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Synthesis, characterization and self aggregation of a new *neo*-pentylamide cholic derivative (Na-*n*-penC)

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Abstract

The self-aggregation in aqueous solution of a new *neo*-pentyl amide of the 3- β -amino derivative of cholic acid (Na-*n*-penC) has been investigated in aqueous solution by surface tension and steady state-fluorescence spectroscopy of pyrene (used as a probe). The nature of the aggregates was determined by transmission electron microscopy (TEM) revealing that vesicles are formed. The structure of the compound in the solid state was resolved by X-ray spectroscopy. The synthesis of the compound is also given.

Introduction

Bile salts are natural biosurfactants segregated into the gallbladder by the liver as main components of bile. They play an important role in the digestive process.¹⁻³ Derivatives substituted at the 3-position have shown important biological activity and enhancement of the aggregation properties respect the natural analogues. For example, the new derivatives are used as hypolipemics,^{4,5} MRI agents,⁶⁻⁸ cholesterol-dissolving agents,⁹ antibacterial agents,¹⁰ antifungic agents,¹¹ or ink additives.¹² Other derivatives form coloured gels in alcohols¹³ in the presence of an acceptor, or are linked to crown ethers.¹⁴

Compounds with an amide bond to link the steroid and the substituent group moieties are very attractive because its stability in different media. A typical synthetic routine involves the preparation of the 3- β -aminoderivative of the bile acid (this group being protected as a methyl ester)^{15,16} and its condensation with any acid or acyl chloride. The hydrolysis of the ester bond and neutralization by NaOH are finally

required to obtain the new steroid derivative in salt form (Figure 1). An example of this methodology can be seen in our recent paper concerning the formation of lamellar structures by an adamantyl amide of the 3- β -amino-cholic acid (Na-AdC).¹⁷

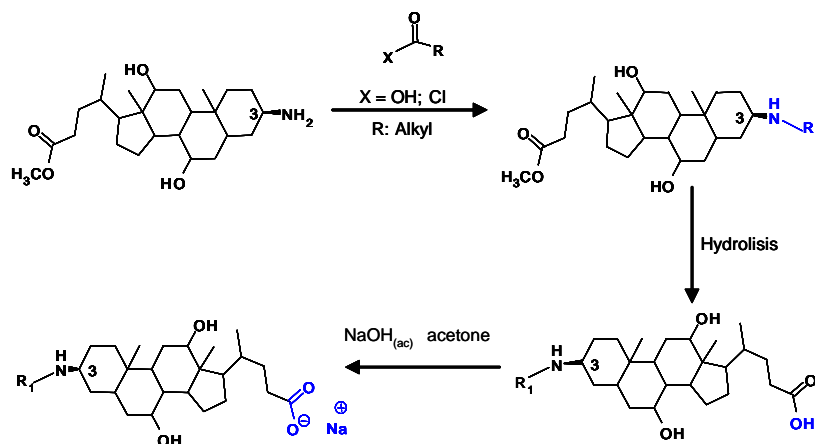


Figure 1.: Synthesis of bile acid derivatives.

The present communication deals with the synthesis, chemical characterization and preliminary study of the self-aggregation behavior of a new *t*-butyl derivative of cholic acid (Na-*n*-penC; Figure 2).

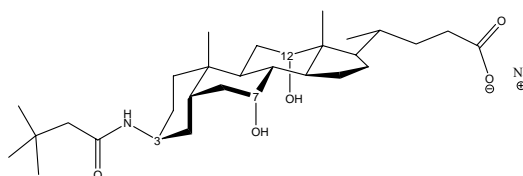


Figure 2.: Structure of Na-*n*-penC.

Experimental

Commercial cholic acid (Sigma-Aldrich) was esterified using the method of Gouin and Zhu.¹⁵ The methyl ester of cholic acid was purified by crystallization from methanol and dried in vacuo at 65°C. The ester was converted in the 3- β -amino derivative by using a modification of the method described by Anelli *et al.*¹⁶ The reaction was carried out in argon atmosphere and THF (dried over sodium/benzophenone).¹⁸ The amine was purified by column chromatography and dried over 8 hours at 65°C in vacuo. The condensation with the *neo*-pentyl chloride was

carried out in dried CHCl_3 under inert atmosphere, in the presence of triethylamine as catalyst. Once the solvent is removed, the product is purified by column chromatography (20:1 ethyl acetate:methanol). The hydrolysis of the ester, and the salt formation was carried out by using procedures described elsewhere.¹⁹ Solutions of Na-tbuC (5 mM and 10 mM) for NMR (one dimension: 1D; two dimensions: 2D) experiments were prepared in D_2O (99,90%, from SDS). Other chemicals were used as provided by Aldrich.

NMR experiments were performed in a Bruker AMX-500 spectrometer (frequency operation ^1H : 500 MHz; ^{13}C : 125 MHz). X ray data were obtained with a Enraf Nonius FR590 at 25°C, diffraction radiation wavelength 0.71069 Å ($\text{MoK}\alpha$). Crystals were obtained from 1:1 acetone/water mixture. Surface tension measurements were made in a K10ST Krüss tensiometer. A Hitachi F3010 fluorimeter was used for fluorescence experiments. TEM images were done at room temperature in a JEOL JEM-1011 instrument, operated at 80 kV, equipped with a MegaView III camera. For the measurements, the samples were prepared by deposition of a drop of a 1mM solution onto carbon-coated copper grids.

Results and discussion.

The product was obtained in 16% (sequence yield) with high purity confirmed by TLC. FAB-Mass spectra confirmed it since a value of $\text{M}+\text{Na}^+$ 550.5 g/mol (theoretical value: 550.71 g/mol) was obtained for the compound.

The partial resolution of the 1-D (^1H , ^{13}C , DEPT) and 2-D (COSY, ROESY, HMQC, HMBC) experiments afforded relevant evidences of the compound structure. Figure 3 shows the principal assignments in the ^1H NMR spectrum. Protons 3, 7, 12, 18, 19, 21 and the methylene and methyl signals from the substituting group were clearly determined by cross peaks analysis of COSY and confirmed by ROESY experiments. Based on these, other proton signals were elucidated.

Previous data, DEPT and HMQC, allowed to assign most of the ^{13}C NMR spectrum (Figure 4). Signal at 187.6 ppm was assigned to the carbonylate group and that one at 177.8 ppm to the amide moiety. HMBC cross peaks are evident from the amide carbonyl and the α -methylene group 26. Other correlations in HMBC were H26-C28, H28-C26, H18-C12, and H18-C17.

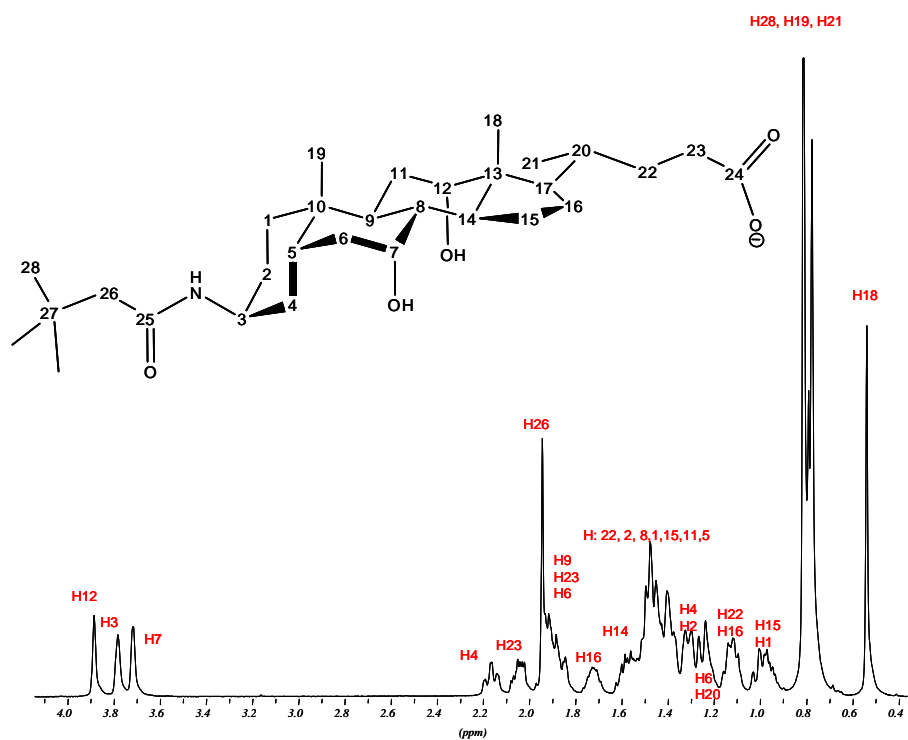


Figure 3: Assignment of ^1H NMR spectrum (principal signals).

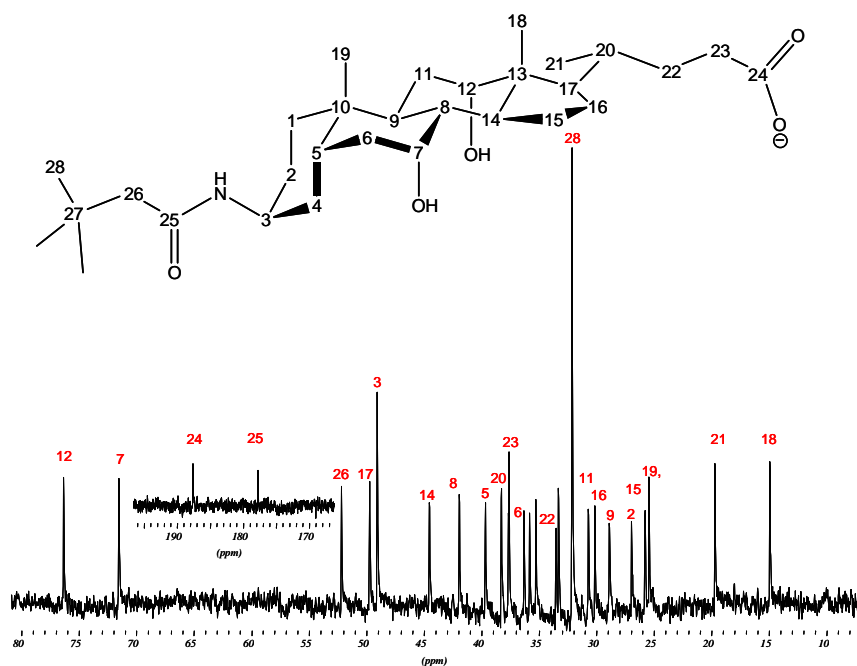


Figure 4: Assignment of ^{13}C NMR spectrum (principal signals).

The crystal confirms the structure of the compound. Table 1 resume the cell parameters of the crystal (colourless) of Na-tbuC in acid form (H-tbuC). Figure 5 resume some significant images of the structure.

Space Group	P212121
Cell Lengths	$a=8.4360(8)$; $b=14.466(3)$; $c=25.164(2)$ Å
Cell Angles	α 90°; β 90°; γ 90°
Cell Volume	3070.89 Å ³
Z,Z'	Z: 4; Z': 0
R-factor	7.04

Table 1: Cell parameters of the H-*n*-penC crystal.

Figure 5 (left) shows the packing of the crystal. Figure 5 (right) shows that the steroid molecules are packed in a back-to-back way evidencing hydrophobic interactions between their β -sides. The hydroxy groups form hydrophilic channels where water molecules are inserted.

The hydrophobic interactions are reinforced by the formation of hydrogen bonds, implying the oxygen and nitrogen atoms of the amide group, the two hydroxyl groups at C7 and C12 and the two oxygen atoms of the carboxylate group of the side chain. Water molecules are also involved in the hydrogen bond network, acting as a bridge between the two hydroxyl groups of the same steroid molecule and having an additional hydrogen bond with the hydroxy group of the carboxylic acid of another molecule.

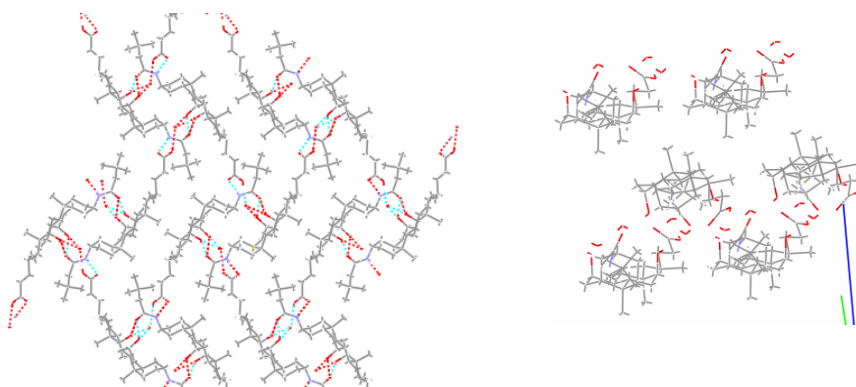


Figure 5: Crystal structure of H-*n*-penC.

The hydrogen bond network evidence two patterns, a ring (Figure 6 left) and a zigzag set (Figure 6 right). The ring involves three steroid molecules and a water molecule linked to the hydroxy group at C12 (O12H) and the hydroxy carboxylic group of two molecules. The ring is closed by the formation of hydrogen bonds of the amide group of the third molecule with former O12H and the carbonyl oxygen of the the

carboxylic group. The zigzag set follows the sequence O12H-water-O7H-O(amide), the hydroxyl groups belonging to the same steroid molecule. The hydrogen bonds simultaneously participate in both patterns.

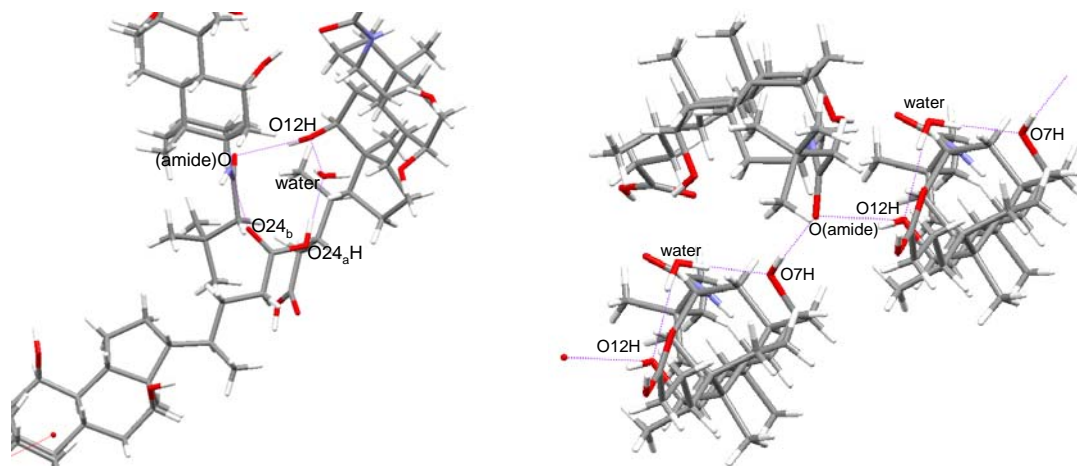


Figure 6. Hydrogen bond patterns. Left: ring implying three molecules. Right: zigzag set.

The self-recognition process between the steroid molecules is different that the observed on for H-AdC. This is due to the different packing of molecules in the crystal mainly due to the bulkier size of the adamantyl group compared with the *neo*-pentyl one. However the α -methylene group linked to the carbonyl amide allows a preferred orientation of the tert-butyl group towards the left side of ring A of the steroid, allowing the formation of a hydrogen bond between the carboxylic group at the side with the amide nitrogen. This is not possible with the adamantyl substituent because of its spherical shape which prevents that hydrogen bond in the H-AdC crystal.

Surface tension measurements (Wilhelmy plate method) show that the plot of γ versus $\ln [\text{Na-}n\text{-penC}]$ is linear until 3.86 mM (Figure 7), reaching a plateau above this concentration. This is the typical behavior for a surfactant in aqueous solution, suggesting that Na-*n*-penC self-aggregates in this solvent. This result was confirmed by fluorescence That critical concentration value is almost 3 times lower than the critical micelle concentration (cmc) of sodium cholate NaC²⁰, indicating that the new compound acts as a better surfactant than the natural bile salt. The value for the critical concentration was confirm from measurements of the ratio I_1/I_3 of the intensities of the vibronic peaks of pyrene I_1 and I_3 . The large limiting value observed at high surfactant concentrations suggest that the aggregated formed is open enough to allow a probe-water interaction.

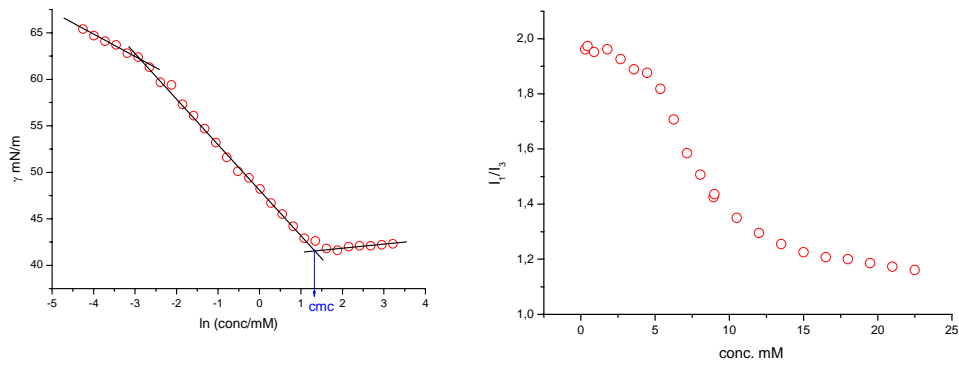


Figure 7: Determination of critical association concentration of Na-*n*-penC by surface tension and fluorescence measurements.

TEM images were obtained to investigate the nature of the aggregates. Typical TEM images are provided in Figure 8. Circular structures with large diameter values (>400 nm) are observed. This shape and the large size of the particles suggest that they probably are vesicles.

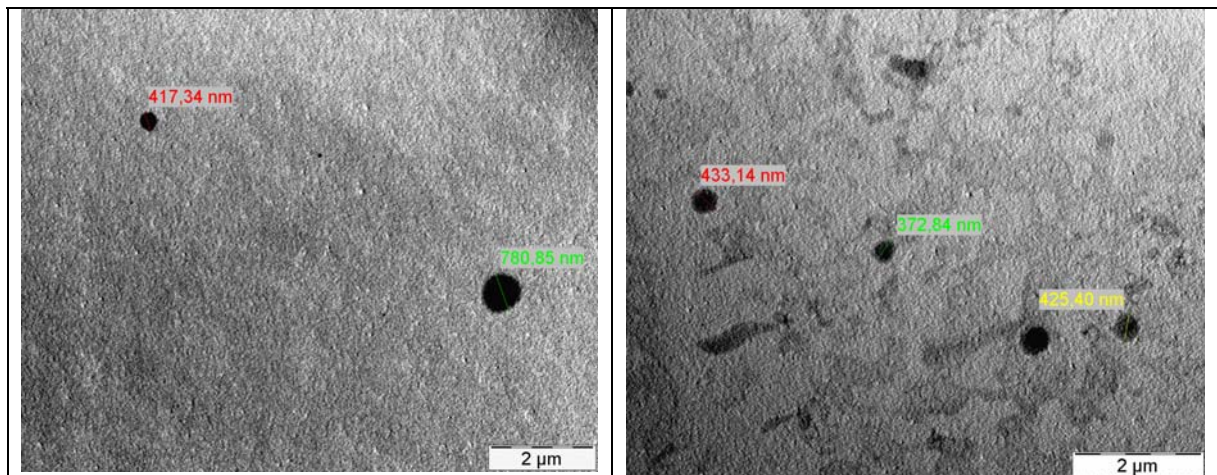


Figure 7: TEM images from a 1mM solution of Na-tbuC.

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