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Assembly of Amphiphilic Baskets into Stimuli-Responsive Vesicles. Developing a Strategy for the Detection of Organophosphorus Chemical Nerve Agents

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Supporting Information

ABSTRACT: We designed basket **1** to comprise a C_3-symmetric hydrophobic cage (477 Å^3) at its southern edge and three polar ammonium caps at the northern edge. This amphiphilic molecule was observed to assemble into large unilamellar vesicles (350 nm, TEM) in water and thereby entrap dimethyl phenylphosphonate (184 Å^3) in its cavity (K_{app} = (1.97 ± 0.02) × 10^3 M^{-1}). The entrapment of the organophosphonate, akin to soman in size (186 Å^3), triggers the transformation of the vesicular material into nanoparticles (100 nm, TEM). Stimuli-responsive vesicles, containing baskets of type **1** in their bilayer membrane, are unique assemblies and important for obtaining novel sensing devices.

Nerve agents of the *G* and *V* types are highly toxic substances, stored in vast quantities around the world and classified as weapons of mass destruction.[1] These organophosphorus (OP) compounds were designed to comprise a labile P−X group (X = F, CN, SR, etc.),[2] such that even a reaction with weakly nucleophilic water occurs at a reasonable rate.[3] After a brief exposure, OP nerve agents inhibit the action of acetylcholinesterase by reacting with the nucleophilic serine in the enzyme's active site,[4] thereby causing respiratory malfunction and death. Developing methods for the unambiguous detection and effective degradation of nerve agents has been a priority for years,[1,5] yet one still has to use expensive and nonportable analytical instrumentation for identifying toxic OPs.[6] A solution to the problem is seen in developing colorimetric chemosensors[7] and stimuli-responsive materials[8] capable of suitable signal transduction upon encountering these substances. Importantly, the colorimetric methodology is prone to false alarms,[9] yet this problem could be overcome by employing an array of sensors (an electronic nose).[10] We reason that developing functional cavitands capable of entrapping *G*/*V* agents[11] not only will permit their unambiguous detection, but also would lead to the development of supramolecular catalysts capable of promoting a rapid degradation of these substances.[12] We recently demonstrated that molecular baskets, with three protonated histamines at the rim,[13] entrap variously sized and shaped organophosphonates (V < 197 Å^3; K_{app} < 310 M^{-1}) in water. In line with these results, we designed and synthesized a more sizable basket, **1** (Figure 1). Will this deeper cavity host (V = 477 Å^3), with three positively charged groups at the northern edge and the larger hydrophobic segment on its southern edge, have sufficient solubility in water to allow the encapsulation and detection of OPs akin to nerve agents?

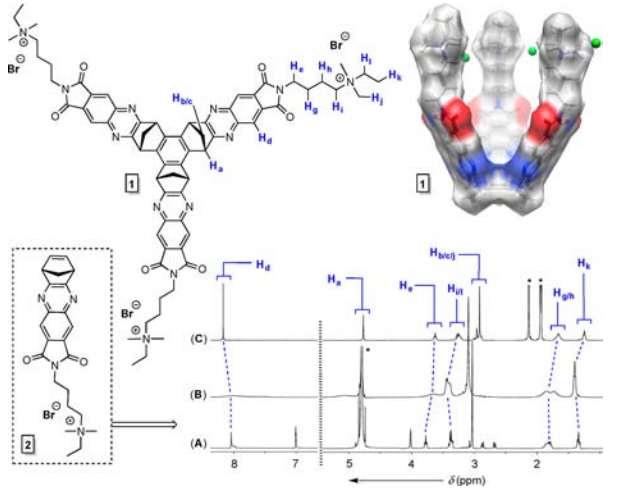

Figure 1. Chemical structure and van der Waals surface of basket **1** (MMFFs, Spartan), and ^{1}H NMR spectra (400 MHz, 298.0 K) of (A) model compound **2** in D_2O (1.0 mM), and basket **1** (1.0 mM) in (B) D_2O and (C) $CD_3CN/CDCl_3$ = 4:1.

Basket **1** is an amphiphilic compound,[14] comprising a tris-norbornadiene base that extends into three quinoxaline-like moieties (Figure 1), each carrying a polar ammonium cap. The semirigid and concave platform of **1** was prepared via tris-annulation of bromostannanes using a Pd(0)/Cu(I) catalytic pair to facilitate the Stille-type coupling (Scheme S1).[15] Conjugation of 4-aminobutan-1-ol to the platform and additional functionalizations were facile to give basket **1** (Scheme S1). Interestingly, the solid-state structure of hexamethylester **3** (Figure 2A) reveals two of these cup-shaped molecules in a perpendicular orientation, with each compound using one side arm to penetrate the inner space of its neighbor. Moreover, the encapsulated ester places its OCH_3 unit against

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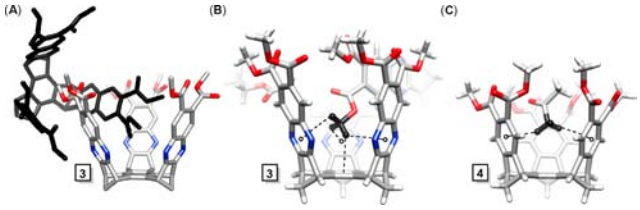

Figure 2. (A) Stick representation of cup-shaped **3** in the solid state forming (B) favorable C−H---π contacts. (C) Stick representation of cup-shaped [**4**⊂CH_3COCH_3] complex in the solid state.[17]

three surrounding pyrazine rings to form favorable C−H---π contacts (2.78−3.20 Å, Figure 2B).[16] In fact, we already observed similar C−H---π interactions within the [**4**⊂CH_3COCH_3] complex in the solid state[17] (Figure 2C) to attest to the propensity of the tris-bicyclic framework for hosting aliphatic groups,[18] which are components of nerve agents.

Basket **1** was soluble in D_2O (0.1−5.2 mM at 298 K), although the ^{1}H NMR spectrum (Figure 1B) showed an extensive broadening of proton resonances. Presumably, aggregation of **1** could have contributed to the ill-defined NMR spectrum with (a) shortening of the transverse magnetic relaxation (T_2) of the proton nuclei and (b) positioning of the protons in different environments to exchange at intermediate rates on the NMR time scale.[19] Interestingly, $\mathbf{H}_e$−$\mathbf{H}_k$ protons of the aliphatic chain in **1** seem to retain the mobility, as these resonances exhibit narrow lines (Figure 1B, $\Delta\nu_{1/2} = 1/\pi T_2$*). When amphiphilic **1** was dissolved in nonpolar $CDCl_3/CD_3CN$ = 1:4 (1.0 mM, Figure 1C), however, the signals became well-resolved, corresponding to a C_{3v}-symmetric molecule. With the assistance of ^{1}H−^{1}H COSY/NOESY and ^{1}H−^{13}C HMBC NMR spectroscopic correlations (Figures S1−S3), we assigned all of the resonances in **1** (Figure 1C). A 10-fold dilution of basket **1** in D_2O (from 5.2 to 0.5 mM, Figure S4) did not considerably affect the appearance of its ^{1}H NMR spectrum to, perhaps, suggest the formation of rather stable aggregate(s). In line with this observation, pulse-field gradient NMR spectroscopic measurements of 0.5−2.0 mM solution of **1** (D_2O, 298.0 K) showed almost identical diffusion coefficients of the aggregates (3.36−3.67 × 10^{-10} m^2/s).[20] In contrast, model compound **2** (Figure 1a) showed no sign of assembly in water with a set of well-resolved ^{1}H NMR resonances that stayed practically unperturbed upon a dilution of its solution from 5.0 to 0.5 mM (Figure S5).

To more closely examine the assembly of amphiphilic **1** and, in particular, evaluate the size distribution of its aggregates (hydrodynamic diameter, D_H), we completed a series of dynamic light scattering (DLS) measurements (Figure 3A).[21] Evidently, basket **1** (1.0 mM) forms particles of 200−500 nm diameter with the distribution centered at D_H = 350 nm; note that the observed distribution was consistent with 0.5−3.0 mM solutions of **1** (Figure S6). The stability of $[\mathbf{1}]_n$ aggregates was further probed with diffusion NMR spectroscopy at variable temperatures (Figure 3B): the change in the apparent hydrodynamic radius R_H of **1** with temperature was sigmoidal, suggesting a cooperative phase transition from less to more dynamic states of the aggregate, and the melting temperature (T_m) was determined to be ∼45 °C. Evidently, amphiphilic basket **1** gives rise to discrete assemblies in water with, perhaps, a gel to liquid-crystalline phase transition at 35−50 °C.[22] In order to obtain additional information about the nature of the

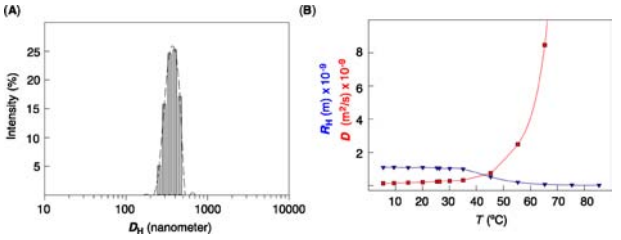

Figure 3. (A) Size distribution of $[\mathbf{1}]_n$ (1.0 mM in H_2O) obtained from dynamic light scattering measurements at 298.0 K. (B) Variation of diffusion coefficients D (m^2/s) and hydrodynamic radii R_H of $[\mathbf{1}]_n$ (1.0 mM in D_2O) with temperature, examined with diffusion NMR spectroscopy.

assembled structures, we completed electron microscopy measurements. An aqueous solution of **1** (1.0 mM) was deposited on copper grids and, after solvent evaporation, was examined with transmission electron microscopy (TEM), which revealed the existence of vesicular entities with a spherical morphology and a diameter of ∼350 nm (Figure 4A)![23] Importantly, the result is in good agreement with our

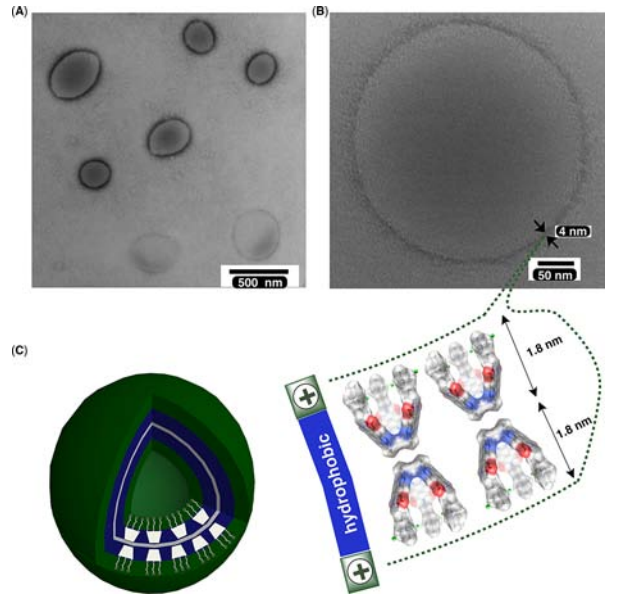

Figure 4. (A,B) TEM images of **1** (1.0 mM in H_2O) deposited on a copper grid and stained with uranyl acetate. (C) Proposed packing of basket **1** in the bilayer of vesicles.

DLS measurements of **1** in solution, denoting the presence of particles with an average size of 350 nm (Figure 3A). In fact, "zooming-in" on a single vesicle allowed us to estimate the thickness of its membrane to be 4 nm (Figure 4B). Given that the length of a single basket **1** is ∼1.8 nm (MMFFs, Spartan), we deduce that two baskets must be packing tail-to-tail (Figure 4C) to form a curved unilamellar membrane and thereby enclose the vesicle's inner space. That is to say, the inner side of the lipid-like bilayer comprises two hydrophobic cages, while the polar ammonium caps reside at the outer edges and contact bulk water (Figure 4C).[24]

An incremental addition of dimethyl phenylphosphonate (DMPP, 184 $Å^3$) to vesicular **1** in D_2O (1.0 mM, Figure 5) caused sharpening of the host's ^{1}H NMR lines as well as perturbation of the magnetic environment of all protons. We surmised that baskets, packed in the bilayer, interacted with

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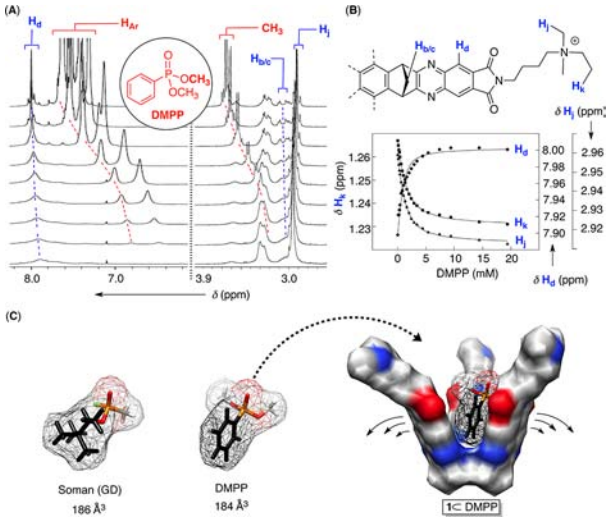

Figure 5. (A) Section of ^{1}H NMR spectra (400 MHz, 298.0 K) of **1** (1.0 mM) obtained upon an incremental addition of DMPP (0−19 mol equiv). (B) Nonlinear least-squares analysis of the binding isotherms, giving K_{app} (R^2 = 0.999). (C) Structure of [**1**⊂DMPP] complex, computed with molecular dynamics.

DMPP, with some reorganization of the vesicular material to enhance the host's dynamics. The method of continuous variation (Figure S7)[25] suggested a 1:1 complexation stoichiometry, while nonlinear least-squares analysis of the binding isotherms gave K_{app} = (1.3 ± 0.2) × 10^3 M^{-1} (Figure 5B);[26] ESI-MS measurements also revealed the formation of a 1:1 complex (Figure S7). The strong binding was additionally corroborated with isothermal titration calorimetry (K_{app} = 1.97 × 10^3 M^{-1}, Figure S8), which revealed that the formation of [**1**⊂DMPP] at 298.0 K is driven by both enthalpy ($\Delta H°$ = −1.06 ± 0.02 kcal/mol) and entropy ($\Delta S°$ = 11.5 ± 0.9 eu). What is the structure of the [**1**⊂DMPP] complex formed from amphiphilic and vesicular **1** trapping the DMPP guest? To address this matter, we noted a different extent of the perturbation of ^{1}H NMR resonances of the guest during its addition to the host (Figure 5A). That is to say, the change in the chemical shift of the $\mathbf{H}_{Ar}$ signals corresponding to the guest's phenyl group ($\Delta\delta$ = 0.80 ppm, Figure 5A) is greater than that of the OCH_3 nuclei ($\Delta\delta \approx$ 0.45 ppm, Figure 5A). Inclusion of the P−C_6H_5 unit in the cavity of the basket accounts for this observation.[27] In fact, the electronic ring currents of the host's aromatic rings must be, via diamagnetic anisotropy, contributing to the apparent shielding of the $\mathbf{H}_{Ar}$ protons from the encapsulated guest (Figure 5B). The positioning of DMPP inside the basket was studied with molecular dynamics and docking computational protocols established in our earlier work (Figure 5C).[13] Most simulations gave rise to a [**1**⊂DMPP] complex having the P-C_6H_5 group oriented in the cavity of **1** (Figure 5C)! A subsequent TEM study of the [**1**⊂DMPP] complex, as deposited on a copper surface, revealed the formation of nanoparticles having a uniform coloration and ∼100 nm diameter (Figure 6A). In addition, DLS measurements of [**1**⊂DMPP] in solution indicated the presence of aggregates for which the size distribution (PDI = 0.4) is centered at D_H = 200 nm (Figure 6B). Evidently, the addition of DMPP to **1** caused a phase transition of the vesicles into nanoparticles. An apparent downfield shift of bridged $\mathbf{H}_{b/c}$ protons (Figure 5A) furthermore denotes an expansion of the carbon framework

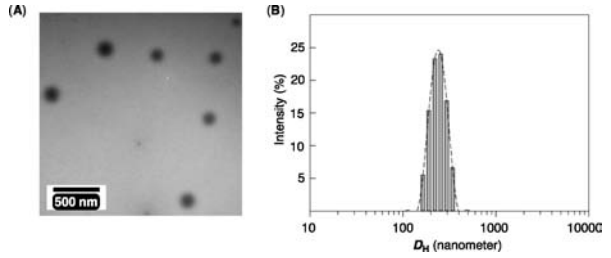

Figure 6. (A) HR-TEM image of H_2O solution of **1** (1.0 mM) containing DMPP (19.4 mM). (B) Size distribution of **1** (1.0 mM in H_2O) containing DMPP (19.4 mM), examined with dynamic light scattering at 298.0 K.

of amphiphilic **1** within the [**1**⊂DMPP] complex.[13] We reason that the change in the shape of **1** affected the packing of these molecules: the critical packing parameter[28] of vesicles, $P \approx 0.59$, decreases to $P \approx 0.32$ (Figure S9) to render the aggregation of [**1**⊂DMPP] into nanoparticles. The packing of host−guest complexes inside nanoparticles remains to be understood, although the absence of a water reservoir (Figure 6A) suggests the formation of a multilayer assembly.

In conclusion, amphiphilic baskets of type **1** were found to assemble into large unilamellar vesicles[29] capable of interacting with organophosphonate molecules akin to soman (186 Å^3, Figure 5C). The insertion of guest compound into the cavity of **1** destabilizes the packing of the vesicular material to promote its transformation into nanoparticles. The stimuli-responsive nature of our basket-containing vesicles[30] is unique and could be of great value for the preparation of functional supramolecular materials capable of reporting on the presence of important analytes.[31]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Additional details of the experimental and computational protocols. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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