



Spectral and microscopic study of self-assembly of novel cationic spermine amides of betulinic acid



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ABSTRACT

Supramolecular characteristics of two spermine amides of betulinic acid (**1** and **2**) were studied by measuring and evaluating their UV–VIS–NIR spectra in aqueous acetonitrile and DOSY-NMR spectra in tetra-deuteromethanol, accompanied by atomic force microscopy (AFM) images, scanning electron microscopy (SEM) micrographs, and transmission electron microscopy (TEM) micrographs. Fibrous supramolecular self-assembly of **1** and **2** was observed by AFM images, as well as by the SEM and TEM micrographs. Bathochromic shifts of the absorbance maximum at 870 nm to 1015–970 nm in the UV–VIS–NIR spectra were observed with increasing water content in the acetonitrile/water systems, indicating formation of fibrous *J*-type aggregates. Variable temperature DOSY-NMR spectral measurement showed non-linear dependence that also suggests self-assembly behavior of the studied systems. Chiral supramolecular structures were formed by self-assembling due to the chirality of the monomeric molecules. Application of aqueous media during self-assembly procedures is an important factor in the development of targeted drug delivery systems.

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1. Introduction

Supramolecular chemistry has become an important scientific area with a deep impact on different scientific disciplines, ranging from biological chemistry/chemical biology to physical and material chemistry. Areas of potential applications for materials capable of self-assembling under given conditions include drug delivery systems, tissue engineering, neuroscience, cosmetics, materials science, optoelectronics, and other technical areas [1,2]. A number of natural products have been proven to undergo self-assembly into supramolecular systems under specific conditions. The supramolecular systems are formed as a result of non-covalent interactions, e.g., mainly by hydrogen bonding, π - π stacking, van der Waals interactions, charge transfer, electrostatic interactions and also metal chelation [3].

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Among the natural products, triterpenoids represent a large group of plant products with a broad variety of biological activity. Triterpenoid acids display many interesting and important properties. Among them, betulinic acid, (3 β)-3-hydroxylup-20(29)-en-28-oic acid, is a plant product found in different quantity in a number of natural sources, e.g., in genera *Betulla*, *Diospiros*, *Paeonia*, *Syzigium*, or *Ziziphus* [4]. This pentacyclic lupane-type triterpenoid bearing a 6-6-6-6-5 system of annulated cycles displays anti-inflammatory, antimalarial, and antiretroviral activities [5,6]. In cancer therapy, betulinic acid has already been used against a variety of tumor cell lines: malignant brain tumor, primitive neuroectodermal tumor, human chronic myelogenous leukemia, and against the most prevalent human cancer types, such as cervical, prostate, breast, lung, or colorectal cancer [7–10]. The main medical disadvantage of betulinic acid for clinical practice is its poor solubility in aqueous media, i.e., in polar solvents used for screening tests, and, more importantly, in blood serum [11]. To augment the solubility of betulinic acid in aqueous media, we have developed a series of derivatives, mostly amides and derivatives bearing simultaneously amide and ester groups in the molecules [12].

Among them, special attention has been devoted to spermine amides of betulinic acid.

The self-assembly properties of betulinic acid can be augmented by suitable modification so that even supramolecular gels can be obtained. Betulinic acid itself is able to form supramolecular system with organic solvents [13]. The accurate definition of supramolecular gel remains still somewhat unclear, but generally it is argued that the non-covalent self-assembly of low molecular weight gelator (LMWG) molecules resulting in the formation of highly entangled network is able to immobilize bulk solvent. Generally, LMWGs form fibrous aggregates to entrap the solvent molecules through self-assembly appearing as a result of non-covalent forces [14]. The response of supramolecular gels to various stimuli can have considerable potential in developing smart and chiral supramolecular systems for e.g. controlled drug delivery [15]. Numerous methods like X-ray diffraction, small-angle X-ray scattering, liquid and solid state NMR spectroscopy, UV–VIS and UV–VIS–NIR spectrometry, and other analytical methods have been used to study LMWGs and the supramolecular gels formed by them [16,17].

The tetramine spermine [*N,N'*-bis(3-aminopropyl)butane-1,4-diamine] is among the main polyamines found in all living cells [18]. Polyamines are positively charged at physiological pH value and this characteristic is connected with their important role in biological systems. They are able to bind to negatively charged DNA macromolecules, and they generally contribute to any interaction where ionic character is present. They are also able to neutralize the negative charge of phosphates. In animals, spermine appears as a steroid conjugate, squalamine, was first isolated from the tissues of shark, *Squalus acanthias*, in which it functions as a strong antibiotic able to act against both Gram-positive and Gram-negative bacteria, as well as against fungi, and causes osmolysis of protozoa [19,20]. Squalamine and several of its analogues inhibit angiogenesis and act against cancer. During angiogenesis, the tumor cells produce growth factors initiating formation of new blood vessels for providing the tumor with nutrients. Squalamine inhibits the vessel formation, which results in tumor death. A number of squalamine conjugates have displayed antimicrobial effects [21–23]. In addition, polyamines play their role in plants by being involved in their response to abiotic stress [18]. Spermine brings a cationic feature to the target molecules, due to which betulinic acid derivatives can be dissolved in aqueous media [12,24].

Within this study, a strong ability of the spermine amides of betulinic acid to self-assemble into supramolecular systems was detected by various methods. A detailed study was performed of the physico-chemical characteristics of the target compounds using a combination of the UV–VIS–NIR and DOSY NMR measurements, to which AFM images and SEM and TEM micrographs were added. The UV–VIS–NIR spectra were recorded for systems consisting of two miscible solvents (water/acetonitrile) with stepwise change of the solvent ratios. The DOSY NMR spectra, for one, were recorded at several temperatures to determine the non-linear dependence of the diffusion coefficient on the temperature to investigate supramolecular changes in the studied system.

2. Experimental

2.1. Synthesis of **1** and **2**

The synthetic protocol, published earlier [12], including all procedures and analytical data on the target compounds **1** and **2**, as well as their synthetic precursors, was also placed into the [Supplementary Material](#).

2.2. AFM images

The atomic force microscopy (AFM) experiments were performed by using a multimode scanning probe microscope (Nanoscope V, Veeco, USA) on a freshly cleaved highly oriented pyrolytic graphite (HOPG) or mica. The compound (**1** or **2**; 5 mg; Fig. 1) was dissolved in water (0.6 mL), put onto the surface of HOPG or mica, and left to evaporate to dryness. AFM images were obtained by scanning the samples in contact mode (Figs. 2 and 3). The measurements were performed in ambient air. The scanning speed was 0.3 or 0.4 Hz with 512 pixels per line.

2.3. SEM micrographs

The samples were prepared by dissolving the compounds **1** (2.1 mg) and **2** (2.5 mg), respectively, in deionized water (1 mL) and then the clear solution was pipetted on a silicon plate placed on a carbon tape over the sample stub. After drying in a desiccator for several days the samples were coated with gold in a JEOL Fine Coat Ion Sputter JFC-1100. Scanning electron micrographs were taken with a Bruker Quantax400 EDS microscope equipped with a digital camera (Figs. 4–7).

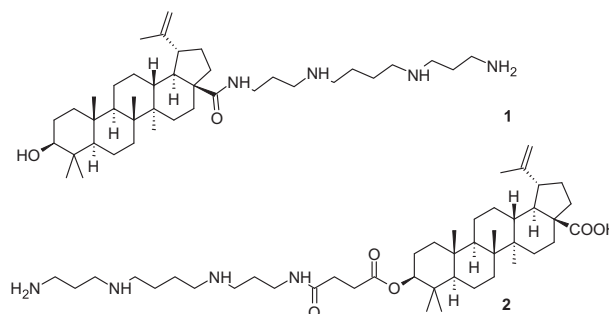


Fig. 1. Structures of compounds **1** and **2**.

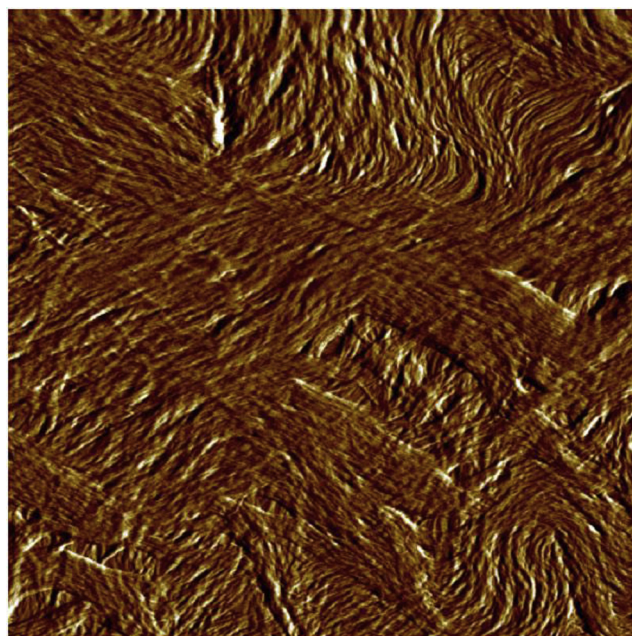


Fig. 2. AFM image of the fibrous supramolecular system formed by compound **1** (15 × 15 μm, 0.4 Hz).

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