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Modern approaches to modification of bile acids for the synthesis of compounds possessing valuable physicochemical and biological properties

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Data published in the period from 2000 to 2015 on chemical transformations of the major human bile acids (cholic, chenodeoxycholic, deoxycholic and lithocholic acids) and ursodeoxycholic acid are integrated and described systematically. Characteristic features of their structures, biosynthesis, main sources and biological functions are considered. The data on chemical transformations of the indicated bile acids include reactions involving modifications of the side chain or the steroid nucleus. The biological activities and applications of these derivatives are surveyed in brief.
The bibliography includes 227 references.

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I. Introduction

The synthesis of new compounds possessing valuable biological or physicochemical properties based on substances of plant and(or) animal origin is a promising research area of organic and medicinal chemistry. Bile acids (BAs) are steroid metabolites found predominantly in bile of various animals. Early investigations of the bile composition date back to the first decade of the 19th century. Over five decades, major BAs of humans and some animals were isolated in the individual state and structurally characterized: cholic (CA), chenodeoxycholic (CDCA), deoxycholic (DCA) and lithocholic (LCA) acids, as well as glycine- and taurine-conjugated BAs, which are formed from BAs under physiological conditions.[1, 2] In the next stage, BAs were isolated from various animals, their structures and metabolism were studied, and their physicochemical and biological properties were investigated in detail. With the development of physicochemical methods of analysis and the assessment of physiological properties of BAs, the research focused on reactivity of these compounds. New derivatives were synthesized and tested for different biological activities.

Chemical modifications, physicochemical properties and biological functions of BAs have been covered in numerous publications. The biological activities of bile acids and their derivatives and application of these compounds in medicinal chemistry are considered in many reviews. However, the data on chemical modifications of BAs were not summar-

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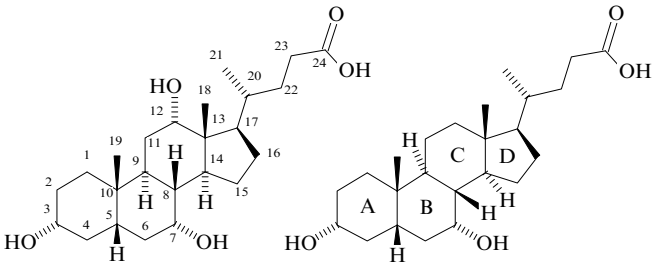
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ized, systematically considered and integrated in relation to chemical transformations. The goal of this review is to summarize the data published in the period from 2000 to 2015 on chemical modifications of major human BAs (CA, CDCA, DCA, LCA) and ursodeoxycholic acid (UDCA), which is present in bile in trace amounts. The emphasis is given to reactions producing low-molecular-weight compounds. The synthesis of supramolecular composites based on BAs is considered in brief. It should be noted that the configurations of chiral centres in BA derivatives are given in accordance with the structures reported in the cited studies.

II. Structure, biosynthesis, main sources and functions of bile acids in the body

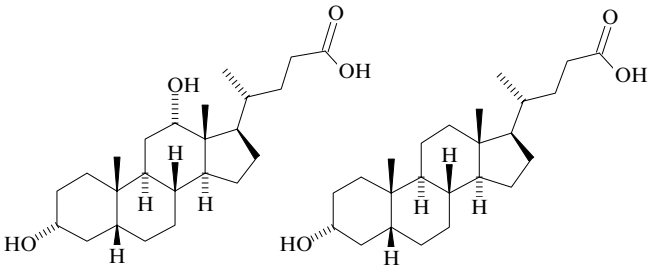
The structures of mammalian BAs include the rigid steroid nucleus containing one to three hydroxyl groups and a short aliphatic side chain bearing the terminal carboxyl group. Major human bile acids — cholic (**1**), chenodeoxycholic (**2**), deoxycholic (**3**) and lithocholic (**4**) — differ in the number and arrangement of hydroxyl groups in the steroid nucleus. Ursodeoxycholic acid (**5**) is the 7β-epimer of CDCA. In allocholic acid (**6**), the mode of ring fusion differs from that in the above BAs.

Structures 1 – 6



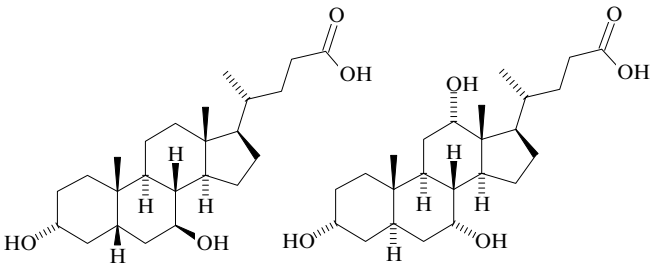
1 (cholic acid)

2 (chenodeoxycholic acid)



3 (deoxycholic acid)

4 (lithocholic acid)



5 (ursodeoxycholic acid)

6 (allocholic acid)

The steroid nucleus of BAs is a saturated tetracyclic hydrocarbon — cyclopentanoperhydrophenanthrene (gonane) consisting of the following fused rings: three six-membered rings (A, B, C) and one five-membered ring (D). The rings A and B can be either *cis*- or *trans*-fused (Fig. 1). Bile acids in higher vertebrates are characterized by *cis*-fusion (β configuration of hydrogen at position 5), and their skeleton is bent. Some BAs in lower vertebrates, known as allocholic acids, contain *trans*-fused rings A and B [α configuration of C(5)–H][3] and, consequently, have a planar structure (see Fig. 1).[1,2]

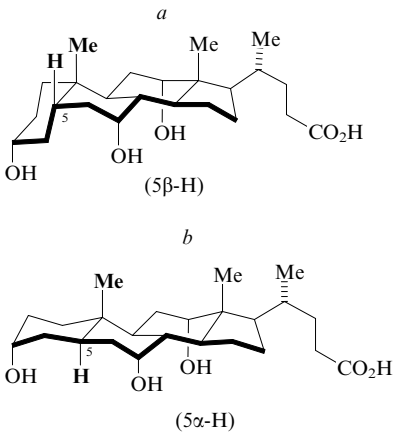


Figure 1. *cis*-Fusion of rings A and B in cholic acid (*a*) and *trans*-fusion of these rings in allocholic acid (*b*).

Bile acids are amphiphilic compounds due to the presence of several hydroxyl groups and their arrangement in the steroid nucleus (Fig. 2). The difference in the number of hydroxyl groups is responsible for the difference in solubility and surface activity of these compounds. The convex surface (β-face) of the steroid nucleus of BA molecules has a more pronounced hydrophobic character, while the concave surface (α-face) is hydrophilic. The simultaneous presence of polar and non-polar surfaces in the molecule determines the behaviour of BAs in the self-association as well as other physicochemical properties at the interface.

In the body, BAs are synthesized from cholesterol in liver hepatocytes. This multi-enzymatic biosynthesis involves a number of complex chemical transformations (Scheme 1).[4–6] In the first biosynthetic step, cholesterol is converted into 7α-hydroxycholesterol and then into the key intermediate **A** — 7α-hydroxycholest-4-en-3-one. The subsequent biosynthesis follows two pathways to form acids **1** and **2**. The hydroxylation at position 12 gives rise to the CA skeleton, and the subsequent stereoselective reduction of 7α-hydroxycholest-4-en-3-one results in the formation of *cis*-fused rings A and B characteristic of BAs in higher mammals and humans. The next step involves the oxidative cleavage of the side chain to form a carboxyl group. The

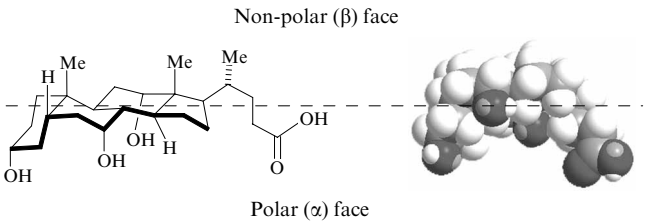
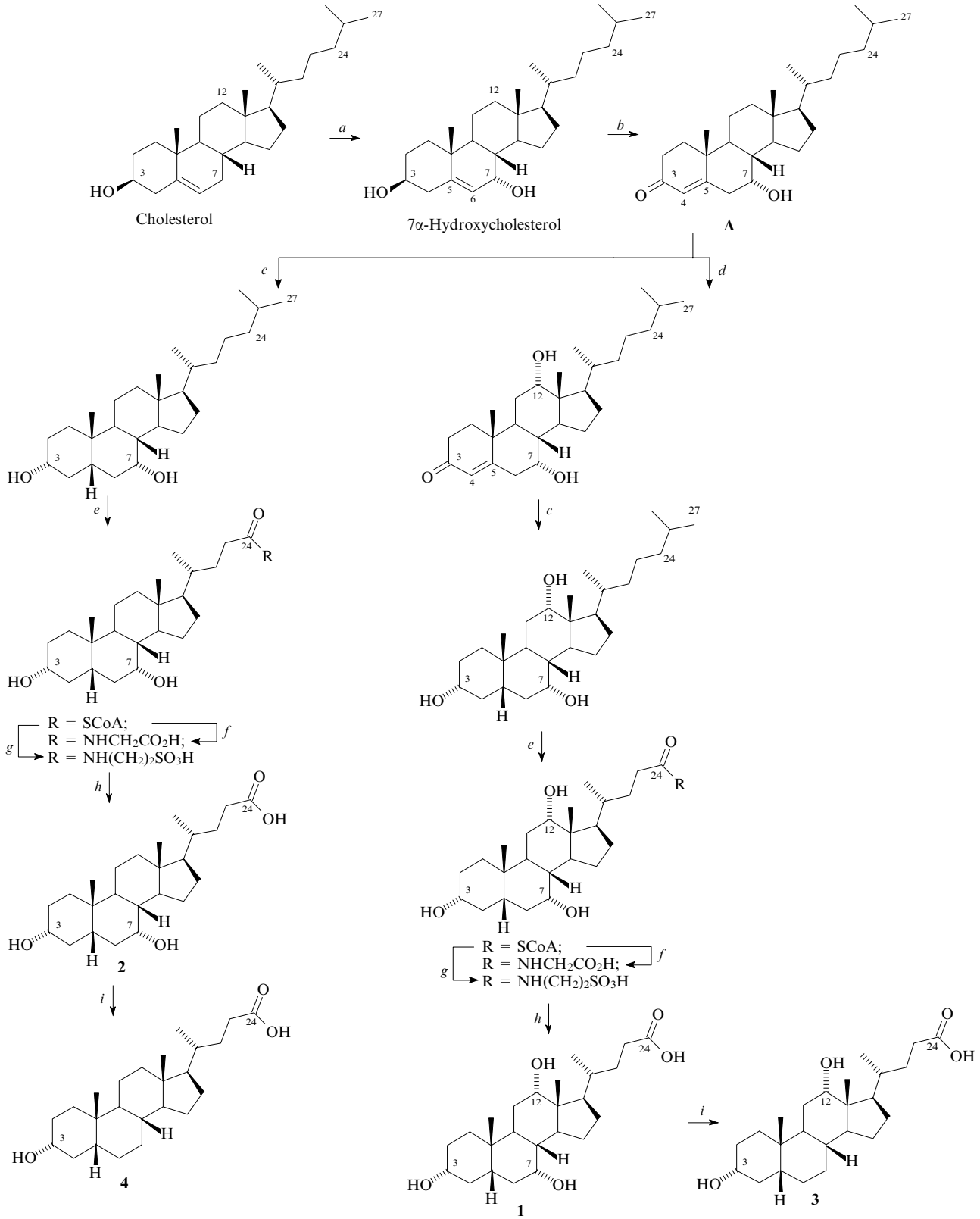


Figure 2. Three-dimensional structure of cholic acid.

transformation of the latter catalyzed by the activating enzyme in liver microsomes gives coenzyme A (CoA) esters. The resulting cholyl- and chenodeoxycholyl-CoA contain α-OH groups at positions 3, 7, 12 and 3, 7, respectively, and

Scheme 1



(*a*) oxysterol 7α-hydroxylase; (*b*) 7β-hydroxy-Δ^5-C_{27}-steroid oxidoreductase; (*c*) 1) Δ^4-3-oxosteroid 5β-reductase, 2) 3α-hydroxysteroid dehydrogenase; (*d*) steroid 12α-hydroxylase; (*e*) 1) side chain oxidation, 2) conjugation; (*f*) glycine, *N*-acetyltransferase; (*g*) taurine, *N*-acetyltransferase; (*h*) deconjugation; (*i*) 7α-dehydroxylation

have the fully saturated steroid nucleus (see Scheme 1). The CoA derivatives of BAs are converted into glycine- and taurine-conjugated forms *via* amidation. Gut microbiota-mediated deconjugation produces CA (**1**) and CDCA (**2**) — primary BAs. Normally, BAs are secreted into bile in the conjugated form. In humans, the ratio of glycine- to taurine-conjugated BAs is *ca.* 3 : 1. This conjugation increases solubility of BAs in acidic media, prevents the precipitation of BAs in the presence of Ca^{2+} ions and renders these bile acids impermeable to cell membranes.[4,7] Since bile contains large concentrations of sodium and potassium ions and has alkaline pH, BAs and their conjugated forms are generally considered as salts (the commonly used term 'bile salts' refers to salts of bile acids).[4]

After biosynthesis in the liver, BAs are secreted into bile, which is stored and concentrated in the gallbladder. The food intake initiates the secretion of bile into the small intestine, where BAs exert their physiological function: solubilize fats, fatty acids and fat-soluble vitamins and facilitate their absorption.

In the gut, primary BAs partially undergo gut microbiota-mediated deconjugation and 7α-dehydroxylation to form secondary BAs. Thus, deoxycholic acid (**3**) is derived from cholic acid; lithocholic acid (**4**), from chenodeoxycholic acid (see Scheme 1).[4] Apart from major BAs in higher mammals and humans (CA, CDCA, DCA, LCA) and their glycine- and taurine-conjugated forms, minor BAs are produced at lower concentrations by gut bacterial microbiota or *via* conjugation to other biomolecules in the liver.[7] Ursodeoxycholic acid (**5**) is one of minor bile acids, which was isolated for the first time from bear bile (ursus means bear in Latin).

Then BAs are absorbed in the distal part of the ileum, return to the liver through the portal vein and reexcreted into bile. This process is known as enterohepatic circulation. A small portion of bile salts is not absorbed and is secreted from the body through the digestive tract in the faeces. Despite the fact that a relatively small amount of BAs is secreted *via* this route, this is the major route for cholesterol excretion from the body. The loss of BAs *via* faecal excretion is compensated by the *de novo* synthesis of BAs from cholesterol in an amount equivalent to that being excreted, due to which the bile acid pool remains essentially constant. This process is regulated *via* a feedback mechanism.[4,5,8]

Bile of mammals (cattle, sheep and pig) is the main natural source of BAs. Since these acids are present in bile in the conjugated form, free BAs are produced by alkaline hydrolysis of the bile solid material. Then dilute hydrochloric acid is added to convert sodium salts of BAs into free acids followed by extraction with organic solvents (diethyl ether, ethyl acetate or lower alcohols). The subsequent purification and separation of BAs are performed by crystallization or chromatography.[9–11] This procedure is mainly applied to obtain CA, CDCA and DCA, because their amounts in bile are rather large. Methods for the synthesis of DCA and LCA from available steroids have been developed.[12,13] Chenodeoxycholic and ursodeoxycholic acids, which are used in medical practice, can be synthesized from cholic acid.[14]

Bile acids have a broad spectrum of biological activities, including anti-inflammatory, antitumour, cytoprotective, neuroprotective, antifungal and antibacterial action.[1,15–19] The main physiological functions of bile acids are the solubilization and transport of lipids and lipid-soluble vitamins, as well as the regulation of lipid, glucose and cholesterol homeostasis.[18] The structural features and physiological functions of BAs have dictated their use in medical practice in early stages of investigation. In particular, cholic acid served for the regulation of bile acid biosynthesis. Chenodeoxycholic and ursodeoxycholic acids were used to dissolve gallstones. Later on, UDCA substituted for CDCA because UDCA exerts the same action but is much less toxic.[1] Ursodeoxycholic acid was employed for the treatment of different biliary and hepatic diseases. It may neutralize the toxic effect of other BAs, exhibits anti-apoptotic activity against both liver and other cells and can serve as a neuroprotectant.[19–21] Taurine-conjugated UDCA prevents development of cardiac fibrosis and has other useful properties.[22–24]

Bile acids also function as hormones that bind to nuclear receptors: farnesoid X receptor (FXR), liver X receptor (LXR), pregnane X receptor (PXR), constitutive androstane receptor (CAR), vitamin D receptor (VDR)[18,25–30] and G-protein-coupled membrane receptor (TGR5 or GP-BAR1).[31] It should be noted that BAs are cytotoxic when present in abnormally high concentrations[26] and can cause cell necrosis or apoptosis.[32] Disorders of bile acid biosynthesis and excessive BA accumulation in organs and cells leads to development of different diseases. The characteristic features of bile acid metabolism and pathophysiology are considered in more detail in monographs[33,34] and the review.[1]

III. Specific features of chemical modifications of bile acids

In past decades, BAs have attracted attention of researchers in different areas of chemistry: medicinal chemistry, supramolecular chemistry, materials science and so on. Due to a broad spectrum of native biological activities, unique physicochemical properties, structural features, high enantiomeric purity, low cost and availability, BAs are ideal starting materials for chemical transformations.[1,2,18]

The chemistry of BAs is based on known organic reactions that allow researchers to synthesize of derivatives at carboxyl and hydroxyl groups and to modify the steroid nucleus by introducing various functional substituents. Combinations of these reactions can be applied to prepare new bile acid derivatives with valuable properties (including biological activity and amphiphilicity), which have a great potential for medicinal and supramolecular chemistry, as well as for some other fields of chemistry.

Since BAs are specifically distributed in the human body due to the involvement in enterohepatic circulation, the synthesis of BA-based hybrid compounds (conjugates) has been extensively investigated. These molecules consist of a biologically active moiety, including a drug, attached either directly or through a linker to carbon atoms at different positions of BAs [most often to C(3), C(7), C(12), C(24)] by forming a covalent bond (ester or amide bond; less frequently, ether bond). The synthesis of such conjugates holds promise for solving problems of selective drug delivery and improvement of bioavailability of poorly soluble pharmaceutical agents, in particular, due to enhancement of intestinal absorption of poorly absorbed or non-absorbed drugs (*e.g.*, peptides). In some cases, the conjugation reduces toxicity of pharmaceutical agents.[35–37]

IV. Transformations of bile acids involving side chain

IV.1. Reactions at the carboxyl group

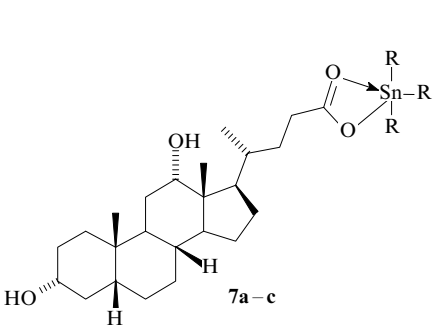
IV.1.a. Synthesis of salts and complexes with metals

Bile acids and their salts are natural surfactants. Due to structure rigidity and the specific arrangement of polar and non-polar parts (surface amphiphilicity), the self-association of BAs and their salts produces quite stable ordered two- or three-dimensional structures (globules, micelles, films and so on) depending on the conditions.[1,2] Because of their strong tendency to self-aggregate and form micellar structures, BAs and their salts with alkali,[38,39] alkaline-earth[40] and transition[41] metals are employed as auxiliary reagents in the synthesis of nanomaterials with different structures and investigations of their properties. Sodium salts of BAs and their derivatives are used to construct nanostructures loaded with biologically active molecules for the targeted drug delivery to organs or tissues or for improvement of bioavailability.[42,43] Complexes of BAs with pharmaceuticals have been described.[44,45] Sodium salts of CA, DCA, CDCA and UDCA are widely applied as surfactants, biological media and drugs.[1,38,39]

Bile acids can act as ligands and form complexes with metals possessing valuable physicochemical or biological properties. For example, tin-containing DCA derivatives **7a – c** exhibit antitumour activity.[46] The heating of CA with $Ru_3(CO)_{12}$ in dry THF followed by the addition of triphenylphosphine gives ruthenium complex **8**.[47] Complexes of UDCA and dehydrocholic (3,7,12-trioxo-5β-cholanic, dehydro-CA) acid with platinum compounds (structures **9** and **10**) possess antitumour activity.[44,45,48–51] These complexes were synthesized by exchange reactions with both Na and silver salts (silver oxide precipitate as the control). Complexes of CA with Ca, Cd, Pb, Bi and transition metals (Ni, Co, Hg, Ag, Zn, Cu) were prepared by reactions with salts of appropriate metals in methanol at room temperature or under reflux; some of these complexes exhibit antifungal properties.[52] Complexes of UDCA with transition metals (Co, Ni, Cu, Zn) were synthesized by the reaction of aqueous solutions of the Na salt of UDCA with appropriate metal nitrates at room temperature.[53] Strontium salts of cholic acid and its derivatives were prepared by reactions of appropriate acids with $Sr(OH)_2$ in water; these compounds were tested for antiosteoporotic activity, and compound **11** proved to be most active. These salts can be considered as promising agents that regulate the loss of bone mass or regeneration of bone tissues.[54]

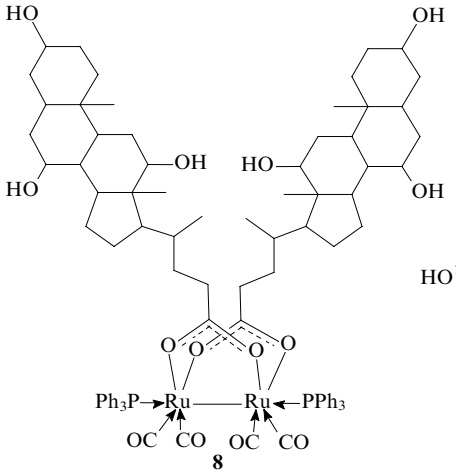
Apart from BA salts with metal ions, BA salts with organic cations, in particular with nicotine derivatives (compounds **12 – 14**), were synthesized by Jasiewicz *et al.*[55] The authors made an attempt to find compounds possessing

Structures 7 – 14

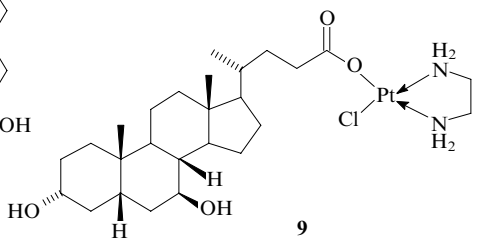


7a – c

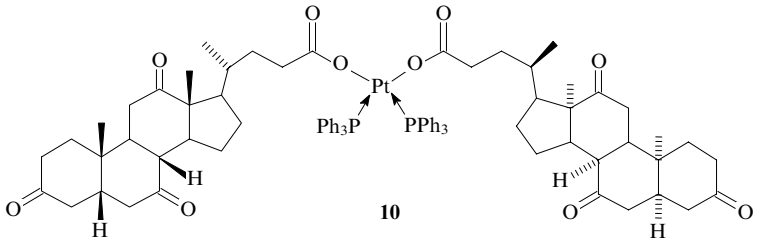
R = Me (**a**), Bu^n (**b**), Ph (**c**)



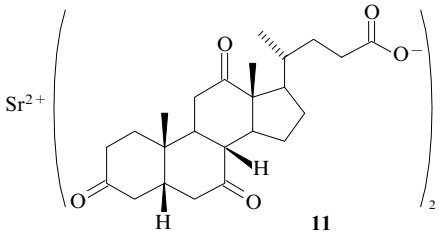
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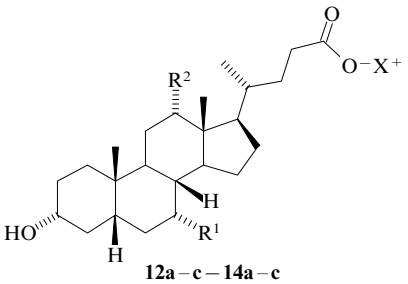
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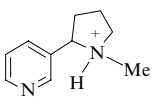
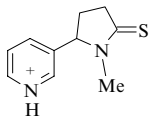
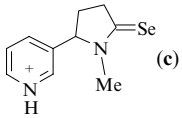


11



12a – c – 14a – c

R^1 = H: R^2 = H (**12**), OH (**13**); R^1 = R^2 = OH (**14**);

X =  (**a**),  (**b**),  (**c**)

hemolytic activity; however, BA derivatives that were synthesized did not exhibit this activity.

Therefore, salts and complexes of BAs with different metals can be synthesized by reactions of aqueous or alcoholic solutions of acids with salts, hydroxides and complexes of appropriate metals at room temperature or on heating or by the exchange reaction of Na salts of appropriate acids with a metal salt (complex).

IV.1.b. Synthesis of esters

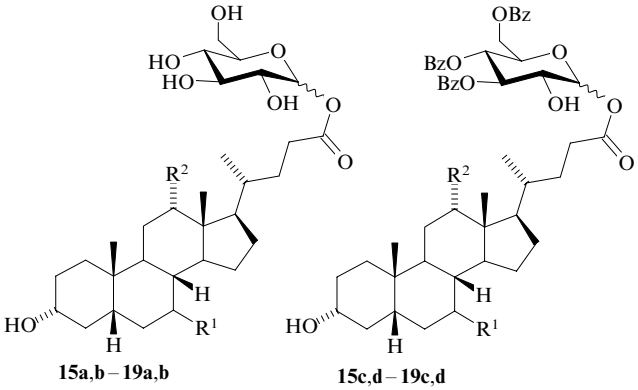
Esters represent an important group of carboxylic acid derivatives and are naturally abundant. The direct esterification of the carboxyl group by alcohols is a conventional method for the preparation of BA esters. This method proved to be efficient for the synthesis of esters of methanol, ethanol and other lower alcohols,[56–60] as well as of normal higher alcohols. This modification provides a tool for varying the hydrophilic – hydrophobic balance of the molecule, which is important for the development of drugs with bioavailability and(or) specified properties.[61] Bile acid esters can be synthesized by reactions of BAs with alkyl halides[56, 60, 62] or tosyl derivatives (tosyl is *p*-toluenesulfonyl, Ts) of alcohols[63] in the presence of bases or by enzymatic esterification of BAs with alcohols, *e.g.*, using commercially available lipases.[64]

Methyl esters are most widespread bile acid esters. They are used for the protection of the carboxyl group in multistep syntheses, because BAs are polyfunctional molecules. These esters are synthesized by the reaction of BAs with methanol either at room temperature or with slight heating in the presence of inorganic acids[56, 58, 59] or by methylation of BAs with a solution of diazomethane in diethyl ether.[58, 65]

The esterification of the carboxyl group of BAs is applied to synthesize BA conjugates with other compounds linked *via* an ester bond. With the aim of finding possible metabolites of BAs, Iida *et al.*[66] prepared esters of CA, CDCA, DCA, LCA, UDCA with glucose and its derivatives (compounds **15** – **19**).

The conjugation of BAs can be applied to change hydrophobicity (hydrophilicity) of the molecule, which in turn influences its bioavailability. For instance, BA conjugates with natural biologically active secondary alcohols, juvenoids (analogues of juvenile hormones of insects, which are potential environmentally friendly insecticides), were synthesized (compounds **20a**,**b** and **21a**,**b**) with the goal of modifying physicochemical properties of the latter.[67, 68] The reaction with the starting juvenoid was performed in the presence of pyridine, the carboxyl group being preactivated with *N*,*N*′-dicyclohexylcarbodiimide (DCC) in the presence of 4-dimethylaminopyridine (DMAP) or through the intermediate formation of acid chloride.

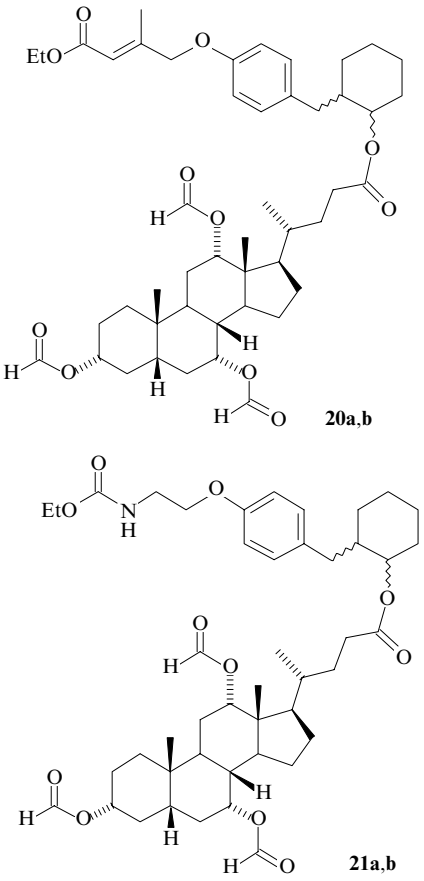
Structures 15 – 19



15a,**b** – **19a**,**b** **15c**,**d** – **19c**,**d**

R^2 = OH: R^1 = α-OH (**15**), H (**16**); R^2 = H: R^1 = α-OH (**17**), H (**18**), β-OH (**19**); epimers: α (**a**, **c**), β (**b**, **d**)

Structures 20, 21



20a,**b**

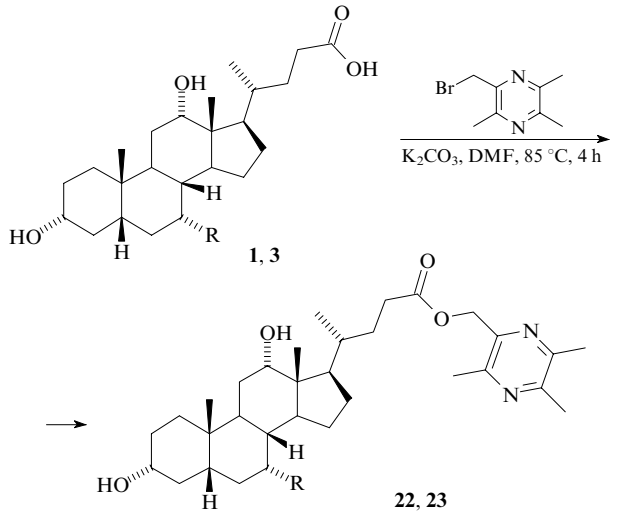
21a,**b**

Isomers: *cis* (**a**), *trans* (**b**)

The conjugation of a biologically active molecule with BAs can lead to enhancement (weakening) of native biological activity or reduction of toxicity of the product compared to the starting molecule. For example, Wang *et al.*[62] synthesized CA and DCA conjugates with ligustrazine (2,3,5,6-tetramethylpyrazine), which exhibits anti-inflammatory activity. Esters **22** and **23** were prepared by heating CA or DCA with 2-bromomethyl-3,5,6-trimethylpyrazine in dimethylformamide in the presence of K_2CO_3 (Scheme 2). The antitumour activity of these derivatives was assessed in five human tumour cell lines (HCT-8, Bel-7402, BGC-823, A-549 and A2780). Derivative of DCA **23** was found to be the most efficient inhibitor of cancer cell proliferation; the half-maximal inhibitory concentration (IC_{50}) in all cell lines varied from 6.619×10^{-6} (for A2780) to 8.012×10^{-6} g ml^{-1} (for Bel-7402).

Bile acids can be employed to prepare esters possessing valuable physicochemical properties.[56, 63] For example, Banerjee *et al.*[63] synthesized polyfluorinated esters **24** – **26** by the reactions of appropriate BA with the tosyl derivative of polyfluorinated alcohol in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) as the base (Scheme 3) and investigated their gelation properties. Organogels and

Scheme 2

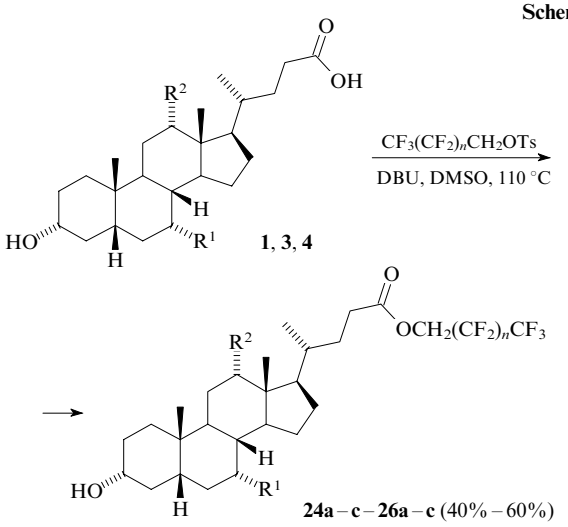


R = OH (**1**; **22**, 55% yield), H (**3**; **23**, 58%)

hydrogels based on BA derivatives can serve as new materials with unique physical properties and can be used for drug delivery.[56, 63]

The carboxyl group can also be modified by esterification with alcohols containing additional functional groups. These products can be employed in subsequent transformations. Two approaches to the synthesis of propargyl ester of BA were considered in relation to CA (Scheme 4). One approach is based on the esterification of cholic acid with propargyl alcohol, the cholic acid being preactivated with 3-ethyl-1-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl) and 1-hydroxybenzotriazole hydrate (HOBt).[69] Another approach involves the reaction of CA with propargyl bromide in the presence of a base (Cs_2CO_3).[60]

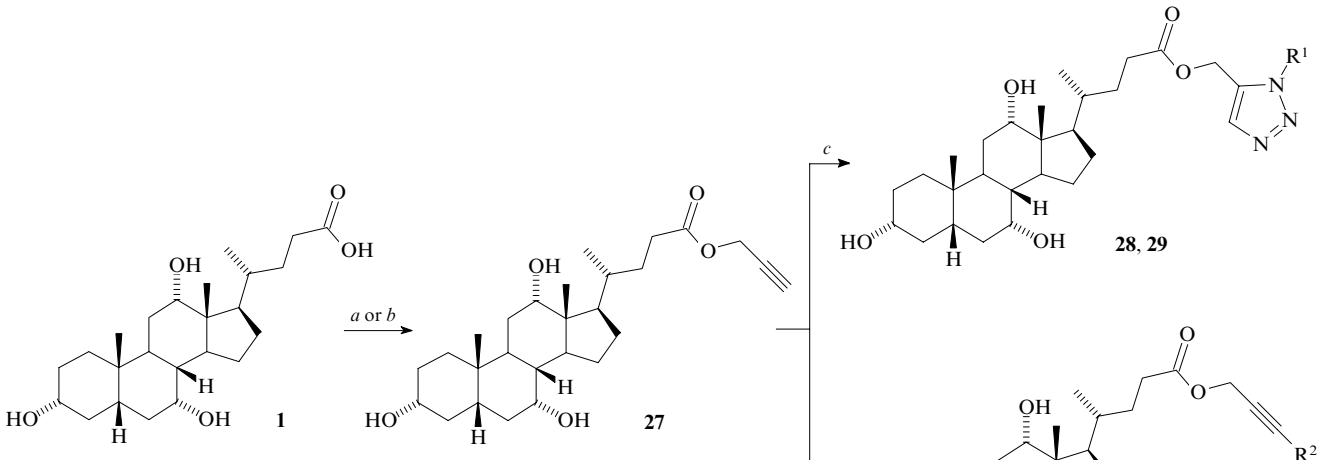
Scheme 3



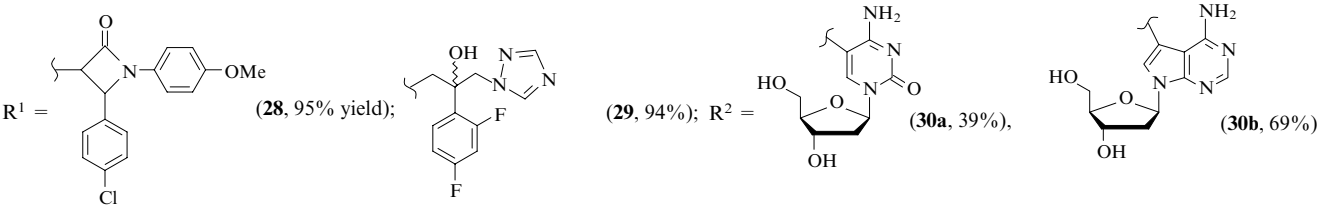
$R^1 = R^2 = OH$ (**1**, **24**), H (**4**, **26**); R^1 = H, R^2 = OH (**3**, **25**); n = 1 (**a**), 6 (**b**), 8 (**c**)

Propargyl esters of BAs are intermediates in the synthesis of conjugates containing the 1,2,3-triazole ring, which serves as a linker between BA and another molecule. An example is the synthesis using propargyl ester **27** as the starting compound, which produces BA conjugates with the

Scheme 4



(*a*) $HC{\equiv}CCH_2OH$, EDC·HCl, HOBt, DMF, 0–25 °C, 12 h (88% yield); (*b*) $HC{\equiv}CCH_2Br$, Cs_2CO_3, DMF; (*c*) R^1N_3, sodium ascorbate, $CuSO_4 \cdot 5H_2O$, DMF–H_2O, MW; (*d*) R^2I, CuI, $Pd(OAc)_2$, $P(C_6H_4SO_3Na\text{-}4)_3$, $EtNPr^i_2$, MeCN–H_2O, 65 °C, 12 min; MW is microwave radiation



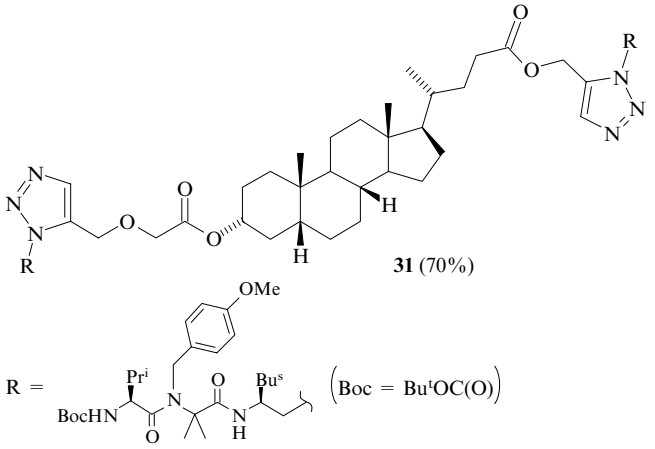
β-lactam antibiotic [69, 70] and an analogue of the antifungal drug fluconazole [71] (compounds **28** and **29**, respectively; see Scheme 4). Apart from 1,3-cycloaddition, the terminal acetylenic group can be involved in cross-coupling. For instance, propargyl esters of CA, DCA and CDCA were subjected to Pd-catalyzed cross-coupling with iodine derivatives of nucleotides and nucleosides in the presence of CuI.[60] Scheme 4 shows examples of the synthesis of conjugates **30a**,**b** based on CA and different nucleosides. Despite the bulky substituent (steroid moiety), these derivatives can be employed in the polymerase chain reaction (PCR) and serve as important building blocks in supramolecular chemistry.

Sokolova *et al.*[59] synthesized dipeptide-containing conjugate **31** based on lithocholic acid through the formation of the corresponding dipropargyl ester.

A series of CA esters with polyfunctional alcohols containing amino groups were described (Scheme 5).[72, 73] These esters were meant for the selective delivery of biologically active compounds to organs and tissues. The activation of the carboxyl group of cholic acid with 1,1′-carbonyldiimidazole (CDI) followed by the reaction with appropriate alcohol in the presence of a base and subsequent deprotection of amino groups gave target esters **32a** – **d**. The reaction of the latter with platinum complexes of different structures produced compounds **33a** – **d** and **34a** – **d**. Biological activity assays demonstrated that compounds **34a** – **d** exhibit cytotoxic activity similar to that of the starting Pt complex.

Therefore, BA esters can be synthesized by esterification with alcohols in an acidic medium or in the presence of enzymes and by the reaction of BAs with alkyl halides or tosyl derivatives of alcohols in the presence of bases. Prior to esterification with polyfunctional alcohols, the carboxyl group of BAs is activated by the reaction with carbodiimides (EDC · HCl, DCC) or 1,1′-carbonyldiimidazole. The reaction is also performed through the intermediate formation of acid chloride. Bile acid conjugates with biologically active compounds can be prepared through the formation of an ester bond. These conjugates facilitate selective drug delivery to organs and tissues or lead to reduction of toxicity of the starting molecule. These conjugates display antiproliferative and cytotoxic activity and have other valuable properties.

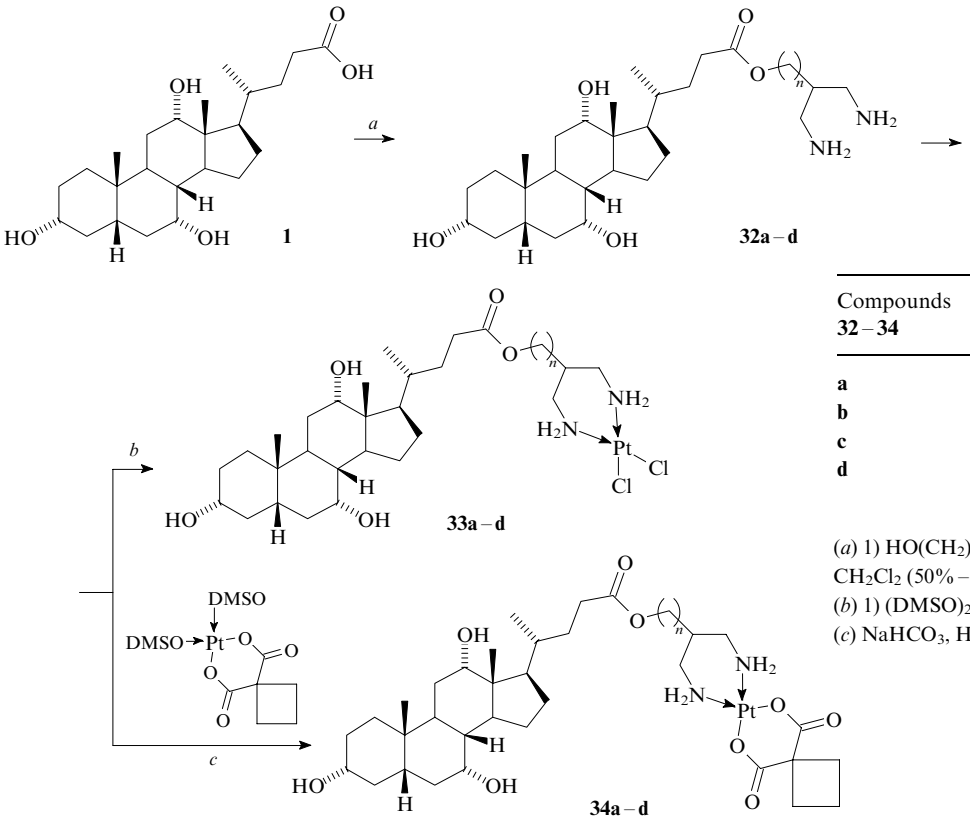
Structure 31



IV.1.c. Synthesis of amides

Amides belong to one of the most extensive classes of BA derivatives. The synthesis of amides involves the preactivation of the carboxyl group either by the reaction with

Scheme 5

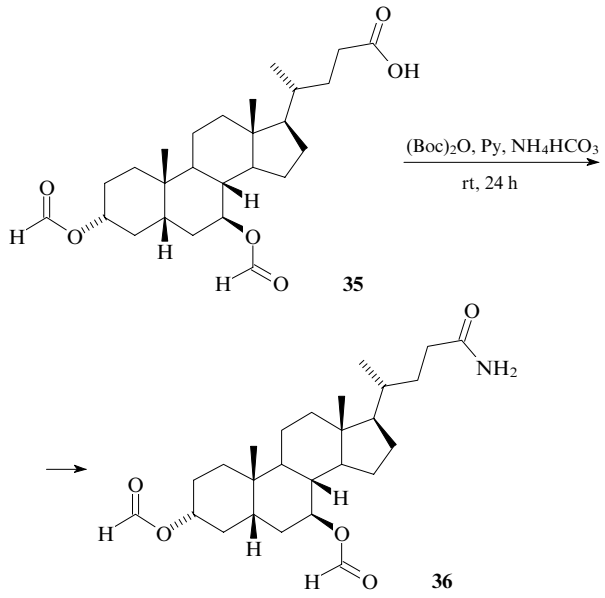


Compounds **32** – **34**	*n*	Yield (%)	
		33	**34**
a	4	58	52
b	6	44	57
c	8	27	54
d	11	58	62

(*a*) 1) $HO(CH_2)_nCH(CH_2NHCO_2Bn)_2$, CDI, DMAP, CH_2Cl_2 (50% – 65% yield); 2) Pd/C, EtOH;
(*b*) 1) $(DMSO)_2PtCl_2$, MeOH; 2) LiCl, H_2O, 80 °C, 2 h;
(*c*) $NaHCO_3$, H_2O, 60 °C, 12 h

$SOCl_2$,[21] ethyl chloroformate,[60,74–76] *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ)[77,78] or hydroxysuccinimide[79–81] in the presence of different carbodiimides or by the treatment with carbodiimides themselves.[69,70,82,83] In some cases, the reaction proceeds through the intermediate formation of ester[84,85] followed by the reaction with amine (aliphatic, aromatic, heterocyclic, polyfunctional) in the presence of a base. The synthesis of amides often requires the preliminary protection of hydroxyl groups to avoid unwanted side reactions. (Methods for the protection of OH groups will be discussed in Section V.1.) Primary amides can be synthesized by the reaction of hydroxyl-protected acids with $(Boc)_2O$ and NH_4HCO_3 in the presence of a base. For instance, this method was applied to synthesize amide **36** from protected acid **35** (Scheme 6).[32]

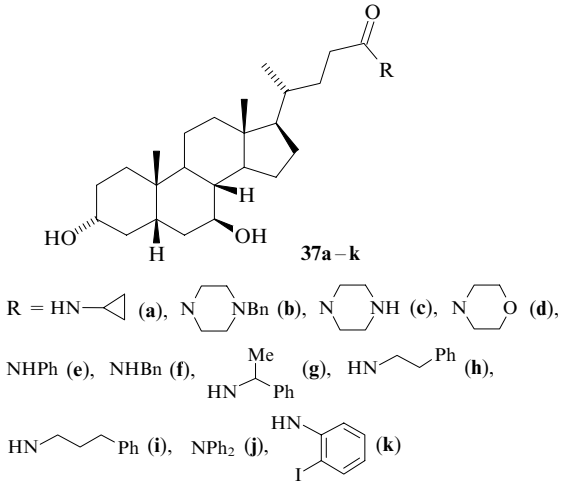
Scheme 6



Py is pyridine

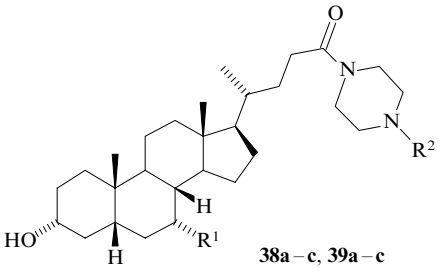
The amidation of UDCA with cyclic polyfunctional amines or amines containing a phenyl or cyclopropyl substituent proceeds through the intermediate formation of acid chloride to give amides **37a–k**. These compounds induce translocation of the glucocorticoid receptor, derivatives **37a–d** exhibiting the highest activity.[21]

Structures 37



Lithocholic and chenodeoxycholic piperazinylamides (**38a–c** and **39a–c**) have an antiproliferative effect on various human cancer cells.[74] Compound **39c** proved to be the most active derivative; the median lethal doses (LD_{50}) were 18.5×10^{-6}, 8.5×10^{-6} and 16.5×10^{-6} mol litre^{-1} against the cell lines GBM, KMS-11 and HCT-116, respectively.

Structures 38, 39

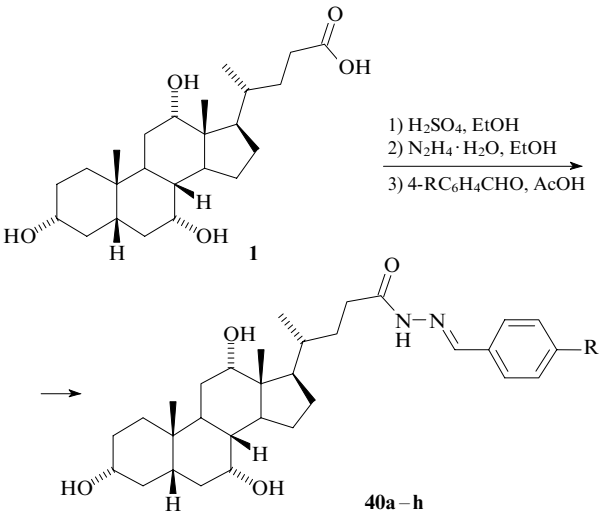


R^1 = H (**38**), OH (**39**);

R^2 = (**a**), (**b**), Ph (**c**)

Hydrazides are another class of BA derivatives containing the C(O)—N bond. These compounds are used as intermediates in the synthesis of biologically active compounds. Cholic acid hydrazone analogues of different structure were synthesized[84] and their antimicrobial activity against Gram-positive and Gram-negative bacteria was assessed. Cholic acid hydrazide was produced from corresponding ethyl ester and was then used in reactions with different aromatic aldehydes. Scheme 7 presents examples of products generated in the reaction of CA hydrazide with *para*-substituted benzaldehydes (compounds **40a–h**). Compounds containing a chlorine- or bromine-substituted benzene ring exhibited the highest antimicrobial activity. The minimum inhibitory concentration (MIC) of compound **40c** against *Escherichia coli* and *E. faecalis* was 3.91 and

Scheme 7



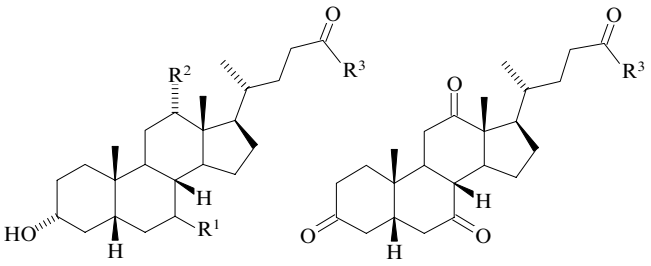
R = H (**a**, 84% yield), F (**b**, 40%), Cl (**c**, 89%), Br (**d**, 80%), Me (**e**, 48%), OMe (**f**, 94%), CN (**g**, 79%), NO_2 (**h**, 90%)

1.96 μg ml^{-1}, respectively; MIC of compound **40d** against both cultures was 3.91 μg ml^{-1}.

Amidation reactions are widely used for the synthesis of BA conjugates with biologically active molecules. For example, conjugates with β-lactam antibiotics[69, 70] and nucleoside derivatives[60] linked *via* the 1,2,3-triazole ring were synthesized from BA propargylamide (in a way similar to reactions with BA propargyl esters, see Scheme 4).

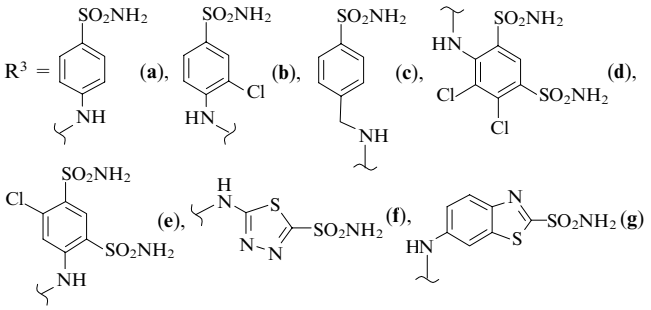
A series of sulfonamide derivatives of BAs were synthesized from CA, DCA, CDCA, LCA, UDCA and dehydro-CA, and their inhibitory activity against carbonic anhydrase was assessed *in vitro* and *in vivo*. This modification resulted in enhancement of inhibitory properties, for example, of compounds **41** – **46** compared to the starting sulfonamides.[75, 82] The synthesis involved the activation of the carboxyl group of the appropriate BA with EDC·HCl followed by the reaction with different aromatic and heterocyclic sulfonamides containing amino groups.

Structures 41 – 46



41a – g – 45a – g **46a – g**

R^1 = α-OH: R^2 = OH (**41**), H (**42**); R^1 = H, R^2 = OH (**43**);
R^2 = H: R^1 = H (**44**), β-OH (**45**);

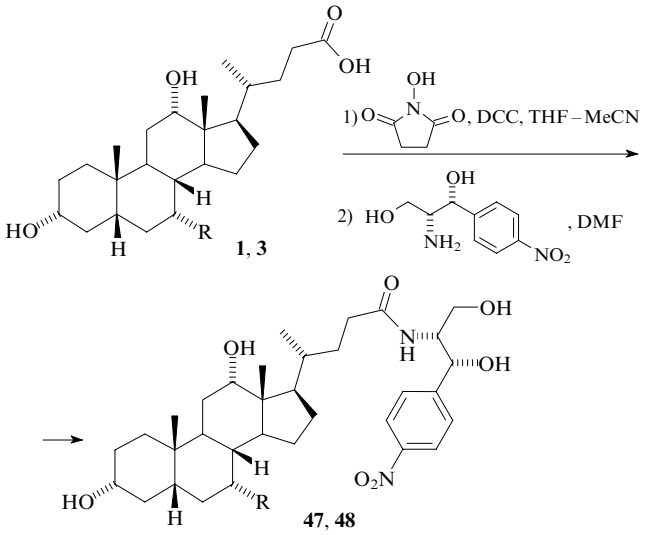


Hazra *et al.*[79] demonstrated that CA and DCA modified at the carboxyl group by chiral amino alcohols (Scheme 8) exhibit antifungal activity. A method was proposed for the preparation of compounds **47** and **48** through the intermediate formation of ester using *N*-hydroxysuccinimide in the presence of DCC followed by the reaction of the latter with appropriate amino alcohol.

Adenine- and adenosine-conjugated cholic acid amides **49a**,**b** were synthesized using the activation of the carboxyl group with EEDQ followed by the reaction with a nitrogen base.[77] These compounds exhibit cytostatic activity against murine and human cancer cell lines at concentrations $> 5 \times 10^{-4}$ mol litre^{-1}.

Adducts of DCA and LCA with chitosan (aminosaccharide with a broad spectrum of native biological activity, including antiinflammatory activity) are examples of conjugated BAs, which facilitate selective drug delivery and reduce their toxicity.[80]

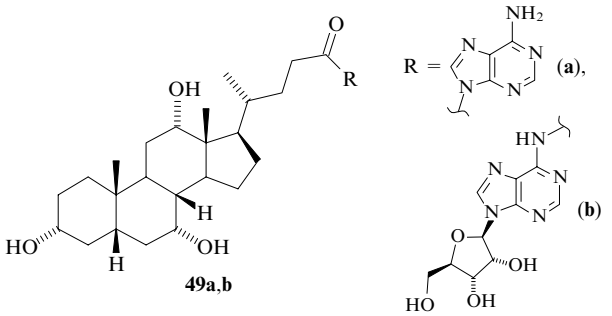
Scheme 8



1, **3** **47**, **48**

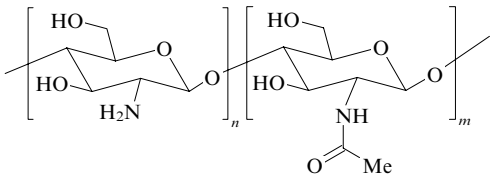
R = OH (**1**; **47**, 77% yield), H (**3**; **48**, 69%)

Structures 49



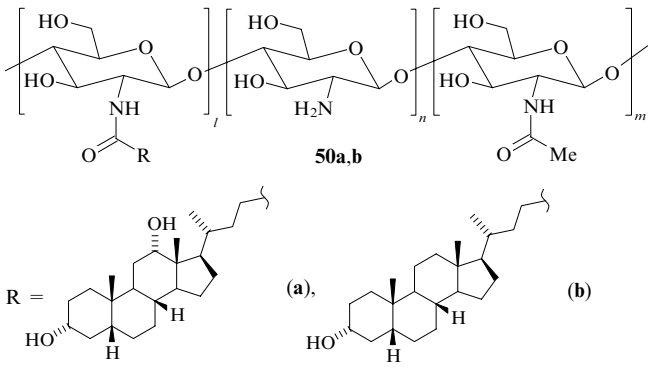
49a,**b**

Chitosan structure



Compounds **50a**,**b** form nanostructures having a hydrophobic cavity loaded with the anticancer drug Paclitaxel. The *in vitro* assessment of antitumour activity of the resulting complex demonstrated that the toxicity of the drug

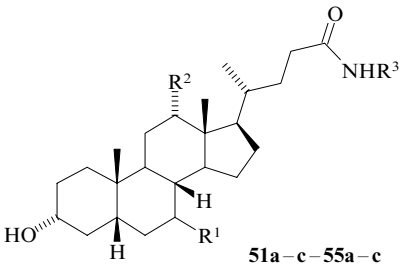
Structures 50



against normal cells is reduced, while it is enhanced against cancer cells.

Bile acid amides **51**–**55** containing polyfunctional aliphatic substituents at the N atom, *e.g.*, 2-sulfanylethylamine (**51a**–**55a**), were tested for the ability to undergo self-association and form gels, which can be employed for pharmacological purposes, primarily, for drug delivery.[76] A series of amides based on 2-aminoethanol and 3-aminopropanol (**51b**,**c**–**55b**,**c**) were synthesized from BA methyl esters, and their crystal structures were determined.[85] These data are of great importance for computer simulation of interactions of BAs and their derivatives with various biological targets.

Structures 51–55

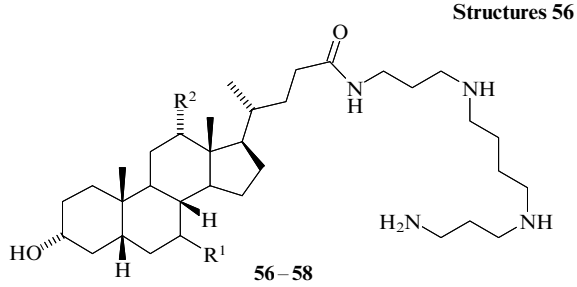


51a–c–55a–c

R^1 = α-OH: R^2 = OH (**51**), H (**52**); R^1 = H, R^2 = OH (**53**); R^2 = H: R^1 = H (**54**), β-OH (**55**); R^3 = $(CH_2)_2SH$ (**a**), $(CH_2)_2OH$ (**b**), $(CH_2)_3OH$ (**c**)

Another important class of BA amides was synthesized by the reaction of BAs with polymethyleneamines of different structure. These derivatives are of interest for gene therapy because polymethyleneamines have high binding affinity for DNA. For example, spermine-conjugated CDCA, DCA and UDCA amides (**56**–**58**) have been synthesized[83] and their relative binding affinities for calf thymus DNA have been assessed. It was found that the binding affinity of the compounds depends both on the structure of the lipid moiety (a BA moiety) and the charge distribution in the polymethyleneamine.

Structures 56–58



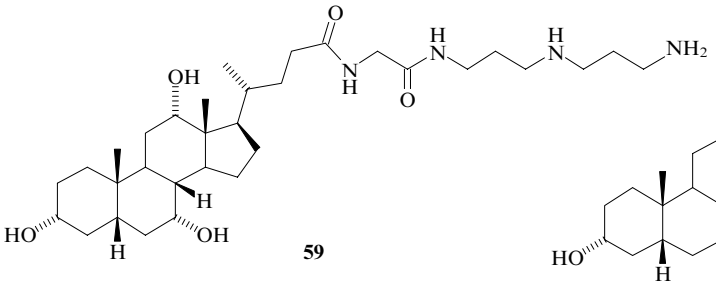
56–58

R^2 = H: R^1 = α-OH (**56**), β-OH (**58**); R^1 = H, R^2 = OH (**57**)

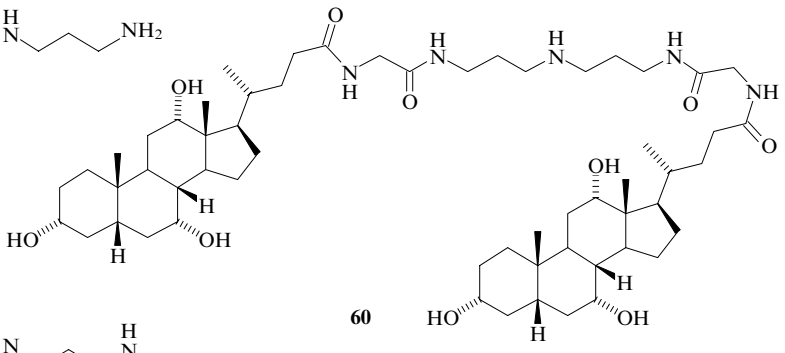
Conjugates with polymethyleneamines can contain either one or several BA molecules.[78,86–90] Vicens *et al.*[78] synthesized compounds **59**–**61**, in which one, two or three cholic acid moieties were cojugated to polymethyleneamine through a glycine linker. The effect of these conjugates on BA transport systems was investigated. These transport systems are involved in hepatic uptake or intestinal absorption, namely, in the inhibition of the apical sodium-dependent bile acid transporter (ASBT) and sodium-taurocholate cotransporting polypeptide (NTCP). Mono- and diprotonated forms of compounds **59** and **60** were shown to be able to inhibit intestinal bile acid absorption.[87] Compounds with such properties are of potential interest for pharmacological studies as regulators of the enterohepatic circulation and, as a consequence, as cholesterol-lowering agents.

Dimers of CA (**62a**) and DCA (**62b**), in which the bile acids are linked to one another by a 1,2-diaminoethylene bridge, exhibit considerable antiproliferative activity. The IC_{50} value for compound **62b** is $(2-3)\times10^{-6}$ mol litre^{-1} against the cell lines HEp-2 and MCF-7.[86,89] Dimer **63**

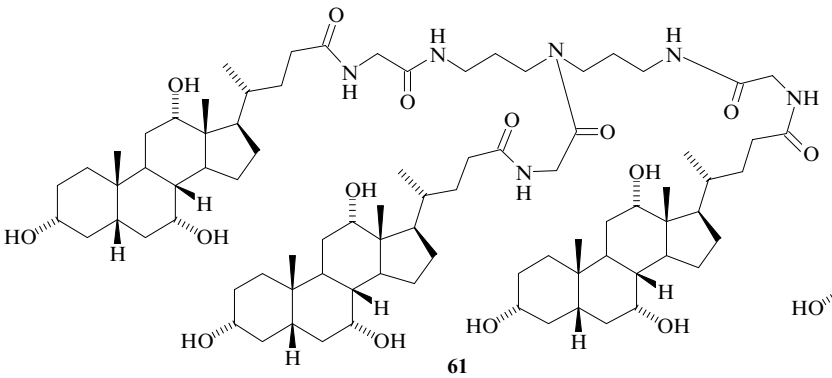
Structures 59–62



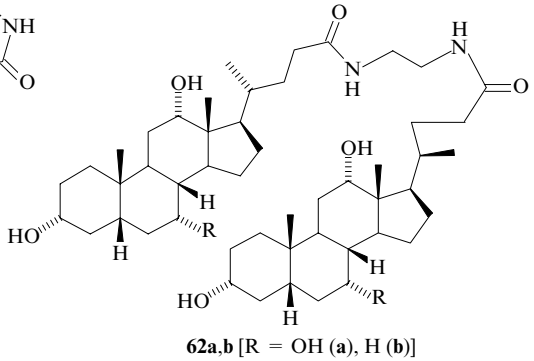
59



60

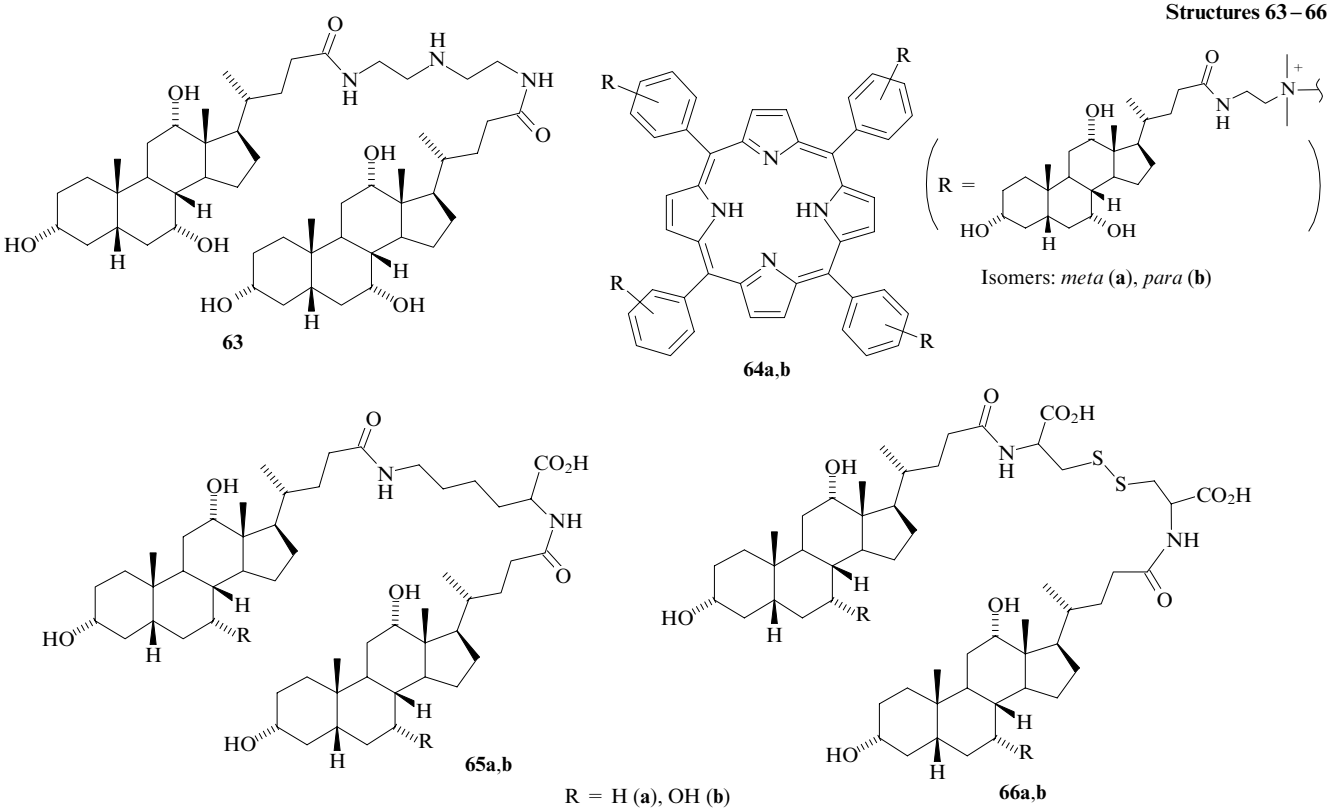


61



62a,b [R = OH (**a**), H (**b**)]

Structures 63 – 66



synthesized by the reaction of CA with diethylenetriamine possesses antifungal activity; MIC = 11.32×10^{-9} mol litre^{-1} against *Candida albicans*, *Benjaminiella poitrasii* and *Yarrowia lipolytica*.[89] These dimers were synthesized by the addition of ethylenediamine or diethylenetriamine to *N*-succinimidyl ester of the appropriate acid.[86, 89]

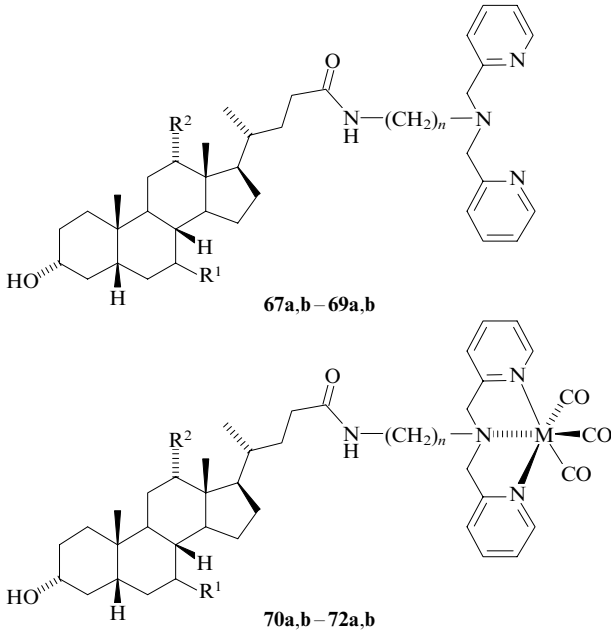
Porphyrin – CA conjugates (**64a**,**b**) were found to be able to bind to biologically important oligosaccharides and were used for recognition of the surface of tumour cells with an altered phenotype. Due to this properties, the compounds can be selectively accumulated in tumour cells and cause their apoptosis or necrosis in response to photodynamic therapy in *in vivo* and *in vitro* experiments.[88]

The dimerization of CA and DCA using the amino acids L-lysine and L-cystine as linkers gave compounds **65a**,**b** and **66a**,**b**, which exhibited moderate antiproliferative activity against the cancer cells MCF-7 (IC_{50} varied from 53×10^{-6} to 61×10^{-6} mol litre^{-1}).[90]

The reactions of CA, CDCA and UDCA methyl esters with 1,2-diaminoethane and 1,6-diaminohexane followed by the reaction of the resulting amides with pyridinecarboxaldehyde produced compounds **67** – **69** exhibiting antifungal activity against *Saccharomyces cerevisiae* (MIC varied from 0.125×10^{-6} to 1×10^{-6} g ml^{-1}).[91] These amides can also be used for the complex formation with Re and Tc compounds.[92] Complexes **70** – **72** with M = ^{99m}Tc are potential radioactive agents for radioisotope diagnosis or radiation therapy.[92]

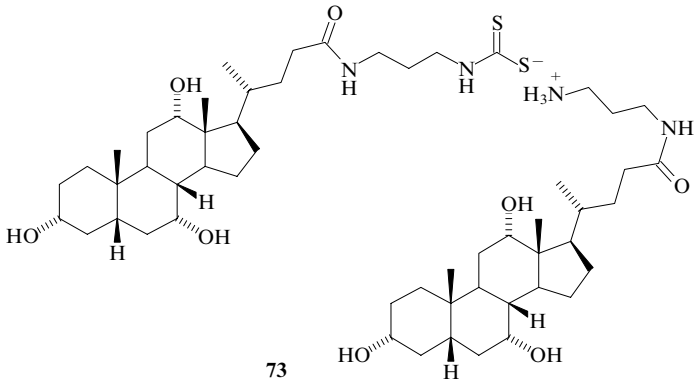
Amino groups of polymethyleneamine – BA conjugates can be subjected to further modifications. For example, the reaction of cholic acid *N*-(3-aminopropyl)amide with carbon disulfide produces ammonium dithiocarbamate derivative **73**.[93] The oxidation of the amino group of *N*-(3-dimethylaminopropyl)amides and *N*-methyl(3-dimethylaminopropyl)amides of CA and DCA with *m*-chloroperoxybenzoic acid (*m*-CPBA) gave the corresponding N-oxides **74a**,**b** and **75a**,**b**, which were tested for the ability to solubilize biological membranes.[94] Compounds capable of solubilizing biological membranes are required for determining the structures of membrane proteins, when it is

Structures 67 – 72

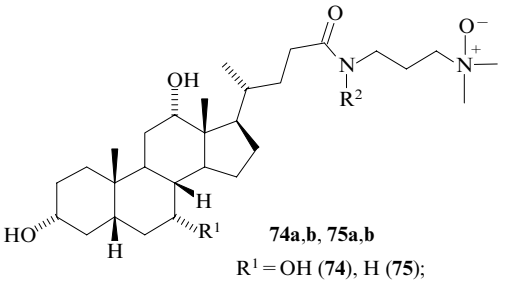


R^1 = α-OH: R^2 = OH (**67**, **70**), H (**68**, **71**);
R^1 = β-OH, R^2 = H (**69**, **72**); *n* = 1 (**a**), 6 (**b**); M = Re, ^{99m}Tc

Structures 73–78

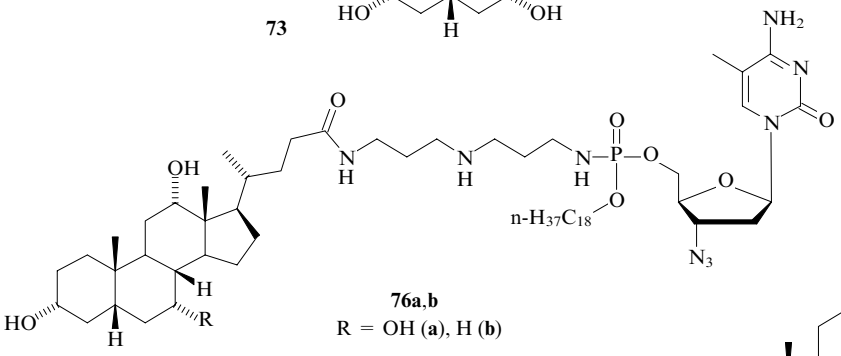


73



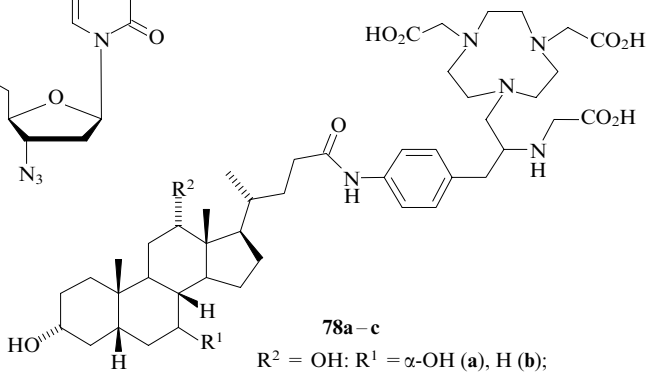
74a,b, 75a,b

R^1 = OH (**74**), H (**75**);
R^2 = H (**a**), Me (**b**)



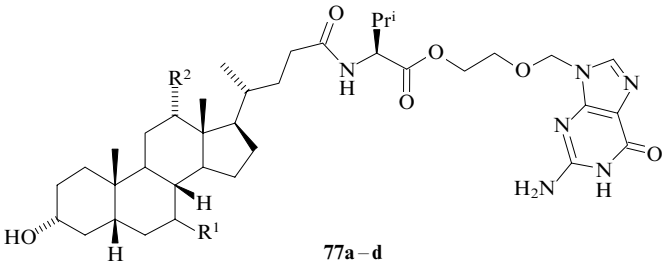
76a,b

R = OH (**a**), H (**b**)



78a–c

R^2 = OH: R^1 = α-OH (**a**), H (**b**);
R^1 = α-OH, R^2 = H (**c**)



77a–d

R^2 = OH: R^1 = α-OH (**a**), H (**b**);
R^2 = H: R^1 = α-OH (**c**), β-OH (**d**)

necessary to extract the proteins from biological materials, without causing denaturation or aggregation, for their subsequent crystallization.

Cholic and deoxycholic acids conjugated to 3′-azido-3′-deoxythymidine through a polymetyleneamine and phosphoric acid ester linkers were shown to substantially inhibit HeLa cancer cell growth: $IC_{50} = 7.2 \times 10^{-6}$ and 6.8×10^{-6} mol litre^{-1} for compounds **76a**,**b**, respectively.[81]

The conjugation of the antiviral drug Acyclovir to moieties of bile acids (CA, CDCA, DCA, UDCA) *via* a valine linker (compounds **77a–d**) resulted in the enhancement of Acyclovir oral bioavailability.[95]

Polyaminocarboxylate–BA conjugates (compounds **78a–c**) exhibit cytotoxicity against cancer cells. The IC_{50} values for compounds **78a–c** are 24.0×10^{-6}, 6.3×10^{-6} and 24.6×10^{-6} mol litre^{-1}, respectively, against the cell line HeLa and 8.3×10^{-6}, 7.5×10^{-6} and 15.5×10^{-6} mol litre^{-1} against the cell line HT29.[96] These compounds can also serve as metal ion chelating agents, thus holding promise for diagnostic purposes.[97]

In this section, we briefly consider the structural diversity of the prepared BA amides. This is actually one of the most extensive classes of BA derivatives, representatives of which are widely employed in different fields of medicine. These are compounds exhibiting biological activity (antimicrobial, antifungal, antitumour and so on), diagnostic and drug delivery agents, chelating and gel-forming agents.

IV.2. Other side chain modifications

Other side chain modifications include further transformations of BA esters and amides, the introduction of functional groups (halogen atoms, hydroxyl and epoxide groups, alkyl substituents, multiple bonds) and radioactive carbon isotopes. The side chain reactions of BAs can be arbitrarily divided into three types according to their final results:

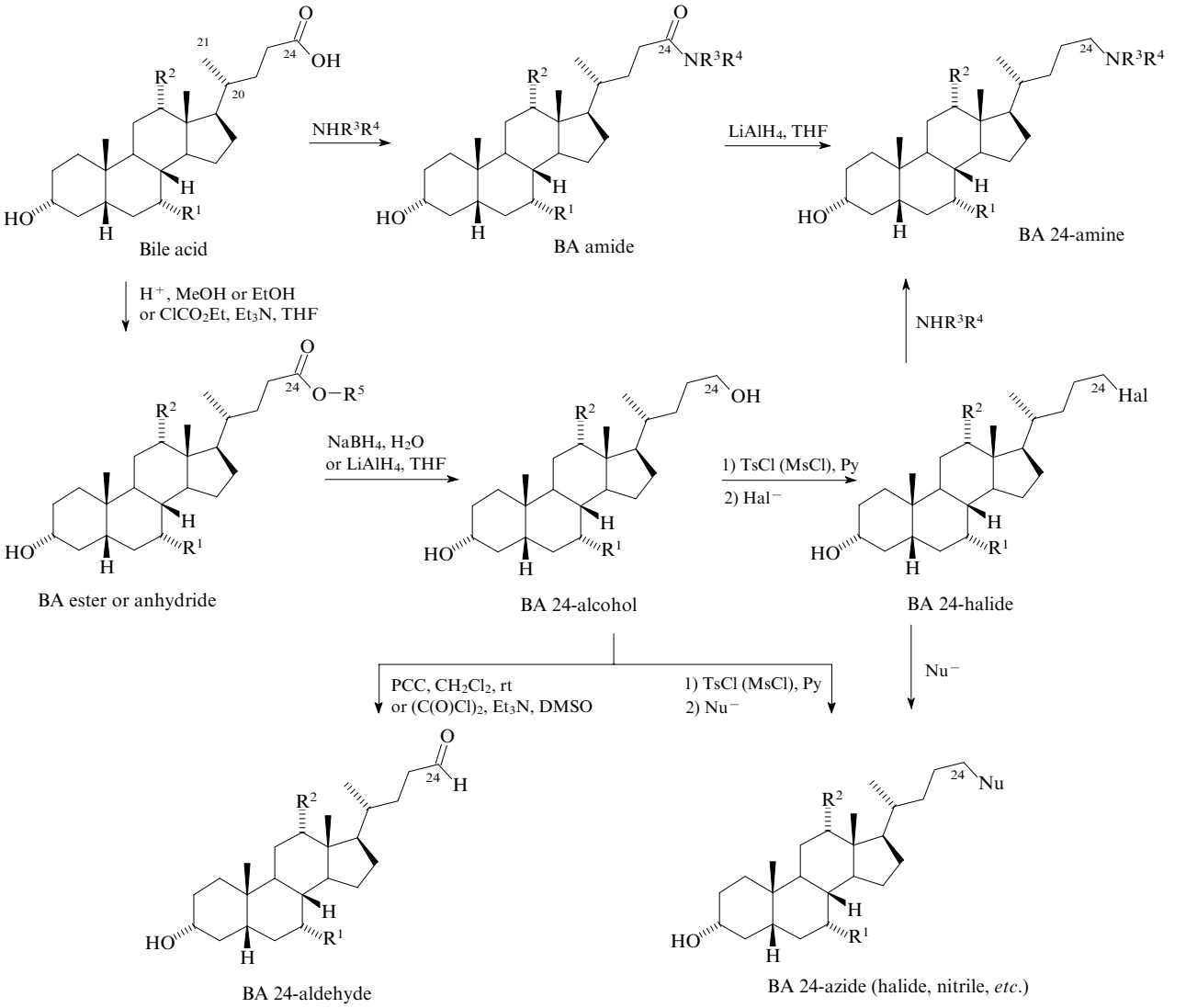
— side chain length is not changed [modification at the C(24) terminal atom],

— side chain shortening {decarboxylation giving nor [C(23)] and dinor [C(22] derivatives},

— side chain elongation giving homo derivatives of BAs [C(25), C(26)] or analogous steroids [C(27)-branched chains].

As mentioned above, bile acids are signalling molecules that interact with many nuclear and membrane receptors [FXR, LXR, GP-BAR1 (TGR5) an so on]. Endogenous BAs display low selectivity for activation of these receptors. Therefore, the synthesis of BA analogues and assessment of their ability to selectively activate a particular receptor have attracted great attention.[58,98,99] The most commonly used

Scheme 9



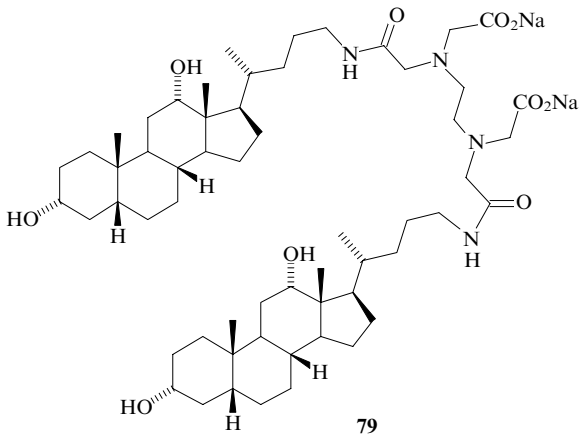
R^1, R^2 = H, OH; R^3, R^4 = H, Alk, Ar, *etc.*; R^5 = Me, Et, CO_2Et; Nu = N_3, Hal, CN, *etc.*; Ms is methanesulfonyl (mesyl)

side chain modifications are based on the insertion of functional groups (CO_2H, CHO, OH, CN, SO_3H) and alterations of the side chain length [C(24) – C(26)].

The general representation of synthetic pathways to different BA derivatives with retention of the C(24) atom is shown in Scheme 9. Bile acid amides are reduced with $LiAlH_4$ in THF to give the corresponding 24-amines,† which can be subjected to amidation.[100] An example is a BA dimer (compound **79**), which is formed through disodium ethylenediaminetetraacetate as a linker. Such compounds are surfactants, which are employed for solubilizing various substances.[100]

Another approach to the synthesis of BA derivatives at the C(24) atom involves the reduction of BA anhydrides or esters to alcohols using the complex hydrides $NaBH_4$,[101, 102] $LiAlH_4$ (Refs 58, 103) (see Scheme 9). The subsequent methylation of the alcohol derived from BA ester with MeI in THF in the presence of NaH is a way of protecting

Structure 79



79

the side chain in multistep syntheses.[104, 105] The activation of the OH group with tosyl[102, 106] or mesyl chloride[32] followed by the nucleophilic substitution can be applied to synthesize halogen,[107, 108] cyano,[108] azido[32, 109] and many

† Hereinafter, the number indicates the position of the carbon atom to which the functional group is attached.

other derivatives of BAs. The Swern oxidation of the side chain OH group to an aldehyde group [$(COCl)_2$, Et_3N, DMSO][58] or the oxidation of the OH group with pyridinium chlorochromate (PCC, Corey reagent)[103, 106] provide numerous routes to side chain modifications using the reaction with phosphorus ylides, condensation with CH-acids and so on.[58, 103, 109]

One of the main approaches to the side chain elongation is based on the nucleophilic substitution of the TsO group by CN in 24-tosyl derivatives of BAs.[108] An alternative route involves the modification of aldehydes derived from BAs, for example, by the condensation with CH-acids. This procedure can be applied to synthesize steroid analogues containing the C(27) atom and a branched side chain.[58, 65, 98, 110] The condensation of halides based on BAs with CH-acids produces C(27)–C(28) steroids with a branched side chain.[111]

Three main approaches are used to shorten the side chain of BAs (Scheme 10). One approach is based on the oxidative decarboxylation of BAs containing protected OH groups in the skeleton with lead tetraacetate $Pb(OAc)_4$ in the presence of different halide ions or halogens [LiCl,[112] I_2 (see Refs 102, 108, 113, 114)], which produces 24-nor-23-halides (the number at the prefix 'nor' indicates the absence of this carbon atom). To perform this reaction, it is necessary to protect OH groups in the starting acid. In another procedure, copper acetate is employed instead of halide, resulting in the formation of the C(22)=C(23) double bond in the side chain.[115] In the third approach, the decarboxylation is accomplished with sodium nitrite, trifluoroacetic acid and trifluoroacetic anhydride to produce 24-nor-23-nitriles of BAs.[98, 108] The mechanism of decarboxylation with sodium nitrite and trifluoroacetic anhydride was proposed and discussed by Schteingart and Hofmann.[116]

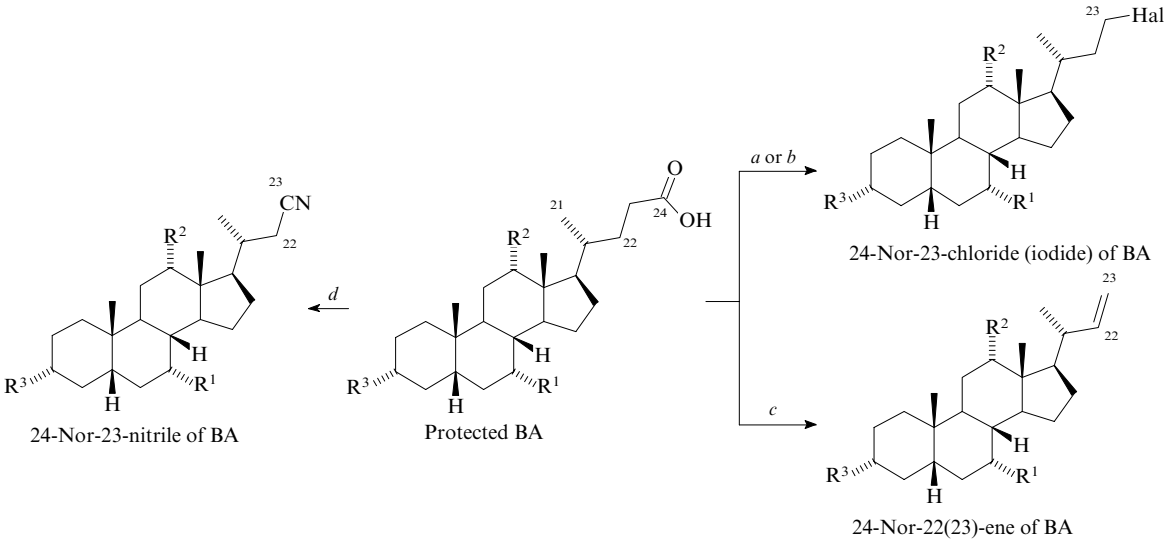
24-Nor- and 23,24-dinor-bile acids are analogues of BAs and can be produced in the body but at very low concentrations. These acids are usually not conjugated during the enterohepatic circulation and were not detected in healthy humans, but can be found in patients with disorders of lipid metabolism. Therefore, investigations of the metabolism of these acids and their effects on the body are of great importance.[117] Since BAs are often used in supramolecular chemistry for the construction of assemblies or inclusion compounds, the determination of the crystal structures of 24-nor-bile acids is of interest for the design of molecular cavities in nanoporous organic materials.[118]

24-Nor-23-nitriles of BAs are difficultly hydrolyzed under very drastic conditions. Therefore, the hydrolysis and subsequent decarboxylation cannot be considered as a convenient strategy for the synthesis of 23,24-dinor-bile acids. An alternative approach is based on the treatment of 24-nor-23-nitrile **80** with a small excess of lithium diisopropylamide (LDA) at low temperatures under an oxygen atmosphere in the presence of 18-crown-6, resulting in the formation of the target 23,24-dinor derivative (Scheme 11) but in low yield (10%–15%). The use of potassium *tert*-butoxide instead of LDA leads to an increase in the yield of product **81** to 75%–90%.[117]

The synthesis of *N*-hydroxypyridine-2-thione esters, so-called Barton esters, is another interesting synthetic approach to decarboxylation of the side chain of BAs (Scheme 12). These esters are employed for the synthesis of heterocyclic derivatives of 24-nor-bile acids. The cleavage of Barton ester is triggered by heating or irradiation with light to give carboxyl and pyridine-2-thiyl radicals. Then the carboxyl radical is converted into the alkyl radical through elimination of CO_2 (indicated by an arrow in the scheme) followed by reactions of the alkyl radical with other substrates and the formation of a new C–C bond.[119–121]

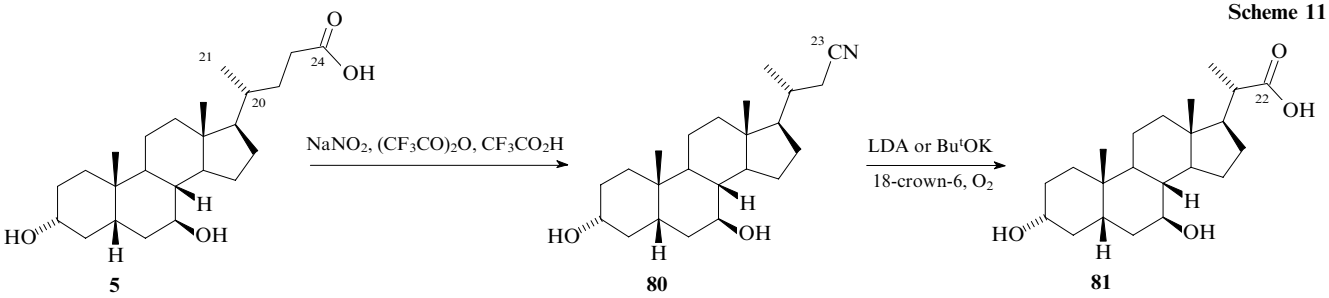
Hybrids of Cinchona alkaloids (quinine and quinidine) and bile acids (compounds **84a**,**b** and **85a**,**b**) were synthesized[119, 120] based on protected LCA and CDCA **82a**,**b** through the intermediate formation of Barton esters **83a**,**b**. These hybrid molecules exhibited rather high *in vitro* antiparasitic activity against *Trypanosoma brucei brucei*, *Leishmania mexicana mexicana* and *Plasmodium falciparum* (3D7). In some cases, the activity of the resulting derivatives

Scheme 10



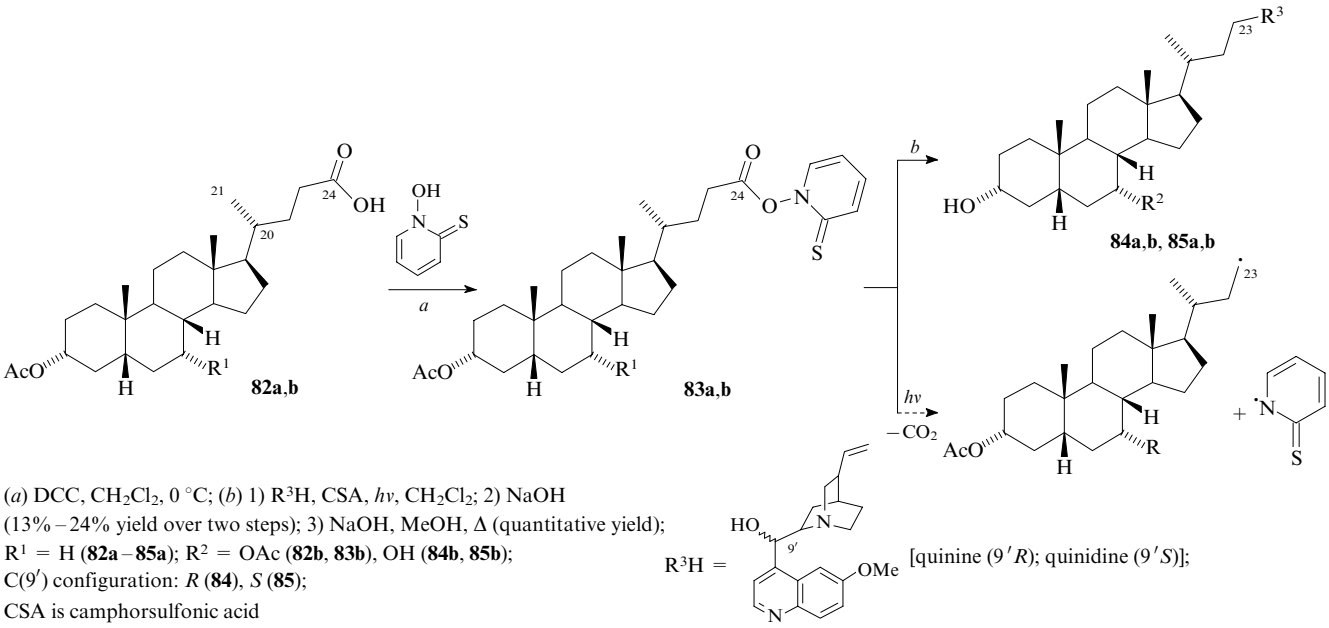
(*a*) $Pb(OAc)_4$, LiCl, PhH (80% yield for $R^1 = R^2 = R^3 = OCHO$, Hal = Cl); (*b*) $Pb(OAc)_4$, I_2, CCl_4, *hv* (61%–99% yield for $R^3 = OCHO$: R^1, R^2 = H, OCHO; Hal = I); (*c*) $Pb(OAc)_4$, $Cu(OAc)_2$, Py, PhH, Δ (45% yield for $R^2 = R^3 = OAc$: R^1 = H, OAc); (*d*) $NaNO_2$, $(CF_3CO)_2O$, CF_3CO_2H (96% yield for $R^1 = R^3 = OCHO$, R^2 = H)

Scheme 11



5 **80** **81**

Scheme 12



82a,**b** **83a**,**b** **84a**,**b**, **85a**,**b**

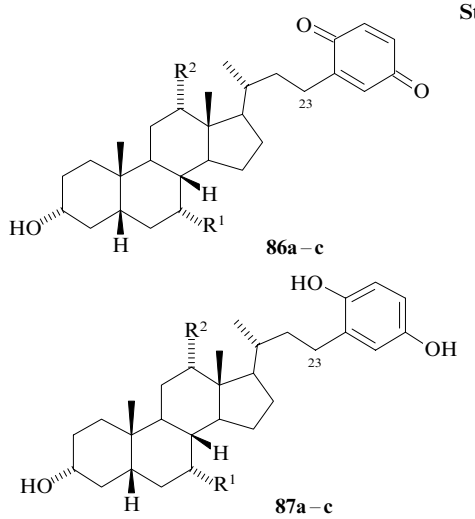
(*a*) DCC, CH_2Cl_2, 0 °C; (*b*) 1) R^3H, CSA, *hv*, CH_2Cl_2; 2) NaOH (13% – 24% yield over two steps); 3) NaOH, MeOH, Δ (quantitative yield);
R^1 = H (**82a** – **85a**); R^2 = OAc (**82b**, **83b**), OH (**84b**, **85b**);
C(9′) configuration: *R* (**84**), *S* (**85**);
CSA is camphorsulfonic acid

R^3H = [quinine (9′*R*); quinidine (9′*S*)];

was higher than that of the starting alkaloids ($IC_{50} < 0.5 \times 10^{-6}$ g ml^{-1}).

Barton esters were also employed in the synthesis of BA derivatives **86a** – **c** and **87a** – **c** containing quinone and hydroquinone moieties.[121] Compound **87c** exhibited higher *in vitro* cytotoxic activity against human pancreatic cancer cells PANC1 ($IC_{50} = 12.1 \times 10^{-6}$ mol litre^{-1}) compared to the commercially available drug Doxorubicin ($IC_{50} = 20.2 \times 10^{-6}$ mol litre^{-1}).

Structures 86, 87



86a – **c**

87a – **c**

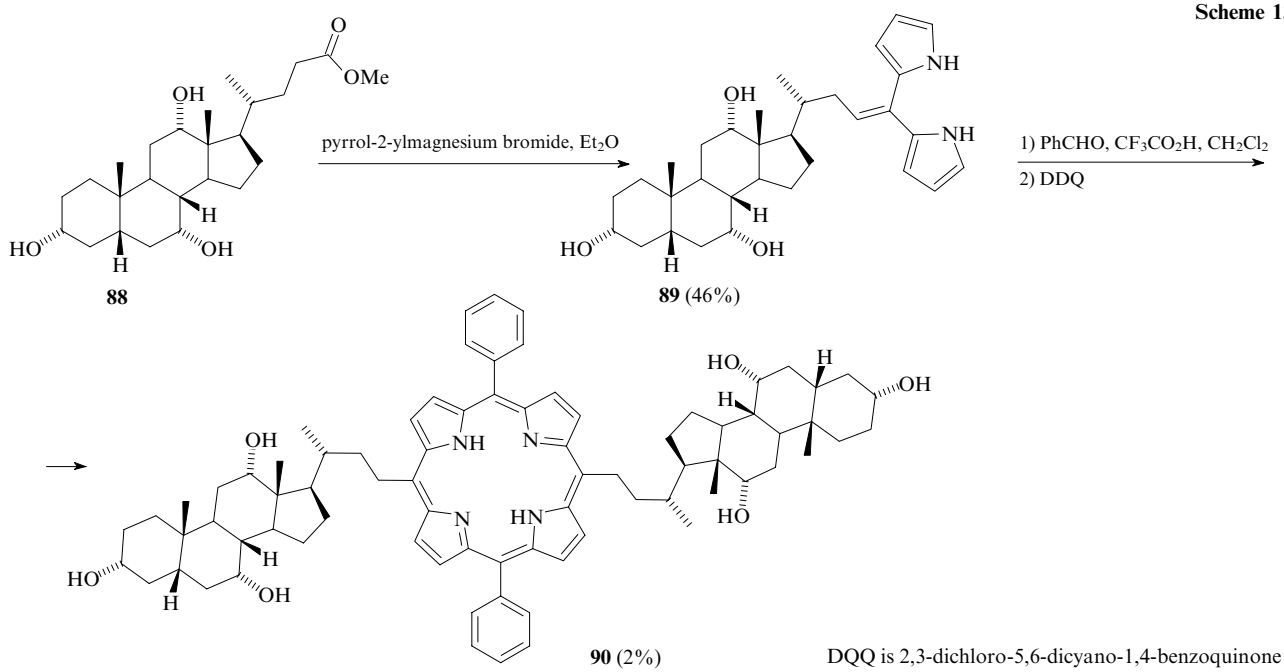
R^1 = OH: R^2 = OH (**a**), H (**b**); R^1 = R^2 = H (**c**)

Bile acid esters can serve as intermediates in the synthesis of derivatives containing heterocyclic moieties.[57, 77, 122] For example, the reaction of CA methyl ester **88** with pyrrol-2-ylmagnesium bromide in diethyl ether gives product **89** (Scheme 13), which can serve as a building block for the synthesis of porphyrins.[57] Porphyrin **90** containing two steroid moieties was prepared in low yield.

The amidation of CA and UDCA with metformin (*N*,*N*-dimethylimidodicarbonimidic diamide) produces BA derivatives containing the 1,3,5-triazine ring. These products exhibited *in vitro* antitumour activity against the cell lines LS 174T, Caco-2, McA-RH7777 and Hepa 1-6. Scheme 14 shows this transformation for UDCA. Compound **91** displays cytotoxic effect ($IC_{50} = 30.3 \times 10^{-6}$ mol litre^{-1}) even on the cell line Hepa 1-6/R, which is partially resistant to the drug cisplatin ($IC_{50} = 68.3 \times 10^{-6}$ mol litre^{-1}).[77]

24-Hydroxy derivatives of BAs are often employed as the starting compounds for the modification of position 24 with functional groups, such as N_3, I, SO_3H, CN and R_3N^+. For instance, the activation of the hydroxyl group at the C(24) atom of compounds **92a**,**b** with mesyl chloride followed by the reaction with sodium azide gives rise to azido derivatives of CA and DCA **93a**,**b** in high yields (Scheme 15).[109] As discussed above (see Sections IV.1.b

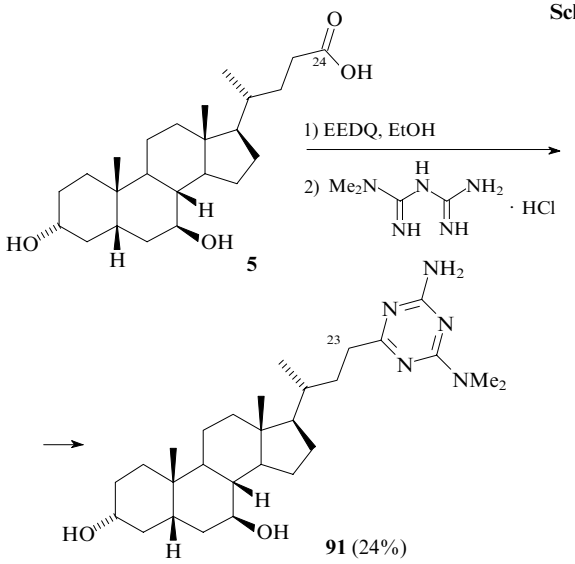
Scheme 13



88 **89** (46%) **90** (2%)

DQQ is 2,3-dichloro-5,6-dicyano-1,4-benzoquinone

Scheme 14



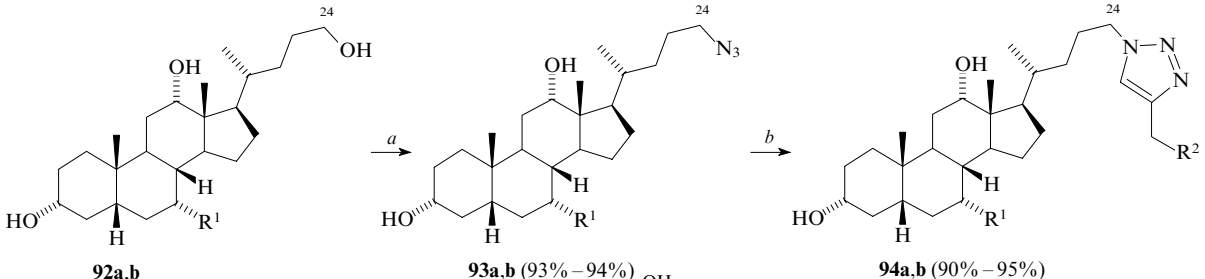
5 **91** (24%)

and IV.1.c), the 1,3-dipolar cycloaddition of terminal alkynes is an important reaction of azido derivatives. This reaction can be applied to synthesize BA conjugates containing the 1,2,3-triazole ring as a linker (see, for example, compound **29** in Scheme 4). In this case, the reactions of azido derivatives **93a**,**b** with terminal alkynes produce conjugates **94a**,**b**.

The reaction of 24-tosyl derivative of LCA **95** with sodium cyanide produces nitrile **96** (Scheme 16). The subsequent alkaline hydrolysis of **96** gives 25-homo derivative of LCA **97** (the reaction proceeds under very drastic conditions [117]); the reaction with sodium azide under reflux in toluene affords heterocyclic derivative **98** containing the tetrazole ring in the side chain.[108] 24-Nitriles of BAs can be produced by the reaction of 24-nor-23-halides with cyanides or by the dehydration of amides with a 1 : 1 mixture of trifluoroacetic anhydride and pyridine [132] or with a solution of $POCl_3$ in pyridine.[108]

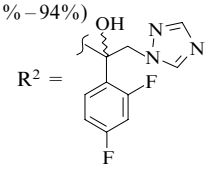
Compounds **96** – **98**, their 24-nor analogues and functional derivatives **99a** – **d** were tested for the ability to activate voltage-gated potassium channels containing a β1 subunit. The activity of these channels provides the outward

Scheme 15

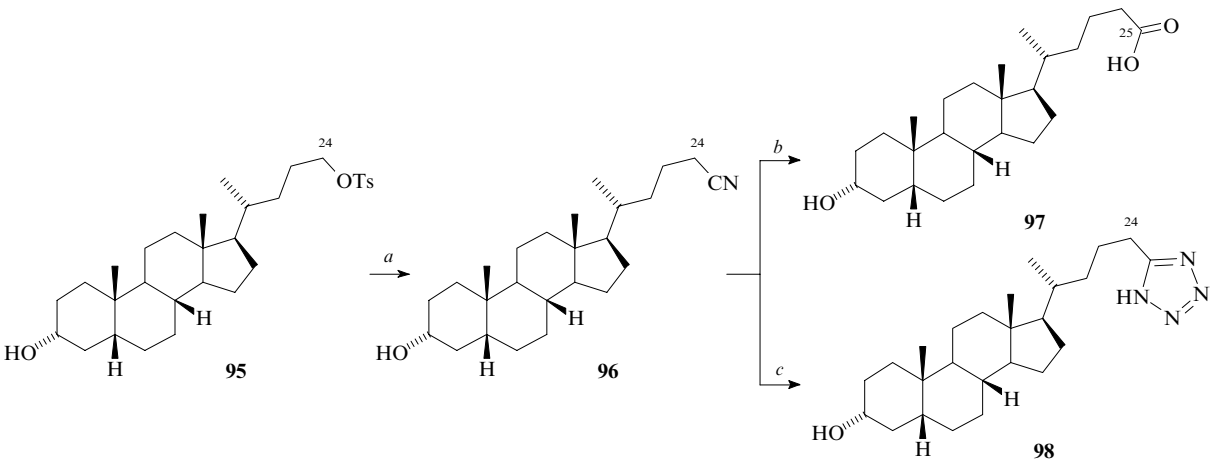


92a,**b** **93a**,**b** (93% – 94%) **94a**,**b** (90% – 95%)

(*a*) 1) MsCl, Et_3N, CH_2Cl_2, 0 °C (for **a**) or MsCl, Py, 0 °C (for **b**); 2) NaN_3, DMF, 60 °C, 3 h; (*b*) $HC{\equiv}CCH_2R^2$, $CuSO_4 \cdot 5H_2O$, sodium ascorbate, DMF, H_2O, MW; R^1 = OH (**a**), H (**b**);

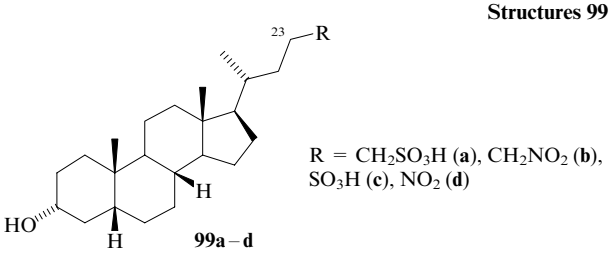


Scheme 16



(*a*) NaCN, DMSO, 90 °C, 2.5 h; (*b*) NaOH, EtOH, Δ, 24 h; (*c*) NaN_3, $Et_3N \cdot HCl$, PhMe, Δ, 24 h

Structures 99



R = CH_2SO_3H (**a**), CH_2NO_2 (**b**), SO_3H (**c**), NO_2 (**d**)

99a – d

K^+ current from the cell and negatively feeds back on Ca^{2+} entry into the cell, opposing smooth muscle cell contraction and promoting myocyte relaxation. Therefore, these channels are an attractive target for drug design for the regulation of smooth muscle tone.[108]

Since 23- and 24-halogen derivatives of BAs readily undergo nucleophilic substitution, they are very convenient intermediates for subsequent side chain modifications. 23-Chloro(iodo)-substituted analogues of bile acids can be synthesized by the decarboxylation reactions considered above (see Scheme 10). 24-Halogen derivatives are generally synthesized by the nucleophilic substitution of 24-tosyl-substituted BAs with sodium iodide in acetone[123] or by the direct reaction of 24-hydroxy-BAs with iodine in the presence of triphenylphosphine.[107]

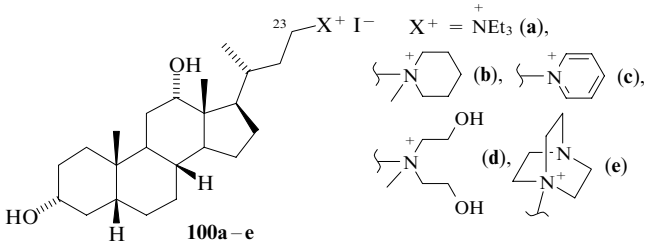
The reaction of BA 24-nor-23-halide with $K^{14}CN$ and subsequent alkaline hydrolysis of the nitrile group produce the acid containing the ^{14}C isotope in the carboxyl group. This modification can be used to synthesize isotopically labelled BA derivatives applied in diagnosis. Such derivatives allow the investigation of the distribution of the corresponding compounds in organs and tissues in the course of metabolism and can serve as internal standards for physicochemical analysis.[112]

The reaction of 24-iodo derivatives of CA, CDCA and UDCA with Na_2SO_3 produced BAs sulfonic-substituted at the side chain[123] with the goal of finding TGR5 nuclear receptor agonists and revealing structure – property relationships.

A series of 23-ammonium derivatives of DCA were prepared by the reaction of the 24-nor-23-iodo derivative of DCA with different tertiary amines (aliphatic, bicyclic, cyclic and polyfunctional), and their gel-forming ability was evaluated. Examples are compounds **100a – e**.[102, 113, 114]

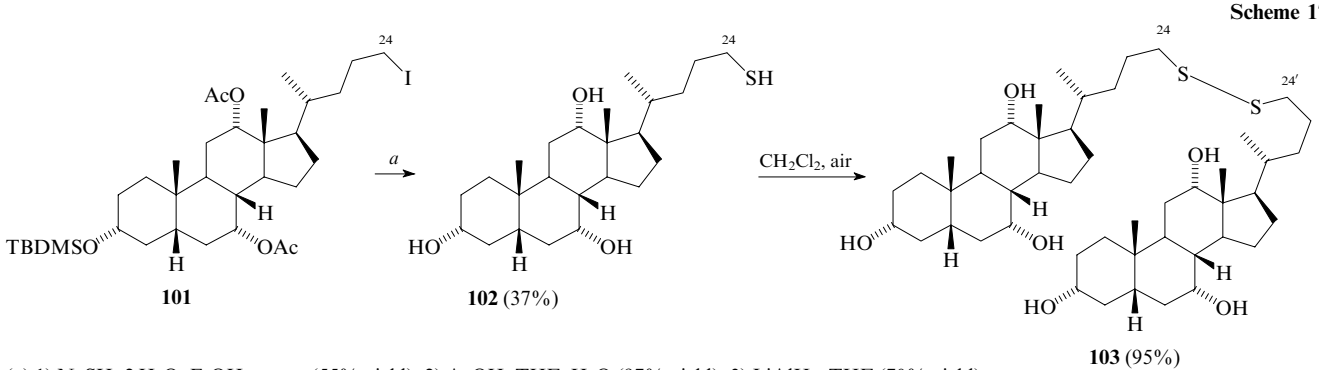
Lotowski and Kulesza[107] synthesize 24-thiol **102** and disulfide **103** starting from 24-iodo derivative of CA **101** (Scheme 17). These compounds are suitable as building

Structures 100



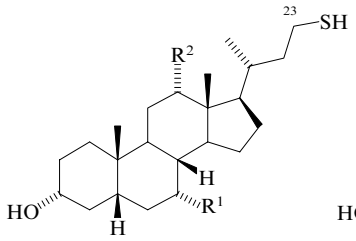
100a – e

Scheme 17



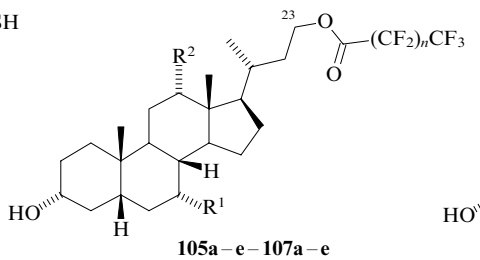
(*a*) 1) $NaSH \cdot 2\,H_2O$, EtOH, argon (55% yield); 2) AcOH, THF, H_2O (97% yield); 3) $LiAlH_4$, THF (70% yield);
TBDMS is *tert*-butyldimethylsilyl

Structures 104 – 112



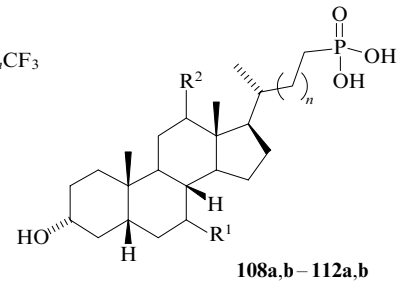
104a – c

$R^1 = R^2 = OH$ (**a**), H (**b**);
$R^1 = H$, $R^2 = OH$ (**c**)



105a – e – 107a – e

$R^1 = R^2 = OH$ (**105**), H (**106**);
$R^1 = H$, $R^2 = OH$ (**107**);
$n = 6$ (**a**), 8 (**b**), 9 (**c**), 10 (**d**), 12 (**e**)



108a,b – 112a,b

$R^1 = R^2 = \alpha$-OH (**108**), H (**109**);
$R^2 = H$: $R^1 = \alpha$-OH (**110**), β-OH (**111**);
$R^1 = H$, $R^2 = \alpha$-OH (**112**); $n = 2$ (**a**), 1 (**b**)

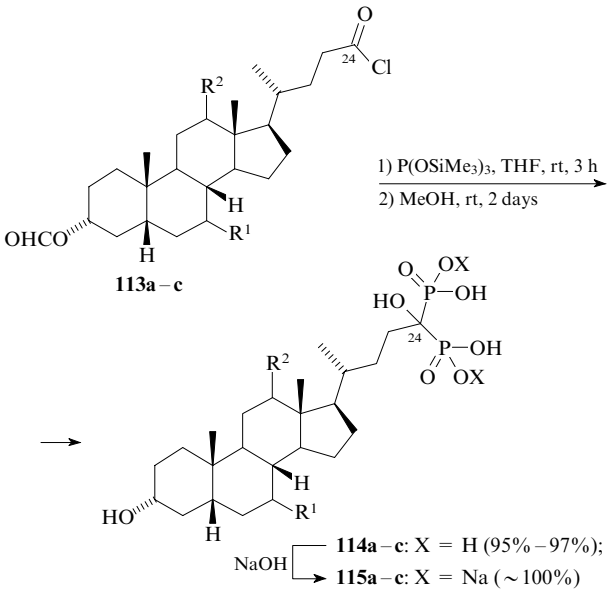
blocks for use in supramolecular chemistry. The nucleophilic substitution of an SH group for iodine in 24-iodo derivative **101** followed by the deprotection of the hydroxyl groups gave target product **102**, which was transformed into compound **103** in high yield by oxidative dimerization in air.

23-Sulfanyl derivatives of CA, DCA and LCA **104a – c** produced from the corresponding 24-nor-23-iodo derivatives of BAs can serve as capping agents for stabilization of gold nanoparticles.[124] Esters **105a – e – 107a – e** were synthesized by the reaction of 24-nor-23-iodo derivatives of CA, DCA and LCA with perfluorinated acids in the presence of DBU in DMF, and their gel-forming properties were investigated.[63] Compounds **108 – 112** containing a carbon – phosphorus bond were prepared from CA, DCA, CDCA, LCA and UDCA.[125, 126] The synthesis involves the reaction of the appropriate 24- or 23-iodo derivatives of BAs with $P(OEt)_3$ followed by the hydrolysis of phosphoric acid ester. The study of the aggregation properties of compounds **108 – 112** and, as a consequence, their ability to dissolve cholesterol (and potentially gallstones) demonstrated that 24-phosphonic acids **108a – 112a** more readily dissolve cholesterol than their 24-nor analogues **108b – 112b**; this comparative analysis is useful for elucidating structure – property relationships.[126]

Biphosphonates belong to another class of phosphorus-containing analogues of BAs. The reaction of bile acid chlorides **113a – c** with $P(OSiMe_3)_3$ in THF followed by the hydrolysis of protecting groups produced bisphosphonates **114a – c** and **115a – c** (Scheme 18).[127] The assessment of biological activity of these compounds included tests for the ability to inhibit osteoclast formation. In the general case, bisphosphonates are taken up by osteoclasts and interfere with bone resorption. In the series of these compounds, CDCA derivative **115b** proved to be more effective and less toxic compared to the drug Neridronate used for the treatment of osteoporosis.

The formation of an aldehyde group in the side chain of BAs offers a great potential for subsequent chemical transformations. For example, such aldehydes can serve as building blocks for supramolecular assemblies or employed in the synthesis of compounds suitable as ligands for complexation with metals[101] (see the synthesis of compound **117** from aldehyde **116**, Scheme 19). The reaction of aldehyde **116** with trimethylsilanecarbonitrile followed by the hydrolysis of silyl ester **118** led to a side chain elongation and the formation of α-hydroxycarboxylic acid **119** (see Scheme 19). The latter is a convenient starting

Scheme 18



113a – c

1) $P(OSiMe_3)_3$, THF, rt, 3 h
2) MeOH, rt, 2 days

NaOH

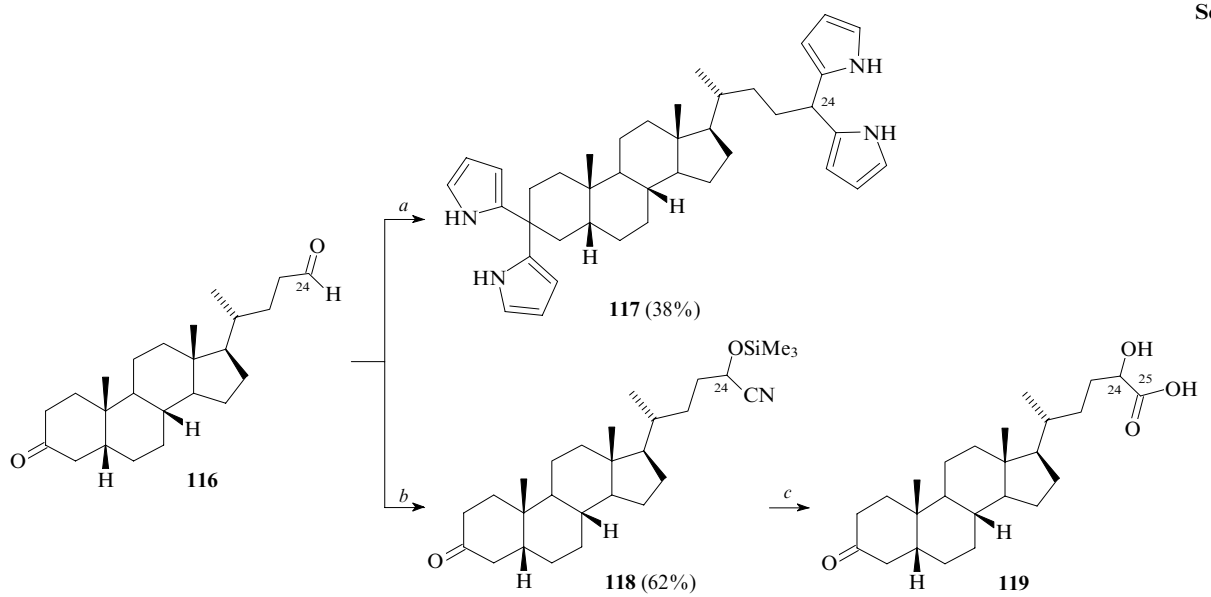
114a – c: X = H (95% – 97%);
115a – c: X = Na (~100%)

113: $R^1 = R^2 = \alpha$-OCHO (**a**); $R^2 = H$: $R^1 = \alpha$-OCHO (**b**), β-OCHO (**c**);
114, **115**: $R^1 = R^2 = \alpha$-OH (**a**); $R^2 = H$: $R^1 = \alpha$-OH (**b**), β-OH (**c**)

compound for the subsequent synthesis of biologically active steroids.[110]

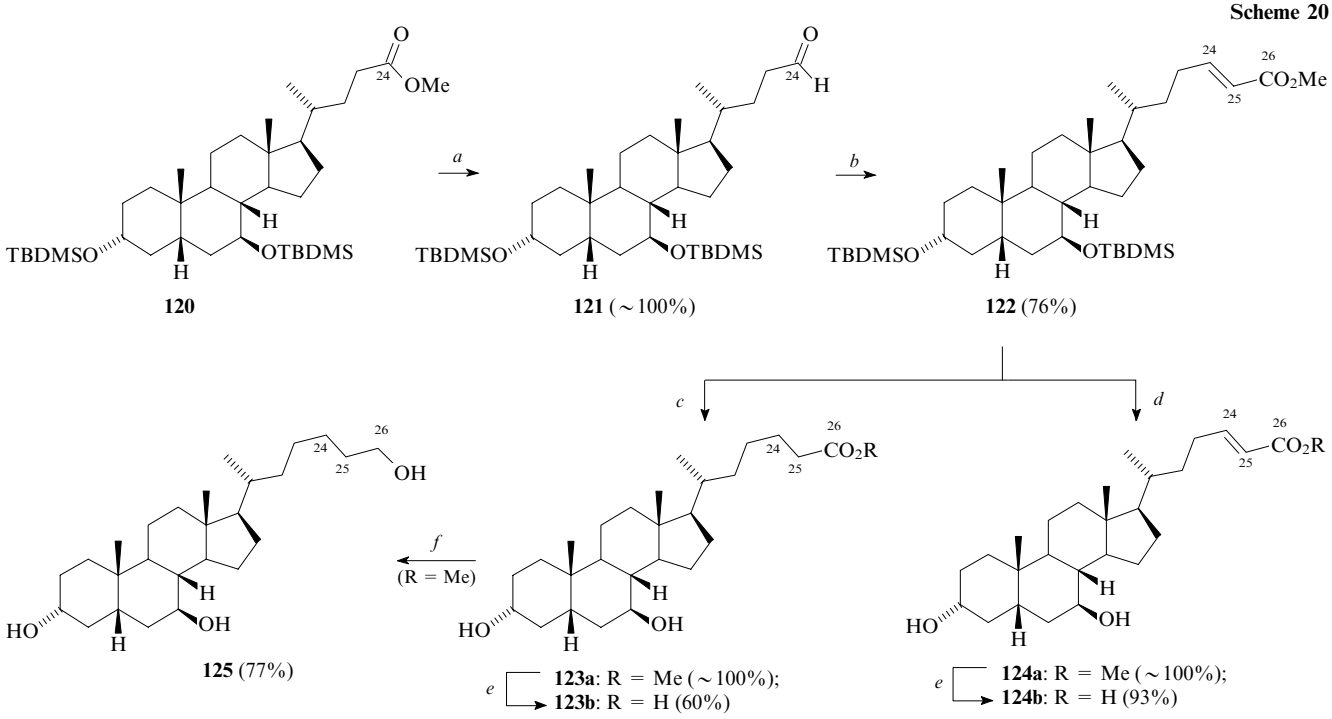
Sepe *et al.*[98] elongated the side chain of BAs and prepared 25,26-bis-homo derivatives of CDCA and UDCA containing carboxyl, hydroxyl and ester groups and a multiple bond in the side chain (Scheme 20 shows UDCA derivatives as examples). The synthesis involves the intermediate formation of 24-aldehyde **121** from protected methyl ester **120** as the key step. The reaction of aldehyde **121** with phosphorus ylide gives bile acid methyl ester **122**, the side chain of which is elongated by two carbon atoms and contains a double bond. The subsequent reduction of the double bond in compound **122** combined with hydrolysis of protecting groups affords methyl ester **123a**, which is hydrolyzed in an alkaline medium to form 25,26-bis-homo-UDCA **123b**. Acid **124b** containing a double bond in the side chain was synthesized by the hydrolysis of protecting groups of compound **122** and the Me group of ester **124a**. The reduction of methyl ester **123a** produced alcohol **125**. According to assessment of biological activity, compound **123b** modulated selectivety the membrane receptor TGR5 in comparison with FXR.

Scheme 19



(*a*) pyrrole, $BF_3 \cdot Et_2O$, rt, 20 h; (*b*) Me_3SiCN, MeCN; (*c*) 1) 25% aq. NH_3, THF, Δ, 18 h (64%); 2) HCl (conc.), MeOH, THF, Δ, 24 h (69% yield)

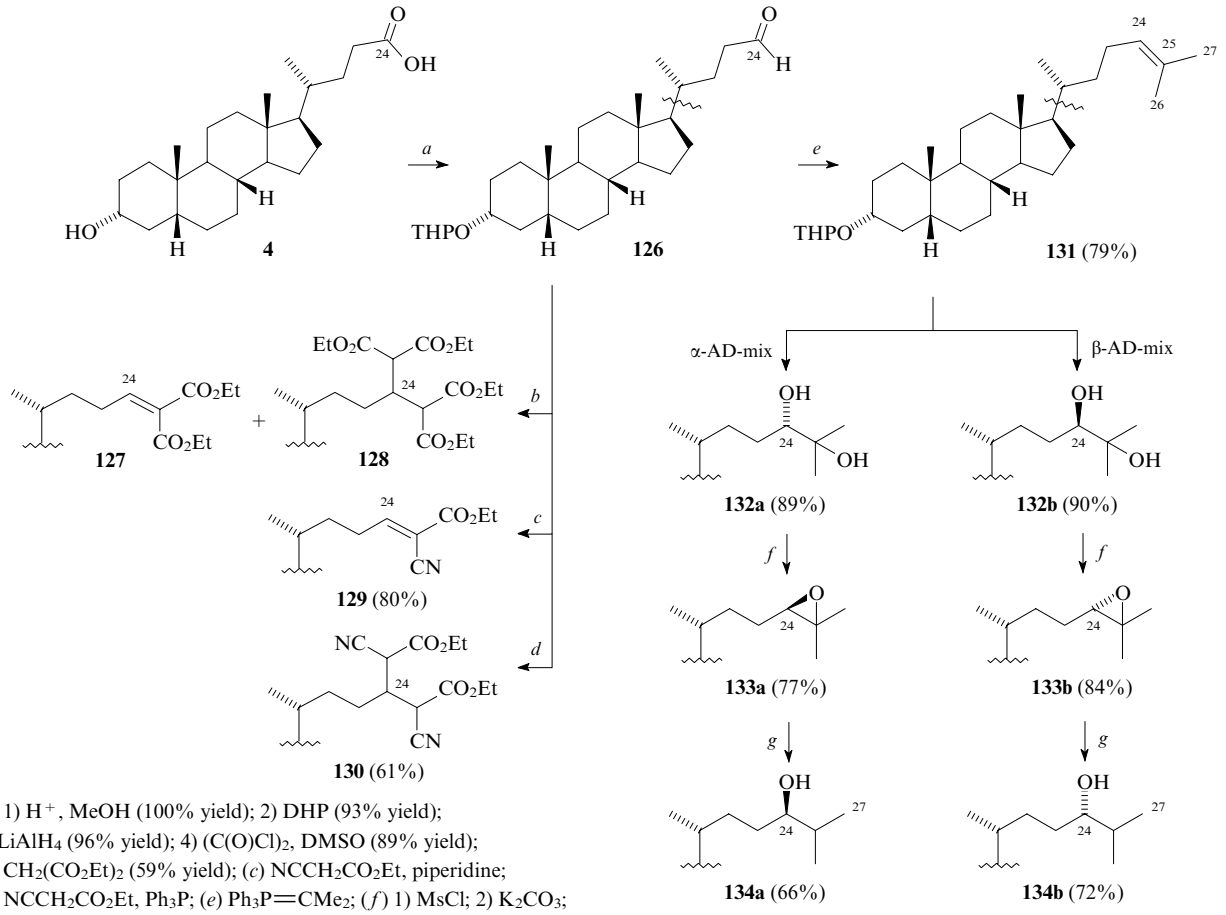
Scheme 20



(*a*) 1) $LiBH_4$, MeOH, THF, 0 °C; 2) $(C(O)Cl)_2$, Et_3N, DMSO, CH_2Cl_2, −78 °C; (*b*) $Ph_3P{=}CHCO_2Me$; (*c*) 1) H_2, $Pd(OH)_2/C$, THF, MeOH; 2) HCl (conc.), MeOH; (*d*) HCl (conc.), MeOH; (*e*) 5% NaOH, MeOH, H_2O; (*f*) $LiBH_4$, MeOH, THF, 0 °C

Many biologically active steroids have side chains functionalized at the C(24) – C(27) atoms. The reactivity of LCA 24-aldehyde **126** in the Knoevenagel reaction with malonic and cyanoacetic esters (Scheme 21) was investigated [65] with the goal of developing new methods for side chain modifications of steroids. These reactions give mono- and bisaddition products **127** – **130**. This approach can be used to synthesize steroids containing the functionalized side chain of different length starting from available and cheap BAs. Khripach *et al.*[58] prepared a series of LCA derivatives with an elongated side chain with the aim of finding potential LXR agonists (see Scheme 21). The reaction of LCA 24-aldehyde **126** with $Ph_3P{=}CMe_2$ produced 27-analogue of LCA **131** with a branched side chain containing a double bond. The subsequent asymmetric dihydroxylation of compound **131** with commercial mixtures of α- and β-AD-mix yielded diols **132a** and **132b**, respectively. Then epoxides **133a**,**b** were prepared by the intramolecular nucleophilic substitution followed by their reduction to 24-hydroxy steroids **134a**,**b**.

Scheme 21



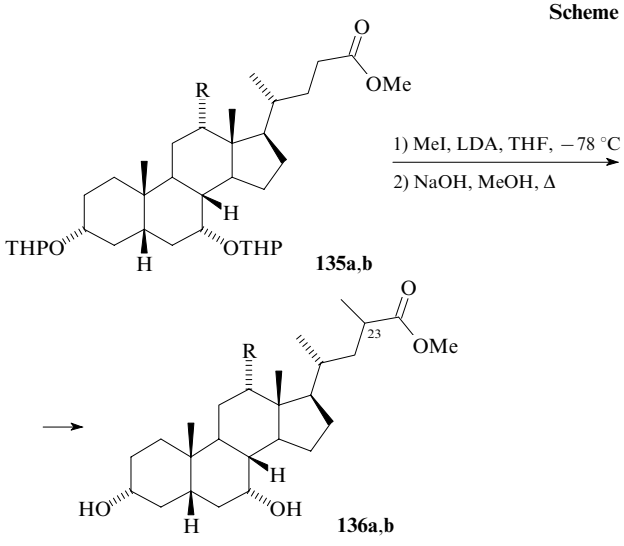
(*a*) 1) H^+, MeOH (100% yield); 2) DHP (93% yield); 3) $LiAlH_4$ (96% yield); 4) $(C(O)Cl)_2$, DMSO (89% yield); (*b*) $CH_2(CO_2Et)_2$ (59% yield); (*c*) $NCCH_2CO_2Et$, piperidine; (*d*) $NCCH_2CO_2Et$, Ph_3P; (*e*) $Ph_3P{=}CMe_2$; (*f*) 1) MsCl; 2) K_2CO_3; (*g*) 1) $NaBH_3CN$; 2) H^+; DPH is 2,3-dihydro-4*H*-pyran, THP is tetrahydropyran-2-yl

The side chain modification of BAs with additional functional groups usually implies the introduction of either a methyl group[128,129] or a halogen atom[115] at the α position with respect to the carboxyl group. 23-Methyl derivatives of CA and CDCA and their analogues were synthesized in order to investigate the structure–property relationship for the activation of the TGR5 nuclear receptor.[128] The reaction of CA and CDCA methyl esters **135a**,**b**, which were protected at the OH groups with the tetrahydropyranyl moiety, with MeI in the presence of lithium diisopropylamide at low temperature followed by saponification of protecting groups and the methyl ester produced a mixture of (23*R*)- and (23*S*)-epimers of acids **136a**,**b** (Scheme 22).

Yu *et al.*[129] synthesized 23-methyl derivatives of BAs (UDCA, CDCA, *etc.*) using (instead of LDA) the bulky base — potassium hexamethyldisilazide (KHMDS) — in a THF–hexamethylphosphoramide (HMPA) mixture at −78 °C. The reaction with MeI in THF was stereoselective and produced only (23*S*)-methyl derivatives of BAs (in a total yield of 60%).

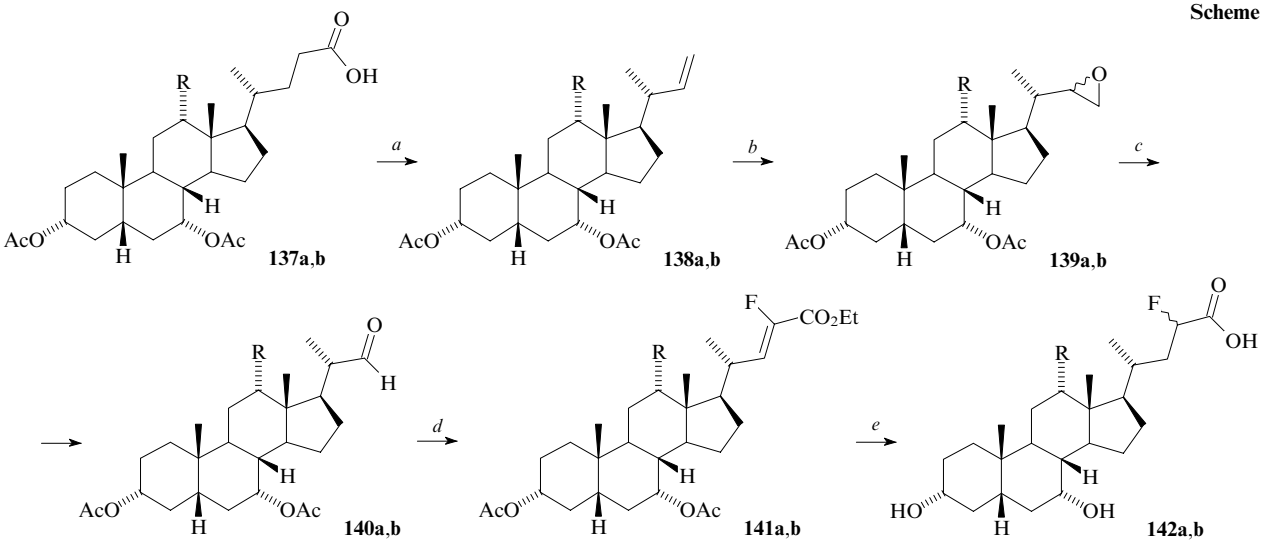
The synthesis of 23-fluoro derivatives of CA and CDCA was described.[115] The sequence of transformations giving the target products is shown in Scheme 23. The oxidative decarboxylation of CA and CDCA peracetates **137a**,**b** with lead tetraacetate in the presence of copper acetate yields 24-nor-22(23)-ene derivatives **138a**,**b**, and the oxidation of the double bond in these compounds with *m*-CPBA gives 24-nor-22,23-epoxy derivatives **139a**,**b**. The subsequent oxidative cleavage with periodic acid (H_5IO_6) affords 23,24-dinor-22-aldehydes **140a**,**b**. Their condensation with ethyl 2-(diethylphosphono)-2-fluoroacetate, the subsequent reduction of the double bond and the hydrolysis of the ester and acetate groups in intermediates **141a**,**b** give a

Scheme 22



R = OTHP (**135a**), OH (**136a**), H (**135b**, **136b**)

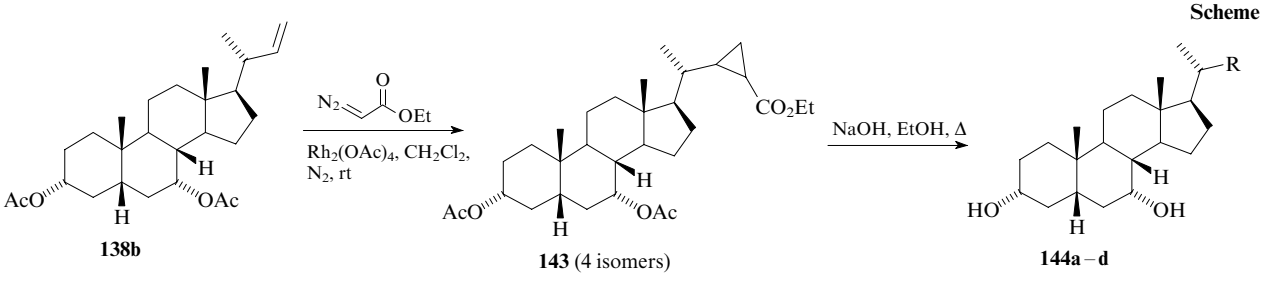
Scheme 23



(*a*) $Pb(OAc)_4$, $Cu(OAc)_2$, Py, PhH, Δ, 16 h; (*b*) *m*-CPBA, $NaHCO_3$, CH_2Cl_2, rt; (*c*) H_5IO_6, THF, rt; (*d*) $(EtO)_2P(O)CHFCO_2Et$, LiCl, DBU, MeCN, rt, 3 h; (*e*) 1) H_2, Pd/C, THF, rt, 10 h; 2) NaOH, MeOH, H_2O, rt, 12 h; R = OH (**142a**), OAc (**137a** – **141a**), H (**137b** – **142b**)

Compound	Yield (%)				
	138	**139**	**140**	**141**	**142**
a	44	85	43	70	87
b	45	83	45	72	83

Scheme 24



mixture of epimeric (23*R*)- and (23*S*)-fluoro derivatives of BAs **142a**,**b**.

Compound **138b** was used to prepare 22,23-methylene derivatives of BAs (Scheme 24) intended for studying the structure – property relationship for the activation of nuclear receptors (TGR5, FXR).[1,23] The reaction of compound **138b** with ethyl diazoacetate at the multiple bond gives a mixture of four isomers **143**. Individual isomers of the acids (**144a** – **d**) were isolated by chromatographic separation of the reaction mixture obtained after alkaline hydrolysis of **143**.

Therefore, the reactions involving the side chain of BAs are related to numerous synthetic transformations including both modifications of the native carboxyl group, which give bile salts, esters and amides, and changes in the length of the side chain and its functionalization with different groups [CN, CHO, OH, SO_3H, $P(O)(OH)_2$, NO_2, Cl, I, *etc*.].

V. Modifications of the steroid nucleus

In rare cases, modifications of the steroid nucleus of BAs do not require preliminary side chain modifications. Generally, in the first step, the carboxyl group is transformed into the ester one, which can be subsequently hydrolyzed to the starting carboxyl group. Therefore, strictly speaking, all transformations of BAs outlined in this section can be considered as combined transformations, in which both the nucleus and the side chain are involved.

Below we consider different approaches to modifications of native hydroxyl groups (esterification, oxidation), resulting in the formation of multiple bonds and the introduction of new functional groups and(or) heteroatoms into the steroid skeleton.

The structure of BAs is such that native hydroxyl groups, as well as other functions that are formed during chemical modification of rings A, B and C of the steroid skeleton, have different reactivity. For example, functional groups of ring C are less reactive than the corresponding substituents in rings A and B due to steric factors. Therefore, reactions suitable for selective modification of individual functional groups of the steroid skeleton are of particular interest.

V.1. Protection of hydroxyl groups

Generally, modifications of the steroid skeleton of BAs involve multistep transformations, which entail the protection of one or several hydroxyl groups. Examples of BA derivatives protected at hydroxyl groups were described in the previous sections (when considering the synthesis of BA halides or 24-nor analogues), but they were not discussed in detail. Different approaches to the protection of native hydroxyl groups are described in the literature concerned with modifications of BAs. The choice of a particular protecting group and the procedure for its removal generally depends on subsequent transformations. According to the published data, the reactivity of OH groups of the steroid skeleton of BAs decreases in the series 3-OH > 7-OH > 12-OH. It should also be noted that, as demonstrated by Broderick *et al.*,[130] the reaction rate of acylation of the axial 7α-OH group is lower than that for the equatorial 7β-OH group.

The esterification with formylating and other acylating agents under different conditions is the approach most commonly applied for the protection of BA hydroxyl groups. Bile acid hydroxyl groups are formylated with formic acid. At room temperature, the reaction takes 3 days for the completion,[57] while the heating to 55 °C (see Refs 113, 114, 125) or the addition of $HClO_4$ (see Refs 21, 98, 112) reduces the reaction time to a few hours. Several procedures were developed for the acylation of bile acid OH groups. The most commonly used procedure involves the reaction of BAs with acetic anhydride in the presence of different bases (Py,[74] the DMAP–Py system,[115] the DMAP–Et_3N system [131, 132]); acetic anhydride in the presence of trimethylsilyl triflate [133] or acetyl chloride [75] are also employed. Examples of enzymatic acylation of BAs are also available in the literature.[64] An advantage of the latter approach is that it does not require the transformation of the carboxyl group in the side chain of BAs into an ester group.

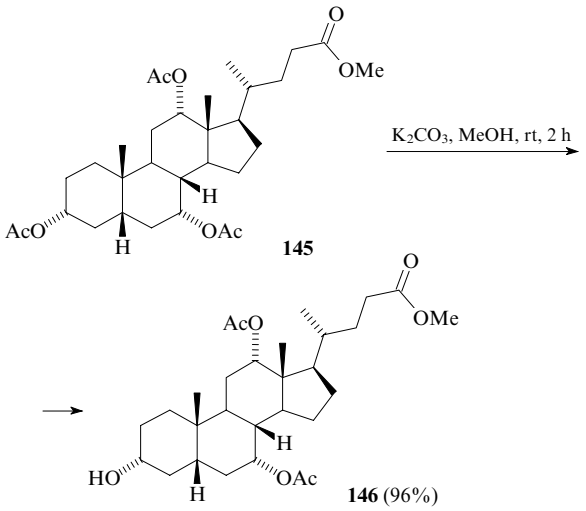
Hydroxyl groups can also be protected by the reaction with benzoyl chloride in the presence of pyridine.[134]

The formyl and acetyl protecting groups are generally removed under alkaline hydrolysis conditions [using NaOH,[112, 115] KOH,[74, 98] NaOMe (see Ref. 21) or K_2CO_3 (see Refs. 113 and 114) in EtOH or MeOH under reflux or at room temperature]. The formyl protection can be removed using acidic hydrolysis [*p*-toluenesulfonic acid (TsOH), Pr^iOH, MW].[57] If alkaline hydrolysis is inapplicable because other functional groups will be involved in the reaction, one can employ the reduction of acyloxy derivatives of BAs with complex hydrides, *e.g.*, $LiAlH_4$, giving hydroxyl groups.[107]

The selective deprotection of the 3-OH group, for example, of compound **145** can be accomplished by performing hydrolysis under mild conditions [weak bases, *e.g.*, $NaHCO_3$ (see Refs 67 and 68) or K_2CO_3 (see Ref 133) in MeOH at room temperature or by treating with acyl chloride in MeOH (see Refs 130 and 135)]; in this case, OH groups at positions 7 and 12 of ester **146** remain protected (Scheme 25).

The protection of all hydroxyl groups followed by incomplete hydrolysis (deprotection of OH groups at positions 3 and 7) gives BA derivatives containing the protected 12-OH group (compound **147**, Scheme 26). Compound **147** containing the trifluoroacetate group at position 12 and the *tert*-butyl ester moiety in the side chain was synthesized [136, 137] by the reaction of CA with trifluoroacetic acid anhydride under argon at −45 °C followed by the reaction with *tert*-butyl alcohol at −57 °C and hydrolysis with sodium hydrogen carbonate in a THF–MeOH mixture at low temperature as the final step. The acylation of the 3-OH group with decanoyl chloride in the presence of bases affords compound **148**. The trifluoroacetyl protection of the 12-OH group was removed by the treatment with aqueous ammonia in a THF–MeOH mixture to form compound **149**. In this case, the ester functions in the side chain and at position 3 remain protected despite the fact that the substituent at the C(3) atom is more reactive than that at the C(12) atom.

Scheme 25

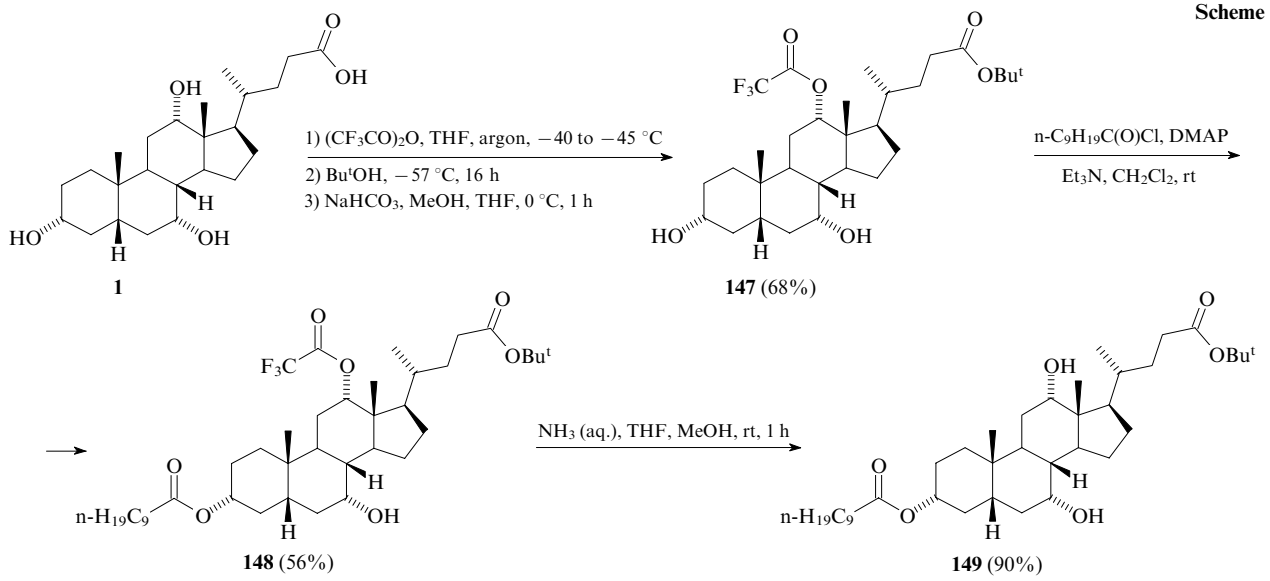


Another approach to the protection of BA hydroxyl groups is based on the formation of acetals, methyl ethers and trialkylsilyl ethers. Acetals are stable in alkaline media, and this protection is removed by means of acid-catalyzed hydrolysis. For example, the reaction of methyl 3-oxocholate **150** with dimethoxymethane and P_2O_5 in dry $CHCl_3$ produces compound **151** (Scheme 27; MOM is methoxymethyl).[138] The acetal protection can be accomplished using the reaction of OH groups of cholic acid methyl ester **88** with methoxyethoxymethyl chloride (MEMCl) in the presence of diisopropylethylamine (DIPEA) in CH_2Cl_2 at room temperature for 24 h, resulting in the formation of product **152**.[103] The protection of BA hydroxyl groups by the reaction with 3,4-dihydropyran in the presence of TsOH in dioxane [128] or in CH_2Cl_2 (see Ref. 58) at room temperature (see compound **153** in Scheme 27) was also described in the literature. However, it should be noted that the reaction gives a mixture of products, in which the asymmetric centre in the tetrahydropyranyl moiety has the *R*- and *S*-configurations.

The protection of hydroxyl groups by their transformation into ether groups is accomplished by the reaction with methyl iodide in the presence of a base giving the corresponding methyl ethers (compound **154**, see Scheme 27). An advantage of these protecting groups is their stability in photolysis. The methoxy protecting groups are removed by the treatment with trimethylsilyl iodide in $CHCl_3$ (see, for example, the study [104]).

Trialkylsilyl protecting groups are also employed in modifications of BAs.[82, 98, 107] For example, the reaction of CA with *tert*-butyldimethylsilyl chloride in the presence of

Scheme 26



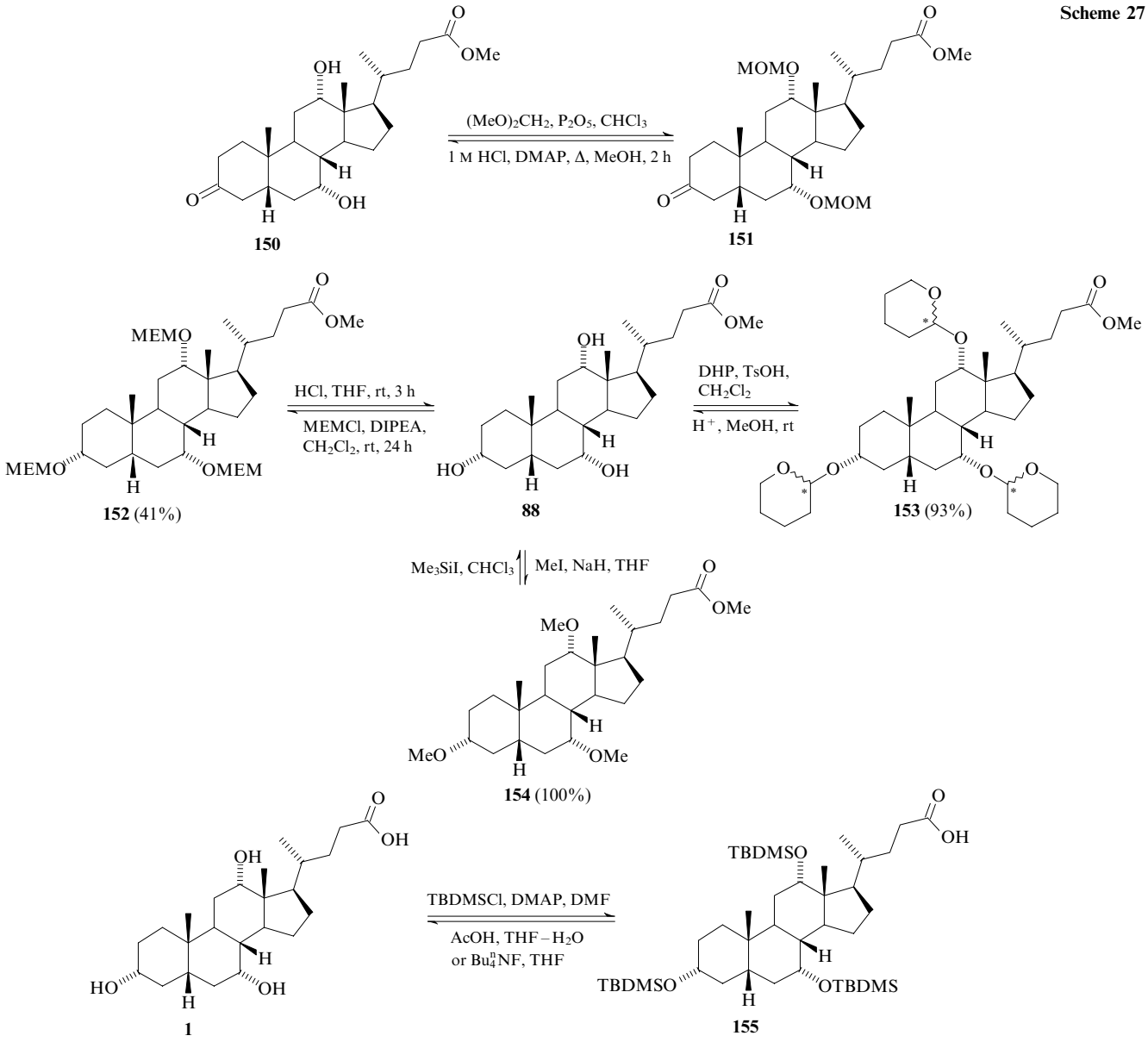
1

147 (68%)

148 (56%)

149 (90%)

Scheme 27



150

151

152 (41%)

88

153 (93%)

154 (100%)

1

155

DMAP in DMF at room temperature[82] yields compound **155** (see Scheme 27). The silyl protection is removed with tetra-n-butylammonium fluoride in THF (see Ref. 82) or by acid-catalyzed hydrolysis (for example, by the treatment with AcOH in a mixture of THF and H_2O).[98, 107]

In summary, the currently used approaches to the protection of native hydroxyl groups of BAs are based on the formation of esters, acetals, methyl and trialkylsilyl ethers. The choice of a particular protecting groups depends on stability of the compound in the steps of the synthesis that are planned to be performed, as well as on the ease of the protection and deprotection.

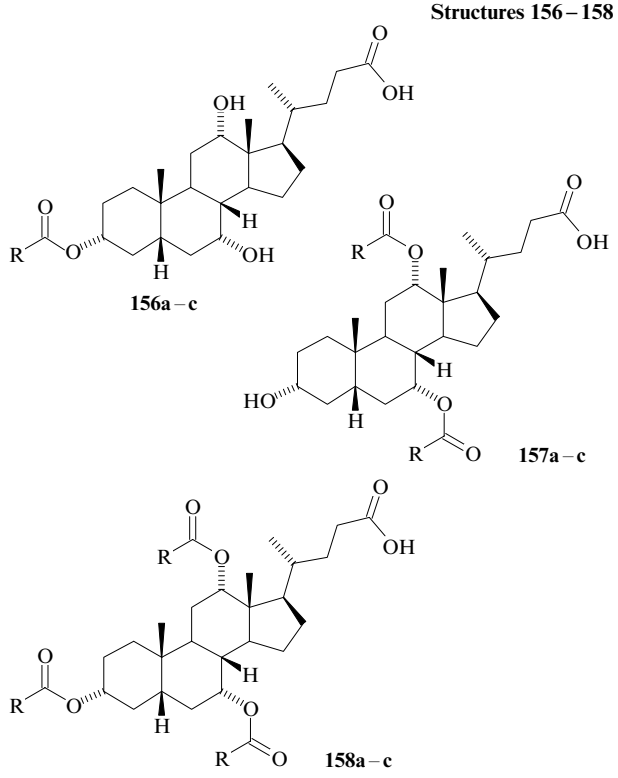
V.2. Synthesis of carboxylic and mineral acid esters and ethers at hydroxyl groups

The synthesis of BA derivatives *via* modifications of native hydroxyl groups of BAs can be used to change physicochemical properties of the molecule (polarity, solubility) and to prepare bile acid conjugates with biologically active compounds and drugs. Modifications can be performed with identical or different moieties.

The introduction of one or several hydrocarbon groups into BAs leads to an increase in lipophilicity of the molecule as a whole. Derivatives **156**–**158** mono-, di- and trisubstituted at hydroxyl groups were synthesized by the reactions of CA with butyryl, caproyl and palmitoyl chlorides.[136] The goal of these modifications was to find compounds that enhance transdermal penetration or intestinal absorption of poorly bioavailable drugs. Lipophilicity and hydrophobicity of the newly synthesized compounds were evaluated and their ability to enhance intestinal and transdermal absorption was tested *in vitro* using theophylline as a model penetrant. Compounds **156b** and **158b** proved to be better transdermal penetration enhancers, while derivative **157b** was a more efficient promoter of intestinal absorption of the model agent. The toxicity assays of these compounds against the human cancer cell lines CEM and MCF7 and the normal cells BJ demonstrated that only compounds **156b**, **156c**, **157b** and **158a** exhibited antiproliferative activity against cancer cells (IC_{50} varied from 16.4×10^{-6} to 34.6×10^{-6} mol litre^{-1}). The other derivatives displayed low toxicity ($IC_{50} > 37 \times 10^{-6}$ mol litre^{-1}) against both cancer and normal cells. Therefore, they can be considered as potential agents that enhance intestinal drug absorption.

Compounds **159a**–**c** are examples of BA derivatives modified with functional moieties with different structures. These derivatives were synthesized by the combined esterification of 7- and 12-OH groups of 3-oxo-CA methyl ester **150** with dihydropyran and benzoyl chloride. These compounds are potential inhibitors of P-glycoprotein — a membrane protein responsible for multidrug resistance.[139]

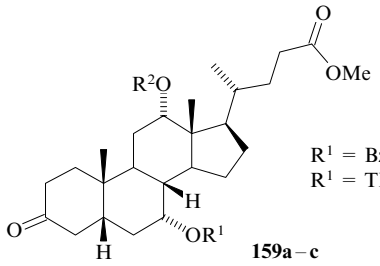
Structures 156–158



156a–c **157a–c** **158a–c**

R = Pr^n (**a**), n-C_9H_{19} (**b**), n-$C_{15}H_{31}$ (**c**)

Structures 159



R^1 = Bz: R^2 = Bz (**a**), THP (**b**);
R^1 = THP, R^2 = Bz (**c**)

159a–c

The sequential esterification of the 3-OH group of compound **160** with oxazolidinecarboxylate **161** and then of the 7-OH group with benzoyl bromide followed by the reduction of the side chain ester group to the carboxyl function in compound **162** produced CA derivative **163** (Scheme 28).[137] This compound is a mimetic of Docetaxel (**164**) — an anticancer drug of the taxane class. In tubulin depolymerization assays, compounds **162** and **163** proved to be inactive compared to Docetaxel; however, $IC_{50} = 3.8 \times 10^{-6}$ and 94×10^{-6} mol litre^{-1}, respectively, were obtained in tubulin polymerization assays, whereas Docetaxel was inactive in the latter assay.

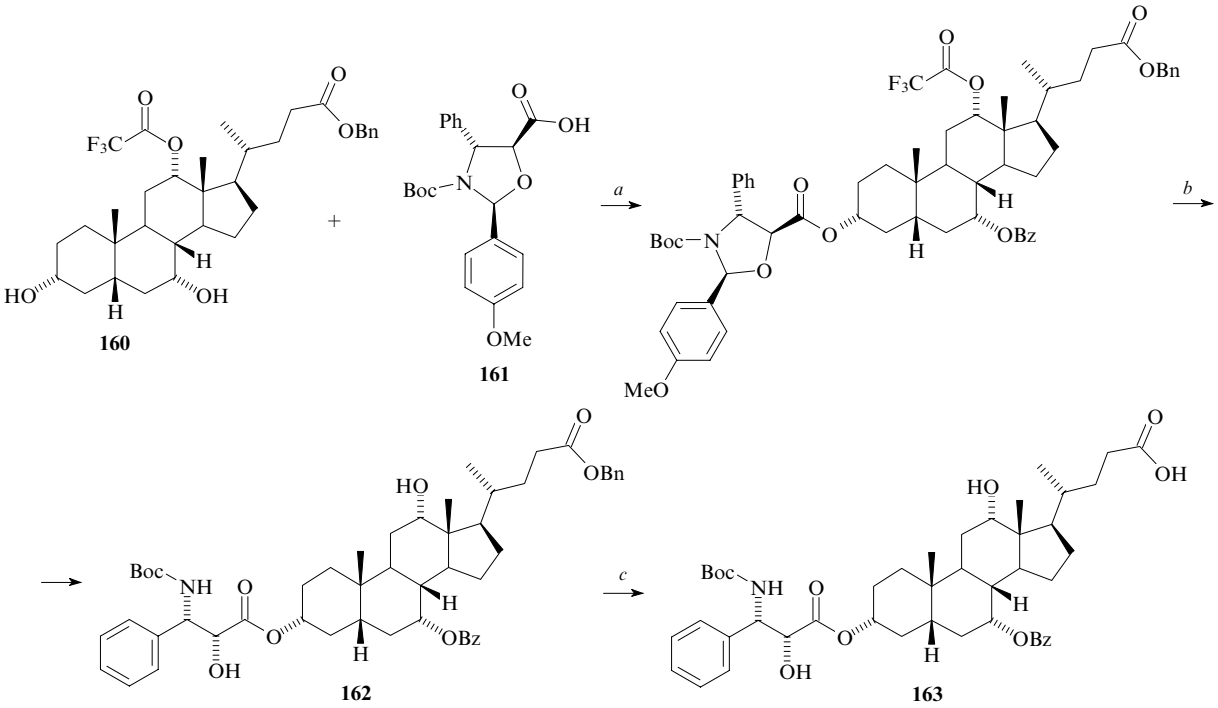
The esterification of BA hydroxyl groups with polyfunctional carboxylic acids produces compounds, which hold promise for subsequent modifications, primarily, for the synthesis of BA dimers and oligomers, as well as BA conjugates with biologically active molecules containing a polyfunctional carboxylic acid moiety as a linker.

For example, pyridine-4-carboxylic acid and CA esters form dimers **165a**,**b** with palladium and platinum ions *via* coordination bonds with the pyridine nitrogen atom. These structures can be considered as analogues of ion channels that are open or closed depending on the presence or absence of the ligand, respectively.[140]

The bile acids conjugated with juvenoids at the carboxyl group, which were discussed in Section IV.1.b, were dimerized *via* the esterification with adipic acid involving the 3-OH group[67] (compounds **166a**,**b**) or subjected to the reaction with stigmasterol using a succinic acid moiety as a linker[68] (compounds **167a**,**b**).

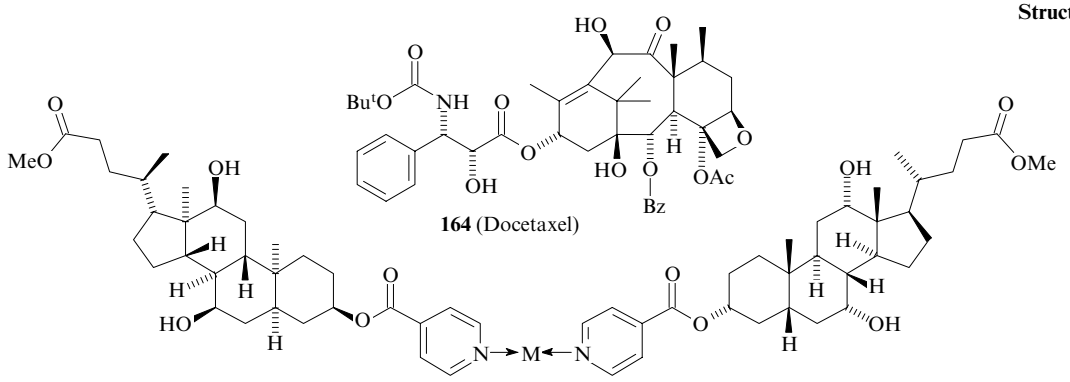
A conjugate with low-molecular-weight heparin (LMWH) — product **168** — was synthesized from taurocholic acid sodium salt using a dicarbamic acid moiety.

Scheme 28



(*a*) 1) DCC, DMAP, PhMe (67% yield); 2) BzBr, DMAP, Py, 80 °C (80% yield); (*b*) 1) TsOH, MeOH (86% yield); 2) NH_4OH, MeOH, THF (68% yield); (*c*) H_2, Pd/C, THF (98% yield)

Structures 164, 165



164 (Docetaxel)

165a,**b** (M = *trans*-$PdCl_2$ (**a**), *cis*-PtI_2 (**b**))

This conjugate inhibits angiogenesis and has been extensively investigated.[141, 142]

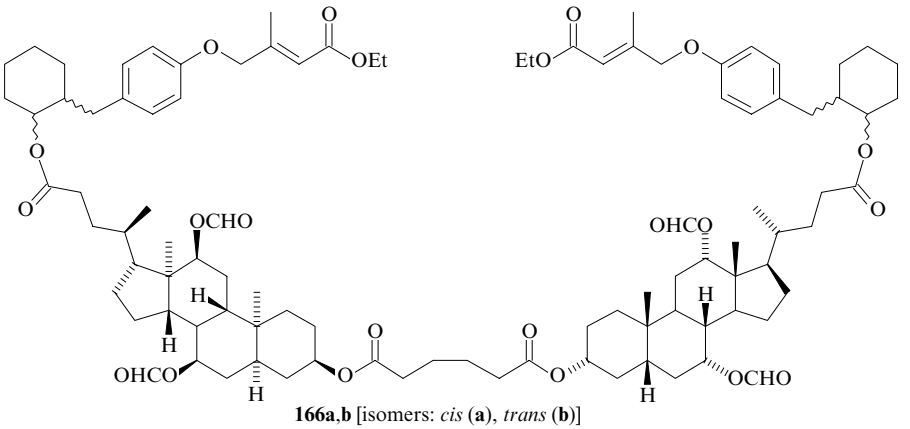
The modification of the BA backbone with carboxylic acid moieties containing the terminal triple bond was applied for the subsequent binding of peptides accompanied by the formation of the 1,2,3-triazole ring (compounds **169a**,**b**).[59] In oligomer **170**, which was synthesized based on CA,[143] the monomeric units are connected by a linker containing 1,2,3-triazole and an ester bond. Cholic acid derivative **171** which contains a propargyl ester group in the side chain and an α-azidoacetic acid moiety at position 3, was the key intermediate in the synthesis.

Bile acid derivatives esterified at hydroxyl groups and containing polyfunctional carboxylic acid moieties (compounds **172** – **175**) can also be employed as chiral auxiliary agents in the stereoselective synthesis.[144, 145] Depending on the nature of functional substituents at the C(7) or C(12) atom, the reaction in question and the nature of the substrate, the enantiomeric excess (*ee*) or diastereomeric excess (*de*) of the products varies from 20% to 98%.

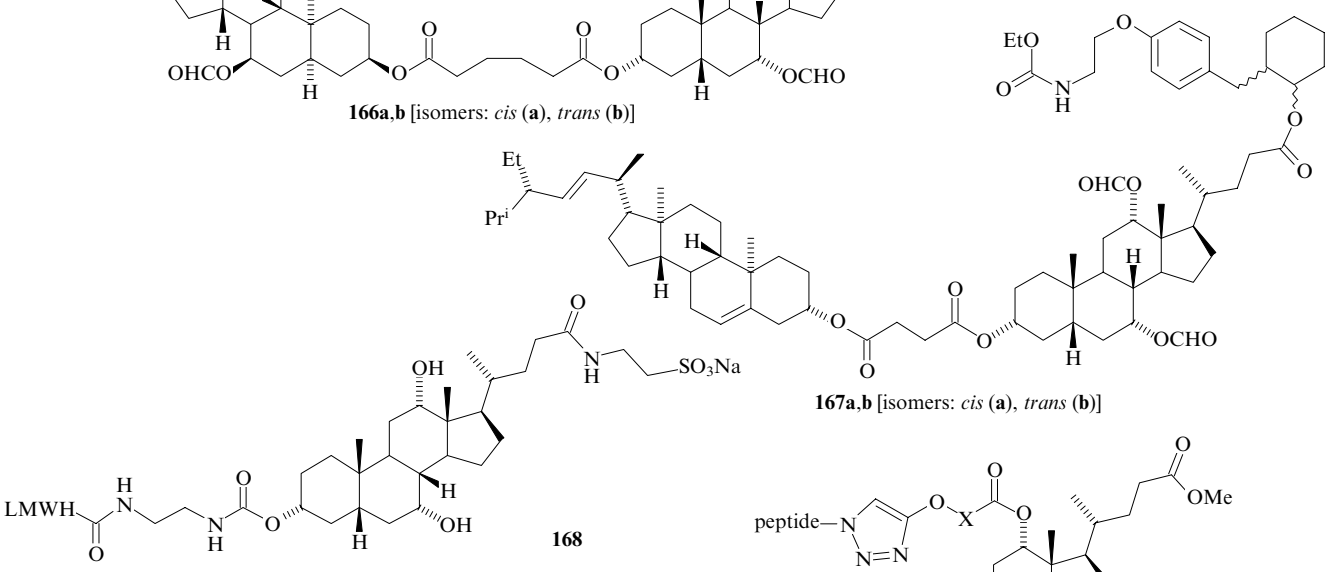
The esterification of BA hydroxyl groups with halocarboxylic acids makes it possible to replace a halogen atom in these compounds with nitrogen-containing functional groups using reactions with amines. Many bile acid derivatives containing amino or ammonium groups exhibit antibacterial activity. The modification of the steroid nucleus with ionogenic groups (*e.g.*, NH_2, CO_2H) can lead to an increase in the surface amphiphilicity of BAs. These surface amphiphiles can be applied in different ways: as ion transporters, membrane permeability enhancers and in combinatorial chemistry.

For example, the esterification of the carboxyl group of CA with higher n-alcohols, the reactions with ω-halocarboxylic acid chlorides at hydroxyl groups of the steroid nucleus and the subsequent reaction with trimethylamine give compounds **176a** – **g** containing three ammonium salt

Structures 166 – 175

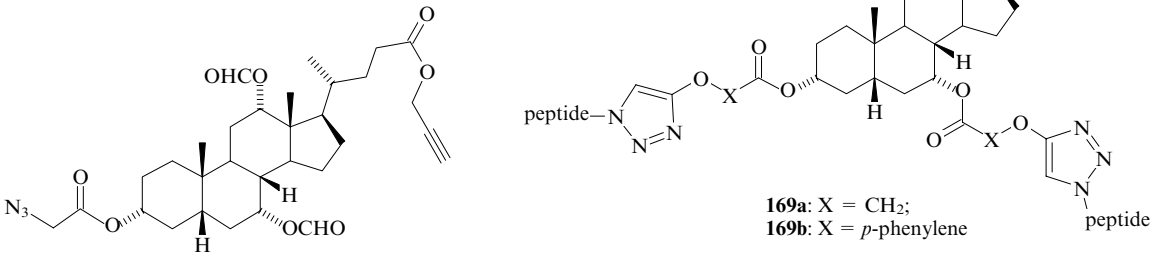


166a,**b** [isomers: *cis* (**a**), *trans* (**b**)]



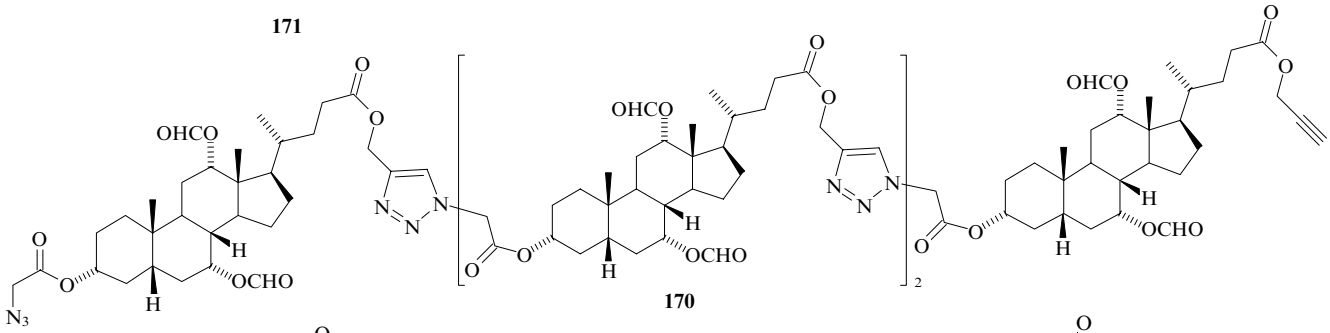
167a,**b** [isomers: *cis* (**a**), *trans* (**b**)]

168

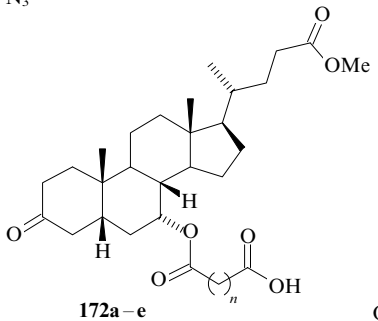


169a: X = CH_2;
169b: X = *p*-phenylene

171

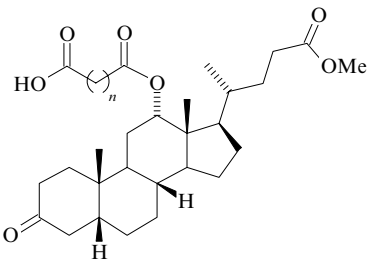


170

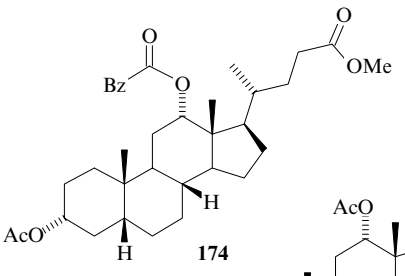


172a – **e**

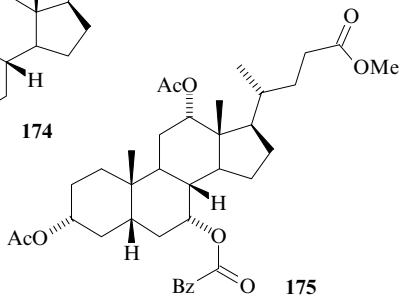
n = 0 (**a**), 1 (**b**), 2 (**c**), 3 (**d**), 4 (**e**)



173a – **e**



174



175

groups. These compounds can form spherical micelles and exhibit antimicrobial activity. For example, MIC for compound **176b** against *E. coli* and *E. faecalis* was 12.5×10^{-6} g ml^{-1}.[146]

Ye *et al.*[147] synthesized CDCA amides **177a – d** containing the pyridinoacyl moiety using a similar procedure for the modification of hydroxyl groups (the reactions with chloroacetyl chloride and then with pyridine) and side chain modifications. Compound **177d** exhibited antimicrobial activity comparable to that of the known drug Ofloxacin: MIC values varied from 0.8×10^{-6} to 1.6×10^{-6} g ml^{-1} for different microorganisms (*Bacillus subtilis*, *Streptosporangium longisporum*, *E. coli*, *Salmonella enteritidis*).

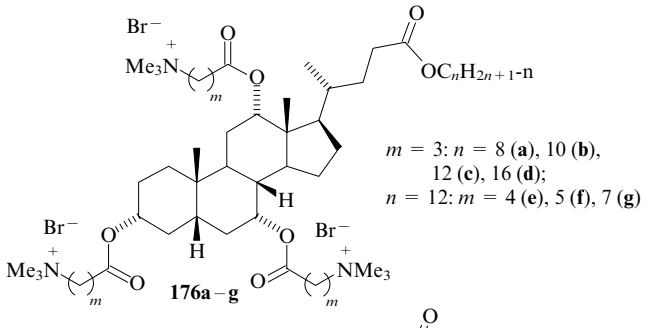
Modifications of the steroid nucleus of BAs with aliphatic amino acids containing the terminal NH_2 group to form compounds **178a – f** have been described.[148] The reaction of Boc-protected amino acids with CA involving hydroxyl groups in the presence of DCC and DMAP was used for this purpose. The treatment with hydrochloric acid in dioxane in the final step of the synthesis afforded target products. The MIC values for these compounds against *S. aureus* and *E. coli* varied from 1.0×10^{-6} to 60×10^{-6} g ml^{-1}.

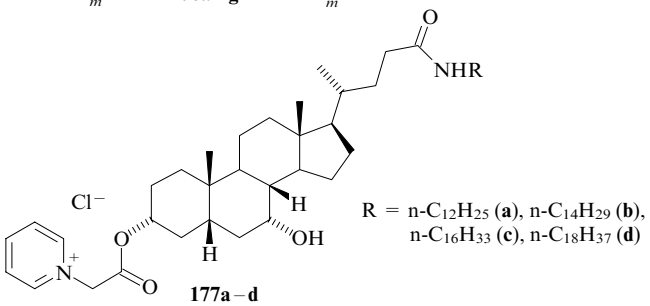
Apart from carboxylic acids, mineral acids are also employed for the esterification of BA hydroxyl groups. 24-Nor-5β-cholane-3α,7β,23-triol derivatives **179a – f** containing OSO_3Na groups in the side chain and(or) at positions 3 and 7 of the steroid nucleus were synthesized from UDCA and were tested as agonists of the nuclear receptors FXR and TGR5.[98]

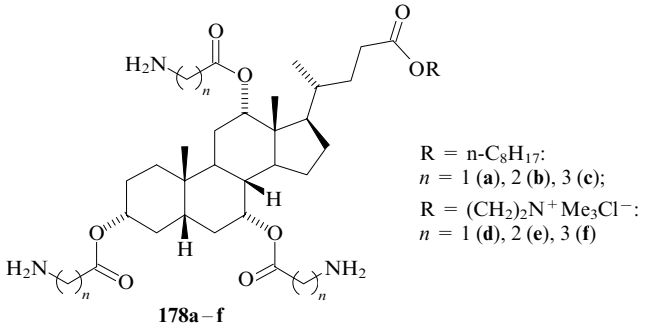
Dosa *et al.*[134] prepared the following phosphate esters of UDCA in order to increase solubility of this acid: phosphates and hydroxymethylphosphates at positions 3 and 7 of the steroid nucleus (compounds **180a**,**b** and **181a**,**b**). Ursodeoxycholic acid possesses numerous valuable biological properties, including anti-apoptotic activity in *in vitro* and *in vivo* models. However, the acid is characterized by low solubility under physiological conditions, which limits its bioavailability and, as a consequence, decreases its therapeutic effect. The assessment of biological activity of the new compounds demonstrated that derivative **180a** displays an anti-apoptotic effect comparable to that of UDCA.

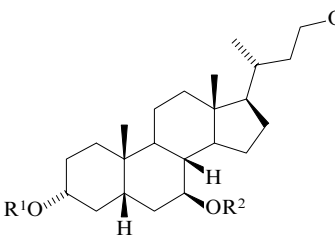
Jin *et al.*[149] synthesized nitrate esters of CA **182a – c** and nitrate esters of UDCA, in which the nitro group is either directly bonded to the BA backbone (compound **183a**) or is separated from the latter by an oligomethylene chain (compounds **183b**,**c**). The nitro group is a NO-releasing moiety. Nitric oxide has useful biological effects on the human body, among which is the protection of the liver from the progress of liver fibrosis and cirrhosis and induced injuries. Due to organotropism of bile acids for the liver, the binding of a NO-releasing moiety to BAs can lead to the design of agents that deliver NO selectively to the liver. Compounds

Structures 176 – 183

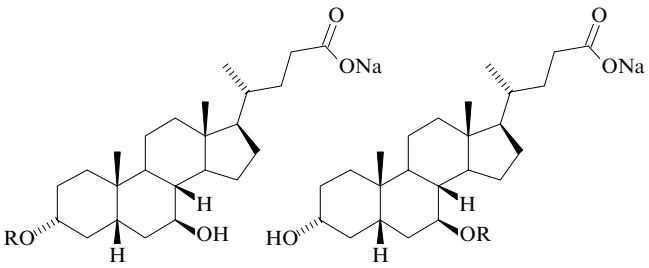




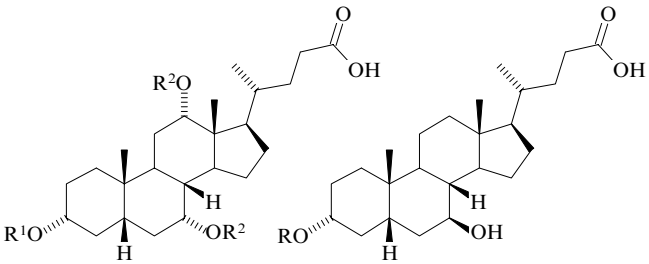




179	R^1	R^2	R^3
a	H	H	SO_3Na
b	SO_3Na	H	H
c	H	SO_3Na	H
d	SO_3Na	H	SO_3Na
e	H	SO_3Na	SO_3Na
f	SO_3Na	SO_3Na	SO_3Na



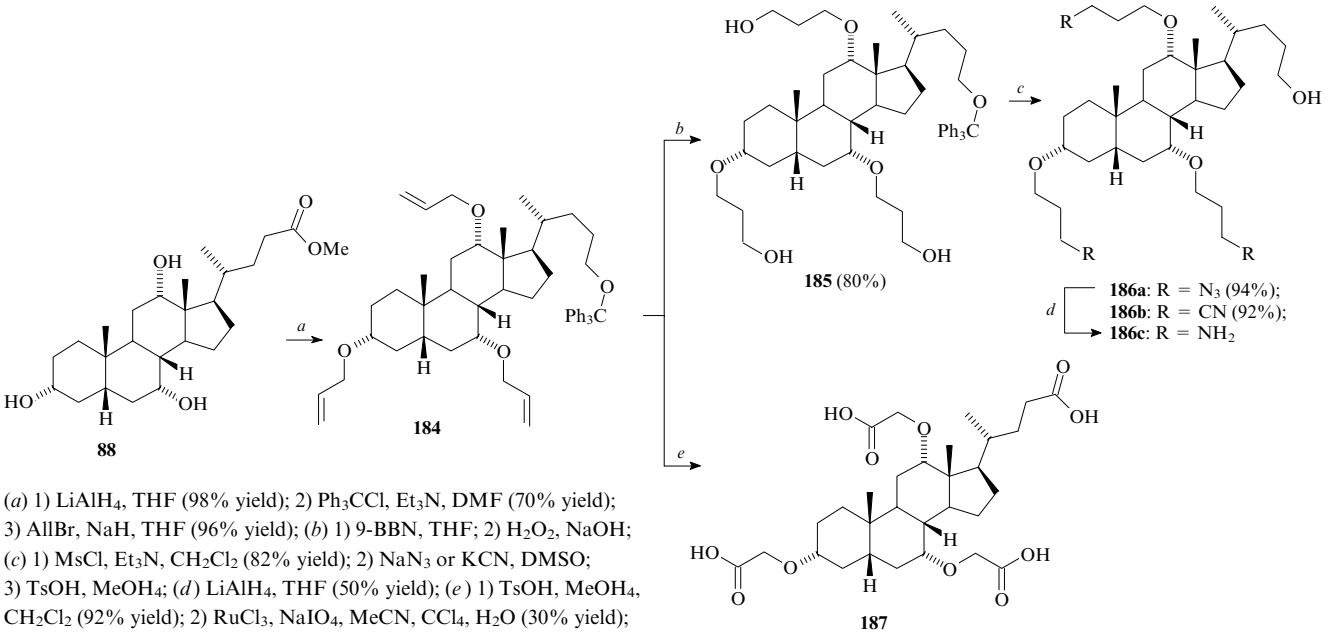
R = $(CH_2O)_nP(O)(ONa)_2$ [*n* = 0 (**a**), 1 (**b**)]



$R^1 = NO_2$; $R^2 = NO_2$ (**a**), H (**b**); R^1 = H, $R^2 = NO_2$ (**c**)

R = NO_2 (**a**), $(CH_2)_2ONO_2$ (**b**), $(CH_2)_4ONO_2$ (**c**)

Scheme 29



(*a*) 1) $LiAlH_4$, THF (98% yield); 2) Ph_3CCl, Et_3N, DMF (70% yield); 3) AllBr, NaH, THF (96% yield); (*b*) 1) 9-BBN, THF; 2) H_2O_2, NaOH; (*c*) 1) MsCl, Et_3N, CH_2Cl_2 (82% yield); 2) NaN_3 or KCN, DMSO; 3) TsOH, $MeOH_4$; (*d*) $LiAlH_4$, THF (50% yield); (*e*) 1) TsOH, $MeOH_4$, CH_2Cl_2 (92% yield); 2) $RuCl_3$, $NaIO_4$, MeCN, CCl_4, H_2O (30% yield); All is allyl

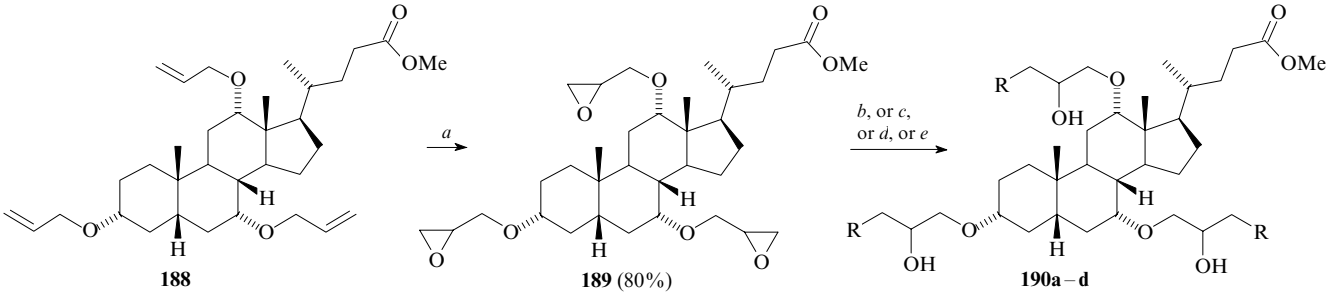
183b,**c** showed the strongest protective effect against *N*-(4-hydroxyphenyl)acetamide- or tetrachloromethane-induced liver injuries in *in vivo* assays.

The steroid nucleus of BAs can also be modified with additional functional groups by means of reactions, resulting in etherification of hydroxyl groups.

One of the most convenient approaches to the synthesis of BA derivatives containing an ether bond and new functional groups is based on the modification of the appropriate allyl ethers. For example, allyl ether **184** was synthesized from derivative **88** by the successive reduction and reaction with Ph_3CCl and allyl bromide (Scheme 29).[150] The subsequent hydroboration of double bonds in compound **184** with 9-boracyclo[3.3.1]nonane (9-BBN) and the treatment with hydrogen peroxide in the presence of NaOH afford key intermediate **185** containing hydroxyl groups connected to the BA backbone through a linker. The activation of OH groups by the reaction with mesyl chloride allows the nucleophilic substitution and modification of the BA backbone with other functional groups. The synthesis of CA derivatives **186a**–**c** containing azido, cyano or amino groups (see Scheme 29) was described in the patent.[150] Apart from the nucleophilic substitution, allyl derivative **184** can undergo oxidative destruction accompanied by the cleavage of the multiple bond with $RuCl_3$ and $NaIO_4$ to form a BA derivative containing carboxyl groups in the backbone (*e.g.*, compound **187**).[151] The modification of the steroid nucleus with polar groups can be used to vary the surface amphiphilicity of BAs and their hydrophobic (hydrophilic) properties. The presence of an ether bond is responsible for stability of the compound in acidic and alkaline media.

The double bond oxidation of allyl derivatives of BAs (*e.g.*, compound **188**) giving rise to the epoxide ring provides an approach to the synthesis of compounds with complex structures and with a variable number of cationic or anionic groups. Thus, the epoxide ring opening in compound **189** with nucleophiles [NaOH, Na_2SO_3, Me_2NH, 3-(methylamino)propane-1,2-diol] gives products **190a**–**d** (Scheme 30).[152] These BA derivatives are potential therapeutic agents, because the introduction of polar groups of different nature and variations in their number provide a tool for changing the hydrophilic–lipophilic balance of the compound, thus entailing the enhancement of the solubilizing and(or) absorbing ability of the products compared to the starting acid.

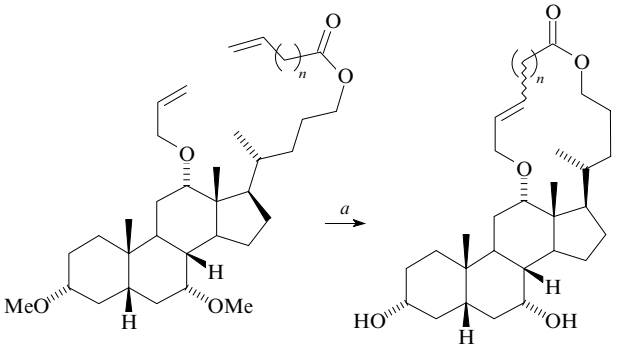
Scheme 30



(*a*) *m*-CPBA, CH_2Cl_2; (*b*) for **190a**: NaOH, H_2O; (*c*) for **190b**: 1) Na_2SO_4, H_2O; 2) Na_2SO_4, MeOH; (*d*) for **190c**: 1) Me_2NH, 2) HCl; (*e*) for **190d**: $MeNHCH_2CH(OH)CH_2OH$; R = OH (**a**, 67% yield), SO_3Na (48%), $N^+HMe_2Cl^-$ (**c**, 74%), $N(Me)CH_2CH(OH)CH_2OH$ (**d**, 36%)

Due to the structural features and polyfunctionality, intramolecular reactions of certain BA derivatives can be accomplished. A synthetic pathway towards pentacyclic steroids **192a – d** starting from compounds **191a – d** was described [153] *via* the metathesis reaction involving the allyl ether moiety at the C(12) atom and the ester group in the side chain containing the terminal double bond (Scheme 31).

Scheme 31



191a – d **192a – d**

(*a*) 1) Cat, PhMe, Δ, 4 h (36% – 63% yield); 2) Me_3SiI, $CHCl_3$; n = 0 (**a**), 1 (**b**), 2 (**c**), 3 (**d**); Cat = (IMES)$(PCy_3)Cl_2Ru{=}CHPh$ (IMES is 1,2-dimesityl-4,5-dihydroimidazol-2-ylidene, mesityl is 2,4,6-trimethylphenyl; Cy is cyclohexyl)

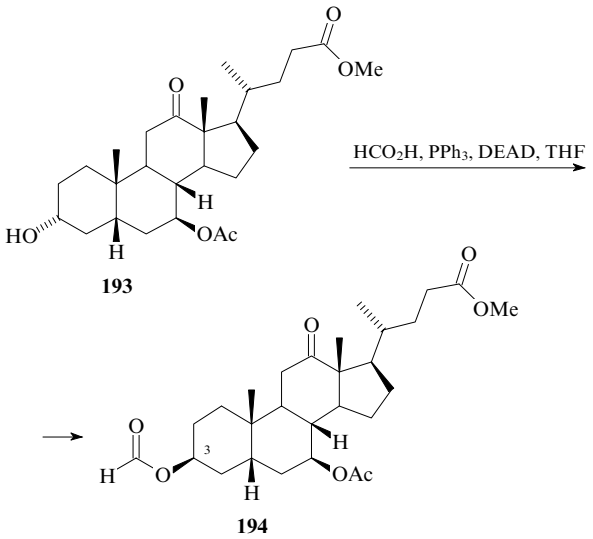
Therefore, the esterification of hydroxyl groups of the steroid nucleus with carboxylic acids, including polyfunctional acids, mineral acids and biologically active molecules, is applicable to the synthesis of a wide range of BA derivatives, such as dimers, oligomers and conjugates having valuable biological and(or) physicochemical properties. The synthesis of BA derivatives containing ether bonds is accomplished through the intermediate formation of allyl ethers followed by modifications of double bonds accompanied by the formation of additional polar functional groups. These transformations can be used to increase the amphiphilicity of the steroid nucleus, while the presence of an ether bond is responsible for stability of the corresponding derivatives in acidic and alkaline media.

V.3. Substitution and elimination reactions of hydroxyl groups

One type of modifications of the steroid nucleus of BAs is based on the replacement of hydroxyl groups by other functions using the nucleophilic substitution reactions, which are generally accompanied by a change in the chiral centre configuration (from α to β). A sequence of transformations involving the double inversion of the chiral centre is employed in order to retain the α configuration of the new functional group.

The Mitsunobu reaction is an example of the replacement of the hydroxyl group accompanied by inversion of the chiral centre configuration. The reaction of compound **193** with triphenylphosphine and diethyl azodicarboxylate (DEAD) in the presence of formic acid produced 3β-formyloxy derivative **194** (Scheme 32).[130] The subsequent hydrolysis of the product yielded the corresponding 3β-hydroxy derivative. This transformation scheme can be

Scheme 32



193 **194**

considered as an approach to the synthesis of β-epimers of BAs.

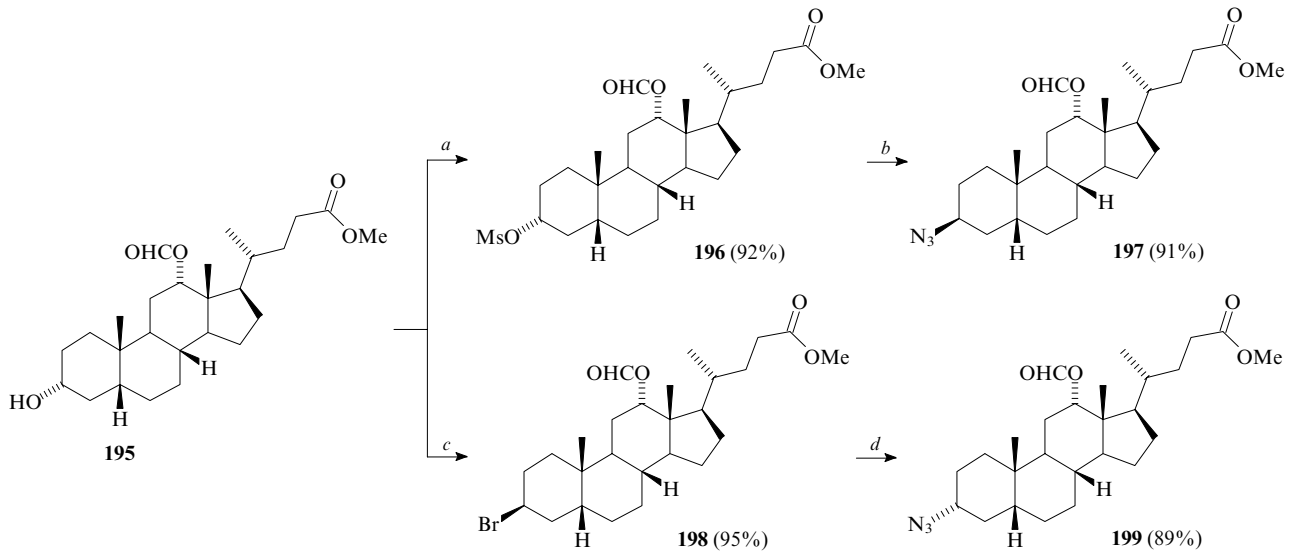
The replacement of the 3α-hydroxyl group with the azido group having the α or β configuration is frequently employed in modifications of BAs. For instance, the 3-OH group in 3α-hydroxy derivative **195** is preactivated with mesyl chloride, and then the nucleophilic substitution reaction of intermediate **196** produces 3β-azido derivative **197** (Scheme 33).

The transformation scheme was proposed for the synthesis of an azido derivative with the 3α configuration.[135] The reaction of compound **195** with *N*-bromosuccinimide (NBS) in the presence of PPh_3 produced 3β-bromo derivative **198**, and its subsequent reaction with sodium azide gave 3α-azide **199** (see Scheme 33). Apart from this sequence of transformations, the successive Mitsunobu reaction and the nucleophilic substitution of the newly formed 3β-functional group by the azido group were also applied for the synthesis of 3α-azido derivatives of BAs.[154 – 156]

The azido group is an important structural unit of biologically active compounds. Different LCA, CDCA and DCA amides containing the azido group at position 3 (compounds **200 – 203**) were synthesized using a combined modification of the steroid nucleus and the side chain.[135] The assessment of biological activity of the resulting compounds demonstrated that they can cause apoptosis in the cancer cells lines Caco-2 and HT1080 at micromolar concentrations. Deoxycholic acid derivatives **202b** and **203b** proved to be most active: IC_{50} against the cell line Caco-2 was 6.3×10^{-6} and 2.3×0^{-6} mol litre^{-1}, respectively; against HT1080, 1.9×10^{-6} and 5.7×10^{-6} mol litre^{-1}.

Azido derivatives of BAs are key intermediates in the synthesis of amino derivatives of BAs. Azido groups can be reduced to amino groups with different reducing agents, for example, with zinc dust in acetic acid,[154] with hydrogen over different catalysts [156, 157] or with triphenylphosphine in acetonitrile followed by the treatment with water.[157] The subsequent modification of both the azido and amino derivatives of BAs allows the synthesis of compounds with different structures possessing valuable biological and(or) physicochemical properties. Several examples of such compounds are given below.

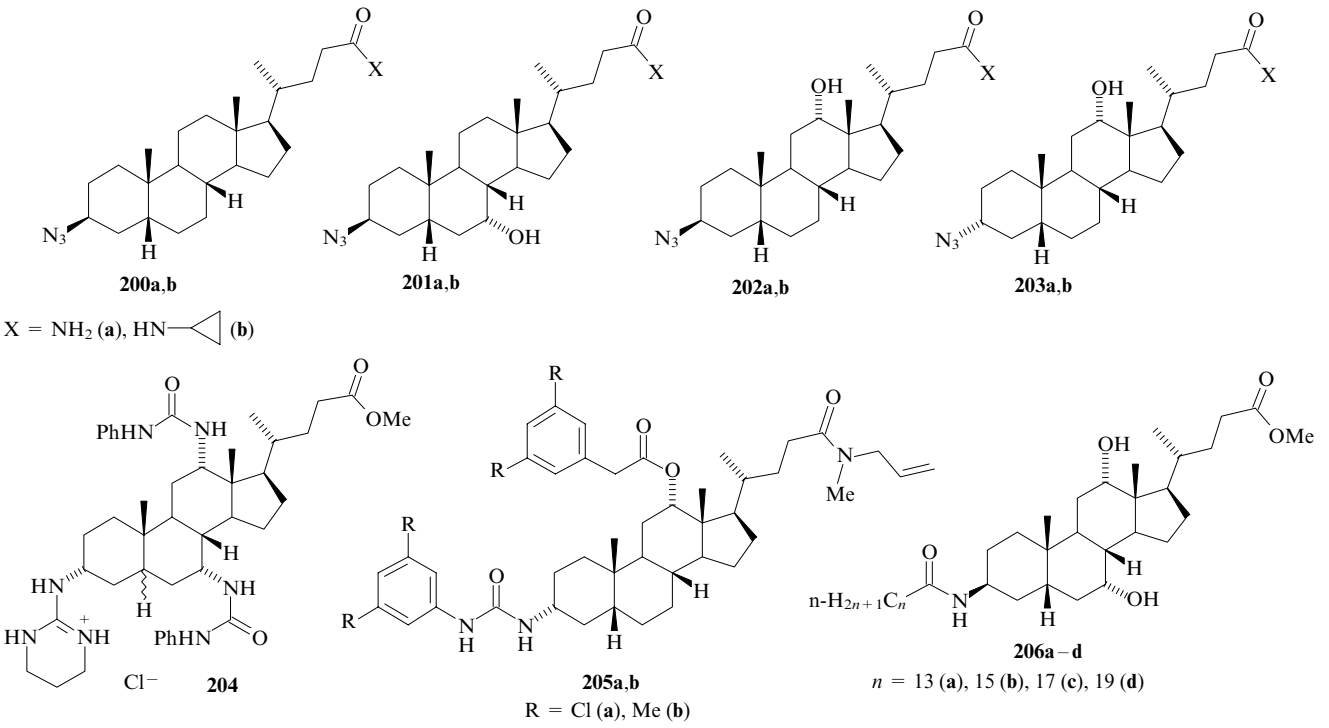
Scheme 33



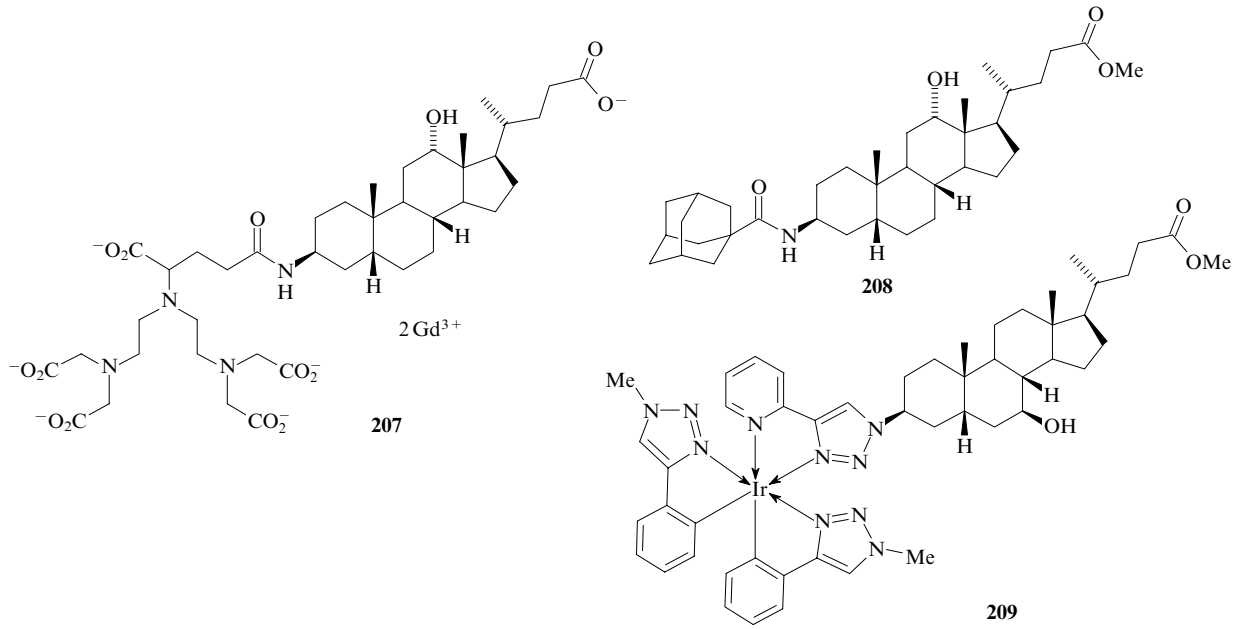
(*a*) MsCl, Et_3N, CH_2Cl_2, 0 °C; (*b*) NaN_3, DMPU, 50 °C, 5 days; (*c*) NBS, PPh_3, THF, −18 °C → rt, 8 h; (*d*) NaN_3, DMPU, rt, 4 days; DMPU is 1,3-dimethyl-3,4,5,6-tetrahydropyrimidin-2(1*H*)-one

Cholic acid derivatives containing guanidine and aromatic amine moieties (*e.g.*, compound **204**) were employed as auxiliary reagents for the enantioselective extraction of chiral carboxylates (*N*-acetyl-α-amino acids) from aqueous solution into an organic medium; the enantiomeric excess was up to 80%.[154, 155] Deoxycholic acid derivatives **205a**,**b** containing aromatic amine moieties and amide groups were used as chiral additives to silica gel with the goal of preparing chiral stationary phases for chromatographic separation of racemic mixtures.[156] Conjugates of a 3β-azido derivative of CA with saturated fatty acids (compounds **206a** – **d**) were synthesized. Biological activity assays demonstrated that these compounds are potential agents that prevent cholesterol crystallization in bile[158] or inhibit the *in vivo* development of atherosclerosis.[159] Bile acid derivatives can serve as ligands for the complex formation with metal atoms, for example with Gd^{III} (compound **207**). These complexes are promising contrast agents for magnetic resonance imaging.[157, 160] Deoxycholic acid derivative **208** containing the adamantyl moiety was employed for stabilization of nanoparticles for the design of bioavailable pharmaceutical formulations.[42] Luminescent iridium(III)

Structures 200 – 206



Structures 207 – 209

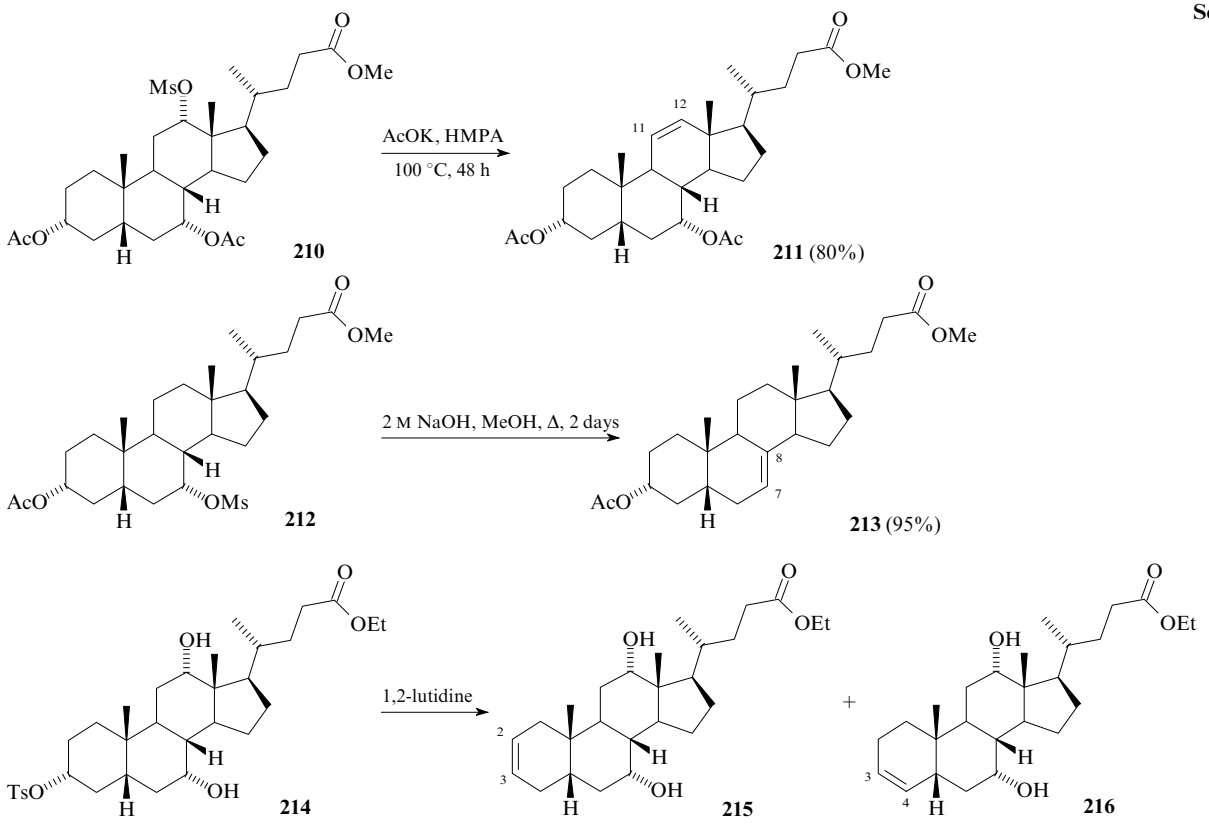


207 **208** **209**

complexes (*e.g.*, compound **209**) were synthesized using modified UDCA.[161]

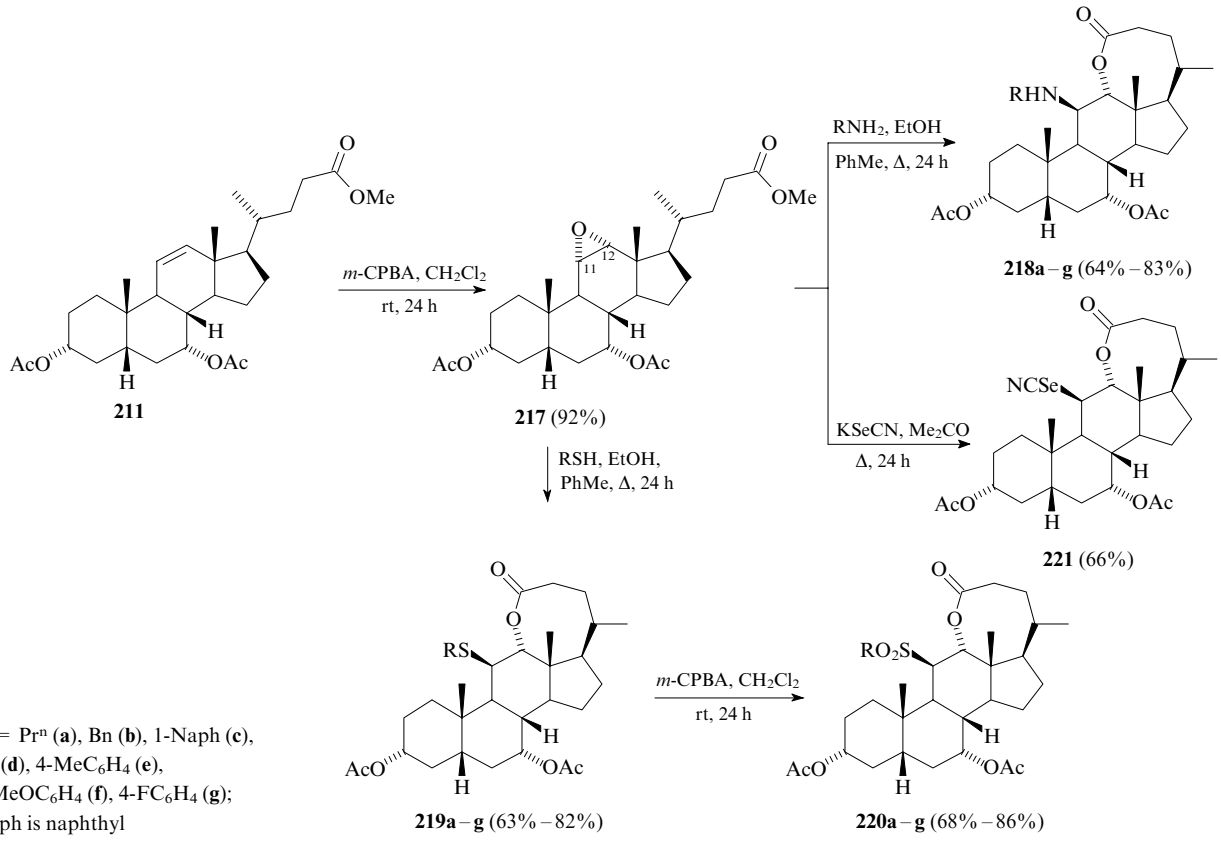
The double bond formation is another type of modifications of the steroid nucleus of BAs. Double bonds are formed in elimination reactions of mesyl and tosyl derivatives of BAs on heating with alkalis or organic bases. Scheme 34 shows examples of such reactions giving rise to unsaturated bonds in rings A, B and C of various BA derivatives. The heating of CA 12-mesylate **210** with potassium acetate in HMPA for 48 h produces compound **211** containing the C(11)=C(12) double bond.[162, 163] The heating of CDCA 7-mesylate **212** under reflux in a 2 M NaOH solution in MeOH affords compound **213** containing the C(7)=C(8) double bond.[135] The treatment of CA 3-tosylate **214** with 1,2-lutidine gives a mixture of compounds **215** and **216** containing C(2)=C(3) and C(3)=C(4) bonds, respectively.[164]

Scheme 34



210 **211** (80%) **212** **213** (95%) **214** **215** **216**

Scheme 35



R = Pr^n (**a**), Bn (**b**), 1-Naph (**c**), Ph (**d**), 4-MeC_6H_4 (**e**), 4-$MeOC_6H_4$ (**f**), 4-FC_6H_4 (**g**); Naph is naphthyl

Multiple bonds of the steroid nucleus can be further modified. For instance, the reaction of compound **211** with *m*-CPBA in CH_2Cl_2 at room temperature produced 11α,12α-epoxide **217** (Scheme 35).[162, 163] The subsequent epoxide ring opening with primary aliphatic and aromatic amines and thiols, as well as with KSeCN, afforded the corresponding products with a nitrogen-, sulfur- or selenium-containing functional group at the C(11) atom. In each case, the epoxide ring opening was accompanied by intramolecular lactonization between the side chain ester group and the hydroxyl group at position 12 to give compounds **218a–g**, **219a–g**. Despite the unusualness of the transformation, the product yields varied from 63% to 86%. Sulfur-containing derivatives **219a–g** were subsequently oxidized with *m*-CPBA to sulfones **220a–g**. Selenium derivative **221** was synthesized from epoxide **217**.

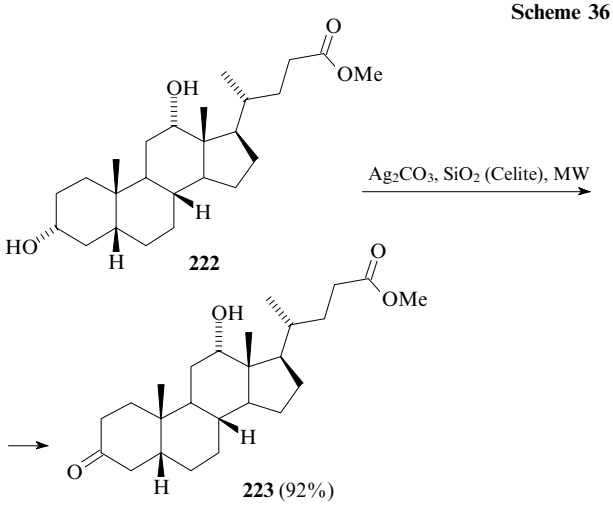
In conclusion, approaches to modifications of the steroid nucleus of BAs based on nucleophilic substitution reactions, which proceed with either the retention or inversion of the configuration at the C(3) atom, are surveyed in this section. The elimination of hydroxyl groups activated with mesyl or tosyl chloride, resulting in the formation of multiple bonds in the BA backbone, and subsequent transformations of such products are considered.

V.4. Oxidation of hydroxyl groups

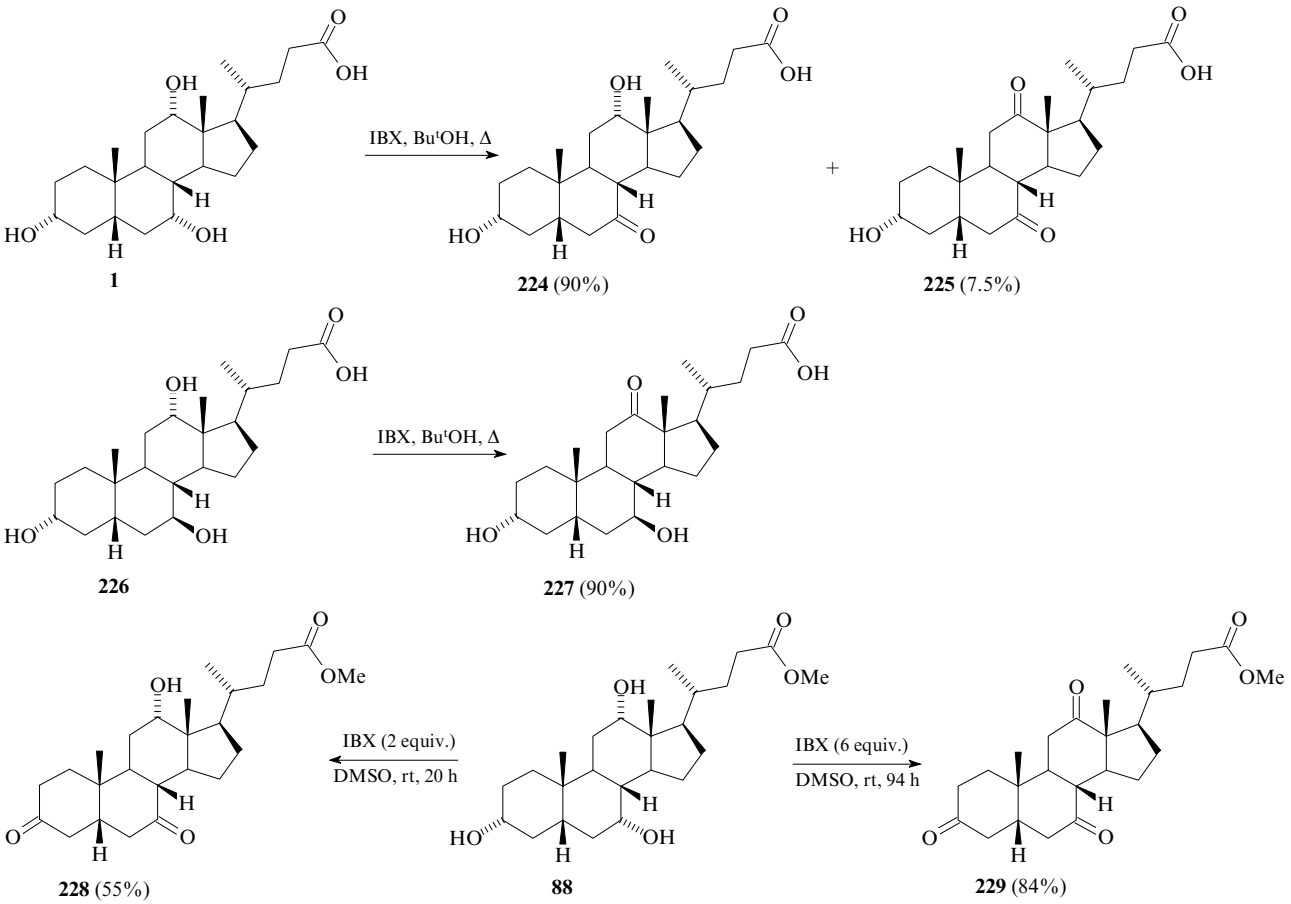
The present section deals with oxidation reactions of hydroxyl groups of the steroid nucleus of BAs to carbonyl functions. This modification has a great potential for further chemical transformations, in particular, for reduction of carbonyl groups to hydroxyl functions (in the α or β configuration), the synthesis of compounds containing α-substituted and α,β-unsubstituted carbonyl moieties in the steroid nucleus, as well as other reactions of carbonyl groups, including reactions with different nucleophiles or oxidation with peroxy acids and hydrogen peroxide.

As mentioned above, BA hydroxyl groups have different reactivity determined by the structural features of the steroid nucleus. Mono, di and trioxo BA derivatives can be synthesized by combining different methods for the protection and oxidation of hydroxyl groups and their sequences. All BA hydroxyl groups can be oxidized with Jones reagent,[132, 165–167] Sarett–Collins reagent (CrO_3–Py in CH_2Cl_2),[57] pyridinium chlorochromate,[168–172] $K_2Cr_2O_7$ in acetic acid,[156] 2-iodobenzoic acid (IBX) in DMSO,[61] $Ca(ClO)_2$ in AcOH.[173] Selective oxidation reactions of individual hydroxyl groups without preliminary protection

Scheme 36



Scheme 37



1 **224** (90%) **225** (7.5%)

226 **227** (90%)

228 (55%) **88** **229** (84%)

of other OH groups are of particular interest. The only selective method for the oxidation of the 3-OH group of BAs is based on the reaction with Ag_2CO_3 on SiO_2 (Celite).[104, 105, 174] For example, the reaction of ester **222** produced ketone **223** (Scheme 36).

The 7-OH group can be selectively oxidized by several procedures. Thus, Sarett – Collins reagent at low temperature,[57] NBS in aqueous[168, 169] or aqueous-organic media[164] and pyridinium chlorochromate on SiO_2 in $CHCl_3$ (see Ref. 175) selectively oxidize only this BA OH group. 2-Iodoxybenzoic acid is also employed in oxidation reactions of BAs. Either the selective oxidation of the OH group at position of 7 or oxidation of several hydroxyl groups takes place depending on the reaction conditions (solvent, temperature, amount of the oxidant). For instance, the oxidation of CA with 2-iodoxybenzoic acid under reflux in Bu^{t}OH gave 3,12-dihydroxy-7-oxo derivative **224** as the major product. 3-Hydroxy-7,12-dioxo derivative **225** is formed as a by-product in a yield of < 7.5% (Scheme 37).[176] It should be noted that IBX selectively oxidizes only the 7α-OH group, while the hydroxyl group in the β configuration remains intact. This is demonstrated in relation to the reaction of 7β-epimer of CA **226** with IBX, which gives product **227** containing only the 12-oxo group (see Scheme 37).[176] The oxidation of CA methyl ester **88** with 2 equiv. of IBX in DMSO at room temperature for 20 h affords compound **228**, in which the 3- and 7-OH groups are oxidized. An increase in the excess of IBX to 6 equiv. and an increase in the reaction time to 4 days resulted in the oxidation of all hydroxyl groups to form dehydrocholic acid methyl ester **229**.[61]

Apart from conventional chemical methods employed for oxidation of hydroxyl groups, the electrochemical oxidation can also be used to modify BAs. For example, the electrochemical oxidation of CA with different anodes[177] produced dehydro-CA, 7-oxo and 7,12-dioxo derivatives of CA. In the past decade, enzymatic redox systems have gained in popularity. These systems are employed for the selective oxidation of 7-OH and(or) 12-OH groups to carbonyl functions.[14, 178 – 181] These transformations are performed using living organisms under aerobic conditions or employing enzymes isolated from these organisms. Advantages of these methods are selectivity, mild conditions and environmental friendliness. Besides, these methods can be easily scaled up to gram amounts.

Consequently, the oxidation of individual hydroxyl groups of the steroid nucleus of BAs can be performed by chemical, electrochemical or enzymatic procedures.

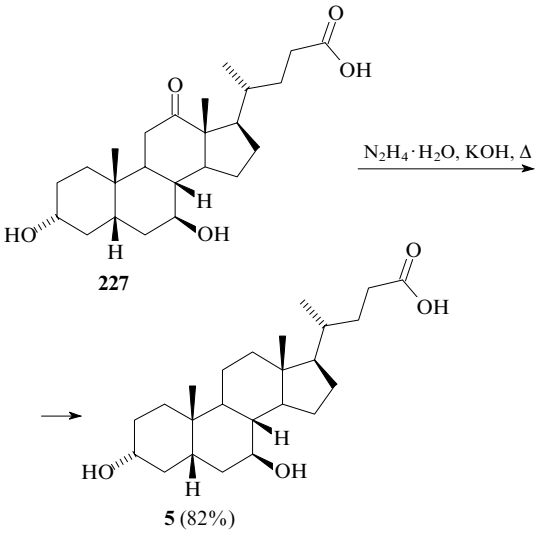
V.5. Reduction of carbonyl groups

Carbonyl groups of oxo derivatives of BAs are reduced to methylene or hydroxyl groups in the α or β configuration.

One of approaches to the synthesis of BAs containing one or two hydroxyl groups is based on the Wolff – Kishner reduction.[14, 176, 180] For instance, UDCA (**5**) was synthesized by the reduction of the 12-oxo group of compound **227** (Scheme 38).[176]

Carbonyl functions of BAs are reduced to α-OH groups by the treatment with sodium borohydride[61] or alkali metal organoboranes (selectrides).[138] For instance, dehydro-CA methyl ester **229** reacts with sodium borohydride in methanol to form 12-oxo derivative **230** (Scheme 39). The

Scheme 38

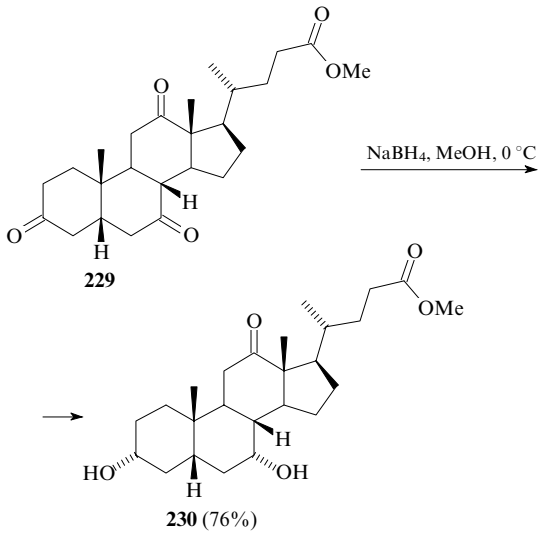


reduction can be performed regioselectively due to different reactivity of carbonyl groups.[61]

The reduction of carbonyl groups of the BA backbone to hydroxyl functions accompanied by a change in the configuration is suitable for the preparation of difficultly accessible derivatives or epimers of available BAs. For instance, the epirimerization of the 7α-OH group is performed through oxidation and subsequent reduction to 7β-OH with alkali metals in alcohol — under reflux with Na in n-butanol,[14,180] n-propanol (Scheme 40; compound **226**)[176] or with K in *tert*-amyl alcohol.[130] Enzymatic redox systems containing nicotinamide adenine dinucleotide (NAD^+/NADH), as well as 7α- or 7β-hydroxysteroid dehydrogenases (HSDH) (an example is the synthesis of compound **231** shown in Scheme 40) are used for this purpose.[14,178–181]

Therefore, diverse reducing systems combined with different reactivity of carbonyl groups of BA derivatives make it possible to perform regio- and stereospecific reduction of oxo groups to methylene or hydroxyl groups.

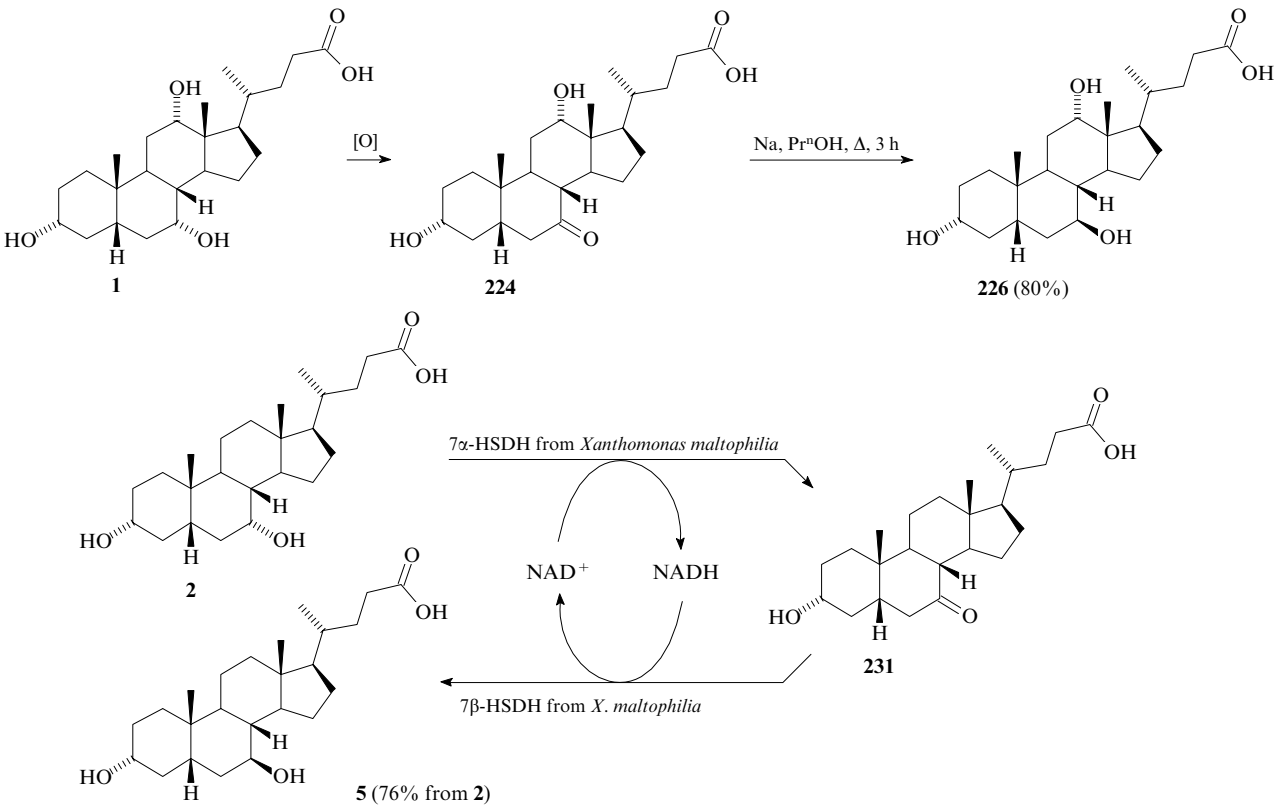
Scheme 39



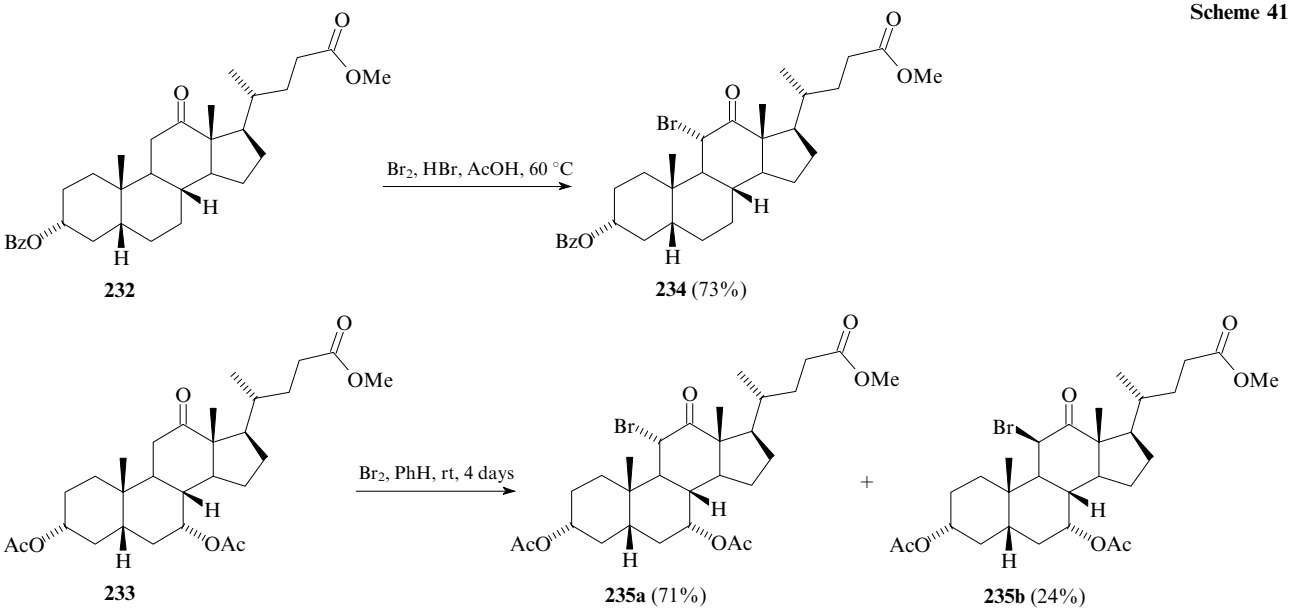
V.6. Synthesis of α-substituted carbonyl derivatives of bile acids

Due to the tautomeric equilibrium between the enol and keto forms of carbonyl groups, oxo derivatives of BAs have

Scheme 40

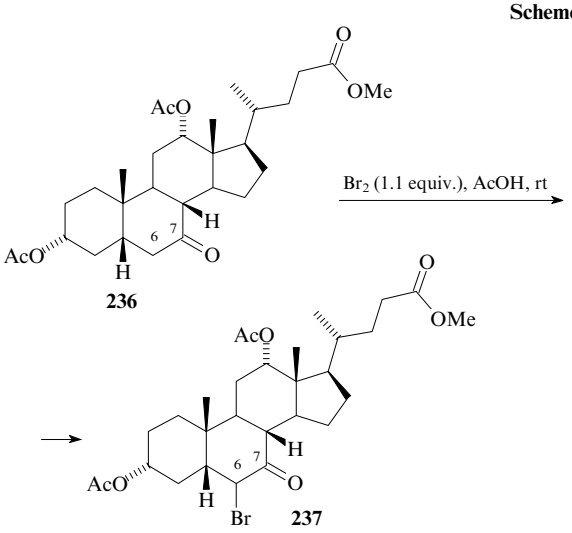


Scheme 41



232 **234** (73%) **233** **235a** (71%) **235b** (24%)

Scheme 42



236 **237**

found wide application in modifications of the steroid nucleus with other functional groups, such as halogen atoms, alkyl groups and so on.

α-Bromo oxo derivatives of BAs are key intermediates in the synthesis of α-substituted carbonyl derivatives containing N_3 or OH groups or the F atom.

The 11-bromo-12-oxo derivative of bile acid was prepared by the reaction of compound **232** with bromine in acetic acid in the presence of catalytic amounts of HBr on heating [182] or by the reaction of compound **233** with bromine in benzene at room temperature (Scheme 41).[183] It should be noted that Harburn *et al.*[182] reported only the isolation of 11α-bromo derivative **234** in 73% yield but did not mention the 11β-epimer, while Salunke *et al.*[183] described the formation of a mixture of 11α- and 11β-bromo derivatives **235a**,**b** in 71% and 24% yields, respectively, from compound **233**.

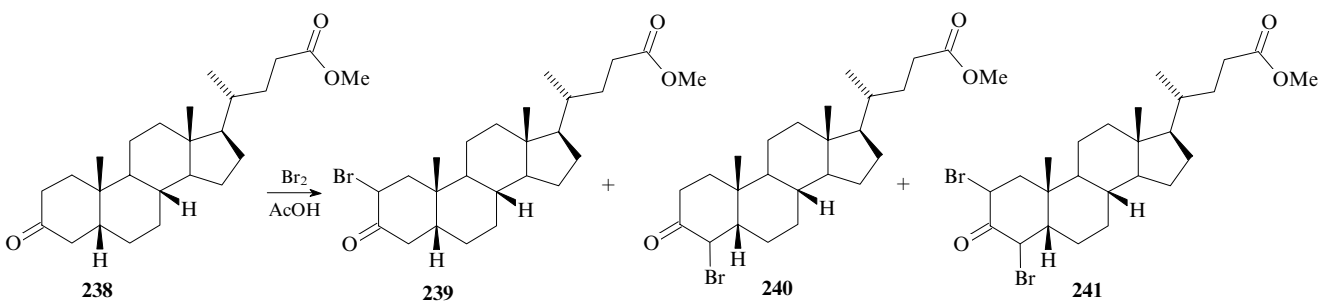
The reaction of 7-oxo BA derivative **236** with bromine gives 6-bromide **237** (Scheme 42).[164]

Unlike 7- and 12-oxo derivatives, 3-oxo derivatives of BAs have two reactive α-positions with respect to the carbonyl group (2 and 4). The bromination of compound **238** with bromine in acetic acid affords a mixture of three products containing bromine atoms at positions 2 (**239**), 4 (**240**) and 2,4 (**241**), with 4-bromo derivative **240** being the major product (Scheme 43).[167, 184]

N-Bromosuccinimide (NBS) is also employed as a brominating agent in BA modifications. For instance, the reaction of LCA with NBS in dioxane [185, 186] produces compound **242** containing the 4β-bromo-3-oxo moiety (Scheme 44).

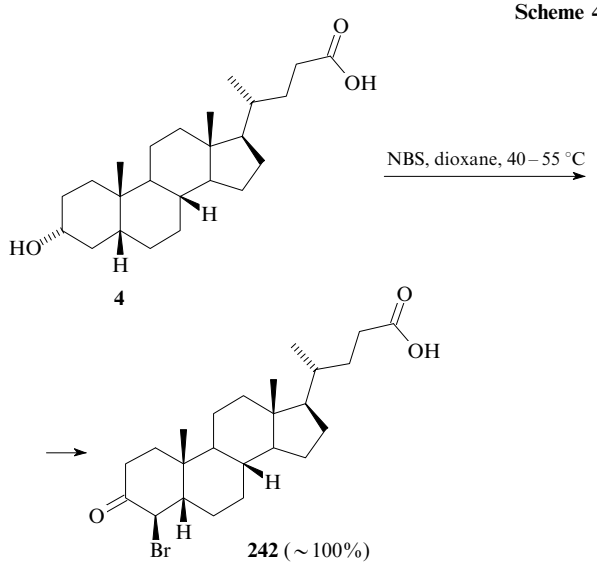
Let us consider examples of using bromo derivatives of BAs in organic synthesis. Many biologically active compounds of the steroid series are functionalized at the C(11) atoms. Hence, derivatives containing different functional groups at position 11 were synthesized based on BAs. For example, an attempt was made to synthesize 11-azido-12-oxo derivative of DCA **243** by the nucleophilic substitution

Scheme 43



238 **239** **240** **241**

Scheme 44

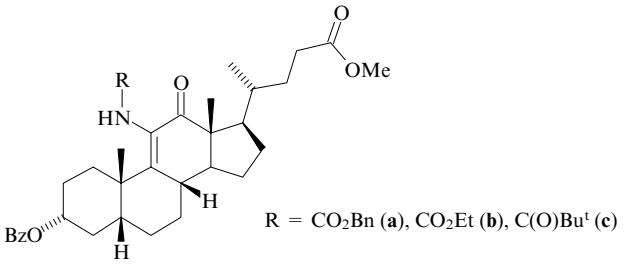


of bromine in compound **234** with the azido group with the aim of searching for inhibitors of HIV-1 protease.[182] The reaction was performed with NaN_3 in DMSO by heating to 100 °C. However, the authors failed to prepare expected product **243** containing the 11-azido-12-oxo moiety; instead, the reaction produced enamino ketone **244** (Scheme 45).

Compound **244** and its amino group-substituted derivatives **245a–c** were tested for the inhibitory activity against HIV-1 protease in infected cells 111B C8166, and the half-maximal effective concentration (EC_{50}) was evaluated at 20×10^{-6}, 8×10^{-6}, 20×10^{-6} and 200×10^{-6} mol litre^{-1}, respectively.[182]

More recently,[183] 11α-bromo- and 11β-bromo-12-oxo derivatives of CA **235a**,**b** were subjected to nucleophilic substitution at lower temperature than that used in the study cited above[182] and prepared compounds **246a**,**b** and **247a** containing 11-azido-12-oxo- and 11-amino-12-oxo moieties in ring C, as well as enamino ketone **248** (Scheme 46).

Structures 245



245a–c

11β-Azide **246b** was prepared by the reaction of 11α-bromo derivative **235a** with 1.2 equiv. of NaN_3 in DMSO at 60 °C. 11α-Azido derivative **246a** was synthesized in several ways: from 11β-bromo derivative **235b** and a fivefold excess of NaN_3 in DMSO at room temperature or from 11α-bromide **235a** and the same reagents in DMF at a temperature elevated to 60 °C. The latter transformation is accompanied by the double inversion of the configuration, as evidenced by the reaction of 11β-azide **246b** with 1.2 equiv. of NaN_3 in DMSO at 60 °C or with 1.2 equiv. of potassium acetate in DMF that gives 11α-azide **246a**.

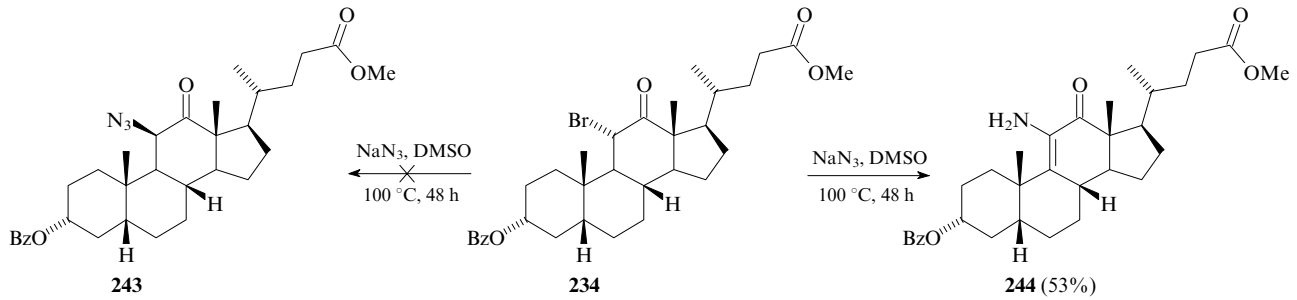
The reduction of 11α-azide **246a** with hydrogen on palladium catalyst produces compound **247a** containing the 11α-amino group. 11β-Azide **246b** is not reduced to amine **247b** under these conditions (only the acetate groups or side-chain ester groups are reduced). Meanwhile, 11β-azide **246b** is reduced with triphenylphosphine in a THF–H_2O mixture to 11α-amine **247a**. Heating of 11α-azide **246a** with a tenfold excess of NaN_3 in DMF to 100 °C gave enamino ketone **248**. Biological activity of these compounds was not assessed.[183]

11-Azido-12-oxo derivatives **246a**,**b** were reduced with $NaBH_4$ in the presence of cobalt chloride and a phase-transfer catalyst to 11-amino-12-hydroxy derivatives **249a**,**b** (see Scheme 46).[132] The assessment of biological activity of compounds **244–249** in the cells CEM-GFP infected with the virus HIV-1 NL4-3 demonstrated that these compounds do not have a marked effect or induce virus replication instead of inhibition.

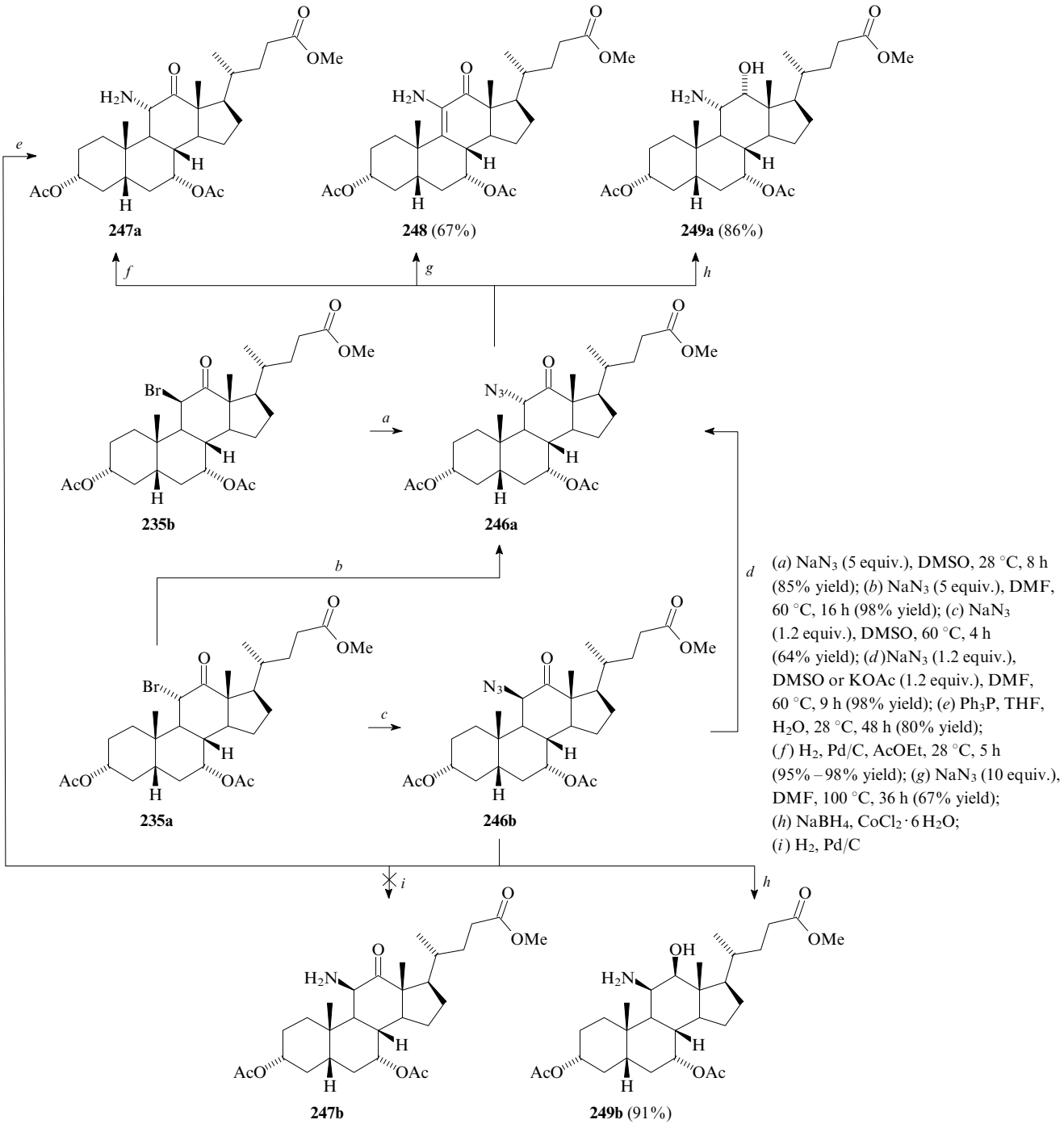
The modification of 11-azido and 11-amino derivatives of BAs can give rise to biologically active compounds. For example, it was shown[187] that 11-azido- and 11-amino-functionalized derivatives generated from compounds **246a** and **247a** exhibit antituberculosis activity against *Mycobacterium tuberculosis* H37Ra. Compounds **250–253** proved to be most active: IC_{50} were 0.64×10^{-6}, 1.08×10^{-6}, 3.62×10^{-6} and 2.70×10^{-6} g ml^{-1}, respectively.

Another type of transformations of α-bromo oxo derivatives of BAs is based on the replacement of the bromine atom with hydroxyl. This approach is applied to synthesize polyhydroxylated BAs with high polarity (hydrophilicity), which are potential agents for the treatment of different bile duct diseases,[164,188,189] as well as difficultly accessible BAs[190] and their possible metabolites.[191] For example, the replacement of bromine in compound **237** with the hydroxy group followed by the reduction of the 7-oxo group and deprotection of intermediate **254** produced BA **255** in 31% yield (with respect to compound **254**) (Scheme 47).[164]

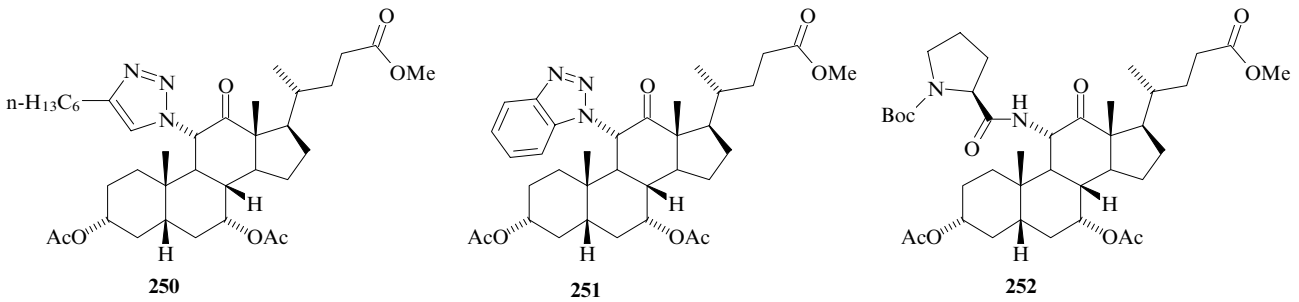
Scheme 45



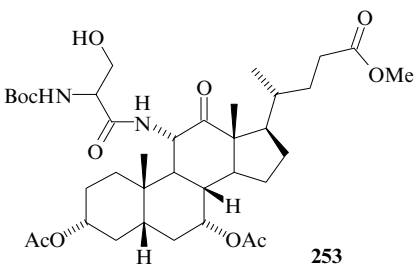
Scheme 46



Structures 250 – 252



Structure 253

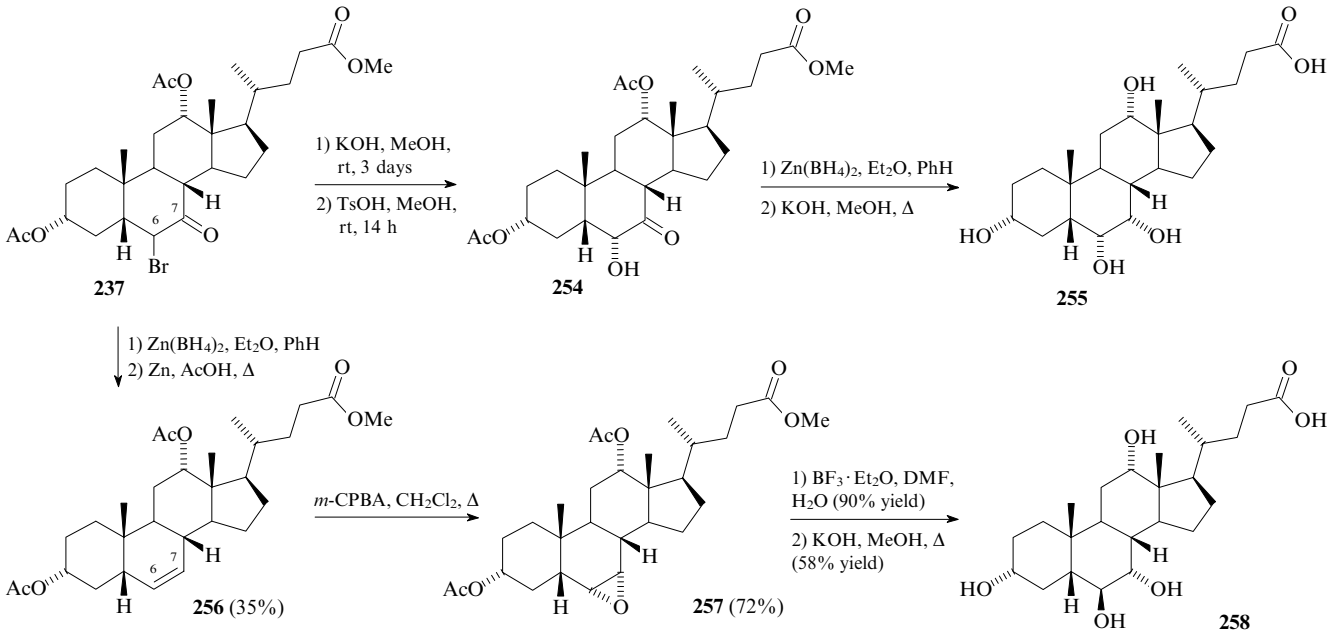


253

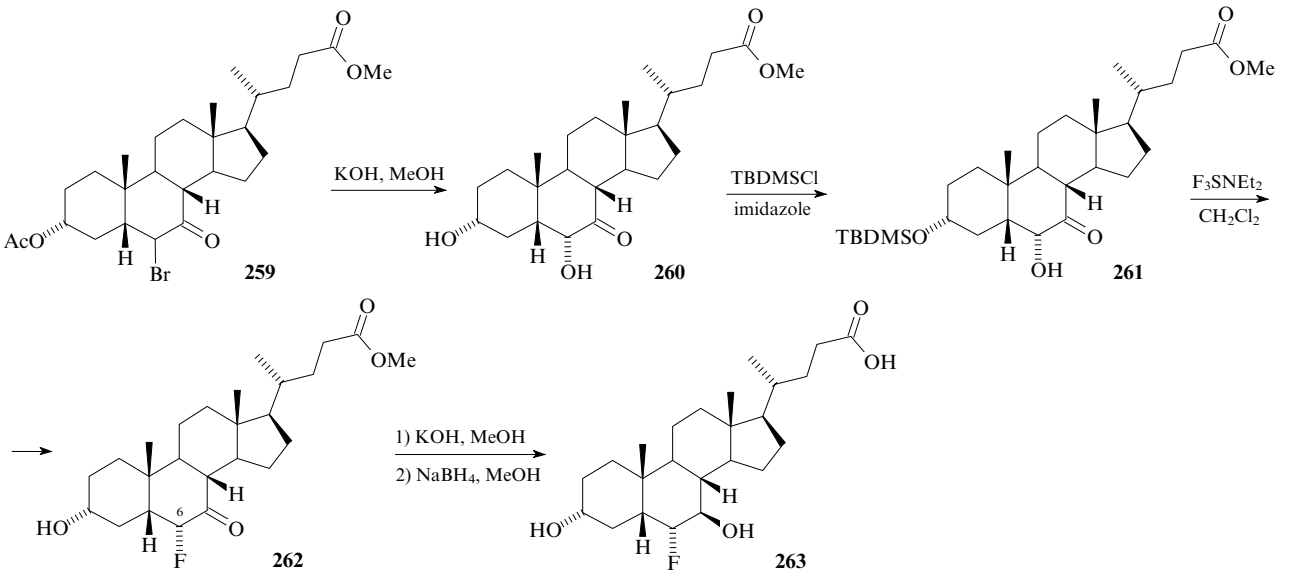
The transformation of the α-bromo-substituted oxo moiety into the double bond is a key step in another approach to the synthesis of polyhydroxylated BAs. Thus, the reduction of 6-bromo-7-oxo derivative of CA **237** with zinc borohydride yielded bromohydrin. The subsequent elimination reaction resulted in the formation of the C(6)═C(7) double bond in the steroid nucleus (compound **256**).[164] The further oxidation of the double bond with *m*-CPBA gave 6α,7α-epoxy derivative **257**. Acid **258** was produced through the epoxide ring opening and deprotection (see Scheme 47). Both these approaches, as well as other transformations, are suitable for the preparation of different isomers of di-, tri- and tetrahydroxylated BAs.[164, 188 – 191]

Bromo derivatives of BAs are also employed in modifications of the steroid nucleus with other halogens, *e.g.*, a fluorine atom. The synthesis of BA fluoride is covered by a patent.[192] The replacement of bromine in compound **259** with the hydroxyl group, the protection of the 3-OH group in intermediate **260** and the subsequent step based on the reaction of compound **261** at the 6α-OH group with diethylaminosulfur trifluoride resulted in the formation of 6α-fluoro derivative **262** (Scheme 48). Acid **263** was synthesized by the hydrolysis of the ester group and reduction of

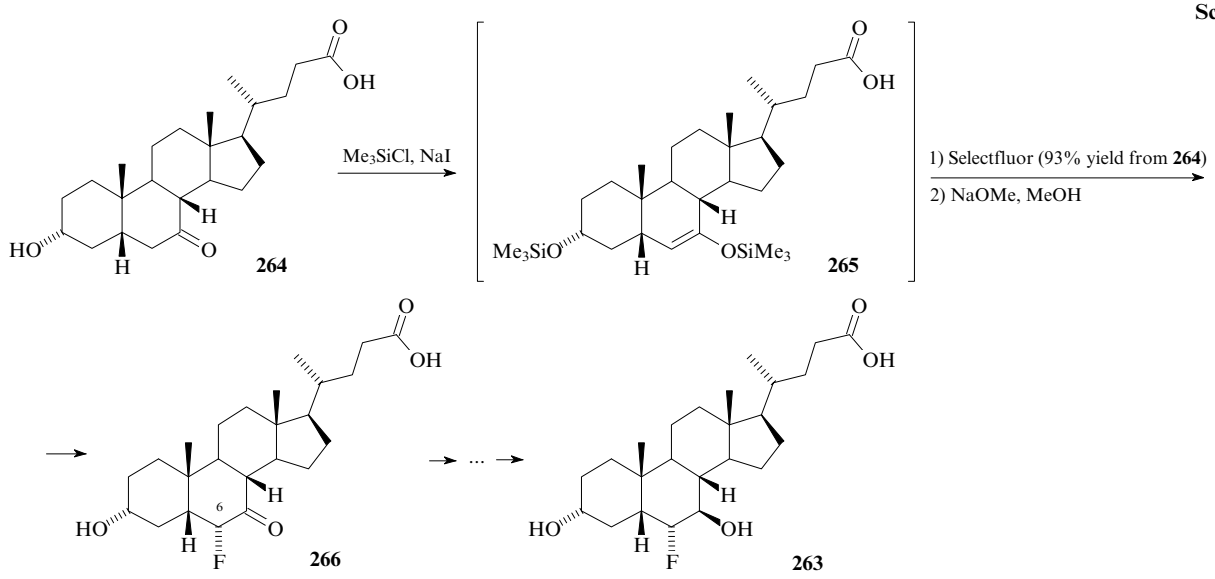
Scheme 47



Scheme 48



Scheme 49



the carbonyl group of compound **262** (the final yield was ~4%).

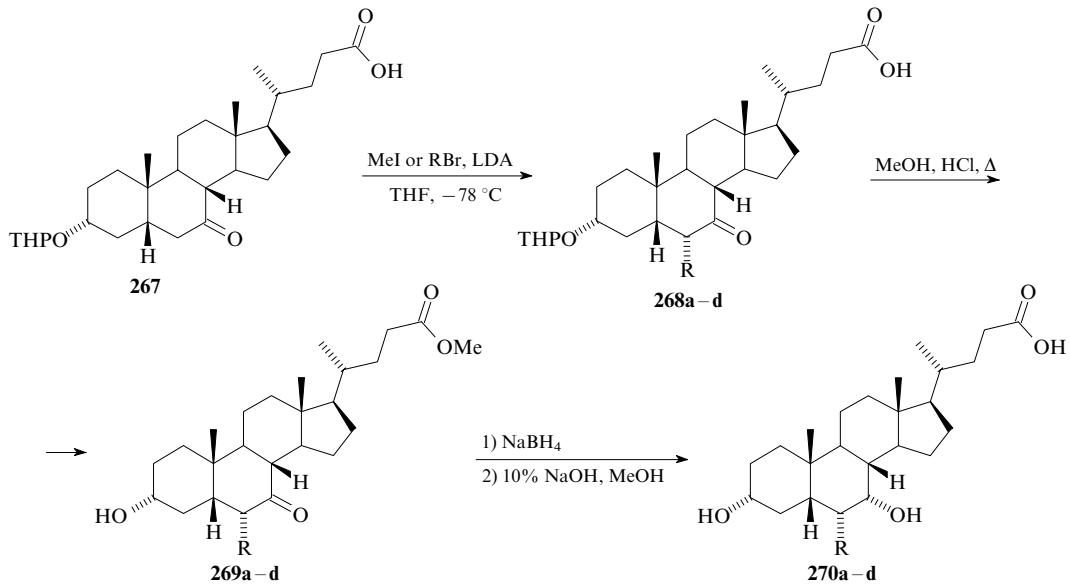
An alternative route to the synthesis of 6-fluoro derivatives of BAs from ketone **264** has been developed[193] (Scheme 49). The key steps are the reaction of silyl enolate **265** with the fluorinating agent Selectfluor and the subsequent reaction with NaOMe in methanol giving 6α-fluoro derivative **266**. Then the 7-oxo group is reduced to 7β-OH, and the sequence of transformations produces fluoro derivative of UDCA **263** in ~36% yield.

Apart from hydroxyl groups and halogen atoms, the BA backbone is modified with alkyl groups. The reaction of oxo derivatives of BAs with alkyl halides in the presence of bases is generally employed for this purpose.[194, 195]

With the aim of finding effective agonists of the nuclear receptor FXR, a research team[194] investigated a series of BAs — DCA, LCA, UDCA and CDCA, the latter acid being the most active one. In the series of CDCA derivatives, the 6-methyl derivative proved to be more active than the initial acid. To reveal the structure–property relationship, CDCA was modified with alkyl groups. Alkyl substituents bulkier than the Me group (ethyl, n-propyl and benzyl) were introduced at position 6 of the steroid nucleus. The alkylation of 7-oxo derivative of CDCA **267** with methyl iodide, alkyl or benzyl bromide in the presence of LDA in THF (Scheme 50) was the key step. It should be noted that the reaction turned out to be strictly stereoselective and produced 6α-alkyl derivatives **268a–d**; however, the yields in these step were low (22%, 12%, 5% and 13% for R = Me, Et, Pr^n and Bn, respectively). The subsequent esterification giving methyl esters **269a–d** and the reduction of the 7-oxo group followed by hydrolysis gave acids **270a–d**.

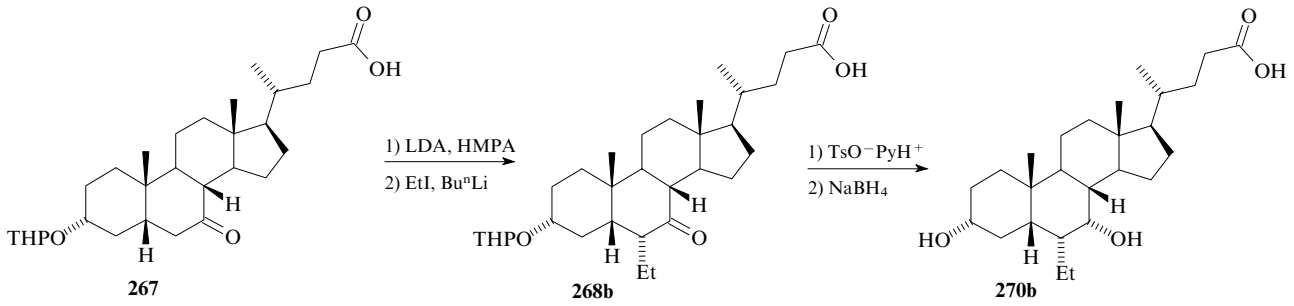
The further study of the ability of these compounds to bind to the receptor FXR demonstrated that 6α-ethylchenodeoxycholic acid (**270b**) (this semisynthetic bile acid was later named obeticholic acid) is the most active agonist of this receptor ($EC_{50} = 0.099 \times 10^{-6}$ mol litre^{-1}; for comparison, $EC_{50} = 8.66 \times 10^{-6}$ mol litre^{-1} for CDCA). Obeticholic acid (**270b**) and CDCA were also tested for *in vivo*

Scheme 50



268–270: R = Me (**a**), Et (**b**), Pr^n (**c**), Bn (**d**)

Scheme 51



267 **268b** **270b**

anti-cholestatic activity. It was found that compound **270b** protects hepatocytes against LCA-induced acute liver necrosis.

More recently, another procedure was developed for the synthesis of 6α-ethylchenodeoxycholic acid (**270b**) (Scheme 51).[195] The employment of several bases (LDA, Bu^{n}Li, HMPA) and ethyl iodide instead of ethyl bromide in the step of alkylation of 7-oxo derivative **267** and the reduction of the number of steps of the synthesis resulted in an increase in the total yield from 2% to 20%.

The discovered ability of 6α-ethylchenodeoxycholic acid (**270b**) to selectively bind to the nuclear receptor FXR and activate the latter has stimulated extensive research on the elucidation of the structure–property relationship for BA derivatives that behave as nuclear receptor agonists. Thus, a library of CDCA derivatives bearing different substituents at position 6 of the steroid nucleus (allyl, 2-hydroxyethyl, propargyl, OH, OMe, F, *etc.*) and a series of side chain-modified analogues of CDCA were synthesized.[196] In current studies aimed at searching for nuclear receptor agonists among BA derivatives, chemical synthesis methods are combined with the preliminary mathematical modelling and quantum chemical calculations, including those taking into account the spatial orientation of substituents (3D QSAR). It was shown that most of biologically active molecules are characterized by the presence of an ethyl substituent at position 6 of the steroid nucleus.[98, 99, 129, 190, 197]

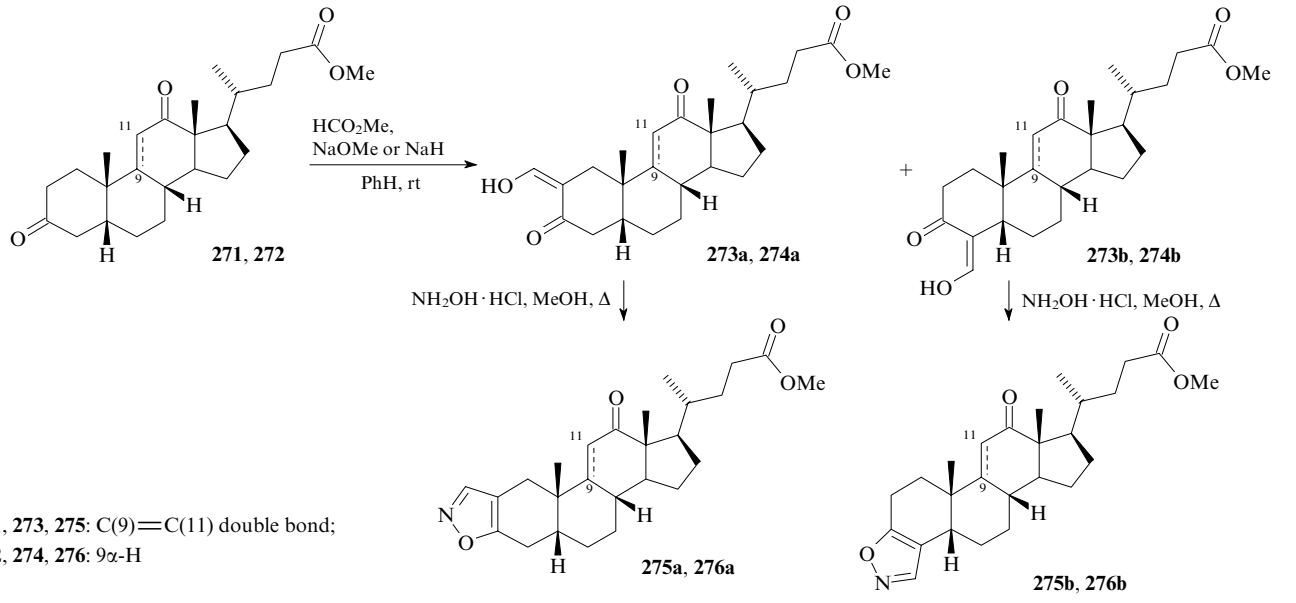
Another important class of reactions of oxo derivatives of BAs existing as keto-enol tautomers involves condensation reactions, in which oxo derivatives of BAs serve as methylene components.

The condensation reactions of 3-oxo derivatives of BAs **271** and **272** bearing two α positions in the steroid nucleus (2 and 4) give compounds functionalized at position 2 as the major products.[167, 198, 199] For example, the reaction of diketon **271** with methyl formate in the presence of sodium methoxide produced a mixture of 2- (**273a**) and 4-hydroxymethylene (**273b**) derivatives in a 10 : 1 ratio in 97% yield (Scheme 52). The similar transformation of compound **272** lacking a double bond in ring C gave products **274a** and **274b** in a 2 : 1 ratio. The use of sodium hydride instead of sodium methoxide resulted in a change in the ratio of the reaction products **274a** and **274b** to 8 : 1 (93% yield).[167]

The subsequent reaction of such 1,3-dicarbonyl derivatives with ambident nucleophiles (*e.g.*, NH_2OH, N_2H_4 and so on) produces pentacyclic BA derivatives containing a heterocyclic moiety. Scheme 52 shows one of possible synthetic routes to BA derivatives containing the isoxazole moiety fused to ring A of the steroid nucleus. The reaction of hydroxymethylene derivatives **273a**,**b** and **274a**,**b** with hydroxylamine hydrochloride in methanol affords isoxazole-containing BAs **275a**,**b** and **276a**,**b** in 94% and 95% yields, respectively.[167]

In general, the synthesis of heterocyclic BA derivatives is accomplished using standard approaches. Most often, the synthesis involves the intermediate formation of compounds

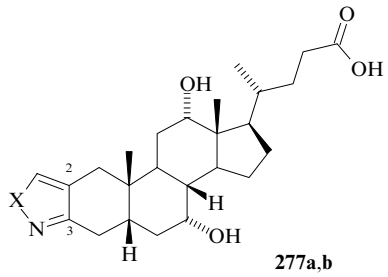
Scheme 52



271, **272** **273a**, **274a** **273b**, **274b** **275a**, **276a** **275b**, **276b**

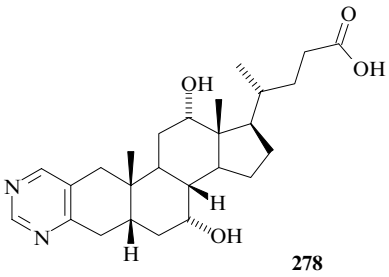
271, **273**, **275**: C(9)=C(11) double bond;
272, **274**, **276**: 9α-H

Structures 277 – 279

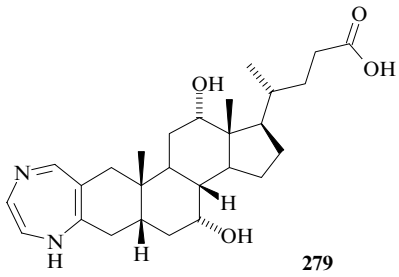


277a,b

X = O (**a**), NH (**b**)



278



279

containing different dicarbonyl or unsaturated carbonyl moieties, which can be transformed into five-, six- or seven-membered heterocycles.[199] A large number of synthetic protocols for the preparation of heterocyclic BA derivatives have been patented.[199] Examples are compounds **277a**,**b**, **278** and **279**.

Bile acid derivatives containing heterocyclic moieties also exhibit biological activity (*e.g.*, cytotoxic or anti-inflammatory effect) and are abile to inhibit protein tyrosine phosphatase 1B (see Refs 167, 184, 200) or serve as carriers for biologically active molecules, thus enhancing their bioavailability.[198, 199]

To sum up, this section deals with bromination reactions of various carbonyl derivatives of BAs and the application of the reaction products in the subsequent synthesis for modifications of the steroid nucleus with functional groups, such as azido and hydroxy groups, and a fluorine atom. The section considers also alkylation reactions of oxo derivatives of BAs at the α position that give compounds with valuable biological properties and condensation reactions producing dicarbonyl derivatives of BAs, which can be used in the synthesis of compounds containing a heterocyclic moiety.

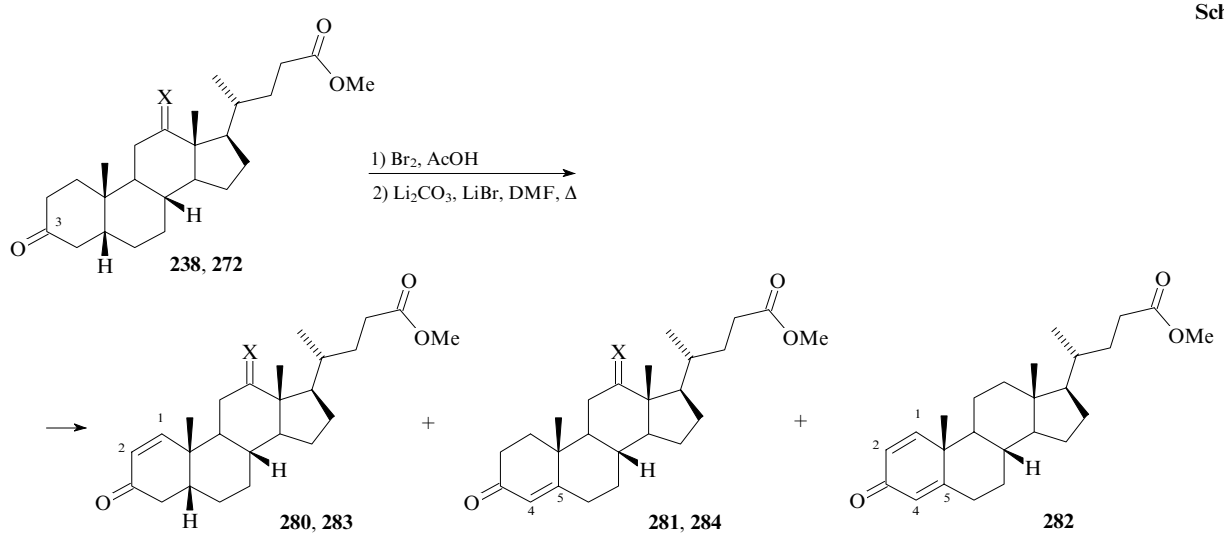
V.7. Synthesis of α,β-unsaturated carbonyl derivatives of bile acids

α,β-Unsaturated carbonyl moieties are important structural units of biologically active steroids, such as corticosteroids and androgenic sex hormones containing the 3-oxo-4(5)-ene moiety in the steroid nucleus. Bile acid derivatives containing an α,β-unsaturated carbonyl moiety are key intermediates in the synthesis of many biologically active compounds of the steroid series.[185, 186, 201 – 203]

Main investigations of the reactivity of oxo derivatives of BAs related to the synthesis of compounds containing α,β-unsaturated carbonyl moieties were performed during the second half of the 20th century. This section is focussed on the currently used methods. One approach to the synthesis of such BAs is based on the formation of a α-bromo-substituted carbonyl derivative followed by its dehydrobromination [165, 167, 184, 185] under reflux with Li_2CO_3 and LiBr in DMF.[167, 184, 185] For example, the bromination of 3-oxo derivative of LCA **238** and subsequent dehydrobromination afford a mixture of compounds **280** – **282** (in a total yield of 48%) containing the 3-oxo-1(2)-ene, 3-oxo-4(5)-ene or 3-oxo-1,4-diene moiety, respectively, with compound **281** being the major product (Scheme 53). Recall that functional groups in rings A and C of the steroid nucleus have different reactivity. Thus, only ring A in 3,12-dioxo derivative of DCA **272** is subjected to bromination followed by dehydrobromination, while ring C remains intact (see compounds **283** and **284** in Scheme 53). The **283** : **284** ratio is 1 : 4.[167]

The main drawback of the synthetic schemes considered above is that they give mixtures of products because 3-oxo derivatives of BAs have two reactive α positions. Therefore, reactions suitable for the synthesis of individual enones without an admixture of other structural isomers are of particular interest for the multiple bond formation in the steroid nucleus. A method for the synthesis of a BA derivative containing the 3-oxo-1(2)-ene moiety from dibromide **285** (Scheme 54) has been patented.[189] This dibromide is a convenient starting compound because it is the only

Scheme 53



238, **272**

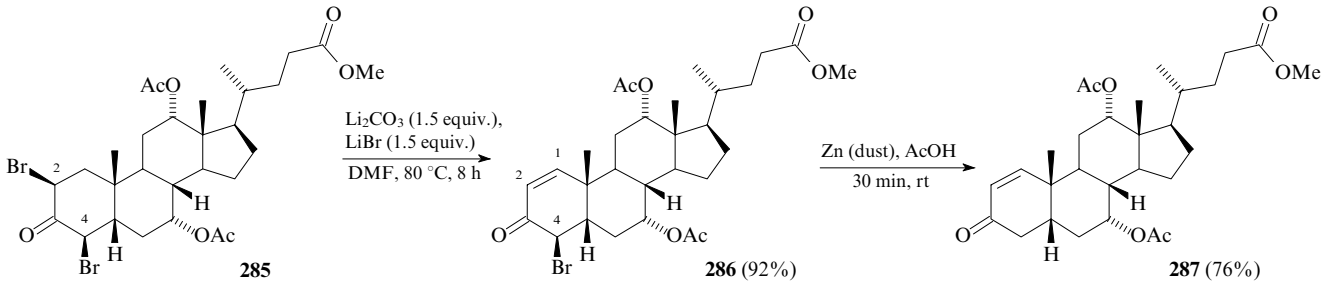
280, **283**

281, **284**

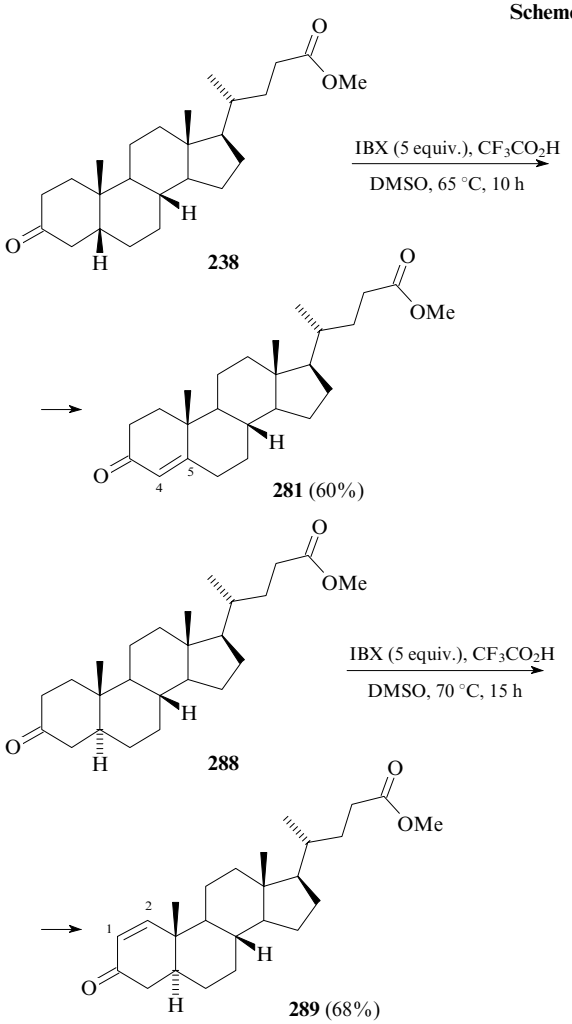
282

X = 2 H (**238**, **280**, **281**), O (**272**, **283**, **284**)

Scheme 54



Scheme 55



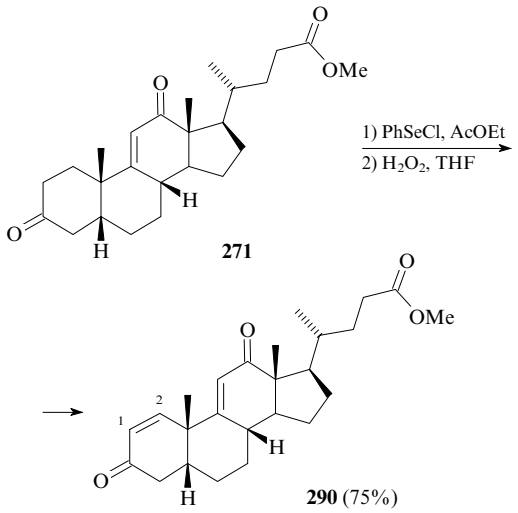
exhaustive bromination product of the corresponding 3-oxo derivative. Upon heating of dibromide **285** with Li_2CO_3 and LiBr in DMF, only the bromine atom at position 2 of the steroid nucleus undergoes dehydrobromination to form derivative **286** containing the 4β-bromo-3-oxo-1(2)-ene moiety. The reduction of compound **286** with zinc dust in acetic acid leads to the elimination of the bromine atom at position 4 to form product **287**.

The reaction of 3-oxo derivatives of BAs with IBX in the presence of catalytic amounts of trifluoroacetic acid in DMSO is another route to the selective introduction of the C(1)=C(2) or C(4)=C(5) double bond into the steroid nucleus of BAs.[201,204] It should be noted that the reaction pathway depends on the structure of BA derivatives, namely, on the fusion mode of rings A and B. Under these reaction conditions, 3-oxo steroids containing *cis*-fused rings A and B (5β-H), which include bile acids and their derivatives, are transformed into compounds containing the 4(5)-double bond (*e.g.*, product **281** in Scheme 55). This reaction with steroids containing *trans*-fused rings A and B, including allocholic acid derivatives, affords products containing the 1(2)-double bond in ring A (*e.g.*, the transformation of compound **288** into product **289**).

Steroidal enones can also be synthesized using selenium-containing reagents. In many cases, the reactions of BA derivatives or other compounds of the steroid series containing the 3-oxo group with Se reagents yield by-products — dienones and trienones.[204] Meanwhile, there are several published examples of the regioselective formation of multiple bonds in BAs using selenium-containing reagents. These reactions produce individual enones without an admixture of their structural isomers.

The reaction of 3,12-dioxo derivative of DCA **271** with PhSeCl in ethyl acetate and the subsequent oxidation of the organoselenium intermediate with hydrogen peroxide accompanied by the elimination of the selenium-containing moiety provides a route to the selective formation of the C(1)=C(2) double bond in the BA backbone (compound **290** in Scheme 56).[167]

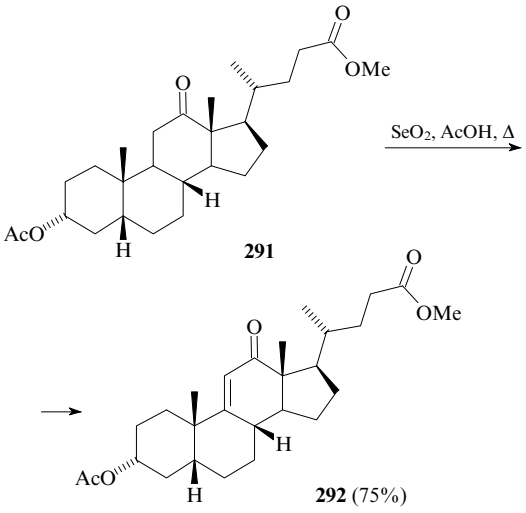
Scheme 56



Functional groups in ring C are less reactive than those in rings A and B. The C(9)=C(11) double bond was introduced into ring C by refluxing 12-oxo derivative of DCA **291** with SeO_2 in acetic acid[167] (compound **292** in Scheme 57).

Bile acid derivatives containing an α,β-unsaturated carbonyl moiety are of interest as both the target compounds and intermediates for subsequent modifications. An impor-

Scheme 57



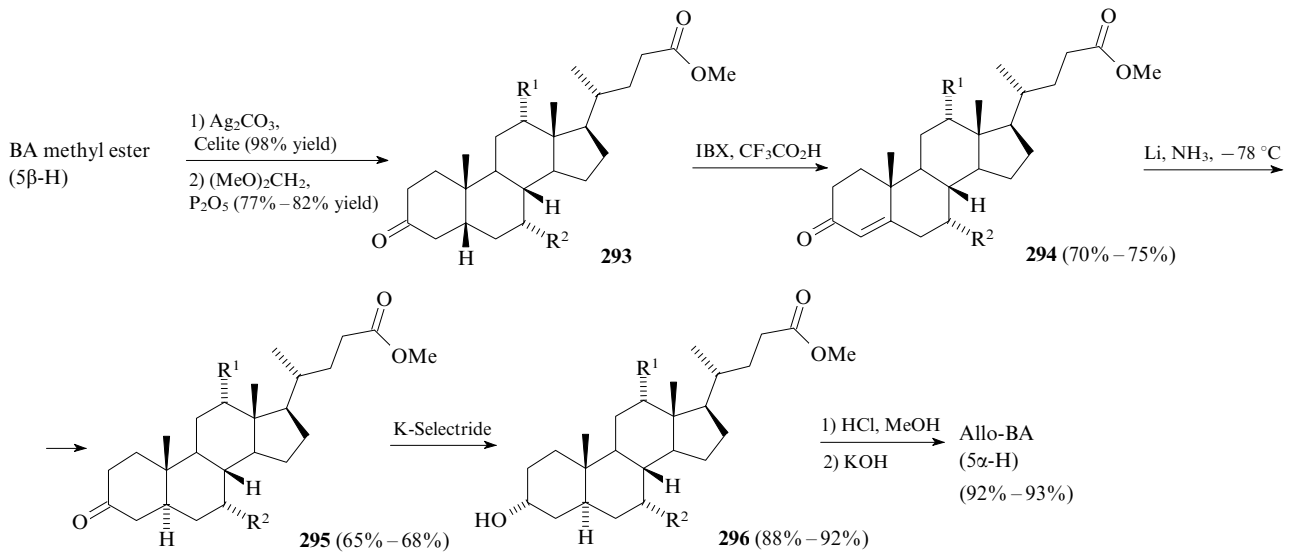
tant type of transformations of 3-oxo-4(5)-ene derivatives of BAs is based on the reduction of the C(4)=C(5) double bond, resulting in the formation of compounds containing *trans*-fused rings A and B in the steroid nucleus. This reaction is a key step in the synthesis of allocholic acids (*trans*-fusion, 5α-H) from BAs (*cis*-fusion, 5β-H) because the natural sources of these acids are rather scarce.[138] Besides, many biologically active steroids are also characterized by the *trans*-fusion of rings A and B.[186,201] The complete synthetic scheme for the preparation of allocholic acids from bile acids is outlined in Scheme 58. The first step affords BA methyl ester followed by the selective oxidation of the 3-OH group to a carbonyl function and the protection of OH groups at positions 7 and(or) 12 if they are present in the starting acid (compound **293**). Then the treatment with IBX in the presence of trifluoroacetic acid produces a derivative containing the C(4)=C(5) double bond (compound **294**). The reduction of the double bond with Li in liquid ammonia at −78 °C yields BA derivative **295** containing *trans*-fused rings A and B. The subsequent selective reduction of the 3-oxo group to 3α-OH with K-Selectride gives compound **296** and the subsequent hydrolysis of protecting and ester groups produces the target 5α-epimer of the starting BA.

Also, 3-oxo-4(5)-ene derivatives of BAs are utilized in the synthesis of lanostane-type compounds. Thus, the reaction of enone **281** with MeI in the presence of Bu^tOK produced *gem*-dimethyl derivative **297** (Scheme 59).[184] The reaction involves the introduction of two methyl groups at position 4 accompanied by the double bond migration. The subsequent condensation of compound **297** with ethyl formate in the presence of a base (MeONa) affords 2-hydroxymethylene-3-oxo derivative **298**, which is further transformed into polycyclic compounds **299a**–**c** and **300a**–**c**. These products contain different five- and six-membered heterocyclic moieties fused to ring A. The yields of compounds **299** were 21% – 66% with respect to intermediate **297**; of a compounds **300**, 40% – 68%.

This modification of the backbone was accomplished in order to prepare structural analogues of naturally occurring pentacyclic triterpenes. Previously, these compounds were found to possess inhibitory activity against protein tyrosine phosphatase 1B (PTP1B). The inhibition of this enzyme is considered as a promising approach to the treatment of type 2 diabetes and obesity. Assays of the inhibitory activity of the synthesized compounds against PTP1 demonstrated that acid **300b** was the most active compound in this series ($IC_{50} = 1.62 \times 10^{-6}$ mol litre^{-1}).

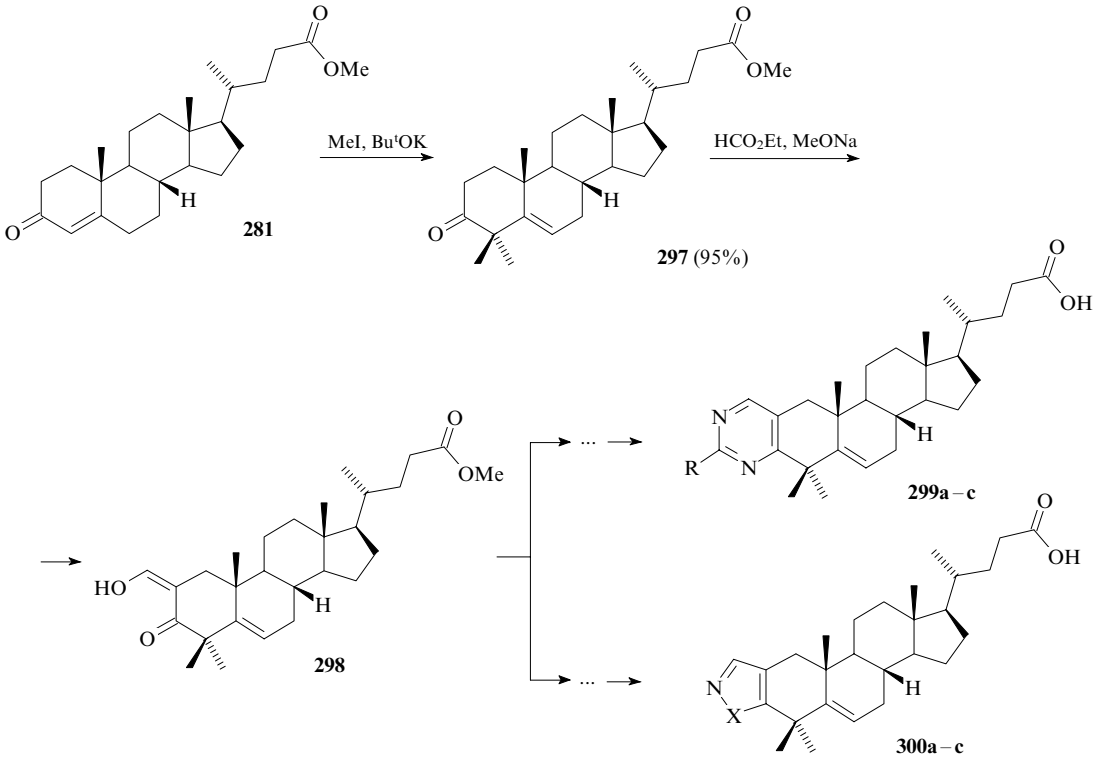
3-Oxo-4(5)-ene derivatives of BAs were also utilized in the synthesis of compounds containing a heteroatom (N or O) at position 4 of the steroid nucleus. The oxidative cleavage of the C(4)=C(5) double bond followed by decarboxylation affords a product containing carboxyl and carbonyl groups (*e.g.*, the transformation of compound **301**, Scheme 60). These products are used for the preparation of derivatives containing the lactam or lactone ring, which, in turn, serve as intermediates in the synthesis of more complex compounds. For example, the oxidative cleavage of the C(4)=C(5) bond in compound **301** and the subsequent treatment of product **302** with liquid ammonia produced amide **303** containing an NH group instead of CH_2 at position 4 of the steroid nucleus (see Scheme 60).[202] Amide **303** served as the starting compound in the synthesis of biologically active steroids **304a**,**b**.

Scheme 58



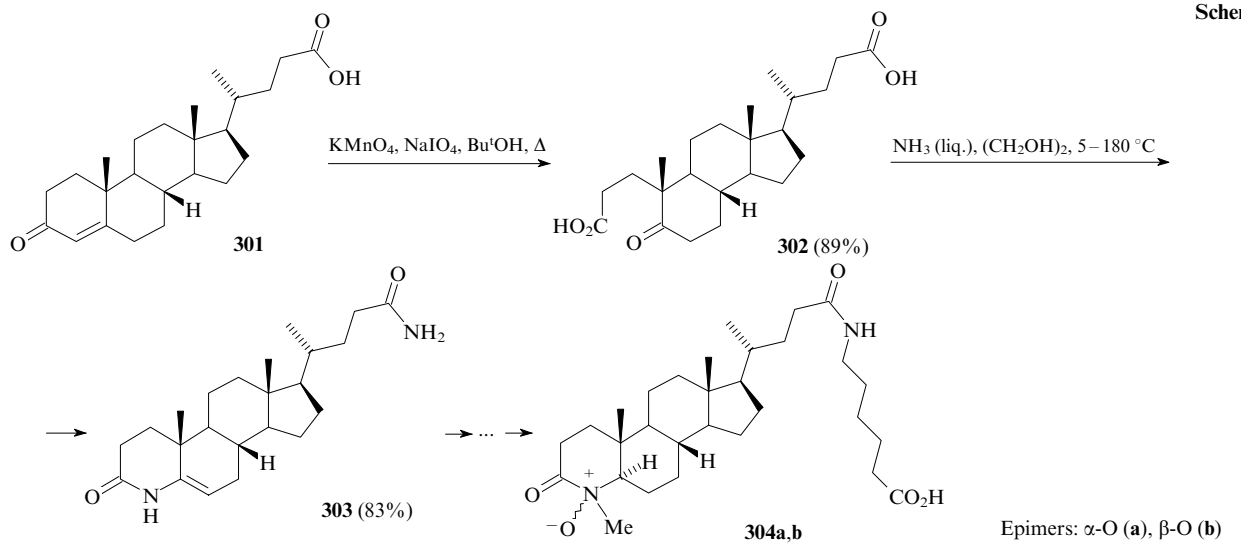
R^1, R^2 = H or OMOM

Scheme 59



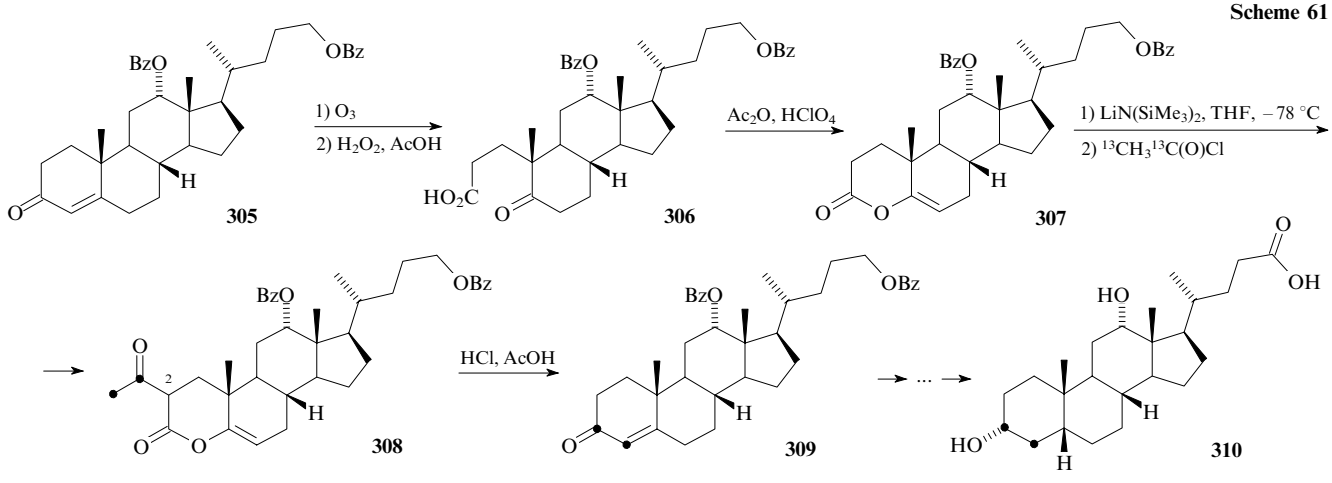
299: R = H (**a**), Me (**b**), NH_2 (**c**); **300**: X = NH (**a**), NPh (**b**), O (**c**)

Scheme 60



Epimers: α-O (**a**), β-O (**b**)

Scheme 61



The formation of the lactone ring in the steroid nucleus was described (Scheme 61).[165] The ozonolysis of compound **305** followed by oxidation yielded acid **306**. The treatment of the latter with acetic anhydride in the presence of $HClO_4$ gave lactone **307** (62% yield with respect to substrate **305**). Bile acid derivatives containing the lactone ring were utilized in the synthesis of ^{13}C isotope-labelled BA analogues (^{13}C atoms are indicated by solid circles in Scheme 61).[165] The acylation of compound **307** at position 2 with ^{13}C isotope-labelled chloroacetic anhydride produced compound **308**. The lactone ring opening and recyclization with acid gave [3,4-$^{13}C_2$]-derivative **309** (58% yield with respect to compound **307**). The latter was transformed into [3,4-$^{13}C_2$]-deoxycholic acid **310** *via* a series of reactions. The [3,4-$^{13}C_2$]-analogues of CA and CDCA were synthesized in a similar way.[165]

To sum up, this section considers procedures for the formation of α,β-unsaturated carbonyl moieties in the BA backbone. Possible fields of application of BA derivatives containing the 3-oxo-4(5)-ene moiety, including the synthesis of allocholic acids, lanostane-type compounds and steroid analogues bearing a heteroatom at position 4, are discussed.

V.8. Nucleophilic addition to the carbonyl group. Oxidation with peroxy acids and peroxides

Oxo derivatives of BAs can be subjected to further chemical transformations involving carbonyl groups, such as the addition of carbon-, oxygen- or nitrogen-centred nucleophiles to the carbonyl carbon atom or oxidation with peroxides or peroxy acids.

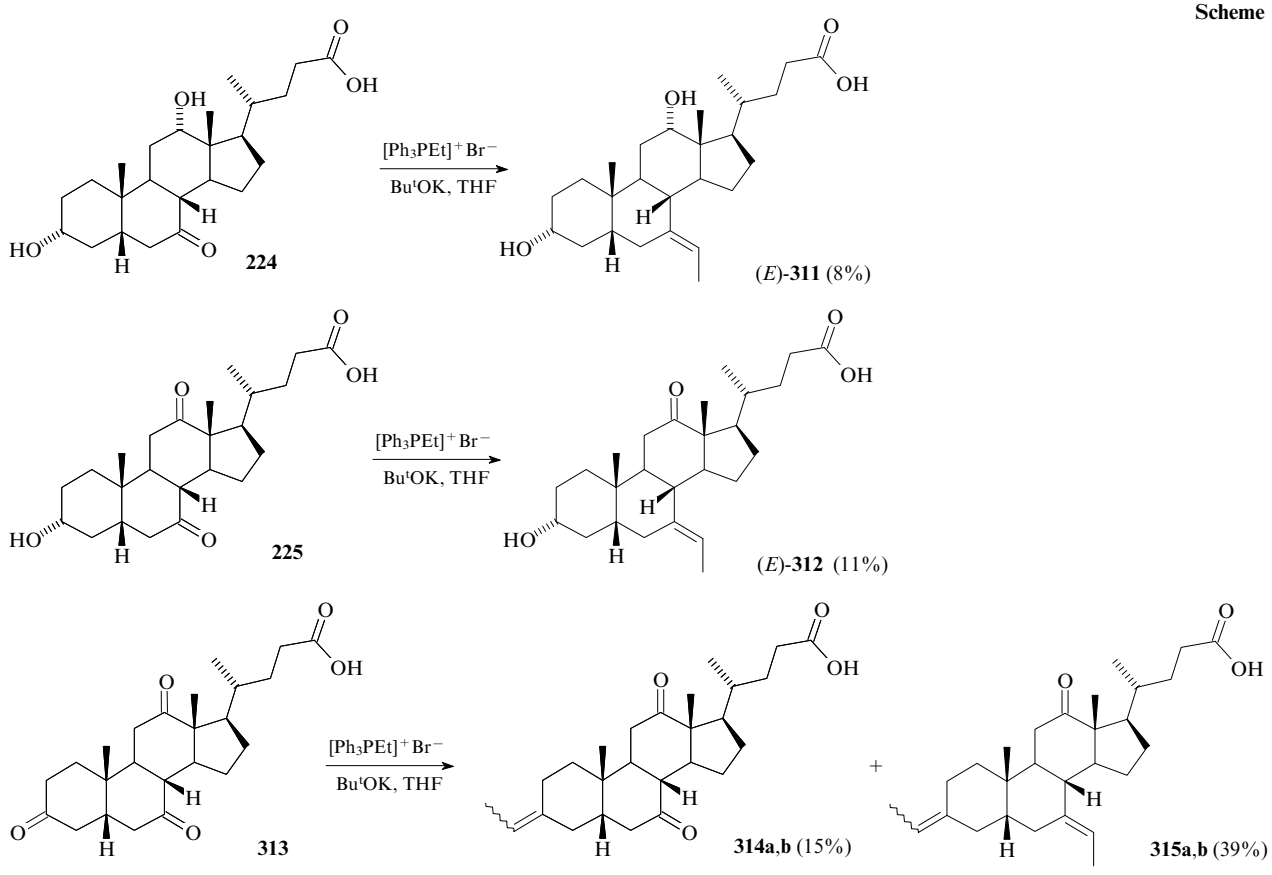
Let us consider Grignard reagents and phosphorus and sulfur ylides as C-nucleophiles. The reaction of carbonyl derivatives of BAs with Grignard reagents can be used to modify the steroid nucleus with alkyl groups at position 3 or 7. The drawback of this reaction is the lack of stereoselectivity, resulting in the formation of mixtures of products containing hydroxyl and alkyl groups in different orientations.[123, 205] Hence, this reaction is of little use in the chemistry of bile acids.

7-Oxo- (**224**) and 7,12-dioxo derivatives of CA (**225**) and dehydro-CA (**313**) were subjected to the Wittig reaction with triphenylethylphosphonium bromide, which was transformed into phosphorus ylide in the presence of Bu^{t}OK in THF (Scheme 62).[206] The reaction with phosphorus ylide at the 7-oxo group gives only *E*-isomers of ethylidene derivatives **311** and **312**. Meanwhile, the reaction with phosphorus ylide at the 3-oxo group was not stereospecific. Thus, the reaction of dehydro-CA (**313**) gave a mixture of mono- (**314a**,**b**) and bis-products (**315a**,**b**) as *E* and *Z* isomers in equal amounts. Under these conditions, the 12-oxo group of compounds **225** and **313** remained intact.

The introduction of ethylidene groups into the CA backbone leads to an increase in the hydrophobicity. The assessment of membranotoxicity of sodium salts of the starting (**224**, **225**, **313**) and resulting acids (**311**, **312**, **314**, **315**) by measuring the haemolytic potential (Lys50 and Lys100 are the concentrations of the compound that cause destruction of 50% and 100% erythrocytes, respectively) demonstrated that these compounds are less toxic (Lys50 varied from 2.95×10^{-3} to 56.00×10^{-3} mol litre^{-1}; Lys100, from 6.10×10^{-3} to 175×10^{-3} mol litre^{-1}) than DCA sodium salt (Lys50 = 2.30×10^{-3} mol litre^{-1}, Lys100 = 5.00×10^{3} mol litre^{-1}).

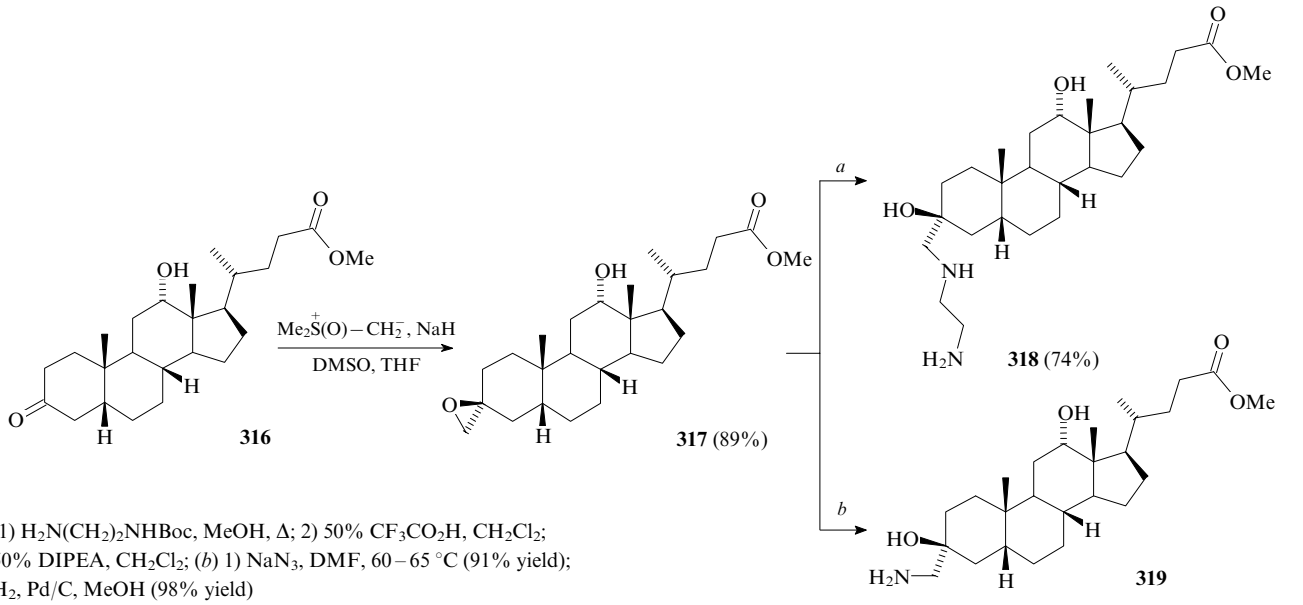
The reaction of oxo derivatives with sulfur ylides gave rise to the epoxide ring, which is a highly reactive structural

Scheme 62



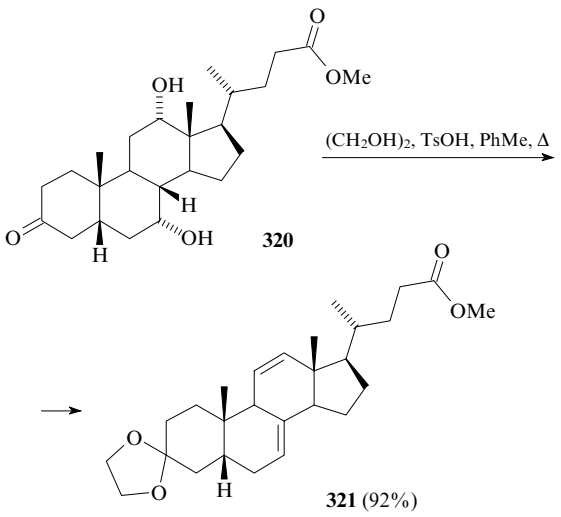
Isomers: *E* (**a**), *Z* (**b**)

Scheme 63



(*a*) 1) $H_2N(CH_2)_2NHBoc$, MeOH, Δ; 2) 50% CF_3CO_2H, CH_2Cl_2; 3) 50% DIPEA, CH_2Cl_2; (*b*) 1) NaN_3, DMF, 60 – 65 °C (91% yield); 2) H_2, Pd/C, MeOH (98% yield)

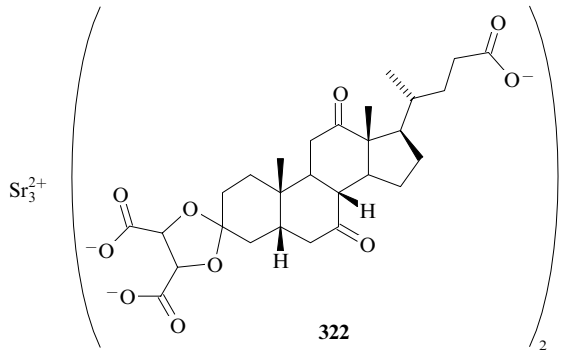
Scheme 64

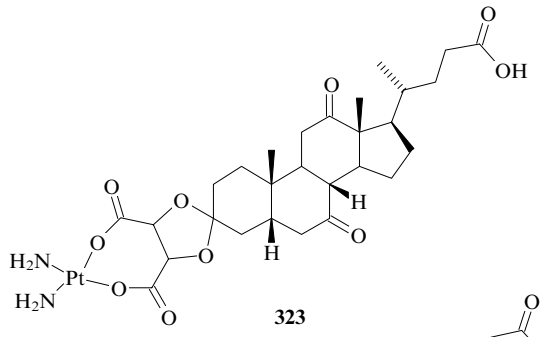


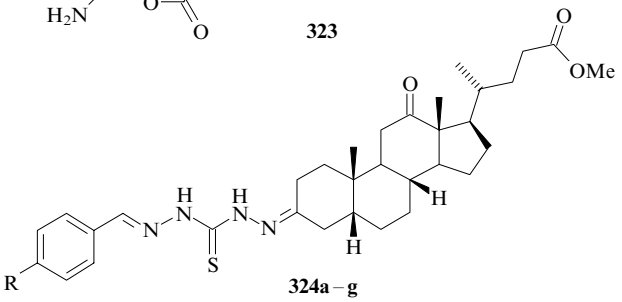
moiety capable of undergoing further transformations. For example, the reaction of 3-oxo derivative of DCA **316** with dimethylsulfoxonium methylide proceeds stereoselectively to form one diastereomer of epoxide **317** (Scheme 63).[174] The subsequent epoxide ring opening with polyfunctional amines (for example, with substituted ethylenediamine) or sodium azide followed by the reduction of the azido group to the amino function gave compounds **318** and **319** containing OH and NH_2 groups. These products were tested for *in vitro* antibacterial and antifungal activity against different cultures. Compound **318** exhibited high antibacterial (IC_{50} against *Staphylococcus aureus* and *Klebsiella pneumoniae* were 5.14×10^{-6} and 5.21×10^{-6} g ml^{-1}) and antifungal activity (IC_{50} against *Sporothrix schenckii*, *Trichophyton mentagrophytes* and *Aspergillus fumigatus* were 3.36×10^{-6}, 6.25×10^{-6} and 8.14×10^{-6} g ml^{-1}, respectively).

Among reactions involving carbonyl groups of oxo derivatives of BAs with O-centred nucleophiles, reactions with polyatomic alcohols and compounds containing several hydroxyl groups are worthy of note. The reactions of oxo derivatives of BAs with polyatomic alcohols giving ketals are applied in multistep synthetic protocols for the protection of carbonyl groups.[104] In some cases, the formation of ketals is accompanied by side processes due to the polyfunctionality of the starting molecule. For example, the reaction of 3-oxo derivative of CA **320** with ethylene glycol

Structures 322 – 324

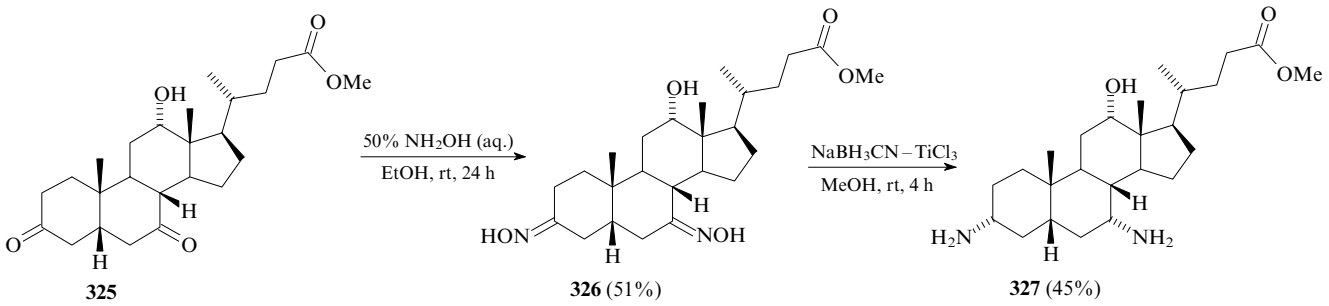






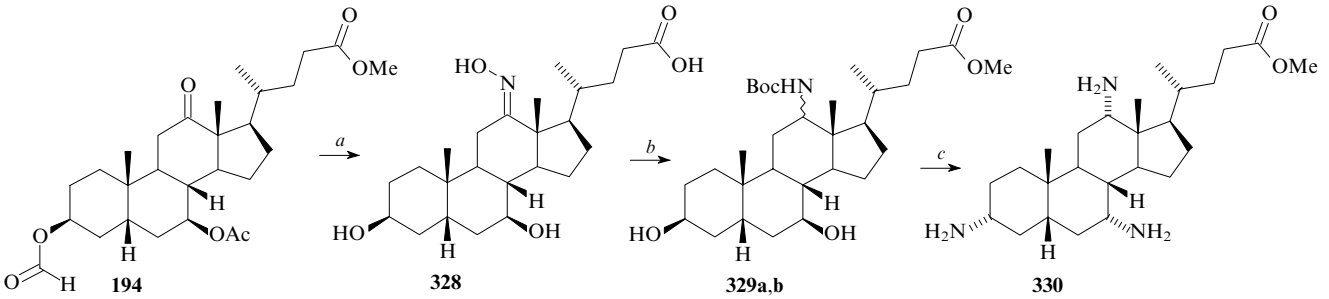
R = H (**a**), Cl (**b**), F (**c**), Br (**d**), OH (**e**), OMe (**f**), NO_2 (**g**)

Scheme 65



325 **326** (51%) **327** (45%)

Scheme 66



194 **328** **329a**,**b** **330**

(*a*) 1) NH_2OH, NaOAc, MeOH; 2) NaOH, MeOH; (*b*) 1) Na, EtOH; 2) Boc_2O; 3) MeI, CsF, DMF; (*c*) 1) MsCl, Et_3N, CH_2Cl_2; 2) NaN_3, DMPU, 80 °C; 3) H_2, PtO_2, Boc_2O; 4) CF_3CO_2H, CH_2Cl_2; epimers: 12-α (**a**), 12-β (**b**)

resulted in the closure of the 1,3-dioxolane ring accompanied by dehydration to form the C(7)=C(8) and C(11)=C(12) double bonds in the steroid nucleus (compound **321**, Scheme 64).[104]

The reaction of oxo derivatives of BAs with tartaric acid is another example of the preparation of ketals for their subsequent application. These reaction products were utilized in the synthesis of the biologically active strontium salt (**322**)[54] and in the complexation with cisplatin (**323**) in order to decrease its cytotoxicity.[44,48]

The reactions of oxo derivatives of BAs with N-nucleophiles produce imines with different structures. For example, a series of bis(thiocarbazones) **324a** – **g** were synthesized from 3,12-dioxo derivative of DCA **272**.[172] Compounds **324a** – **c** were tested for *in vitro* antibacterial activity against *S. aureus*, *Streptococcus pyogenes* and *E. coli* (MIC varied from 32×10^{-6} to 256×10^{-6} g ml^{-1}).

Oximes are an important class of BA derivatives, which are widely used in the synthesis of various nitrogen-containing BAs. Oximes can be synthesized by the reaction of oxo derivatives of BAs with hydroxylamine in methanol or ethanol.[61,166,171,173] The subsequent reduction of these compounds to the corresponding amino derivatives can be performed using several procedures: with sodium under reflux in n-propanol,[6] with the $NaBH_3CN - TiCl_3$ system in methanol[61] or with hydrogen in the presence of platinum catalyst.[173] For instance, the reaction of dioxo derivative **325** produced dioxime **326** and then diamine **327** (Scheme 65).[61] These methods lead to the formation of α-amino groups, thus making it possible to retain the surface amphiphilicity of the molecule.

The reduction of the 12-oxime group is the only way of introducing the amino group at position 12 of the steroid nucleus of BAs.3α,7α,12α-Ttriamine was synthesized using a combination of methods for the reduction of the oxime and azido groups (Scheme 66).[130] The reaction of compound **194** with hydroxylamine followed by alkaline hydrolysis produced acid **328**. Then the reduction of the oxime group with sodium in ethanol to the 12-amino group, the subsequent reaction of the intermediate amine with Boc_2O and esterification of the carboxyl group gave 12α-isomer **329a** as the major product (**329a** : **329b** was 85 : 15, the yield was 32% with respect to compound **194**). The nucleophilic substitution of azido groups for the activated hydroxyl groups at positions 3 and 7 with their reduction to amino groups and the removal of Boc protection yielded the target 3α,7α,12α-triamino analogue of CA — compound **330**.

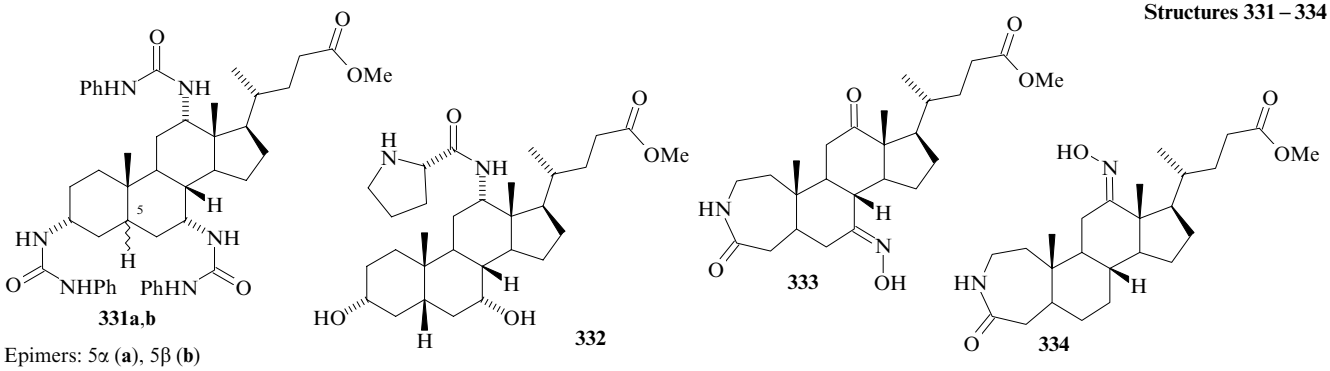
Bile acid derivatives at the amino group are suitable chiral auxiliary agents for the stereoselective synthesis, extraction or chromatographic separation. The binding constants of compounds **331a**,**b** to different anions were evaluated.[207,208] The binding constants of compound **331a** to anions were two to five times higher than the corresponding constants for compound **331b**.

12-(D-Prolinoyl)amino derivative of CA **332** was employed as a catalyst for asymmetric aldol condensation of cyclic ketones and aromatic aldehydes; the enantiomeric excess was up to 98%.[209]

Bile acid oximes are utilized, in particular, in lactam synthesis. In the presence of acidic dehydrating agents (*e.g.*, thionyl chloride[171]), BA oximes undergo the Beckmann rearrangement, resulting in the formation of the seven-membered lactam ring. A series of lactam ring-containing CA and DCA derivatives were synthesized[171] and their cytotoxic activity against different cell lines (MGC 7901, SMMC 7404, HeLa) was assessed. Compounds **333** and **334** exhibited the highest cytotoxic activity against the cell line HeLa ($IC_{50} = 14.3 \times 10^{-6}$ and 24.3×10^{-6} mol $litre^{-1}$) comparable to that of cisplatin ($IC_{50} = 20.6 \times 10^{-6}$ mol $litre^{-1}$).

Another type of modifications of oxo derivatives of BAs is based on oxidation reactions, for example, the Baeyer –

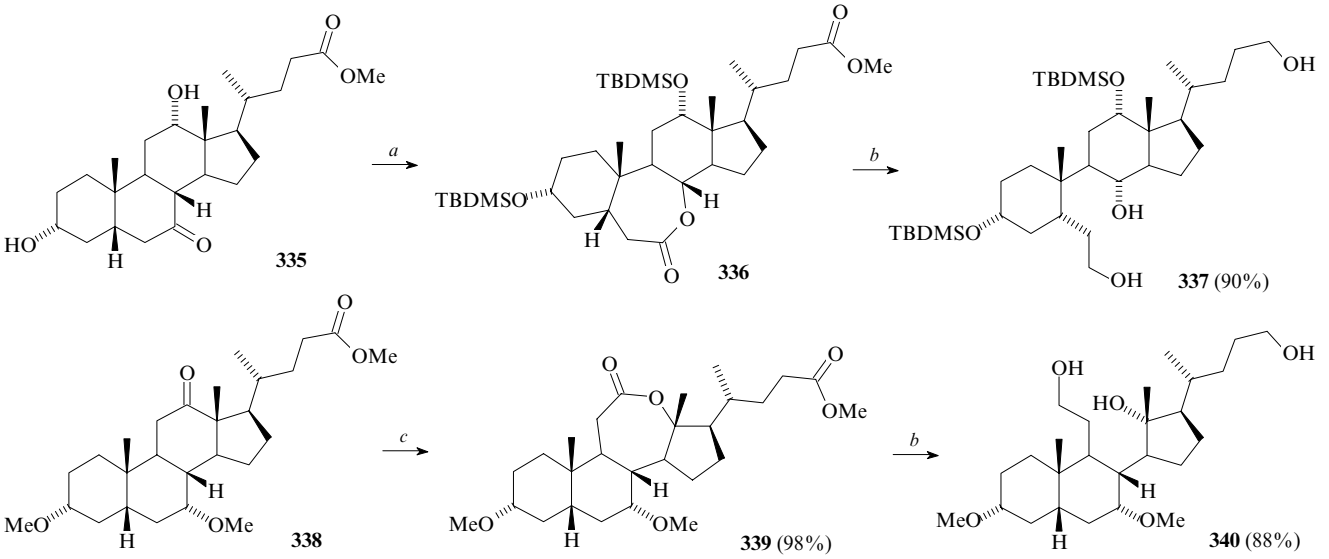
Structures 331 – 334



331a,**b** **332** **333** **334**

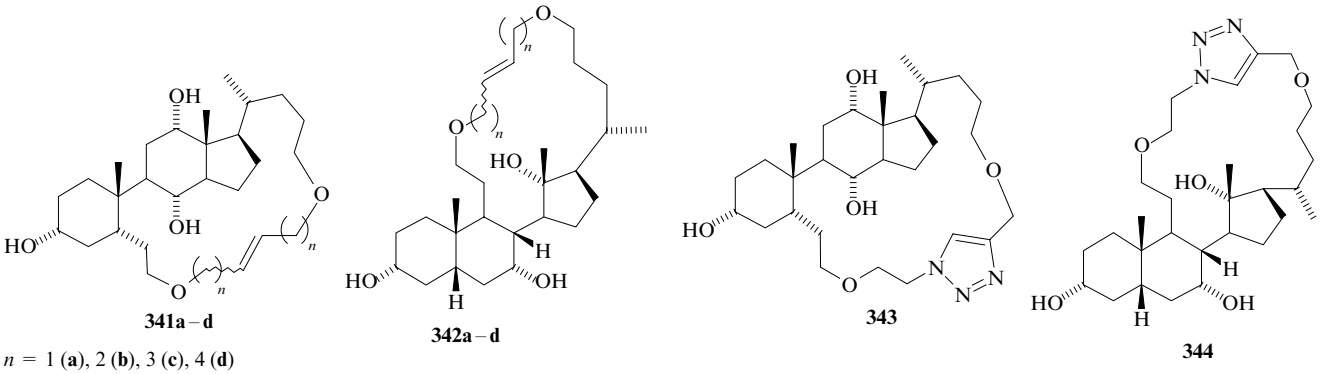
Epimers: 5α (**a**), 5β (**b**)

Scheme 67



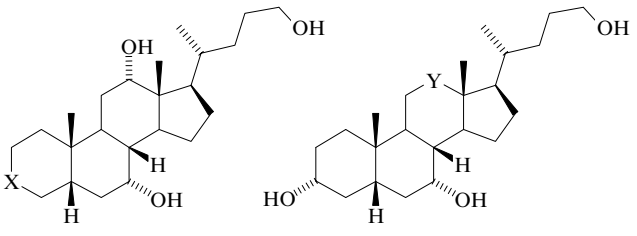
(*a*) 1) *m*-CPBA, TsOH, CH_2Cl_2, rt, 24 h (82% yield); 2) TBDMSCl, DMF, rt, 12 h (91% yield); (*b*) $LiAlH_4$, THF, 0 °C → rt, 12 h;
(*c*) *m*-CPBA, TsOH, CH_2Cl_2, rt, 24 h

Structures 341 – 346



341a – **d** **342a** – **d** **343** **344**

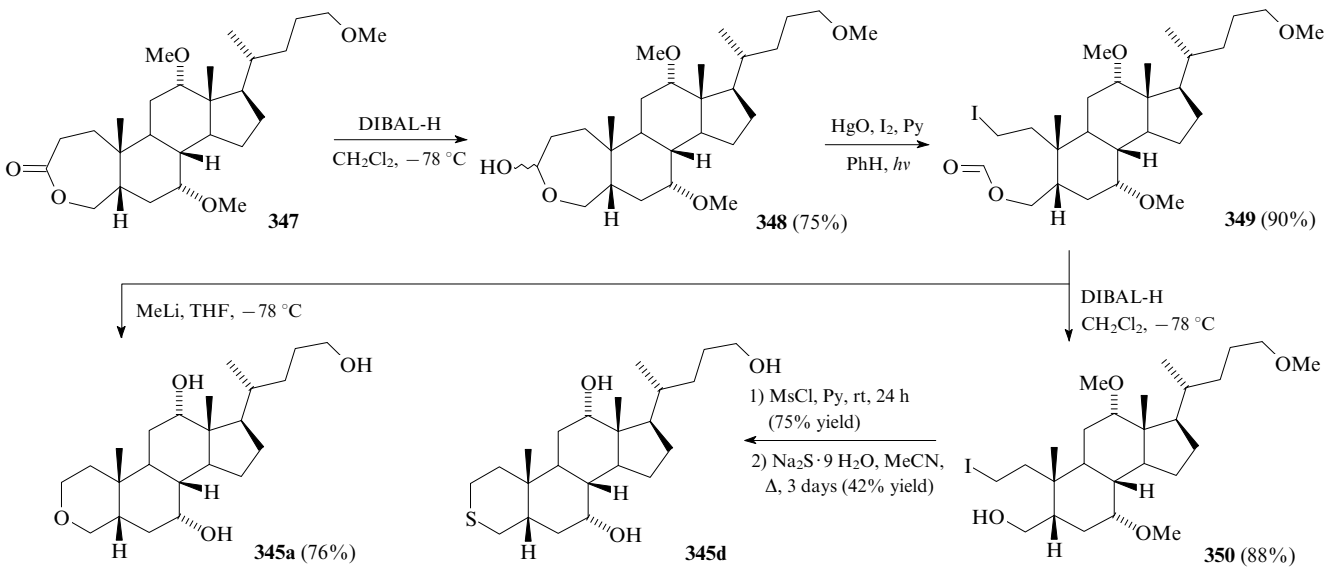
n = 1 (**a**), 2 (**b**), 3 (**c**), 4 (**d**)



345a – **d** **346a** – **e**

X = Y = O (**a**), SO (**b**), SO_2 (**c**), S (**d**); Y = NBn (**e**)

Scheme 68



Villiger oxidation,[104, 105, 168–170, 210] resulting in the ring expansion and the formation of lactone. These lactones are key intermediates in the synthesis of secosteroids and macrocyclic structures based on BAs,[168–170] as well as derivatives containing heteroatoms in the steroid nucleus.[104, 105]

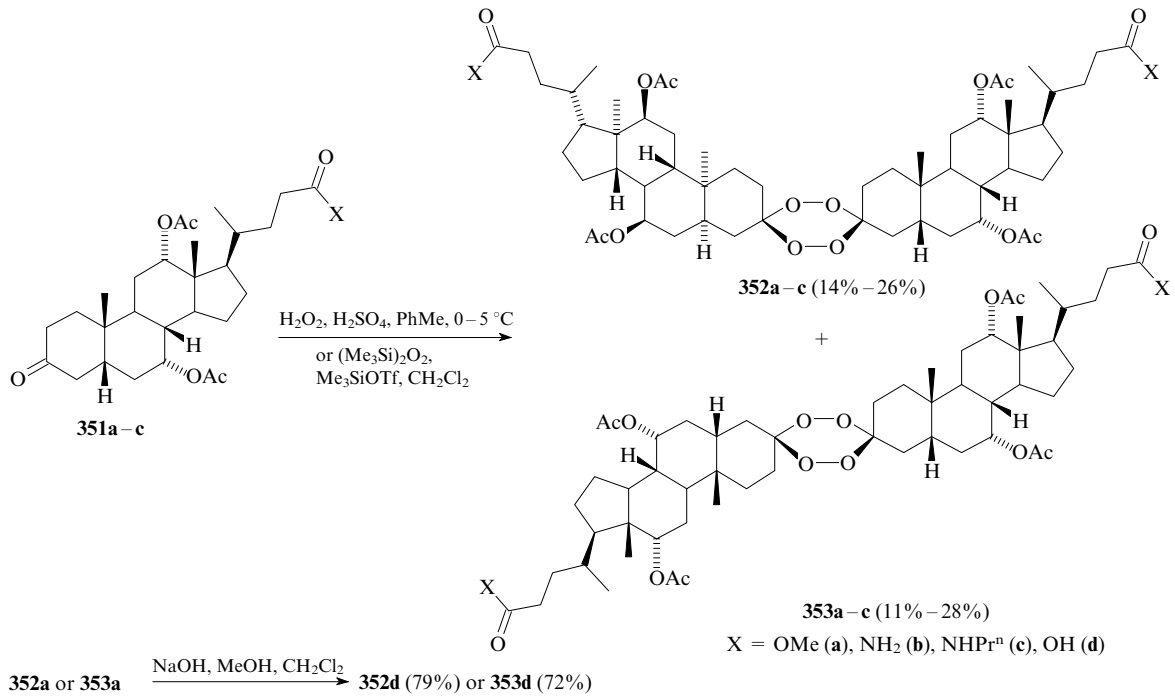
The oxidation of 7-oxo derivative of CA **335** with *m*-CPBA produced lactone **336**, and its reduction with lithium aluminium hydride in THF gave 7,8-secosteroid **337** (Scheme 67).[168–170] Similar reactions were performed for 12-oxo derivative **338**; the reduction of lactone **339** afforded 12,13-secosteroid **340**.

Products **337** and **340** can be employed for further modifications. For instance, the sequence of transformations, including the metathesis or 1,3-cycloaddition reaction, produced macrocyclic compounds **341a–d**, **342a–d**, **343** and **344**.[168–170]

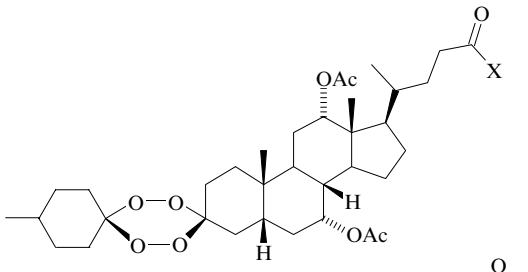
The replacement of one or several methylene groups in the steroid nucleus with heteroatoms (O, N, S) can lead to changes in the native biological activity and(or) the appearance of new biological activity. A synthetic approach comprising the intermediate formation of the lactone derivative of BAs was developed for the introduction of heteroatoms at positions 3 and 12 of the steroid nucleus.[104, 105] This method was applied for the preparation of compounds **345a–d** and **346a–e**.

Scheme 68 shows a sequence of reactions giving compounds **345a**,**d**. The reduction of lactone **347** with diisobutylaluminium hydride (DIBAL-H) affords lactol **348**, and its oxidative cleavage yields key intermediate **349**. The reaction of compound **349** with methyllithium in THF results in the formation of the tetrahydropyran ring to give product **345a** containing an oxygen atom at position 3 of the steroid nucleus. The introduction of a sulfur atom at

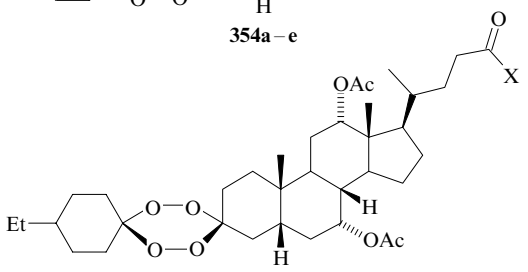
Scheme 69



Structures 354, 355



354a–e



355a–e

X = OH (**a**), NH_2 (**b**), NHMe (**c**), NHEt (**d**), $NHPr^n$ (**e**)

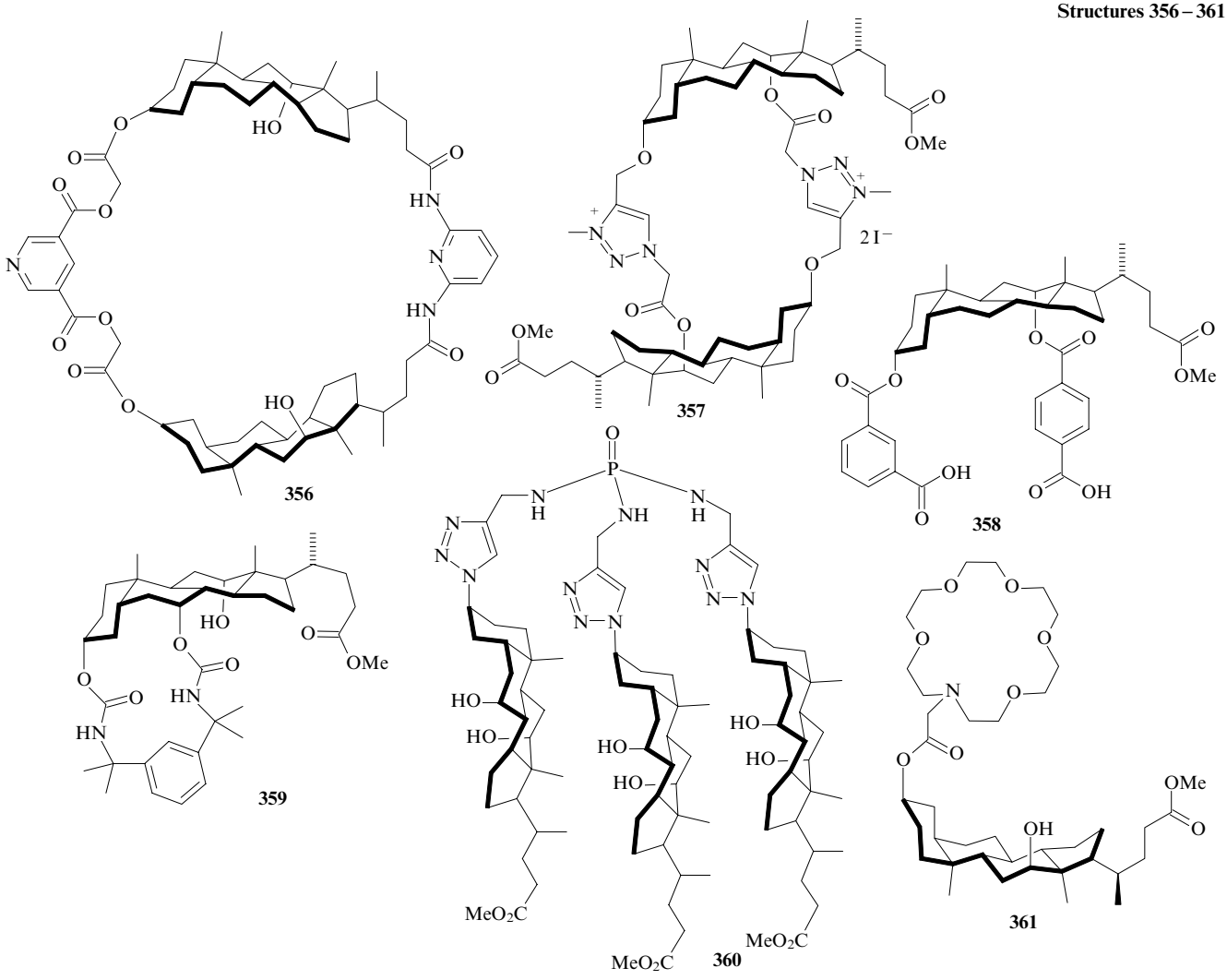
this position is performed through an additional sequence of transformations, including the reduction of the formyloxy group in intermediate **349** to the hydroxyl group (compound **350**) and the subsequent reactions with mesyl chloride and sodium sulfide.

The oxidation of carbonyl derivatives of BAs with peroxides produces 1,2,4,5-tetraoxanes. For instance, the reaction of 3-oxo derivatives of CA **351a–c** with hydrogen peroxide in the presence of an acidic catalyst at low temperature or with $(Me_3Si)_2O_2$ in the presence of trimethylsilyl triflate (Me_3SiOTf) affords dimeric tetraoxanes **352a–c** and **353a–c** (Scheme 69).[133,211] It should be noted that the dimerization of one stereoisomer of chiral ketone **351** yields two diastereomers (**352** and **353**).

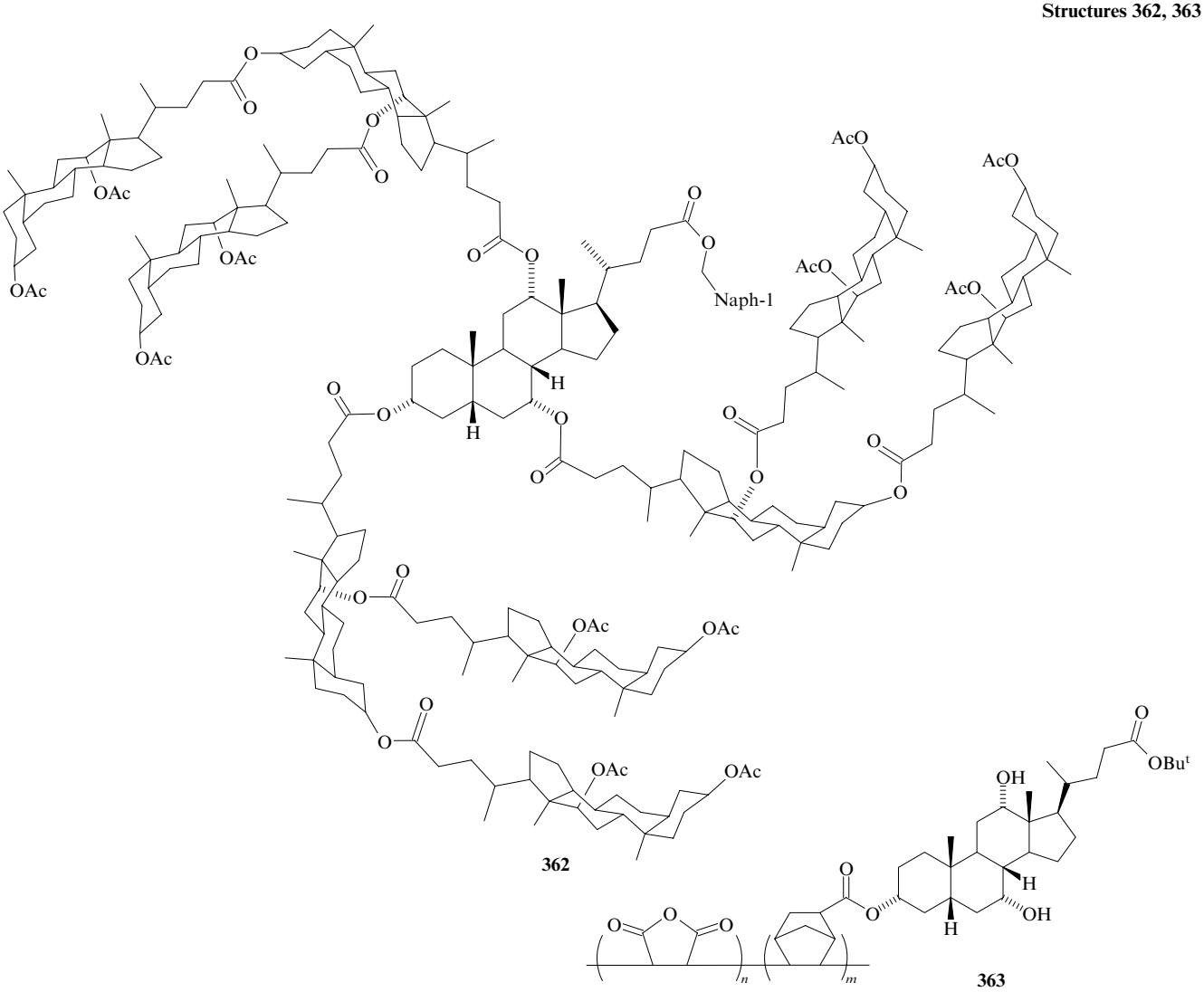
The synthesized compounds exhibited *in vitro* antimalarial activity against *Plasmodium falciparum D6* and *P. falciparum W2*[133,211] and antiproliferative activity against the cancer cell lines FemX and HeLa.[133] n-Propylamide **352c** proved to be the most active and, at the same time, the least toxic ($IC_{50} = 9.29 \times 10^{-9}$ mol litre^{-1} against *P. falciparum D6*); the antimalarial drug Artemisinin was used as the standard ($IC_{50} = 8.6 \times 10^{-9}$ mol litre^{-1}).[133,211] Compounds **351b**,**d** and **352b** exhibited the highest antiproliferative activity against the cancer cell lines FemX and HeLa comparable to that of cisplatin (IC_{50} varied from 3.1×10^{-6} to 6.2×10^{-6} mol litre^{-1} *versus* $IC_{50} = 3.5 \times 10^{-6}$ mol litre^{-1} for cisplatin against the cells HeLa).[133]

The oxidation of compound **351a** with hydrogen peroxide and the subsequent reaction of the intermediate with different cyclic ketones were used to synthesize mixed 1,2,4,5-tetraoxanes **354a–e** and **355a–e**.[212] These compounds exhibited *in vitro* (see Ref. 212) and *in vivo* (see Ref. 213) antimalarial activity. Mixed tetraoxanes possess

Structures 356–361



Structures 362, 363



362

363

higher antimalarial activity against *P. falciparum D6* and *P. falciparum W2* than dimeric tetraoxanes prepared based on BAs or from cyclic ketones.[212] Compounds **354a**,**b** proved to be the most active: IC_{50} against *P. falciparum D6* were 1.53×10^{-9} and 1.17×10^{-9} mol litre^{-1}; against *P. falciparum W2*, 0.98×10^{-9} and 0.58×10^{-9} mol litre^{-1}, respectively.

To sum up, this section deals with reactions of carbonyl groups of oxo derivatives of BAs with different C-, O- and N-nucleophiles that give compounds suitable for subsequent modifications. The Baeyer – Villiger oxidation of oxo derivatives of BAs to give lactone-containing compounds is considered. These lactones are key intermediates in the synthesis of secosteroids, macrocyclic structures and compounds containing a heteroatom in the steroid nucleus. The oxidation reactions of oxo derivatives of BAs with hydrogen peroxide in an acidic medium, which are utilized in the synthesis of dimeric or mixed tetraoxanes, are discussed.

VI. Bile acids in supramolecular chemistry

The synthesis of supramolecular composites based on bile acids is an emerging field of chemistry of BAs. Some examples of building blocks for assembly of supramolecular structures or composites themselves were considered above (see compounds **50**, **60** – **64**, **90**, **117**, **170**, **192**, **204**, **207**).

The synthesis and properties of supramolecular and polymer derivatives of BAs are considered in a number of reviews.[2, 214 – 219] Macrocylcic and acyclic dendritic molecules and polymer structures (compounds **356** – **363**) were synthesized by a combined modification of native functional groups of the steroid nucleus and the side chain of BAs. The esterification of hydroxyl and carboxyl groups and amidation of the latter groups are generally the key steps in such synthetic protocols.[220 – 225] Metal-catalyzed reactions,[221, 222, 225 – 227] the Mitsunobu reaction[221] and some other, which were discussed in the previous sections, are also appllied for this purpose.

Supramolecular assemblies based on BAs are used as amphiphilic compounds and drug delivery systems, catalysts for different processes, for ion and molecular recognition and in many other fields.[2, 217 – 219]

VII. Conclusion

This review covers a broad spectrum of various chemical reactions of bile acids giving compounds with biological activity or valuable physicochemical properties. The specific structural features of BAs, their biosynthesis, biological functions and main sources are considered.

Side chain modifications of BAs include numerous synthetic transformations related primarily to the synthesis

of derivatives at native carboxyl groups (salts, esters and amides) and those associated with changes in the side chain length and introduction of different functional groups.

The synthesis of bile salts with different metals is based on exchange reactions or reactions with oxides, hydroxides, salts or complexes of appropriate metals. Examples of BA salts with organic cations are given. Esters and amides are the most extensive classes of BA derivatives at the carboxyl group. The use of mono- and polyfunctional alcohols and amines in the synthesis allows the variation of physicochemical properties of the molecules, such as hydrophobicity and hydrophilicity. Many BA derivatives are biologically active compounds possessing antiproliferative, antitumour, antimicrobial, antifungal, anti-osteoporosis and some other activity. It should be noted that the amidation and esterification of carboxyl groups are commonly applied for the preparation of bile acid conjugates with biologically active molecules in order to employ them for targeted drug delivery to particular organs associated with specific distribution of bile acids through the body. In some cases, this conjugation leads to a considerable decrease in toxicity and reduces other unwanted side effects of the initial drug.

Variations in the side chain length and(or) side chain modifications with new functional groups [OH, OR, NH_2, NR_2, SR, CHO, SO_3H, $P(O)(OH)_2$, CN, NO_2, Cl, F, I and so on], including isotopically labelled groups, provide routes to biologically active steroids. In particular, bile acids were employed to prepare modulators of nuclear receptors (FXR, TGR5 and some other), gallstone-solubilizing agents, osteoporosis agents (interfering with bone resorption), gel-forming agents and building blocks for supramolecular chemistry.

Modifications of the steroid nucleus are based on transformation of native hydroxyl groups, including the esterification, substitution, elimination and oxidation reactions. The structure of the steroid nucleus is responsible for different reactivity of both the native hydroxyl groups and other functional moieties of rings A, B and C, due to which a particular group in BA molecules can be selectively modified.

Oxo derivatives of BAs are an important class of compounds widely employed in organic synthesis. Apart from the reduction of carbonyl groups to hydroxyl functions in the β configuration, resulting in the formation of epimers of BAs, chemical lability of carbonyl derivatives associated with keto-enol tautomerism makes it possible to perform profound transformations of the steroid nucleus.

Special emphasis is given to 6α-alkyl derivatives of BAs, in particular, 6α-ethylchenodeoxycholic acid, which efficiently activates the nuclear receptor FXR. It was also shown that oxo derivatives of BAs can act as methylene components in condensation reactions, resulting in the formation of different 1,3-dicarbonyl moieties. Dicarbonyl derivatives are, in turn, employed in the synthesis of biologically active compounds containing a heterocyclic moiety fused to ring A of the steroid nucleus. α-Bromo-substituted oxo derivatives of BAs serve as key intermediates in modifications of the steroid nucleus with functional groups, such as F, OH, N_3 (nucleophilic substitution of bromine) and in the multiple bond formation (elimination reactions).

In the series of α,β-unsaturated carbonyl derivatives of BAs, compounds containing the 3-oxo-4(5)-ene moiety are of particular value. These derivatives are intermediates in the synthesis of allocholic acids, lanostane-type compounds, biologically active steroids, including those containing a heteroatom (O or N) at position 4, and BAs containing the ^{13}C isotope-labelled nucleus.

Reactions of carbonyl derivatives with different nucleophilic agents are suitable for modifications of the steroid nucleus with functional groups, such as epoxide, alkylidene, oxime and so on, that are promising for subsequent transformations. The Baeyer–Villiger oxidation of carbonyl derivatives affords compounds with the annulated seven-membered lactone ring, which are widely used in the synthesis of secosteroids and macrocyclic structures based on BAs, as well as of BA derivatives containing O and S atoms or SO, SO_2 and NPh groups instead of CH_2 groups at positions 3 and 12 of the steroid nucleus.

Due to diversity of modifications of bile acids, the chemistry of this class of compounds continues to attract considerable attention.

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