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Towards new nanoporous biomaterials: self-assembly of sulfopillar[5]arenes with vitamin D_3 into supramolecular polymers

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Novel water-soluble, deca-substituted pillar[5]arenes containing thiosulfate and thiacarboxylate fragments were synthesized and characterized. UV-vis, 2D ^{1}H-^{1}H NOESY and DOSY NMR spectroscopy revealed the ability of pillar[5]arenes containing thiasulfate fragments to form an inclusion complex with cholecalciferol (vitamin D_3) in a 1: 2 ratio (lgK_{ass} = 2.2). Using DLS and SEM it was found that upon concentration and/or evaporation of the solvent, the supramolecular polymer (pilar[5]arene/vitamin D_3 (1: 2)) forms a porous material with an average wall diameter of 53 nm. It was shown that the supramolecular polymer is stable during photolysis by UV radiation ($k_1 = 1.7 \times 10^{-5}$ s^{-1}).

Introduction

The D vitamins D (vitD) are indispensable for human health.[1a] The most important vitDs are two fat-soluble forms: cholecalciferol (vitamin D_3, $vitD_3$) and ergocalciferol (vitamin D_2, $vitD_2$).[1b, c] $VitD_3$ is the main physiologically active form and is found in a range of foods including egg yolk, oily fish meat and fish oil.[1c] $VitD_2$ is of plant origin and is present in small amounts in some mushrooms. $VitD_2$, being less active than $vitD_3$, is rarely present in commercial preparations and fortified foods.[1d, e] It should be noted that $vitD_3$ is mainly obtained from the residual endogenous synthesis of 7-dehydrocholesterol in human skin after exposure to the sun.[1c] Vitamins of the group are used to prevent or treat rickets in children, osteomalacia, osteoporosis, bone fractures and secondary hyperparathyroidism in adults.[1e] In the literature there are examples of the use of vitD in the treatment of cancer [1f], diabetes [1g] and cardiovascular diseases [1h].

In the modern world, due to insufficient sun exposure, vitD deficiency is a significant problem.[1c] Fortunately it can be addressed through the use of vitamin supplements in food, or application to the skin, to maintain the recommended level of vitD in the blood (30-60 $ngml^{-1}$).[1] However, the low solubility of vitD in water and the high activity of $vitD_3$ associated with a constant intake of vitamin supplements can lead to hypervitaminosis of vitD, and then to hypercalcemia and hypercalciuria. [2a]

To mitigate against these effects the use of drug delivery systems (DDS) to increase the bioavailability, water solubility and controlled release of vitD has been proposed [1c] Among vitD delivery systems, those consisting of polymer,[2b] lipid,[2c] surfactants[2d] nano- or micron sized particulates[2e] are used. A common disadvantage of these systems is the complexity of the preparation and, as a rule, the toxicity of the DDS components themselves. Another disadvantage of polymer DDS is the poor protection of the encapsulated vitD from ultraviolet radiation, which makes it impossible to apply vitD to the skin.[2f]

A special place among DDS is occupied by systems based on macrocyclic compounds. The presence of a macrocyclic cavity of a preorganized structure makes these compounds universal components in DDS. However, due to the complicated preparation and low selectivity of vitD binding, macrocyclic receptors are practically not used in vitD delivery systems.[1c, 2g, h]

In recent years, researchers have been particularly interested in macrocyclic systems based on pillar[5]arenes, which consist of units of hydroquinone linked by methylene bridges in para positions.[3] Unlike other classes of macrocyclic compounds, pillar[5]arenes have several advantages, such as: ease of preparation and functionalization of the macrocyclic platform [4a, b], controlled size of the macrocyclic cavity by varying the length of substituents,[4c, d] low toxicity,[4e] the ability to dissolve in water.[4f] Due to their unique characteristics, pillar[5]arenes can act as components of $vitD_2$ delivery systems.[5a] Thus, Diao et al.[5b] showed the formation of suprastructures between the pillar[5]arenes and the volumetric, lipophilic β-carotene interacting between the host and the guest.

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In this article, we report on the first example of the use of water-soluble derivatives of pillar[5]arenes as self-assembled biomedical delivery systems vitD$_3$.

Results and discussion

It has been shown[6] that macrocyclization of 1,4-bis(2-bromoethoxy) benzene proceeds to the pillar[5]arenes platform with a good yield, where the bromoethoxyl fragment is able to react with thiols under soft conditions and with high yields.[4f] In this regard, we obtained compound **1** (Fig. 1) from commercially available reagents according to the literature method.[6b, c] Then macrocycle **1** was reacted with 2-mercaptoethanesulfonic acid sodium salt and 2-mercaptoacetic acid. 2-Mercaptoethanesulfonic acid sodium salt and 2-mercaptoacetic acid were selected as commercially available bifunctional reagents, to extend the macrocyclic pseudo-cavity of the pillar[5]arenes by increasing the length of the substituents,[4c, d] as well as to make the final products soluble in water due to the carboxylate and sulfate groups. The reaction was carried out in DMF in the presence of NaH at a temperature of 90 ° C for 12 hours (Fig. 1, ESI,† S2- S5). The yields of the target macrocycles **2** and **3** were 90% and 61%, respectively. The structures of the obtained products **2** and **3** were confirmed by ^{1}H NMR and ^{13}C, IR spectroscopy and mass spectrometry. Their compositions were confirmed by elemental analysis (ESI,† S2-S5).

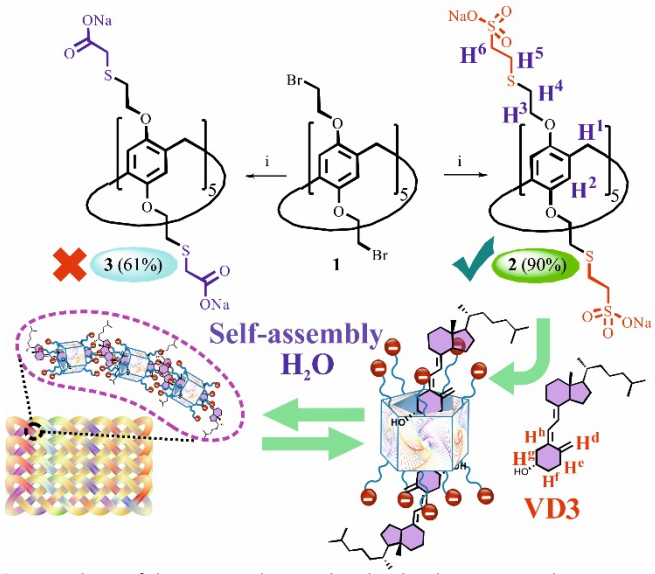

Fig. 1 Synthesis of the macrocycles **2** and **3**: the sketch represents the estimated structure of the complex **2**/vitD$_3$ and self-assembly of complex **2**/vitD$_3$. Reagents and conditions: i – 2-mercaptoethanesulfonic acid sodium salt (2-mercaptoacetic acid), NaH, DMF, 100 °C.

The ability of macrocycles **2** and **3** to interact with vitD$_2$ and vitD$_3$ in water was subsequently studied by UV-vis, ^{1}H 2D NMR spectroscopy and dynamic light scattering (DLS) (Fig. 1). Due to the poor solubility of vitD$_2$ and vitD$_3$ in water, the UV-vis experiments were carried out in H_2O/C_2H_5OH (100:1) by dispersing an alcoholic solution of vitD$_2$ and vitD$_3$ in water (4×10^{-5} M). Only during the interaction of macrocycle **2** with vitD$_3$ were the spectral changes significant, which made it possible to establish quantitative characteristics of the binding. The study of complexation by the isomolar series method made it possible to establish a 1:2 stoichiometry for the **2**/vitD$_3$ system (ESI,† S6). The association constant was determined based on spectrophotometric titration of a system in which the concentration of macrocycle **2** (3.33×10^{-6}- 4×10^{-5} M) was varied at a constant concentration of vitD$_3$ (4×10^{-5} M) (ESI,† S6). The linearization of the curve using $lg(C_{complex}/C_{guest})$ - $lg(C_{host})$ allowed us to calculate a lgK_{ass} of 2.2 (ESI,† S6).

We selected 2D ^{1}H-^{1}H NOESY and 2D DOSY NMR spectroscopy methods to confirm the formation of the **2**/2 vitD$_3$ complex and its structure. Analysis of the experimental data obtained from ^{1}H NMR spectroscopy did not allow us to determine the nature of the interaction through chemical shifts of the guest and the host since vitD$_3$ did not dissolve in the necessary concentration (5×10^{-3} M). However, in the presence of pillar[5]arene **2**, vitD$_3$ dissolved in D_2O/CD_3OD, which made it possible to prepare the **2**/vitD$_3$ system in a stoichiometric ratio of 1:2 (5×10^{-3} M). Cross peaks between the protons of the thiaethylsulfonate fragment **H^5** and the protons **H^d** of the vitD$_3$ multiple bonds were observed in the 2D ^{1}H-^{1}H NOESY NMR spectrum from **2**/2 vitD$_3$ (5×10^{-3} M) in D_2O/CD_3OD (Fig. 1, ESI,† S7).

To gain an insight into the interactions between the **2**-vitD$_3$ complex, models of the pillar[5]arene and its complex with vitamin D$_3$ were constructed. Their geometries optimized using molecular mechanics in the gas phase. The results show that electrostatic interactions between sulfonate groups in uncomplexed **2** leaves the macrocyclic cavity available to guest molecules (ESI SX). Addition of vitD$_3$ guests leads to the formation of a 1:2 complex as indicated in Fig. 1. The vitD$_3$ cyclohexyl moieties are held outside the opening of the macrocyclic cavity, possibly stabilized by their hydroxyl groups interacting with sulfonate oxygens at 1.82 and 1.87 Å.

The formation of the **2**/2 vitD$_3$ complex was additionally confirmed by two-dimensional diffusion ordered spectroscopy (DOSY) (ESI,† S10). The diffusion coefficients of **2** and **2**/2 vitD$_3$ at 298 K (10^{-3} M) were determined. The DOSY spectrum of system **2**/vitD$_3$ shows the presence of complex signals lying on a straight line with a diffusion coefficient, D, of 0.76×10^{-10} m^2 s^{-1}, which is much lower than the self-diffusion coefficient of macrocycle **2** (D = 4.83×10^{-10} m^2 s^{-1}) under the same conditions.[7] These results clearly indicate the formation of the **2**/2 vitD$_3$ complex. The results obtained are in good agreement with published data.[5b]

The resulting **2**/2 vitD$_3$ complex (Fig. 1) is characterized by two distinct lipophilic vitD$_3$ residues and ten hydrophilic sulfonate moieties from macrocycle **2** which makes it function as a supramolecular amphiphile.[8a-c] Similar systems are capable of self-association and aggregation in water.[8d, e] To confirm this hypothesis, we used dynamic light scattering (DLS) to study the **2**/vitD$_3$ macrocycle systems in ratios of 1:1, 1:2, 1:5 and 1:10 over the concentration range of 10^{-3}-10^{-5} M. Macrocycle **2** itself does not form stable associates over this concentration range (ESI,† S8). Particles with a hydrodynamic diameter of 70 nm (ESI,† S8) were observed in aqueous vitD$_3$ solutions at a concentration of 5×10^{-5} M in the absence of macrocycle **2**. In this case, a fairly stable monodispersed system (ζ = -31.10 mV)

was formed with a polydispersity index (PDI) of 0.19 (ESI,† S8). However, it was not possible to study the system at higher concentrations of vitD$_3$, as it precipitates. A decrease in the concentration of vitD$_3$ (10^{-6}-10^{-7} M) leads to the formation of a polydisperse system. In the case of the pillar[5]arene **2**/vitD$_3$ system, an increase in the concentration of vitD$_3$ (10^{-3}-10^{-5} M) in ratios of 1: 1, 1: 2, 1: 5 and 1: 10, led to an increase in the average hydrodynamic diameter of the aggregates formed (ESI,† S8). The largest average hydrodynamic diameter (D = 236nm) was observed at a ratio of **2**/vitD$_3$ of 1: 5 (10^{-3} M) with a PDI of 0.23. It is worth noting that with an increase in the concentration of **2**/2 vitD$_3$ from 10^{-5} M to 10^{-3} M, a monotonic increase in the size of aggregates (Fig. 2, ESI,† S8) from 53 nm to 202 nm is observed, while the value of the PDI (0.16) is practically unchanged. The most stable system is formed at a **2**/vitD$_3$ concentration of 10^{-4} M based on the data of the ζ potential (ζ = -50.50 mV).

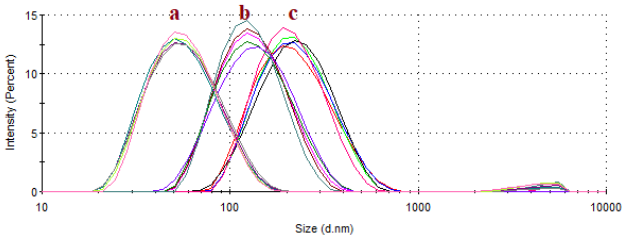

Fig. 2 Size distribution of the particles by intensity for **2**/vitD$_3$ in water with an increase in both components in a stoichiometric mixure: a) 1:2 (10^{-5} M+ 20 mkl 10^{-3} C_2H_5OH) (d=53.13 ± 1.14nm, PDI= 0.18 ± 0.01), b) 1:2 (10^{-4} M+ 20mkl 10^{-2} C_2H_5OH) (d=123.70 ± 0.75 nm, PDI= 0.16 ± 0.01), c) 1:2 (10^{-3} M+ 20mkl 10^{-1} C_2H_5OH) (d=201.80 ± 1.41 nm, PDI= 0.16 ± 0.01).

The results obtained, namely an increase in the diameter of the aggregates formed with increasing concentration of **2**/2 vitD$_3$, obviously indicate the formation of supramolecular polymers characteristic of pillar[5]arenes.[9] Consequently we proposed that, by increasing the concentration or removing the solvent it is possible to form nanostructured supramolecular polymer films.[10] The ability to aggregate macrocycle **2**, vitD$_3$ and associate **2**/2 vitD$_3$ into ordered structures was investigated by scanning electron microscopy (SEM).

According to SEM data (Fig. 3, ESI,† S9- S10), macrocycle **2** (Fig. 3a) does not form aggregates; only a silicon substrate is observed in SEM images. In the case of vitD$_3$ (Fig. 3b), irregularly shaped aggregates are seen in SEM images. As expected, in the case of **2**/2 vitD$_3$, aggregates are formed from supramolecular polymers, shaping a porous material with an average wall diameter of 53 nm (Fig. 3c, d). Thus, we can conclude that during the concentration and/or removal of the solvent (H_2O/C_2H_5OH), the **2**/2 vitD$_3$ complex forms a supramolecular polymer, which is prone to the formation of nanostructured porous films.

It is known that such systems have a number of unique properties, such as the ability to self-regeneration,[9,10] resistance to various types of radiation,[10d] etc. It is also worth noting that the main disadvantage of vitD-based vitamin supplements is their low photochemical stability. Long-term exposure to ultraviolet radiation on vitD$_3$ leads to the isomerization of vitD$_3$ into biologically inactive forms.[11] It was therefore of interest to study the resulting water-soluble complex **2**/vitD$_3$ for its resistance to ultraviolet radiation. Freshly prepared samples of vitD$_3$ (2.5×10^{-5} M) and **2** (2.5×10^{-5} M)/vitD$_3$ (5×10^{-5} M) were placed in a lightproof chamber and exposed to ultraviolet light with a wavelength of 352 nm to measure UV stability

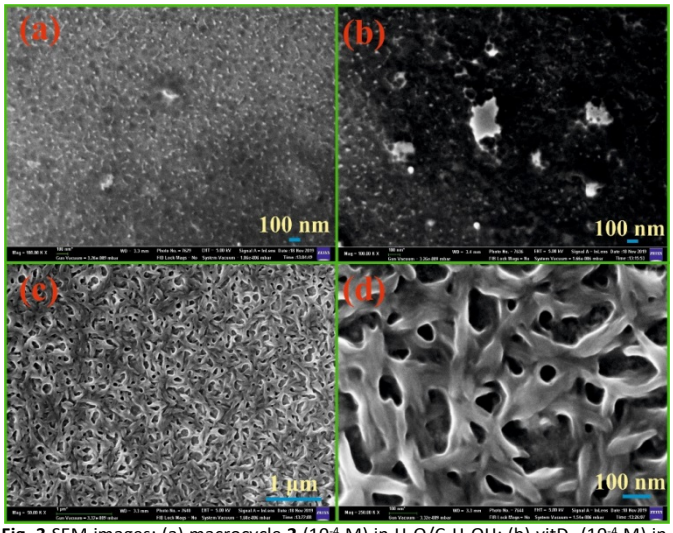

Fig. 3 SEM images: (a) macrocycle **2** (10^{-4} M) in H_2O/C_2H_5OH; (b) vitD$_3$ (10^{-4} M) in H_2O/ C_2H_5OH; (c) and (d) system **2** vitD$_3$ (10^{-4} M) in H_2O/C_2H_5OH.

Under the influence of thermal and UV radiation, vitD$_3$ is in equilibrium with the form of pre-vitamin D3 (pre-vitD$_3$). Thus, with an increase in the UV exposure time (0-90 min) on the vitD$_3$ sample (Fig. 4a, ESI,† S7), a regular decrease in the absorption intensity is observed at the vitD$_3$ absorption maxima (217, 254, 265 and 280 nm). This is consistent with the transition of the UV-active form of vitD$_3$ to the cyclic, inactive form - lumisterol (Fig. 4b).[11d, e] However, under the action of UV radiation on the complex of macrocycle **2** with vitD$_3$, a slight hypochromic effect occurred (ESI,† S7), which indicates the binding of pillar[5]arene **2** to the active form of vitD$_3$ through the conjugate multiple bonds (ESI,† S7)[5b] and therefore, with stabilization of vitD$_3$.

To quantify the photochemical stability of vitD$_3$ and the macrocycle **2**/vitD$_3$ complex, we used the kinetic constant of the photochemical decomposition rate (k_1).[12] The photochemical decomposition rate constant was determined in accordance with the linear correlation between ln (C/C_0) and UV exposure time t (1) (ESI). [12]

$$\ln(C/C_0) = k_1 t \qquad (1)$$

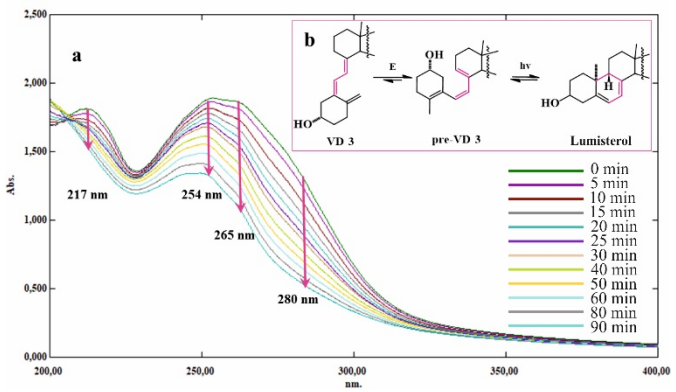

Fig. 4 a) Time-dependent UV–vis spectra of the UV-destruction vitD$_3$ (1×10^{-5}M), b) UV vitD$_3$conversion.

Figure 5 shows the kinetic dependences of the UV decomposition of vitD$_3$ in the absence and presence of pillar[5]arene **2** with k_1 values of 2.34 ×10^{-4} s $^{-1}$ for vitD$_3$ and 1.7 ×10^{-5} s $^{-1}$ for **2**/vitD$_3$. It can be concluded that the resulting **2**/2 vitD$_3$ supramolecular system is stable in the presence of UV radiation. Resistance to photochemical degradation of vitD$_3$ in the **2**/2 vitD$_3$ complex is an order of magnitude higher than that of free vitD$_3$. The results obtained on the stability of vitD$_3$ are superior to the previously published results in the literature.[11c]

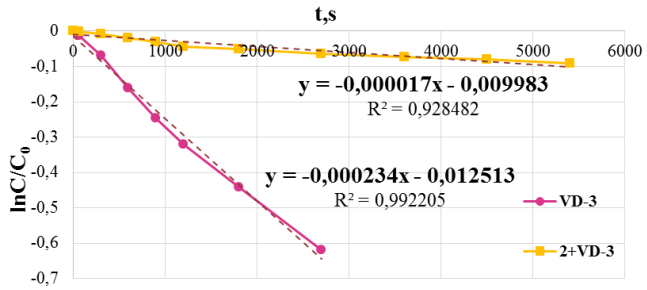

Fig. 5 Plot ln(C/C$_0$) versus reaction time for the UV-destruction of vitD$_3$ and **2** (2.5×10^{-5} M)/vitD$_3$ (5×10^{-5} M). C$_0$ and C were the absorption peak at 295 nm initially and at time t.

The photochemical stability of vitD$_3$ is the main disadvantage for its use in vitamin supplements taken orally or transdermally. A possible mechanism for the protection of a macroscopic capsule based on pillar[5]arene **2** from photochemical degradation of vitD$_3$ may be its extensive conjugation that can absorb ultraviolet light and, therefore, reduce the absorption of ultraviolet vitD$_3$.[5b] However, the **2**/vitD$_3$ system is not applicable for use in living systems due to the toxicity of the DDS components themselves, namely, the macrocyclic platform pillar[5]arene **2**.

In this regard, a series of experiments to assess the viability of A549 model cells treated with pillar[5]arenes **2** and **3** was performed. The ability of the pillar[5]arenes to inhibit the viability and proliferative activity of model A549 cells was studied by the MTT test[7a] after incubation in the presence of test compounds for 24 hours. Cell viability was expressed in relative units compared to a control not treated with pillar[5]arenes. Compounds **2** (0.5 - 50 μgml^{-1}) and **3** (0.5 - 50 μgml^{-1}) were studied in a wide range of concentrations. It was found that, in the entire range of studied concentrations (0.5 – 50 μgml^{-1}), A549 cells' viabilities were unaffected by pillar[5]arenes **2** and **3** (ESI,† S11).

Conclusions

In summary, for the first time, water-soluble deca-substituted pillar[5]arenes containing thiasulfate and thiacarboxylate *O*-substituents were synthesized. The ability of thiosulfate pillar[5]arene derivative **2** to interact with vitD$_3$ was demonstrated by UV-vis spectroscopy. The stoichiometry of the 1:2 **2** / vitD$_3$ complex with a logK_{ass} of 2.2 was established by Job's method. The structure of the resulting complex was confirmed by 2D ^{1}H-^{1}H NOESY and DOSY NMR spectroscopy. DLS showed that if in the case of pillar[5]arenes **2** no stable self-associates are formed in aqueous solutions, then it was typical for the associate to form stable monodisperse dispersions with a ζ of -50.50 mV. The polydispersity index of the system of 0.16 was essentially unchanged with increasing concentrations of **2**/2 vitD$_3$ in the range of 10^{-5} to 10^{-3} M, as was the average hydrodynamic particle diameter range of 53 nm to 202 nm. It was shown that **2**/2 vitD$_3$ associated in aqueous solutions to generate supramolecular polymers capable of forming nanostructured porous films with an average wall diameter of 53 nm. The photochemical stability of the **2**/vitD$_3$ system was studied and shown to be stable in the presence of UV irradiation with a k_1(**2**/vitD$_3$) of 1.7 ×10^{-5} s^{-1}. The cytotoxic effect of pillar[5]arenes **2** and **3** on A549 cells was determined by an MTT assay and it was established that they did not reduce the viability of A549 cells over the concentration range of 0.5 to 50 μgml^{-1}. These results offer great opportunities in the development of new non-toxic biomedical materials containing group D vitamins that are resistant to external conditions.

Experimental

General methods

^{1}H NMR, ^{13}C NMR spectra were obtained on a Bruker Avance-400 spectrometer (^{13}C{^{1}H} - 100 MHz and ^{1}H - 400 MHz). Chemical shifts were determined against the signals of residual protons of deuterated solvent ($CDCl_3$, D_2O, CD_3OD-d_4). The concentration of sample solutions was 3-5 %. Attenuated total internal reflectance IR spectra were recorded with Spectrum 400 (Perkin Elmer) Fourier spectrometer. The IR spectra from 4000 to 400 cm^{-1} were considered in this analysis. The spectra were measured with 1 cm^{-1} resolution and 64 scans co-addition. Elemental analysis was performed with Perkin Elmer 2400 Series II instrument. Electrospray ionization mass spectra (ESI) were obtained on an AmazonX mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany). The measurements were carried out in the regime of positive ions registration in the range of m/z from 100 to 2800. The voltage on the capillary was -4500 V. Nitrogen was used as the gas-drier with a temperature of 300 °C and a flow rate of 10 L·min^{-1}. Compounds were dissolved in acetonitrile at a concentration of 10^{-6} gL^{-1}. Data was

processed using DataAnalysis 4.0 (Bruker Daltonik GmbH, Bremen, Germany). Additional control of the purity of compounds and monitoring of the reaction were carried out by thin-layer chromatography using Silica G, 200 μm plates, UV 254.

Spectroscopic studies

^{1}H diffusion ordered spectroscopy (DOSY) spectra were recorded on a Bruker Avance 400 spectrometer at 9.4 tesla at a resonating frequency of 400.17 MHz for ^{1}H using a BBO Bruker 5 mm gradient probe. The temperature was regulated at 298 K and no spinning was applied to the NMR tube. DOSY experiments were performed using the STE bipolar gradient pulse pair (stebpgp1s) pulse sequence with 16 scans of 16 data points collected. The maximum gradient strength produced in the z direction was 5.35 Gmm^{-1}. The duration of the magnetic field pulse gradients (δ) was optimized for each diffusion time (Δ) in order to obtain a 2% residual signal with the maximum gradient strength. The values of δ and Δ were 1.800 μs and 100 ms, respectively. The pulse gradients were incremented from 2 to 95% of the maximum gradient strength in a linear ramp.[13a]

UV-vis spectra were recorded using the Shimadzu UV-3600 spectrometer; the cell thickness was 1 cm, slit width 1 nm. Deionized water with a resistivity >18.0 MΩ cm was used to prepare the solutions. Deionized water was obtained from a Millipore-Q purification system. Recording of the absorption spectra of the mixtures of vitD$_3$ with pillar[5]arenes **2** and **3** at 4 × 10^{-5} M was was carried out after mixing the solutions at 298 K. The 10^{-4} M solution of pillar[5]arene **2** (100, 150, 200, 220, 250, 280, 300, 400, 500, 600, 70, 800, 900, 1000 and 1200 μl) in water was added to 12 μl of the solution of guest (vitD$_3$) (10^{-2} M) in ethanol and diluted to final volume of 3 ml with water. The UV spectra of the solutions were then recorded. The stability constant of complexes were calculated as described below. Three independent experiments were carried out for each series. Student's *t*-test was applied in statistical data processing. Experiment was carried out according to the literature method. The stoichiometries of complexes were determined by Job's plots.

Photochemical stability measurement.

Photochemical stability was studied by UV-vis spectroscopy. Freshly prepared macrocycle **2**, together with a vitD$_3$ dispersion in water as a control, were used for vitD$_3$ stability measurements. A vitD$_3$ dispersion was prepared by dissolving 30 μl of 10^{-2} M vitD$_3$ in ethanol followed by dispersion into water, with the final concentration of vitD$_3$ equivalent to macrocycle **2**. Samples of **2** in transparent glass vials were placed in a light-proof cabinet and exposed to two 15 W UV light bulbs at 352 nm for up to 1.5 h. At exposure time intervals of 1, 2, 5, 10, 15, 20, 25, 30 , 45, 60 and 90 min, 600 μl 10^{-4} M of sample **2** with 30 μl 10^{-2} M vitD$_3$ was withdrawn from each treatment and then 30 μl 10^{-2} M vitD$_3$ was extracted and measured according to the method described above. All measurements were performed in triplicate.[2f]

The processing of the obtained kinetic date were carried out in accordance with first-order kinetics (1).[12]

$$\lg(C/C_0) = k_1 t \qquad (1)$$

Accordingly, the kinetic rate constant k_1 as an activity parameter was defined according to the linear correlation between $\ln(C/C_0)$ and reaction time t (1), which has been utilized to evaluate the photostability (**2** + vitD$_3$ and only vitD$_3$). In Equation (1), C_0 and C were the UV–vis absorption at 295 nm initially and at time t.

Scanning electron microscopy (SEM).

Morphological structures of samples were observed by a SEM (Carl Zeiss Auriga Cross Beam). Samples were first diluted with water to final concentration 1 × 10^{-4} g/ml, the resulting suspension were placed on silicon pan, which was then dried in vacuum desiccator for 1 hour.

Dynamic light scattering (DLS).

The particle sizes and zeta potentials were determined by a Zetasizer Nano ZS at 20 °C. The instrument contains a 4 mW He-Ne laser operating at a wave length of 633 nm and incorporated noninvasive backscatter optics (NIBS). The measurements were performed at the detection angle of 173° and the software automatically determined the measurement position within the quartz cuvette. The 10^{-3} - 10^{-5} M aqueous solutions of **2**, vitD$_3$ (dissolved in ethanol 1 × 10^{-3}, 1 × 10^{-2} and 1 × 10^{-1} M) and vitD$_3$ with macrocycle **2** complex were prepared. The concentration ratio of pillar[5]arene **2** and vitD$_3$ in the complex was 1:1, 1:5, 1:10 and 2:1. Electrophoretic mobility of different samples was using a fold capillary cuvette (Folded Capillary Cell DTS1060, Malvern, U.K.). The experiments were carried out for each solution in triplicate.

Computational chemistry.

The structures of 2 and vitD3 were constructed using Spartan '18.[14] Their gas phase geometries, and that of **2**/2 vitD$_3$, were opitimized by molecular mechanics using the Merck Molecular Force Field.

The study of cytotoxicity.

Characterization of the lung adenocarcinoma A549 cell (Russian cell culture collection) viability changes under the action of pillar[5]arenes **2**, **3** was performed by the MTT test.[13b] Cells were cultured at 37°C in DMEM medium (Invitrogen, USA) supplemented with penicillin/streptomycin (100 units mL^{-1}), 2 mM L-glutamine and 10% fetal bovine serum in an humidified atmosphere with 5% CO_2. A cell suspension of 150 μl per well was seeded in a 96-well plate at a final concentration of 1 × 10^4 cells per well. After 24 hours the media from the wells was aspirated and replaced by fresh supplemented with test compounds in a volume of 100 μl. After 24 hours of incubation in the presence of pillar[5]arenes **2, 3**, 10 μl of MTT (3-(4,5-dimeth-ylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Sigma-Aldrich) solution (5 mg ml^{-1}) was added to the wells. Plates were incubated at 37 °C in a humidified atmosphere with 5% CO_2 for 4 hours. Visually, the formation of formazan crystals in the wells was detected with microscopy. Then the medium was aspirated

from the wells and 100 µl of dimethyl sulfoxide (Tatkhimpharmpreparat, Russia) was added to dissolve the crystals in each well. Probes were incubated at 37°C for 5 minutes. The absorbance was measured at 570 nm at a reference length of 630 nm using xMark spectrophotometer (BIO-RAD, USA). The optical density of the solution is proportional to the integral activity of mitochondrial dehydrogenases of the cells in the well, and, consequently, to their number. Mathematical processing of the results was carried out using the non-parametric Cramer-Welch criterion as a reliability criterion. $p \leq 0.05$ was taken as a reliable level of significance. The calculation of the standard deviation of the results of the experiments, as well as a comparison of the two data groups, was carried out in the MS-Excel.

Pillar[5]arene **1** was synthesized according to the literature procedure.[6b, c]

Detailed information (NMR and MS spectra, quantum chemical calculations, biological assays) is given in the ESI.†

Synthesis of the compounds 2 and 3

To a mixture of NaH 0.45 g (18.75 mmol) in 4 ml of DMF, a solution of 4.3 mmol of 2-mercaptoethanesulfonic acid sodium salt (or 2-mercaptoacetic acid) in 4 ml of DMF was added while cooling in an ice bath. The mixture was stirred for 5 minutes. Then, a solution of 0.4 g (0.24 mmol) of macrocycle **1** in 12 ml of DMF was added to the reaction mixture. Next, the reaction mixture was heated to 90 ° C for 12 hours. Then the reaction mixture was poured into ethanol, a white precipitate was filtered off and washed with ethanol, then purified by recrystallization from ethanol. The resulting precipitate was dried over P_2O_5.

4,8,14,18,23,26,28,31,32,35-deca-[(tioetansulfonate)ethoxy)]-pillar[5]arene sodium salt (2)

Yeld 0.54 g (90%), mp= 278°C with decomposition. NMR^{1}H (D_2O, δ, ppm. J/Hz): 2.78 t (20H,-OCH_2-CH_2-S-$\underline{CH_2}$-CH_2-SO_3Na, $^3J_{HH}$=5.51 Hz), 2.86-3.01 m (20H,-OCH_2-$\underline{CH_2}$-S-CH_2-CH_2-SO_3Na), 3.09-3.23 m (20H,-OCH_2-CH_2-S-CH_2-$\underline{CH_2}$-SO_3Na), 3.83 s (10H,-CH_2), 6.79 s (10H, H_{Ph}.). NMR ^{13}C (D_2O, δ, ppm.): 26.44, 30.14, 31.16, 51.22, 68.84, 116.61, 129.57, 150.10. IR , ν/sm^{-1}: 3438 (-OH), 2926 (-C_{Ph}-H), 2866 (-CH_2-), 1652 (C_{Ph}-C_{Ph}), 1499 (-CH_2-), 1464 (C_{Ph}-C_{Ph}), 1406 (-SO_3^-), 1277 (C_{Ph}-O-CH_2-), 1174 (-SO_3^-), 1047 (C_{Ph}-O-CH_2-, -SO_3^-). MS (ESI): calc. [M+] m/z =2512.68, found [M-3Na]$^{3-}$ m/z =813.6. Found (%): C, 36.12; H, 4.25; S, 24.93. Calc. for $C_{75}H_{100}O_{40}S_{20}Na_{10}$. (%): C, 35.85; H, 4.01; S, 25.52.

4,8,14,18,23,26,28,31,32,35-deca-[(acetatsulfanediyl)ethoxy)]- pillar[5]arene sodium salt (3).

Yeld 0.26 r (61%), mp.=220°C with decomposition. NMR ^{1}H (D_2O, δ, ppm. J/Hz): 3.24-3.28 m (20H,-OCH_2-$\underline{CH_2}$-S-CH_2-COONa), 3.41 br. s (20H,-OCH_2-CH_2-S-$\underline{CH_2}$-COONa), 3.79 s (10H, -CH_2-), 3.90-4.00 m (20H,-$\underline{OCH_2}$-CH_2-S-CH_2-COONa),6.86 s (10H, H_{Ph}.). NMR ^{13}C (D_2O, δ, ppm.): 29.14, 31.67, 37.09, 42.72, 68.22, 116.28, 129.40, 149.98, 177.85. IR , ν/sm^{-1}: 3412 (-OH), 2926 (-C_{Ph}-H), 2868 (-CH_2-), 1653 (C_{Ph}-C_{Ph}), 1579 (-COO^-), 1576 (-COO^-), 1498 (-CH_2-), 1464 (C_{Ph}-C_{Ph}), 1405 (-COO^-), 1383 (-CH_2-COO^-), 1202 (C_{Ph}-O-CH_2-), 1042 (C_{Ph}-O-CH_2-). MS (ESI): calc [M+] m/z = 2010.09, found [M-3Na]$^{3-}$ m/z =647.04. Found (%):C, 51.26; H, 4.42, S, 18.21. Calc. for $C_{75}H_{80}O_{30}S_{10}Na_{10}$. (%): C, 50.55; H, 4.53; S, 17.99.

Conflicts of interest

There are no conflicts to declare.

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