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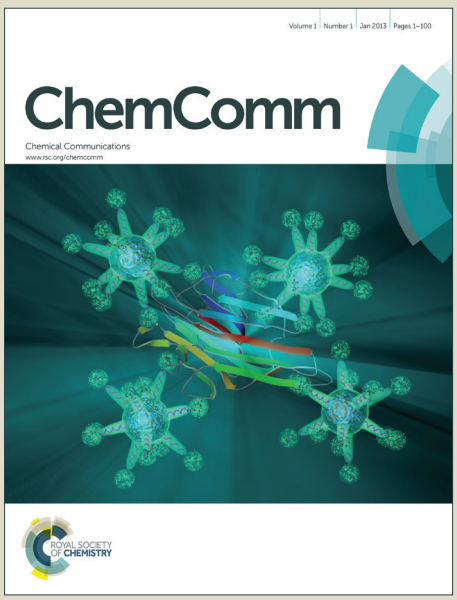

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ARTICLE TYPE

Glycoligand-targeted core-shell nanospheres with tunable drug release profiles from calixarene-cyclodextrin heterodimers

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Stable core-shell nanospheres have been self-assembled in water from heterodimers encompassing a hydrophobic calix[4]arene moiety and a hydrophilic β-cyclodextrin head; their potential to encapsulate and provide sustained release of the anticancer drug docetaxel and undergo surface post-modification with glycoligands targeting the macrophage mannose receptor is discussed.

The development of well-ordered functional nanostructures filled with the capacity to assemble under dilute conditions in water or buffer media continues to be one of the more fascinating challenges facing modern chemistry, with relevance in areas like imaging, diagnostics, tissue engineering or drug delivery, among others.[1] Many of those channels require the supramolecular system to be capable of encapsulating a cargo, targeting specific cell surface receptors and allowing the gradual release of the payload. Although polymeric materials have concentrated most of the efforts in the field, *de novo* design of monodisperse building blocks appears as a better suited approach for a detailed investigation of the structural features governing self-assembly.[2] Due to their capacity to form host-guest superstructures, macrocyclic cavity-featuring members of the calixarene[3] (CA) and cyclodextrin[4] (CD) families are privileged platforms towards this end. In this work we report the successful construction of nanospheres entangling an inner core formed by hydrophobic calix[4]arene (CA_4) units and an external hydrophilic shell exposing β-cyclodextrin (βCD) motifs by the self-assembly in water of amphiphilic CA_4—βCD heterodimers (**1** and **2**). The CA_4 scaffold in its cone structure is very well suited to promote tight packing of lipophilic chains installed at the lower rim, providing a lipid matrix where entrapped hydrophobic drugs can undergo sustained release, whereas the presence of βCD at the nanosphere surface allows host-guest directed post-modifications, e.g. for targeting purposes. As a proof of concept, the antimitotic agent docetaxel[5] (DXT) and the macrophage mannose receptor (MMR), for which trivalent mannosyl dendrons such as **3** have shown a remarkable affinity,[6,7] were selected as the drug and target, respectively (Fig. 1). DXT, primarily used to treat prostate, breast, neck and lung cancer, can induce selective apoptosis in myeloid-derived suppressor cells overexpressing the MMR, so that targeting is expected to improve cancer immunotherapies.[8]

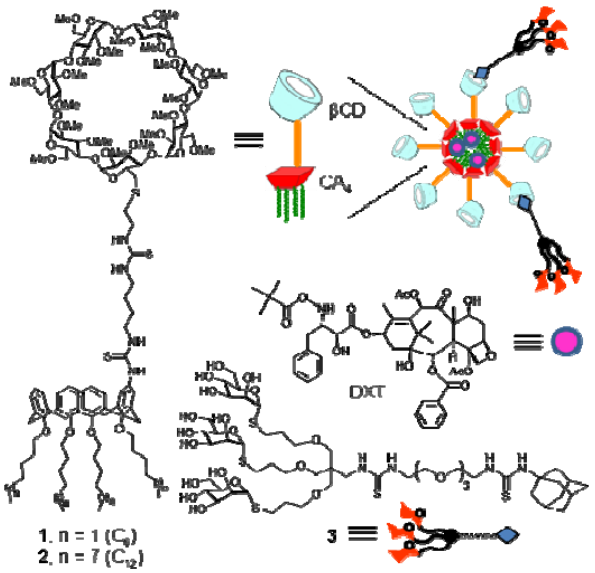
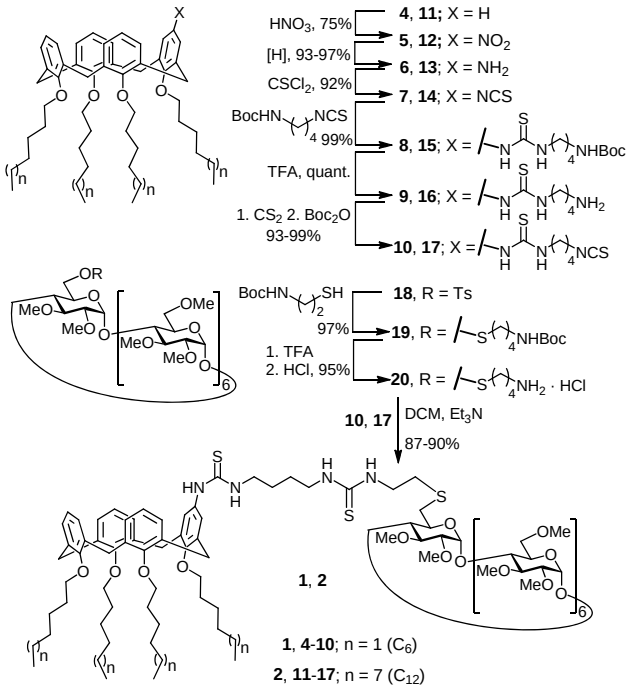


Figure 1 Structure of the calix[4]arene—β-cyclodextrin heterodimers **1** and **2** prepared in this work and schematic representation of their self-assembly in water to afford core-shell nanospheres that can be loaded with a drug (docetaxel; DXT) and/or coated with targeting ligands (**3**).

There have been several reports on the use of CAs as scaffolds for positioning CD hosts, e.g. for sensing purposes.[9] In some cases the formation of vesicular or fiber-like aggregates from CA-CD couples was observed, but in those examples the CD cavity was uniformly occupied due to intra- or intermolecular inclusion phenomena.[10] In our heterodimer prototype, the molecular geometry was conceived to avoid self-inclusion, whereas the compact arrangement of the four hydrophobic tails onto the CA scaffold also prevents the formation of monodimentional oligomers. The driving force that makes the single amphiphiles to self-assemble into core-shell nanospheres arises then from strong hydrophobic interactions involving exclusively the multi-tail CA_4 platform, leaving the external βCD moieties fully accessible to participate in further supramolecular events. The heterodimers **1** and **2** were synthesized in a convergent way. The isothiocyanate-armed CA_4 building blocks **10** and **17** and the amine-functionalized βCD derivative **20** were prepared and coupled through thiourea-forming ligation chemistry in the



Scheme 1. Synthesis of the CA_4-βCD heterodimers **1** and **2**.

last reaction. The key step in the synthesis of the singly functionalised CA4 derivatives is the mononitration of the corresponding tetra-hexyl and -dodecyl ethers **4** and **11**, which proceeded in 75% after treatment with concentrated nitric acid in a mixture of dichloromethane and glacial acetic acid.[11] Sequential reduction to the corresponding amines, isothiocyanation, thiourea coupling with mono-Boc-protected butylenediamine, carbamate hydrolysis and final isothiocyanation (→**5**-**10** and **12**-**17**) proceeded with over 90% yield in every step. The βCD amine counterpart **20** was prepared in 92% yield from the known mono-O6-tosyl-derivative **18**[12] after reaction with Boc-protected cysteamine (→**19**) and final hydrolysis. Thiourea conjugation of **20** with the CA_4 partners **10** and **17** provided the target heterodimers **1** and **2** in 87 and 90% yield, respectively (Scheme 1).

Blank (unloaded) nanospheres of the two amphiphilic CA_4-βCD heterodimers **1** and **2** were prepared by the interfacial solvent displacement method.[13] Dynamic light scattering (DLS) monitoring of nanoparticle size revealed unimodal distributions of submicronic aggregates of 129 and 189 nm hydrodynamic diameter, respectively (Supplementary information, Table S1). Such colloidal dispersions remained stable for more than 30 days at rt and at 37 ºC. Atomic force microscopy (AFM) observation showed an average size of the aggregates of 100 nm diameter (Figure 2). An ultra-thin structure was inferred in some cases, suggesting that those objects are composed at their turn of smaller entities of about 22-25 nm. Assuming a conical shape for the molecules and a compact hexagonal arrangement filling 90% of the nanosphere volume, an aggregation number of 17-25 molecules per nanosphere can be estimated from geometrical considerations,[14] the higher aggregages being composed of about 60 nanospheres. No collapsing or significant flattening of the nanoparticles was observed even after prolonged drying

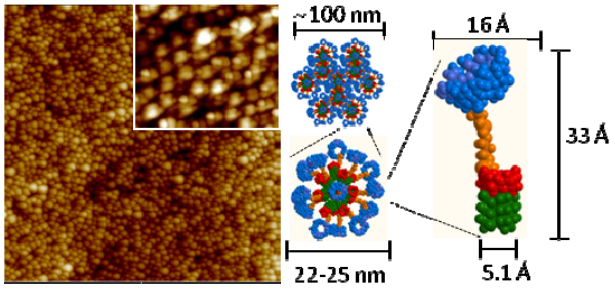


Figure 2. Tapping mode AFM image (5 x 5 μm) of the nanospheres obtained from **1** (left). The insert (0.7 x 0.7 μm) shows a zoom were the ultra-thin structure can be appreciated. Schematic representations of the aggregates and the individual nanospheres and a 3D molecular model of **1** with the estimated geometry are also depicted (right; CD moiety in blue, CA moiety in red, thiourea connector in orange, aliphatic chains in green).

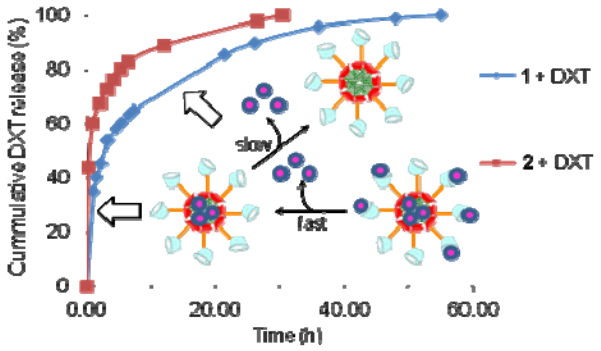


Figure 3. Release profiles of docetaxel (DXT) from loaded nanospheres prepared from **1** and **2** in water. A schematic representation of the processes involved in the initial burst (dissociation from the β-cyclodextrin cavities in the external shell) and the further sustained release (diffusion from the calix[4]arene core) is shown.

times, discarding a vesicle-type structure and supporting instead their solid lipid nanosphere nature.

Nanoprecipitation of the heterodimers **1** and **2** in the presence of DXT afforded assemblies of 35 and 20 nm hydrodynamic diameter, respectively, that remained stable in water (Supplementary Information, Table S2 and Figure S1). The release profiles of the drug from the loaded nanospheres showed an initial burst followed by sustained delivery over 50 or 30 h, respectively (Figure 3). It is presumed that DXT is encapsulated in the nanospheres in two different compartments, the hydrophobic core formed by the substituted CA moieties and the CD cavities. Actually, the aromatic rings in DXT have been shown to promote divalent interactions with βCD derivatives, leading to stable inclusion complexes.[6] The observation of significant differences in the ζ-potential between blank and loaded nanospheres, from -31 to -13 mV (Supplementary information, Tables S1 and S2), is consistent with nanoparticle surface modification upon DXT loading. Probably, release of DXT into bulk water out from the CD cavity is faster as compared with diffusion from the CA core.[15] The prolonged release of DXT from the nanospheres prepared with the hexyloxy (C_6) derivative as compared with the dodecyloxy analogue (C_{12}) might then relate to the differences in particle size and compaction of the CA_4-templated chains in the core. The data illustrate how the drug

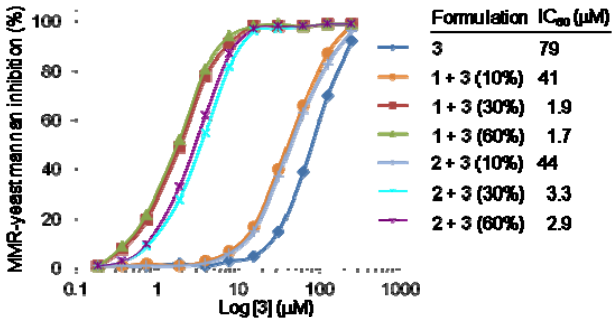


Figure 4. Inhibition of the adhesion of human MMR to immobilized yeast mannan in the presence of increasing concentrations of **3** and of nanosphere formulations prepared from **1** and **2** after post-functionalization with 10, 30 and 60% of **3**. The corresponding IC_{50} values (in μM referred to **3**) are indicated. Control experiments with unmodified nanospheres showed no inhibition at the highest assayed concentration.

release rate can be adjusted by judicious design of the heterodimer component.

The accessibility of the βCD cavity in the nanospheres was further exploited for supramolecularly anchoring the trimannosyl dendron **3** at their surface, taking advantage of the strong affinity of adamantane moieties towards the βCD cavity.[16] Incorporation of the glycoligand antenna (10, 30 and 60% in molar bases with respect to **1** or **2**) in water was monitored by measuring the increase in the hydrodynamic diameters (about +10 nm) and ζ-potential (about +4 mV) of the aggregates (Supplementary information, Table S3). Through this sequential self-assembling process, a glycocalyx-like corona was created around the core-shell nanospheres. The availability of the mannopyranosyl glycotopes to participate in protein recognition events was confirmed by monitoring the binding affinity towards the human MMR, following a modified enzyme-linked lectin assay (ELLA).[17] The results evidenced a strong affinity increase of the **3**-decorated nanospheres for the MMR as compared to the individual trimannosyl ligand **3**, up to 27- or 46-fold for nanospheres formulated from **1** or **2**, respectively, on a mannose molar basis (Figure 4). The data are consistent with the expected reinforcement of the interaction between the sugar and the MMR lectin after multivalent presentation of the former onto the nanoparticle. Interestingly, the MMR binding affinity of the nanosphere-glycodendron formulations was drastically decreased in the presence of a 10-fold excess of adamantane carboxylate sodium salt, a competitor of **3** for the CD cavity (Supplementary information, Figure S2). This supports the notion that reversible inclusion of the adamantane moiety of **3** in the βCD cavity is the main mechanism at play.[18] The rather small difference in MMR affinity for 30 and 60% dendron-loaded materials reflects the existence of a plateau on the sugar ligand content-dependent multivalent effect. This is interesting since it might allow optimizing simultaneously the content of different functional elements, e.g. for visualization, biocompatibility, furtivity in the blood circulation or cell internalization.

In conclusion, we have prepared a new type of core-shell self-assembled nanospheres stable in water solution consisting of only one molecular species that combines a hydrophobic multi-tail calix[4]arene moiety and a hydrophilic β-cyclodextrin head. The results illustrate the potential of CA-CD heterodimer-based delivery systems in nanomedicine applications where a guest molecule has to be stabilized, targeted and/or protected in aqueous media.

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Notes and references

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† Electronic Supplementary Information (ESI) available: Protocols for blank and DXT-loaded nanosphere preparation, characterization by AFM and DLS, determination of DXT release profiles and affinity measurements of glycoligand-decorated nanospheres towards the human MMR, as well as synthetic procedures and copies of the ^{1}H and ^{13}C NMR spectra for compounds **1**-**3** and their precursors. See DOI: 10.1039/b000000x/

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