1.0, MeOH); ^{1}H NMR (500 MHz, CD_3OD) δ 3.46 (tt, $J = 10.9$, 4.7 Hz, 1H), 2.82-2.76 (m, 1H), 2.35-2.15 (m, 4H), 2.06-1.98 (m, 1H), 1.94-1.79 (m, 4H), 1.77-1.60 (m, 6H), 1.58-1.40 (m, 4H), 1.34-1.09 (m, 3H), 1.19 (s, 3H), 0.99 (d, $J = 6.3$ Hz, 3H), 0.97-0.88 (m, 3H), 0.87 (d, $J = 6.8$ Hz, 3H), 0.82 (t, $J = 7.4$ Hz, 3H), 0.77 (dt, $J = 11.3$, 13.2 Hz, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 215.52, 177.91, 71.77, 57.76, 57.00, 52.92, 52.11, 44.84, 43.52, 41.87, 37.98, 36.68, 35.30, 33.43, 33.18 (2C), 32.90, 32.65, 31.56, 30.73, 24.08, 23.67, 21.34, 20.07, 17.17, 12.31; HRMS (ESI) *m/z* calcd for $C_{26}H_{42}O_4Na^+$ 441.2975, found 441.2973.

6α-Ethyl-3α,7α-dihydroxy-12β-methyl-17-*epi*-18-*nor*-5β-cholan-24-oic acid (43). Employing the same protocol used for the preparation of **34**, crude **42** (3.13 g, 7.48 mmol) was dissolved in a 4:1 mixture of tetrahydrofuran (80 mL) and water (20 mL) and was treated with sodium borohydride (849 mg, 22.4 mmol) at 0 °C to furnish 2.27 g (72%) of **43** as colorless needles after being recrystallized from methanol. $[\alpha]_D^{20} = -43.4$ (*c* 0.5, MeOH); Mp 207–211 °C (DSC); ^{1}H NMR (500 MHz, CD_3OD) δ 3.76-3.73 (m, 1H), 3.35-3.27 (m, 1H), 2.34-2.19 (m, 3H), 1.98-1.82 (m, 5H), 1.81-1.69 (m, 3H), 1.69-1.58 (m, 2H), 1.58-1.31 (m, 8H), 1.27 (dt, $J = 13.1$, 3.6 Hz, 1H), 1.11-0.93 (overlapping signals: m, 4H and 0.97, d, $J = 6.3$ Hz, 3H), 0.93-0.87 (overlapping signals: 0.90, t, $J = 7.1$ Hz, 3H and 0.88, d, $J = 6.8$ Hz, 3H), 0.85 (s, 3H), 0.67 (dt, $J = 11.6$, 12.4 Hz, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 177.98, 73.31, 70.90, 57.73, 49.46, 47.17, 44.61, 43.00, 42.87, 37.20, 36.85, 36.61, 34.66, 34.46, 33.59, 33.36, 33.23, 32.98, 31.40, 30.34, 24.11, 23.68, 23.56, 21.77, 17.23, 12.09; HRMS (ESI) *m/z* calcd for $C_{26}H_{44}O_4Na^+$ 443.3132, found 443.3136.

***N*-(6α-Ethyl-3α,7α-dihydroxy-12β-methyl-17-*epi*-18-*nor*-5β-cholan-24-oyl)taurine sodium salt (18).** Applying the same method as described for the preparation of compound **35**, bile acid **43** (303 mg, 0.724 mmol) dissolved in dry tetrahydrofuran (14 mL) was activated

with isobutyl chloroformate (IBCF; 0.12 mL, 0.925 mmol) in the presence of triethylamine (TEA; 0.12 mL, 0.861 mmol) and subsequently treated with taurine (102 mg, 0.815 mmol) and a second addition of triethylamine (0.12 mL) to give 287 mg (72%) of **18** as a colorless powder. $[\alpha]_D^{20} = -28.6$ (*c* 1.0, H_2O); ^{1}H NMR (500 MHz, CD_3OD) δ 3.76-3.72 (m, 1H), 3.63-3.52 (m, 2H), 3.35-3.27 (m, 1H), 2.96 (t, $J = 7.0$ Hz, 2H), 2.26-2.14 (m, 3H), 1.98-1.78 (m, 5H), 1.78-1.69 (m, 3H), 1.69-1.59 (m, 2H), 1.59-1.32 (m, 8H), 1.27 (dt, $J = 13.2$, 3.4 Hz, 1H), 1.10-0.92 overlapping signals (m, 4H and 0.97, d, $J = 6.3$ Hz, 3H), 0.92-0.86 (m, 6H), 0.84 (s, 3H), 0.66 (ap q, $J = 12.1$ Hz, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 176.19, 73.15, 70.79, 57.59, 51.41, 49.34, 47.00, 44.49, 42.88, 42.72, 37.08, 36.75, 36.51, 36.48, 35.39, 34.50, 34.29, 34.20, 33.21, 33.08, 31.29, 30.26, 24.12, 23.62, 23.42, 21.82, 17.21, 12.08; HRMS (ESI) *m/z* calcd for $C_{28}H_{48}NNaO_6SNa^+$ 572.2992, found 572.3004

3α-Hydroxy-12β-methyl-7-oxo-25-*homo*-18-*nor*-5β-cholan-25-oic acid (46). Using the same procedure as outlined for the preparation of compound **28** according to Method A, bleach (2 M, 3.46 mL) was added dropwise to a mixture of compound **44** [47] (2.38 g, 5.85 mmol) and potassium bromide (35.0 mg, 0.294 mmol) in a 1:1 mixture of acetic acid (11 mL) and ethyl acetate (11 mL) at 10 °C (over the course of 1 h) to give 1.77 g (75%) of **46** as a colorless foam. In addition, 363 mg (15%) of the corresponding 3,7-dioxo by-product **45** was recovered.

3α-Hydroxy-12β-methyl-7-oxo-25-homo-18-nor-5β-cholan-25-oic acid (**46**). $[\alpha]_D^{20} = -13.1$ (*c* 0.635, MeOH); ^{1}H NMR (125 MHz, $CDCl_3$) δ 3.60 (tt, $J = 11.0$, 4.7 Hz, 1H), 2.84 (dd, $J = 12.6$, 6.0 Hz, 1H), 2.41-2.26 (m, 2H), 2.25-2.18 (m, 1H), 2.14 (ap t, $J = 10.9$ Hz, 1H), 1.99-1.91 (overlapping signals: m, 1H and 1.96, dd, $J = 12.7$, 2.2 Hz, 1H), 1.91-1.83 (m, 2H), 1.81-1.63 (m, 5H), 1.63-1.53 (m, 2H), 1.53-1.45 (m, 1H), 1.45-1.13 (m, 7H), 1.16 (s, 3H), 1.06-0.95 (overlapping signals: m, 2H and 0.97, d, $J = 6.6$ Hz, 3H), 0.91 (d, $J = 6.8$ Hz, 3H), 0.81-0.69 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 212.39, 179.14, 70.94, 54.57, 52.46, 48.00, 46.17, 46.13, 45.07, 42.64, 37.78, 37.28, 36.96, 34.98 (2C), 34.37, 34.15, 29.87, 29.85, 28.96, 24.23, 23.28, 23.05, 21.09, 19.76; HRMS (ESI) *m/z* calcd for $C_{25}H_{40}O_4Na^+$ 427.2819, found 427.2826.

12β-Methyl-3,7-dioxo-25-homo-18-nor-5β-cholan-25-oic Acid (**45**). $[\alpha]_D^{20} = -19.6$ (*c* 0.81, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.87 (dd, $J = 12.9$, 5.5 Hz, 1H), 2.43-2.17 (m, 9H), 2.16-2.09 (m, 1H), 2.04 (td, $J = 11.9$, 3.6 Hz, 1H), 1.98 (dd, $J = 13.0$, 2.3 Hz, 1H), 1.83-1.66 (m, 3H), 1.66-1.37 (m, 7H), 1.37-1.29 (m, 1H), 1.27 (s, 3H), 1.16-0.98 (overlapping signals: m, 2H and 1.00, d, $J = 6.6$ Hz, 3H), 0.92 (d, $J = 6.8$ Hz, 3H), 0.85-0.74 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 211.01, 210.27, 178.56, 54.62, 52.48, 47.99, 47.72, 46.06, 44.73, 42.95, 42.64, 37.84, 37.24, 36.83, 35.46, 35.32, 35.05, 34.20, 29.88, 29.01, 24.28, 23.30, 22.46, 21.13, 19.78; HRMS (ESI) *m/z* calcd for $C_{25}H_{38}O_4Na^+$ 425.2662, found 425.2661.

Methyl 3α-hydroxy-12β-methyl-7-oxo-25-*homo*-18-*nor*-5β-cholan-25-oate (47). Deploying the same protocol as for the preparation of compound **29**, a solution of compound **46** (1.28 g, 3.16 mmol) in methanol (60 mL) was refluxed in the presence of a catalytic amount of *p*-toluenesulfonic acid monohydrate (100 mg, 0.526 mmol) overnight. After being cooled to room temperature the reaction was quenched with pyridine (2 mL, 24.7 mmol), concentrated and purified by automated flash column chromatography (silica gel, ethyl acetate/petroleum ether 5–50%) to provide 1.15 g (87%) of **47** as a colorless foam. $[\alpha]_D^{20} = -12.1$ (*c* 1.10, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.67 (s, 3H), 3.59 (tt, $J = 10.9$, 4.7 Hz, 1H), 2.84 (dd, $J = 12.5$, 6.0 Hz, 1H), 2.37-2.18 (m, 3H), 2.13 (ap t, $J = 10.9$ Hz, 1H), 1.98-1.84 (m, 4H), 1.82 (s_{br}, 1H, OH), 1.79-1.63 (m, 5H), 1.63-1.52 (m, 2H), 1.52-1.44 (m, 1H), 1.44-1.33 (m, 3H), 1.33-1.20 (m, 3H), 1.20-1.12 (overlapping signals: m, 1H and 1.16, s, 3H), 1.04-0.92 (overlapping signals: m, 2H and 0.97, d, $J = 6.6$ Hz, 3H), 0.90 (d, $J = 6.8$ Hz, 3H), 0.80-0.67 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 211.92, 174.20, 70.94, 54.59, 52.50, 51.41, 48.04, 46.12 (2C), 45.12, 42.59, 37.79, 37.44, 37.00, 35.01, 34.94, 34.46, 34.16, 30.01, 29.87, 29.00, 24.22, 23.50, 23.07, 21.13, 19.75; HRMS (ESI) *m/z* calcd for $C_{26}H_{42}O_4Na^+$ 441.2975, found 427.2979.

Methyl 12β-methyl-3α,7-di(trimethylsilyloxy)-25-*homo*-18-*nor*-5β-cholan-6(7)-en-25-oate (48). Applying the same procedure as for the synthesis of compound **30**, a solution of **47** (1.14 g, 2.72 mmol) in dry tetrahydrofuran (8 mL) was introduced dropwise into a solution of lithium diisopropylamide (2 M in THF/heptane/ethyl-benzene; 9.20 mL, 18.4 mmol) and trimethylsilyl chloride (2.90 mL, 22.9 mmol) in dry tetrahydrofuran (15 mL) at −78 °C. After being stirred for another 40 min at this temperature the reaction mixture was treated with triethylamine (0.57 mL, 4.09 mmol) to yield 1.39 g (91%) of **48** as a colorless oil. $[\alpha]_D^{20} = +64.5$ (*c* 0.67, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.70 (dd, $J = 5.9$, 1.7 Hz, 1H), 3.65 (s, 3H), 3.50 (tt, $J = 10.9$, 4.5 Hz, 1H), 2.36-2.22 (m, 2H), 2.12-2.05 (m, 1H), 1.84-1.65 (m, 6H), 1.65-1.23 (m, 11H), 1.20-1.11 (m, 1H), 1.11-0.96 (m, 3H), 0.94 (d, $J = 6.6$ Hz, 3H), 0.88 (d, $J = 6.8$ Hz, 3H), 0.80-0.70 (overlapping signals: 0.79, s, 3H and m, 2H), 0.15 (s, 9H), 0.09 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.18, 151.58, 108.39, 71.56, 55.15, 51.33, 51.22, 47.72, 47.12, 42.79, 40.83, 39.27, 38.79, 35.50, 35.22, 34.63, 34.44, 33.02, 32.33, 30.67, 29.03, 24.83, 23.53, 22.43, 21.47, 19.76, 0.35 (3C), 0.20 (3C); HRMS (ESI) *m/z* calcd for $C_{29}H_{50}O_4SiNa^+$ 513.3371, found 513.3372, and calcd for $C_{32}H_{58}O_4Si_2H^+$ 563.3946, found 563.3960.

Methyl 6-ethyliden-3α-hydroxy-12β-methyl-7-oxo-25-*homo*-18-*nor*-5β-cholan-25-oate (49). According to the same procedure as for the preparation of compound **31**, a solution of compound **48** (1.14 g, 2.03 mmol) in dry dichloromethane (10 mL) and acetaldehyde (0.23 mL, 4.10 mmol) was treated with a solution of boron trifluoride diethyl etherate (1.00 mL, 8.10 mmol) in dry dichloromethane (2 mL) at -60 °C by dropwise addition *via* cannula and the resulting reaction mixture was stirred at -60 °C for 2 h before being allowed to warm to room temperature. The crude reaction product was re-dissolved in dichloromethane (15 mL) and subsequently stirred vigorously in the presence of 3 M aqueous hydrochloric acid solution (10 mL) at 0 °C for 1 h to afford 190 mg (21%) of **49** as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.15 (q, $J = 7.1$ Hz, 1H), 3.66-3.56 (overlapping signals: 3.63, s, 3H and m, 1H), 2.54 (dd, $J = 13.1$, 4.2 Hz, 1H), 2.49-2.41 (m, 1H), 2.34-2.21 (m, 2H), 2.18 (s_{br}, 1H, OH), 2.05 (td, $J = 11.9$, 3.5 Hz, 1H), 2.01-1.91 (m, 2H), 1.78-1.68 (m, 3H), 1.66 (d, $J = 7.1$ Hz, 3H), 1.65-1.21 (m, 11H), 1.14 (td, $J = 14.3$, 3.2 Hz, 1H), 1.02-0.85 (overlapping signals: m, 3H; 0.96, d, $J = 6.6$ Hz, 3H; 0.94, s, 3H and 0.88, d, $J = 6.8$ Hz, 3H), 0.71 (ap q, $J = 10.3$ Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 204.42, 174.17, 143.18, 129.97, 70.44, 53.72, 53.59, 51.36, 47.76, 47.62, 45.62, 38.99, 37.75, 37.52, 36.42, 34.94, 34.49 (2C), 34.40, 30.95, 29.76, 28.92, 24.33, 23.46, 22.76, 21.10, 19.71, 12.65; HRMS (ESI) *m/z* calcd for $C_{28}H_{44}O_4Na^+$ 467.3132, found 467.3141.

Methyl 6-ethyl-3α-hydroxy-12β-methyl-7-oxo-25-*homo*-18-*nor*-5β-cholan-25-oate (50). Following the same procedure as for the synthesis of **32**, compound **49** (191 mg, 0.430 mmol) dissolved in ethanol (15 mL) was hydrogenated over 10% palladium on charcoal (53 mg) under atmospheric pressure and at room temperature overnight to afford 172 mg (90%) of **50** as a colorless oil. $[\alpha]_D^{20} = +9.1$ (*c* 0.53, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.67 (s, 3H), 3.61-3.52 (m, 1H), 2.38-2.24 (m, 3H), 2.21-2.14 (m, 1H), 1.99-1.84 (m, 3H), 1.81-1.35 (m, 15H), 1.33-1.13 (overlapping signals: m, 4H and 1.18, s, 3H), 1.05-0.93 (overlapping signals: m, 2H and 0.97, d, $J = 6.6$ Hz, 3H), 0.91 (d, $J = 6.8$ Hz, 3H), 0.85 (t, $J = 7.3$ Hz, 3H), 0.80-0.69 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 215.26, 174.22, 70.75, 61.86, 52.39, 51.43, 50.74, 49.61, 48.19, 46.01, 42.92, 39.94, 37.71, 36.85, 35.43, 35.39, 34.96, 34.50, 29.93, 29.72, 29.05, 26.50, 26.05, 24.14, 23.53, 21.16, 19.76, 13.06; HRMS (ESI) *m/z* calcd for $C_{28}H_{46}O_4Na^+$ 469.3288, found 469.3289.

6α-Ehyl-3α-hydroxy-12β-methyl-7-oxo-25-*homo*-18-*nor*-5β-cholan-25-oic acid (51). Using the same procedure as outlined for the preparation of compound **33**, 8 M aqueous potassium hydroxide solution (0.22 mL, 1.8 mmol) was added to a solution of compound **50** (160 mg, 0.358 mmol) in methanol (2.5 mL) and the resulting reaction mixture was stirred at room temperature overnight to give 145 mg

(94%) of **51** as colorless foam. $[\alpha]_D^{20} = -34.6$ (*c* 0.324, MeOH); ^{1}H NMR (500 MHz, CD_3OD) δ 3.47 (tt, J = 10.8, 4.7 Hz, 1H), 2.85-2.78 (m, 1H), 2.34-2.22 (m, 3H), 2.18-2.11 (m, 1H), 1.95 (td, J = 11.9, 3.4 Hz, 1H), 1.91-1.82 (m, 2H), 1.82-1.53 (m, 8H), 1.52-1.43 (m, 3H), 1.43-1.32 (m, 1H), 1.32-1.20 (overlapping signals: m, 3H and 1.21, s, 3H), 1.20-1.08 (m, 1H), 1.07-0.98 (overlapping signals: m, 2H and 1.01, d, J = 6.6 Hz, 3H), 0.93 (d, J = 6.8 Hz, 3H), 0.85-0.73 (overlapping signals: m, 3H and 0.82, t, J = 7.5 Hz, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 215.58, 177.66, 71.79, 56.35, 53.78, 53.11, 52.19, 49.63, 47.73, 45.19, 39.40, 38.35, 36.74, 36.44, 35.31, 35.28, 32.64, 30.78, 30.66, 30.20, 25.36, 24.72, 24.02, 21.68, 20.27, 20.05, 12.29; HRMS (ESI) *m/z* calcd for $C_{27}H_{44}O_4Na^+$ 455.3132, found 455.3135.

6α-Ethyl-3α,7α-dihydroxy-12β-methyl-25-*homo*-18-*nor*-5β-cholan-25-oic acid (52). Employing the same procedure as described for the preparation of **34**, compound **51** (140 mg, 0.324 mmol) was dissolved in a 4:1 mixture of tetrahydrofuran (7.6 mL) and water (1.9 mL) and subsequently reduced by adding sodium borohydride (61.2 mg, 1.62 mmol) in one portion at 0 °C to yield 91.0 mg (65%) of **52** as a colorless amorphous solid. $[\alpha]_D^{20} = +16.2$ (*c* 0.437, MeOH); ^{1}H NMR (500 MHz, CD_3OD) δ 3.78-3.75 (m, 1H), 3.36-3.28 (m, 1H), 2.35-2.23 (m, 2H), 1.98-1.81 overlapping signals (1.94, td, J = 11.7, 3.6 Hz, 1H and m, 3H), 1.80-1.69 (m, 4H), 1.65 (dt, J = 13.1, 3.5 Hz, 1H), 1.63-1.34 (m, 10H), 1.34-1.26 (m, 2H), 1.24 (td, J = 11.0, 3.2 Hz, 1H), 1.09-0.96 (overlapping signals: m, 2H and 0.99, d, J = 6.6 Hz, 3H), 0.95-0.89 (m, 7H), 0.88 (s, 3H), 0.84-0.76 (m, 2H); ^{13}C NMR (125 MHz, CD_3OD) δ 177.73, 73.31, 71.13, 54.58, 50.77, 49.04, 47.85, 47.02, 42.94, 39.94, 37.46, 36.85, 36.61 (2C), 35.34, 34.74, 34.47, 31.36, 30.39, 29.84, 25.42, 24.78, 23.97, 23.53, 21.98, 20.32, 12.08; HRMS (ESI) *m/z* calcd for $C_{27}H_{46}O_4Na^+$ 457.3288, found 457.3293.

***N*-(3α,7α-Dihydroxy-12β-methyl-25-*homo*-18-*nor*-5β-cholan-25-oyl)taurine sodium salt (53).** Conjugation of **52** (14 mg, 32.2 μmol) with taurine (**22**) was carried out according to Method B employed for the preparation of **23** by deploying DPPA as the coupling reagent. The crude product was purified by reversed-phase flash chromatography on C-18 silica gel eluting with 20–80% methanol in water to yield 10 mg (55%) of conjugate **53** as a white powder. ^{1}H NMR (500 MHz, CD_3OD) δ 3.78-3.75 (m, 1H), 3.59 (td, J = 6.8, 1.1 Hz, 2H), 3.35-3.28 (m, 1H), 2.96 (t, J = 6.9 Hz, 2H), 2.26-2.11 (m, 2H), 1.97-1.81 (m, 4H), 1.80-1.69 (m, 4H), 1.68-1.21 (m, 14H), 1.06-0.96 overlapping signals (m, 2H and 0.98, d, J = 6.6 Hz, 3H), 0.94-0.88 overlapping signals (m, 1H; 0.93, d, J = 6.7 Hz, 3H and 0.91, t, J = 7.2 Hz, 3H), 0.88 (s, 3H), 0.84-0.75 (m, 2H); ^{13}C NMR (125 MHz, CD_3OD) δ 176.03, 73.25, 71.09, 54.56, 51.51, 50.69, 49.00, 47.78, 46.93, 42.84, 39.88, 37.70, 37.41, 36.79, 36.67, 36.58, 36.55, 34.68, 34.41, 31.31, 30.54, 29.78, 25.61, 25.40, 23.93, 23.46, 21.97, 20.28, 12.03; HRMS (ESI) *m/z* calcd for $C_{29}H_{50}NO_6S^-$ 540.3364, found 540.3360.

3α-Hydroxy-12β-methyl-7-oxo-17-*epi*-25-*homo*-18-*nor*-5β-cholan-25-oic acid (55). Employing Method A as described for the preparation of compound **28**, bleach (2 M, 3.5 mL) was introduced slowly into a mixture of compound **20** [47] (2.51 g, 6.17 mmol) and potassium bromide (39.6 mg, 0.33 mmol) in a 1:1 mixture of acidic acid (40 mL) and ethyl acetate (40 mL) at 10–14 °C to afford 2.14 g (86%) of **55** as a colorless foam along with 296 mg (12%) of the corresponding diketone by-product **54**.

3α-Hydroxy-12β-methyl-7-oxo-17-epi-25-homo-18-nor-5β-cholan-25-oic acid (**55**). $[\alpha]_D^{20} = -66.0$ (*c* 0.565, MeOH); $[\alpha]_D^{20} = -68.8$ (*c* 0.555, $CHCl_3$); ^{1}H NMR (500 MHz, CD_3OD) δ 3.54 (tt, J = 10.9, 4.8 Hz, 1H), 2.96 (dd, J = 13.1, 6.2 Hz, 1H), 2.29-2.16 (overlapping signals: 2.26, t, 7.5 Hz, 2H and m, 2H), 2.06 (t, J = 10.7 Hz, 1H), 1.96-1.85 (m, 4H), 1.80 (sext d, J = 6.8, 2.5 Hz, 1H), 1.75 (dt, J = 12.9, 3.3 Hz, 1H), 1.71-1.57 (m, 6H), 1.57-1.46 (m, 2H), 1.35-1.11 (overlapping signals: m, 5H and 1.17, s, 3H), 1.01 (d, J = 6.3 Hz, 3H), 0.99-0.88 (m, 3H), 0.86 (d, J = 6.8 Hz, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 214.99, 177.63, 71.57, 57.12, 57.07, 47.43, 45.98, 43.94, 43.50, 41.98, 38.27, 37.87, 37.82, 36.07, 35.24 (2C), 33.18, 33.15, 31.73, 30.82, 24.17, 23.72, 23.62, 21.40, 17.43; HRMS (ESI) *m/z* calcd for $C_{25}H_{40}O_4Na^+$ 427.2819, found 427.2827.

12β-Methyl-3,7-dioxo-17-epi-25-homo-18-nor-5β-cholan-25-oic acid (**54**). $[\alpha]_D^{20} = -69.6$ (*c* 0.51, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.85 (dd, 13.1, 5.4 Hz, 1H), 2.36-2.11 (m, 10H), 2.05-1.94 (m, 3H), 1.81-1.72 (m, 2H), 1.72-1.48 (m, 7H), 1.29-1.11 (overlapping signals: m, 2H and 1.24, s, 3H), 1.05-0.88 (overlapping signals: m, 3H and 1.00, d, J = 6.3 Hz, 3H), 0.85 (d, J = 6.8 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 210.86, 210.25, 178.71, 56.04, 55.71, 47.61, 44.55, 43.02, 42.16, 41.96, 40.56, 36.89 (2C), 36.52, 35.48, 35.23, 34.11, 31.88, 31.81, 30.73, 22.79, 22.77, 22.51, 20.98, 17.02; HRMS (ESI) *m/z* calcd for $C_{25}H_{38}O_4Na^+$ 425.2662, found 425.2663.

Methyl 3α-hydroxy-12β-methyl-7-oxo-17-*epi*-25-*homo*-18-*nor*-5β-cholan-25-oate (56). Using the same protocol as outlined for the preparation of **29**, compound **55** (1.50 g, 3.71 mmol) was dissolved in dry methanol (100 mL) and heated under reflux in the presence of a catalytic amount of *p*-toluenesulfonic acid monohydrate (72 mg, 0.379 mmol) for 2.5 h. After being cooled to room temperature the reaction was quenched with pyridine (1.3 mL, 16.1 mmol) and concentrated. The reaction product was subsequently dried under high vacuum to yield 1.46 g (94%) of the corresponding methyl ester **56**. $[\alpha]_D^{20} = -65.5$ (*c* 0.56, $CHCl_3$); $[\alpha]_D^{20} = -59.6$ (*c* 0.56, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.67 (s, 3H), 3.65-3.57 (m, 1H), 2.82 (dd, J = 12.9, 5.9 Hz, 1H), 2.33-2.25 (overlapping signals: m, 1H and 2.28, t, J = 7.6 Hz, 2H), 2.14 (tt, J = 8.5, 3.3 Hz, 1H), 1.96 (dd, J = 12.9, 2.3 Hz, 1H), 1.93-1.82 (m, 4H), 1.79-1.41 (m, 11H), 1.36-1.09 (overlapping signals: m, 5H and 1.13, s, 3H), 0.96 (d, J = 6.3 Hz, 3H), 0.94-0.81 (overlapping signals: m, 3H and 0.84, d, J = 6.8 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 211.47, 174.24, 71.05, 56.02, 55.83, 51.42, 45.98, 44.93, 42.14, 41.99, 40.52, 37.41, 36.70, 36.64, 34.92, 34.38, 34.16, 31.87, 31.76, 30.81, 30.07, 23.14, 23.03, 22.78, 21.03, 17.00; HRMS (ESI) *m/z* calcd for $C_{26}H_{42}O_4Na^+$ 441.2975, found 441.2974.

Methyl 12β-methyl-3α,7-di(trimethylsilyloxy)-17-*epi*-25-*homo*-18-*nor*-5β-cholan-6(7)-en-25-oate (57). According to the same method as outlined for the preparation of compound **30**, a solution of **56** (1.28 g, 3.06 mmol) in dry tetrahydrofuran (6 mL) was added dropwise to a solution of lithium diisopropylamide (2 M in THF/heptane/ethylbenzene; 10.1 mL, 20.2 mmol) and trimethylsilyl chloride (3.15 mL, 24.8 mmol) in dry tetrahydrofuran (15 mL) at −78 °C. After being stirred for another 45 min at this temperature the reaction mixture was treated with triethylamine (0.68 mL, 4.88 mmol) to yield 1.54 g (90%) of **57** as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.72 (dd, J = 5.8, 1.2 Hz, 1H), 3.67 (s, 3H), 3.52 (tt, J = 11.0, 4.5 Hz, 1H), 2.28 (t, J = 7.6 Hz, 2H), 2.21-2.08 (m, 2H), 1.85 (dt, J = 14.3, 3.1 Hz, 1H), 1.80-1.09 (m, 18H), 1.05 (td, J = 14.3, 3.0 Hz, 1H), 0.97-0.86 (overlapping signals: 0.95, d, J = 6.3 Hz, 3H and m, 1H), 0.79 (s, 3H), 0.77 (d, J = 6.7 Hz, 3H), 0.64 (dt, J = 11.1, 12.7 Hz, 1H), 0.17 (s, 9H), 0.11 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.28, 151.82, 108.53, 71.79, 57.72, 51.41, 48.48, 46.74, 42.86, 40.76, 39.77, 38.75, 36.52, 34.80 (2C), 34.44, 33.15, 32.85, 32.16, 31.72, 30.72, 23.04, 22.69, 22.54, 21.31, 16.77, 0.41 (3C), 0.23 (3C); HRMS (ESI) *m/z* calcd for $C_{29}H_{50}O_4SiNa^+$ 513.3371, found 513.3365, and calcd for $C_{32}H_{58}O_4Si_2H^+$ 563.3946, found 563.3952.

Methyl 6-ethyliden-3α-hydroxy-12β-methyl-7-oxo-17-*epi*-25-*homo*-18-*nor*-5β-cholan-25-oate (58). Applying the same method as for the synthesis of **31**, compound **57** (1.43 g, 2.54 mmol) in dry dichloromethane (6.3 mL) and acetaldehyde (0.27 mL, 4.81 mmol) was treated with a solution of boron trifluoride diethyl etherate (1.16 mL, 9.40 mmol) in dry dichloromethane (4 mL) at -60 °C by dropwise addition *via* cannula. The resulting reaction mixture was stirred at -60 °C for 2 h before being allowed to warm to room temperature. The crude reaction product was re-dissolved in dichloromethane (12 mL) and subsequently stirred vigorously in the presence of 3 M aqueous hydrochloric acid (5.3 mL) at 0 °C for 1 h to afford 689 mg (61%) of **58** as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.26 (q, J = 7.1 Hz, 1H), 3.72-3.63 (overlapping signals: m, 1H and 3.67, s, 3H), 2.56 (dd, J = 13.1,

4.1 Hz, 1H), 2.54-2.46 (m, 1H), 2.28 (t, $J = 7.6$ Hz, 2H), 2.16-2.09 (m, 1H), 2.06-1.96 (overlapping signals: 2.03, td, $J = 12.0$, 3.2 Hz, 1H and 1.99, dt, $J = 14.3$, 3.2 Hz, 1H), 1.81-1.71 (m, 4H), 1.69 (d, $J = 7.2$ Hz, 3H), 1.66-1.55 (m, 6H), 1.55-1.44 (m, 2H), 1.43-1.30 (m, 2H), 1.27-1.06 (m, 4H), 0.98 (d, $J = 6.3$ Hz, 3H), 0.94 (s, 3H), 0.90 (dt, $J = 8.3$, 11.9 Hz, 1H), 0.85-0.76 (overlapping signals: m, 1H and 0.82, d, $J = 6.8$ Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 203.82, 174.24, 142.81, 130.42, 70.63, 56.85, 55.16, 51.40, 45.45, 43.63, 40.14, 38.59, 37.51, 36.60, 36.02, 34.53, 34.49, 34.37, 31.88, 31.82, 31.69, 29.80, 23.01, 22.78, 22.74, 21.04, 16.97, 12.73; HRMS (ESI) *m/z* calcd for $C_{28}H_{44}O_4Na^+$ 467.3132, found 467.3139.

Methyl 6-ethyl-3α-hydroxy-12β-methyl-7-oxo-17-*epi*-25-*homo*-18-*nor*-5β-cholan-25-oate (59). Using the same protocol as outlined for the preparation of **32**, compound **58** (630 mg, 1.42 mmol) was dissolved in a 1:1 mixture of methanol (12 mL) and tetrahydrofuran (12 mL) and hydrogenated over 10% palladium on charcoal (35 mg) under atmospheric pressure and at room temperature overnight to yield 559 mg (88%) of **59** as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.67 (s, 3H), 3.62-3.53 (m, 1H), 2.31-2.20 (overlapping signals: 2.28, t, $J = 7.6$ Hz, 2H and m, 1H), 2.18-2.12 (m, 1H), 2.07 (dd, $J = 11.8$, 9.8 Hz, 1H), 1.96 (ddd, $J = 9.8$, 5.7, 1.2 Hz, 1H), 1.92-1.81 (m, 2H), 1.81-1.41 (m, 13H), 1.38 (d, $J = 4.6$ Hz, 1H; OH), 1.33-1.10 (overlapping signals: 1.28, dt, $J = 11.4$, 13.1 Hz, 1H; m, 4H and 1.14, s, 3H), 0.96 (d, $J = 6.3$ Hz, 3H), 0.95-0.80 (overlapping signals: m, 6H and 0.85, d, $J = 7.0$ Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 214.90, 174.25, 70.79, 61.44, 55.82, 52.34, 51.42, 49.30, 42.41, 42.00, 40.75, 39.98, 36.70, 36.46, 35.37, 35.28, 34.39, 31.87, 31.68, 30.87, 29.77, 26.45, 26.01, 23.04, 22.79, 21.05, 17.05, 13.03; HRMS (ESI) *m/z* calcd for $C_{28}H_{46}O_4Na^+$ 469.3288, found 469.3290.

6α-Ethyl-3α-hydroxy-12β-methyl-7-oxo-17-*epi*-25-*homo*-18-*nor*-5β-cholan-25-oic acid (60). Deploying the same method as for the preparation of compound **33**, a solution of **59** (474 mg, 1.06 mmol) in methanol (10 mL) was treated with 8 M aqueous potassium hydroxide solution (0.8 mL) at room temperature overnight to give 450 mg (98%) of crude **60** which was used in the next reaction step without further purification. $[\alpha]_D^{20} = -75.3$ (*c* 0.32, MeOH); ^{1}H NMR (500 MHz, CD_3OD) δ 3.47 (tt, $J = 10.9$, 4.7 Hz, 1H), 2.83-2.77 (m, 1H), 2.26 (t, $J = 7.4$ Hz, 2H), 2.24-2.16 (m, 2H), 2.06-1.98 (m, 1H), 1.94-1.87 (m, 2H), 1.87-1.77 (m, 2H), 1.77-1.57 (m, 8H), 1.57-1.45 (m, 2H), 1.34-1.08 (m, 5H), 1.19 (s, 3H), 1.01 (d, $J = 6.3$ Hz, 3H), 0.99-0.87 (m, 3H), 0.86 (d, $J = 6.8$ Hz, 3H), 0.83 (t, $J = 7.4$ Hz, 3H), 0.78 (dt, $J = 11.3$, 13.1 Hz, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 215.53, 177.63, 71.77, 57.78, 57.04, 52.93, 52.12, 44.85, 43.53, 42.01, 37.98, 37.82, 36.68, 35.30, 35.23, 33.20, 33.15, 32.65, 31.57, 30.74, 24.17, 24.08, 23.74, 21.39, 20.07, 17.42, 12.31; HRMS (ESI) *m/z* calcd for $C_{27}H_{44}O_4Na^+$ 455.3132, found 441.3142.

6α-Ethyl-3α,7α-dihydroxy-12β-methyl-17-*epi*-25-*homo*-18-*nor*-5β-cholan-25-oic acid (61). Following the same method as described for the preparation of compound **34**, a solution of crude **60** (405 mg, 0.936 mmol) in a 4:1 mixture of tetrahydrofuran (10 mL) and water (2.5 mL) was treated with sodium borohydride (109 mg, 2.88 mmol; added in one portion) at 0 °C for 1 h to yield 214 mg (53%) of **61** as a colorless foam. $[\alpha]_D^{20} = -42.5$ (*c* 0.36, MeOH); ^{1}H NMR (500 MHz, CD_3OD) δ 3.76-3.73 (m, 1H), 3.36-3.27 (m, 1H), 2.29-2.20 (overlapping signals: 2.26, t, $J = 7.4$ Hz, 2H and m, 1H), 1.97-1.70 (m, 8H), 1.70-1.48 (m, 8H), 1.44-1.33 (m, 2H), 1.31-1.11 (m, 3H), 1.11-0.92 (overlapping signals: m, 4H and 0.99, d, $J = 6.3$ Hz, 3H), 0.91 (t, $J = 7.2$ Hz, 3H), 0.88 (d, $J = 6.8$ Hz, 3H), 0.85 (s, 3H), 0.68 (dt, $J = 11.6$, 12.4 Hz, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 177.70, 73.32, 70.91, 57.78, 49.47, 47.17, 44.62, 43.16, 42.87, 38.00, 37.20, 36.85, 36.61, 35.28, 34.66, 34.46, 33.37, 33.17, 31.41, 30.33, 24.21, 24.11, 23.76, 23.56, 21.83, 17.48, 12.10; HRMS (ESI) *m/z* calcd for $C_{27}H_{46}O_4Na^+$ 457.3288, found 457.3286.

***N*-(6α-Ethyl-3α,7α-dihydroxy-12β-methyl-17-*epi*-25-*homo*-18-*nor*-5β-cholan-25-oyl)taurine sodium salt (19).** The conjugation of **61** (28 mg, 64.4 μmol) with taurine (**22**) was carried out according to Method A employed for the preparation of **23** using EEDQ as the coupling reagent. The crude product was purified by automated reversed-phase chromatography on C-18 silica gel eluting with 20–80% methanol in water to furnish 18 mg (50%) of conjugate **19** as a white powder. $[\alpha]_D^{20}$ −30.2 (*c* 0.63, MeOH); ^{1}H NMR (500 MHz, CD_3OD) δ 3.76-3.73 (m, 1H), 3.59 (t, $J = 6.9$ Hz, 2H), 3.36-3.26 (m, 1H), 2.96 (t, $J = 6.9$ Hz, 2H), 2.26-2.19 (m, 1H), 2.19-2.13 (m, 2H), 1.97-1.70 (m, 8H), 1.70-1.47 (m, 8H), 1.44-1.33 (m, 2H), 1.31-1.24 (m, 1H), 1.24-1.12 (m, 2H), 1.09-0.97 overlapping signals (m, 3H and 0.98, d, $J = 6.3$ Hz, 3H), 0.97-0.88 overlapping signals (m, 1H and 0.91, t, $J = 7.1$ Hz, 3H), 0.87 (d, $J = 6.8$ Hz, 3H), 0.85 (s, 3H), 0.67 (dt, $J = 11.5$, 12.4 Hz, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 176.02, 73.29, 70.85, 57.72, 51.51, 49.43, 47.15, 44.57, 43.13, 42.81, 38.07, 37.58, 37.15, 36.81, 36.60, 36.56, 34.60, 34.43, 33.33, 33.23, 31.38, 30.29, 24.98, 24.05, 23.73, 23.52, 21.81, 17.44, 12.04; HRMS (ESI) *m/z* calcd for $C_{29}H_{50}NO_6S^-$ 540.3364, found 540.3358.

3α-Formyloxy-12β-methyl-7-oxo-17-*epi*-18-*nor*-5β-cholan-24-oic acid (62). To a solution of **37** (3.78 g, 9.68 mmol) in a 10:1 mixture of neat formic acid (38 mL) and water (3.8 mL) was added 70% perchloric acid (0.35 mL, 4.06 mmol) at 40 °C. Then the temperature was raised to 60 °C and the mixture stirred for 4.5 h. After this time a water bath was added to maintain a reaction temperature below 40 °C while introducing acetic anhydride (7 mL, 74.1 mmol) dropwise. Eventually, effervescence occurred, and the temperature began to drop. The water bath was removed, and the reaction allowed to stir at room temperature for 1 h before being poured into an excess of cold water. The precipitate was filtered off and re-dissolved in ethyl acetate. The organic solution was washed with water and brine, dried over $MgSO_4$ and concentrated under reduced pressure to give 3.95 g (98%) of crude **62** which was used in the next step without further purification. $[\alpha]_D^{20}$ −47.6 (1.01, $CHCl_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 211.43, 179.80, 160.49, 72.87, 55.83, 55.57, 45.64, 44.53, 42.06, 41.78, 40.38, 36.53, 34.81, 33.62, 32.93, 32.10, 31.72, 31.59, 31.53, 30.57, 26.08, 22.97, 22.64, 20.75, 16.59; HRMS (ESI) *m/z* calcd for $C_{25}H_{38}O_5Na^+$ *441.2611*, found 441.2613.

3α-Formyloxy-12β-methyl-7-oxo-17-*epi*-18,24-*bisnor*-5β-cholane-23-nitrile (63). Following the same procedure as for the preparation of **S10** in [47], crude **62** (3.87 g, 9.25 mmol) was dissolved in a mixture trifluoroacetic acid (TFA; 27 mL) and trifluoroacetic anhydride (TFAA; 8 mL) at −10 °C and then treated portionwise with sodium nitrite (645 mg, 9.35 mmol; the reaction temperature was maintained below −5 °C during the addition and before subsequent heating to 40 °C) to yield 1.92 g (54%) of **63** as a colorless solid. The reaction product (**63**) was purified by automated column chromatography on silica gel gradient eluting with ethyl acetate/toluene. $[\alpha]_D^{20}$ −34.3 (*c* 1.0, $CDCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.99 (s, 1H), 4.88-4.79 (m, 1H), 2.84 (dd, $J = 12.9$, 5.8 Hz, 1H), 2.36-2.14 (m, 5H), 2.01-1.84 (m, 5H), 1.82-1.58 (m, 5H), 1.53-1.40 (m, 3H), 1.36 (dt, $J = 12.1$, 13.0 Hz, 1H), 1.25 (td, $J = 14.4$, 3.2 Hz, 1H), 1.16 (s, 3H), 1.05-0.85 (overlapping signals: 1.03, d, $J = 7.5$ Hz, 3H; 1.01, d, 6.9 Hz, 3H and m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 210.85, 160.42, 119.35, 72.83, 55.75, 55.41, 45.66, 44.62, 42.11, 41.70, 40.37, 36.48, 34.91, 33.70, 33.05, 31.74, 30.38 (2C), 26.17, 24.84, 23.03, 22.59, 20.80, 16.82; HRMS (ESI) *m/z* calcd for $C_{24}H_{35}NO_3Na^+$ 408.2509, found 408.2511.

3α-Hydroxy-12β-methyl-7-oxo-17-*epi*-18,24-*bisnor*-5β-cholan-23-oic acid (64). Following a similar protocol as described for the synthesis of **27b** in [47], a 20% (w/v) aqueous potassium hydroxide solution (30 mL) was added to a solution of nitrile **63** (1.97 g, 5.12 mmol) in ethanol (100 mL). The mixture was heated to 90 °C for 7 d to afford bile acid **64** (1.10 g, 57%) after purification. $[\alpha]_D^{25}$ −74.4 (*c* 0.85, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.59 (tt, $J = 11.0$, 4.7 Hz, 1H), 2.84 (dd, $J = 12.8$, 6.0 Hz, 1H), 2.37 (sext d, $J = 6.9$, 2.7 Hz, 1H), 2.33-2.24 (m, 1H), 2.24-2.09 (m, 3H), 1.98-1.82 (m, 5H), 1.75-1.57 (m, 5H), 1.55-1.43 (m, 2H), 1.35-1.14 (m, 3H), 1.13 (s, 3H), 1.02 (d, $J = 6.2$ Hz, 3H), 0.95-0.82 overlapping signals (m, 3H and 0.92, $J = 6.8$ Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 212.66, 176.27, 70.50, 55.86, 55.55, 45.91,

44.81, 42.20, 41.68, 41.62, 40.43, 36.88, 36.55, 34.80, 33.96, 31.65, 30.52, 29.54 (2C), 23.00, 22.65, 20.67, 16.78; HRMS (ESI) *m/z* calcd for $C_{23}H_{36}NO_4Na^+$ 399.2506, found 399.2514.

3α,7α-Dihydroxy-12β-methyl-17-*epi*-18,24-*bisnor*-5β-cholan-23-oic acid (65). To a solution of **64** (225 mg, 0.598 mmol) in a 4:1 mixture of tetrahydrofuran (15 mL) and water (3.8 mL) was added sodium borohydride (69.2 mg, 1.83 mmol) portionwise at 0 °C. After being stirred for 1 h at 0 °C the tetrahydrofuran was evaporated under reduced pressure. The residue was re-dissolved in ethyl acetate and treated with 1 M aqueous hydrochloric acid until acidic. The organic phase was separated, washed with brine, dried over $MgSO_4$ and concentrated. The crude product was purified by automated column chromatography [silica gel, acetone/dichloromethane (+1% acetic acid) 15–50%] to furnish 206 mg (91%) of **65** as a colorless foam. The spectral data of the **65** was consistent with that reported for the same compound in [47].

***N*-(3α,7α-Dihydroxy-12β-methyl-17-*epi*-18,24-*bisnor*-5β-cholan-23-oyl)taurine sodium salt (66).** According to the same method as described for the preparation of compound **26**, bile acid **65** (166 mg, 0.439 mmol), dissolved in dry tetrahydrofuran (9 mL), was activated with isobutyl chloroformate (IBCF; 0.07 mL, 0.540 mmol) in the presence of triethylamine (TEA; 0.07 mL, 502 mmol) at 0 °C and subsequently treated with triethylamine (TEA; 0.12 mL, 0.861 mmol) and an aqueous solution of taurine (78.4 mg, 0.627 mmol) in water (1.2 mL) followed by aqueous 2 M sodium hydroxide (1.32 mL) to give 120 mg (54%) of **66** as a colorless powder. The reaction product was purified by automated reversed-phase column chromatography (C18-silica gel, water/acetone 0–50%). $[\alpha]_D^{20}$ −38.7 (*c* 1.0, water); ^{1}H NMR (500 MHz, D_2O) δ 4.04 (s_{br}, 1H), 3.73-3.65 (m, 1H), 3.65-3.51 (m, 2H), 3.16 (t, J = 7.1 Hz, 2H), 2.47-2.36 (m, 1H), 2.24-2.10 (m, 4H), 2.09-1.84 (m, 5H), 1.82-1.61 (m, 7H), 1.55-1.42 (m, 2H), 1.16-1.03 (overlapping signals: m, 3H and 1.09, d, J = 5.5 Hz, 3H), 1.03-0.95 (overlapping signals: m, 1H and 0.97, d, J = 6.3 Hz, 3H), 0.94 (s_{br}, 3H), 0.77-0.64 (m, 1H); ^{13}C NMR (125 MHz, D_2O) δ 175.66, 71.71, 67.85, 56.44, 50.00, 47.09, 43.60, 42.79, 41.61, 41.55, 38.45, 35.86, 35.47, 35.14, 34.84, 34.11, 32.65, 31.64, 30.43, 29.91, 29.30, 23.36, 22.62, 21.29, 16.16; HRMS (ESI) *m/z* calcd for $C_{25}H_{42}NO_6S^-$ 484.2738, found 484.2733.

6α-Ethyl-3α-formyloxy-12β-methyl-7-oxo-18-*nor*-5β-cholan-24-oic Acid (67). Employing the same protocol as for the synthesis of **S4** in [47], the 6α-ethyl-7-oxo-bile acid **33** (1.19 g, 2.84 mmol) in formic acid (30 mL) was treated with 70% perchloric acid (0.01 mL, 0.116 mmol) and stirred for at 55 °C for 6 h. Upon further reaction with acetic anhydride (24 mL, 254 mmol) as described, 889 mg (70%) of **67** was isolated as a colorless foam. The reaction product was purified by automated column chromatography on silica gel gradient eluting with acetone/toluene. The crude compound was used in the next reaction step without further purification. $[\alpha]_D^{20}$ −32.2 (*c* 1.0, $CDCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.98 (d, J = 0.8 Hz, 1H), 4.83-4.75 (m, 1H), 2.73-2.67 (m, 1H), 2.45 (ddd, J = 15.4, 10.0, 5.5 Hz, 1H), 2.28-2.18 (m, 2H), 2.14 (ap t, J = 10.9 Hz, 1H), 1.96-1.84 (m, 3H), 1.83-1.67 (m, 6H), 1.66-1.58 (m, 1H), 1.55 (dt, J = 13.0, 3.3 Hz, 1H), 1.49-1.36 (m, 3H), 1.34-1.22 (m, 3H), 1.20 (s, 3H), 1.16-1.06 (m, 1H), 1.06-0.94 overlapping signals (m, 2H and 0.96, d, J = 6.6 Hz, 3H), 0.92 (d, J = 6.7 Hz, 3H), 0.84-0.72 overlapping signals (m, 2H and 0.81, t, J = 7.4 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 212.28, 180.05, 160.56, 73.20, 55.04, 52.35, 51.65, 50.47, 47.80, 46.11, 43.41, 37.79, 37.00, 35.58, 34.86, 33.79, 32.54, 29.68, 27.69, 26.08, 24.64, 24.23, 23.47, 20.96, 19.38, 18.73, 11.97; HRMS (ESI) *m/z* calcd for $C_{27}H_{42}O_5Na^+$ 469.2924, found 469.2930.

6α-Ethyl-3α-formyloxy-12β-methyl-7-oxo-18,24-*bisnor*-5β-cholane-23-nitrile (68). Using the same procedure as for the preparation of **S10** in [47], **67** (630 mg, 1.41 mmol) was dissolved in a mixture trifluoroacetic acid (TFA; 2.2 mL) and trifluoroacetic anhydride (TFAA; 0.6 mL) at 0 °C and then treated portionwise with sodium nitrite (98.3 mg, 1.42 mmol) to yield 393 mg (67%) of **68** as a colorless amorphous solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.98 (d, J = 0.8 Hz, 1H), 4.82-4.74 (m, 1H), 2.73-2.67 (m, 1H), 2.38 (dd, J = 16.6, 4.1 Hz, 1H), 2.31-2.22 (m, 2H), 2.12 (ap t, J = 11.0 Hz, 1H), 2.07 (dd, J = 16.6, 10.1 Hz, 1H), 1.96-1.63 (m, 8H), 1.59 (dt, J = 13.0, 3.4 Hz, 1H), 1.50-1.21 overlapping signals (m, 4H and 1.25, td, 14.4, 3.3 Hz, 1H), 1.20 (s, 3H), 1.16-1.06 overlapping signals (m, 1H and 1.13, d, J = 6.9 Hz, 3H), 1.04-0.94 overlapping signals (m, 2H and 1.01, d, J = 6.6 Hz, 3H), 0.81 (t, J = 7.4 Hz, 3H) 0.78-0.63 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 211.69, 160.34, 119.86, 72.96, 54.76, 52.92, 51.50, 50.26, 46.33, 45.94, 43.19, 37.45, 36.76, 35.44, 33.64, 33.27, 29.40, 27.58, 25.96, 23.79, 23.29, 20.89, 20.02, 18.62, 18.32, 11.86; HRMS (ESI) *m/z* calcd for $C_{26}H_{39}NO_3Na^+$ 436.2822, found 436.2827.

6α-Ethyl-3α-hydroxy-12β-methyl-7-oxo-18,24-*bisnor*-5β-cholan-23-oic acid (69). Following the same protocol as described for the preparation of **27b** in [47], nitrile **68** (387 mg, 0.936 mmol) dissolved in ethanol (6.5 mL) was reacted with a solution of potassium hydroxide (2.6 g, 46.3 mmol) in water (6.5 mL) at 92 °C to yield 379 mg (quant.) of **69** as a colorless foam. The compound was purified by automated column chromatography [silica gel, acetone (+1% acetic acid)/dichloromethane (+1% acetic acid) 5–80%]. $[\alpha]_D^{20}$ −58.8 (*c* 1.005, MeOH); ^{1}H NMR (500 MHz, CD_3OD) δ 3.51-3.43 (m, 1H), 2.84-2.79 (m, 1H), 2.43-2.31 (m, 2H), 2.27 (ap t, J = 10.9 Hz, 1H), 2.21-2.14 (m, 1H), 2.00-1.82 (m, 4H), 1.80-1.57 (m, 6H), 1.47-1.35 (m, 2H), 1.35-1.01 overlapping signals (m, 5H; 1.21, s, 3H and 1.04, d, J = 6.7 Hz, 3H), 0.98 (d, J = 6.8 Hz, 3H), 0.86-0.70 overlapping signals (m, 3H and 0.83, t, J = 7.4 Hz, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 215.41, 177.94, 71.78, 56.23, 54.16, 53.07, 52.15, 48.58, 47.65, 45.14, 39.26, 38.25, 36.74, 36.28, 35.31, 34.08, 32.63, 30.68, 30.66, 25.41, 24.02, 21.49, 20.83, 20.04, 12.31; HRMS (ESI) *m/z* calcd for $C_{25}H_{40}O_4Na^+$ 427.2819, found 427.2832.

6α-Ethyl-3α,7α-dihydroxy-12β-methyl-18,24-*bisnor*-5β-cholan-23-oic acid (70). According to the same method as outlined for the preparation of **65**, the 6α-ethyl-7-oxo-bile acid **69** (68 mg, 0.168 mmol) dissolved in a 4:1 mixture of tetrahydrofuran (4 mL) and water (1 mL) was reduced with sodium borohydride (20 mg, 0.529 mmol) to afford 55 mg (81%) of **70** as a colorless solid. The reaction product was purified by automated column chromatography [silica gel, acetone/dichloromethane (+1% acetic acid) 5–40%]. Mp 204–206 °C (DSC); $[\alpha]_D^{20}$ −5 (*c* 0.3, MeOH); ^{1}H NMR (500 MHz, CD_3OD) δ 3.78-3.75 (m, 1H), 3.36-3.29 (m, 1H), 2.41 (dd, J = 14.5, 3.0 Hz, 1H), 2.38-2.30 (m, 1H), 1.99-1.71 (m, 7H), 1.69-1.22 (m, 12H), 1.06-0.86 overlapping signals (m, 2H; 1.02, d, J = 6.6 Hz, 3H; 0.98, d, J = 6.8 Hz, 3H; 0.91, t, J = 7.2 Hz, 3H and 0.88, s, 3H), 0.81 (ap q, 12.3 Hz, 1H), 0.73 (ap q, J = 10.2 Hz, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 178.00, 73.24, 71.05, 54.96, 49.71, 48.94, 47.77, 46.96, 42.88, 39.76, 37.36, 36.84, 36.59, 36.46, 34.69, 34.41, 34.22, 31.32, 29.74, 25.46, 24.03, 23.49, 21.82, 20.87, 12.13; HRMS (ESI) *m/z* calcd for $C_{25}H_{42}O_4Na^+$ 429.2975, found 429.2977.

***N*-(6α-Ethyl-3α,7α-dihydroxy-12β-methyl-18,24-*bisnor*-5β-cholan-23-oyl)taurine sodium salt (71).** Application of the same method as described for the synthesis of **26**, bile acid **70** (86 mg, 0.212 mmol) dissolved in dry tetrahydrofuran (4 mL) was activated with isobutyl chloroformate (IBCF; 0.035 mL, 0.270 mmol) in the presence of triethylamine (TEA; 0.06 mL, 0.431 mmol) at 0 °C and subsequently treated with triethylamine (TEA; 0.2 mL, 1.44 mmol) and an aqueous solution of taurine (40 mg, 0.32 mmol) in water (1 mL) followed by aqueous 2 M sodium hydroxide (0.63 mL) to give 75 mg (67%) of **71** as a colorless powder. The reaction product was purified by automated reversed-phase column chromatography (C18-silica gel, water/acetone 0–40%). $[\alpha]_D^{20}$ −1.7 (*c* 1.0, water); ^{1}H NMR (125 MHz, D_2O) δ 3.90 (s_{br}, 1H), 3.72-3.59 (m, 2H), 3.55-3.46 (m, 1H), 3.16 (t, J = 7.1 Hz, 2H), 2.44-2.33 (m, 2H), 2.06-1.66 (m, 10H), 1.66-1.41 (m, 7H), 1.41-1.34 (m, 1H), 1.28 (ap t, J = 10.6 Hz, 1H), 1.17-1.03 (overlapping signals: m, 1H and 1.12, d, J = 6.2 Hz, 3H), 1.02-0.89 (overlapping signals: 0.99, t, J = 7.3 Hz, 3H; 0.98, d, J = 6.5 Hz, 3H; 0.95, s_{br}, 3H and m, 1H), 0.84 (ap q, J = 11.7 Hz, 1H), 0.74 (ap q, J = 9.9 Hz, 1H); ^{13}C NMR (125 MHz, D_2O) δ 175.94, 72.01, 70.24, 53.49, 50.06, 47.99, 47.52, 46.27, 45.29,

41.31, 37.98, 37.22, 36.13, 35.60, 35.37, 35.15, 33.28, 32.87, 32.61, 29.73, 28.56, 24.14, 23.76, 22.08, 21.25, 18.87, 11.69; HRMS (ESI) *m/z* calcd for $C_{27}H_{46}NO_6S^-$ 512.3051, found 512.3049.

6α-Ethyl-3α-formyloxy-12β-methyl-7-oxo-17-*epi*-18-*nor*-5β-cholan-24-oic acid (72). Employing the same protocol as for the synthesis of **S4** in [47], the 6α-ethyl-7-oxo-bile acid **42** (822 mg, 1.96 mmol) in formic acid (8 mL) was treated with 70% perchloric acid (0.074 mL, 0.858 mmol) and acetic anhydride (6.8 mL, 71.9 mmol) to give 883 mg (quant.) of **72** as a colorless foam. The crude compound was used in the next reaction step without further purification. $[\alpha]_D^{25}$ −54.0; (*c* 1.01; $CHCl_3$); ^{1}H NMR (500 MHz, $CHCl_3$) δ 7.99 (s, 1H), 4.83-4.74 (m, 1H), 2.68 (dd, J = 12.5, 6.0 Hz, 1H), 2.41-2.31 (m, 2H), 2.31-2.22 (m, 1H), 2.18-2.11 (m, 1H), 1.95-1.82 (m, 4H), 1.82-1.39 (m, 12H), 1.24 (td, J = 14.4, 3.3 Hz, 1H), 1.18 (s, 3H), 1.16-1.05 (m, 1H), 1.05-0.84 (overlapping signals: 1.00, dt, J = 12.0, 14.3 Hz, 1H; 0.96, d, J = 6.3 Hz, 3H; m, 3H and 0.87, J = 6.8 Hz, 3H), 0.82 (t, J = 7.4 Hz, 3H); ^{13}C NMR (125 MHz, $CHCl_3$) δ 212.19, 179.91, 160.54, 73.24, 56.49, 55.65, 51.38, 50.34, 43.01, 41.93, 40.52, 36.72, 35.50, 33.78, 32.16, 31.80, 31.70, 31.62, 30.48, 27.68, 26.12, 23.54, 22.74, 20.82, 18.71, 16.64, 11.92; HRMS (ESI) *m/z* calcd for $C_{27}H_{42}O_5Na^+$ 469.2924, found 469.2926.

6α-Ethyl-3α-formyloxy-12β-methyl-7-oxo-17-*epi*-18,24-*bisnor*-5β-cholane-23-nitrile (73). Employing the same method as outlined for the preparation of **S10** in [47], **72** (800 mg, 1.79 mmol) was dissolved in a mixture trifluoroacetic acid (TFA; 5.6 mL) and trifluoroacetic anhydride (TFAA; 1.6 mL) at 0 °C and then treated portionwise with sodium nitrite (136 mg, 1.97 mmol) to yield 538 mg (73%) of **73** as a colorless amorphous solid. $[\alpha]_D^{25}$ −53.1; (*c* 1.0; $CHCl_3$); ^{1}H NMR (500 MHz, $CHCl_3$) δ 7.99 (d, J = 0.75 Hz, 1H), 4.79 (tt, J = 11.3, 4.9 Hz, 1H), 2.72-2.66 (m, 1H), 2.33-2.25 (m, 2H), 2.25-2.15 (m, 3H), 1.97-1.83 (m, 4H), 1.80-1.61 (m, 6H), 1.53-1.39 (m, 3H), 1.25 (td, J = 14.4, 3.4 Hz, 1H), 1.19 (s, 3H), 1.17-1.06 (m, 1H), 1.04-0.85 (overlapping signals: 1.02, d, J = 6.4 Hz, 3H; 1.01, d, J = 6.4 Hz, 3H and m, 4H), 0.82 (t, J = 7.4 Hz, 3H); ^{13}C NMR (125 MHz, $CHCl_3$) δ 211.88, 160.38, 119.27, 73.05, 56.19, 55.28, 51.29, 50.20, 42.90, 41.65, 40.32, 36.48, 35.42, 33.65, 31.64, 30.27, 30.08, 27.61, 26.03, 24.72, 23.41, 22.51, 20.70, 18.62, 16.69, 11.83; HRMS (ESI) *m/z* calcd for $C_{26}H_{39}NO_3Na^+$ 436.2822, found 436.2823.

6α-Ethyl-3α-hydroxy-12β-methyl-7-oxo-17-*epi*-18,24-*bisnor*-5β-cholan-23-oic acid (74). To a solution of nitrile **73** (404 mg, 0.978 mmol) in ethanol (6 mL) were added solid potassium hydroxide (1.94 g, 34.6 mmol) and water (2 mL). After dissolution, the mixture was heated at 90 °C overnight. The reaction was worked-up as described for compound **27b** in [47] and the crude reaction product purified by automated column chromatography [silica gel, acetone/dichloromethane (+1% acetic acid) 10–25%] to give 345 mg (87%) of **74** as a colorless foam. $[\alpha]_D^{25}$ −84.8 (*c* 1.0; $CHCl_3$); ^{1}H NMR (500 MHz, $CHCl_3$) δ 3.55 (tt, J = 11.0, 4.7 Hz, 1H), 2.65 (dd, J = 12.1, 6.5 Hz, 1H), 2.37 (sext, J = 7.0, 2.4 Hz, 1H), 2.32-2.11 (m, 4H), 1.91-1.84 (m, 3H), 1.81-1.60 (m, 7H), 1.55-1.42 (m, 2H), 1.35-1.25 (m, 1H), 1.22-1.09 (overlapping signals: 1.15, s, 3H and m, 2H), 1.01 (d, J = 6.3 Hz, 3H), 0.95-0.78 (overlapping signals: 0.92, d, J = 6.8 Hz, 3H; m, 4H and 0.81, t, J = 7.4 Hz, 3H); ^{13}C NMR (125 MHz, $CHCl_3$) δ 212.52, 178.43, 71.22, 56.46, 55.64, 51.44, 50.46, 43.02, 41.85, 41.67, 40.58, 36.75, 35.48, 34.17, 31.74, 31.59, 30.46, 29.85, 29.59, 23.57, 22.77, 20.82, 18.72, 16.95, 11.90; HRMS (ESI) *m/z* calcd for $C_{25}H_{40}O_4Na^+$ 427.2819, found 427.2822.

6α-Ethyl-3α,7α-dihydroxy-12β-methyl-17-*epi*-18,24-*bisnor*-5β-cholan-23-oic acid (75). Method A: Using the same reaction conditions as outlined for the preparation of **65**, the 6α-ethyl-7-oxo-bile acid **74** (306 mg, 0.756 mmol) dissolved in a 4:1 mixture of tetrahydrofuran (19 mL) and water (4.8 mL) was treated with sodium borohydride (88 mg, 2.33 mmol) to furnish 291 mg (95%) of **75** as a colorless foam.

Method B: To a solution of nitrile **78** (150 mg, 0.338 mmol) in ethanol (5 mL) was added an 7 M aqueous solution of potassium hydroxide (5 mL, 35.0 mmol) and the mixture was heated at 90 °C over 5 days. After being cooled to room temperature, the pH was adjusted to 1 by introducing slowly an ice cold 10% aqueous solution of hydrochloric acid. The mixture was subsequently extracted with ethyl acetate (3 ×) and the combined organic fractions were washed with brine, dried over $MgSO_4$ and concentrated. The residue was purified by automated column chromatography on silica gel, gradient eluting with acetone/dichloromethane (+1% acetic acid) to furnish 88 mg (64%) of **75** as a colorless foam. $[\alpha]_D^{25}$ −43.4 (*c* 1.0, MeOH); ^{1}H NMR (500 MHz, CD_3OD) δ 3.77-3.73 (m, 1H), 3.36-3.28 (m, 1H), 2.45-2.36 (m, 1H), 2.25-2.06 (m, 3H), 2.00-1.82 (m, 4H), 1.82-1.47 (m, 9H), 1.46-1.33 (m, 2H), 1.32-1.24 (m, 1H), 1.12-0.88 overlapping signals (m, 4H; 1.02, d, J = 6.2 Hz, 3H; 0.93, d, J = 6.8 Hz, 3H and 0.91, t, J = 7.1 Hz, 3H), 0.86 (s, 3H), 0.68 (ap q, J = 12.1 Hz, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 177.23, 73.29, 70.87, 57.64, 49.39, 47.14, 44.47, 43.13, 43.06, 42.85, 37.19, 36.85, 36.60, 34.66, 34.44, 33.41, 31.40, 31.14, 30.25, 24.13, 23.71, 23.55, 21.67, 17.41, 12.11; HRMS (ESI) *m/z* calcd for $C_{25}H_{42}O_4Na^+$ 429.2975, found 429.2980.

***N*-(3α,7α-Dihydroxy-6α-ethyl-12β-methyl-17-*epi*-18,24-*bisnor*-5β-cholan-23-oyl)taurine sodium salt (76).** According to the same protocol as described for the synthesis of **26**, bile acid **75** (141 mg, 0.347 mmol) dissolved in dry tetrahydrofuran (7 mL) was activated with isobutyl chloroformate (IBCF; 55 μL, 0.424 mmol) in the presence of triethylamine (TEA; 58.3 μL, 0.418 mmol) at 0 °C and subsequently treated with triethylamine (TEA; 97.2 μL, 0.697 mmol) and an aqueous solution of taurine (62 mg, 0.495 mmol) in water (1 mL) followed by aqueous 2 M sodium hydroxide (1.1 mL) to give 82 mg (44%) of **76** as a colorless powder. The reaction product was purified by automated reversed- phase column chromatography (C18-silica gel, water/acetone 3–50%) followed by automated normal phase column chromatography (silica gel, methanol/water 10–100%). $[\alpha]_D^{20}$ −41.2 (*c* 1.0, water); ^{1}H NMR (500 MHz, D_2O) δ 3.89 (s_{br}, 1H), 3.74-3.66 (m, 1H), 3.65-3.57 (m, 1H), 3.54-3.45 (m, 1H), 3.17 (t, J = 7.1 Hz, 2H), 2.47-2.38 (m, 1H), 2.25-2.13 (m, 3H), 2.09-1.44 (m, 15H), 1.41-1.32 (m, 1H), 1.16-1.04 (overlapping signals: 1.10, d_{br}, J = 4.9 Hz, 3H and m, 3H), 1.04-0.90 (overlapping signals: m, 4H; 0.98, d_{br}, J = 6.2 Hz, 3H and 0.93, s_{br}, 3H), 0.76-0.62 (m, 1H); ^{13}C NMR (125 MHz, D_2O) δ 175.64, 72.05, 70.22, 56.54, 50.00, 47.95, 45.49, 43.66, 42.93, 41.68, 41.43, 36.02, 35.73, 35.42, 35.14, 33.14, 32.68, 31.62, 30.38, 29.87, 29.23, 23.90, 22.68, 22.17, 21.29, 16.15, 11.79; HRMS (ESI) *m/z* calcd for $C_{27}H_{46}NO_6S^-$ 512.3051, found 512.3047.

6α-Ethyl-3α,7α-diformyloxy-12β-methyl-17-*epi*-18-*nor*-5β-cholan-24-oic acid (77). Deploying the same synthetic method as for the preparation of **S4** in [47], bile acid **43** (1.96 g, 4.66 mmol) in formic acid (8 mL) was treated with 70% perchloric acid (7.5 μL) and stirred for at 60 °C for 4 h. Following subsequent reaction with acetic anhydride acetic anhydride (6.4 mL, 67.7 mmol) at 40 °C for 1 h as described, 1.2 g (54%) of **77** was isolated as a colorless foam. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.14 (s, 1H), 8.04 (d, J = 0.8 Hz, 1H), 5.33-5.30 (m, 1H), 4.71 (tt, J = 11.2, 4.5 Hz, 1H), 2.40-2.29 (m, 2H), 2.21-2.14 (m, 1H), 1.96 (dt, J = 14.5, 3.3 Hz, 1H), 1.87 (dt, J = 11.9, 13.1 Hz, 1H), 1.83-1.69 (m, 7H), 1.67-1.58 (m, 1H), 1.57-1.24 (m, 8H), 1.22-1.00 (m, 4H), 0.99-0.93 overlapping signals (m, 1H and 0.94, d, J = 6.3 Hz, 3H), 0.93-0.87 overlapping signals (0.899, t, J = 7.4 Hz, 3H and 0.893, s, 3H), 0.81 (d, J = 6.8 Hz, 3H), 0.67 (dt, J = 11.7, 12.4 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 179.84, 160.70, 160.46, 74.49, 73.01, 56.14, 45.85, 44.80, 43.01, 41.31, 40.30, 35.41 (2C), 34.99, 33.93, 32.14, 31.80 (2C), 31.58, 29.50, 28.95, 26.76, 23.25, 22.45, 22.15, 20.98, 16.64, 11.45; HRMS (ESI) *m/z* calcd for $C_{28}H_{44}O_6Na^+$ 499.3036, found 499.3037.

6α-Ethyl-3α,7α-diformyloxy-12β-methyl-17-*epi*-18,24-*bisnor*-5β-cholane-23-nitrile (78). According to the same method as reported for the preparation of **S10** in [47], compound **77** (542 mg, 1.14 mmol) dissolved in a mixture trifluoroacetic acid (TFA; 1.5 mL) and trifluoroacetic anhydride (TFAA; 0.4 mL) was reacted portionwise with sodium nitrite (91.0 mg, 1.32 mmol) to give 300 mg (60%) of **78** as a colorless foam. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.14 (s, 1H), 8.05-8.03 (m, 1H), 5.34-5.30 (m, 1H), 4.71 (tt, J = 11.3, 4.4 Hz, 1H), 2.34-2.28 (m, 1H),

2.28-2.11 (m, 3H), 1.96 (dt, J = 14.5, 3.1 Hz, 1H), 1.92-1.64 (m 8H), 1.58-1.07 (m, 10H), 1.06-0.97 overlapping signals (m, 1H and 1.00, d, J = 6.3 Hz, 3H), 0.96 (d, J = 6.6 Hz, 3H), 0.93-0.87 overlapping signals (0.90, t, J = 7.4 Hz, 3H and s, 3H), 0.69 (ap q, J = 12.1 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 160.58, 160.30, 119.29, 74.33, 72.79, 55.85, 45.67, 44.72, 42.79, 41.28, 40.24, 35.38, 35.26, 34.92, 33.90, 31.78, 30.28, 29.20, 28.91, 26.72, 24.84, 23.18, 22.28, 22.09, 20.92, 16.65, 11.40; HRMS (ESI) m/z calcd for $C_{27}H_{41}NO_4Na^+$ 466.2928, found 466.2937.

3α,7α-Diformyloxy-12β-methyl-17-*epi*-18,24-*bisnor*-5β-chol-22 (23)-ene (80). To a solution of **79** [47] (914 mg, 2.04 mmol) in dry toluene (12 mL) were added copper(II) acetate (74.0 mg, 0.408 mmol) and dry pyridine (0.15 mL, 1.86 mmol). The resulting mixture was heated to reflux before bis(acetoxy)iodobenzene was introduced in five portions (BAIB; 5 × 406 mg, 1.26 mmol) once every hour. Upon complete addition, the reaction was heated at reflux for another 2–4 h. After being cooled to room temperature, the mixture was washed three times with 10% aqueous hydrochloric acid solution then water and brine, before the organic phase was dried over $MgSO_4$ and concentrated. The crude reaction product was purified by automated column chromatography (silica gel, ethyl acetate/petroleum ether 3–50%) to afford 524 mg (64%) of **80** as a crystalline solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.08-8.06 (m, 1H), 8.03 (d, J = 0.8 Hz, 1H), 5.77 (ddd, J = 17.2, 10.3, 6.7 Hz, 1H), 5.19-5.15 (m, 1H), 4.95-4.86 (m, 2H), 4.74 (tt, J = 11.4, 4.6 Hz, 1H), 2.48-2.41 (m, 1H), 2.24-2.10 (m, 2H), 2.05-1.98 (m, 1H), 1.96 (dt, J = 14.5, 3.4 Hz, 1H), 1.88 (td, J = 11.8, 3.4 Hz, 1H), 1.81-1.71 (m, 2H), 1.69-1.43 (m, 9H), 1.24-1.18 (m, 1H), 1.14-1.01 (m, 2H), 1.01-0.92 overlapping signals (m, 1H; 0.98, d, J = 6.3 Hz, 3H and 0.95, d, J = 6.8 Hz, 3H), 0.89 (s, 3H), 0.69 (dt, J = 11.6, 12.3 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 160.59, 160.50, 145.29, 111.74, 74.07, 70.76, 56.23, 45.07, 42.54, 42.35, 40.97, 36.73, 35.37, 34.73, 34.63, 34.48, 33.85, 31.98, 31.27, 28.51, 26.80, 23.68, 22.80, 21.12, 16.59; HRMS (ESI) m/z calcd for $C_{25}H_{38}O_4Na^+$ 425.2662, found 425.2661.

Mixture of diastereoisomers of 3α,7α-diformyloxy-12β-methyl-17-*epi*-18,24-*bisnor*-5β-cholan-22,23-diol (81). To a solution of **80** (495 mg, 1.23 mmol) in acetonitrile (10 mL) were added a catalytic amount of osmium tetroxide (15.7 mg, 0.062 mmol), 4-methylmorpholine *N*-oxide (NMO; 238 mg, 2.03 mmol) and water (1.2 mL) and the resulting reaction mixture was stirred for 4 h at room temperature. After completed dihydroxylation (TLC analysis), the reaction was quenched with a 1 M aqueous solution of sodium thiosulfate and stirred for another 30 min. Then the reaction was extracted with ethyl acetate (3 ×) and the combined organic fractions were washed with brine, dried over $MgSO_4$ and concentrated. The crude reaction product was purified by automated column chromatography (silica gel, ethyl acetate/petroleum ether 10–80%) to give 527 mg (98%) of the diol **81** as a colorless foam. ^{1}H NMR (125 MHz, $CDCl_3$) δ 8.07 (s_{br}, 1H), 8.03 (d, J = 0.6 Hz, 1H), 5.19-5.15 (m, 1H), 4.73 (tt, J = 11.4, 4.4 Hz, 1H), 3.75-3.69 (m, 1H), 3.51-3.43 (m, 1H), 3.38 (td, J = 7.9, 2.8 Hz, 1H), 2.62-2.55 (m, 1H), 2.14 (dt, J = 11.4, 13.1 Hz, 1H), 2.05-1.84 (m, 4H), 1.81-1.60 (m, 6H), 1.58-1.44 (m, 5H), 1.24-1.17 (m, 1H), 1.14-0.92 overlapping signals (m, 3H and 0.98, d, J = 6.3 Hz, 3H), 0.90 (s, 3H), 0.82 (d, J = 7.0 Hz, 3H), 0.75-0.66 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$; major isomer) δ 160.66, 160.55, 75.26, 74.05, 70.79, 64.68, 55.83, 44.95, 43.51, 40.88, 37.16, 35.32, 34.89, 34.65, 34.57, 34.40, 33.73, 31.89, 31.19, 29.05, 26.74, 23.07, 22.74, 21.00, 12.89; ^{13}C NMR (125 MHz, $CDCl_3$; minor isomer) δ 76.44, 70.75, 64.93, 56.12, 43.19, 38.42, 35.26, 31.83, 29.13, 23.46, 20.87; HRMS (ESI) m/z calcd for $C_{25}H_{40}O_6Na^+$ 459.2717, found 459.2719.

3α,7α-Diformyloxy-12β-methyl-17-*epi*-18,23,24-*trisnor*-5β-chol an-22-oic acid (82). To a solution of diol **81** (496 mg, 1.14 mmol) in acetonitrile (8 mL) were added 2,2,6,6-tetramethylpiperidine 1-oxyl (TEMPO; 38.3 mg, 0.245 mmol), bis(acetoxy)iodobenzene (BAIB; 2.0 g, 6.21 mmol) and water (2.7 mL) and the reaction was stirred at room temperature overnight. After complete conversion (TLC analysis), *iso*-propanol (0.5 mL) was added. The resulting mixture was then left to stir at 40 °C for 20 min to 1 h before being concentrated. Upon co-evaporation with toluene (2–3 ×) to remove excess acetic acid, the crude was purified by automated column chromatography (silica gel, ethyl acetate/petroleum ether 20–50%) to afford 222 mg (47%) of **82** as a colorless amorphous solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.08 (s, 1H), 8.03 (d, J = 0.8 Hz, 1H), 5.19-5.15 (m, 1H), 4.74 (tt, J = 11.4, 4.6 Hz, 1H), 2.69-2.61 (m, 2H), 2.13 (q, J = 12.7 Hz, 1H), 2.05-1.91 (m, 1H), 1.94 (dt, J = 14.6, 2.3 Hz, 1H), 1.88 (td, J = 11.7, 3.3 Hz, 1H), 1.81-1.44 (m, 11H), 1.24 (td, J = 10.7, 3.3 Hz, 1H), 1.18-0.96 (overlapping signals: m, 3H), 1.13 (d, J = 6.7 Hz, 3H), 0.99 (d, J = 6.3 Hz, 3H), 0.89 (s, 3H), 0.71 (q, J = 12.1 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 183.38, 160.84, 160.68, 74.16, 70.81, 56.14, 45.04, 42.59, 41.05, 40.54, 38.86, 35.47, 34.84, 34.76, 34.59, 33.98, 32.03, 31.36, 28.18, 26.92, 24.45, 22.91, 20.97, 14.54; HRMS (ESI) m/z calcd for $C_{24}H_{36}O_6Na^+$ 443.2404, found 443.2410.

3α,7α-Dihydroxy-12β-methyl-17-*epi*-18,23,24-*trisnor*-5β-cholan-22-oic acid (83). To a solution of **82** (202 mg, 0.480 mmol) in methanol (3 mL) was added a 2 M aqueous solution of sodium hydroxide (3 mL) and the resulting reaction mixture was heated at 90 °C overnight. After complete deprotection (TLC analysis) methanol was evaporated, and the remainder poured into 10 times the volume of an ice-cold solution of a 1 M aqueous solution of hydrochloric acid, upon which a colorless precipitate forms. The precipitate is filtered off and washed with water to yield 163 mg (93%) of **83** as a colorless crystalline solid. Mp 228–229 °C (DSC); $[\alpha]_D^{20}$ −49.7 (c 0.35, MeOH); ^{1}H NMR (500 MHz, CD_3OD) δ 3.91-3.86 (m, 1H), 3.38 (tt, J = 11.1, 4.4 Hz, 1H), 2.69-2.56 (m, 2H), 2.26 (dt, J = 11.8, 13.1 Hz, 1H), 2.00-1.86 (m, 4H), 1.79-1.60 (m, 6H), 1.57-1.46 (m, 2H), 1.42-1.32 (m, 2H), 1.17-1.06 overlapping signals (m, 2H and 1.12, d, J = 6.8 Hz, 3H), 1.04-0.95 overlapping signals (m, 2H and 1.00, d, J = 6.3 Hz, 3H), 0.87 (s, 3H), 0.70 (dt, J = 11.7, 12.3 Hz, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 181.28, 72.92, 68.72, 57.69, 48.38, 43.81, 43.25, 42.15, 40.36, 40.02, 36.91, 36.60, 36.06, 35.67, 34.25, 33.23, 31.46, 29.25, 25.43, 23.71, 21.62, 15.16; HRMS (ESI) m/z calcd for $C_{22}H_{36}O_4Na^+$ 387.2506, found 387.2509.

***N*-(3α,7α-Dihydroxy-12β-methyl-17-*epi*-18,23,24-*trisnor*-5β-cho lan-22-oyl)taurine sodium salt (84).** Conjugation of **83** (20 mg, 54.9 μmol) with taurine (**22**) was carried out according to Method B employed for the preparation of **23** by using DPPA. The crude product was purified by automated reversed-phase chromatography on C18 silica gel (20–80% methanol in water) to give 15 mg (55%) of conjugate **84** as a white powder. ^{1}H NMR (500 MHz, CD_3OD) δ 3.91-3.86 (m, 1H), 3.58 (t, J = 7.0 Hz, 2H), 3.37 (tt, J = 11.1, 4.5 Hz, 1H), 3.02-2.91 (m, 2H), 2.49-2.37 (m, 2H), 2.25 (dt, J = 11.9 Hz, 12.9 Hz, 1H), 2.00-1.84 (m, 4H), 1.78-1.59 (m, 6H), 1.57-1.44 (m, 2H), 1.43-1.28 (m, 2H), 1.18-1.04 overlapping signals (m, 2H and 1.09, d, J = 6.6 Hz, 3H), 1.03-0.89 overlapping signals (m, 2H and 0.96, d, J = 6.1 Hz, 3H), 0.86 (s, 3H), 0.71-0.61 (m, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 179.98, 72.93, 68.82, 58.03, 51.41, 48.18, 43.21, 43.11, 42.80, 41.40, 40.36, 36.88, 36.56 (2C), 36.01, 35.65, 34.22, 33.21, 31.45, 28.52, 26.13, 23.61, 21.85, 16.55; HRMS (ESI) m/z calcd for $C_{24}H_{40}NO_6S^-$ 470.2582, found 470.2576.

6α-Ethyl-3α,7α-diformyloxy-12β-methyl-17-*epi*-18,24-*bisnor*-5β -chol-22(23)-ene (85). Using the same procedure as outlined for the preparation of **80**, the formyl-protected 6α-ethyl-bile acid **77** (687 mg, 1.44 mmol) in dry toluene (9 mL) was treated with five portions of bis (acetoxy)iodobenzene (BAIB; 5 × 288 mg, 0.894 mmol) in the presence of copper(II) acetate (52.4 mg, 0.288 mmol) and dry pyridine (0.11 mL, 1.36 mmol) to yield 230 mg (37%) of **85** as a colorless amorphous solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.14 (s, 1H), 8.04 (d, J = 0.8 Hz, 1H), 5.76 (ddd, J = 17.2, 10.4, 6.8 Hz, 1H), 5.34-5.30 (m, 1H), 4.94-4.86 (m, 2H), 4.72 (tt, J = 11.3, 4.5 Hz, 1H), 2.46-2.39 (m, 1H), 2.23-2.17 (m, 1H), 1.96 (dt, J = 14.5, 3.4 Hz, 1H), 1.93-1.70 overlapping signals (1.89, dt, J = 11.8, 13.1 Hz, 1H and m, 6H), 1.65-1.51 (m, 3H), 1.51-1.36 (m, 3H), 1.35-1.25 (m, 1H), 1.24-1.01 (m, 4H), 1.00-0.94 overlapping signals (m, 1H; 0.98, d, J = 6.3 Hz, 3H), 0.94-0.87 overlapping signals (0.92, d, J = 6.8 Hz, 3H; 0.902, t, J = 7.3 Hz, 3H and 0.895, s, 3H), 0.67 (dt, J = 11.6,

12.4 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 160.60, 160.37, 145.26, 111.73, 74.45, 73.00, 56.34, 45.93, 44.81, 42.50, 42.28, 40.31, 36.77, 35.40, 35.38, 34.98, 33.98, 31.87, 28.94 (2C), 26.77, 23.68, 23.23, 22.14, 21.09, 16.59, 11.44; HRMS (ESI) *m/z* calcd for $C_{27}H_{42}O_4Na^+$ 453.2975, found 453.2997.

Mixture of diastereoisomers of 6α-ethyl-3α,7α-diformyloxy-12β-methyl-17-*epi*-18,24-*bisnor*-5β-cholan-22,23-diol (86). Applying of the same synthetic method as for the preparation of **81**, compound **85** (230 mg, 0.534 mmol) dissolved in acetonitrile (4 mL) and water (1 mL) was dehydroxylated in the presence of osmium tetroxide (6.8 mg, 0.027 mmol) and 4-methylmorpholine *N*-oxide (NMO; 103 mg, 0.879 mmol) at room temperature overnight to furnish 200 mg (81%) of **86** as a colorless foam. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.15-8.13 (m, 1H), 8.04 (d, $J = 0.5$ Hz, 1H); 5.34-5.29 (m, 1H), 4.71 (tt, $J = 11.3$, 4.5 Hz, 1H), 3.75-3.69 (m, 1H), 3.51-3.35 (m, 2H), 2.60-2.23 (m, 3H), 1.99-1.61 (m, 10H), 1.58-0.94 (m, 11H and 0.97, d, $J = 6.3$ Hz, 3H), 0.94-0.73 (m, 9H), 0.73-0.63 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$; major isomer) δ 160.67, 160.43, 75.28, 74.45, 73.03, 64.69, 55.98, 45.84, 44.74, 43.49, 40.25, 37.12, 35.37, 35.35, 34.93 (2C), 33.89, 31.80, 29.48, 28.91, 26.73, 23.20, 23.06, 22.10, 20.99, 12.86, 11.41; ^{13}C NMR (125 MHz, $CDCl_3$; minor isomer) δ 76.44, 72.97, 64.95, 56.25, 43.19, 38.31, 35.29, 31.74, 29.57, 23.45, 20.86, 12.92; HRMS (ESI) *m/z* calcd for $C_{27}H_{44}O_6Na^+$ 487.3030, found 487.3035.

6α-Ethyl-3α,7α-diformyloxy-12β-methyl-17-*epi*-18,23,24-*trisnor*-5β-cholan-22-oic acid (87). Using the same procedure as outlined for the preparation of **82**, the diol **86** (200 mg, 0.430 mmol) dissolved in acetonitrile (3 mL) and water (1 mL) was treated with 2,2,6,6-tetramethylpiperidine 1-oxyl (TEMPO; 13.6 mg, 0.087 mmol) and bis(acetoxy) iodobenzene (BAIB; 707 mg, 6.21 mmol) to give 157 mg (81%) of **87** as a colorless foam. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.15 (s, 1H), 8.04 (d, $J =$ 0.7 Hz, 1H), 5.34-5.31 (m, 1H), 4.71 (tt, $J = 11.2$, 4.5 Hz, 1H), 2.66-2.59 (m, 2H), 1.95 (dt, $J = 14.4$, 3.2 Hz, 1H), 1.92-1.68 (m, 8H), 1.63-1.08 (m, 10H), 1.10 (d, $J = 6.7$ Hz, 3H), 1.07-0.99 (m, 1H), 0.98 (d, $J =$ 6.3 Hz, 3H), 0.93-0.87 overlapping signals (0.90, t, $J = 7.3$ Hz, 3H and 0.896, s, 3H), 0.68 (dt, $J = 11.7$, 12.3 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 183.20, 160.69, 160.38, 74.35, 72.75, 56.07, 45.69, 44.69, 42.34, 40.31, 40.24, 38.67, 35.33, 35.28, 34.90, 33.91, 31.70, 28.87, 28.40, 26.69, 24.26, 23.15, 22.06, 20.76, 14.36, 11.38; HRMS (ESI) *m/z* calcd for $C_{26}H_{40}O_6Na^+$ 471.2717, found 471.2722.

6α-Ethyl-3α,7α-dihydroxy-12β-methyl-17-*epi*-18,23,24-*trisnor*-5β-cholan-22-oic acid (88). Employing the same method as described for the preparation of **83**, the carboxylic acid **87** (157 mg, 0.350 mmol) dissolved in methanol (3 mL) was treated with a 2 M aqueous sodium hydroxide solution (3 mL) at 90 °C overnight to afford 105 mg (76%) of **88** as a colorless foam. $[\alpha]_D^{20}$ −35.8 (*c* 0.45, MeOH); ^{1}H NMR (500 MHz, CD_3OD) δ 3.76-3.73 (m, 1H), 3.36-3.27 (m, 1H), 2.69-2.57 (m, 2H), 1.98-1.59 (m, 10H), 1.59-1.45 (m, 3H), 1.45-1.33 (m, 2H), 1.28 (dt, $J =$ 13.1, 3.4 Hz, 1H), 1.17-0.97 overlapping signals (m, 4H; 1.11, d, $J = 6.9$ Hz, 3H and 1.00, d, $J = 6.3$ Hz, 3H), 0.91 (t, $J = 7.2$ Hz, 3H), 0.86 (s, 3H), 0.69 (dt, $J = 11.6$, 12.4 Hz, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 181.32, 73.27, 70.87, 57.74, 49.32, 47.11, 43.90, 42.86, 42.18, 40.04, 37.06, 36.83, 36.59, 34.70, 34.44, 33.24, 31.38, 29.24, 25.44, 24.10, 23.55, 21.59, 15.12, 12.10; HRMS (ESI) *m/z* calcd for $C_{24}H_{40}O_4Na^+$ 415.2819, found 415.2824.

***N*-(6α-Ethyl-3α,7α-dihydroxy-12β-methyl-17-*epi*-18,23,24-*trisnor*-5β-cholan-22-oyl)taurine sodium salt (89).** Conjugation of **88** (17 mg, 43.3 μmol) with taurine (**22**) was carried out according to the protocol employed for the preparation of **23** using DPPA. The crude product was purified by automated reversed-phase chromatography on C-18 silica gel eluting with 20–80% methanol in water to yield 14 mg (62%) of conjugate **89** as a colorless powder. ^{1}H NMR (500 MHz, CD_3OD) δ 3.76-3.73 (m, 1H), 3.59 (t, $J = 6.8$ Hz, 2H), 3.34-3.26 (m, 1H), 3.01-2.91 (m, 2H), 2.50-2.39 (m, 2H), 1.96-1.82 (m, 4H), 1.80-1.59 (m, 6H), 1.58-1.45 (m, 3H), 1.45-1.32 (m, 2H), 1.28 (dt, $J = 13.5$, 3.8 Hz, 1H), 1.16-1.04 overlapping signals (m, 2H and 1.09, d, $J = 6.7$ Hz, 3H), 1.02-0.93 overlapping signals (m, 2H and 0.96, d, $J = 6.3$ Hz, 3H), 0.91 (t, $J = 7.1$ Hz, 3H), 0.85 (s, 3H), 0.71-0.60 (m, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 179.96, 73.30, 70.97, 58.07, 51.41, 48.83, 47.08, 43.26, 42.85, 42.79, 41.44, 37.05, 36.79, 36.58, 36.54, 34.65, 34.41, 33.22, 31.39, 28.59, 26.08, 24.01, 23.51, 21.83, 16.41, 12.03; HRMS (ESI) *m/z* calcd for $C_{26}H_{44}NO_6S^-$ 498.2895, found 498.2888.

4.3. Reporter assay conditions

Cell culture. Human embryonic kidney cells (293T/17; CRL 11268), purchased from the American Type Culture Collection and stored in liquid nitrogen in 10% (v/v) DMSO, were thawed and sub-cultured a minimum of three times. Once thawed, cells were transferred into specialized culture media and kept in a humidified 37 °C incubator in 5% CO_2. For maintenance, cells were grown in a complete growth medium comprising of Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) foetal bovine serum (FBS) and 1% (v/v) penicillin and streptomycin sulfate (pen/strep). For all experiments, cells were transferred to a stimulation medium comprising of DMEM supplemented with 1% (v/v) pen/strep. After reaching 60–80% confluency (every 2–3 days), cells were sub-cultured using trypLE select enzyme at a 1:10 dilution prior to each experiment. Prior to each experiment, all cells were at an early passage number (<20) when they reached their final 60–80% confluency before being set-up in experimental plates.

Reverse transfection of HEK293 cells with human TGR5 receptor. Following counting on a haemocytometer using 50% (v/v) Trypan blue stain, 293T/17 cells were plated on TGR5 Reverse Transfection strips (TGR5 (GP-BAR1) Reporter Assay kit; Cayman Chemical) at a density of 3.8×10^4 cells/well in 200 μL complete growth media for 20 h to enable cell adherence and growth to 75% confluency. Hereafter, it was assumed all 293T/17 cells were stably expressing the human TGR5 receptor.

Stimulation of TGR5 with test samples. The complete growth media was aspirated from all wells containing 293T/17 cells and replaced with 150 μL of pre-warmed stimulation media. Each well was stimulated with various concentrations of synthetic BAs in 50 μL of pre-warmed stimulation media containing 1% (v/v) DMSO. Each concentration of each bile acid screened was performed in triplicate. Negative control wells received 50 μL of pre-warmed stimulation media containing 1% (v/v) DMSO alone. Each TGR5 reporter assay experiment included positive control wells which contained a 7-point (1:4 serial dilution) LCA standard curve starting at 10 μM concentration. After the addition of test compounds, the transfection plate was incubated on the bench for 30 min, before being transferred to the 37 °C, 5% CO_2 incubator for 7 h.

SEAP secretion analysis *via* luminescence quantification. Following incubation, a 10 μL media sample was transferred from each well to a 96-well solid assay plate (white). The assay plate was covered and heated at 65 °C for 30 min to inactivate endogenous alkaline phosphatase. Thereafter, 50 μL of secreted alkaline phosphatase (SEAP) substrate was added and the assay plate was shaken briefly to mix and then incubated at room temperature for 5 min. A luminescence plate reader was used to measure relative light units (RLU) in each well.

Determination of 50% effective concentration (EC_{50}) and efficacy. Each triplicate RLU measurement was averaged and the mean basal (untreated cells) RLU response subtracted. Efficacy was calculated as a percentage of the RLU response produced by the highest reference standard concentration (10 μM of LCA). The data was plotted as a function of percentage response against logarithmic concentrations of test compounds. Four parameter nonlinear regressions without constraints were used to plot best fit sigmoidal dose response curves using Prism: GraphPad Software. Log EC_{50} values were extrapolated from the dose response curves and converted to the anti-log for comparison between test compounds.

Molecular modeling of 12β-methyl-18-*nor*-BA analogues to

TGR5. The structure of human TGR5 was taken from the Cryo-EM structure of the INT-777-bound GPBAR-Gs complex (PDB 7CFN) and prepared using protein preparation wizard [60,61]. Missing side chains were built in, hydrogens were added and PROPKA predicted the protonation states of ionizable amino acids at pH 7.5. To relieve close contacts and relax the structure, the protein was refined using restrained minimization with the OPLS4 Force Field and heavy atom convergence was set with RMSD equal to 0.30 Å. The prepared structure was used to generate a receptor grid for docking calculations using Glide [61,62]. The center of the receptor grid was defined as the centroid of the INT-777 molecule bound in the orthosteric site. Ligand structures were built in Maestro and prepared using LigPrep [61]. The ligands prepared were modelled into the receptor binding site using Glide with XP mode. The output poses from the Glide docking were further optimized using MMGBSA calculations, during which an implicit membrane was employed to mimic the membrane environment of the receptor [63].

5. Associated content

Supporting information

Additional figures and tables, ^{1}H and ^{13}C NMR spectra and LCMS chromatograms for compounds as well as crystallographic data and structure refinement tables for compounds **43**, **70** and **83** (PDF). CCDC 2191713–2191715 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre *via* www.ccdc.cam.ac.uk/structures .

Notes

Anthony D. Woolhouse: Deceased (31.12.2014).

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Andreas Luxenburger reports financial support was provided by the New Zealand Ministry of Business, Innovation and Employment (MBIE) and New Zealand Pharmaceuticals (now part of ICE Pharma). Alex Weymouth-Wilson reports a relationship with ICE Pharma that includes: employment.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmech.2023.115143.

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