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Water-soluble octahedral polyammonium nanocapsules: synthesis and encapsulation studies

Xuejun Liu, Junling Sun, Ralf Warmuth *

Rutgers, The State University of New Jersey, Department of Chemistry and Chemical Biology, Piscataway, NJ 08854, USA

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Dedicated to the memory of Dmitry M. Rudkevich

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ABSTRACT

The syntheses of two water-soluble octahedral polyammonium nanocapsules **5e** and **5f** are described. Nanocapsule **5e** was prepared via the TFA-catalyzed condensation reaction of six MOM-protected 4-hydroxybutyl-footed tetraformyl cavitands **3c** with 12 ethylene diamines, followed by reduction and acidic hydrolysis of the intermediate octahedral polyimino nanocapsule. Nanocapsule **5f** was prepared from **5e** via the oxidation of the 4-hydroxybutyl feet to 3-carboxypropyl feet. Both nanocapsules are soluble in water below pH 5. **5f** is also water-soluble above pH 7. In acidic aqueous solution, nanocapsule **5e** encapsulates small negatively charged, partially hydrophobic guests, such as *p*-toluenesulfonic acid, *N*-BOC-aspartic acid, or 4-methylumbelliferyl phosphate. The single site microscopic binding constants for the latter guests are 150±30, 510±50, 1550±150 M^{-1}, respectively and were determined from ^{1}H NMR titrations and DOSY experiments by assuming a 6:1 binding model with six identical, independent binding sites per nanocapsule (statistical binding). **5e** doesn't bind small neutral hydrophobic molecules in aqueous solution. However, both nanocapsules bind to nucleotides, such as ATP, dAMP, dGMP or TTP. NMR experiments support that nucleotides are not encapsulated, but bind to the outside of the nanocapsules.

1. Introduction

Molecular container compounds are spherical hosts with cavities that are large enough to accommodate one or more small organic guests.[1] They have been first reported by Collet[2] and Cram[3] and have opened opportunities for novel applications. Most notable is the use of molecular containers as nanoreactors,[4] in which reactive intermediates can be generated and attain unusual longevity,[5] reactions are accelerated[6] and product distributions altered.[7] Other potential applications that are currently explored are container-based devices for hydrocarbon and gas storage,[8] drug delivery,[9] separation technology,[10] molecular sensing,[11] and solar energy capture.[12] Many of these applications will benefit from the availability of molecular capsules with nanometer-sized cavities. Recently, covalent syntheses[13–15] and self-assembly approaches utilizing hydrogen bonding,[16,17] metal coordination chemistry,[18,19] ion-pairing[20] and hydrophobic interactions[9a,21,22] have been developed for the construction of such capsules with cavities large enough to accommodate multiple or very large guest molecules. An example is the multi-step synthesis of the first covalent six-cavitand container molecule **1** by Sherman et al. (Fig. 1a).[13b]

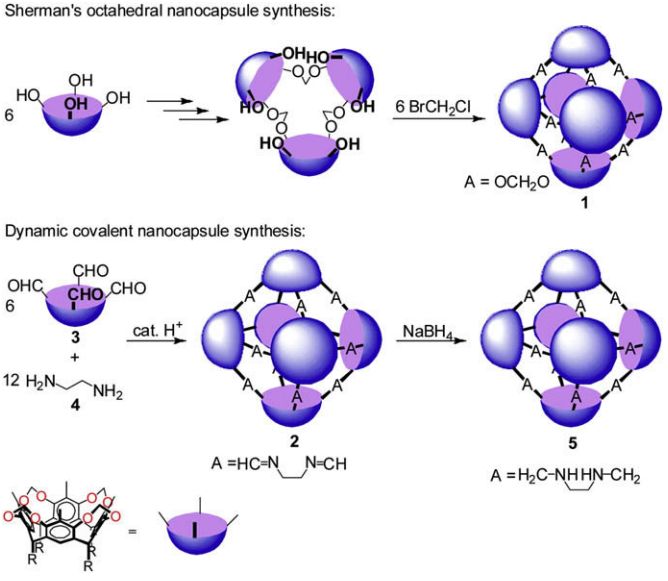

Figure 1. (a) Sherman's stepwise synthesis of a six-cavitand nanocapsule **1**.[13b] (b) Dynamic covalent synthesis of six-cavitand nanocapsule **2** and reduction of **2**.[14a,b]

This nanocontainer accommodates seven DMSO solvent molecules, which are permanently trapped and acted as 'multimolecule

* Corresponding author. Tel.: +1 732 445 8432; fax: +1 732 445 3213.
E-mail address: warmuth@rutgers.edu (R. Warmuth).

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template' in the final shell closing step. Using dynamic covalent chemistry, we recently showed that the structurally related polyimino nanocapsule **2** can be assembled almost quantitatively in a one-pot reaction from six tetraformyl cavitands **3** and 12 ethylenediamine linker units **4**.[14a,b] The high yield is a consequence of the dynamic nature of the imine bonds that link capsule building blocks together. The reversibility of the imine bond formation provided an error correction and proof reading mechanism, similar to other self-assembly processes, and ultimately furbished the thermodynamically most stable condensation product **2a**.[23]

Another very useful feature of the imine bond is the ease of its reduction to an aminomethylene group. This reduction allows one to 'fix' the product of a dynamic covalent synthesis. In addition, the generated secondary amines are useful functional groups for molecular recognition, to increase the polarity of the product by protonation and for further functionalization. Reduction of all imine bonds of **2** gave polyamino nanocapsule **5**, which has a somewhat hydrophobic inner cavity of approximately 1700–2000 Å^3 and is expected to encapsulate nonpolar guests in aqueous solution. Unfortunately, protonation of all amines was not sufficient to render **5** soluble in water in order to test its binding properties. This motivated the current work. Here, we describe the synthesis of related water-soluble octahedral polyammonium nanocapsules and some preliminary binding studies in aqueous solution, that show good binding properties for negatively charged organic guest molecules.

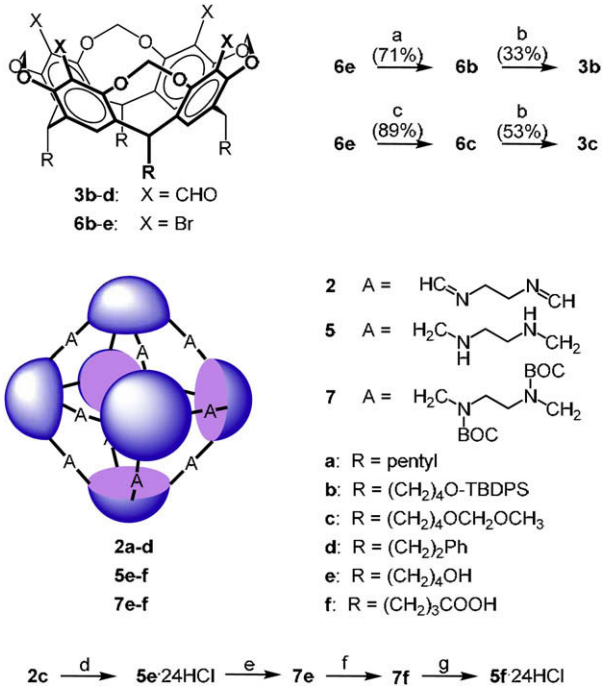
2. Results and discussion

2.1. Synthesis of water-soluble nanocapsules

In order to increase the hydrophilicity of the octahedral nanocapsule **5**, we introduced hydroxyl groups to the capsule's feet by replacing the pentyl groups of **5a** with 4-hydroxybutyl groups. 4-Hydroxybutyl-footed cavitand **6e** was prepared as described by Sherman and co-workers.[24] The hydroxyl groups of **6e** were protected as TBDPS ethers (Scheme 1). Lithiation of **6b** with *n*-BuLi followed by quench with DMF gave the TBDPS-protected tetraformyl cavitand **3b** in 30% yield.

To our surprise, the trifluoroacetic acid (TFA) catalyzed condensation reaction of **3b** with ethylene diamine gave only approximately 35% hexamer **2b** after 2.5 days, even though the same reaction with pentyl-footed cavitand **3a** produced **2a** in up to 82% yield.[14a] On the other hand, when we protected the hydroxyl groups of **6e** as MOM ethers (Scheme 1), the condensation reaction of **3c** with ethylene-diamine gave hexamer **2c** in 75% yield, which falls in between the yield obtained with **3a** and the phenethyl-footed cavitand **3d**.

Thus, hexamer yield decreased in the order **2a**>**2c**>**2d**>>**2b**, which scales with the size of the appending feet (Table 1). Gel permeation chromatography indicated that the condensation reaction between **3b** and ethylenediamine produced a larger amount of smaller capsules (tetrameric and pentameric) as compared to the reaction of **3a**, **3c** and **3d**. A possible explanation for this trend may be a feet crowding effect, that would stronger destabilizes a larger hexameric capsule with smaller curvature, but less so a smaller tetrameric and pentameric capsule, which have higher curvature.



Scheme 1. Octahedral nanocapsules **2**, **5** and **7** and synthesis of capsules **5e** and **5f** and of cavitands **3b–c**. Conditions: (a) TBDMSCl, NEt_3; (b) (1) BuLi, THF, −78 °C −>0 °C, (2) DMF; (c) CH_3OCH_2Cl, NEt_3; (d) (1) $NaBH_4$, (2) HCl concd/CH_3OH (90% yield); (e) BOC_2O/NEt_3 (95% yield); (f) TEMPO; NaOCl (94% yield); (g) (1) TFA, (2) 2 N HCl aq/CH_3OH (91% yield).

Table 1
Yields of octahedral nanocapsule **2a–d** in the acid-catalyzed condensation of **3a–d** with two molequivalents of ethylenediamine **4**[a,b]

Entry	Nanocapsule	Feet	Yield[a]
1	**2a**	$(CH_2)_4CH_3$	82%[c]
2	**2b**	$(CH_2)_4$OTBDPS	35%
3[b]	**2c**	$(CH_2)_4OCH_2OCH_3$	75%
4	**2d**	$(CH_2)_2$Ph	70%

[a] Yields determined from the integration of selected nanocapsule signals in the ^{1}H NMR spectra of the crude products.
[b] Reaction condition: $CHCl_3$; room temperature; 2.5 days; 0.1 mol % TFA catalyst.
[c] Ref. 14a.

Reduction of all imine bonds of crude hexamer **2c** with $NaBH_4$ followed by hydrolysis of the resulting boramines and MOM-protecting groups in methanol/concd HCl gave polyammonium nanocapsule **5e** in 70% yield based on cavitand **3c**. During the hydrolysis, **5e** slowly crystallized and could be obtained in excellent purity (>95%)[25] without the need for reversed phase chromatographic purification (Fig. 2a). Hexamer **5e** is soluble in water below pH 5 in the millimolar concentration range, but becomes increasingly less soluble upon raising the pH and insoluble above pH 7.

In order to increase the solubility above pH 7, we oxidized the terminal hydroxymethylene groups of **5e** to carboxylic acids (Scheme 1). Firstly, the amino groups of **5e** were BOC-protected by reacting **5e** with excess BOC_2O in refluxing methanol until MALDI-TOF MS indicated an increase in molecular weight that corresponds to 24 BOC groups. Subsequently, the terminal hydroxymethylene groups of **7e** were oxidized with TEMPO/NaOCl.[26] Again, the progress of the reaction was monitored by ^{1}H NMR spectroscopy and MALDI-TOF MS to ensure complete oxidation. Deprotection of the amino groups of **7f** with neat TFA followed by trifluoroacetate/chloride exchange gave **5f** as its HCl salt in 81% yield based on **5e**. Nanocapsule **5f** shows excellent water-solubility in acidic or basic solution, but is essentially insoluble at its isoelectric point just below pH 7 (Fig. 3a).

We also attempted to increase the water-solubility of hexamer **5a**, by acylation of the amines with carboxyl-containing acyl groups (Scheme 2). Firstly, we acylated the amines of **5a** with excess succinic acid monomethyl ester/diisopropylcarbodiimide (DIC). Saponification of intermediate hexamer **8a** with NaOH in methanol gave **9a**. Even though **9a** carries up to 24 negative charges at high pH, it is still not soluble in water. To increase the number of negative charges, we reacted **5a** with **12**. Unfortunately, this reaction didn't reach completion and only an average of 23 groups could be attached to **5a**. Most likely, steric congestion caused the reaction to

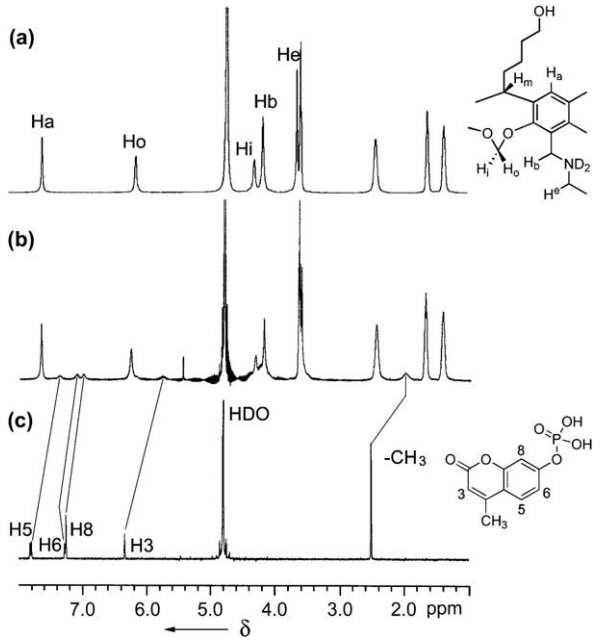


Figure 2. ^{1}H NMR spectra (500 MHz, D_2O, pD 4.2) of hexamer **5e** (a), of **5e** and 4.6 equiv of 4-MUP (b) and of 4-MUP alone (c).

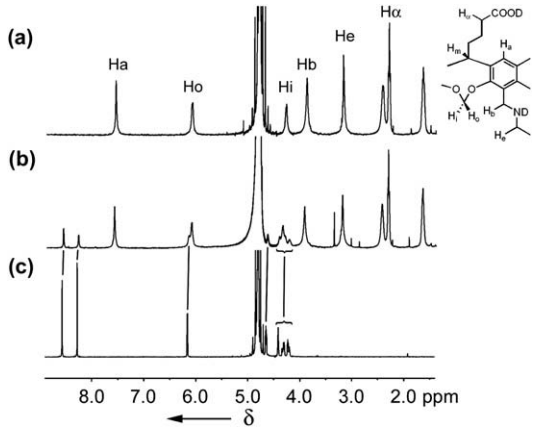
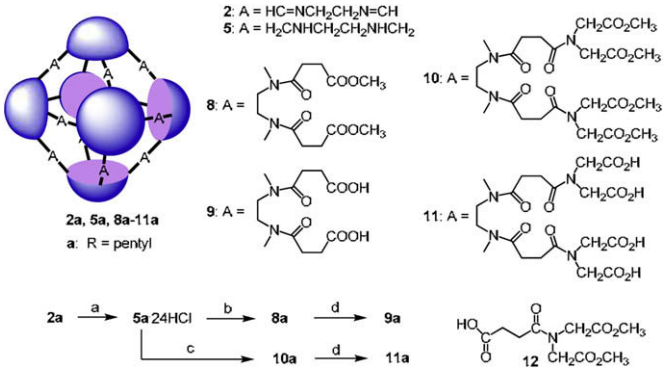


Figure 3. ^{1}H NMR spectra (400 MHz, D_2O, pD 7.55) of hexamer **5f** (a), of **5f** and 4.5 equiv of ATP (b) and of ATP alone (c).

become extremely slow once about 20 groups were attached. Saponification of the product mixture, gave hexamers **11a**, which were soluble in D_2O at pD 12. Strong line broadening in the ^{1}H NMR spectrum of D_2O solutions of **11a** suggested that this host may be strongly aggregated in water, perhaps due to its ambiphilic outer surface with alternating hydrophilic and hydrophobic areas.

2.2. Binding studies

We investigated the molecular recognition properties of **5e** in acidic aqueous medium (pH 1–5). Binding of small neutral and charged guest molecules was evaluated by NMR spectroscopy. Typically the encapsulation of guest molecules inside cavitand-based molecular containers is accompanied by large complexation induced upfield shifts (CIS) of guest protons. Protons, that are preferentially located in the cavity of a cavitand may be shifted as much as 4.5 ppm upfield and for aliphatic protons may appear in the negative region of the ^{1}H chemical shift scale.[10a] If the exchange between encapsulated and free guests is slow on the NMR time



Scheme 2. Synthesis of octahedral nanocapsules **9a** and **11a**. Conditions: (a) (1) $NaBH_4$, $CH_3OH/CHCl_3$, (2) HCl concd/CH_3OH; (b) $HO_2C(CH_2)_2CO_2CH_3$/DIC/NEt_3; (c) **12**/DIC/NEt_3; (d) (1) KOH/CH_3OH, (2) HCl aq.

scale (typically, if $\Delta G^{\ddagger}$>15–16 kcal/mol at ambient temperature), two sets of guest signals will be observed with that for the encapsulated guests being strongly upfield shifted.[14e,27,28] In the event of fast exchange, only average signals will be seen, whose upfield shifts depend on the relative amount of complexed and free guest. The latter was the situation for all encapsulated guests described here, most likely due to the large size of the openings of **5e** relative to the smallest guest's cross-section and the flexibility of the linkers, which allows passage of the guest through the host's skin with little or no barrier.

Addition of small, neutral, hydrophobic molecules, such as toluene, *p*-cresol, adamantane or anthracene to solutions of **5e** in D_2O at pD 4–5 didn't lead to characteristic CIS of guest protons, from which we conclude that these molecules are not encapsulated. However, guests such as *p*-toluenesulfonic acid (*p*-TsOH), *N*-BOC-aspartic acid (BOCAspOH) or 4-methylumbelliferyl phosphate (4-MUP), which are ambiphilic in nature and possess a hydrophobic and, at the experimental pD 1–5, a negatively charged moiety, were encapsulated as judged by the upfield shifts of guest protons in the ^{1}H NMR spectra of D_2O solutions of **5e** containing 1–7 equiv of guest (Figs. 2b, c and 4). Upfield shifts were largest for guest protons that are furthest away from the charged polar functional groups. For example, in the case of 4-MUP, CIS were largest for the methyl and the vinyl protons, and smallest for the aryl protons *ortho* to the negatively charged phosphate substituent. This suggests a binding orientation, in which the strongest upfield shifted protons are closest to the shielding cavity of a host's cavitand, allowing ion-pairing and H-bonding interaction between the phosphate and ammonium groups in the central and less shielding part of the cavity (Fig. 5).

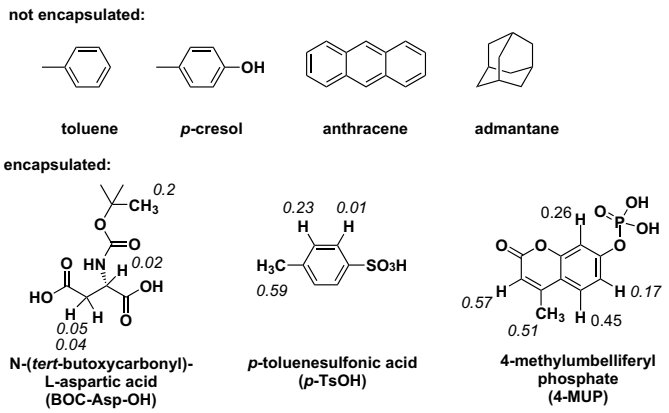


Figure 4. Neutral and acidic organic molecules that are or are not encapsulated inside **5e** in D_2O at pD 1–5 and complexation induced upfield shifts (CIS) for guest protons (italics): BOCAspOH/**5e**=4.6:1; *p*-TsOH/**5e**=0.6:1; 4-MUP/**5e**=4.6:1.

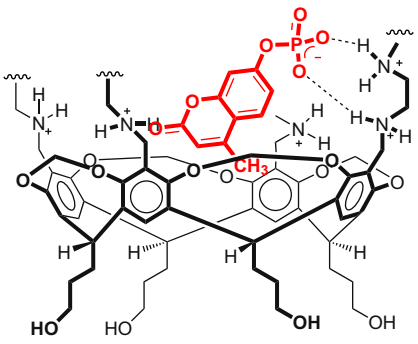


Figure 5. Proposed binding orientation of 4-MUP inside **5e** leading to a maximal CIS for the methyl and vinyl protons of the guest.

Guest molecules, such as ATP, dAMP, dGMP and TTP, which are negatively charged at pH >3, but lack a hydrophobic moiety, were not encapsulated inside **5e** or **5f**. For example, addition of ATP to a solution of **5f** in D_2O at pD 7.55 (Fig. 3b, c) or to **5e** in D_2O at pD 4.5 didn't lead to upfield shifts on the magnitude observed for the encapsulated guests described earlier. However, strong line broadening of all guest protons indicated that these nucleotides bind to the outer host surface, most likely via ion-pairing interactions with ammonium groups. Binding was further supported by diffusion NMR studies (DOSY).[29] From the diffusion constants of ATP ($D'_{ATP}=1.97\times10^{-10}$ m^2 s^{-1}) and of **5e** ($D'_{5e}=1.36\pm0.05\times10^{-10}$ m^2 s^{-1}) measured in a D_2O solution of 1 mM **5e** and 1.8 mM ATP at pD 3.6 and that of ATP alone ($D_{ATP}=3.77\pm0.05\times10^{-10}$ m^2 s^{-1}) in D_2O at pD 3.6, one can estimate that about 75% of the ATP is bound under these conditions. The binding of nucleotides to the polyammonium capsules was to be expected, since in the past, polyaza macrocyclic hosts have been used successfully in the design of synthetic hosts for nucleotide recognition and as artificial ATPases.[30,31]

In order to determine binding constants for the encapsulation of BOCAspOH, 4-MUP and *p*-TsOH inside **5e**, we used a combination of NMR titrations, in which we measured the observed CIS at different guest concentrations, and diffusion NMR experiments, in which we determined the diffusion constants of host and guest in these mixtures. Diffusion NMR spectroscopy is an excellent technique to determine the fraction of free and complexed guest for host–guest equilibria, in which the exchange between bound and free guest is fast on the NMR time scale.[29] The limitation of using diffusion NMR experiments is that the binding affinity has to be moderate, so that considerable amounts of free and complexed guest are present in the concentration range needed to observe host and guest in such experiments. This was the case of the three encapsulated guests.

In the event of fast exchange, the measured guest diffusion constant D'_{guest} is the weighted average of the diffusion constant of the free guest D_{guest} and that of the host–guest complex $D_{complex}$:

$$D'_{guest} = x_{free}\times D_{guest} + x_{complexed}\times D_{complex} \quad (1)$$

$$x_{free} + x_{complexed} = 1 \quad (2)$$

x_{free} and $x_{complexed}$ are mole factions of free and complexed guest. If the host is much larger than the guest, which is the case for **5e**, the diffusion properties of the host will change very little upon complexing/encapsulating one or more guests. Therefore, it is common to replace $D_{complex}$ in Eq. 1 with the diffusion constant of the host D'_{host} measured in the presence of the guest:

$$D'_{guest} = x_{free}\times D_{guest} + x_{complexed}\times D'_{host} \quad (3)$$

We used $x_{complexed}$, which was determined from Eqs. 2 and 3, to calculate the chemical shift of the encapsulated guest δ_{encaps} from the chemical shift of the free guest δ_{free} and that of the guest measured the diffusion NMR sample δ':

$$\delta_{encaps} = \left(\delta' - \delta_{free}\times(1-x_{complexed})\right)\Big/ x_{complexed} \quad (4)$$

The estimated volume of the cavity of **5e** and the van der Waals volumes of the guests (Table 2),[32] suggests that up to six guest molecules are encapsulated inside one nanocapsule. Thus, by determining δ_{encaps} as described above, we made the assumption that each of the guests experiences approximately the same upfield shift. δ_{encaps} allowed us to evaluate simple NMR titration data and to extract $x_{complexed}$ from δ_{free} and δ' using Eq. 4. In order to determine binding constants from these NMR titration data, we assumed a 6:1 binding model with six identical binding sites inside **5e**. We believe that this assumption is reasonable, since **5e** is built up from six identical cavitands, of which each can interact with the hydrophobic part of one guest. Furthermore, filling the cavity with six guest molecules yields cavity packing coefficients *P* that are close to the ideal packing coefficients *P*=0.55–0.65 for spherical capsules (Table 2).[33] For the sake of simplicity, we also assumed that all six binding sites are independent. The maximal CIS for the methyl group of the encapsulated guests, $\Delta\delta(CH_3)=\delta_{free}-\delta_{encaps}$, is smaller than those observed for methyl groups buried inside cavitands.[10] This suggests that the methyl groups are less deeply immersed inside the cavitands of **5e**, possibly due to the necessity of ion-pairing interactions with the host's ammonium groups, which may not allow the former (Fig. 5).

The stepwise encapsulation of six guests can be described by the following sequential binding equilibria:[34]

$$G + \mathbf{5e} \leftrightarrows \mathbf{5e}\odot G;\ G + \mathbf{5e}\odot G \leftrightarrows \mathbf{5e}\odot 2G;\ \ldots,\ G + \mathbf{5e}\odot 5G \leftrightarrows \mathbf{5e}\odot 6G$$

Table 2
Computed guest volume *V*,[32] computed packing coefficient *P* for six guests occupying the cavity of **5e**, single site binding constant *Q* for guest encapsulation inside **5e**, guest diffusion constant in the absence (D_{guest}) and presence of **5e** (D'_{guest}) at a **5e**:guest ratio of [H]:[G], diffusion constants of **5e** in the presence of guest (D'_{host}) at a **5e**:guest ratio of [H]:[G], chemical shift of the guest's methyl group ($\delta_{free}(CH_3)$), chemical shift of guest's methyl group in the presence of **5e** ($\delta'(CH_3)$) at a **5e**:guest ratio of [H]:[G], and CIS for methyl group of the encapsulated guest ($\Delta\delta(CH_3)$) in D_2O at 25 °C

Guest	V[a] Å^3	P[b]	Q(pD)[c] M^{-1}	D_{guest}[d] 10^{-10} s^{-1} m^2	D'_{guest}[e] 10^{-10} s^{-1} m^2	D'_{host}[d] 10^{-10} s^{-1} m^2	$\delta_{free}(CH_3)$	$\delta'(CH_3)$	[H]:[G][f]	$\Delta\delta(CH_3)$[g]
BOCAspOH	201	0.60–0.71	510±50 (*4.7*)	5.15	3.91	1.36	1.488	1.328	1:7.2	0.34
4-MUP	192	0.58–0.68	1550±150 (*4.2*)	5.10	2.83	1.36	2.53	2.04	1:3	0.95
p-TsOH	135	0.41–0.48	150±30 (*1.0*)	7.42	6.35	1.40	2.39	2.13	1:5.7	1.48

[a] Computed for energy-minimized structures (Amber*).
[b] Calculated for an estimated cavity volume of **5e** V_{cav} ~1700–2000 Å^3. $P=6\times V/V_{cav}$.
[c] Single site microscopic binding constant for a 6:1 binding model with identical, independent binding sites. The stepwise binding constants K_i are related to *Q* via $K_i=Q\times(6-i+1)/i$ (i=1, 2, …, 6).
[d] Mean average of the diffusion constant of each host (guest) signal; $\Delta D=\pm0.05\times10^{-10}$ s^{-1} m^2.
[e] Mean average of the diffusion constant of each guest signal that is clearly separated from the host signals; $\Delta D=\pm0.05\times10^{-10}$ s^{-1} m^2.
[f] [**5e**]=0.22 mM.
[g] $\Delta\delta(CH_3)=\delta_{free}(CH_3)-\delta_{encaps}(CH_3)$.

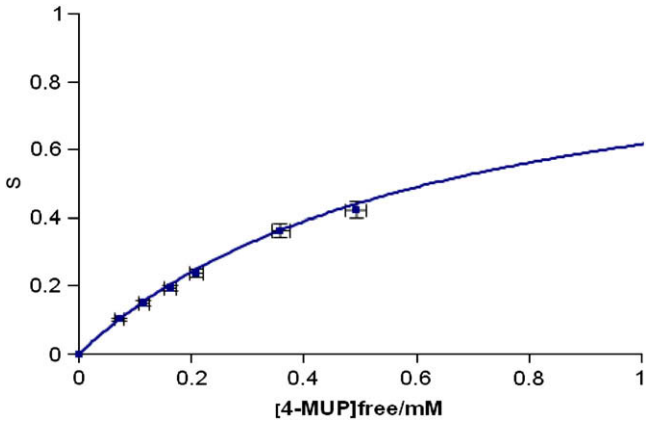


Figure 6. Saturation of binding sites *S* as function of free 4-MUP concentration at pD 4.2 for the encapsulation of 4-MUP inside **5e** calculated by assuming a 6:1 binding model with identical and independent binding sites and best fit (blue line; r^2=0.999). At guest concentrations higher than those shown, complex started to precipitate.

The individual stoichiometric formation constants for the individual binding steps K_i are defined as:

$$K_i = [\mathbf{5e} \odot iG]/[\mathbf{5e} \odot (i-1)G][G]_{\text{free}} \quad (5)$$

For identical and independent binding sites, K_i can be related to a single site microscopic binding constant *Q*, which is defined as:

$$Q = [\text{occupied single site}]/[\text{free single site}][G]_{\text{free}} \quad (6)$$

$$Q = iK_i/(6-i+1),\ i = 1,2,\ldots,6 \quad (7)$$

Q and K_i can be obtained by fitting titration data with the Bjerrum complex formation function, which correlates the binding site saturation *S* to the free guest concentration $[G]_{\text{free}}$, and which for statistical binding takes a very simple form:[34]

$$S = Q[G]_{\text{free}}/\left(1+Q[G]_{\text{free}}\right) \quad (8)$$

Q values determined from fitting binding isotherms (*S* versus $[G]_{\text{free}}$, Fig. 6) to Eq. 8 are listed in Table 2.

The measured binding constants are in the range of binding constants that have been measured for the interaction of similar negatively charged small organic guest molecules with water-soluble, single-cavitand hosts that have positively charged groups at their upper rim.[35,36] Thus, as host for small guest molecules, **5e** or **5f** don't offer much advantage in terms of single site binding strength. Stronger binding of small hydrophobic guest molecules is observed with deeper cavitand molecules, such as those developed by Gibb[37] and by Rebek,[38] and with hemicarcerand-type hosts.[28] These hosts possess extended hydrophobic, inner surfaces. These extended surfaces allow for an increased contact with a bound hydrophobic guest as compared to the more shallow cavitand building blocks of **5**, which provides a large driving force for binding in water.

Nevertheless, the architecture of **5** and its large inner cavity offers the opportunity for strong and selective multi-site interactions with larger guests that are flexible enough to enter the inner cavity of **5** and that have multiple hydrophobic and charged parts/moieties, which are able to interact with a set of binding sites inside **5**. Examples would be short polypeptides composed of acidic and hydrophobic residues.[39] Molecular recognition studies along these lines are currently underway in this laboratory.

3. Summary and conclusions

In summary, we have developed the multi-component synthesis of two different water-soluble octahedral polyammonium nanocapsules. These capsules have inner volumes of approximately 2000 Å^3 and have the potential to encapsulate multiple smaller guest molecules. Molecular recognition studies suggest that encapsulation requires that the guest is negatively charged and posses a hydrophobic group. Neutral guests or negatively charged guests that lack the hydrophobic part are not encapsulated or are only bound to the outside. This underlines the importance of both hydrophobic and ion pairing interactions for the encapsulation of a guest inside these polyammonium nanocapsules.

4. Experimental section

4.1. General

All reactions were conducted under argon. Reagents and chromatography solvents were purchased from Aldrich and used without further purification except that chloroform was passed through K_2CO_3 prior to use. ^{1}H NMR spectra recorded in $CDCl_3$, D_2O or DMSO-d_6 were referenced to residual $CHCl_3$, HDO and $CD_3S(O)CD_2H$ at 7.26 ppm, 4.80 ppm and 2.50 ppm, respectively. ^{13}C NMR spectra recorded in $CDCl_3$ or DMSO-d_6 were referenced to $^{13}CDCl_3$ at 77.0 ppm and $^{13}CD_3S(O)CD_3$ at 39.51 ppm, respectively. Mass spectra were recorded on an Applied Biosystems Voyager DE-Pro mass spectrometer (MALDI-TOF). 2′,4′,6′-Trihydroxylacetophenone (THAP) was used as matrix. Gel permeation chromatography (GPC) was performed on a Varian *prostar* 210 HPLC system equipped with dual wavelength UV/Vis detector (280 nm), Eppendorf CH-30 column heater and two Jordi GPC columns (cross linked DVB; 10^3 Å pore size; MW cutoff ~25,000; 7.8 mm×30 cm) with CH_2Cl_2/1% NEt_3 as mobile phase at a flow of 1 mL/min. Approximate molecular weights of analytes were determined from a semi logarithmic calibration plot (Ln(MW) against retention time) using the following molecular weight standards: benzene (MW 78); cavitand **3a** (MW 928); a NMP hemicarceplex (MW 2348),[10a] and polyaminonanocapsules (MW 3941, 5912 (**5a**) and 7882).[14a,c]

4.2. NMR complexation studies

Acetate buffer (pD 4.7, 103 mM) was prepared from acetic acid-d_4 and 40 wt% NaOD/D_2O. A BocAspOH solution (87.8 mM) was prepared by dissolving BocAspOH in acetate buffer. **5e**·24HCl (1.06 mg) was dissolved in 0.7 ml acetate buffer (0.22 mM) and placed into an NMR tube. This solution was titrated with the BocAspOH solution. Before and after each addition, ^{1}H NMR spectra were recorded on a 400 MHz Varian FT-NMR instrument. DCl buffer (pD 1.0, 100 mM) was prepared by diluting conc. DCl/D_2O solution. A *p*-TsOH solution (87.9 mM) was prepared by dissolving *p*-TsOH in the DCl buffer. A solution of **5e**·24HCl in DCl buffer (1.06 mg in 0.7 ml solution, 0.22 mM) was placed in an NMR tube and titrated with *p*-TsOH solution as described for BocAspOH. A solution of 4-MUP was adjusted to pD 4.5 with NaOD/D_2O (final concentration 78.6 mM). This solution was used to titrate **5e**·24HCl (1.17 mg in 0.7 ml D_2O, 0.24 mM, pD 4.2) as described for BocAspOH.

4.3. DOSY experiments

DOSY NMR experiments were performed on a 400 MHz Varian spectrometer equipped with a gradient system capable of producing magnetic field pulse gradients in the *z*-direction of about 50 G^{-1}/cm. A 5 mm broadband probe was used to carry out all the measurements. Samples were prepared as described in the previous section and were loaded into a 5 mm NMR tube. Temperature was controlled at 298 K. Samples were equilibrated at least 10 min before the measurement started. The diffusion experiments were performed using the pulse sequence Dbppste

(Bipolar Pulse Pair Stimulated Echo Experiment),[40] which is implemented in the NMR software VnmrJ. The diffusion delay (del, δ) was set at 0.05 s. The gradient pulse strength (gzlvl1, G_z) was varied from 400 to 25,000 G/cm. For all the other parameters, the default values were used. The field gradients were calibrated with the ^{1}H signal in a D_2O sample containing 1% H_2O. The literature value of D(HDO)=(1.902±0.002)×10^{-5} cm^2 s^{-1} was used for the self-diffusion rate of HDO at 25 °C.[41] For the diffusion studies involving free guest and free host, the mean average of the diffusion constants of each individual guest or host signal, respectively was calculated and is reported in Table 2. For the experiments with host–guest mixtures, only those signals that are clearly separated from other host/guest signals were used for the averaging.

4.4. Syntheses

4.4.1. TBDPS-protected tetrabromocavitand **6b**

Tetrabromocavitand **6e**[24] (1.0 g, 0.876 mmol) was dissolved in 20 ml DMF under argon. Imidazole (0.954 g, 14.0 mmol) was added into the solution. After 10 min, *tert*-butyl diphenylsilyl chloride (2.69 ml, 10.5 mmol) was added and stirring continued at room temperature for 24 h. Then, DMF was removed under reduced pressure. The residue was dissolved in 50 ml CH_2Cl_2 and washed with 30 ml water three times. It was dried over $MgSO_4$ and concentrated. The brownish residue was dried under high vacuum at room temperature overnight. The crude was purified by flash column chromatography (SiO_2, CH_2Cl_2/hexane 1:1) to yield **6b** as a white foam (1.30 g, 71%). ^{1}H NMR ($CDCl_3$, 25 °C, 400 MHz), δ_H: 7.61 (m, 16H), 7.36 (m, 8H), 7.33 (m, 16H), 6.96 (s, 4H), 5.98 (d, J=7.5 Hz, 4H), 4.84 (t, J=8.0 Hz, 4H), 4.40 (d, J=7.5 Hz, 4H), 3.62 (t, J=6.2 Hz, 8H), 2.14 (m, 8H), 1.61 (m, 8H), 1.37 (m, 8H), 1.01 (s, 36H). ^{13}C NMR ($CDCl_3$, 25 °C, 100 MHz), δ_C: 152.1, 139.1, 135.5, 133.9, 129.5, 127.6, 119.0, 113.6, 98.5, 63.6, 37.3, 32.2, 29.3, 26.8, 23.8, 19.2. MS (MALDI-TOF): m/z (M+K$^+$, 100%), calcd for $C_{112}Br_4Si_4H_{124}O_{12}$: 2134.451; found: 2134.470.

4.4.2. TBDPS-protected tetraformyl cavitand **3b**

TBDPS-protected tetrabromocavitand **6b** (700 mg, 0.334 mmol) was dried under high vacuum at 110 °C overnight. Then, it was dissolved in 18 ml dry THF and cooled down to −78 °C while protected under argon. *n*-BuLi (2.5 M hexane solution, 1.6 ml, 4.0 mmol) was syringed into the flask. The solution was stirred at −78 °C. After 20 min, it was warmed up to 0 °C for 30 min and recooled to −78 °C. Dry DMF (1.55 ml, 20.0 mmol) was added by syringe. After stirring at −78 °C for 10 min, the solution was warmed up to room temperature and stirred for 1 h. 0.5 ml 5 wt % NH_4Cl aq was added and stirring continued for 5 min. Then 20 ml water was added into the flask. The mixture was extracted with EtOAc (1×40 ml and 2×20 ml). The organic layers were combined, washed with 20 ml saturated $NaHCO_3$ aq, 20 ml brine, dried over $MgSO_4$ and concentrated. The yellow residue was dried under high vacuum. The crude product was purified by silica gel column chromatography (CH_2Cl_2/EtOAc 95:5) to give TBDPS-protected tetraformyl cavitand **3b** as a white powder (208 mg, 33%). ^{1}H NMR ($CDCl_3$, 25 °C, 400 MHz), δ_H: 10.26 (s, 4H), 7.61 (m, 16H), 7.36 (m, 8H), 7.33 (m, 16H), 7.22 (s, 4H), 5.91 (d, J=7.4 Hz, 4H), 4.90 (t, J=8.2 Hz, 4H), 4.47 (d, J=7.5 Hz, 4H), 3.63 (t, J=6.3 Hz, 8H), 2.18 (m, 8H), 1.64 (m, 8H), 1.41 (m, 8H), 1.00 (s, 36H). ^{13}C NMR ($CDCl_3$, 25 °C, 100 MHz), δ_C: 189.8, 154.7, 139.0, 135.5, 133.8, 129.6, 127.6, 124.7, 124.4, 100.1, 63.6, 35.5, 32.2, 29.0, 26.9, 23.8, 19.2. MS (MALDI-TOF): m/z (M+Na$^+$, 100%), calcd for $C_{116}Si_4H_{128}O_{16}$: 1911.818; found: 1911.823.

4.4.3. MOM-protected tetrabromocavitand **6c**[24]

Tetrabromocavitand **6e**[24] (1.0 g, 0.876 mmol) was dried overnight at high vacuum at 110 °C. It was dissolved in 20 ml DMF under argon. Diisopropylethyl amine (2.32 ml, 14.0 mmol) was added into the solution. After 10 min, chloromethyl methyl ether (0.8 ml, 10.5 mmol) was added and stirring continued at room temperature for 5.5 h. Then, DMF was removed under reduced pressure. The residue was dissolved in 50 ml EtOAc and washed twice with 50 ml 0.2 M HCl aq. The aqueous layer was further extracted with 30 ml EtOAc. The organic layers were combined, washed with 20 ml saturated $NaHCO_3$ aq, 30 ml brine, dried with $MgSO_4$ and concentrated. After drying the product under high vacuum at room temperature overnight, **6c** was obtained as slightly yellowish foam (1.03 g, 89%). ^{1}H NMR ($CDCl_3$, 25 °C, 300 MHz), δ_H: 7.03 (s, 4H), 5.95 (d, J=7.4 Hz, 4H), 4.89 (t, J=8.0 Hz, 4H), 4.61 (s, 8H), 4.39 (d, J=7.4 Hz, 4H), 3.54 (t, J=6.5 Hz, 8H), 3.35 (s, 12H), 2.26 (m, 8H), 1.73 (m, 8H), 1.46 (m, 8H). ^{13}C NMR ($CDCl_3$, 25 °C, 75 MHz), δ_C: 152.1, 139.1, 118.9, 113.7, 98.4, 96.5, 67.6, 55.2, 37.5, 29.6, 24.4. MS (MALDI-TOF): m/z (M+Na$^+$, 100%), calcd for $C_{56}H_{68}O_{16}Br_4$: 1339.1098; found: 1339.1225. Elemental analysis: calcd for $C_{56}H_{68}O_{16}Br_4$: C, 51.08; H, 5.21; found: C, 51.47; H, 5.42.

4.4.4. MOM-protected tetraformyl cavitand **3c**

Tetrabromocavitand **6c** (0.5 g, 0.38 mmol) was dried under high vacuum at 110 °C overnight. Then, it was dissolved in 20 ml dry THF and cooled down to −78 °C while protected under argon. *n*-BuLi (2.5 M hexane solution, 1.22 ml, 3.04 mmol) was syringed into the flask. The solution was stirred at −78 °C. After 20 min, it was warmed up to 0 °C for 30 min and recooled to −78 °C. Dry DMF (1.18 ml, 15.2 mmol, dried over 3 Å molecular sieves for 24 h) was added by syringe. After stirring at −78 °C for 10 min, the solution was warmed up to room temperature and stirred for one additional hour. 10 ml 5 wt % NH_4Cl aq was added and stirring continued for 10 min. The mixture was extracted with EtOAc (1×40 ml and 2×20 ml). The organic layers were combined, washed with 30 ml saturated $NaHCO_3$ aq, 30 ml brine, dried over $MgSO_4$ and concentrated. The yellow residue was dried under high vacuum. The crude product was purified by silica gel column chromatography (Et_2O/CH_2Cl_2/Et_3N 8:1:0.036 gradient to Et_2O/CH_2Cl_2/Et_3N 3:2:0.036) to give **3c** as a white powder (0.225 g, 53%). ^{1}H NMR ($CDCl_3$, 25 °C, 300 MHz), δ_H: 10.25 (s, 4H), 7.29 (s, 4H), 5.90 (d, J=7.6 Hz, 4H), 4.94 (t, J=8.1 Hz, 4H), 4.62 (s, 8H), 4.47 (d, J=7.6 Hz, 4H), 3.56 (t, J=6.4 Hz, 8H), 3.36 (s, 12H), 2.29 (m, 8H), 1.75 (m, 8H), 1.48 (m, 8H). ^{13}C NMR ($CDCl_3$, 25 °C, 125.7 MHz), δ_C: 189.8, 154.7, 139.0, 124.6, 124.5, 100.1, 96.5, 67.5, 55.2, 35.8, 29.6, 29.3, 24.4. MS (MALDI-TOF): m/z (M+Na$^+$, 100%), calcd for $C_{60}H_{72}O_{20}$: 1135.4515; found: 1135.4581. Elemental analysis: calcd for $C_{60}H_{72}O_{20}$: C, 64.74; H, 6.52; found: C, 64.94; H, 6.12.

4.4.5. 4-Hydroxybutyl-footed nanocapsule **5e**

Hexamer **5e** was prepared according to a procedure developed for hexamer **5a**.[14a,b] Tetraformyl cavitand **3c** (569 mg, 0.511 mmol), ethylenediamine **4** (63.4 mg, 1.05 mmol) and TFA (6.1 μl, 0.0821 mmol) were dissolved in 47 ml chloroform (passed through a pad of K_2CO_3). The slightly yellow solution was stirred at room temperature under argon for 41 h. To the stirred solution, $NaBH_4$ (4.04 g, 107 mmol) was added. After 3 min, 0.4 ml dry methanol (dried over 3 Å molecular sieves for 24 h) was added. After 5 min, additional 4.3 ml dry methanol was added and the mixture was stirred at room temperature overnight. The solvents were removed under reduced pressure. The white residue was sonicated with 30 ml water for 10 min and the mixture was filtered. The white residue was transferred into a 250 ml round bottom flask and stirred with 170 ml methanol. Then, 17 ml concentrated HCl was added drop wise into the flask. Stirring was continued at room temperature under argon for 3 days (white precipitates gradually formed after 1 day). The mixture was filtered and the white residue was washed three times with 1 ml cold methanol. The residue was dried under high vacuum overnight to give **5e**·24HCl as a slightly

yellowish powder (409 mg, 70% yield based on **3c**). ^{1}H NMR (0.2 wt % DCl/D_2O, 25 °C, 500 MHz), δ_H: 7.72 (s, 24H), 6.22 (s, 24H), 4.37 (s, 24H), 4.24 (s, 48H), 3.70 (s, 48H), 3.64 (t, J=6.0 Hz, 48H), 2.49 (s, 48H), 1.70 (m, 48H), 1.44 (s, 48H). ^{13}C NMR (0.2 wt % DCl/D_2O, 25 °C, 125 MHz), δ_C: 153.4, 138.8, 123.9, 117.9, 100.4, 61.9, 42.8, 41.5, 37.0, 31.2, 28.8, 23.6. MS (MALDI-TOF) for $C_{336}H_{432}N_{24}O_{72}$: m/z ($M+Na^+$), calcd 5978.08; found: 5978.32.

*4.4.6. Synthesis of nanocapsules **2b** and **2d***

The condensation reaction between cavitands **3b** and ethylenediamine (**4**) and between **3d**[15a] and **4** was carried out under identical conditions described for the condensation between **3c** and **4** (Section 4.4.5). The yield of nanocapsules **2b** and **2d** was determined from the integral of the imine protons at δ 8.40 (**2b**) and δ 8.36 (**2d**) in the ^{1}H NMR spectrum ($CDCl_3$) of the reaction mixtures.

*4.4.7. Nanocapsule **5f**·24HCl*

*4.4.7.1. BOC protection of **5e** to form **7e**.* Triethylamine (303 μl, 2.17 mmol) was added into the suspension of **5e** 24HCl (206.6 mg, 0.0302 mmol) in 35 ml methanol. The mixture was stirred for 20 min. $(BOC)_2O$ (317 mg, 1.45 mmol) was added and the mixture was heated to reflux. The mixture was kept refluxing under argon for 3 days while additional 6 portions of $(BOC)_2O$ (~300 mg each, 1.38 mmol) was added during the reaction. Then the solvent was removed under reduced pressure. The white foam-like residue was sonicated with 10 ml Et_2O and filtered. The residue was washed with additional 1 ml Et_2O three times and 1 ml H_2O three times. It was redissolved in methanol. The solvent was evaporated and the off-white residue was dried under high vacuum for 30 min to give **7e** (241.3 mg, 95%). MALDI-TOF MS for $C_{456}H_{624}N_{24}O_{120}$, calcd 8384.91 ($M+Na^+$), found 8384.17.

*4.4.7.2. TEMPO oxidation of **7e** to form **7f**.* To the suspension of **7e** (201.3 mg, 0.0241 mmol) in 10 ml acetone, 2.5 ml 5% $NaHCO_3$ aq and 10 mg KBr was added. The mixture was cooled to 0 °C. TEMPO (150 mg, 0.96 mmol) was added. Then 5 ml ~5% NaClO aq was added into the mixture drop wise over 10 min. The mixture was stirred at 0 °C under argon for 1 h. A deep yellow mixture was obtained. Then additional 5 ml ~5% NaClO aq was added and continued stirring for another hour. The white mixture was quenched with 25 ml H_2O. Then 1 M H_2SO_4 aq was added to adjust pH ~2. The white precipitate was filtered and washed with enough water until neutral. The residue was redissolved in methanol and the solvent was evaporated. The slightly yellow residue was dried under high vacuum (235.8 mg). The crude product was then subjected to hydrolysis to break possible ester bonds formed in the oxidation process. It was dissolved in 20 ml 1 M NaOH aq. The yellow solution was heated to reflux and stirred for 2 h. Then it was cooled down and acidified with 1.2 ml conc. HCl to pH ~7 and then with 1 M H_2SO_4 aq to pH ~3. The white precipitate was filtered and washed with enough water until neutral. The residue was redissolved in methanol and the solvent was evaporated. The remaining off-white solid **7f** was dried under high vacuum (197.7 mg, 94%) and used without further purification for the next step.

*4.4.7.3. Deprotection of **7f**.* A solution of crude **7f** (197 mg, 0.0226 mmol) in 20 ml TFA was stirred at room temperature for 1 h. TFA was removed under reduced pressure. The yellow residue was dried under high vacuum to give crude **5f**·24TFA (251.7 mg). Crude **5f**·24TFA (230 mg) was dissolved in methanol (4 mL). Diluted aqueous HCl (2 N, 0.2 mL) was added and the solvent evaporated at reduced pressure. This procedure was repeated (four times) until no ^{19}F signal could be detected in the ^{19}F NMR spectrum of a solution of the residual solid in D_2O. Finally the product was dried at high vacuum to give **5f**·24HCl as pale yellow solid (136 mg, 91% yield). ^{1}H NMR (500 MHz, NaOD/D_2O, 25 °C), δ_H (ppm): 7.43 (s, 24H, H_{aryl}), 6.04 (d, J=5.8 Hz, 24H, H_{outer}), 4.71 (t, J=8.0 Hz, 24H, $H_{methine}$), 4.21 (d, J=5.2 Hz, 24H, H_{inner}), 3.51 (s, 48H, –ArCH_2NH–), 2.69 (s, 48H, –NHCH_2CH_2NH–), 2.36 (m, 48H), 2.28 (t, J=7.5 Hz, 24H, $–CH_2CO_2^-Na^+$), 1.58 (m, 48H). ^{13}C NMR (D_2O, pH 9.7, 25 °C, 125 MHz), δ_C: 183.5, 153.4, 138.5, 123.4, 121.7, 100.2, 46.3, 41.7, 37.4, 36.9, 29.0, 24.2, 12.0. ESI-MS (NH_3 H_2O/MeOH) for $C_{336}H_{384}N_{24}O_{96}$, calcd 1572.7 $(M-4H)^{4-}$, 1258.0 $(M-5H)^{5-}$; found, 1572.8, 1258.7.

*4.4.8. Nanocapsule **9a***

Triethylamine (43 μl, 0.309 mmol) was added to a suspension of **5a**·24TFA[14a] (100 mg, 0.0116 mmol) in 30 ml dichloromethane. The solution was stirred at room temperature for 20 min. Then, monomethyl succinate (74 mg, 0.560 mmol) and *N,N′*-diisopropylcarbodiimide (DIC, 95 μl, 0.614 mmol) was added and stirring continued for 7.5 days. The clear, slightly yellow solution was washed with 1 M HCl aq (30 ml), satd $NaHCO_3$ aq (30 ml), H_2O (30 ml) and brine (30 ml). The solution was concentrated and the crude product was dried under high vacuum to give **8a** as a slightly yellow oil (144.5 mg). MALDI-TOF MS for $C_{480}H_{624}N_{24}O_{120}$ calcd 8672.35 ($M+Na^+$), found 8672.34. Crude **8a** was subjected to saponification without purification. **8a** (81.2 mg) was dissolved in 10 ml THF and 1 M KOH aq (1.4 ml, 1.4 mmol) was added. The mixture was stirred at room temperature for 2 days, after which it was concentrated. The residue was partitioned between 10 ml EtOAc and 5 ml 1 M HCl aq. The organic layer was separated, dried over $MgSO_4$ and concentrated. **9a** was obtained as a slightly yellowish solid (27.6 mg, 51% yield based on **5a**). ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C), δ_H (ppm): 12.0 (br s, 24H), 7.54 (br s, 24H, H_{aryl}), 5.73 (br s, 24H, H_{outer}), 5.21 (br s, 24H, $H_{methine}$), 4.55 (br s, 48H, ArCH_2N–), 4.06 (br s, 24H, H_{inner}), 2.70 (br s, 96H, –NCOCH_2CH_2-COOH), 2.18 (br s, 48H), 1.30 (br s, 144H), 0.85 (br s, 72H). ^{13}C NMR (100 MHz, DMSO-d_6, 25 °C), δ_C (ppm): 174.2, 171.1, 153.5, 138.1, 123.3, 122.0, 99.5, 43.8, 36.7, 31.5, 31.3, 29.5, 29.0, 27.5, 27.0, 22.2, 14.0. MS (MALDI-TOF): m/z ($M+Na^+$, 100%), calcd 8334.97 for $C_{456}H_{576}N_{24}O_{120}$; found: 8334.71 (100%).

*4.4.9. Compound **12***

Triethylamine (370 μl, 2.65 mmol) was added to a suspension of dimethyl iminodiacetate hydrochloride (500 mg, 2.53 mmol) in 20 ml acetonitrile. The solution was stirred at room temperature for 30 min. Succinic anhydride (278 mg, 2.78 mmol) was added and stirring continued for 1 day. The mixture was concentrated and the residue was partitioned between 10 ml EtOAc and 5 ml 1 M HCl aq. The organic layer was washed with brine 10 ml, dried over $MgSO_4$ and concentrated. **12** was obtained as a slightly yellowish oil (280 mg, 42% yield), which slowly solidified over time at room temperature. ^{1}H NMR ($CDCl_3$, 25 °C, 300 MHz), δ_H: 4.21 (s, 2H, –CONCH_2–), 4.19 (s, 2H, –CONCH_2–), 3.79 (s, 3H, –COCH_3), 3.73 (s, 3H, –COCH_3), 2.77–2.64 (m, 4H, $HO_2CCH_2CH_2$CON–). ^{13}C NMR ($CDCl_3$, 25 °C, 75 MHz), δ_C: 176.5, 172.3, 169.5, 169.0, 52.6, 52.3, 50.0, 48.1, 29.7, 29.1, 27.6. MS (MALDI-TOF): m/z ($M+Na^+$, 100%), calcd for $C_{10}H_{15}NO_7$: 285.0780; found: 285.0783.

*4.4.10. Nanocapsule **11a***

Triethylamine (22 μl, 0.0158 mmol) was added to a suspension of **5a**·24TFA[14a] (50 mg, 0.00578 mmol) in 10 ml dichloromethane. The solution was stirred at room temperature for 20 min. **12** (73 mg, 0.279 mmol) and *N,N′*-diisopropylcarbodiimide (DIC, 47 μl, 0.304 mmol) were added and stirring continued for 3.5 days. The clear, slightly yellow solution was washed with 1 M HCl aq (10 ml), satd $NaHCO_3$ aq (10 ml), and brine (10 ml). It was dried over $MgSO_4$, concentrated and dried under high vacuum. Crude **10a** was obtained as an oil (102.3 mg) and was subjected to saponification without purification. It was dissolved in 8.5 ml THF and 1 M KOH aq (3.0 ml, 3.0 mmol) was added. The mixture was stirred at room

temperature for 2.5 days. It was concentrated and partitioned between 20 ml EtOAc and 5 ml 1 M HCl aq. The organic layer was dried over $MgSO_4$ and concentrated NaOH aq (10 ml, 1 M) was added to the residue. The suspension was sonicated and filtered. The white precipitate containing the sodium carboxylate of **11a** was washed with CH_2Cl_2 to remove diisopropyl urea and then dried under high vacuum (42.2 mg, 65% yield based on **11a**). ^{1}H NMR (400 MHz, D_2O, pD 9, 25 °C), δ_H (ppm): 7.47 (br s, 24H, H_{aryl}), 6.00 (br s, 24H, H_{outer}), 5.32 (br s, 24H, $H_{methine}$), 4.71 (br s, 24H, H_{inner}), 3.94 (br s, 96H, –NCOCH_2CH_2CON–), 2.81 (br s, 96H, –NCOCH_2CO$^-$Na$^+$), 2.37 (br s, 48H), 1.39 (br s, 144H), 0.91 (br s, 72H).

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