Materials Science and Engineering C 38 (2014) 143–150

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

# Amphiphilic macrocycles bearing biofragment: Molecular design as factor controlling self-assembly

L.Ya. Zakharova *, V.E. Semenov, V.V. Syakaev, M.A. Voronin, D.R. Gabdrakhmanov, F.G. Valeeva, A.S. Mikhailov, A.D. Voloshina, V.S. Reznik, Sh.K. Latypov, A.I. Konovalov

*A.E. Arbuzov Institute of Organic and Physical Chemistry of the Russian Academy of Sciences, 8, ul.Akad. Arbuzov, Kazan 420088, Russia*

ARTICLE INFO

*Article history:*
Received 8 October 2013
Received in revised form 26 January 2014
Accepted 30 January 2014
Available online 7 February 2014

*Keywords:*
Uracilophane
Amphiphile
Self-assembly
Geometry and energetic factors

ABSTRACT

Two novel macrocyclic 6-methyluracilic amphiphiles (uracilophanes) with four (**UP1**) and two (**UP2**) uracil moieties and ammonium groups have been synthesized. Tetracationic multi-uracilophane is composed of two macrocyclic units bridged each other with an external methylene spacer, while in the cryptand-like dicationic uracilophane pyrimidinic moieties are connected with an internal methylene spacer. This internal spacer provided a conformational rigidity to the macrocycle. The self-assembly of the uracilophanes is studied and compared with a reference dicationic uracilophane (**UP3**) with no spacer fragment. Compounds **UP1** and **UP3** are capable of aggregating, which is characterized by the analogous critical micelle concentration of 1 mM, although the former has four decyl tails versus two decyl tails in **UP3** molecule. NMR self-diffusion, fluorimetry and DLS techniques revealed that bimodal size distribution occurs in the **UP1** solution, with small (≤2 nm) and large (ca. 30–50 nm) aggregates contributed. Unexpectedly, the cryptand-like uracilophane **UP2** with the same hydrophobicity as **UP3** does not form aggregates. The balance of the geometry and energetic factors was analyzed and compared with those contributing to the aggregation of the reference compound **UP3**. It was established that it is the geometry that controls the packing of the cryptand-like uracilophanes upon aggregation, while hydrophobic effect plays a minor role. In contrast, both factors control the aggregation of oligomeric macrocycle, with energetic factor prevailing. These findings are of importance for (i) the understanding the diverse structural behavior of bioamphiphiles that have very similar chemical structure, but different conformations; and (ii) the design of amphiphiles with controlled model of self-assembly. Supramolecular systems studied can be recommended for biotechnological applications.



## 1. Introduction

Amphiphiles are related to biomimetic compounds [1,2], and therefore their investigation may provide insight into the elementary mechanism of structural behavior of their prototypes, lipid molecules. The exploration of amphiphiles bearing biological fragments (i) further emphasizes the biomimetic aspect of these systems and (ii) bring them into focus of food industry and biotechnology [3]. Pyrimidinic moiety is a component of nucleotide bases, i.e. structural units of DNA. Therefore, on the one hand, the self-organization of pyrimidinic amphiphiles is of interest from the viewpoint of the DNA–bioamphiphile interactions [4], while on the other, it may be of practical importance for the rational design of nonviral vectors for gene delivery. The presence of pyrimidinic bases, in particular nucleotide bases makes it possible to involve complementary interactions and stacking effect into self-assembling behavior and binding mechanism [5,6]. The combination of pyrimidinic fragment, cationic groups and hydrophobic tails in a molecule is an effective approach for the design of carriers capable of complexing the polyanionic biospecies and their transferring through the cell membrane. Meanwhile, pyrimidine-based amphiphiles are scarcely studied [7,8]. In our recent works, cationic surfactants with natural fragments were explored as nanocontainers for model guests and nucleic acids [9–11]. Role of the hydrophobicity, the nature of counterions and morphological behavior in their binding capacity toward oligonucleotide and integration with lipid bilayer have been elucidated. The introduction of macrocyclic scaffold may markedly change the so-called packing parameter, association model and therefore, morphology of aggregates [12–16]. For instance, the formation of large particles based on macrocyclic pyrymidinic bolaamphiphiles instead of micelle-like aggregates was documented [17]. Importantly, macrocyclic platform have attracted much attention in recent works [18–24] as advanced building block for nanotechnological applications and biomedicine, e.g. as synthetic ion channels.

Herein self-assembling behavior of novel amphiphilic macrocycles bearing nucleotide base derivative, namely 6-methyluracil moieties

* Corresponding author at: 8, ul.Akad. Arbuzov, Kazan 420088, Russia. Tel.: +7 843 2 73 22 93; fax: +7 843 2 73 22 53.
*E-mail address:* lucia@iopc.ru (L.Y. Zakharova).

0928-4931/$ – see front matter © 2014 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.msec.2014.01.057

(uracilophanes **UP1** and **UP2**) covalently bound with an external (**UP1**) or internal (**UP2**) methylene spacer is reported (Fig. 1). Unbound uracilophane (**UP3**) studied earlier [17] is used as a reference compound. In these uracilophanes, topology is varied by introduction of the intra- or intermolecular methylene spacers between $C^5$ of uracil units. Actually, uracilophane **UP3** is a conformationally labile compound in solutions, and introduction of internal methylene spacer between uracil moieties significantly limits a set of conformations [25]. Coupling of two molecules of "monomeric" uracilophanes affords nanoscale multiuracilophane with self-assembling behavior in aqueous solution different from "monomeric" counterparts **UP3** and **UP2**. In our opinion, this series of amphiphilic uracilophanes presents the opportunity to determine a contribution of geometry and energetic factors to the aggregation of the uracil based amphiphiles in aqueous solution. Moreover, to the best of our knowledge this is the first example of self-assembly of amphiphilic multimacrocycle, in particular multiuracilophane compared with its "monomeric" units. This type of macrocycles, i.e., multimacrocycles, are of interest, since they are expected to possess unique inclusive, aggregative and sensing properties due to the presence of several proximate cavities [26].

The following points were addressed upon the design of these compounds: (i) the above mentioned biomimetic nature; (ii) the multimacrocyclic structure of molecules favoring the multicentered electrostatic interactions; (iii) analysis of the association model and aggregation capacity, with the geometry and energetic factors compared.

## 2. Materials and methods

### 2.1. Synthesis

The details of synthesis of uracilophanes **UP1**–**UP3** are given in the Supplementary material section.

### 2.2. General procedure for NMR experiments

All NMR experiments were performed on a Bruker AVANCE-600 spectrometer operating at 600.1 MHz for the $^1$H. The spectrometer was equipped with a Bruker multinuclear z-gradient inverse probe head capable of producing gradients with strength of 50 G cm$^{-1}$. All experiments were carried out at 30 ± 0.2 °C. Chemical shifts were reported relative to HDO (4.70 ppm) as an internal standard. The description of the technique and some details of NMR self-diffusion experimental and post processing are given in Supplementary data section and in Refs. [27–30].

### 2.3. Surface tension measurements

Surface tension was measured using the du Nouy ring detachment method. Based on the surface tension isotherms, quantitative parameters characterizing the adsorption of uracilophanes at the air/water interface and their micellization were estimated. The surface excess $\Gamma_{max}$ and the surface area per a molecule, $A_{min}$, have been calculated using the Gibbs equation:

$$\Gamma_{max} = \frac{1}{2.3nRT} \lim_{C \to cmc} (d\pi / d\log C) \quad (1)$$

$$A_{min} = 10^{18}/(N_A \times \Gamma_{max}) \quad (2)$$

were $R = 8.31$ J mol K$^{-1}$ (gas constant), π is the surface pressure obtained from the surface tension of water minus the surface tension of the surfactant solution, and $T$ is the absolute temperature in K. $N_A$ is Avogadro number ($6.02 \times 10^{23}$ mol$^{-1}$). The parameter $n$ represents the number of species at the interface the concentration of which changes with surfactant concentration. The constant $n$ takes the value 2 for an ionic surfactant where the surfactant ion and the counterion are univalent, while the values 3 or 5 are taken for the dicationic and tetracationic surfactants respectively. The standard free energy of micellization per mole of a monomer unit and the standard free energy of interfacial adsorption at the air/saturated monolayer interface were evaluated by applying Eqs. (3) and (4).

$$\Delta G_m = (1 + \beta)RT\ln(cmc) \quad (3)$$

here β is the degree of counterion binding to aggregates;

$$\Delta G_{ad} = \Delta G_m - (\pi_{KKM}/\Gamma_{max}). \quad (4)$$

### 2.4. Electrode potential measurements

The counterion binding (β) was measured by potentiometry. The Nernst equation is known to describe the relation between the electrode potential (Δ$E$) and the activity of bromide ion ($a_{Br}$):

$$\Delta E = -\frac{RT}{F} \log(a_{Br}) + const \quad (5)$$

where $F$ is the Faraday constant and the ideal slope ($RT/F$) is 59.2 mV/equiv at 298.2 K. The measurements were performed using an ion meter I-160MI, with a Br-selective electrode ELIS-131Br and a

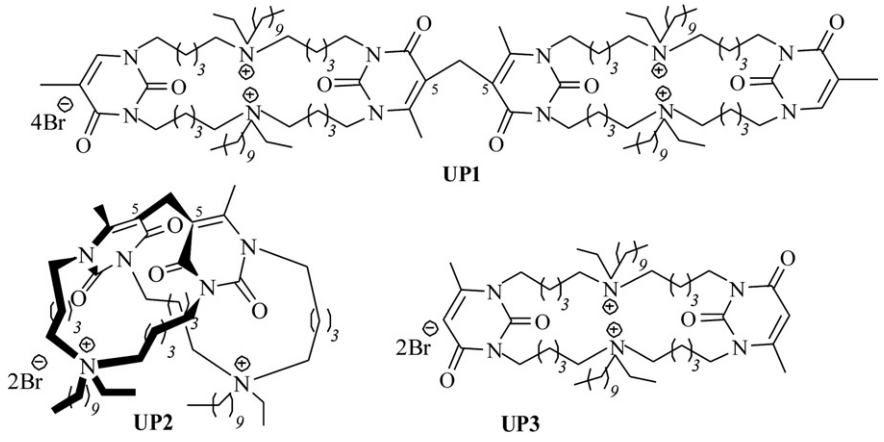


**Fig. 1.** Chemical structures of multiuracilophane **UP1**, cryptand-like uracilophane **UP2** and "monomeric" uracilophane **UP3**.