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A tryptophan-substituted cholic acid: Expanding the family of labelled biomolecules

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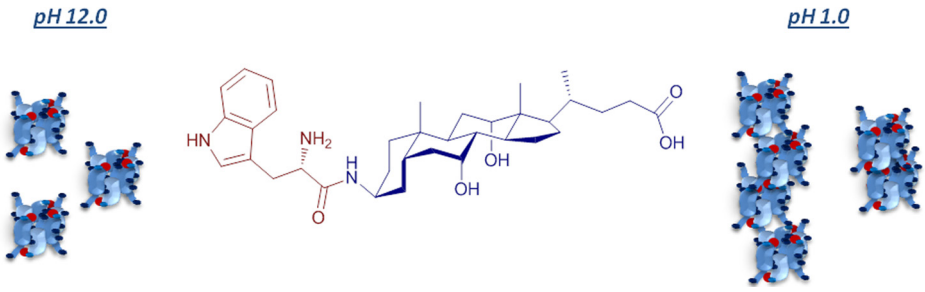
HIGHLIGHTS

- We synthesized a novel bile acid derivative labelled with tryptophan.
- We characterized the self-assembly of the derivative in aqueous solution at high and low pH.
- Ellipsoidal micelles are formed in alkaline and acid solutions.
- A supramolecular gel is formed in acid solution by lowering temperature.
- Tryptophan experiences a polar environment irrespectively of the aggregate structure.

GRAPHICAL ABSTRACT



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ABSTRACT

The synthesis of a novel cholic acid derivative bearing in the C-3 position a residue of tryptophan linked through an amide bond is herein described. Acidic or basic conditions are needed for the solubilization of the derivative in water. In alkaline solutions the molecule shows a self-association similar to the one of its natural precursor leading to the formation of ellipsoidal micelles which does not involve significant Trp–Trp interactions. On the contrary, in acidic conditions strong interactions between the tryptophan moieties occur, leading to the formation of a gel at low temperature. These interactions are broken upon heating and small micelles similar to those observed at high pH are formed. In both cases, fluorescence spectra suggest a polar environment for the amino acid fluorophore not remarkably affected by the self-assembly.

1. Introduction

Bile acids (BAs) are biological surfactants, they are found mainly in the bile of mammals and other vertebrates and they are widely involved in various biological processes *e.g.* emulsification of fats and fat-soluble vitamins, elimination of cholesterol and catabolites, such as bilirubin [1,2], and regulation of receptors [3,4] and cell signalling pathways [5]. They are also important in various applications, such as the preparation of selective culture media for bacteria [6] or liposomes from phospholipid-bile salt mixed micelles [7–9] and the modifications of hydrophobic/hydrophilic balance of microfiltration membranes [10]. Most

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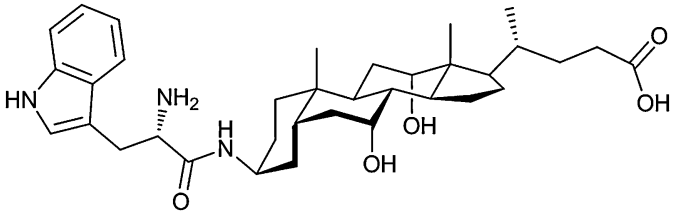

Fig. 1. Molecular structure of the β-L-TrpC derivative.

of their functions rely on their unique amphiphilic structure, which is characterized by a curved and rigid steroidal skeleton in most cases endowed with a hydrophobic and a hydrophilic face.

Due to their peculiar molecular features, BAs in aqueous media show a complex landscape of self-assembled, ordered structures [11]. Tubules [12,13], fibrils [14] and globular [15–18] or elongated micelles [19–21] were extensively reported in the literature. Moreover, BAs are widely used as starting materials in the preparation of synthetic derivatives. By properly choosing the BA and the substituent, it is indeed possible to tune the hydrophobic–hydrophilic balance of the molecule and consequently their self-assembly properties. It follows that it is possible to obtain new assemblies, not formed by the natural BAs, which are therefore of remarkable interest for advanced nanotechnological applications. For example, the functionalization of both the side chain and the tetracyclic system is observed to lead to efficient gelators in organic solvents as well as in aqueous solutions [22], and the possibility to enhance their efficiency by using catanionic mixtures of the derivatives was demonstrated [23], whereas the BAs functionalized in their rigid backbone, self-assemble into lamellae or tubules [24–30]. In particular, the selective substitution of one of the steroid hydroxyl groups by an adamantly group gives derivatives that self-organize in lamellar arrays [24], whereas the introduction of an aromatic residue gives compounds able to self-associate into tubular structures [26–34] showing sometimes unique features such as extremely narrow cross sections [27,28], thermo- [33,34] or pH-responsive aggregations [32] and catanionic controlled compositions [31]. Tubular structures are also formed by BA molecules substituted with monosaccharide residues through an unusual mechanism involving scrolls as intermediates [25], whereas vesicles are given by some Gemini derivatives [35]. In addition, derivatives with specific surfactant and recognition properties are synthesized to be exploited as protein or drug stabilizers [36,37], dispersing agents for carbon nanotubes [38], efficient anion and carbohydrate receptors [39,40], antimicrobial agents [41,42] and drug carriers [43,44].

Hereafter the synthesis and the self-assembly characterization of a BA derivative, labelled by introducing a L-tryptophan (Trp) residue in a cholic acid (HC) molecule, will be described. The substitution of the hydroxyl group in the C-3 position of the BA allowed for the obtainment of the β-L-TrpC derivative (Fig. 1). The β-L-TrpC belongs to a new family of BA-based molecules bearing a hydrophobic amino acid residue on the steroid nucleus [27,28]. In particular, its synthesis and self-assembly characterization follow the one of a tryptophan-substituted deoxycholic acid recently reported [45], thus extending the family of Trp-substituted BA molecules. These derivatives are active to UV, Circular Dichroism (CD) and fluorescence spectroscopies, therefore constituting interesting probes in unravelling the inner working of BAs in biological and nanotechnological systems [46]. We are particularly interested in using them as tracers of the BAs binding to proteins and in their effects on the unfolding of proteins [47].

2. Materials and methods

2.1. Synthesis

The novel β-L-TrpC derivative was prepared according to the synthetic route depicted in Scheme 1. The *N-tert*-butyloxycarbonyl-L-tryptophan and the methyl 3β-amino-7α,12α-dihydroxy-5β-cholan-24-oate **1** were obtained from HC by the procedures reported in the literature [48–50].

All commercially available reagents and solvents were purchased from Sigma–Aldrich with reagent grade and used as received. Dry solvents, namely dichloromethane and methanol, were distilled before use according to standard procedures. Reactions and chromatographic separations were monitored by thin layer chromatography (TLC) on 0.25 mm silica gel plates (Merck Kieselgel 60 F254). Phosphomolybdic acid 12% (w/v) solutions in EtOH, I_2 vapour and UV light (254 nm) were used as revealing agents. Column chromatography was carried out on silica-gel (Merck Kieselgel60, 70–230 mesh, 0.063–0.20 mm). Melting points were determined using a Mettler FP 80 apparatus, interfaced with a Microstar IV microscope. $[\alpha]_D$ values were determined using a JASCO DIP-370 polarimeter (1 cm path length quartz cell). IR spectra of the products were recorded with a Shimadzu IR 470 ($CHCl_3$, 10 mg/mL, 0.5 mm path length NaCl cells) and with a Philips PU-9512 spectrometer (KBr pellet). ^1H and ^{13}C NMR spectra were recorded on a Varian XL 300 Mercury spectrometer using 5 mm tubes and chloroform-*d* ($CDCl_3$), methanol-d_4 (CD_3OD), dimethyl sulfoxide-d_6 ($(CD_3)_2SO$) as solvents. Chemical shifts δ are reported in parts per million (ppm) with residual protons of the deuterated solvent as the reference standard. Signal splitting is described as singlet (s), broad singlet (br s), doublet (d) and multiplet (m). High resolution ESI mass spectra were carried out on a Q-Tof Micro mass spectrometer operating in a positive ion mode. The characterization data of the compounds are reported in the Supplementary information.

2.1.1. Methyl 3β-(2′-(S)-(tert-butoxycarbonyl)amino-3′-indolpropanamido)-7α,12α-dihydroxy-5β-cholan-24-oate (**2**)

Et_3N (0.23 mL, 1.65 mmol) was added to a solution of **1** (406 mg, 1.0 mmol), Boc-Trp-OH (326 mg, 1.0 mmol), HOBt (203 mg, 1.5 mmol) and EDCI (287 mg, 1.5 mmol) in dry CH_2Cl_2 (15 mL) and the mixture was stirred overnight under N_2 atmosphere. CH_2Cl_2 (30 mL) was added to the mixture and the organic layer washed with a 2 M aqueous solution of. citric acid (30 mL × 2), satd aq. $NaHCO_3$ (30 mL × 2), brine (30 mL × 2) and dried over anhydrous Na_2SO_4. After solvent removal under reduced pressure, the crude residue was purified on a silica-gel column eluted with a $CHCl_3$–MeOH 98:2 (v/v) mixture affording the pure **2** as a white solid (602 mg, 85%).

2.1.2. Methyl 3β-(2′-(S)-amino-3′-indolpropanamido)-7α,12α-dihydroxy-5β-cholan-24-oate (**3**)

TFA (2 mL) was added dropwise at 0 °C to a stirred solution of **2** (691 mg, 1.0 mmol) in dry CH_2Cl_2 (18 mL). The mixture was stirred at r.t. for 4 h and then washed with satd aq. $NaHCO_3$ (3 × 25 mL), water (2 × 20 mL) and brine (2 × 20 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Purification on a silica-gel column eluted first with EtOAc and then with MeOH/Et_3N 95:5 (v/v) allowed **3** to be obtained as a white solid (425 mg, 70%).