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Hydrazides of glycine-containing decasubstituted pillar[5]arenes: synthesis and encapsulation of Floxuridine

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ABSTRACT

Hydrazides of glycine-containing decasubstituted pillar[5]arenes were synthesized and characterized. Dynamic light scattering (DLS) and transmission electron microscopy (TEM) showed that self-assembly into monodisperse spherical nanoparticles (28 nm) was typical in water for pillarene hydrazides containing glycylglycide fragments (1×10^{-3} M). Binding of the antitumor drug Floxuridine in water by the substituents of the macrocycle was established by NMR spectroscopy. It was shown by DLS and TEM, that heating of the macrocycle-Floxuridine system in a 1:1 ratio at 1×10^{-4} M led to its self-organization into monodisperse spherical particles 132 nm in diameter.

Introduction

The problem of increasing efficacy of antitumor therapy has recently become acute due to the growth in the number of oncological diseases [1]. Surgical therapy, chemotherapy and immunotherapy are the main methods used in cancer treatment [2a] yet the high toxicity, low bioavailability, poor water solubility, low therapeutic indices, and non-targeted delivery of antitumor drugs to cancer cells remain the main disadvantages of antitumor therapy [2b]. In this regard, development of new water-soluble molecular capsules capable of increasing the solubility and reducing the toxicity of antitumor drugs is an urgent task for supramolecular chemistry to address [2c]. Supramolecular complexes based on such receptors can be applied to a number of problems related to bioavailability and targeted action of the therapeutic agents [2]. In the last decade [3], several macrocyclic structures have been developed and successfully incorporated in supramolecular drug delivery systems [4]. However, poor aqueous solubility, which significantly narrows the therapeutic window, remains a main problem in the application of macrocyclic hosts [3]. Amphiphilic macrocycles capable of association and aggregation are considered to be the main solution to the above problem [5]. Therefore, we propose to use water-soluble decasubstituted pillar[5]arene derivatives incorporating fragments of glycine. It is well known [6] that structural proteins such as elastin and collagen contain a high proportion of glycine residues and that collagen fibrils are components of the intercellular matrix which binds cells in tissues [6b]. In this work, we report the first example of the use of water-soluble pillar[5]arene derivatives containing peptide fragments as self-assembling biomimetic systems [7] for drug delivery.

Previously, we developed a technique [8] to introduce glycine fragments into the pillar[5]arene structure. Macrocycles **3** and **4** were obtained by hydrazinolysis, in yields of 78% and 82%, respectively, from decaethers **1** and **2**. Hydrazide fragments were employed as they may allow the target macrocycles to form additional intra- and intermolecular hydrogen bonds [6c]. The polarity of the amide groups and the ability to protonate the terminal amino groups will increase the solubility of the substituted macrocycles in water.

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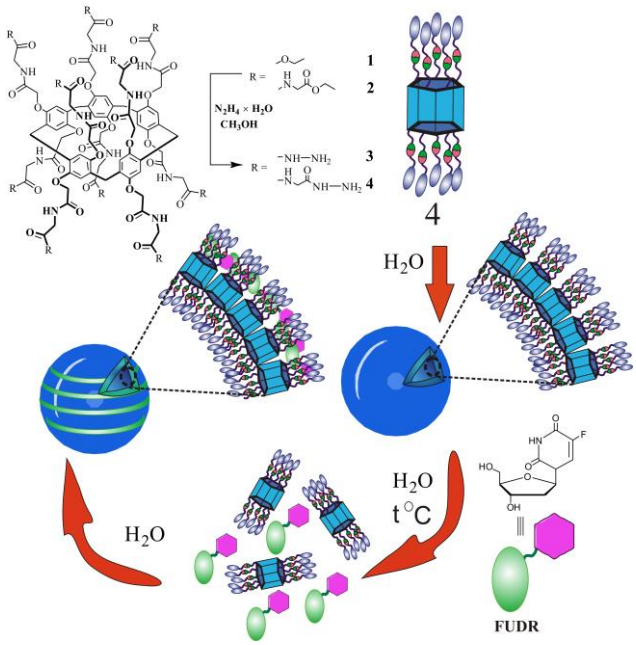


Figure 1. Synthesis of the macrocycles **3** and **4**: the sketch represents pillar[5]arene **4** self-assembly and self-organization of the **4** / **FUDR** system into monodisperse spherical particles

The reactions were carried out in a 3:1 mixture of methanol / DMF at ambient temperature for 72 hours. Then self-association of macrocycles **3** and **4** and their aggregation with the antitumor drug 5-Fluorouracil (**5-FU**) and its derivative 5-fluoro-2'-deoxyuridine (Floxuridine) (**FUDR**) were studied. These drugs are used to treat colorectal cancer, liver and stomach cancer [9], but their high toxicity causes a large number of side-effects, e.g. mouth ulcers, nausea, vomiting, hair loss, stomach ulcers, yellowing of the skin and eyes.

Self-association studies of macrocycles **3** and **4** were carried out in water using DLS. Introduction of ten acetohydrazide fragments, in the case of **3**, appeared insufficient for dissolution of the macrocycle in water but the introduction of ten acetamidacetohydrazide fragments (**4**) led to formation of a homogeneous aqueous system. Macrocycle **4** self-associated in water in a concentration range of 1×10^{-3} to 1×10^{-5} M with monodisperse associates (PDI = 0.11, hydrodynamic diameter of 27.5 nm) were observed at 1×10^{-3} M. According to the TEM data, self-associated aggregates of **4** were tightly packed and characterized by a spherical shape with an average particle diameter of 28 nm (Fig. 2a and 2c).

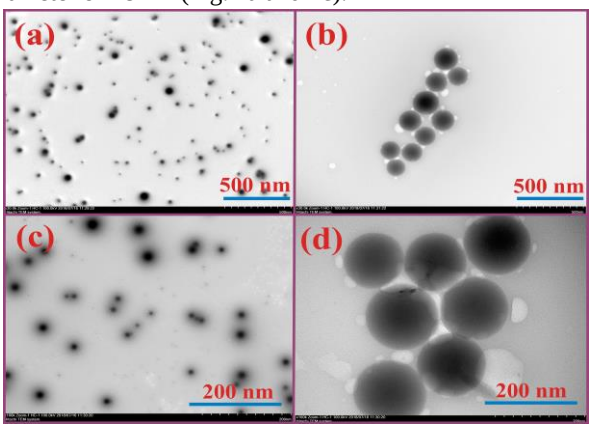


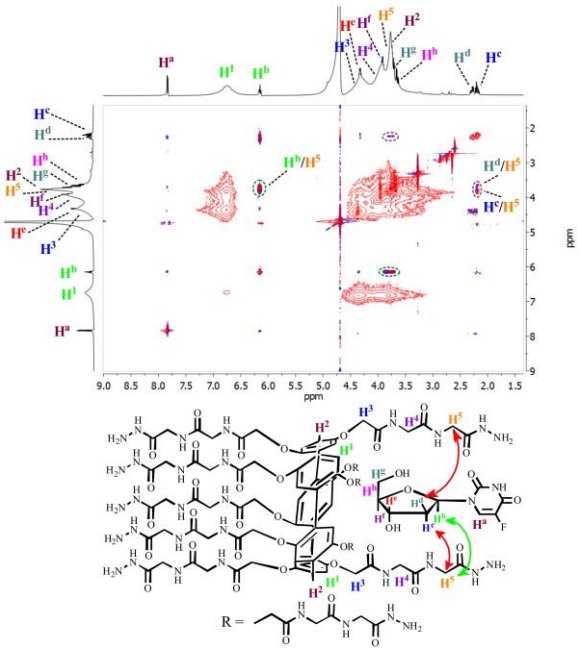
Figure 2. TEM images of pillar[5]arene **4** self-associated aggregates ((a) and (c)) and of pillar[5]arene **4** / **FUDR** associates ((b) and (d)) in water (1×10^{-3}M). (a) and (b): 500 nm scale, (c) and (d): 200 nm scale.

The interaction of pillar[5]arene **4** with **5-FU** and **FUDR** was studied by DLS, UV-vis and ^{1}H, ^{13}C, 2D NMR spectroscopy. No shifts of the absorption maxima were observed after the addition of the macrocycle **4** to 5-**FU** or **FUDR** by UV-vis spectroscopy. In the case of **FUDR**, a hypochromic effect in the region of 295 nm was found. The phenomena are complicated by light scattering, resulting in a rising baseline, making interpretation of the UV-vis data more difficult. This might be due to the aggregation of **FUDR** with pillar[5]arene **4**.

The DLS investigations of the aggregates showed that a polydisperse system was formed from the **4** / **FUDR** system in ratios of 1:1, 1:5, 1:10, 5:1, and 10:1 over a concentration range of 1×10^{-3} to 1×10^{-5} M. However, monodisperse (PDI = 0.11) aggregates with a hydrodynamic diameter of 131.8 nm were formed for both compounds after 30 min of heating the **4** / **FUDR** system (1:1 ratio, 1×10^{-4} M)) at 50 °C (ESI). Thus, self-organization of the pillar[5]arene **4** with **FUDR** into monodisperse particles was observed only if the mixture was heated. With the concentration increased to 1×10^{-3} M and the ratio of the macrocycle **4** / **FUDR** changed to 1:5, 1:10, 5:1, and 10:1, the aggregate diameter increased to 268.8 nm and PDI sharply to 0.50. A polydisperse colloidal system was formed at a concentration of 1×10^{-2} M for the 1:1 mixture of **4** and **FUDR**.

Unfortunately, the study of the interaction of macrocycle **4** with **FUDR** by ^{1}H NMR spectroscopy did not allow the nature of the interaction to be determined because of the changing position of the chemical shifts assigned to the guest and host. This is mainly due to signal overlap and broadening spectral lines from the formation of the association of macrocycle **4**. Therefore, 2D ^{1}H-^{1}H NOESY NMR (Fig.3) and 2D DOSY spectroscopy and as ^{19}F NMR spectroscopy of the antitumor drugs, alone and in mixtures with macrocycle **4** were used to confirm the formation of the associate.

Figure 3. The 2D ^{1}H-^{1}H NOESY NMR spectrum of the **4** / **FUDR** complex (1:1, 1×10^{-3} M) in D_2O at 25 °C and the proposed structure of the complex.



Cross-peaks between the $\mathbf{H^5}$ protons (methylene groups of acetohydrazide fragments) of pillar[5]arene **4** and $\mathbf{H^b}$, $\mathbf{H^d}$ and $\mathbf{H^c}$ protons of the deoxyribose fragment of **FUDR** were observed in the 2D ^{1}H-^{1}H NOESY NMR spectrum of the 1:1 mixture of **4** and **FUDR** (1×10^{-3} M) (Fig. 3). The formation of the **4** / **FUDR**

associate was additionally confirmed by 2D DOSY spectroscopy (ESI). Diffusion coefficients of 1×10^{-3} M **4**, **FUDR** and **4** / **FUDR** were determined at 298 K (ESI). The DOSY spectrum of the 1:1 mixture of **4** and **FUDR** (1×10^{-3} M) showed the presence of only a single species of particles with a diffusion coefficient below that of the macrocyclic ligand **4** and the antitumor drug **FUDR**. A significant decrease in the diffusion rate of **4** / **FUDR** is an additional argument in favor of the formation of stable associates between **4** and **FUDR**.

According to the TEM data, associates of pillar[5]arene **4** with **FUDR** are characterized by a spherical shape and average diameter of 130 nm (Fig.2b and 2d.). No cross-peaks in the 2D ^{1}H-^{1}H NOESY NMR spectrum between the protons of macrocycle **4** and proton (δ = 8.02 ppm) of the fluorouracil fragment in **FUDR** was observed. This made it possible to propose the formation of the associate due to hydrogen bonds between the fragments of deoxyribose in **FUDR** and the amide and hydrazide groups of the pillar[5]arene **4**. The absence of significant displacements of the **FUDR** proton signals in the ^{1}H NMR spectrum indicated that the substrate molecules did not enter the hydrophobic cavity of the macrocycle **4** [10a]. Studying the ^{19}F NMR spectra with proton decoupling of **5-FU**, **FUDR** and **4** / **5-FU** and **4** / **FUDR** mixtures is a convenient way to confirm this hypothesis because **5-FU** and **FUDR** molecules contain a single fluorine atom that is detected by ^{19}F NMR spectroscopy. If **5-FU** and **FUDR** molecules are included in the cavity of pillar[5]arene **4**, the fluorine atom is shielded and its signal shifts to a strong field [10 b, c]. Study on the **4** / **5-FU** system in a 1:1 ratio (1×10^{-3} M) showed no displacement of the ^{19}F signals vs. initial value of the fluorine signal of **5-FU** (Δδ ~ 0.02 ppm) (Fig. 4). However, in case of the **4** / **FUDR** system (1:1 ratio, 1×10^{-3} M), the fluorine signal was shifted by Δδ ~ 1.30 ppm (Fig.4) against that in free **FUDR**. Based on this, it can be concluded that the downfield shift of the fluorine signal in the system of **4** / **FUDR** indicates no inclusion of the **FUDR** molecule in the cavity of the macrocycle **4**. De-shielding of the fluorine atom is caused by formation of the hydrogen bonds of the deoxyribose moiety and fluorine atom with the amide groups of pillar[5]arene **4**.

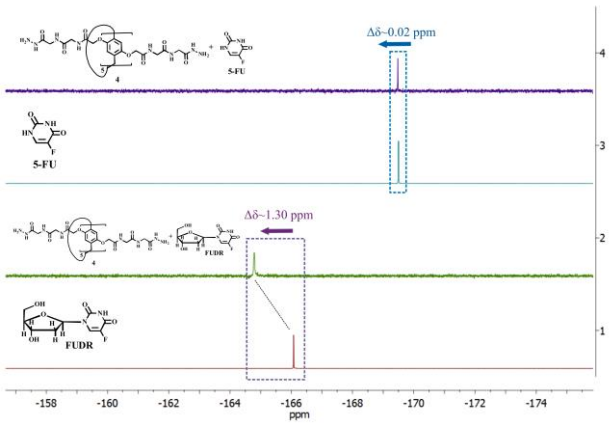


Figure 3. ^{19}F NMR spectra with proton decoupling (1×10^{-3} M) in D_2O at 25 °C: 1) **FUDR**; 2) **4** / **FUDR** in the 1:1 ratio; 3) **5-FU**; 4) **4** / **5-FU** in the 1:1 ratio.

Further confirmation of this hypothesis was found in the additional study of the 2D ^{1}H-^{1}H NOESY NMR spectrum of the 1:1 mixture of **4** and deoxyribose (1×10^{-3} M) (ESI). Cross-peaks between protons $\mathbf{H^5}$, $\mathbf{H^4}$ and $\mathbf{H^3}$ (the methylene groups of acetohydrazide and acetamide fragments) of pillar[5]arene **4** and the deoxyribose protons in the 2D 1H-1H NOESY NMR spectrum of the 1:1 **4**/deoxyribose mixture (1×10^{-3} M) undoubtedly indicate formation of the **4**/deoxyribose associate by intermolecular hydrogen bonds between amide fragments of pillar[5]arene **4** and deoxyribose protons. The orientation of the **FUDR** molecule inside pillar[5]arene **4** was studied by computational methods and found to be in agreement with the NMR interpretation (ESI).

A series of experiments to assess the viability of cells under treatment with pillar[5]arenes **3** and **4** was carried out. Survival was determined after incubation of the cells with the pillar[5]arenes for 24, 48 and 72 h. In the samples, cells survival was expressed in relative units against the control with no pillar[5]arenes. In the study of compound **3** (25 and 50 μg / ml), an appropriate amount of deionized water was added to the control. Compound **3** appeared to be more toxic than **4** with a decreased survival at a concentration of 25-50 μg/ml. Pillar[5]arene **4** (250 and 500 μg/ml) was non-toxic throughout the 24 h treatment. A small concentration-dependent viability suppression was found for incubation times of 48 h and 72 h. In general, the toxicity of **4** is weak even at high concentration (ESI).

In conclusion, water-soluble decasubstituted pillar[5]arenes containing hydrazide fragments of glycine and glycylglycine were synthesized for the first time. DLS showed that 1×10^{-3} M pillar[5]arene **4** containing acetamidacetohydrazide fragments formed monodisperse associates in aqueous solution with a PDI of 0.11 and a hydrodynamic diameter of 27.5 nm. Association of macrocycle **4** with the antitumor drug 5-fluoro-2'-deoxyuridine (**FUDR**) was demonstrated by DLS, UV-vis and ^{1}H, ^{13}C, 2D NMR spectroscopy. As a result of 1:1 association, monodisperse nanosized particles with a PDI of 0.11 and a hydrodynamic diameter of 131.8 nm at 1×10^{-4} M were formed by both compounds. The formation of nano-sized particles with a diameter of 130 nm was shown by TEM. The formation of the **4** / **FUDR** associates occurred as "head to head" species with the fluorine atom directed towards the macrocyclic cavity of the pillar[5]arene and the deoxyribose **FUDR** fragment outwards as followed from the 2D ^{1}H-^{1}H NOESY and ^{19}F NMR spectroscopy.

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Supplementary Material

Supplementary data associated with this article can be found in the online version, at