Chemistry and biology of bile acids

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This review makes an attempt to combine the insights gained into the biochemistry and physiology of bile acids with the elegant supramolecular systems designed from them. Bile acids are cholesterol-derived facial amphiphiles responsible for the solubilization of cholesterol and fat through mixed micelle formation with phospholipids. An intriguing aspect of bile acids is that their chemical structure has been postulated to correlate with vertebrate evolution. However, the etiology of molecular evolution of bile acids is still poorly understood. There has been a steady progress in the studies aimed at elucidating physiological functions and developing pharmacological applications of bile acids. In recent years, bile acids and their analogues have been extensively utilized as supramolecular receptors for various types of guest molecules and ions. Under certain defined conditions, the supramolecular association of bile acids and their derivatives leads to gel formation. Thus, modified bile acids might find use in the design of futuristic materials.

DESPITE having a history of more than a century, bile-acid science (cholanology) continues to have importance in biology and medicine[1–3]. Bile salts are steroidal detergents, which together with lipids/fats/cholesterol form mixed micelles in the intestine to enable fat digestion and absorption through the intestinal wall[1]. They are biosynthesized from cholesterol in the liver and stored in the gall bladder (Figure 1). Bile is secreted through the bile duct into the intestine when food passes from the stomach to the duodenum. Most of the bile salts secreted into the upper region of the small intestine are absorbed along with dietary lipids at the lower end of the small intestine. They are separated from the dietary lipid and returned (more than 85%) to the liver for re-circulation. This movement of bile salts is termed as enterohepatic circulation (Figure 1 *b*). The most abundant bile salts in humans are cholate, chenodeoxycholate and deoxycholate, and they are normally conjugated with either glycine (75%) or taurine (25%). Conjugation increases the aqueous solubility of bile salts under physiological conditions. The chemical structures of free and conjugated cholic acid (taurocholate and glycolate) are discussed later in the article. All primary bile acids appear to have three features in common: (i) they are the major end-products of cholesterol metabolism, (ii) they are secreted into the bile largely in a conjugated form, and (iii) these conjugates are membrane-impermeable, water-soluble, amphiphilic molecules having a powerful ability to transform lamellar arrays of lipids into mixed micelles[1,2].

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Historical perspectives

Studies on the chemistry of compounds present in the bile had begun in the early nineteenth century[3]. Remarkable experiments performed by Thenard and Berzelius led to the identification of choleic acid and bilin[4]. The isolation of nitrogen-free bile acid (cholic acid) was achieved by Demarcay[4]. A crystalline sample of a bile acid was first prepared by Platner[5], but any structural insight was not possible at that time. However, painstaking efforts were taken by chemists, Strecker and Mylius to work out the molecular formulae of bile acids (both free and conjugated forms)[6]. Wieland and Sorage[7] first demonstrated the formation of cholic acid–fatty acid complex (choleic acid). Wieland and Windaus performed detailed structural studies on bile acids[8]. After Bernal elucidated the cyclopentanoperhydrophenanthrene structure of cholesterol by X-ray in 1932, Rosenheim and King were able to propose the correct structure of bile acids[9]. The current scientific phase of bile acid research began in the 1950s. Isolation and characterization of bile acids from different species, extensive metabolic studies and detailed physico-chemical studies were embarked upon during this period.

Chemical structure of bile acids

All bile acids consist of two connecting units, a rigid steroid nucleus and a short aliphatic side chain (Figure 2)[2]. The steroid nucleus of bile acids has the saturated tetracyclic hydrocarbon perhydrocyclopentanophenanthrene, containing three six-member rings (A, B and C) and a five-member ring (D). In addition, there are angular methyl groups at positions C-18 and C-19. In higher vertebrates, the bile acid nucleus is curved (beaked) because the A and the B rings are in a *cis*-fused configuration. Some bile acids in lower vertebrates, known as *allo*-bile acids, are flat because of an A/B *trans*-fusion (5α-stereochemistry). The side chain structure determines the class of the compound (bile acids or bile alcohols). There are four different types of bile alcohols (C_{27}, C_{26}, C_{25} and C_{24}) and they occur in the less evolved forms of life. There are two major classes of bile acids depending on the length of the side chain: C_{27} and C_{24} bile acids. In higher vertebrates, C_{24} bile acids consti-

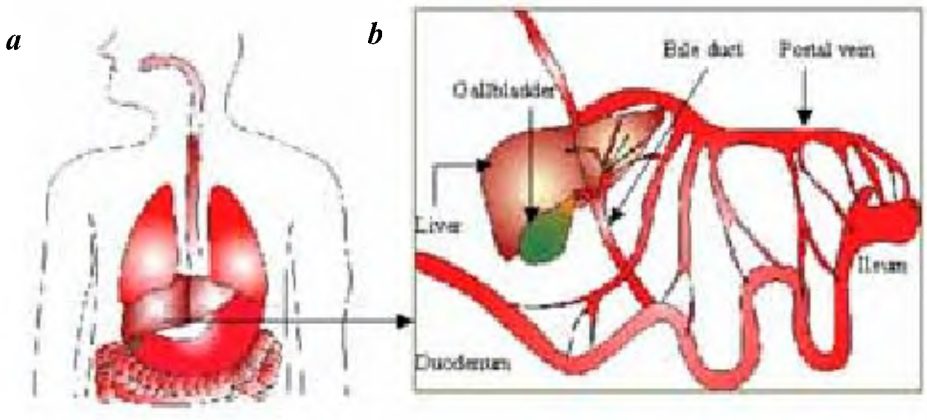


Figure 1. *a*, Digestive system; *b*, Enterohepatic circulation.

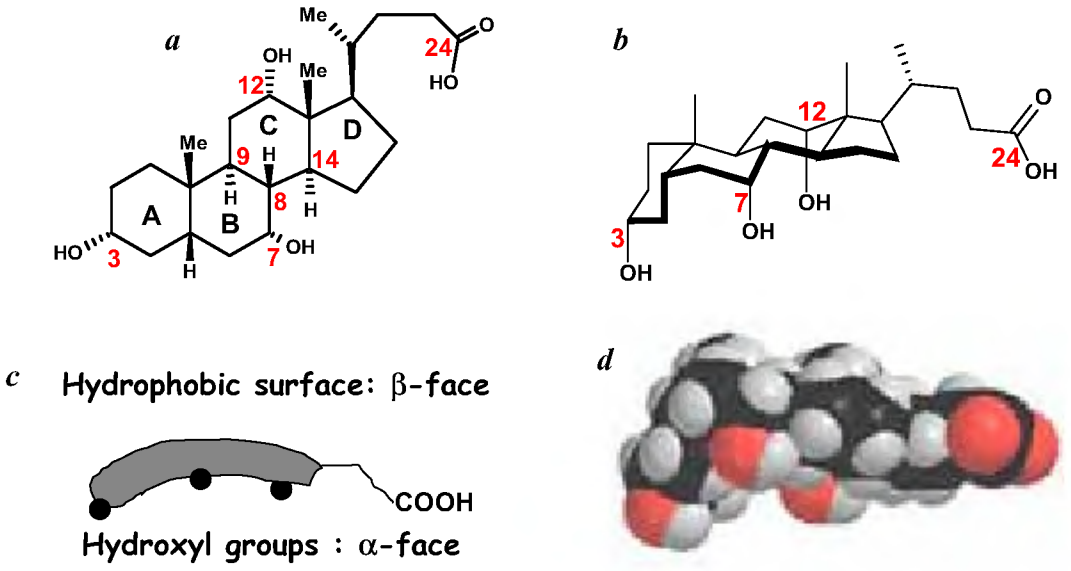


Figure 2. Structure of cholic acid. *a*, Chemical structure; *b*, Perspective structure; *c*, Cartoon representation (as introduced by Small); *d*, Space-filling model.

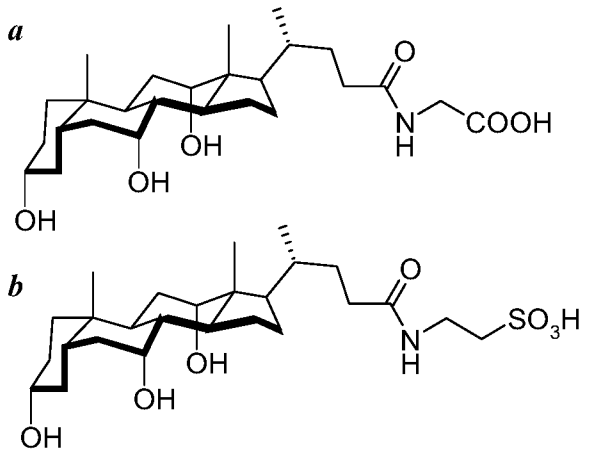


Figure 3. Structure of conjugated bile acids. *a*, Glycocholic acid; *b*, Taurocholic acid.

tute a major part of the bile. They are conjugated to glycine or taurine to yield the conjugated form of bile acids (Figure 3). Bile acids are facially amphipathic, i.e. they contain both hydrophobic (lipid soluble) and hydrophilic (polar) faces.

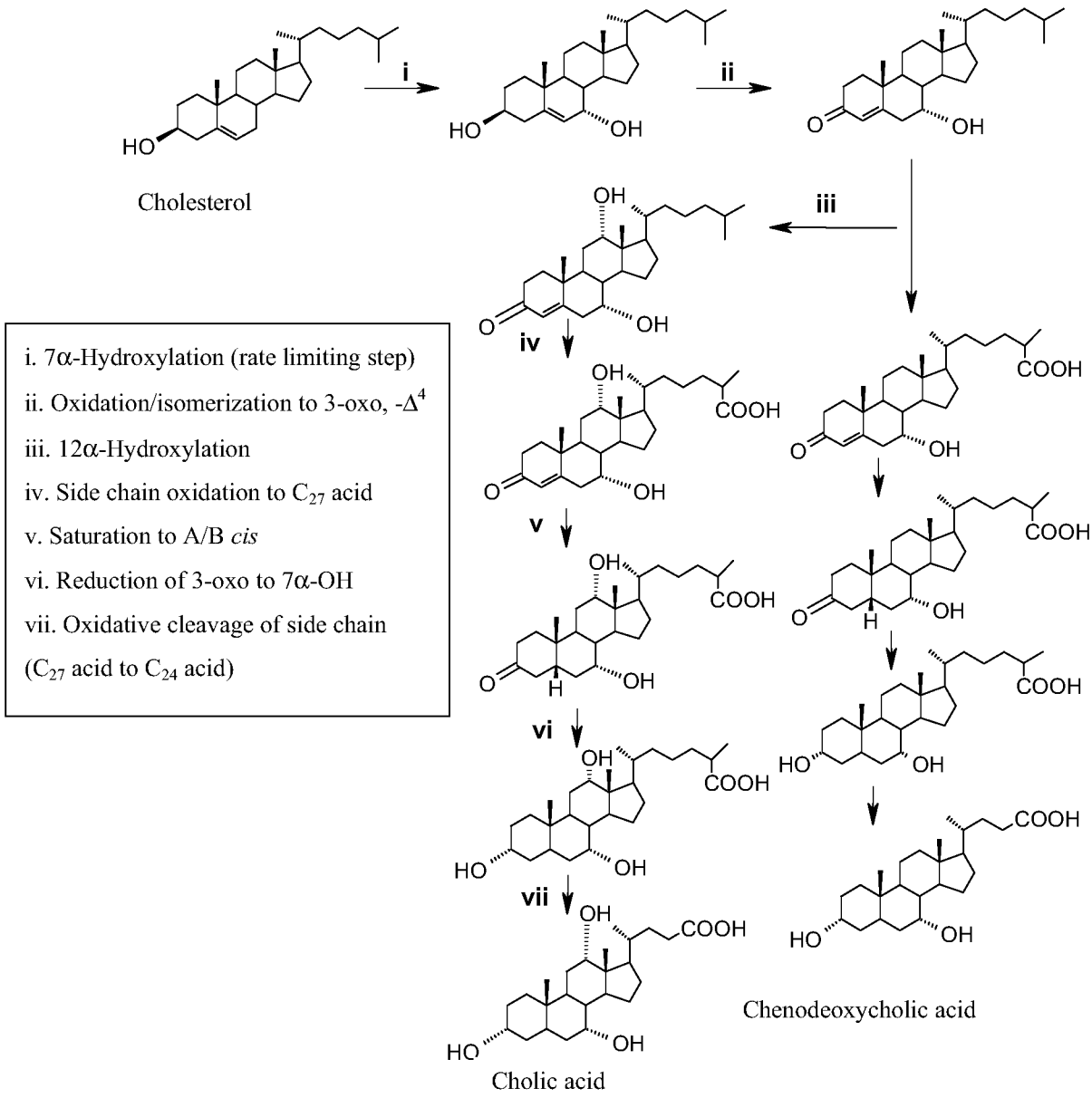
Chemical structure of bile acids and evolution

In contrast to most of the small molecules found in vertebrates, bile acids are strikingly diverse in a structural sense. Bile acids from different species chemically differ in three respects: (i) side-chain structure, (ii) stereochemistry of the A/B ring fusion, and (iii) the distribution of the number, position and stereochemistry of hydroxyl groups in the steroid nucleus. Several decades ago, Haslewood[10] addressed the issue of considering the bile acid structure as an aid to

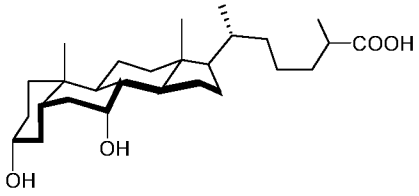
the understanding of the evolutionary processes. It has been noted that the bile acid structure shows a pattern of progressive molecular development along the line of vertebrate evolution. There is a clear evidence of evolution of bile acids through the stages: C_{27} alcohols → C_{27} acids → C_{24} acids. Bile alcohols act as bile salt after conjugation with sulphate (which increases water solubility), C_{27} acids are conjugated with taurine and C_{24} acids exist in the bile as taurine and glycine conjugates. It has been suggested that the most evolved mammalian bile acids have a 5β-configuration with hydroxyl groups at 3α, 7α and 12α. Names and structures of bile acids in vertebrate bile are given in Chart 1 (approximately in the descending order of evolution). Bile alcohols are not discussed, although they are major components in lower vertebrates.

Biosynthesis of bile acids

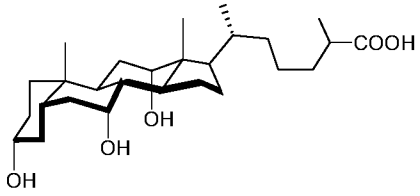
Bile acids are synthesized from cholesterol involving a number of complex steps in both the steroid nucleus and the side chain (Scheme 1)[1,2]. This biosynthesis involves at least five steps each on the nucleus and the side chain. Since these steps may in principle occur in any order, elucidation of individual steps of bile acid biosynthesis is not straightforward. In the first step, believed to be the rate-limiting step, cholesterol is oxidized to 7α-hydroxycholesterol by cholesterol 7α-hydroxylase. 7α-Hydroxy cholesterol is then converted to the key intermediate cholest-7α-hydroxy-Δ^4-3-one through the action of an isomerase and a reductase. This unsaturated oxo derivative is the branching point for cholic and chenodeoxycholic acid biosynthesis.



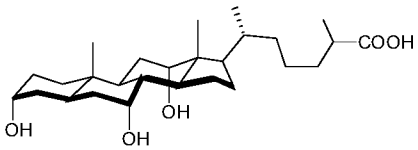
Scheme 1. Biosynthesis of bile acids from cholesterol.



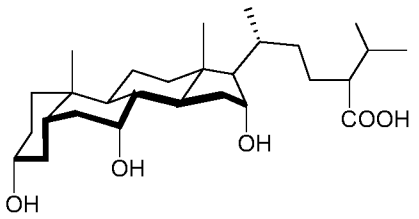
3α, 7α-Dihydroxycoprostanic acid:
Rattites, alligator, Andean condor



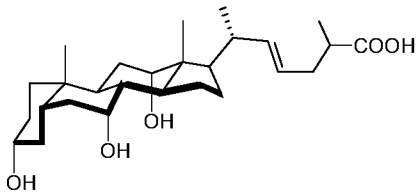
3α, 7α, 12α-Trihydroxycoprostanic acid:
Hornbill, alligator, frog



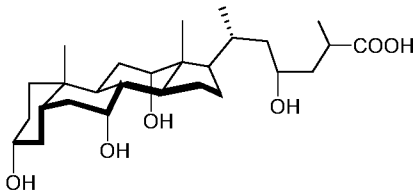
3α, 7α, 12α-Trihydroxycholestanic acid:
Iguana, frog



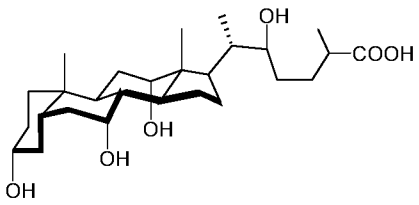
3α, 7α, 16α-Trihydroxycoprost-24-carboxylic acid: **Andean condor**



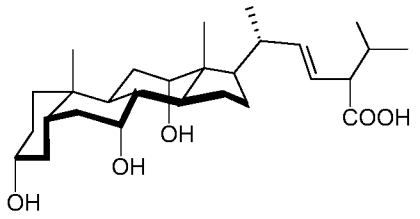
3α, 7α, 12α-Trihydroxy-25α-coprost-23-enic acid: **Toad**



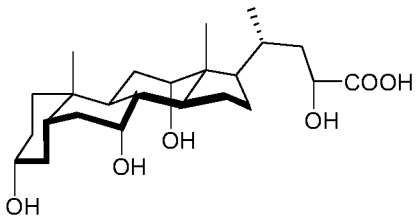
3α, 7α, 12α, 24-Tetrahydroxycopro-stanic acid (Varanic acid): **Lizard**



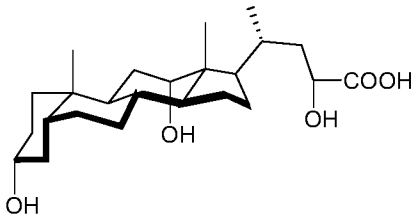
3α, 7α, 12α, 22-Tetrahydroxycoprostanic acid: **Turtle**



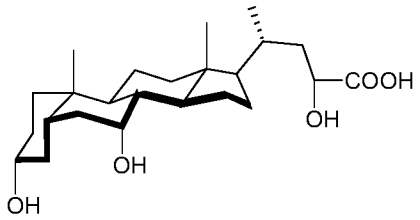
3α, 7α, 12α-Trihydroxycoprost-22-ene-24-carboxylic acid: **Toad**



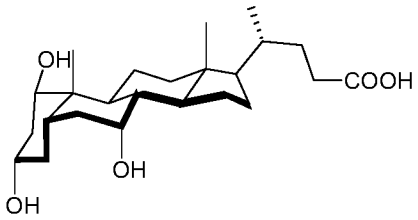
23R-hydroxycholic acid:
Seal, snake



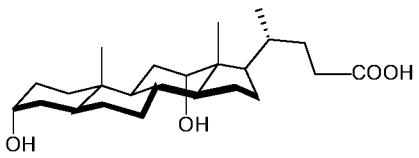
Bitocholic acid:
Snake



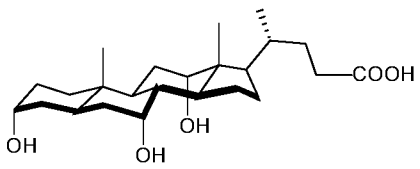
Phocaecholic acid:
Seal, walrus



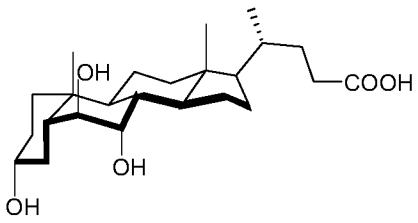
1α-Hydrohychenodeoxycholic acid:
Australian marsupials



Allodeoxycholic acid:
Rabbit



Allocholic acid:
Gigi fish



α-Muricholic acid:
Rat

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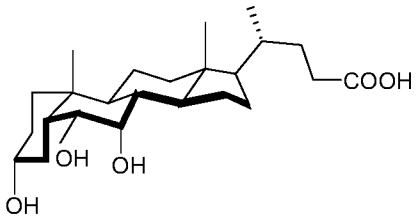
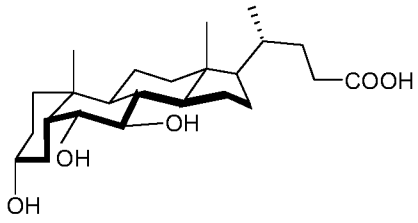
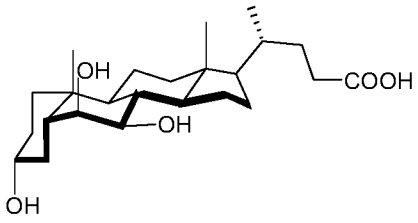


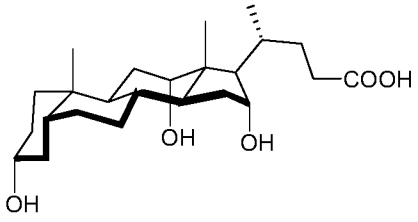
α-Hyocholic acid:
Pig



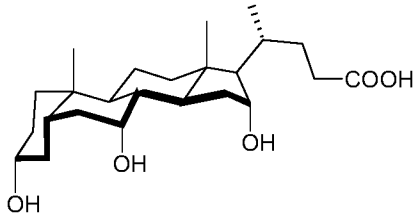
β-Hyocholic acid (ω-Muricholic acid):
Rat



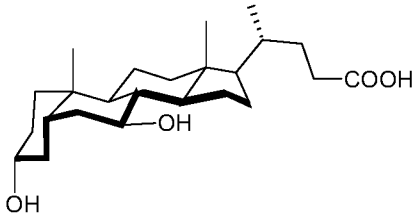
β-Muricholic acid:
Rat



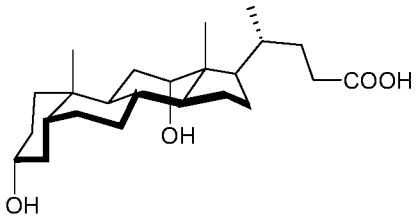
Pythocholic acid:
Snakes



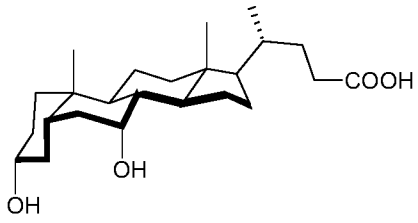
Avicholic acid:
Birds



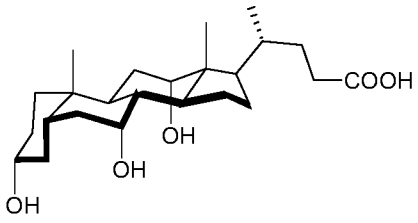
Ursodeoxycholic acid:
Bear, nutria



Deoxycholic acid: **Human** (bacterial 7-dehydroxylation product).



Chenodeoxycholic acid:
Human



Cholic acid:
Human

Chart 1. Chemical structure of bile acids in vertebrate bile.

Hydroxylation at C-12 leads to the formation of the cholic acid backbone. This oxo derivative is then stereoselectively reduced to afford the 5β-bile acid skeleton. The second key metabolic step, 27-hydroxylation followed by the formation of a C_{27} carboxylic acid, is believed to occur in the mitochondrion mediated by a P-450 hydroxylase. Then the oxidative cleavage of the side chain (C_{27} acid to C_{24} acid) mediated by peroxisomal enzymes affords the mature C_{24} bile acids. Bile acids are bio-transformed into glycine and taurine conjugates by the amidation reaction catalysed by an acyltransferase. During the past decade, significant developments have been achieved in the genetics of bile acid synthesis[11]. The synthesis of full complement bile acids requires the participation of 17 enzymes. The expression of selected enzymes is tightly regulated by nuclear hormone receptors and other transcription factors. Mutations in bile-acid biosynthesis genes responsible for several human diseases involving liver disorder and progressive CNS neuropathy have been identified[11].

Chemical synthesis of rare bile acids

To the best of our knowledge, the total synthesis of any bile acid has not been documented in the literature so far[12]. Considerable efforts, however, have been made to obtain rare bile acids starting from readily available bile acids, enabling one to study the physico-chemical properties of unusual bile acids. Several decades ago, *allo*-bile acids were obtained by treating the methyl ester of 5β-bile acids in boiling *p*-cymine[13]. Subsequently, *allo*-bile acids were prepared using different routes involving the Δ^4-3-oxo derivative as the key intermediate[14]. Chemical syntheses of several rare bile acids (in vertebrates) have been achieved by Iida and coworkers[15]. Stereoselective oxyfunctionalization by dimethyldioxirane has been shown as a convenient way of generating new bile acids[16].

An unusual bile acid, 16α-chenodeoxycholic acid, has recently been isolated from certain species of storks and herons[17]. This bile acid was named avicholic acid to signify

that, to date, this bile acid has been isolated only from avian species. The first chemical synthesis of avicholic acid was achieved by Iida *et al.*[15] from chenodeoxycholic acid using stereoselective oxyfunctionalization route. The overall yield of avicholic acid was <1%, clearly suggesting the need for an improved synthetic route in order to study its aggregation behaviour in aqueous media. We developed a synthetic route for avicholic acid. It was possible to prepare (in 9% overall yield) this rare bile acid from readily available chenodeoxycholic acid using Breslow's biomimetic remote functionalization in a key step[18]. This strategy can possibly be extended in order to synthesize other rare bile acids.

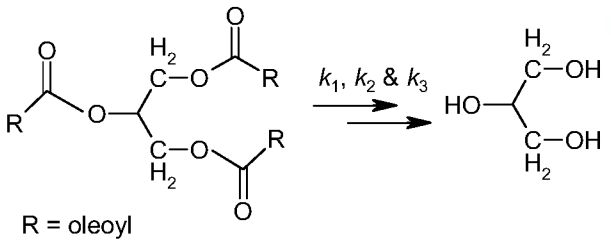
Physiological functions

Solubilization and transport of lipids

Bile acids emulsify dietary fat droplets through the formation of mixed micelles (discussed later in the article). This significantly increases the surface area of fat, making it available for digestion by lipases, which otherwise cannot access the interior of lipid droplets. Bile acids are lipid-carriers and are able to solubilize many lipids by forming mixed micelles with fatty acids, cholesterol and monoglycerides. These micelles are responsible for the solubilization and absorption of fat-soluble vitamins[19] such as vitamin E.

Bile salt-activated lipase

The intestinally located pancreatic enzyme, bile salt-activated lipase (BAL), possesses unique activities for digesting different types of lipids. The reaction scheme for the conversion of trioleoylglyceride to glycerol catalysed by BAL can be described by consecutive first-order reactions comprising three pseudo-first-order rate constants (k_1, k_2 and k_3: Scheme 2). BALs differ from other lipases in their requirement of bile salts for activity. Recently, the crystal structures of bovine BAL and its complex with taurocholate have been determined at 2.8 Å resolution (Figure 4)[20]. Two bile salt binding sites were found in each BAL molecule within the BAL–taurocholate complex structure. Bile salts activate BAL by binding to a relatively short 10-residue loop near the active site, and stabilize the loop in an open conformation. Presumably, this conformational change leads to the formation of the substrate-binding site, as suggested from kinetic data.



Scheme 2. Conversion of triglycerides into glycerol catalysed by human milk BAL.

Cholesterol homeostasis

The hepatic synthesis of bile acids accounts for the majority of cholesterol breakdown in the body. In humans, every day ca. 500 mg of cholesterol is converted to bile acids. This route for the elimination of excess cholesterol is probably important in all animals. It has recently been discovered that bile acids can also act as hormones, which bind to nuclear receptors and subsequently modulate the expression of proteins involved in cholesterol homeostasis[21]. A number of nuclear receptors have been shown to bind bile acids (cholic and chenodeoxycholic acids), including the farsenoid X receptor (FRX), the LXR-alpha receptor and the CPA receptor. The resulting bile acid–receptor complexes have been shown to be capable of binding to promoter regions of specific genes and either stimulating or suppressing their transcription.

Pathophysiology of bile acids

A number of hepato-biliary diseases have been identified which are caused by defects in bile acid biosynthesis[22]. The formation of 3β-hydroxy (instead of 3α-hydroxy) bile acids was observed in cholestasis[2]. In some cases, hepato-

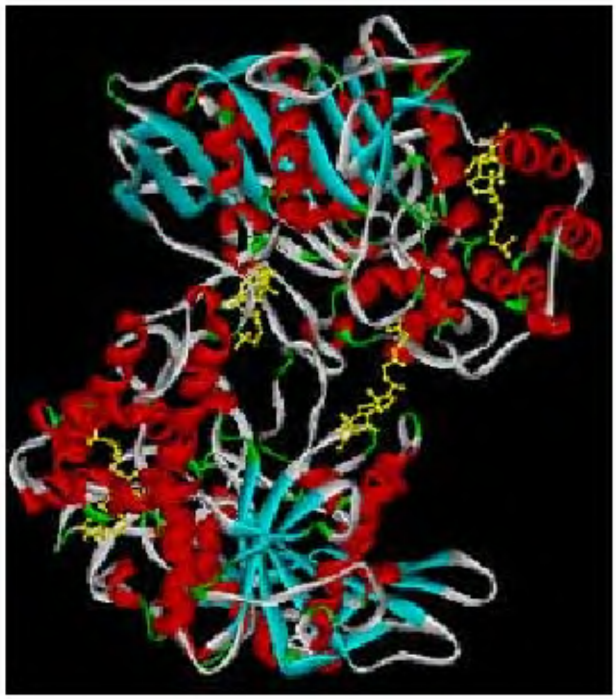
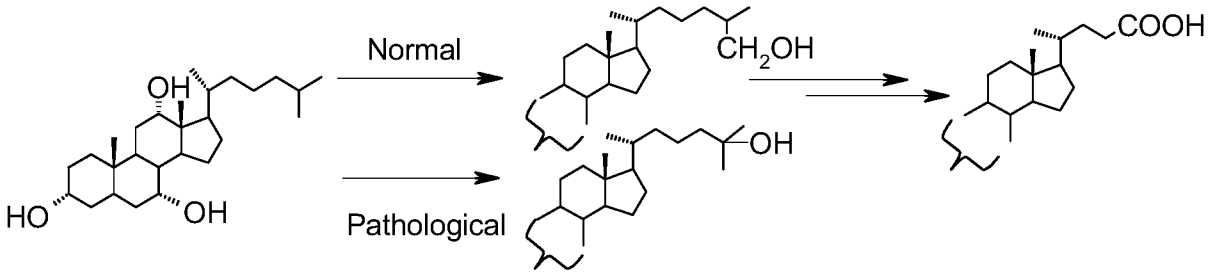


Figure 4. Crystal structure of bovine bile salt-activated lipase (dimer) showing bound taurocholate molecules (ball and stick, yellow).



Scheme 3. Mechanism for biosynthesis in CTX disease.

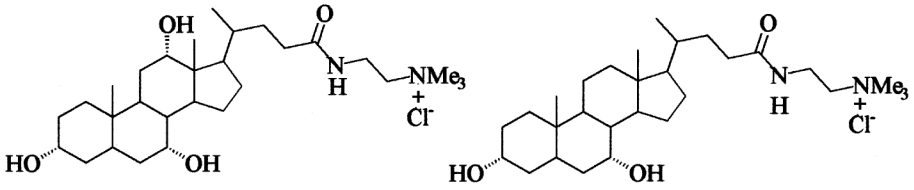


Figure 5. Bacterial swelling agent that inhibits protein synthesis.

biliary diseases were associated with a plausible deficiency of 12α-hydroxylase. A defect in mitochondrial 27-hydroxylation leads to a genetic disease called cerebrotendinous xanthomatosis (CTX), which is characterized by progressive neurological dysfunction, cataracts, xanthomatosis, etc. 25-Hydroxylation (instead of 27-hydroxylation) leads to disruption in the feedback inhibition of cholesterol biosynthesis, which increases the biosynthesis of cholesterol and subsequent accumulation of bile alcohols (Scheme 3).

Zellweger's syndrome is associated with defects in the side chain oxidation, as a result of which C_{27} bile acids, and surprisingly C_{29} bile acids (for unknown reasons), accumulate[2]. Improvements have been noted when patients were treated with a mixture of cholic and chenodeoxycholic acids.

It has been noticed that bile acids play a significant role in the etiology of intestinal cancer[23]. Studies have indicated that certain dietary habits affect bile acid metabolism in a way that may have a role in carcinogenesis. It has also been shown that the ingestion of large amounts of fats, particularly of animal origin, is an important element responsible for intestinal cancer. Earlier, cholic and deoxycholic acids were considered to act as mutagens or co-carcinogens[24]. Recently, several epidemiological studies have indicated that the possible mechanism of action is bile acid-induced DNA binding and transactivation of activator protein-1 (AP-1) by cooperative activation of extracellular signal-regulated kinases (ERKs) and protein kinase C (PKC) signalling[25]. Therefore, bile acids are now considered to act as tumour promoters, and not as mutagens.

Gallstones are formed by the accumulation of cholesterol, bilirubin and calcium carbonate in the gall bladder. Cholesterol gallstones are preponderantly found compared to other types of gallstones. The aqueous solubility of cholesterol in bile principally depends on the formation of mixed micelles by biliary bile salts and phospholipids. Thus, either an increased concentration of cholesterol or a reduced concentration of bile salts would result in the precipitation of cholesterol as gallstone[26]. It has been observed that gallstone patients have both increased cholesterol synthesis and reduced conversion of cholesterol to bile acids. Chenodeoxycholic acid has been used as a gallstone-dissolving agent for quite some time. However, during the last decade, ursodeoxycholic acid has become most useful for non-surgical treatment for gallstone diseases because of its reduced cytotoxicity[27].

Pharmacological applications of bile acid analogues

During the last few decades, there has been considerable interest in the synthesis and study of cationic surfactants from bile derivatives[28]. In most of these studies, a diamine is attached to the bile acid through an amide bond, and the other amine is converted to a quaternary ammonium salt. Fini and coworkers[29] reported comparative studies between the acid and basic derivatives. Bernheim and Lack[30] studied a series of cholanic-acid derivatives, and showed that the cationic bile salts (Figure 5) are potent in accelerating bacterial swelling[30]. In addition to bacterial swelling (which even the anionic ones accomplish), these are shown to inhibit protein synthesis. Antiviral[31] and antifungal[32] properties of some bile acid derivatives have been evaluated. Quaternary ammonium salts derived from bile acids (Figure 6) act as cholesterol dissolution agents[33]. These steroid derivatives are known to accelerate the dissolution of choles-

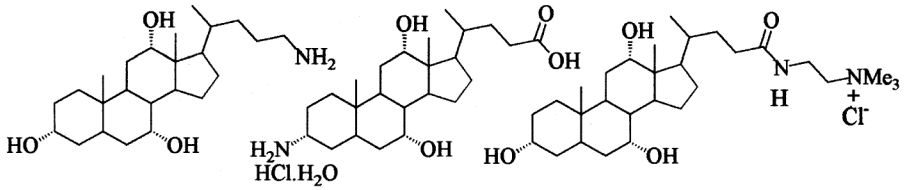


Figure 6. Kwan's cholesterol solubilizing accelerators.

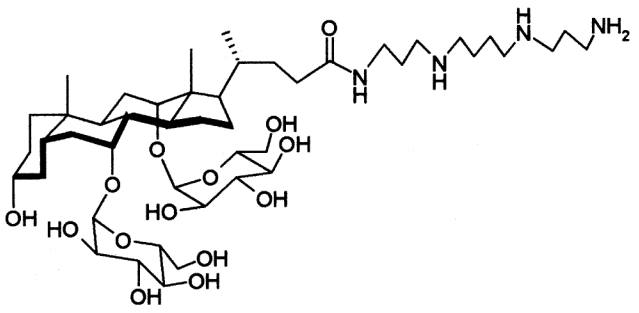


Figure 7. DNA transfecting agent.

terol monohydrate pellets in synthetic bile (11 mM NaC–32 mM lecithin), even at relatively low concentrations. Nor- and homo-bile acid derivatives have been added to some medicaments in order to improve their absorption[34]. Cationic amphiphiles derived from bile acids conjugated with polyamines were shown to dramatically increase the cellular uptake of DNA[35]. The transfection activity of bile acid conjugated polyamines (Figure 7) in combination with dioleoyl phosphatidyl-ethanolamine (DOPE) ranged three to seven times the optimal activity of lipofectin (DOTMA (*N*-[1,2,3-dioleyloxy)propyl]-*N*,*N*,*N*-trimethylammonium chloride) + DOPE), the first cationic lipid based gene delivery agent. Efficient calf thymus DNA condensation upon binding with bile acid polyamine amides has also been reported[36].

It has recently been realized that the high specificity and capacity of bile acid transport systems during their enterohepatic circulation might form the basis of current research on drug–bile acid conjugates for specific drug targetting to the liver and for improving the intestinal absorption of poorly absorbed or non-absorbed drugs, such as peptides[37].

Micelle formation in aqueous solutions

In aqueous environments, bile salts aggregate to form micelles[38]. These micelles, under physiological conditions, are transformed into mixed-micelles (see later in the article) with lecithin and glycerides, which are responsible for fat/cholesterol solubilization in the small intestine. The physical chemistry of micellization of bile salts has been, and still is, an active area of research. A variety of state-of-the-art techniques have been employed in order to gain more insights into the structure/size/shape of bile-salt micelles. This section will deal with both concepts and techniques (classical and advanced) used to understand structure and dynamics of bile-salt micelles.

Unlike conventional surfactant molecules, bile salts possess a rigid steroid backbone having polar hydroxyl groups on the concave α-face and methyl groups on the convex β-face (Figure 2). This arrangement creates a unique facial amphiphilicity for this class of molecules, enabling them to aggregate in aqueous media in a manner different from conventional detergents. Aggregation of bile salts in aqueous solution is largely driven by the hydrophobic association of apolar β-faces of steroid backbones, while further aggregation occurs through hydrogen bonding interactions (Figure 8). Critical micellar concentrations (CMCs) of dihydroxy bile salts are typically below 5 mM, whereas trihydroxy bile salts have higher CMC[1] ranging from 10 to 15 mM. The higher CMC of trihydroxy bile salts is attributed to their higher solubility in water. The aggregation number of these bile-salt micelles ranges from 2 to 10 for globular primary aggregates, which increases as a function of the bile-salt concentration (and added salt concentration) to form larger secondary aggregates. These have been suggested to be rod-like by small-angle X-ray scattering (SAXS) and small-angle neutron scattering (SANX) studies[38]. A model for the primary and the secondary aggregation was first proposed by Small (Figure 8)[38]. The aggregation number of several bile salts (free and conjugated) was determined by ultracentrifugation and quasi-elastic light scattering (QLS) techniques. Trihydroxy bile salt (sodium cholate) in pure water forms smaller micelles (with aggregation number 2). The aggregation number increases with increasing added salt (NaCl). Increase in the aggregation number is more pronounced in the case of dihydroxy bile salts (deoxycholic acid and chenodeoxycholic acid). A lowering of pH (from 9 to 7.3) further increases the aggregation number (>500) for dihydroxy bile salts, which is responsible for thickening of the aqueous solution to afford gel or gel-like materials[39].

QLS-studies have suggested that the hydrodynamic radius increases from 10 to 16 Å for smaller primary micelles to about 100 Å for larger secondary micelles. It was also found that the bile salt micelles are polydispersed (~20 and ~50% for small and large micelles respectively)[38,40].

We have recently described a convenient way to determine the average micellar size by monitoring the rotational

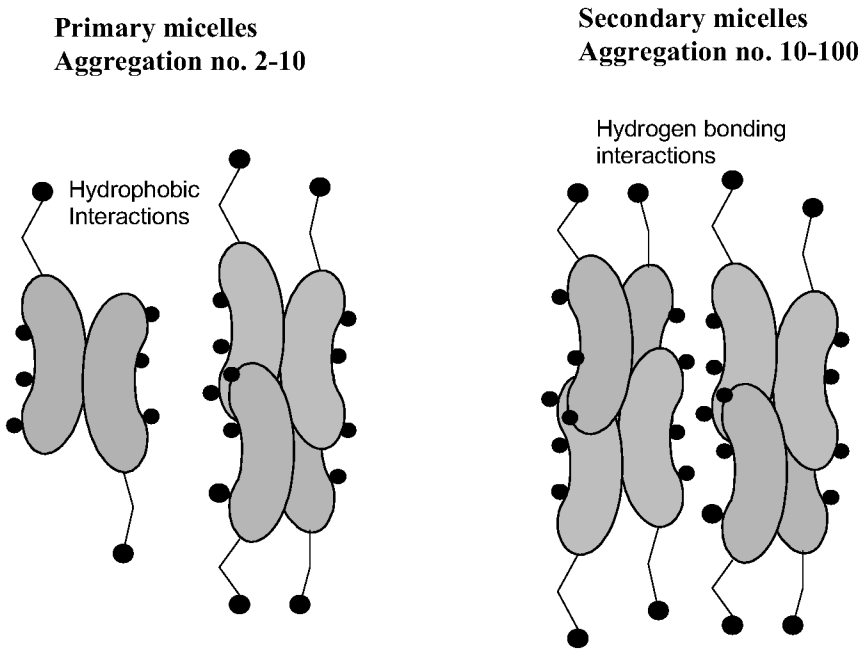


Figure 8. Cartoon representations of bile salt micelles (primary and secondary aggregation model) as introduced by Small.

diffusion (by pico-second time-resolved fluorescence anisotropy decay) using fluorescent probes (DPH: 1,6-diphenyl-1,3,5-hexatriene and ANS: 1-anilinonaphthalene-8-sulphonic acid) complexed to bile salt micelles. DPH showed a single rotational correlation time (about 2 ns in sodium cholate and 3.5 ns in sodium deoxycholate micelles)[41]. The DPH–bile-salt micelle system was the first one to show a single rotational correlation time (φ) among all other micelle-probe combinations[42]. This is due to the fact that nonpolar DPH intercalates tightly to the micellar interior of a bile-salt. Thus the observed rotational dynamics reports the global micellar tumbling of the bile-salt micelle. Thus the Stokes–Einstein equation, $\varphi = \eta V/kT$ (η is viscosity and V is hydrodynamic volume)[42] can be used to determine the average size of bile-salt micelles. The rotational correlation time of DPH in sodium deoxycholate micelle increased from 3.5 (no salt) to 7.5 ns in the presence of 0.3 M NaCl[43], due to increase in micellar size (aggregation number) with added salt[12]. Interestingly, a partly water-soluble probe such as ANS showed two rotational correlation times, which could be due to the distribution of amphiphilic dye in the hydrophobic phase and in the aqueous phase (possibly micelle–water interface). This probe may be useful to uncover finer details of the interfacial water molecules in bile-salt micelles. Similar results were obtained when coumarin 480 was used to probe the dynamics of bile-salt micelle[44]. In mixed micelles (bile salt–Triton X 100 and bile salt–CTAB), the slower dynamics may arise due to increase in size and microviscosity.

Solvation dynamics of a few fluorescent probes in bile-salt micelles has also been determined using time-resolved emission spectra (TRES)[45]. Usually the solvation time (re-orientation time of solvent molecules in response to the instantaneously created dipole by excitation) in bulk water is very fast (<1 ps), whereas in organized assemblies (like membrane, micelles, etc.), it is (ultra) slow and complex[46]. Interpretation of TRES data is not always straightforward in complex systems[47]. However, the average solvation time ranges from 1 to 2 ns in bile salt micelle[48]. The substantially slow solvation dynamics of water in the vicinity of sodium deoxycholate micelle arises from trapped water molecules (bound to the hydroxyl groups, carboxylates and sodium ions). Extension of this work to bile salt–Triton X and bile salt–CTAB mixed micelle using coumarin 480 as a fluorescent dye also revealed slow solvation dynamics in the Stern layer of the mixed micelles[44]. The above study shows the presence of strongly bound water molecules in the bile-salt micellar surface.

Mixed micelle formation

Emulsification of fat through mixed micelle formation is one of the significant properties of bile salts. Bile acids perform almost all physiological functions (discussed earlier) in the form of mixed-micelles. Bile-salt micelles can solubilize cholesterol, lecithin, monoglycerides, etc., which are intrinsically water-insoluble[49]. The aqueous solubility of cholesterol (~1 nM) can increase more than a million fold in the presence of bile-salt micelles[50]. The cholesterol solubilization ability is far better with dihydroxy bile-salts than with trihydroxy bile-salts. It further increases in the

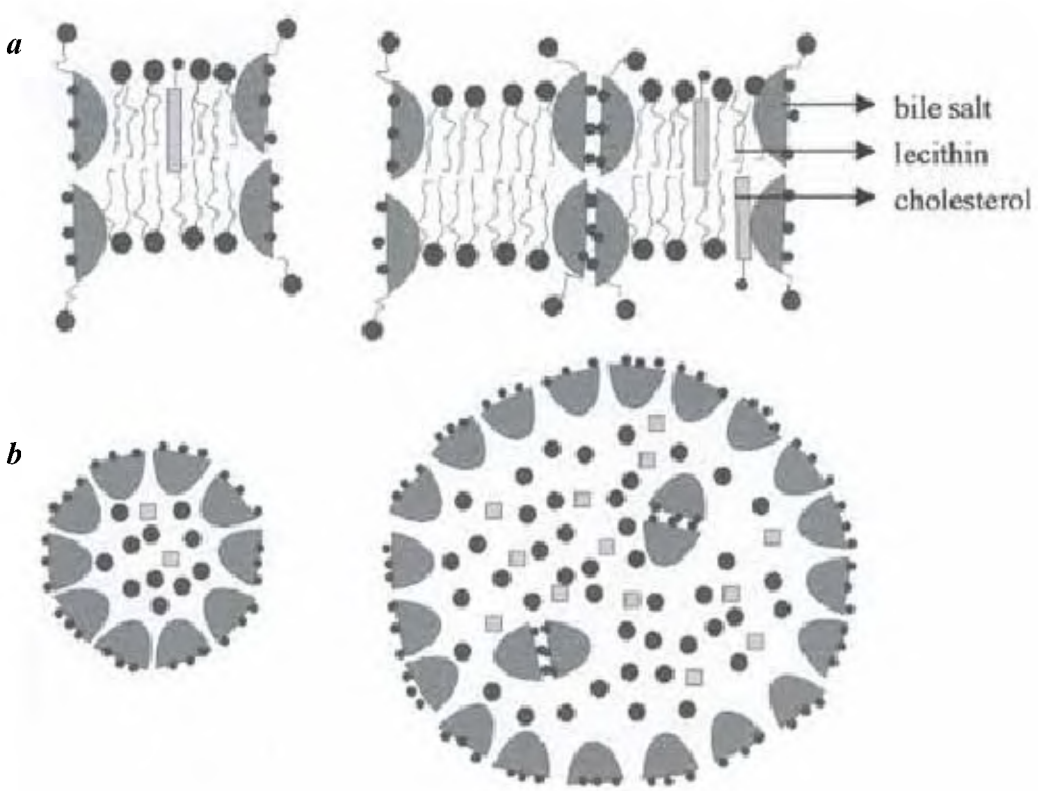


Figure 9. Proposed molecular arrangements of bile salt–lecithin–cholesterol mixed micelles. ***a***, Longitudinal view; ***b***, Cross-sectional view.

presence of lecithin because of the formation of mixed micelles (Figure 9). The average size of mixed micelles is much larger compared to pure bile-salt micelles, and the hydrodynamic radius can go up to several nanometres[50]. In addition to their physiological roles, bile-salt mixed-micelles are promising systems for drug delivery. The solubilization of drugs by bile-salt micelles (through the formation of mixed-micelles) has been examined[50].

Supramolecular chemistry of bile acids

This section deals with relatively newer aspects (supramolecular chemistry and molecular recognition) of bile acids. Because of the unique structural elements, bile acids constitute one of the most prominent classes in the study of molecular recognition, host–guest chemistry, biomimetic chemistry, etc[51]. Some of the bile acids and their analogues act as potent gelators (gel-forming agents) in both organic and aqueous media. These gels may hold promise for future biomaterials.

Molecular recognition and biomimetic chemistry

The bile acid skeleton plays an important role as a chiral building block to construct artificial receptors and supramolecular architectures. Cholic acid is a convenient building block for biomimetic systems because of the following features: (i) rigidity of the steroid 5β-framework (*cis* A/B ring fusion) ensures the formation of a cavity; (ii) the two faces of the steroid differ dramatically in their properties – the α-face displays three hydrogen-bonding groups, while the β-face is entirely hydrophobic; (iii) the hydroxyl groups are directed toward the centre of the concave face; (iv) the side-chain carboxylate can be readily derivatized, and (v) it is chiral. The crystal structure of bile-acid derivatives often shows the presence of channels with guest or solvent molecules (Figure 10)[52]. The tendency to form channels in the solid state arises as a result of the facially amphiphillic nature of bile acids. In solution, bile acids and their conjugates have been utilized to solubilize nonpolar substances in water[53]. Burrows and co-workers[54] used dimeric bile acid derivatives for binding monosaccharides and DNA. A variety of macrocyclic bile acids known as cholaphanes were shown to bind diverse types of guests. Diastereo- and enantioselective binding of octylglucosides by a tetrahydroxycholaphane has been reported[55]. Davis and co-workers[56] reported cholaphanes that bind alkyl glucosides and anions, depending on the size of the cavity. A new generation of cholaphanes (Figure 11) with externally directed alkyl chains (for solubility in organic solvents) was synthesized and shown to form a 1 : 1 complex with a β-D-glucoside with a mode-

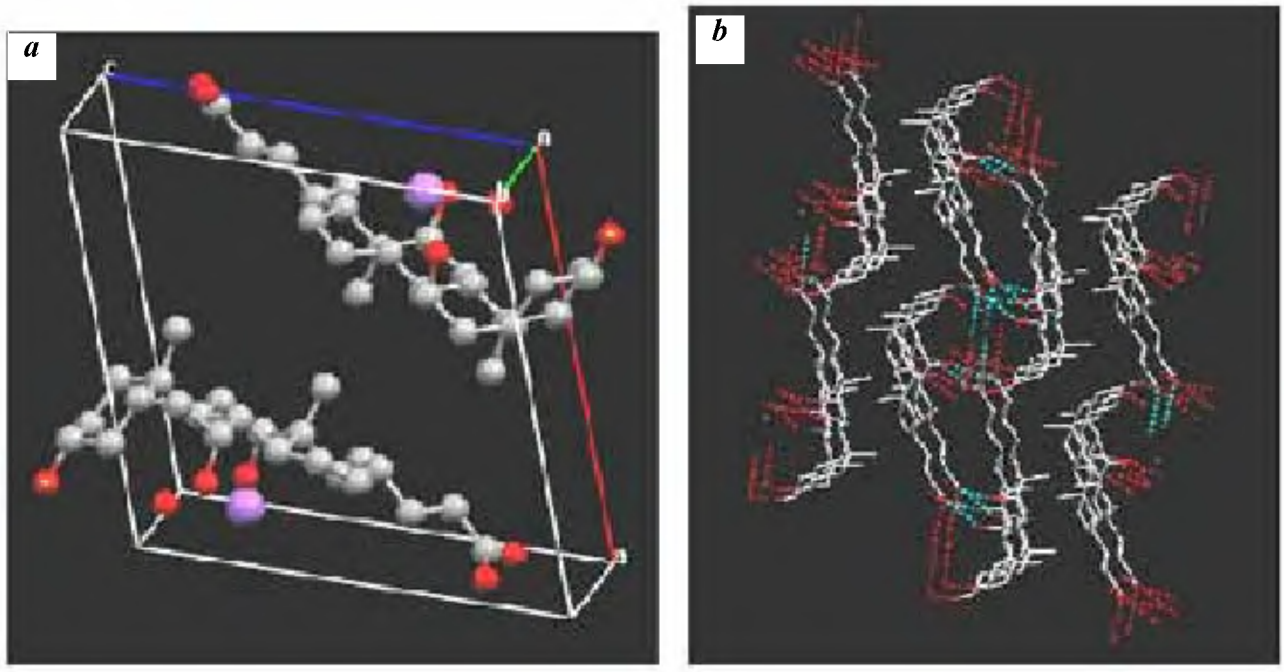


Figure 10. Arrangements of sodium cholate molecules in solid state. ***a***, Packing of two sodium cholate molecules. ***b***, Expanded view showing hydrogen-bonding interactions with solvent molecules.

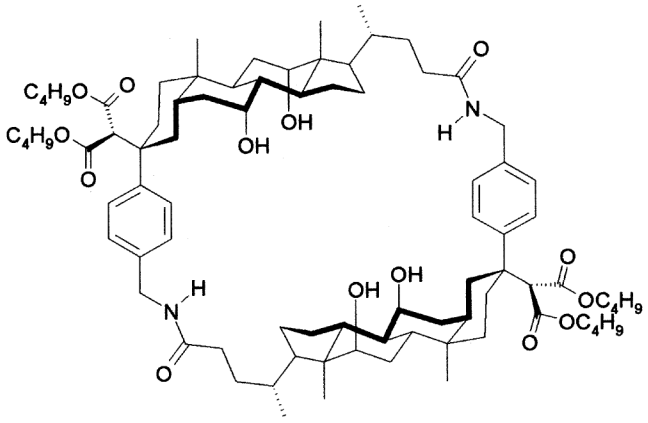


Figure 11. Cholaphane: A receptor of carbohydrate nuclei.

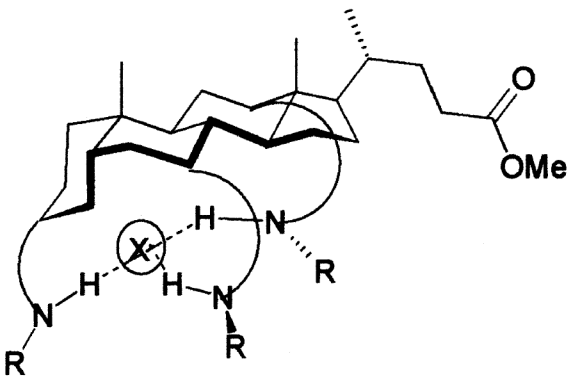


Figure 12. Acyclic cholic acid-based receptor for anions.

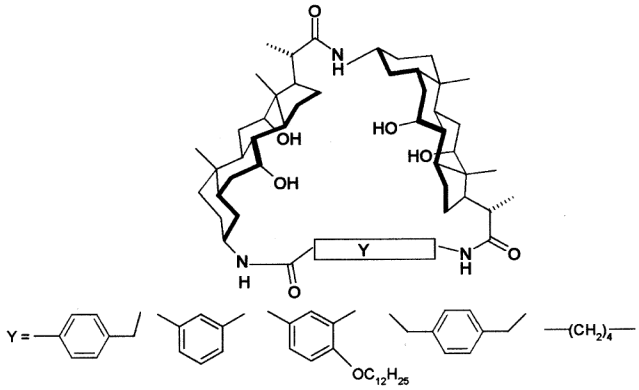


Figure 13. Bile acid-based macrocycles for biomimetic recognition.

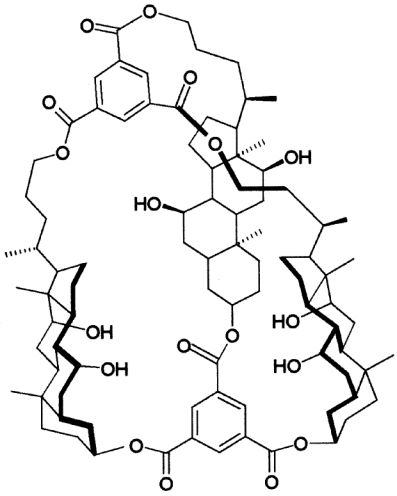


Figure 14. Cholic acid-based triply-bridged cholaphane.

rately high association constant in $CDCl_3$. It was also possible to demonstrate the extraction of β-D-glucoside to an organic layer from aqueous solutions.

The selective binding of fluoride ion by an acyclic cholic acid-based receptor was demonstrated by Davis and coworkers (Figure 12)[57]. Bile acid-based macrocycles of varying sizes (*Y* is variable spacer) and flexibility were synthesized (Figure 13)[58]. Cholic acid-based triply-bridged cyclophane (Figure 14)[59] possesses a hydrophilic cavity and can recognize hydrophilic hosts in organic solvents. It was shown that such molecules bind nitrophenols, triethanolamine, amino acids and octyl-glucosides.

Bonar-Law and Sanders[60] reported a bile acid-based ionophore having a MEM-protected cholaphane, which was found to bind alkali metal ions (Figure 15). This group also designed and synthesized a range of porphyrin-conjugated cyclocholates such as porphyrin-capped cyclocholates[61] and porphyrin bowls (Figure 16)[62].

A novel artificial ion channel consisting of a macrocyclic cholic-acid derivative (Figure 17) was synthesized by Yoshino *et al.*[63] Two- and four-walled molecular umbrellas based on cholic acid have been synthesized (Figure 18), which are able to transport hydrophilic molecules (peptides, thiolated AMP and ATP) across liposomal membranes[64].

From our laboratory the utility of bile acids is in the construction of macrocycles (Figure 19 *a*)[65], receptors for adenine/biotin (Figure 19 *b*)[66], dentritic species (Figure 20)[67] and alkali metal ions (Figure 19 *c*)[68]. Semi-rigid molecular tweezers for electron-deficient aromatic compounds were built by appending aromatic surfaces on the rigid bile acid scaffold (Figure 21)[69]. Recently, bile acid-based dendrons were synthesized from 2,2-bis(hydroxymethyl) propionic acid (bis-MPA) and lithocholic acid by a convergent method[70]. These molecules hold promise as potential drug carriers.

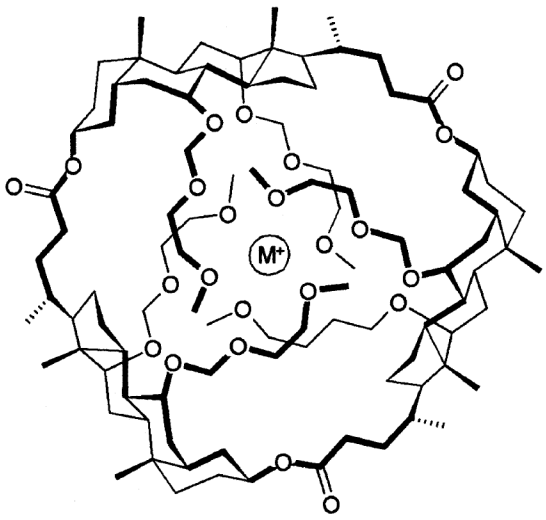


Figure 15. MEM-protected cholaphane.

Supramolecular association leading to gelation of organic fluids

Miyata and co-workers[71] reported that *N*-isopropylamide of cholic acid (Figure 22) is an efficient gelator of aromatic solvents in the presence of methanol. Transmission electron microscopic (TEM) studies of the gel showed the presence of fibres of ca. 200 nm diameter. It was serendipitously discovered by us that a bile-acid derivative with a pyrene

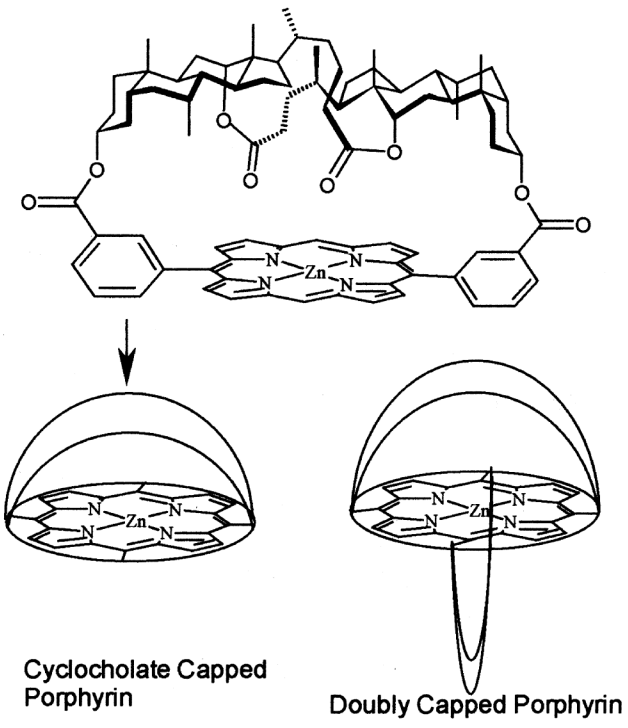


Figure 16. Porphyrin conjugated cyclocholates.

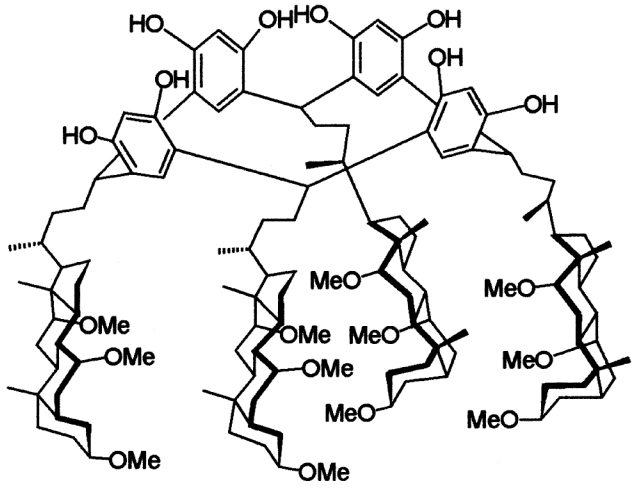


Figure 17. Cholic acid based artificial ion channel.

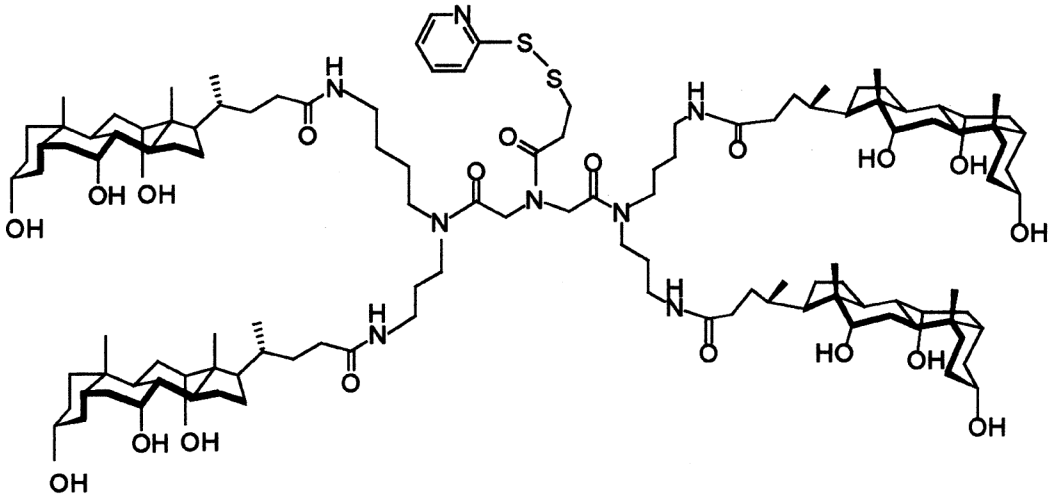


Figure 18. Tetra-walled molecular umbrella derived from cholic acid.

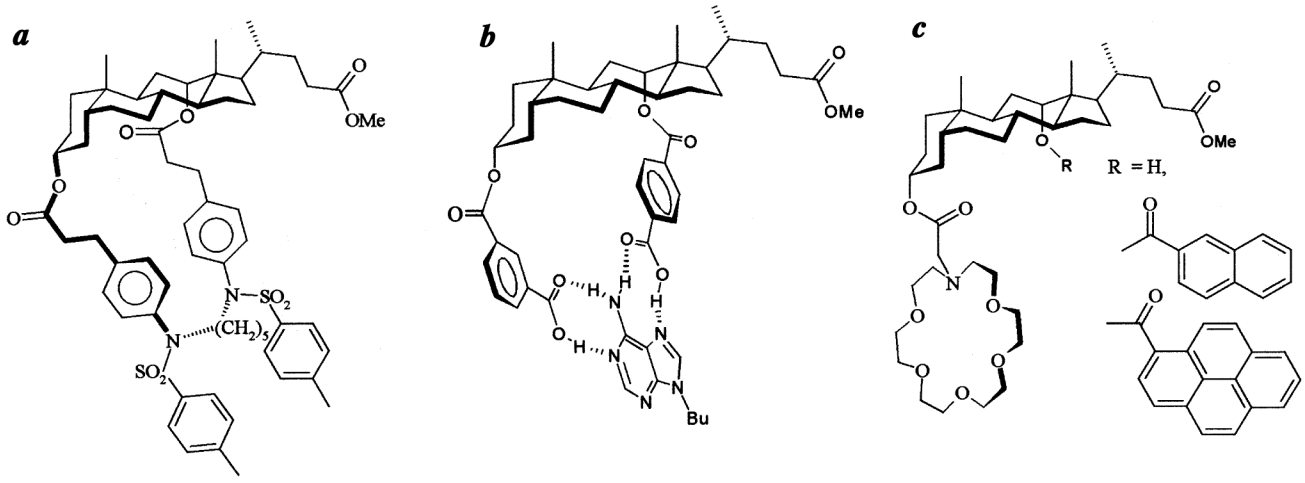


Figure 19. Bile acid-based macrocycle (*a*), receptor for adenin/biotin (*b*), receptor for alkali metal ions (*c*).

moiety attached to C-3 through an ester linkage (Figure 23), formed stable gels in the presence of trinitrofluorenone (TNF) in a mixture of chloroform and ethanol. These gels were coloured due to the charge-transfer interaction between the electron-rich pyrene unit and electron deficient TNF[72].

Recently, a number of *N*-cholyl amino acid alkyl esters (Figure 24) were shown to form stable organogels in aromatic solvents and in cyclohexane[73]. Hydrogen-bonding interactions were reported to be responsible for the self-assembly leading to gelation. The chiral centre of the amino acid component seems to play an important role in gelation.

Supramolecular association leading to hydrogelation

It was mentioned earlier that under certain defined conditions bile acids/salts form gels in water. This unusual behaviour of bile acids was known for a long time[74]. Sodium cholate, sodium deoxycholate and sodium lithocholate were shown to form gels in water. The gelation was found to be pH-dependent (optimal at pH ~ 7), and the gels were thixotropic in nature. However, some of these interesting observations remained unnoticed by several researchers in this area[75]. X-ray diffraction studies performed by Rich and Blow on the deoxycholate gel revealed that the (supra) molecular complex formed a helical structure with 36 Å diameter. The complex formation (gelation) was favoured at lower pH and higher ionic strength[12].

From our laboratory we have demonstrated efficient gelation of predominantly aqueous fluids by a cholic acid trimer (tripodal cholamide, Figure 25)[76]. These gels were transparent (Figure 26 *a*), and formed at remarkably low gelator concentrations (0.02% w/v, 0.15 mM, i.e. one gelator molecule immobilizing $>10^5$ water molecules). A cryo-

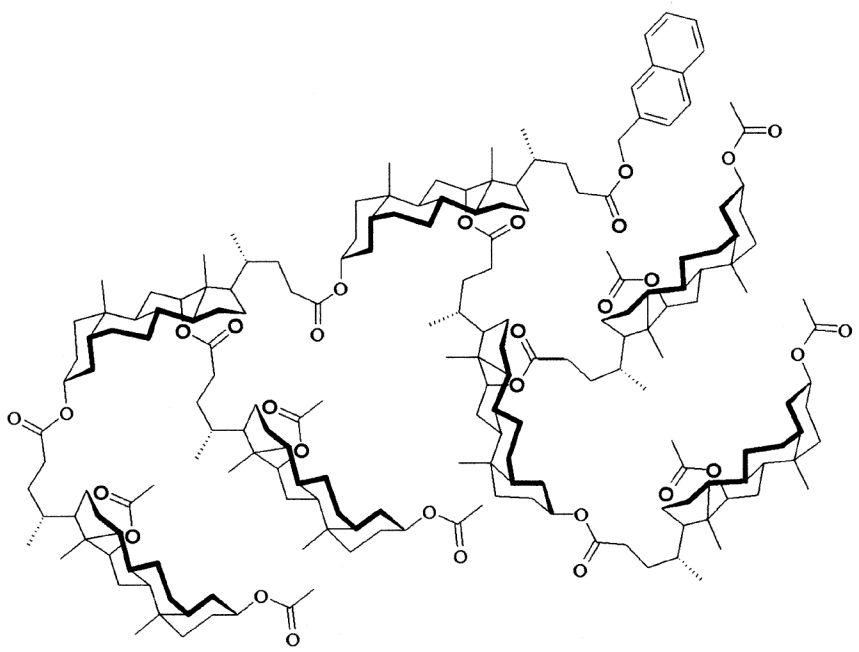


Figure 20. Bile acid based dendrimer.

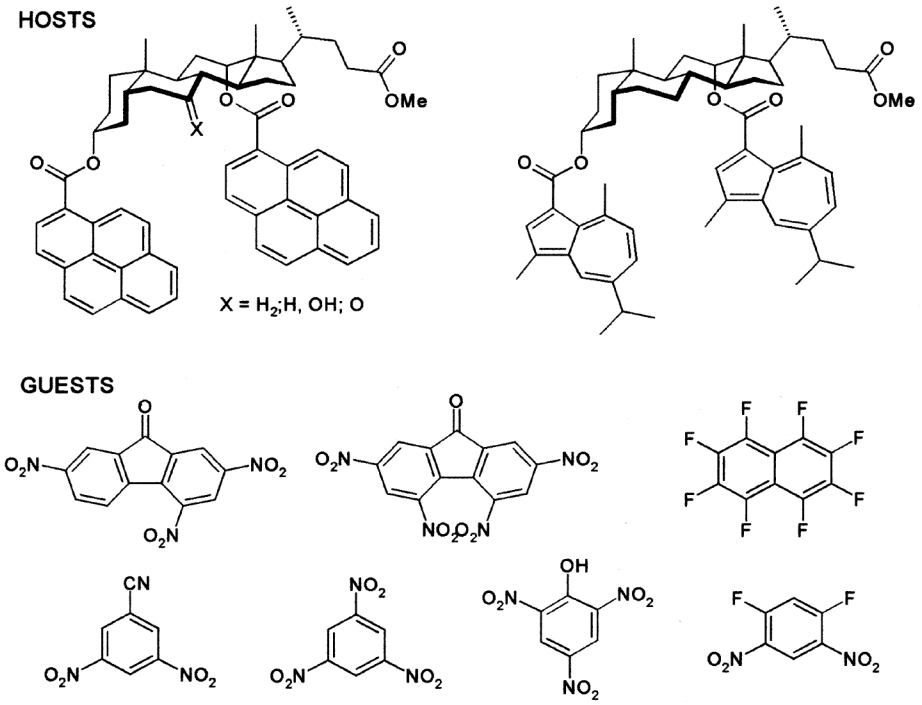


Figure 21. Molecular tweezers based on bile acids.

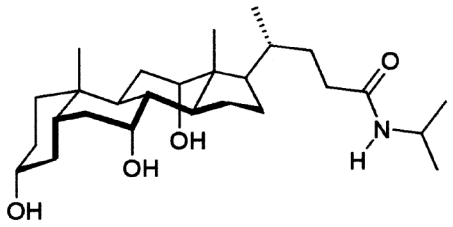


Figure 22. Cholic acid-based organogelator.

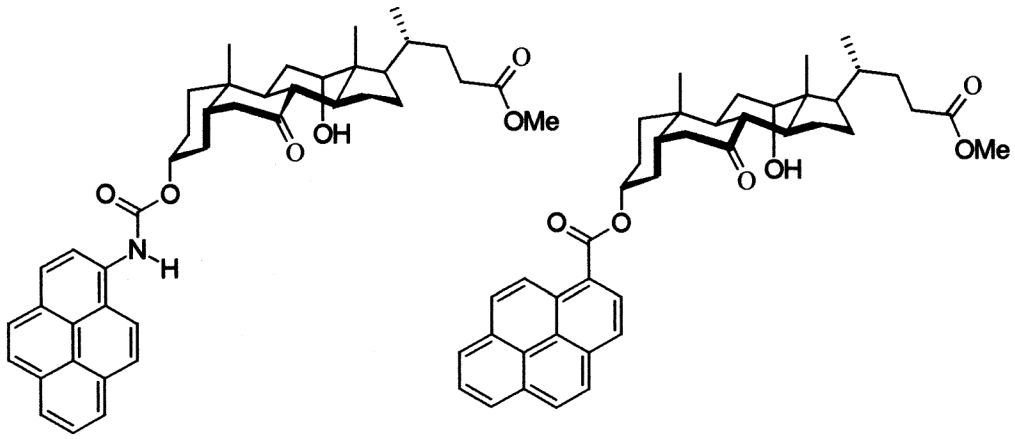


Figure 23. Bile acid-based organogelators.

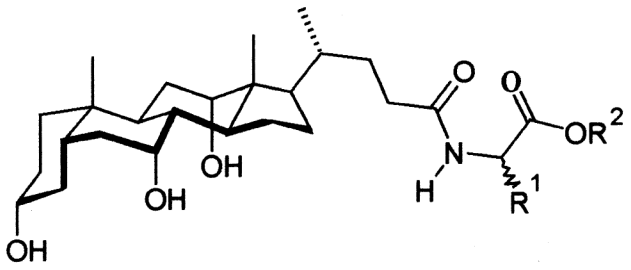


Figure 24. *N*-cholyl amino acid alkyl esters as organogelators. R^1, Amino acid side chain; R^2, Alkyl groups.

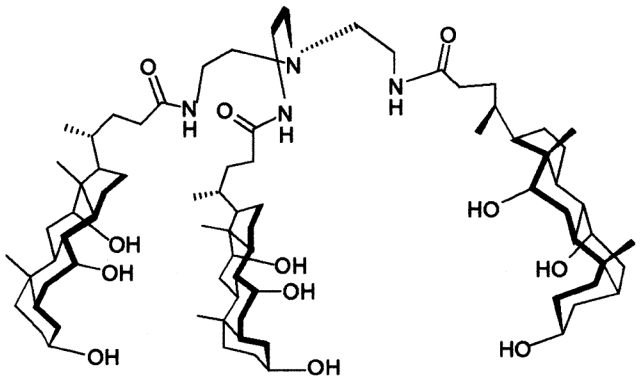


Figure 25. Tripodal cholamide gelator.

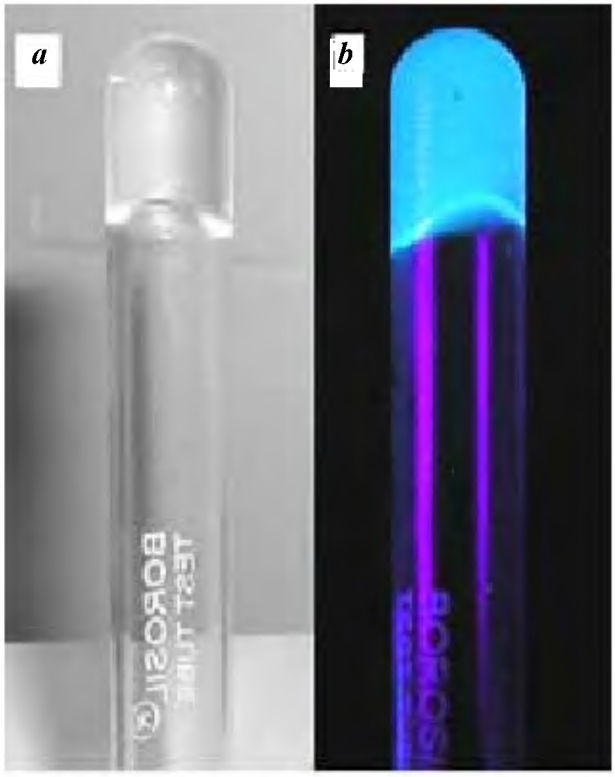


Figure 26. Photograph of gels from tripodal cholamide (5 mM in 20% AcOH–water) in the presence of 30 μM ANS. ***a***, Transparent gel, and ***b***, Luminescent gel.

TEM image of the gel showed the presence of nanofibres (Figure 27). The formation of hydrophobic 'pockets' during gelation was inferred using ANS as a polarity-sensitive probe. ANS became highly fluorescent in the gel state (Figure 26 *b*). A thermochromic gel was developed using bromophenol blue as a dye. The rotational dynamics of polarity-sensitive fluorescent dyes (ANS and DPH) in an aqueous gel derived from tripodal cholamide **1** was studied using picosecond time-resolved fluorescence technique[15]. ANS in the gel showed two rotational correlation time (φ) components, ca. 13 ns (ANS bound to the hydrophobic region of the gel) and ca. 1 ns (free aqueous ANS); whereas DPH showed only one component (ca. 5 ns) characteristic of a single population of the dye in the hydrophobic pockets of the gel. The second shorter component for ANS may be due to its partitioning into the aqueous phase of the gel-network. It is interesting to notice about ten-fold dampening ($\varphi \sim 1$ ns) of the dynamics in the aqueous phase of the gel

network compared to ANS in water (~0.1 ns). This observation is attributed to partial immobilization of water molecules around the nanofibres of the gel, which increases the microviscosity of the aqueous phase experienced by ANS. These gels could act as excellent materials for future applications because of their remarkable water-holding ability and efficient dye-solubilization property. However, we have demonstrated an application of one of these gels (composed of networked fibre of nanometric dimension) in creating

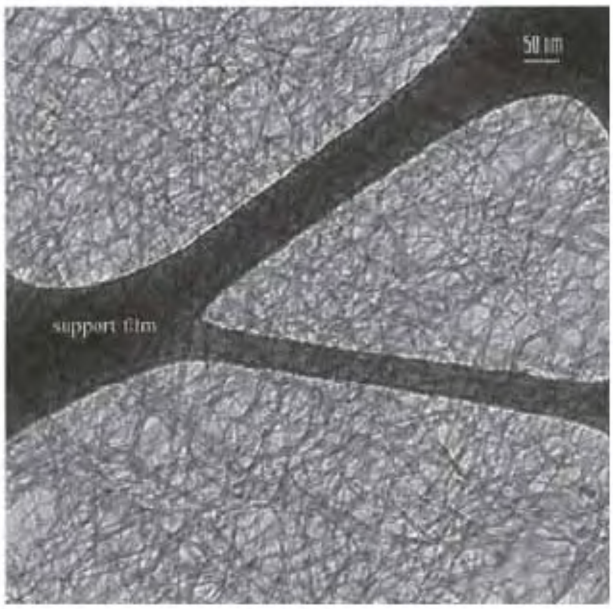


Figure 27. Cryo-TEM image of gel derived from tripodal cholamide.

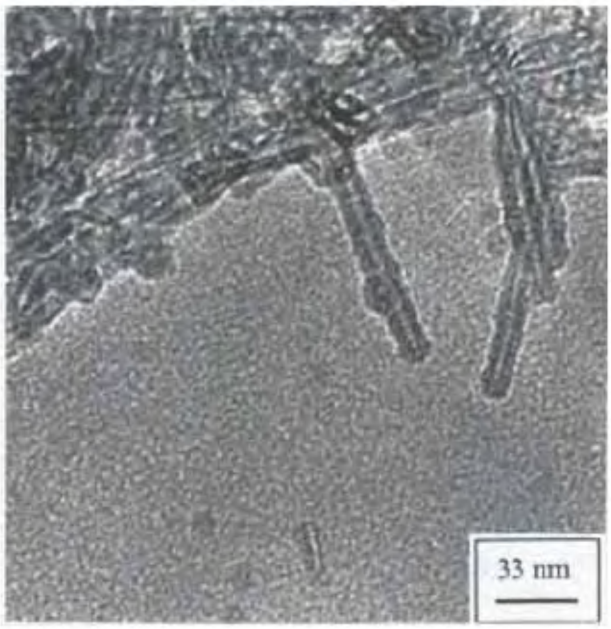


Figure 28. Titania nanotubes made from hydrogel derived from tripodal cholamide gelator (Figure 25).

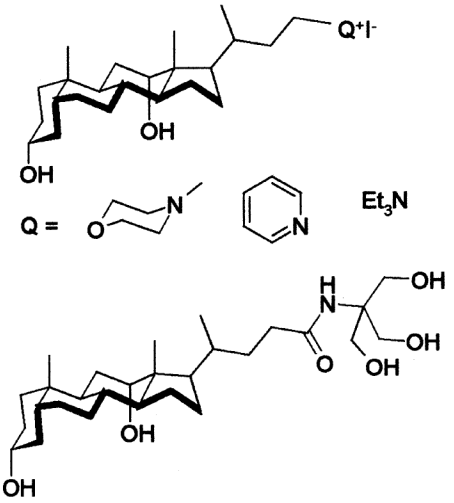


Figure 29. Cationic and neutral analogues of bile acids.

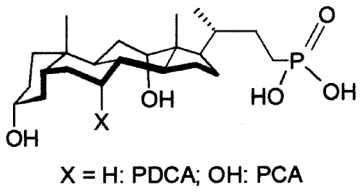


Figure 30. Phosphonobile acids.

inorganic nanostructures. The gel derived from tripodal cholamide was used as an organic template to prepare inorganic nanotubes by employing sol-gel template technique (Figure 28)[77].

Simpler monomeric cationic and neutral analogues of bile acids were also synthesized and evaluated for gelation. It was found that several cationic dihydroxy bile salt analogues form gels in aqueous fluids (Figure 29)[78]. Gelation behaviour of phosphonobile acids (where CO_2H of the steroid is replaced by a PO_3H_2; Figure 30) in acidic pH has also been observed recently[79]. Potential biological applications of these monomeric cationic and anionic bile acid analogues are currently being evaluated in our laboratory.

Concluding remarks

This review encompasses several classical and emerging aspects of chemistry and biology of bile acids. We have described the utility of bile acids both in physiology and in structural chemistry. The diverse and unique properties pertaining to both chemistry and biology arise from their structural uniqueness. The facial amphiphilicity with rigid steroid backbone seems to be most important in the expression of their chemical/supramolecular/biological/physiological properties. Additionally, hydrogels derived from bile acids are of considerable interest due to their excellent

water-holding ability. Dye-intercalation studies on gels show that they could be potential materials for small molecule (drug) delivery. It is noteworthy that these hydrogels are thermoreversible and biodegradable, unlike traditional polymeric gels. It remains to be seen whether these bile acid-based artificial receptor/surfactant/gelator molecules are promising candidates for pharmacological applications. We believe that the information contained in this article will be useful to develop various bile acid based molecular and supramolecular systems with widely different goals.

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