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ARTICLE



## Calixarene-based pure and mixed assemblies for biomedical applications

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### ABSTRACT

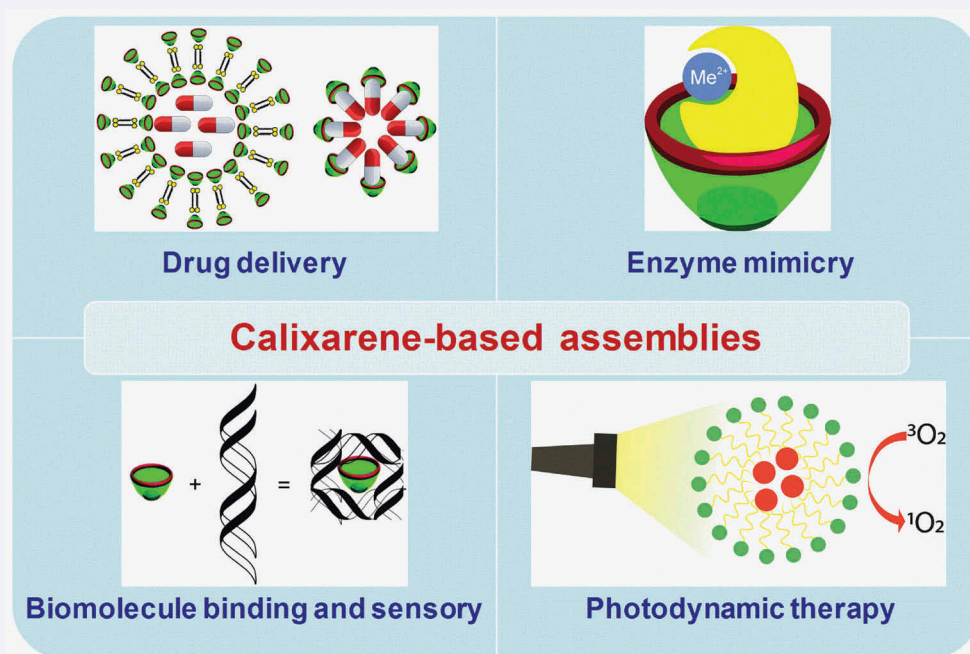
The spontaneous preparation of well-defined functional nanoscale systems is very important, since this formulation process requires additional resources, external supply of energy and time. One of the widely used methods of their spontaneous formation is the self-assembling process using of various calixarene building blocks. The relative simplicity of the synthesis of initial calixarene skeleton, the possibility of modifying both of its rims with a wide range of chemical groups, and the presence of a hydrophobic aromatic cavity allow the use of calixarenes to create both individual and mixed aggregates. This article summarises different types of self-assembled calixarene-based amphiphilic aggregates with useful properties. Potential applications of these aggregates in biological nanotechnology are also described.

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Calixarenes; self-assembly; amphiphiles; supramolecular chemistry



### Introduction

A fundamentally new approach to design new nanoscale materials based on self-assembly with the participation of molecular units in supramolecular interactions is currently being developed. The spontaneous organisation of molecular and macromolecular building blocks relative to each other allows creating complex functional materials and systems with more other functions than those performed by individual components. The examples of self-assembly due to weak non-covalent forces are such fundamental

properties of living matter as transcription of genetic information into DNA, secondary (and higher) protein structures, enzymatic functions, reaction of the immune system, etc. An important function in a living cell of the body is performed by membrane lipids, which line up in bilayers, placing their polar groups towards the aquatic environment and lipophilic chains towards the inside of the bilayer due to amphiphilic nature of these lipids.

The amphiphiles include substances having both hydrophilic and hydrophobic moieties linked by

covalent bonds. The interest in amphiphilic compounds is caused by their ability to self-assemble into such specific aggregates as micelles, vesicles, nanotubes, nanorods, nanoribbons that can be used in the creation of nanodevices, drug and gene delivery, template synthesis, cell imaging, etc. [1–7]. The structures and properties of such aggregates formed by amphiphiles are determined by their architecture (geometric parameters of the hydrophilic and hydrophobic parts) and may vary depending on the solvent, concentration, temperature, pH and ionic strength. In recent years, there has been increased interest in amphiphilic derivatives of calixarenes, which can be used not only as biomimetic model compounds [8,9] but also to create new materials for nanotechnology [10,11].

Calixarenes are macrocyclic compounds comprising four or more phenolic moieties connected to a cyclic array in a meta-position using methylene bridges. The calixarene family can be divided into two main categories depending on the structure of the initial phenol derivative: (i) calix[n]arenes  $C[n]A$  derived from  $n$  phenols, and (ii) calix[n]resorcinols  $C[n]R$ , or resorcin [4]arenes, created from  $n$  resorcinols. Owing to the relative simplicity of synthesis and excellent ability to functionalise, calixarenes have proven to be the third generation of supramolecules (after crown ethers and cyclodextrins) with a wide potential for complexation of cations, anions, and even neutral molecules [12]. Their unique molecular architecture and customisable internal cavity size make calixarenes very attractive building blocks for supramolecular chemistry, where combination of certain size, conformation, preliminary organisation of molecular shape and rigidity can be useful for the suitable non-covalent accommodation of certain molecules.

Despite the fact that many differently functionalised water-soluble calixarenes are known, the sulphonated analogues synthesised in the eighties [13,14] are the most intensively studied water-soluble calixarenes, and they have attracted considerable attention because of their high water-solubility, selective binding ability, catalytic properties and obvious biocompatibility [15–19]. The directed functionalization of calixarenes by other groups and imparting amphiphilic properties to them are necessary conditions for the construction of covalent hybrid conjugates and the self-assembly of different supramolecular assemblies. These ensembles can be formed due to various types of non-covalent interactions, such as electrostatic (ion-ion, ion-dipole, dipole-dipole, dipole-induced dipole and higher order interactions), hydrogen bonds,  $\pi$ -stacking,  $CH-\pi$ , dispersion interactions and hydrophobic effect [20]. As a rule, one weak interaction is not enough to ensure a site-specific

self-assembly of at least two molecules, so usually several types of interactions act together to achieve energy-positive cooperation, as well as to increase the stability of aggregates.

The concepts of supramolecular and colloid chemistry are based on the phenomena of molecular recognition and self-assembly, respectively: molecules (hosts) recognise their complementary regions in other molecules (guests) and combine into larger objects (supermolecules) through weak non-covalent interactions. Therefore, the junction of supramolecular and colloidal chemistry has led to the formation of new objects of primary research attention – supramolecular amphiphiles. Unlike conventional amphiphiles, supramolecular amphiphiles, also known as the superamphiphiles, are built through non-covalent interactions or dynamic covalent bonds. Like the principles of superamphiphilic aggregation, calixarenes can be used to bind such large molecules as polymers, proteins, and small non-amphiphilic organic molecules. The complexes of calixarenes thus formed are not only capable of forming new nanostructures, but can be used to modify systems to improve the required characteristics. This non-covalent approach avoids time-consuming synthetic procedures in the creation of useful nanomaterials with a high degree of structural complexity. In addition, the dynamic and reversible nature of non-covalent interactions gives the supramolecular architectures with superior characteristics that respond to external stimuli. Since the substrate specificity of calixarenes in molecular recognition made these molecules interesting objects of study, the characterisation of self-assembly properties, as well as the conformation and biological activity of aggregates in aqueous solution, are fundamental in this direction. Before proceeding to consideration of formation of supramolecular structures from single and mixed systems based on calixarenes, we first consider their conformational behaviour and biological properties of these macrocycles.

### Conformational properties of calixarenes

The various useful functions of calixarenes are caused not only by the wide range of possible chemical modifications of both rims but also by conformational diversity. The conformational flexibility of calixarenes is explained by the rotation of aromatic groups relative to the bridged methylene group. Calixarenes are conformationally mobile compounds, and an increase in the number of aromatic units is accompanied by an increase in the conformational flexibility of macrocycles.  $C[4]A$  **1**, consisting of four phenolic fragments (Figure 1), form a rather rigid truncated cone-shaped molecule with

corresponding modification by relatively large fragments that fix the structure and prevent the benzene rings from rotating [21,22]. The conformation of C[4]A **2** (Figure 1) can be also fixed by modifying its cup with a substituent in volume exceeding the ethyl group [23,24]. At the same time, large C[6]A **3** and C[8]A **4** (Figure 1) exchange between several possible conformations, which is due to the unimpeded rotation of benzene rings through the annular space [18,25]. Basically, C[6]A in the monomeric state takes a pseudo-1,2,3-alternative conformation, and C[8]A is very conformationally mobile [26].

Conformational transitions in calixarenes containing free hydroxyl groups on both the upper and lower rims are hampered by the formation of intramolecular hydrogen bonds [27–30]. Hence, calixresorcinols molecules with fragments on methylene bridges in the fully cis position or with unsubstituted methylene bridges are found only in the conical conformation, both in solution and in crystals [31]. This conformation is the most thermodynamically stable due to hydrogen bonds realised between hydroxyl groups inside the macrocycle molecule [32].

### Biological properties of calixarenes

Calixarenes can be used in biological and medical applications due to its non-toxicity and non-immunogenicity [33–35]. In vitro studies showed that p-sulphonated C[4]A **5**

(Figure 2), the most widely studied in terms of its biological effects, are not toxic to various tumour cell lines [36] and does not have haemolytic toxicity at concentrations up to 5 mM [37]. Among sodium salts of sulphonated calixarenes with different numbers of aromatic units, cyclic tetramers to a much lesser extent destroy red blood cells than their hexa- and octamer counterparts [37]. However, the effect of the number of aromatic fragments in the sulphonated calixarene cup on neutrophils (cells of the non-specific immune system of the human body) was not detected. Compared to the control sample, the proportion of cells that died as a result of apoptosis and necrosis does not change in the presence of macrocycles regardless of the size of their cup. In addition, there is evidence of the absence of the influence of these calixarenes on protein kinase activating NADPH oxidase, which in turn is involved in the cellular antimicrobial defence system. Therefore, these macrocycles are biocompatible and do not cause a non-specific immune response [33]. In another study [38], the toxicity of unmodified sulphonated C[4]A **5** (Figure 2) labelled with radioactive sulphur ( $S^{35}$ ) was studied in mice. This in vivo study showed that this macrocycle is non-toxic in the concentration range up to 100 mg/kg, does not accumulate in the liver and spleen, does not penetrate the brain and is rapidly excreted in the urine.

In addition, calixarenes can exhibit their own biological activity. For example, methods for treating HIV, herpes, and influenza were patented using calixarenes **5–7**

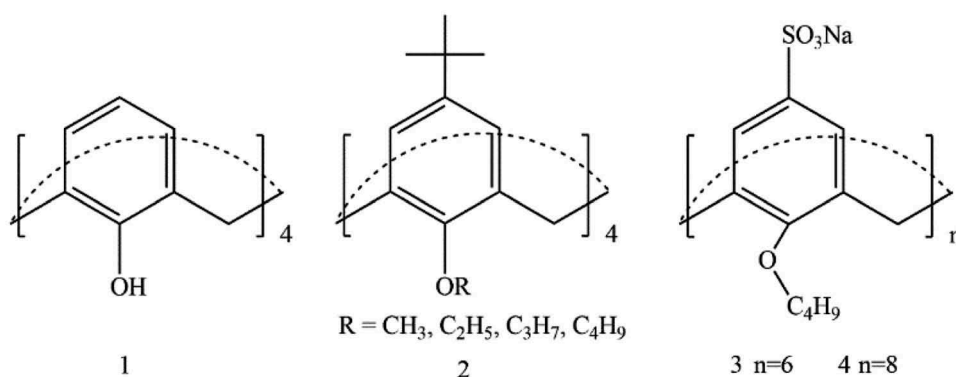


Figure 1. Molecular structures of CAs.

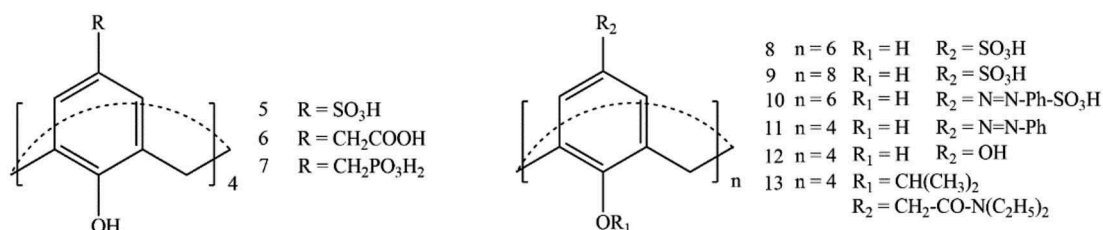


Figure 2. Molecular structures of non-toxic and biologically active C[4]As.

(Figure 2) having various polar (sulphonate, carboxylate, and phosphate) substituents [39]. Tetra-, hexa- and octameric sulphonated calixarenes **5**, **8–13** (Figure 2) effectively inhibit gram-positive *Corynebacterium* and also have selective fungicidal activity against fungal strains of *Fusarium solani* f. sp. *Mori*, *Rosellinia necatrix* and *Colletotrichum dematium* [40]. In an in vitro study of antithrombotic activity, the octameric analogue **9**, **14**, **15** (Figures 2 and 3) showed the greatest effect [41]. Hexameric sulphate C[6]A **8** (Figure 2) is more able to inhibit the formation of amyloid fibrils of insulin molecules than tetrameric macrocycle **5** [42]. C[4]A-containing N-methyldiethanol ammonium groups **16** (Figure 3) show antibacterial activity against gram-positive and gram-negative bacteria, and in combination with tetracycline not only increases the stability of the antibiotic in aqueous solution but also enhances its effect against gram-negative *P. aeruginosa* due to the additive effect [43]. Regarding resorcinarenes, these macrocycles are also non-toxic [44–47] like calixarenes, and only one work reported cytotoxicity data against human cancer cells [48]. In this work, it was shown that modification of the resorcinarene platform with PAMAM-dendrimer **17**, **18** (Figure 3) leads to an increase in anticancer activity, which depends on the nature of the cell line, but these studies were carried out in DMSO due to their low solubility in water.

## Calixarene-based systems

### Micelles and vesicles based on calixarenes

Calixarenes can act as amphiphiles by attaching ionic fragments to one rim and hydrophobic alkyl chains to the opposite rim. The intrinsic conical shape in this type of macrocycle molecule is a prerequisite for self-aggregation [49,50], and a relatively rigid structure of the calixarene framework can increase the stability of aggregates [51]. Shinkai et al. first described sulphonated C[6]A with hexyl tails at the lower rim **19** (Figure 4), for which the formation of spherical micelles with critical micelle concentration (CMC) of 0.6 mM in water was demonstrated [13,14]. Further, they showed a relationship between aggregation ability and alkyl tail length. Calixarenes without long alkyl chains (**5**, **8**, **9**, **21**, Figures 2 and 4) do not form micellar structures, but macrocycles **22–24** with butyl and longer chains self-assemble into micelles [18].

The influence of the alkyl chain length and the number of monomer units in the calixarene framework was also studied by Basilio et al. [26]. They showed that CMC value of p-sulphonated C[4]A derivatives in the conical conformation decreases with an increase in the length of the alkyl chains due to stronger hydrophobic interactions. The free Gibbs energy of micelle formation with an increase in the length of the hydrophobic tail becomes more negative [52], which is consistent with the trend

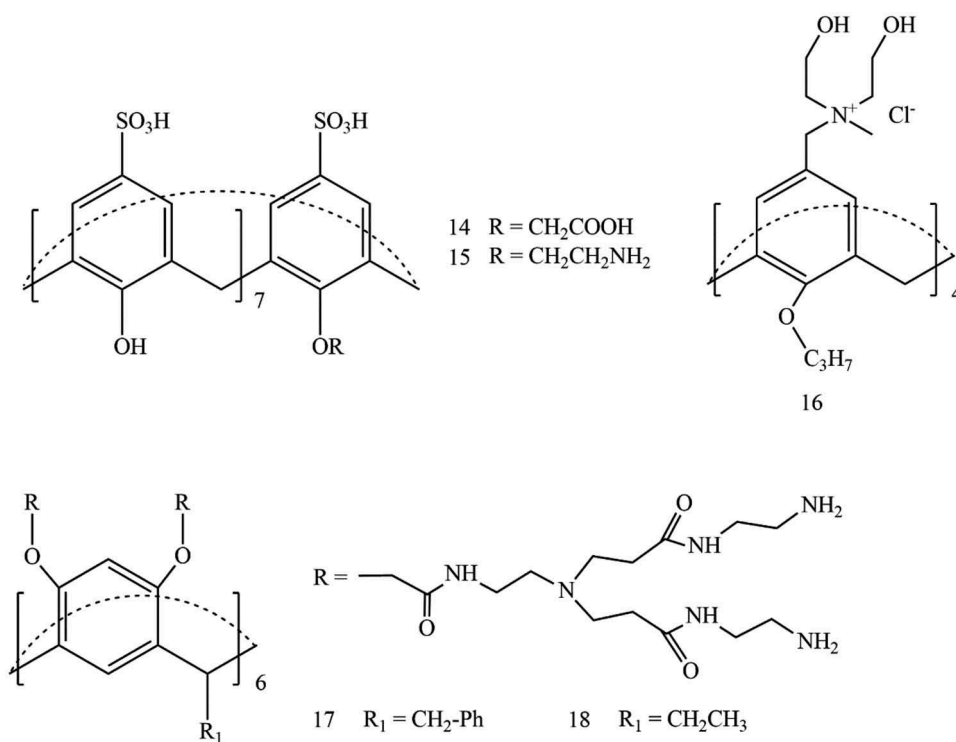


Figure 3. Molecular structures of biologically active C[4]As and C[6]Rs.



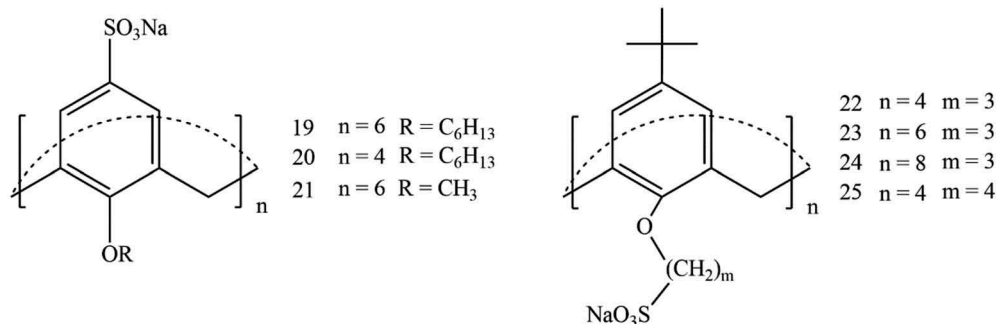


Figure 4. Molecular structures of CAs containing sulfo groups.

observed for ordinary surfactants. On the other hand, CMC increases with an increase in the number of monomer units. These results can be explained by the fact that substituted C[4]A, as mentioned earlier, are in a conical conformation, which is favourable for the formation of globular aggregates, and the derivatives of C[6]A and C[8]A are in other conformations. Changing these conformations to a cone conformer in aggregates implies additional energy costs [26,52].

Sulphonated C[4]A with hexyl tails **24** (Figure 4) forms micelles capable of solubilising hydrophobic paclitaxel and then releasing it slowly [53]. The work [54] describes micelles formed by sulphonated C[4]A **25** (Figure 4), in which the sulphonate groups are located at the end of the alkyl chain on the lower rim, i.e. the hydrophobic micelle core is formed by p-tert-butyl fragments of the upper rim, and tetra-O-butyl sulphonate groups stick out. The molecules of this macrocycle are staggered in the micelle, as can be seen from the cross-peaks in 2D NOESY spectra, indicating the proximity of tert-butyl hydrogen atoms at upper rim and side residues of butyl sulphonate groups at the lower rim of neighbouring calixarene molecules. The hydrophobic core created in this process significantly increases the water solubility of drugs such as naproxen and flurbiprofen.

The amphiphilic calixarenes containing phosphate groups act as mimics of phospholipids and can form

different aggregates with polar groups outward towards the polar solvents [55–58]. Phosphated C[4]A with octyl tails **26** (Figure 5) in an aqueous solution forms spherical micelle with a diameter of 4–5 nm, which are stable over a wide pH range and can also intercalate curcumin as a model antioxidant. The micelles obtained from this macrocycle demonstrate a lower toxicity to rat pheochromocytoma cells (line PC12) when compared with monomeric analogues, which makes them possible candidates for targeted drug delivery [59]. The phosphonate macrocycle with octadecyl tails **27** (Figure 5) aggregates into nanofibers with a diameter of 6 nm, which are able to encapsulate the fluorophore, with possible implications for tissue scaffolding [58]. Methylphosphated C[4]As with hexyl **28** [56] and dodecyl **29** [57] fragments at the lower rim (Figure 8) aggregates in an aqueous solution into vesicles with a diameter of 107 nm. These aggregates are stable at physiological pH, but rapidly degrade at  $pH < 6$ , and can release an encapsulated drug [56]. The aggregates formed by **28** can be used for the joint delivery of hydrophobic anticancer drugs paclitaxel and carboplatin [59]. In this case, carboplatin is located in the macrocycle cavity, and paclitaxel is solubilised in a hydrophobic layer formed by macrocycle alkyl tails. This composition was effectively absorbed by two colon cancer cell lines and showed greater cytotoxicity than a simple mixture of two drugs.

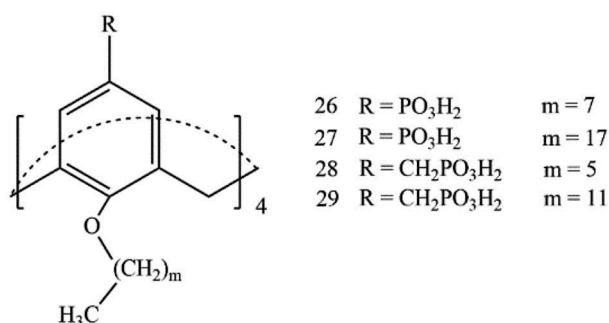


Figure 5. Molecular structures of phosphate C[4]As.

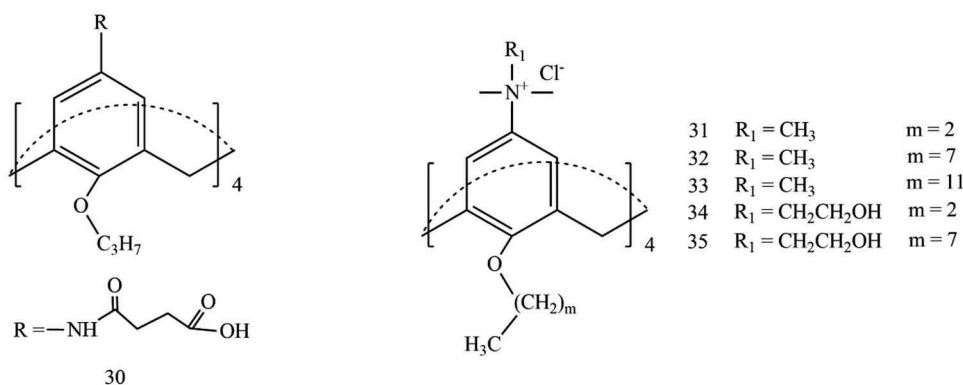


Figure 6. Molecular structures of ammonium-C[4]As.

The joint solubilisation of two types of drugs of different solubility was realised by Granata et al. [60]. C[4]A **30** (Figure 6) containing succinyl groups on the upper rim and dodecyl chains on the lower rim forms multilayer vesicles in an aqueous solution. Due to the poor solubility of this macrocycle in water, the vesicular particles were obtained by evaporation of the solvent from the emulsion. According to computer modelling, macrocycle molecules form four-layer vesicles, in which the interlayer interactions are held due to hydrogen bonds between carboxyl groups. At the same time, the formation of each layer is carried out due to hydrophobic interactions of aliphatic tails, lateral  $\pi$ - $\pi$ -stacking and hydrogen bonds between adjacent carboxyl groups. The size and polydispersity index of the obtained colloidal dispersion did not change for more than 2 years. Such good stability is attributed to a surface zeta potential of  $-40$  mV, which is sufficient to generate repulsive forces between the aggregates and prevent further aggregation between them. Thus, the obtained amphiphilic succinyl C[4]A-based vesicles have an advantage over traditional liposomes, which can be hydrolysed or oxidised.

The aggregation of amphiphilic calixarenes with alkyl ammonium groups at the upper rim was studied [51,61,62]. Strobel et al. found that C[4]As **32**, **33** (Figure 6) form micelles that are less than 5 nm in diameter and then increase in size at higher concentrations into rectangular lyotropic liquid crystals [61]. Later Eggers et al. studied aggregation of **33** (Figure 6) and confirmed that this compound forms micelles with hydrodynamic diameter of 5.4 nm [57]. However, if this macrocycle is mixed with C[4]A **29** identical in the lower rim, but having phosphonate groups on the upper rim, then the vesicular particles with a size of about 100 nm are formed, which can be wrapped in a peptide-glycol shell for targeted in vivo drug delivery.

Mchedlov-Petrosyan et al. also noted the similarity of the aggregation behaviour of tetraalkylammonium

tetrapropoxy C[4]A **34** (Figure 6) with conventional ionic surfactant [62]. It was assumed that shifts of the absorption and emission bands of indicator dye in C[4]A aqueous solutions, as well as changes in the acid dissociation constant values indicate the aggregation, or micelle formation, of cationic C[4]A-like cationic surfactants. DLS studies showed that aggregates appear in water at a concentration of C[4]A above  $6 \times 10^{-3}$  M, their diameter is approximately 3–4 nm, and the zeta potential is  $+66$  mV [62]. Later Ukhatskaya et al. supplemented these studies and determined that vesicular aggregates from **31**, **34**, and **35** (Figure 6) are formed already at concentrations above  $8.79 \times 10^{-6}$  M,  $367 \times 10^{-6}$  M and  $47.8 \times 10^{-6}$  M, respectively [63], and the coexistence of vesicles with aggregates of a different type (presumably micelles) was observed in more concentrated solutions of **34** and **35**. For such calixarene-based surfactants, the transition of vesicles into micelles with increasing concentration is known [64] and is explained by the fact that interactions between hydrophobic tails intensify with increasing concentration of surfactant; therefore, they are more densely packed. Thus, the conformation of the molecule changes from 1,3-alternate to cone, which results in the formation of micelles.

Lee et al. described not only the effect of the length of the hydrophobic tail on size and structure of the C[4]A-based aggregates, but also the structural transition of the vesicle-to-micelle with a change in pH of the aqueous medium of amphiphilic C[4]As with decyl chains at the lower rim and different lengths of the oligo-ethylene oxide chain attached to the amino groups at the upper rim (Figure 7) [51]. If C[4]A with long oligo-ethylene oxide chain **38** is assembled into spherical micelles, the diameters of the aggregates formed by calixarenes **36** and **37** with shorter oligo(ethylene oxide) chains exceeded the corresponding doubled molecular length; therefore, aggregates of **30** and **31** are most likely

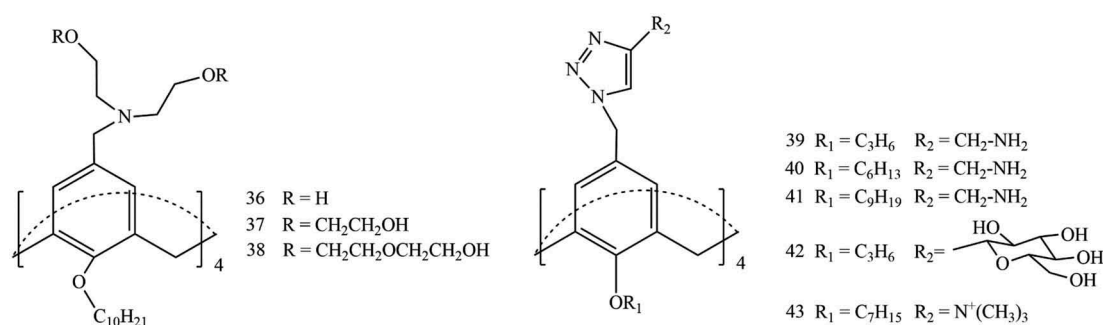


Figure 7. Molecular structures of amino derivatives of C[4]A.

vesicular formations. In this case, the vesicles significantly decrease in diameter with an increase in the length of hydrophilic chain, and a further increase in the chain length causes the transition of the vesicles to spherical micelles. A decrease in pH leads to protonation of the amino groups, which increases the surface area of the hydrophilic head group and, therefore, increases the taper of the molecule. A change in pH from 7 to 5 for **37** causes a sharp decrease in the diameter of aggregates from 36 to 6 nm, i.e. a transition from vesicles to micelles is observed. This phase transition is accompanied by the release of the drug previously encapsulated in the vesicles.

The aggregation of C[4]A-containing primary amino groups on the upper rim and alkyl fragments of various lengths (n-propyl, n-hexyl-) in an aqueous medium with different pH values was studied by Fujii et al. [65]. Macrocycles **39** and **40** (Figure 7) form spherical micelles in an acidic medium due to the strong repulsion of protonated amino groups. With increasing pH (partial deprotonation of amino groups), the phase transitions of spherical micelle-to-cylindrical micelle for **39** and of spherical micelle-to-cylindrical micelle-to-single-layer vesicle for **40** are observed, which is explained by a change in the conicity of macrocycle molecules. An increase in pH to 10 in **39** solution leads to the formation of network structures and gels. For a macrocycle with longer n-nonyl chains **41**, the cylindrical micelles are formed even at acidic pH, and the alkalisation of the solution leads to precipitation. In further studies of macrocycle **39** [66], Sakamoto et al. found that mixing of chiral C[4]A-containing galactose fragments on the upper rim **42**, which forms individual vesicular particles, with achiral **39** causes morphological rearrangement of the vesicles into filiform cylindrical structures with enhanced chirality. These cylinders are assembled into ribbon-like flat structures at the stoichiometric composition of macrocycles, and with an excess of A, the achiral cylinders from individual A begin to form in the solution, and the amount of mixed **39–42** cylinders decreases.

Similarly, cylindrical structures are formed after mixing ammonium C[4]A **43** with sulphonated C[4]A **44** (Figure 8) [67]. However, in contrast to [65], the sphere-to-cylinder transition in the mixed **43–44** system is realised due to electrostatic attraction between oppositely charged upper rims rather than through the van der Waals interactions. These macrocycles have similar heptyl chains on the lower rim and, in an individual state, form monodisperse micelles with a low aggregation number. At equimolar mixing of **43** and **44**, their charges are completely neutralised, and 100-nm vesicles are formed in the mixed system.

Alanine-functionalised C[4]A molecules **45** (Figure 8) forms in an acidic medium at pH 3 spherical aggregates with a diameter of 200–250 nm [68]. With an increase in pH to a neutral value (pH 7), the formation of 500-nm necklace-like aggregates is observed due to strong intermolecular hydrogen bonds. Further alkalisation to pH 9 leads to the destruction of these aggregates. The dendritic three-dimensional nanostructures in which silver was deposited on the surface of obtained calixarene aggregates were also obtained. Thus, assemblies based on amphiphilic calixarene functionalised with amino acid fragments can be used as stabilisers and form regulators in metal reduction reactions from their salts.

Of particular interest are calixarenes modified with carbohydrate moieties. Modification of the amphiphilic C[4]A with large carbohydrate, namely cellobiotic, fragments **46** (Figure 8) leads to the formation of micelles with a small aggregation number [69]. Due to hydrogen bonds between saccharide fragments, the volume of macrocycle head group becomes smaller, which leads to the formation of cylindrical aggregates. However, the splitting of hydrogen bond by changing the temperature or pH causes a morphological transition of the cylindrical structure to the spherical one. Micellar aggregates based on amphiphilic C[4]As with a low aggregation number were also obtained [70,71]. The completely monodispersed micelles formed in this case have the potential for practical application in the field of development of drug delivery systems and nano/microreactors.



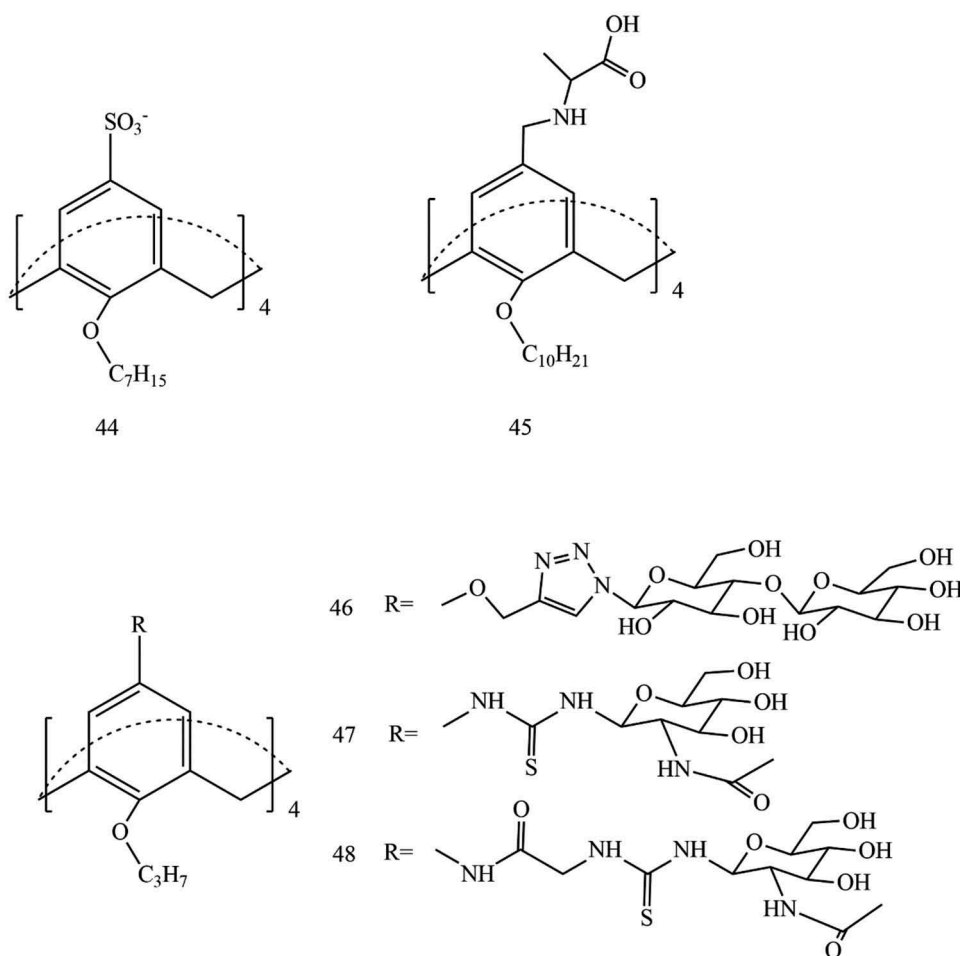


Figure 8. Molecular structures of C[4]As.

C[8]A molecules modified with *n*-propyl groups on the lower rim and glucosamino groups on the upper rim **47**, **48** (Figure 8) are in an aggregated form in an aqueous medium over a wide pH range. They adopt a vesicular structure in a neutral and alkaline medium, but at pH below 4.5, when nitrogen is protonated, a transition from the vesicle to the micelle occurs due to a change in surface charge density [72]. This pH-sensitive behaviour of C[8]A aggregates, together with their molecular recognition ability, opens up prospects for their use as systems for the selective delivery of hydrophilic drugs (for example, in infections or tumours with an acidic environment). Gallego-Yerga et al. received self-assembling nanocapsules and nanospheres with internal and hydrophobic regions from amphiphilic systems synthesised by click-heterodimerization between hydrophilic  $\beta$ -cyclodextrin and hydrophobic C[4]As **49**, **50** (Figure 9) [73]. These aggregates well encapsulate the drug docetaxel and thereby increase its antitumor activity.

Strobel et al. showed that the nature of ionic group in amphiphilic calixarenes is a key parameter for controlling their aggregation in an aqueous solution. If carboxylated

C[4]A formed bilayer structures (vesicles and lamellar liquid crystals), whereas C[4]A with trimethylammonium groups most likely form small micelles with a diameter of less than 5 nm, which aggregate at higher concentrations into rectangular lyotropic liquid crystals [61]. In another work [49], amphiphilic dendroC[4]A **51** (Figure 9) was synthesised, which independently assembled into absolutely homogeneous and structurally stable micelles. Seven molecules of this macrocycle fold into small and highly curved aggregates, which in turn did not form larger aggregates of low curvature. The micellar form of these aggregates, the formation driving force for which is probably hydrogen bonding, is confirmed by the encapsulation of water-insoluble derivatives of porphyrin and fullerene. Other dendrimer conjugates based on PEG, benzamide and amphiphilic C[4]A-containing various alkyl chains (*n*-propyl-, *n*-pentyl- and *n*-decyl-) **52–54** (Figure 9) also form micellar structures in an aqueous solution [74]. However, hydrophobic anti-inflammatory drugs naproxen and ibuprofen are not encapsulated in the hydrophobic core of these micelles, but inside the macrocycle cavities due to hydrogen bonds and  $\pi$ - $\pi$

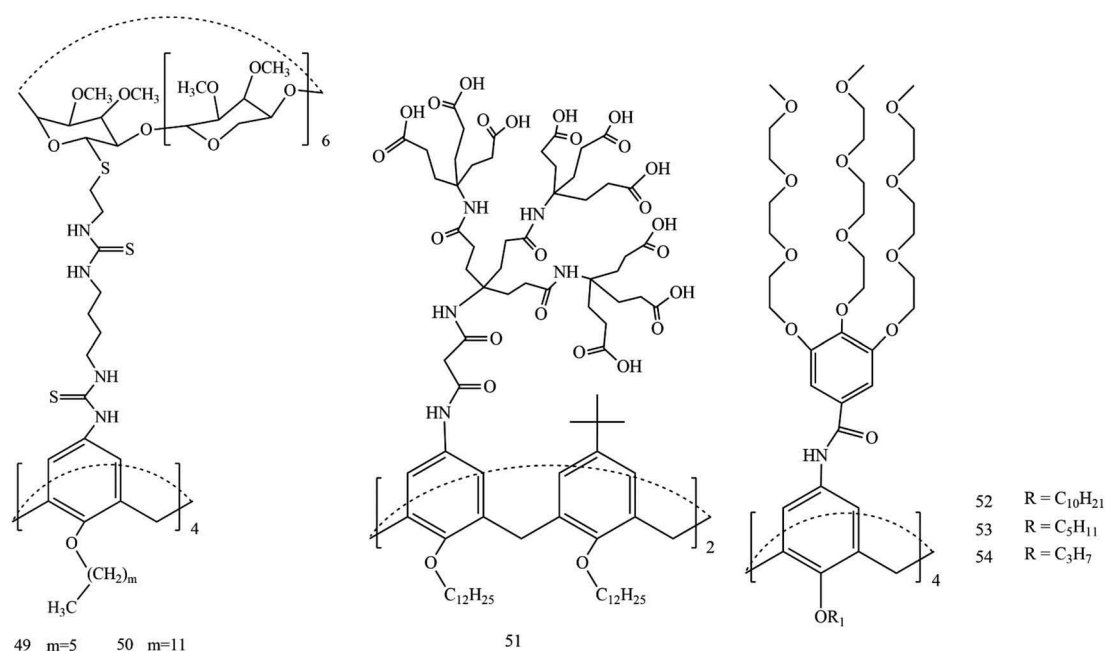


Figure 9. Molecular structures of C[4]As.

stacking. The guest–host interaction between drug and macrocycle pushes the substituents on the upper rim and initiates the formation of hollow micelles of larger diameter. Interestingly, an increase in the length of the alkyl tail on the lower rim reduces the stability of the complexes. This effect is explained by the fact that longer alkyl fragments repel benzamide substituents more strongly, which narrows the cavity for encapsulation of drugs. Accordingly, the shorter the alkyl tail on the macrocycle lower rim, the more amount of drug is encapsulated.

### Calixarene-based capsules and stacked structures

For CA macrocycles it is possible to realise the formation of not only spherical aggregates but also the creation of dimers mainly due to hydrogen bonds. A considerable number of capsules formed for various guests are shown by examples of resorcinarenes ([28], [75–79]). However, in the case of amphiphilic resorcinarenes instead of encapsulation of guests, the individual self-assembly of macrocycles into dimeric ones or stacked structures is observed [80]. Due to the formation of hydrogen bonds between hydroxyl groups, amphiphilic C[4]R **55** (Figure 10) can form capsules consisting of 6 macrocycle molecules and 8 water molecules. These capsules can be used as nanoreactors for cyclisation reactions [81,82], bipolar cycloaddition reactions [83], and oxidation of thioethers to sulphoxides [84]. The possibility of carrying out these reactions without the presence of catalysts, while maintaining a high product yield and greater selectivity, including stereoselectivity, attracts great attention to these macrocyclic systems.

Capsules can be formed by non-covalent interaction of oppositely charged calixarenes **62** and **63** (Figure 11). Corbellini et al. showed the formation of such capsules in an equimolar mixture of these macrocycles in polar solvents as a result of multiple ionic interactions. The resulting capsules have an internal cavity that can accommodate cationic guests such as acetylcholine, tetramethylammonium and N-methylquinuclidinium. A similar system was obtained in a mixture of viologen C[4]R **60** and sulphonated C[4]R **56** (Figure 10). Complete reduction of the viologen macrocycle led to the destruction of the capsule, i.e. the stability of this capsule depends on the oxidation state [85].

C[5]A molecules with phosphonic groups on the upper rim **64** (Figure 11) form 2-nm bis-molecular capsules that are stable in aqueous solution over a wide pH range [2–10], but are destroyed in the presence of a polar aprotic solvent (N, N-dimethylformamide). The capsule structure is also disturbed by vigorous stirring, which in turn allows the anticancer drug carboplatin to be encapsulated with an encapsulation efficiency of 85%. Removing water from this system leads to the release of the encapsulated drug and the formation of nanofibers of 2 nm in size, in which the cavity of one C[5]A is embedded in the cavity of another macrocycle [86]. Another study also demonstrated the formation of stack nanocontainers of drugs. C[8]A with a negatively charged upper rim from sulphate groups and a positively charged lower rim from ammonium groups **65** (Figure 11) in pH region 7.05–7.58 forms stack-type head-to-tail structures due to electrostatic interactions.

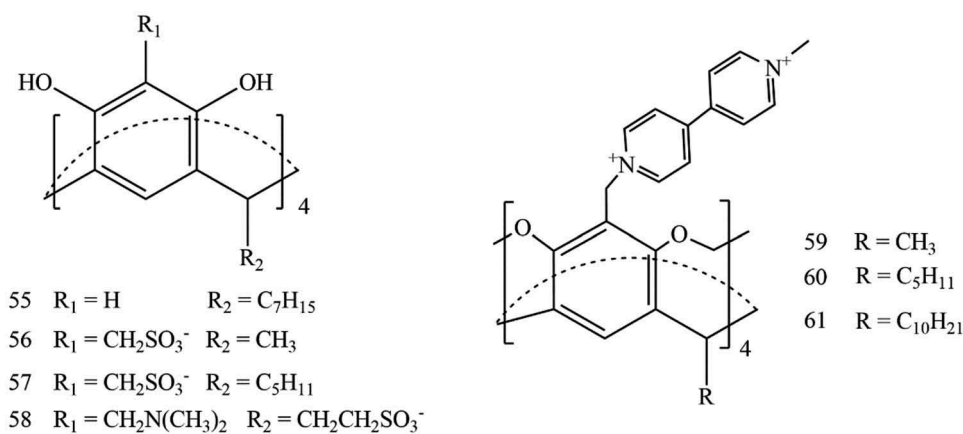


Figure 10. Molecular structures of C[4]Rs.

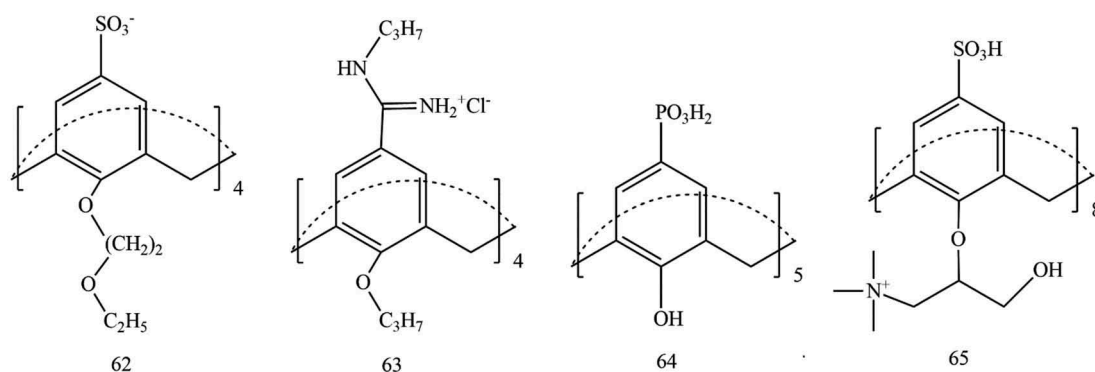


Figure 11. Molecular structures of CAs forming stack aggregates.

The resulting fibres are also capable of solubilising hydrophobic preparations, and a change in the pH (acidification or alkalisation) leads to the destruction of the aggregates with the subsequent release of the encapsulated drug [87].

A similar non-covalent polymerisation of monomer units based on C[4]R with oppositely charged rims **58** (Figure 10) was realised by Ziganshina et al. [88]. The supramolecular oligomer formed through electrostatic interaction can bind a hydrophobic guest. However, the acidification of this mixture led not only to the protonation of all four amino groups of the upper rim but also to the appearance of cationic guest form. In an acidic environment, the guest cations were tightly bound to the lower sulphonate rim, which destroyed the polymer nature of mixed aggregates with the formation of individual host-guest complexes.

Aggregation of calixarene molecules in the head-to-tail manner can be realised through cooperative effect of weak hydrophobic interactions. Syakayev et al. studied the dependence of the types of C[4]R-based aggregates on the length of the alkyl tail. While macrocycle with

methyl groups is not aggregated, the macrocycle with heptyl groups is aggregated in water and water-methanol solutions into a conventional micelle. However, the head-to-tail packing is observed C[4]R with pentyl fragments **57** (Figure 10) due to the effect of CH- $\pi$  or  $\pi$ - $\pi$  interactions. The binding of guests (tetramethylammonium and N-methylpyridinium) leads to additional stabilisation of these aggregates, and the stack packaging is preserved [89,90]. It was shown that the binding of guest molecules by aggregates of C[4]R is more effective than individual macrocycles, because in this case, the guest molecules enter a capsule formed by two macrocyclic molecules [90,91].

Kashapov et al. investigated the aggregation of tetra- viologen C[4]R in aqueous media and also showed the effect of the length of hydrophobic tail on the lower rim on the structure of aggregates [92]. Macrocycle with methyl fragments **59** did not form aggregates, and cavitand with decyl fragments **61** assembles in spherical aggregates of probably vesicular structure (Figure 10). The formation of stack structures by head-to-tail type was observed for C[4]R with pentyl tails **60** (Figure 10),

which is unusual for typical surfactants, but as can be seen from the above examples, this type of aggregation is characteristic of C[4]R with pentyl tails on the lower rim.

### Calixarene-based gels

One of the promising types of nano-structural aggregates is hydrogels. Hydrogels are three-dimensional polymer hydrophilic networks capable of absorbing a large amount of biological fluids or water [93]. Hydrogel systems play an important role as scaffolds for tissue engineering, biosensors, biological microelectromechanical systems and drug carriers. They are formed by crosslinking a gelling agent through covalent or non-covalent interactions. Chemically cross-linked gels have greater strength, but gels based on non-covalent interactions have the properties of good stimulus sensitivity, reversibility, and self-healing.

Song et al. described gelation of amphiphilic C[4]A with carboxyl groups on the upper rim of **30** and **66** (Figures 6 and 12) by dissolving them in ethanol and then adding water [94]. Unlike most other supramolecular gels obtained by cooling a hot solution, the hydrogels based on these macrocycles were formed in an aqueous medium with pH 6 at room temperature. During this gel formation, unstable nanospheres ranging in size from 50 to 140 nm were tuned into stable monodisperse nanofibers with a diameter of about 10 nm for 12 h.

Sharma et al. synthesised macrocycles containing octyloxy chains embedded in the ester derivatives of oxadiazole and thiadiazole on the lower rim **67** and **68** (Figure 12), which formed gels in solvents such as decane and dodecane [95]. Moreover, stable gels with very low critical gel concentrations (less than 2 wt.%) were formed in the last solvent. Interestingly, the **67** and **68** macrocycles exhibit reversible thermal gelling

properties, which is reflected in the fluorescence spectra. A decrease in temperature from 60°C (sol) to 20°C (gel) increases in the emission intensity of macrocycle and vice versa.

### Calixarene-based mixed compositions

In modern supramolecular chemistry, the most widely used approach to the formation of ensembles with useful properties is the combination of several components of different structures by their non-covalent self-assembly. This approach made it possible to create a large number of systems with the properties of nanocontainers [96], nanoreactors [97], and molecular devices [98]. The supramolecular approach can make the synthesis of complex structures simpler and highly reversible compared to the covalent modification. In this vein, the creation of supramolecular amphiphiles (superamphiphiles) is one of the actively developing areas. Unlike traditional amphiphilic compounds, superamphiphiles contain hydrophilic and hydrophobic fragments preorganized by non-covalent interaction [99]. The dynamic nature of the formation of non-covalent bonds can give the system as a whole a reversible change in physicochemical parameters, which is a key characteristic of supramolecular systems, since it allows controlling the process of self-assembly under the influence of external stimuli and, therefore, using superamphiphiles as intellectual nanomaterials. In addition, this section discusses the phenomena of mixed aggregation of calixarenes and potential application of calixarene-based mixed systems in practice, with special emphasis on drug delivery and other aspects of biomedicine.

### Interaction with surfactants

Calixarenes and resorcinarenes can be used as building blocks for superamphiphiles, namely, as a hydrophilic

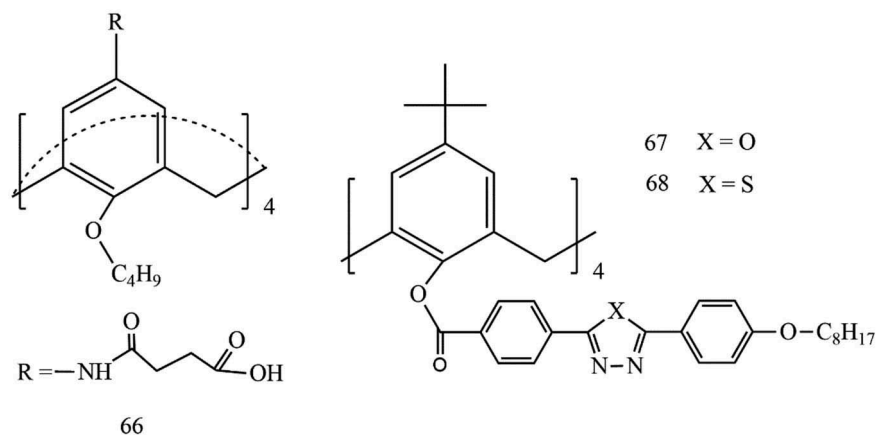


Figure 12. Molecular structures of CAs forming gels.

fragment exhibiting receptor ability for another molecule with a hydrophobic fragment [100,101]. A number of studies are devoted to the formation of aggregates when mixing sulphonated macrocycles with cationic surfactants. Y. Zhou et al. investigated the 1:1 complexes formed upon mixing C[4]A **5** and C[6]A **8** with CTAB, which can form micelle-like aggregates [102]. When non-aggregating and surface-inactive C[6]A **21** is added to cationic surfactant with same head group and dodecyl tail, the supramolecular amphiphiles are formed, which also create micellar aggregates [103]. In the absence of calixarene, the CMC of this surfactant determined by tensiometry is 14 mM, but in the presence of 5 mM calixarene the first breakpoint in the concentration dependence of surface tension is observed at 0.2 mM, i.e. up to this concentration there are guest-host complexes in a ratio of 1:1, and after this concentration, the supramolecular micelles are formed. Further increase in the concentration of surfactants decreases the surface tension values, which reaches values of an individual surfactant at a concentration of 30 mM. Taking into account that there are six sulphonate groups in the macrocycle structure that dissociate in an aqueous medium with the formation of six negative charges, the inflection observed at surfactant:calixarene ratio of 6:1 probably corresponds to complete charge compensation in the system, and after 30 mM, the micelles formed to exhibit a solubilising effect to calixarene molecules [103,104].

When mixing sulphonated C[4]A **5** with trimethylammonium surfactant-containing tetradecyl tail, the formation of unilamellar vesicles, rather than micelles, was identified. This mixed macrocycle-surfactant system at a ratio of 1:2.5 after sonication of an aqueous dispersion with subsequent lyophilisation and rehydration forms stable vesicles that can be stored for a long time without changing the size and shape [105]. The reason for the formation of aggregates of non-micellar structure in this work is probably due to the use of a smaller macrocycle cavity. The presence of sulphonated C[4]A as a hydrophilic fragment gives a cylindrical rather than conical shape to superamphiphile, which leads to the formation of bilayer structures.

The complexation of the cationic surfactant with sulphonate calixarenes leads to the formation of surfactants with other surface-active properties that cause aggregation at lower concentrations than the pure surfactant. It should be noted that CMC in the presence of calixarene decreases by 70 times [103]. In another work, a decrease in critical concentrations of aggregation of gemini surfactants by a factor of 1000 was demonstrated in the presence of sulphonated C[4]A **5** [106]. For comparison, we can give examples of a decrease in CMC in the presence of cryptands by 5 times, and cyclodextrins,

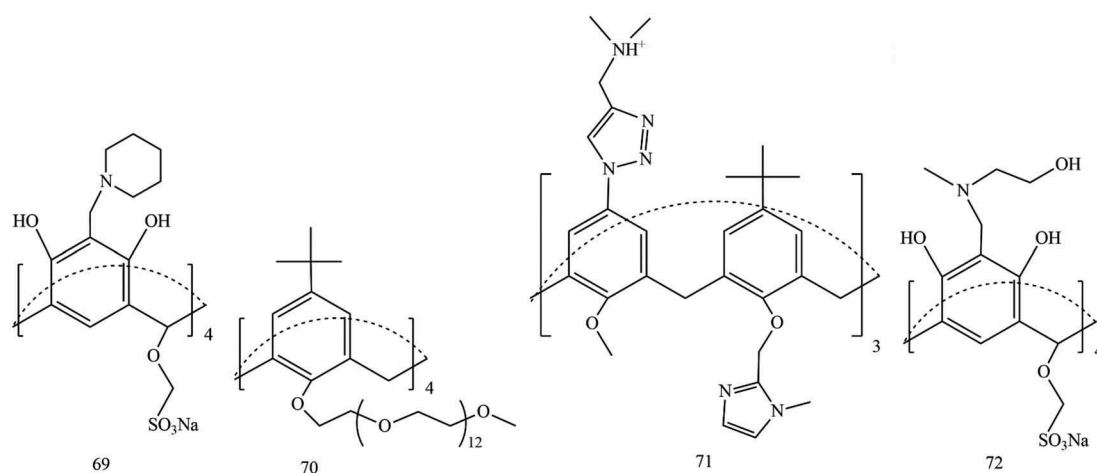
on the contrary, increase CMC of surfactant. Moreover, when comparing the complexation of cyclodextrins, cucurbiturils, and sulphonated calixarenes with an ammonium derivative of tetraphenylethylene, it was found that, in addition to the electrostatic factor, the prearranged cyclic frameworks and the encapsulating ability of calixarenes internal cavity play a decisive role in the effective mixed aggregation of the guest in the presence of a macrocycle [107].

A decrease in CMC value for surfactant in the presence of C[4]R was also shown by Kashapov et al. [108,109]. In these works, aminomethylated derivatives of sulphonated C[4]R **58**, **69** (Figures 10 and 13) and cationic DABCO surfactants with hexadecyl tails were involved. Where there is excess of the macrocycle, the mixed aggregates with surfactant are formed under the action of electrostatic forces, and where there is excess of surfactants in the solution, the formation of mixed micelles is observed due to intermolecular dispersion forces that occur when non-polar parts of the surfactant come into contact. Varying the ratio of components in these supramolecular systems allows controlling the process of binding and release of hydrophobic substrates. Similar changes in supramolecular structures are realised in the mixed system based on sulphonated C[4]A **5** and hexadecyl surfactant with serine head group [110]. The addition of a small amount of calixarene to the surfactant solution first causes the formation of small micellar aggregates, and as the macrocycle content in the solution increases, tubular structures and vesicles appear due to a decrease in the taper of supramolecular amphiphiles.

The paper [101] demonstrated the formation of a guest-host complex between a hydrophobic phenyl palmitate and a hydrophilic conjugate based on C[4]A and PEG **70** (Figure 13), which can then aggregate into various aggregates depending on the ratio of components in the aqueous medium. In the presence of an excess of macrocycle, these superamphiphiles aggregate into vesicular particles with a diameter of 270 nm. An increase in the amount of hydrophobic surfactant to an equimolar ratio shifts the equilibrium from vesicles to micellar particles with a diameter of 130 nm, and an excess of surfactant leads to the formation of network aggregates. Thus, the release of the encapsulated formulations can be controlled by changing the environmental conditions.

The mixed aggregation of sulphonated C[4]R containing amino groups on the upper rim **69** with anionic surfactant sodium dodecyl sulphate at neutral or alkaline pH values is absent or weakly expressed [111]. However, a decrease in pH to acidic values causes protonation of the amino groups in the macrocycle structure and its



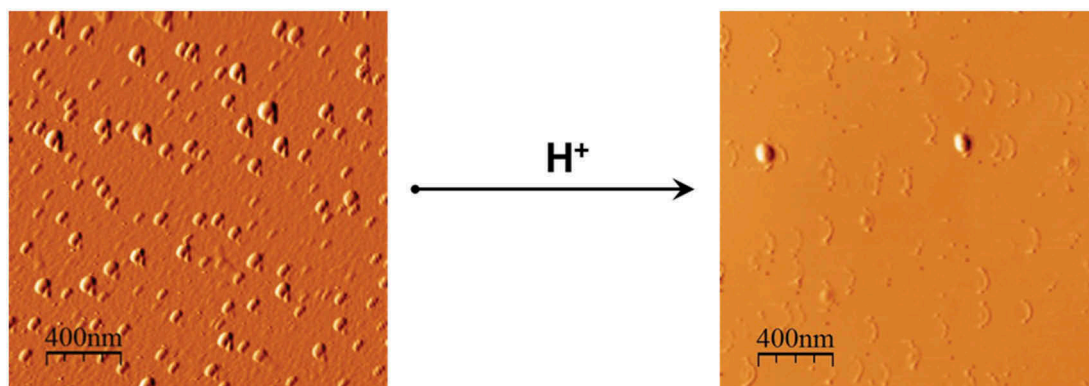


**Figure 13.** Molecular structures of macrocycles forming superamphiphiles with surfactants.

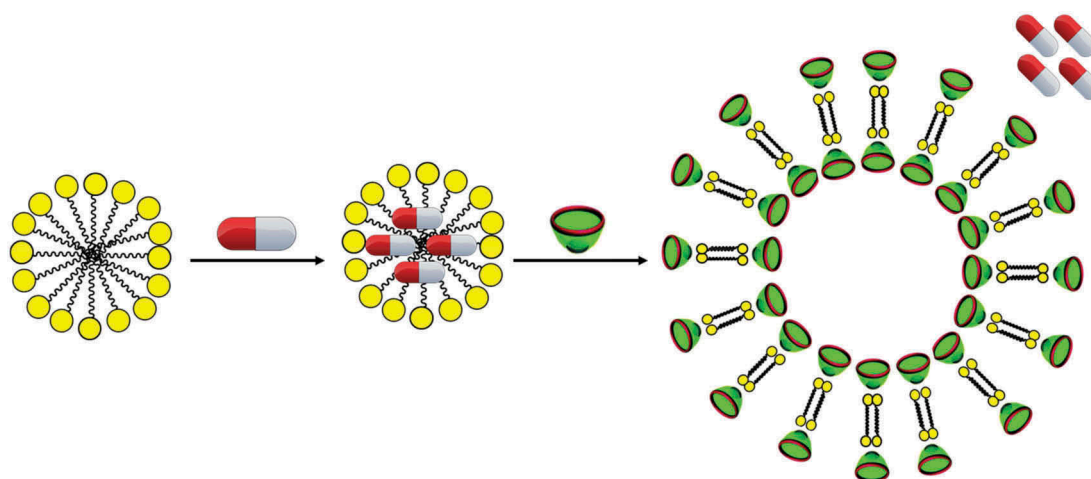
electrostatic interaction with negatively charged surfactant sulphate groups. As a result of the formation of these mixed aggregates, a morphological transition of spherical micelles to mixed rod-like micelles occurs (Figure 14). This morphological rearrangement of spherical particles into rod-like aggregates is accompanied by an increase in the encapsulation of the hydrophobic flavonoid quercetin.

Wintgens et al. presented studies on the effect of macrocycle size on the aggregation of supramolecular amphiphiles based on sulphonated C[4]A **5** and C[8]A **9** with 1-methyl-3-tetradecylimidazole in aqueous neutral solutions [112]. If a macrocycle with four sulfonatophenol units forms large nanoparticles when interacting with a surfactant, then superamphiphiles with C[8]A aggregate into micelles, which are able to rearrange into nanoparticles in the presence of 50 mM sodium chloride. Further, the same group of scientists investigated the effect of changes in the composition of the

surfactant head group on the self-assembly of supramolecular amphiphiles. When the hydrophobic component in the head group of the surfactant dominates, then superamphiphiles with C[6]A **8** line up into multilayer nanoparticles. Surfactant molecules containing more hydrophilic head groups with a higher charge density are able to assemble with the macrocycle not only into nanoparticles but also into supramolecular micelles depending on the ratio of components, salt concentration and temperature [113]. The study of the complexation of sulphonated C[4]A **5** and C[6]A **8** with 1-alkyl-3-methylimidazolium bromide concluded that macrocycles form a 1:1 complex with a surfactant molecule due to the charge- $\pi$  and  $\pi$ - $\pi$  interactions between the imidazolium fragment of guest and aromatic cavity of macrocycles [114]. Moreover, the guest complex with C[4]A has greater stability in contrast to that with C[6]A, but the stability of the latter complex increased through elongation of the alkyl fragment (from methyl to decyl)



**Figure 14.** pH-sensitive morphological transition observed in mixed **69**–sodium dodecyl sulphate system.



**Figure 15.** Schematic representation of drug release from CTAB micelles under the action of C[4]R.

in the guest structure. Therefore, the formation of stable spherical and multilayer particles in an aqueous medium of C[6]A with similar guests having a longer alkyl tail (from dodecyl to hexadecyl) was shown by the methods of cryo-TEM and small-angle neutron scattering [115].

Bize et al. reported spontaneous formation of vesicles by combining aminoC[6]A **71** (Figure 14) with surfactant-containing sugar moieties and carboxyl groups at different ends of the alkyl chain [116]. The vesicles formed by this superamphiphile possessed remarkable stability over time in a wide pH range (from 4 to 11). The diameters of the vesicular particles ranged from 300 to 600 nm and stored for a month. However, the strongly acidic medium led to the destruction of the vesicles due to protonation of amino groups of macrocycle and carboxylic groups of surfactant. Peng et al. constructed the dynamic vesicle from superamphiphiles built on the electrostatic interaction of (dodecyloxybenzyl) tripropargylammonium and sulphonated C[4]A **5**, then this dynamic vesicle was crosslinked by a click reaction [117]. The vesicle crosslinked in this way has improved stability compared to the dynamic one and encapsulates the hydrophilic drug hydroxysafflor yellow A, but the encapsulated drug can be released only by destroying the vesicle using special strong trigger molecules. Since calixarenes have a high affinity for surfactants, these macrocycles can also act as effective triggers for the release of drugs encapsulated in surfactant micelles (Figure 15). Adding an excess of macrocycle **72** to a micellar CTAB medium saturated with a solubilised substrate leads to a phase transition of micelles into vesicular aggregates, which can initiate the release of an encapsulated hydrophobic drug [118].

A multistimuli-sensitive system for delivery and controlled release of doxorubicin was developed by Wang

et al. based on the aggregation of sulphonated C[4]A **5** with viologen surfactant with dodecyl tail in a ratio of 1:2 [119]. The formed vesicles consisted of superamphiphiles formed by the electrostatic interaction of four negatively charged sulfo groups of one macrocycle with four positively charged head groups of two surfactants. The addition of an excess of the macrocycle disrupted the vesicles into separate 1:1 guest-host complexes. Since the thermal effect of complexation is the result of an exothermic process, the mixed aggregates were destroyed at high temperature. The addition of hydrophilic  $\beta$ -cyclodextrin led to complexation with surfactant, which also destroyed the vesicles. The reduction of the viologen group to neutral form facilitated the process of excluding surfactants from calixarene cavities and dissociation of aggregates.

The attention of scientists in the study of calixarene complexes was given to the binding of choline derivatives in order to create biocompatible aggregates for drug delivery, which will release the encapsulated drug under the action of enzymes in the human body. Murayama and Aoki as early as 1997 showed the possibility of binding the ammonium part of the neurotransmitter acetylcholine with aromatic rings of C[4]R due to multiple cation- $\pi$  interactions [120]. Liu et al. obtained vesicular aggregates from sulphonated C[4]A **5** and natural myristoylcholine, which can be cleaved by cholinesterase enzymes overexpressing in patients with Alzheimer's disease. Although hydrophobic drugs can also be loaded into micelles from an individual amphiphilic myristoylcholine in an aqueous medium, they cannot be released, since myristoylcholine is cleaved to myristic acid without destroying the aggregates. However, the combination of C[4]A with myristoylcholine at a molar ratio of 1:10 gives binary vesicles formed

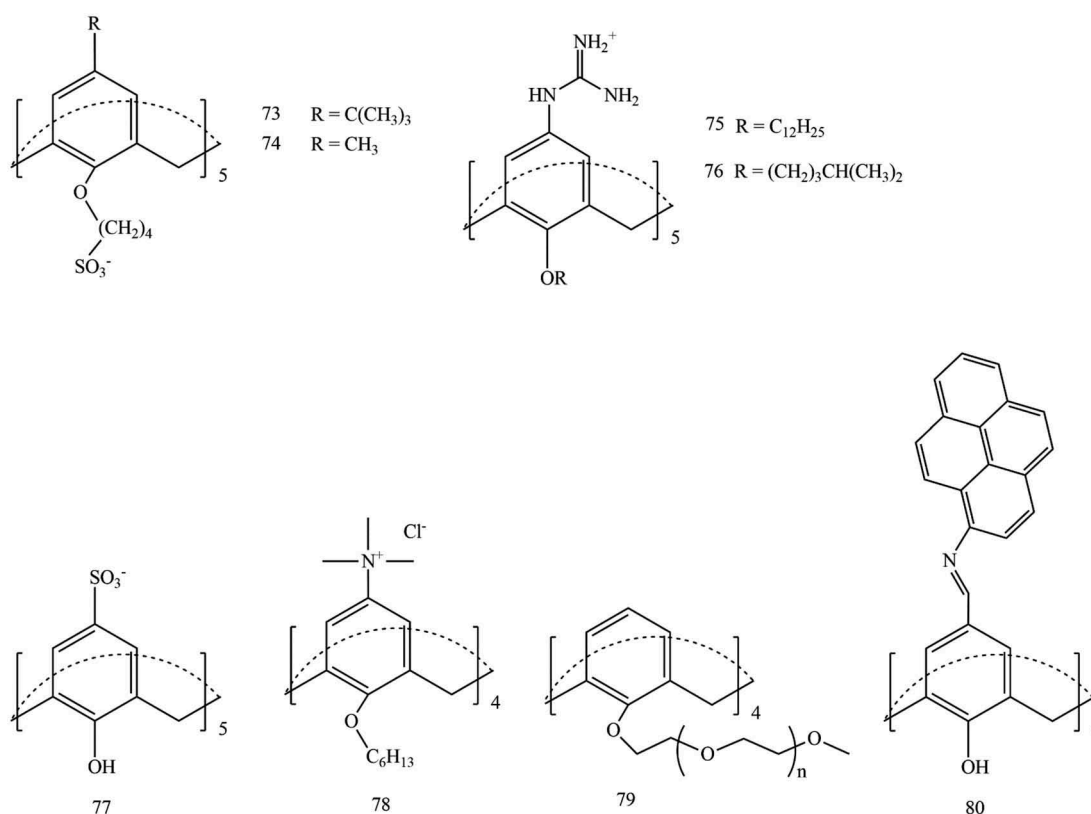


Figure 16. Molecular structures of CAs.

from host-guest complexes. Such aggregates decompose under the action of cholinesterase, since the macrocycle does not form complexes with myristic acid [121].

In another study, superamphiphile based on same sulphonated C[5]A **73** (Figure 16) involved encapsulation of water-insoluble drug and was investigated the change in the morphology of this macrocycle upon addition to cationic bola-amphiphiles [122]. The supramolecular bola-amphiphile obtained from these components more than 1000 times increases the solubility of the antitumor drug tamoxifen in water. Depending on the length of the bolaamphiphile molecule, the formation of not only mixed vesicles but also capsules with C[5]A molecules is possible. Pisagatti et al. noted the selectivity of the formation of such capsules and self-sorting in the mixed solutions containing C[5]As **73** and **74** with p-methyl and p-tert-butyl groups, respectively, and bola amphiphiles of different lengths [123]. When surfactant with decyl chain was mixed with an equimolar mixture of **73** and **74**, one bolaamphiphile molecule formed a capsule with only two molecules of macrocycle **73** having bulky tert-butyl groups on the upper rim. The results of the DOSY NMR experiment in this solution excluded the interaction of this surfactant with the second macrocycle **76** present in the solution. The formed capsules 2:1 with **73** were very

stable at temperatures above 50°C and with fivefold dilution. Subsequent addition of another bolaamphiphile with octyl chain to this mixture resulted in capsules with two **74** molecules, and none of the other eight probable complexes formed even when both surfactants were simultaneously added to the mixture of two macrocycles.

The principles of superamphiphilic aggregation of calixarenes with surfactant molecules can be used to create biosensors. Zheng et al. use amphiphilic C[5]A **76** (Figure 16) molecules modified with guanidinium fragments along the upper rim for targeting to lysophosphatidic acid [124]. Lysophosphatidic acid is a biologically active phospholipid and is considered an ideal biomarker for early detection of ovarian cancer and other gynaecological cancers. Thus, the molecular recognition of calixarene macrocycles with surfactant molecules can be a major advantage in biomedical applications.

### Interaction with aromatic compounds

Spontaneous formation of mixed aggregates is also possible by mixing calixarenes with molecules in which the hydrophobic part is represented not by surfactant alkyl tail, but by an aromatic fragment. Supramolecular vesicles can be formed through complexation between oppositely charged

sulphonated C[5]A **77** (Figure 16) and 1-pyrenemethylamine. The formation of these aggregates occurs at a ratio of host:guest = 1:4, and an excess of calixarene leads to the destruction of aggregates and the formation of 1:1 inclusion complex. The obtained vesicles have thermal reversibility and can decompose when the temperature rises to 35–40° C, releasing the doxorubicin hydrochloride preliminarily loaded into them [19].

A photolysed superamphiphilic system was created using sulphonated C[4]A **5** and anthracene derivative in the ratio 1:1, which independently aggregate into nanoparticles with an average diameter of 266 nm [125]. Upon irradiation with UV light with a wavelength of 365 nm, anthracene guest slowly decomposed into anthraquinone and alkanol, but its photodecomposition rate increases noticeably in the aggregated state with C[4]A, which is accompanied by the disruption of the superamphiphilic system. In addition, these aggregates can undergo effective photolysis under the influence of visible light with a wavelength of 520 nm in the presence of eosin Y as an exogenous photosensitiser. This approach can be used to create various photoreactive self-assembling materials, which makes calixarene-induced aggregation a promising strategy for creating systems for photodynamic therapy and photodegradation of pollutants.

The phosphatase-sensitive supramolecular vesicles formed from superamphiphiles based on amphiphilic C[4]A-containing cationic amino groups on the upper rim **78** (Figure 16) and adenosine triphosphate were reported [126]. Under the influence of phosphatase, nucleoside triphosphate decomposes to adenosine monophosphate, which cannot form a stable complex with the macrocycle, as a result of which the mixed aggregates disintegrate. Since phosphatase is overexpressed in many tumour cells, calixarene–adenosine triphosphate aggregates sensitive to this enzyme can be used in delivery systems of antitumor drugs.

The mixed vesicles based on macrocycle and drug can be also used for drug delivery. Qin et al. constructed the aggregates by interaction between sulphonated C[4]A **5** the antipsychotic drug of cationic nature chlorpromazine in two different ways: (i) from superamphiphiles formed by the electrostatic attraction between the charged amino groups of drug and the sulphonate groups of macrocycle molecules, while the phenothiazine rings of drug are connected with each other by  $\pi$ -stacking; (ii) from ion pairs rather than the guest-host complex as in the first case. In both cases, the large multilayer spherical micelles are formed, which can be also non-covalently modified with such a targeted agent as trimethylated chitosan. However, in the first case, the drug loading efficiency is 61%, and in the second – 46% [127].

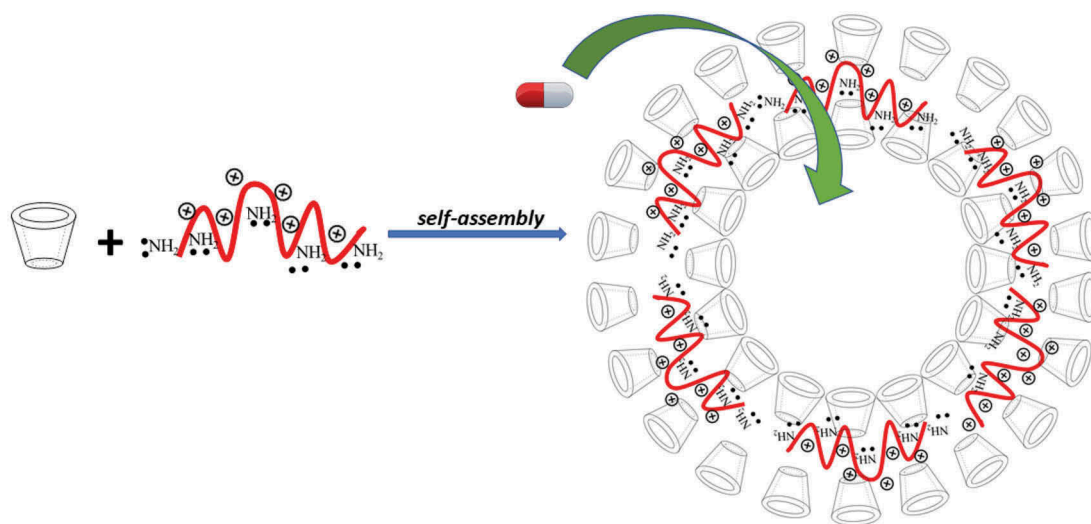
Similarly, the mixed aggregates based on amphiphilic C[4]A-containing hexyl tails on the lower rim **20** and anticancer drugs irinotecan and mitoxantrone were obtained [128]. Nanoparticles obtained by electrostatic and hydrophobic interactions between the macrocycle and drugs have high loading efficiency (65.2% for irinotecan composition and 43% for mitoxantrone composition) and prevent alkalisation of drugs. The surface of these nanoparticles can be non-covalently modified with the pyridinium derivatives of hyaluronic acid (for aggregates with irinotecan) and biotin (for aggregates with mitoxantrone). Thus, the supramolecular strategies revealed in this work allow creating the containers for drugs and probes for visualisation with targeted action, which relevant for effective diagnosis and therapy.

The aromatic cavity of C[4]A molecules can be used to encapsulate hydrophobic guest molecules, which in turn can initiate mixed aggregation. Supramolecular aggregation of superamphiphile based on hydrophobic photosensitiser and C[4]A modified with hydrophilic polyethylene glycol fragments at the lower rim **79** (Figure 16) was realised [129]. The unmodified wide upper rim of this macrocycle encapsulates the chlorin e6 molecule into the cavity with the formation of superamphiphiles, which aggregate in aqueous solution into polymer micelles with a diameter of less than 200 nm suitable for passive target delivery. Encapsulation of the hydrophobic choline e6, which is used in the photodynamic therapy of cancer, allows it to be delivered to tumour tissues with higher quality.

Dinda et al. synthesised C[4]A **80** (Figure 16) containing fluorescent pyrene fragments on the upper rim [130]. This macrocycle selectively binds trinitrophenol (in solution, in solid form, on the surface of silica gel and cellulose), and a several-fold increase in fluorescence is observed with this complexation. The formation of the host–guest complex occurs due to the capture of trinitrophenol by two adjacent pyrene fragments through  $\pi$ – $\pi$ -stack interactions. In the solution, the macrocycle **80** exists in the form of spherical particles with a diameter of  $272 \pm 42$  nm, and the addition of trinitrophenol leads to a decrease in the size of aggregates by about three times and their coalescence in elongated chain aggregates of  $98 \pm 22$  nm in size.

### Interaction with polymers

It's known that polymers as well as surfactant molecules can form globular particles, but unlike surfactant micelles, the polymer aggregates have greater stability. Supramolecular polymer vesicles were created by Peng et al. by complexation of sulphonated C[4]A **5** and chitosan [131] (Figure 17). The main driving force behind the



**Figure 17.** Schematic illustration of formation of supramolecular polymeric vesicle based on C[4]A and chitosan.

formation of aggregates is the numerous electrostatic interactions between the sulphonate groups of the macrocycle and the amino groups of chitosan. These superamphiphilic systems are disassembled under the influence of pH, since the partial deprotonation of chitosan occurs with increasing pH. Addition of a competing guest can also destroy the mixed aggregates of calixarene with the polymer. According to the same principle, the formation of mixed aggregates between same sulphonated C[4]A **5** and cationic protamine polypeptide was observed in the aqueous medium [132]. Cellular experiments and fluorescence imaging in mice showed that the vesicles formed between them are non-toxic and can be cleaved by the trypsin enzyme with controlled release of encapsulated hydrophilic drugs. Thus, the presented system can increase the drug effectiveness in cells with overexpression of trypsin and at the same time minimise unwanted side effects.

The influence of external factors on the mixed aggregation of sulphonated calixarenes and chitosan was also studied [133]. In mixed systems with C[4]A **5** the smaller particles are formed than in systems with larger macrocycles C[6]A **8** and C[8]A **9**. An increase in the concentration of chitosan leads to an increase in the diameter of these nanoparticles, while the concentration of the macrocycle does not affect the particle size. In the mixed system of C[8]A due to its large cavity, an additional binding to alkaloid molecules is provided. Thus, this macrocycle can replace the commonly used tripolyphosphate crosslinking agent with the additional benefit of forming inclusion complexes with drug molecules. Similar patterns of mixed aggregation and encapsulation of alkaloids were identified by Wintgens et al. that involved different sulphonated calixarenes and dextran molecules with methylimidazole side groups [134].

Pegylated superamphiphilic systems can be used for phototeranostics, which implements both selective imaging of tumours and targeted *in vivo* therapy. Gao et al. used mixed micelles based on an equimolar mixture of C[5]A **75** (Figure 16) containing guanidine fragments on the upper rim and dodecyl tails on the lower rim and PEG modified with the same alkyl tail for targeted delivery of photosensitisers to the cancer tumour [135]. The anionic photosensitiser molecule binds to the macrocycle, which initiates almost complete quenching of fluorescence and decrease in photoactivity of the bound guest due to the photoinduced electron transfer mechanism. However, at the same time, the high affinity of the macrocycle is identified to adenosine triphosphate molecules, which are overexpressed in tumour tissues, remains. Accordingly, on tumour cell contact, adenosine triphosphate displaces the sensitiser from the complex with the macrocycle, which restores its fluorescence and photoactivity. Compared to a free photosensitiser, the macrocycle–polymeric surfactant composition is not only capable of selectively visualising a tumour in real time but also provides a much more effective removal of cancer.

Instead of natural polymers, the genetic polymer can be used to create supramolecular systems with calixarenes. Sansone et al. functionalised a series of calixarenes with guanidinium groups and tested their ability to bind DNA. C[4]As with hexyl **83** and octyl **84** (compared to propyl **82**) tails on the lower rim (Figure 18) turned out to be the most effective in transfection, which is in good agreement with the confirmed data obtained for cationic surfactants widely used in this field. Unlike the data for C[4]A **81** in cone conformation, the conformationally mobile C[6]A **85** and C[8]A **86** with methyl tails (Figure 18) do not form



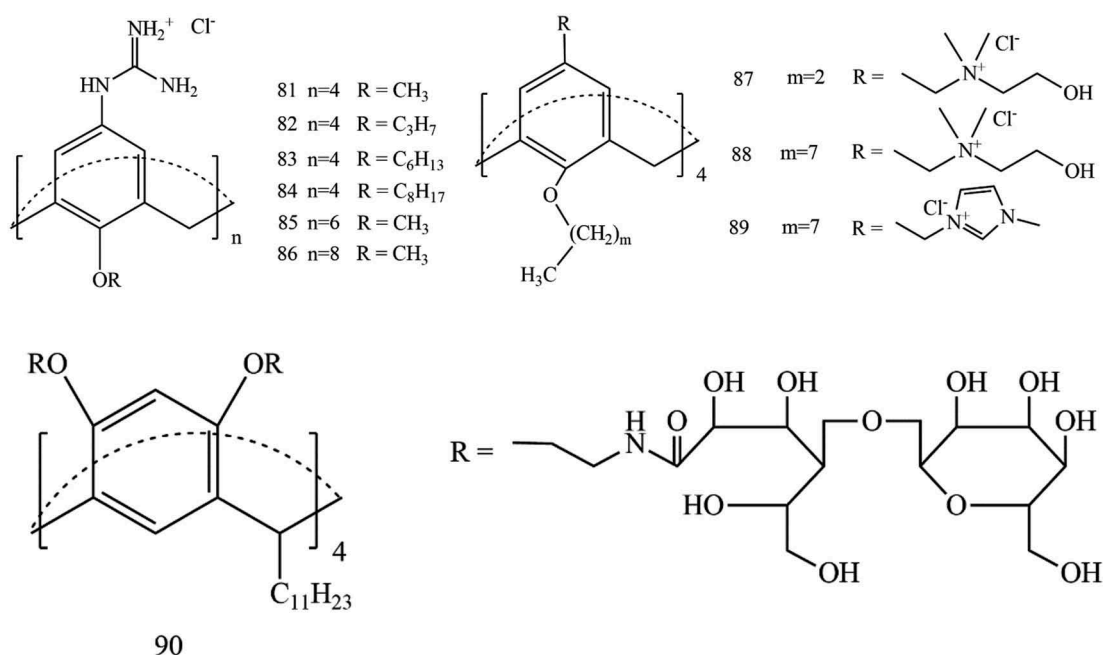


Figure 18. Molecular structures of CAs and C[4]R able to bind DNA.

intramolecular DNA condensates, but simultaneously interact with several DNA strands, forming intermolecular aggregates [136]. Later Rodik et al. synthesised non-toxic amphiphilic C[4]As with ammonium and imidazole groups on the upper rim and alkyl chains on the lower rim (Figure 18) forming micelles with a diameter of 3–4 **87** and 6 nm (**88** and **89**) [137]. C[4]A with propyl chains on the lower rim **87** showed low transfection efficiency, while the interaction of **88** and **89** with DNA was determined by both electrostatic and hydrophobic interactions. Moreover, micelles formed by **88** and **89** were able to condense DNA into nanoparticles with average sizes of 65 and 55 nm, respectively. Compared to lipid-based vectors, a key advantage of cationic calixarenes is the precise control of their aggregation into small nanostructures. The lipid systems interact with DNA to form relatively large aggregates. Compared to conventional cationic surfactants, which also form small particles in the form of micelles, the calixarene-based systems give much more stable complexes with DNA due to their rigid aromatic framework.

Amphiphilic C[4]R with saccharide fragments as hydrophilic groups on the upper rim and undecyl chains on the lower rim **90** (Figure 18) formed micellar aggregates with a diameter of 4–6 nm in water [138]. These aggregates have demonstrated good stability mainly due to intermolecular hydrogen bonds between the side saccharide moieties. When these micellar aggregates interacted with the DNA plasmid, 50-nm particles were formed, which makes them excellent candidates as non-viral vectors [139].

Bono et al. synthesised amphiphiles based on C[4]A platform containing oligomeric aminoglycosides [140].

The obtained macrocycles demonstrated a high ability to bind and pack DNA with the formation of stable mixed aggregates of about 150 nm in size. Interestingly, such mixed systems not only showed good transfection efficiency but also have low or negligible cytotoxicity and exhibit noticeable antimicrobial activity against gram-negative bacteria, even greater than that of individual macrocycles. Thus, the revealed patterns indicate the potential of calixarenes as promising means of delivery of nucleic acids with unique antibacterial properties.

#### Interaction with proteins, peptides and amino acids

Protein recognition studies using non-covalent interactions are of great interest. Complexation of calixarenes with proteins occurs by linking relatively short peptide sequences through linear or three-dimensional recognition. Accordingly, the directed functionalization of macrocycles contributes to the specific recognition and binding of proteins, peptides and amino acids.

Various derivatives of (thia)C[4]As and sulphonyl C[4]As bearing phosphonate groups on the upper rim (**91–100**, Figure 19) exhibit inhibitory activity against type 1B protein tyrosine phosphatase (PTP1B), which is one of the therapeutic targets for the treatment of type 2 diabetes [141–143]. It was found that the introduction of a second bisphosphonate group in the structure of C[4]A [92,94] or the presence of hydroxyl groups [93,94] leads to a decrease in inhibitory activity compared to monosubstituted analogues [141]. Compounds **91**, **93**, and **98** exhibit inhibitory activity not only for PTP1B but also interact with another

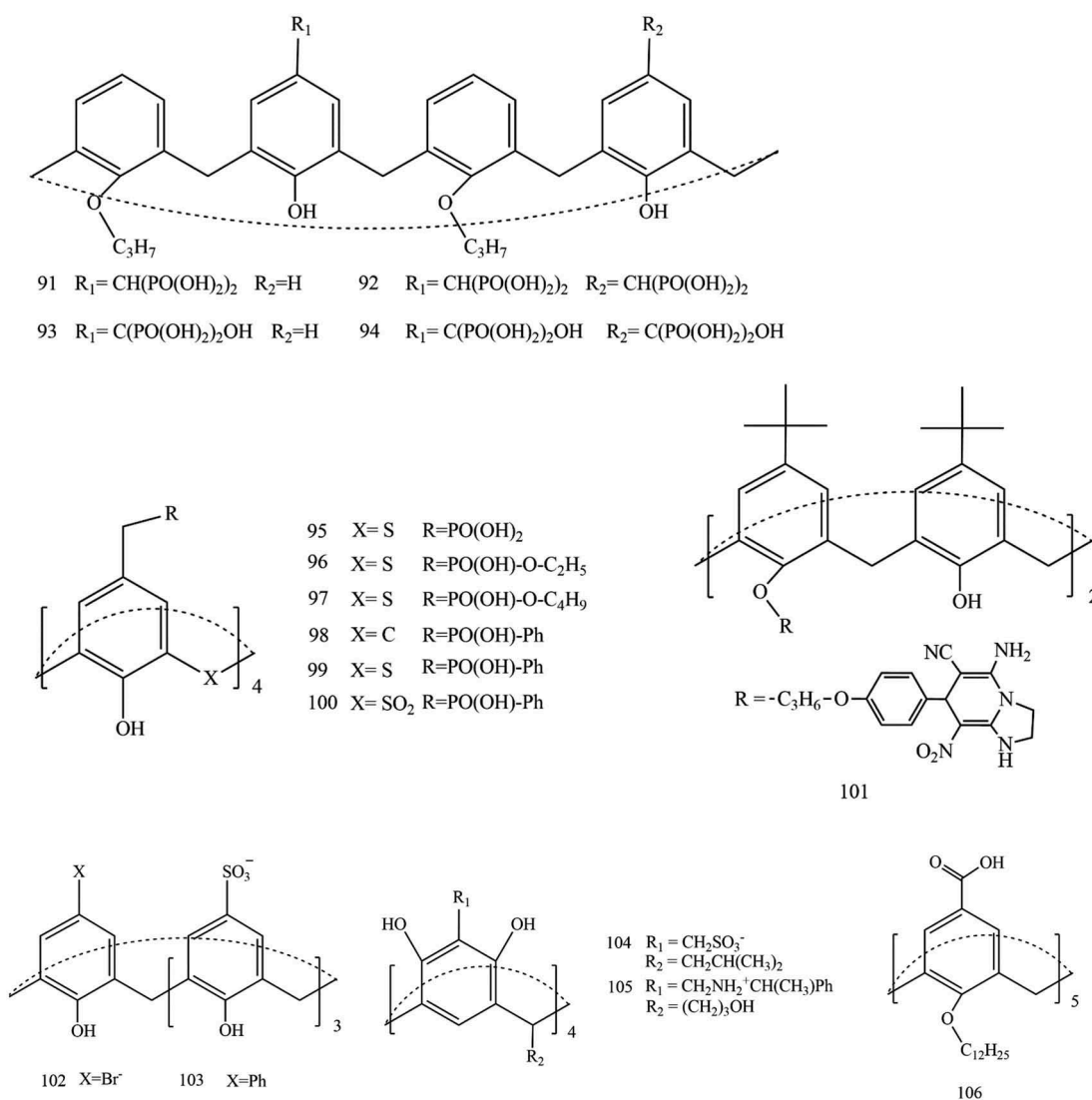


Figure 19. Molecular structures of CAs and C[4]R able to bind proteins.

phosphotyrosine phosphatase SHP2, which is considered the first oncogene in the protein tyrosine phosphatase superfamily. Replacing the methylene bridge with a sulphide **95–97, 99** or sulphonate group **100** in the macrocycle structure leads to a more effective inhibition of enzymes. In addition, the thiaC[4]As **96, 97**, and **99** showed an order of magnitude higher selectivity for PTP1B compared to unsubstituted macrocycles [142,143].

Rostami et al. were able to bind  $\beta$ -lactoglobulin using C[4]A **101** (Figure 19), while the protein structure remains almost unchanged [144]. The binding of this protein can occur through several mechanisms, including van der Waals forces, hydrophobic interactions, and hydrogen bonds. In the free state, proteins have their own fluorescence due to the presence of aromatic amino acid residues, but fluorescence quenching is observed during the formation of equimolar complexes with macrocycle.

Crowley et al investigated the ability of sulphonate C[4]A **5** to form complexes with another protein, cytochrome C, in a crystalline state. Substitution of one sulpho group with bromine **102** or phenyl **103** (Figure 19), i.e. decrease in macrocycle symmetry, slightly affects the mechanism of protein binding. However, it was found that these introduced functional groups enhance the interaction with the protein [145]. A greater interaction of cytochrome is observed for **103**, since, in addition to lysine binding by the hydrophobic cavity of the macrocycle due to the formation of salt bridges,  $\text{CH}-\pi$  and cation- $\pi$  interactions, the phenyl group interacts with a hydrophobic site consisting of alanine and tyrosine moieties. An increase in the number of units in the macrocycle cavity leads to better protein binding [146]. C[6]A **8** is more conformationally mobile and takes the 1,2-alternate configuration, due to which it can simultaneously bind two lysine molecules, facilitating the dimerisation of cytochrome C. C[8]A **9** molecule can be in

different conformational states that bind large surface of a protein molecule. With an increase in the concentration of **9** in the solution, spontaneous formation of cytochrome c tetramers is observed, and then their disassembly occurs with a ratio of **9**:protein more than 2:1.

Regardless of the number of aromatic units in the calixarene structure, sulphonated CAs are able to bind cationic proteins with antifungal activity, in particular against *Penicillium* [147]. Despite the different size and conformational flexibility, all three macrocycles C[4]A **5**, C[6]A **8** and C[8]A **9** similarly bind the same protein site, consisting of proline, lysine and phenylalanine. Macrocycles form lysine-sulphonate salt bridges as well as non-covalent CH- $\pi$  and  $\pi$ - $\pi$  bonds with proline and phenylalanine fragments, respectively. Moreover, the larger the size of the macrocycle cavity, the greater part of the protein surface binds. In particular, C[8]A acts as a bidentate ligand, which facilitated the dimerisation of fungicidal protein. Thus, calixarenes can be used to control the protein assembly, modulate the activity of proteins, detect and separate them due to supramolecular interactions.

Sulphonate C[4]R **104** (Figure 19) molecule is capable of forming complexes with two molecules of derivatives of basic amino acids (dihydrochlorides of methyl esters of lysine, arginine and histidine) due to electrostatic interactions in methanol and methanol/water mixture [148]. The chirality of the guests is transmitted to the whole complex during complex formation. Given a fairly strong interaction with amino acids, this macrocycle can be used as a modulator of protein crystallisation. Similar patterns of chiral binding and transfer are observed when **104** capsules are formed with chiral phenylethylaminomethylated C[4]R **105**.

Stoikov et al. modified thiaC[4]A scaffold with oligolactic acid, which form micelle-like and vesicular aggregates in solution depending on the macrocycle conformation [149]. The thiaC[4]A in the cone conformation forms micelles, while macrocycles with conformation of a partial cone and 1,3-alternate tend to form elongated vesicles. All obtained compounds show the ability to bind proteins (BSA, haemoglobin, lysine), forming spherical (for BSA and haemoglobin) and dendrimeric (for lysine) structures. It was found that the best binding of proteins is provided by the oligolactated macrocycle in the partial cone conformation.

Amphiphilic C[5]A **106** (Figure 19) and  $\beta$ -cyclodextrin containing the same dodecyl chains, when mixed in an aqueous solution, form mixed vesicular aggregates [150]. Since these macrocycles have different binding sites, the surface of the resulting aggregates is a heteromultivalent platform on which two types of receptors alternate. If the cyclodextrin cavity encapsulates an aromatic amino acid tyrosine, the C[5]A molecule containing carboxyl groups binds such positively charged

amino acids as lysine and arginine. Mixed C[5]A-cyclodextrin aggregates bind model peptides containing alternating tyrosine and lysine fragments better than vesicular aggregates of individual macrocycles due to the synergistic action of the two binding sites. In addition, the inhibitory effect of C[5]A-cyclodextrin aggregates on fibrillation of amyloid- $\beta$ -peptides, which is a hallmark of Alzheimer's disease, was found.

### Enzyme mimics

The creation of biomimetic systems, inter alia, on the basis of calixarenes, which will reproduce the properties of enzymes, is of great interest. Bakirci et al. presented models of supramolecular metal enzymes in aqueous solutions based on dynamic self-assembly between sulphonate C[4]A **5**, hydrophobic molecule of 2,3-diazabicyclo [2.2.2] oct-2-ene and metal ion [151]. In the obtained ternary system, the organic molecule is retained in the cavity C[4]A due to hydrophobic interactions, and the metal ion is electrostatically bonded to the sulfo groups of the macrocycle. Moreover, the guest molecule forms complexes with **5** in a 1:1 ratio with a high binding constant, and when transition metal ions ( $\text{Zn}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Mn}^{2+}$ ) are added, a hypsochromic shift in the UV spectra is observed, indicating the formation of a triple complex where the azo group functions as a monodentate ligand. At the same time, in the absence of C[4]A **5**, the guest molecule forms rather weak complexes with metal ions, that is, a macrocycle promotes metal-ligand binding. It is worth noting the high selectivity of the formation of ternary complexes: the size of the organic guest molecule should be optimal for its inclusion in the cavity C[4]A and for ensuring the electrostatic interaction of metal cations with sulfo groups of the macrocycle. Later Francisco et al. [152] found the possibility of same triple complexation with 2-chloropyridine and metal (sodium and copper) ions in equimolar amounts. If a 2-chloropyridine molecule interacts with copper ions in the presence of a macrocycle, in the case of sodium there is no formation of a metal-ligand bond.

Conformationally flexible sulphonated C[6]A **8** and C[8]A **9** form equimolar complexes with lucigenin and yield approximately the same binding constants, which strongly suppressed the fluorescence of the bound guest [153]. Interestingly, the C[6]A framework is fixed in the 1,2,3-alternate conformation upon binding of two luminescent reagent molecules. Quenching of lucigenin fluorescence upon binding to calixarenes can be used to detect competitive complexation with metal cations. The formation of ternary complexes begins at a concentration above 10  $\mu\text{M}$  and are characterised by a higher binding constant compared to binary systems.

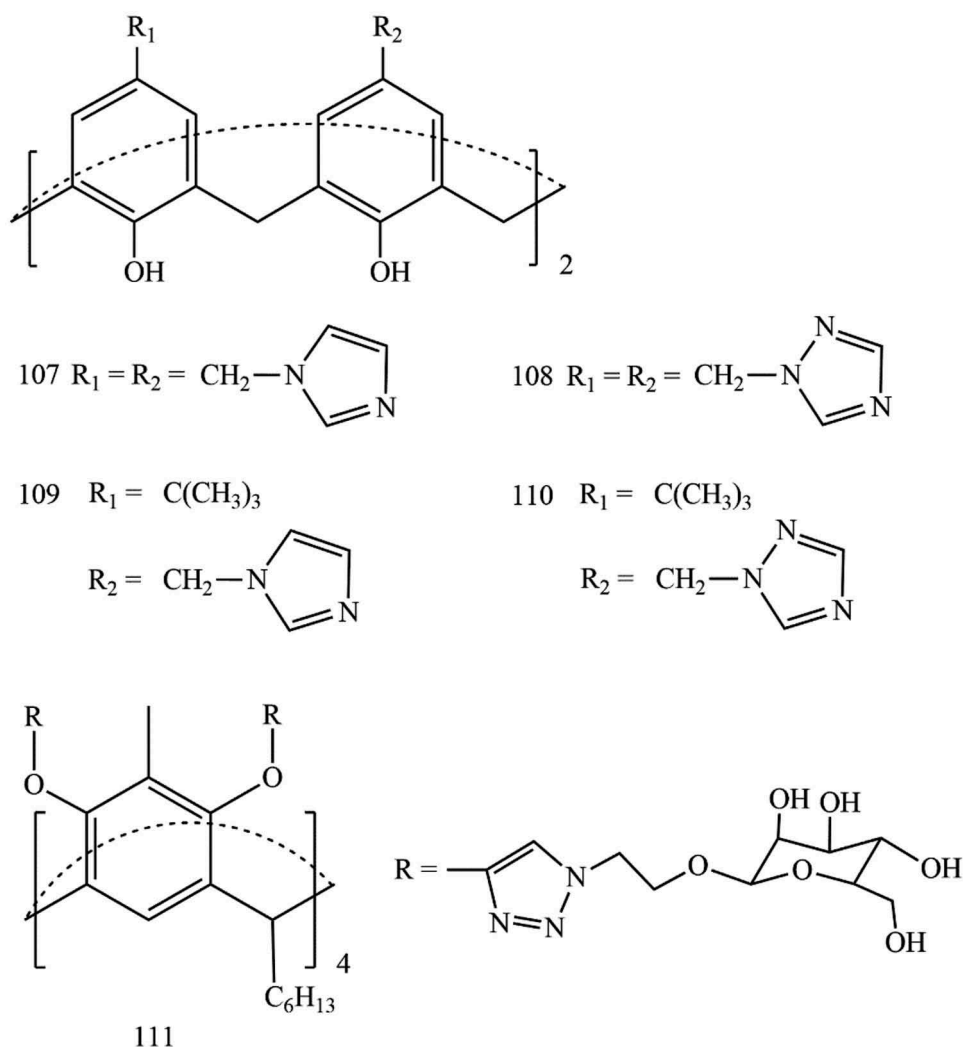


Figure 20. Molecular structures of C[4]As and C[4]R.

Basílio et al. investigated the mechanism and kinetics of catalytic hydrolysis of picolinate ester in the presence of nickel ion and various sulphonated C[4]A **5**, C[6]A **8**, C[8]A **9** [154]. Molecules C[4]A and C[6]A accelerate this reaction due to the simultaneous complexation of metal cation and substrate, and the molecule C[8]A accelerates to a greater extent due to weakly basic phenolate groups involved in basic or nucleophilic catalysis. However, due to the ionic nature of these macrocycles, autoinhibition of the reaction is observed at higher concentrations (more than 10 mM) due to the binding of sodium counterions that compete with catalytically active nickel complexes.

Tabakci et al. synthesised C[4]A-containing imidazole **107**, **108** and triazole groups **109**, **110** (Figure 20), which were then polymerised [155]. Both monomeric and polymeric C[4]As can be used as acyltransferase imitators for the hydrolysis of p-nitrophenyl acetate, the benzene ring of which is located in the macrocycle cavity. The kinetics of hydrolysis of this substrate was

studied in phosphate buffer pH 6.3, in which half of the imidazole or triazole groups are in the protonated state, promoting the binding of the ester due to hydrogen bonds with oxygen of the carbonyl group. At the same time, the unprotonated nitrogen atoms of macrocycles carried out a nucleophilic attack on the substrate carbon atom and thereby induced its transformation into a phenolate anion.

Hexyl-chain C[4]R modified by glucose fragments along the upper rim **111** (Figure 20) that formed a pseudo-saccharide cavity can also be used to accelerate click reactions of the Huisgen-cycloaddition [156]. The essence of this reaction is the [3 + 2] dipolar cycloaddition of azide catalysed by copper ions to an alkyne to form a 1,2,3-triazole ring. Alkyne and azide substrates are encapsulated in the pseudo-β-D-glucopyranoside cavity of the macrocycle due to stacking and are at a favourable distance with copper. Thus, the methods of click-chemistry using the calixarene platform can be widely used due to

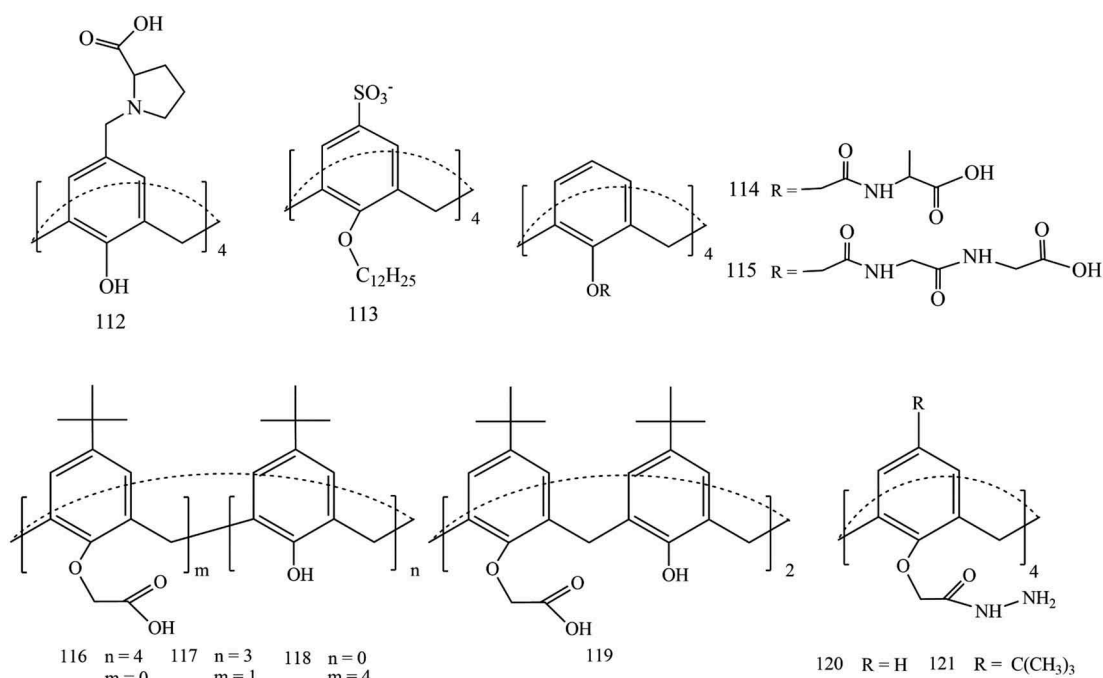


Figure 21. Molecular structures of C[4]As able to gels formation.

their simplicity, versatility, and mild reaction conditions with practically quantitative yield.

### Formation of gels

By transposing the study of calixarene-based aggregates to a higher aggregation level, different gels based on them have attracted much research and development attention in recent years. Using binary systems, it is possible to successfully adapt the gel properties by replacing one of the two components. In particular, the use of biocompatible gelling agents such as amino acids is more advantageous than that of synthetic gelatins.

Zhang et al. constructed a series of binary hydrogels based on tetra-proline-based C[4]A **112** (Figure 21) and a number of amino acids (arginine, histidine, and lysine) [157]. The critical aggregation concentration of this macrocycle determined using circular dichroism is 1.2 mM. In an aqueous medium with a macrocycle concentration of 5 mM used in further gelation with basic amino acids, the spherical aggregates from 40 to 200 nm in size are formed. The amino acids protonated in an acidic medium interact with these C[4]A micelles to form a hydrogel matrix. The shape of the mixed aggregates depends on the amino acid used: xerogels with arginine have a fibrillar structure, xerogels with histidine are a porous material with stacks of sheets, and xerogels with lysine have a lamellar structure. All gels are stable at room temperature, capable of encapsulating doxorubicin and releasing it when immersed in water due to diffusion.

A similar mechanism is the formation of polymer hydrogels based on sulphonate C[4]A with dodecyl tails **113** (Figure 21) and polyvinyl alcohol modified with viologen fragments [158]. The CMC of this macrocycle in an aqueous medium is 0.02 mM, at which the aggregates with a diameter of 14 nm begin to form. After mixing and shaking equal volumes of macrocycle micelles and polymer solution for several seconds, the aqueous solution turns into a soft hydrogel, which does not flow down in an inverted vial. It is likely that **113** micelles are grafted onto polymer chains due to multiple host-guest interactions between macrocycle cavities and viologen fragments of the polymer, forming a non-covalent three-dimensional polymer network. An important advantage of the obtained hydrogel is its good response to temperature changes, namely the temperature-dependent gel-sol transition. Since the bonding between the components in this system during gelation occurs through charged viologen moieties, a change in the ionic strength of the solution (addition of salts) or a change in the redox potential leads to a weakening of the interaction between **113** and polymer. Hence, the stimulus sensitivity and ease of preparation of these hydrogels open up great prospects for their use in biomedicine and industry.

The formation of hydrogels by mixing chiral C[4]As (**114**, **115**, Figure 21) with non-chiral derivatives of bipyridine was studied by Choi et al. [159,160]. In this case, chiral C[4]A molecules can transfer their chirality to achiral guest molecules during the formation of



supramolecular complexes. Supramolecular inversion of chirality causes significant changes in morphology, namely, folding of flat structures into tubular ones. Inversion of chirality and change in morphology are mainly due to intermolecular interactions of hydrogen bonds between achiral and chiral molecules. Twisted fibres in hydrogels are characterised by improved mechanical properties compared to linear structures [160]. This *in situ* gel preparation method can be further used to develop intelligent materials.

Yang et al. studied the gelation of *p*-tert-butyl-C[4]A derivatives with various organoalkoxysilanes [161]. In the C[4]A series with one, two or four carboxyl groups, and also without substituents on the lower rim **116–119** (Figure 21), only the compound with the highest number of carboxy-groups **118** at a concentration of 2% has gel-like properties. In particular, the gel formed due to the intermolecular hydrogen bonds of this macrocycle with trimethoxyphenylsilane demonstrates not only the thermoreversible properties of the phase transition but also the fast and completely reversible thixotropic properties at room temperature. In addition, a gel containing 6% macrocycle is stable for more than 4 months at room temperature under the condition that the gel is stored in a dry place.

Lee et al. investigated the gelation of the macrocycle **120** (Figure 21) in the conformation of 1,3-alternate and diphenyl aldehydes with different positions of substituents [162]. The gelation time depended on the location of the aldehyde group: the fastest (1 h) gel formation occurs when C[4]A **120** interacts with para-aldehyde, the longer the time (2 days) required with the meta-aldehyde, and gelation does not occur for the ortho derivative. The hydrazide group of the macrocycle bound to the aldehyde groups in the para position through the formation of hydrazone bonds, which led to the formation of a three-dimensional network capable of encapsulating up to 2 equivalents of the drug gossypol. The formation of organogels with loaded drug occurred in DMSO, but the replacement of DMSO by immersion in water for 24 h resulted in a hydrogel. Thus, when replacing the solvent, a gradual release of the drug was observed as a result of morphological changes.

Similarly, a gel is formed upon the interaction of **120** with hydrophobic derivative of diphenyl terephthalate [163]. Initially, the gel was also prepared in DMSO, then a solvent was replaced to reduce toxicity, and as a result, a hydrogel was obtained from the organogel. The network structure of the organogel was transformed into a lamellar structure after the formation of the hydrogel in water, which contributed to the improvement of mechanical properties and lower cytotoxicity. Such

a favourable rearrangement of the gel fibre can occur due to an increase in the intermolecular hydrogen bond as a result of the formation of additional groups of hydroxyl groups that arise when immersed in water.

Self-healing gels were obtained by Yang et al. from hydrazide-derivatised C[4]A **121** and linear PEG derivative with benzaldehyde-terminated groups [164]. As a result of the formation of a dynamic acylhydrazone bond between them, a gel was obtained that exhibits self-healing, excellent mechanical properties, and sensitivity to pH and temperature. The structure of this gel makes it possible to dissipate stress under the action of an external force due to the rigidity of the macrocyclic framework, and the reversibility of the dynamic acylhydrazone bond provides self-healing of the structure in case of damage.

The controlled gel was obtained on the basis of the same macrocycle **120** and the derivative of photosensitive stilbene [165]. The properties of the formed gel can be changed under the influence of temperature and UV radiation. Stilbene fragments in the gel form H-aggregates that exhibit blue fluorescence. Heating at a temperature of 60°C for 1 h leads to a partial rearrangement of H-aggregates into J-aggregates with of green fluorescence. An increase in temperature enhances the molecular motion of the solvent, which, in turn, causes this change in aggregation. Similarly, there is a loss of blue fluorescence after irradiation with UV light, which promotes [2 + 2] cycloaddition of stilbenes in the form of H-aggregates with the formation of cyclobutane units, which increases the mechanical strength of the gel. Thus, by adjusting the temperature and exposure to UV light, it is possible to obtain a gel fluorescent coating with a certain number of cross-links inside the gel and, therefore, with specified mechanical properties. Such supramolecular systems from calixarene-based gels with controlled stiffness have the potential for use in tissue engineering.

### Modification of quantum dots

Calixarenes can be used to modify the surface of hydrophobic quantum dots. Macrocyclic molecules can non-covalently attach to the surface of quantum dots coated with trioctylphosphine oxide due to hydrophobic interactions. Carrillo-Carrión et al. obtained CdSe/ZnS quantum dots modified by C[8]A with tert-butyl groups **122** (Figure 22) [166]. The coating from this C[8]A retains the intensity of the emission of quantum dots and their small diameter, and the immobilised macrocycle is capable of molecular recognition. The large cavity of the eight-membered macrocycle is ideal for binding the C60 fullerene molecule. Fullerenes are electron acceptors;

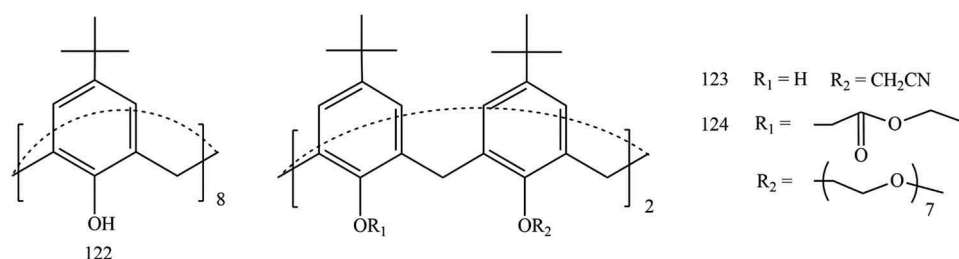


Figure 22. Molecular structures of C[4]As.

therefore, upon complexation, photo-induced electron transfer from quantum dot to C60 is observed and, as a consequence, fluorescence quenching. Such an optical nanosensor based on quantum dots and C[8]A for determining C60 fullerene has a detection limit of 5  $\mu\text{g/L}$ .

The CdSe quantum dots were similarly modified with tert-butyl C[4]A **123** (Figure 22) [167]. The resulting system was investigated as a sensor for a number of polynuclear aromatic hydrocarbons and showed selective sensitivity to fluorene. Tert-butyl calixarenes can also be used to modify oleic acid-coated quantum dots. Molecules C[4]A-containing tert-butyl groups on the upper rim and pegylated fragments on the lower rim **124** (Figure 22) were used to modify hydrophobic CdSe quantum dots [168]. Semiconductor nanocrystals coated in this way were used as sensors for tyrosine, which was bound via hydrogen bonds to the ester groups of the macrocycle. The surface of quantum dots can also be modified with vinylpyrrolidone fragments that bind to the hydroxyl groups of sulphonate C[4]A **5** due to hydrogen bonds [169]. This macrocycle located on the surface of ZnS quantum dots selectively takes a hydrophobic vitamin K molecule into its cavity.

Thus, modifying the surface of quantum dots with calixarenes not only enhances the stability of the systems as a whole and obscures the fluorescence efficiency of the sensors but also expands their capabilities by adding active sites. A macrocycle fixed on the surface does not lose the ability to include guest molecules in its cavity, which determines the principle of operation of these sensors with quenching of their fluorescence during guest–host interaction.

## Conclusions

The supramolecular approach presents an alternative way to create various nanostructures with useful properties. Since such structures are held together by several weak and, therefore, reversible interactions, supramolecular aggregates based on calixarenes are well-malleable objects that easily change their shapes and morphologies under the influence of external factors.

The results obtained in the field of the creation of single and mixed compositions based on calixarenes allow understanding not only the essence of the formation of supramolecular aggregates based on these macrocycles and expand the range of substrates for the creation of systems sensitive to various factors based on them but also show the beauty of supramolecular chemistry in that two or more components can independently assemble into higher-order structures that have unique properties and functions, which cannot be up to stigmatised by a separate monomeric molecular unit. Individual and mixed aggregation of calixarenes serves as a universal strategy for creating a multifunctional nanoplateform, which can be hierarchically modified with targeted agents for increasing therapeutic activity of the encapsulated drug, binding a wide range of biomolecules, creating enzyme mimics, etc. Thus, the existing supramolecular self-assembly approaches involving calixarene-based compositions open up new possibilities for the delivery of significant guest molecules with the desired efficiency and the rational search for optimal compositions for the needs of medicine and biology.

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