JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

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A Carbohydrate-Conjugated Deep Cavitand Permits Observation of Caviplexes in Human Serum

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Supporting Information

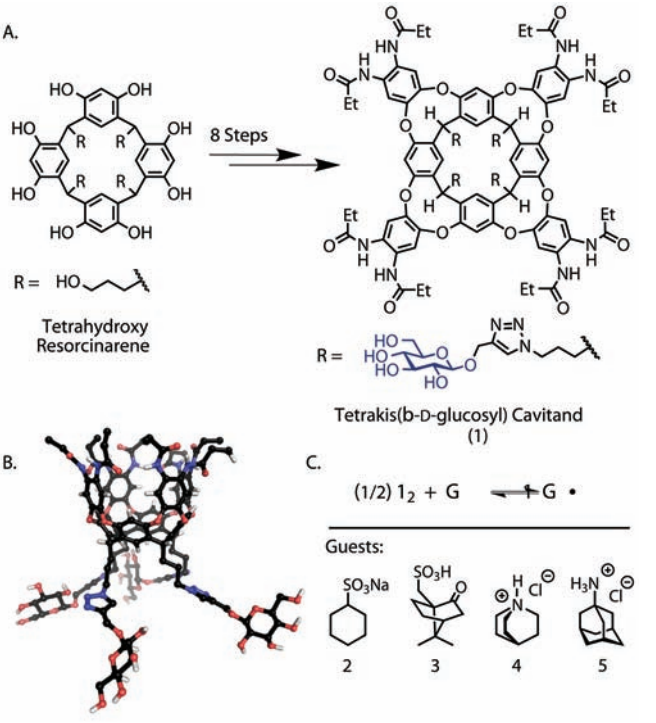
ABSTRACT: A deep cavitand was covalently modified with carbohydrates to provide solubility in biologically relevant environments and to investigate its receptor function. Specifically, a tetrakis(β-D-glucosyl) cavitand (**1**) that was soluble in neutral water or acid/base-buffered solutions was synthesized, and it formed complexes with hydrophobic small molecules. Extraction of the cavitand into aqueous sodium dodecyl sulfate micelles as simple membrane mimetics increased the scope of guests bound by **1** beyond that observed in only aqueous media. Complex formation was also detected in human serum. The findings show the functional compatibility of the receptor in both micelle-bound and serum-soluble forms.

Synthetic deep cavitands are receptors for small molecules that have been used as sensors of biomolecules,[1] but a biocompatible version has yet to be identified.[2] A common strategy for this purpose is conjugation with carbohydrates to impart solubility,[3] and we applied that method here. A cavitand that is soluble in buffered salt solutions was prepared, and its host functions in aqueous media, sodium dodecyl sulfate (SDS) micellar membrane mimetics, and human serum are reported.

Deep cavitands are constructed on a resorcinarene platform with aromatic substituents that act as foldable walls of the binding cavity. Their host−guest complexes generally show low rates of guest exchange on the NMR time scale,[4] and magnetic shielding by the polyaromatic cavity imparts large upfield shifts that provide structural details of the host−guest complex in solution. The first-generation water-soluble cavitands[1a,5] were charged and required pH's some distance from neutrality, but a second-generation tetrakis(tetraethylene glycol)-derivatized cavitand that overcame this limitation and showed pH independent solubility was recently introduced.[6] Carbohydrate-conjugated cavitands offer additional, though still potential, advantages for recognition by lectins[7] or derivatization with carbohydrate-based antigens,[8] receptors,[9] therapeutics,[10] and nucleic acid binding agents.[11] Earlier work with calixarene and resorcinarene glycoconjugates provides precedent for this surmise.[12]

The tetrakis(β-D-glucosyl) cavitand **1** was accessed by a late-stage azide−alkyne "click" reaction between a lower-rim-functionalized tetraazido cavitand and a propargyl glycoside (Scheme 1A).[13] Cavitand **1** is water-soluble (140 μM at 37 °C), but its solutions have a tendency to foam at the air−water interface. The splitting of diagnostic resonances (e.g., *H*-triazole or aromatic protons) in the ^{1}H NMR spectrum of **1** in D_2O (Figure 1A) indicated that **1** has a low-symmetry form, and its complexation thermodynamics showed **1** to be dimeric, probably a kite-conformation velcraplex.[14] This arrangement minimizes solvent-exposed hydrophobic surfaces (see the Supporting Information for a model). Even so, addition of guests such as sodium cyclohexanesulfonate (**2**), (1*R*)-(−)-10-camphorsulfonic acid (**3**), quinuclidine hydrochloride (**4**), or adamantylamine hydrochloride (**5**) induced caviplex formation through the vase (C_4) conformation (Scheme 1B,C). Characteristic upfield chemical shifts for the bound guest were observed by ^{1}H NMR spectroscopy.[15] Guests **2**−**5** are water-soluble and of appropriate size to fill the cavity of **1** with hydrophobic alkane surfaces, and accordingly, no occupation of

Scheme 1. (A) Carbohydrate-Conjugated Cavitand 1 Derived from a Resorcinarene; (B) Model of the Caviplex Vase Conformation (Guest Omitted); (C) Equilibrium Expression for 1 in Water and Structures of Its Guests (G)



Received: June 15, 2011
Published: November 03, 2011

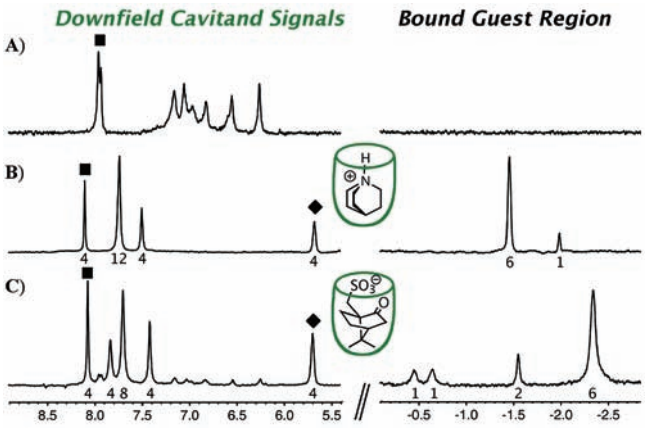


Figure 1. ^{1}H NMR detection of **1** and its complexes at 37 °C, showing downfield and upfield regions: (A) **1** in D_2O; (B) **1**·**4** in 50 mM PB, pD 6.1; (C) **1**·**3** in 50 mM PB, pD 12.6. Labeled resonances of **1**: ■, *H*-triazole; ◆, C*H*-methine of the vase conformation. For binding experiments, PB was saturated with **1** (1.2 mg/mL); $C(\mathbf{4})_{total}$ = 10 mM and [**1**·**4**] = 180 μM; $C(\mathbf{3})_{total}$ = 5 mM and [**1**·**3**] = 170 μM.

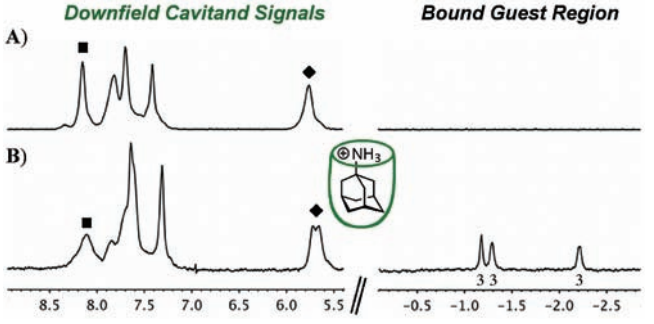


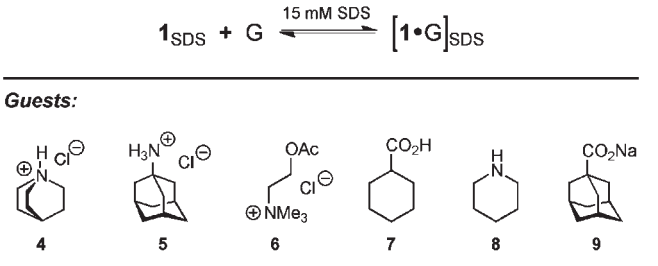
Figure 2. ^{1}H NMR detection of **1** in 15 mM SDS(aq) at 37 °C, showing downfield and upfield regions: (A) **1** in 15 mM SDS(aq); (B) **1**·**5** in 15 mM SDS(aq). Labeled resonances of **1**: ■, *H*-triazole; ◆, C*H*-methine of the vase conformation. For the binding experiment, $C(\mathbf{1})_{total}$ = 1.2 mM, $C(\mathbf{5})_{total}$ = 1.4 mM, [**1**·**5**] = 0.36 mM (30% occupancy).

the cavity by the carbohydrate was observed.[16] The rate of guest exchange was measured by an exchange spectroscopy (EXSY) experiment,[17] which for quinuclidine hydrochloride provided a guest dissociation barrier of 13.6 kcal/mol at 37 °C.

Cavitand **1** (as the dimer $\mathbf{1}_2$) was also soluble in 50 mM phosphate buffer (PB) in deuterium oxide at pD 6.1, and it formed a caviplex with the quinuclidinium ion (**4**; Figure 1B). However, quinuclidine was not bound at pD 12.6 (>99% free base), which indicates that the guest forms a soluble caviplex in its ionic form. In PB at pD 12.6, **1** formed a caviplex with **3** (Figure 1C).

To explore the response of $\mathbf{1}_2$ to membranes, the cavitand was studied in the presence of SDS micelles, which have elsewhere been used as membrane mimetics for the in vitro study of membrane-bound proteins.[18] Previously we saw that cavitands could be extracted into aqueous SDS micelles in which a molecule of SDS binds reversibly in the cavity.[19] For **1**, the use of SDS (15 mM) above its critical micelle concentration (~8 mM)[20] also resulted in its complete extraction, as evidenced by ^{1}H NMR and diffusion-ordered spectroscopy (DOSY) experiments.[21] The diffusion coefficient decreased from $D = 4.1 \times 10^{-10}$ m^2 s^{-1} for $\mathbf{1}_2$ in water to $D = 7.4 \times 10^{-11}$ m^2 s^{-1} for micelle-bound **1**. Although

Scheme 2. Guests for SDS Micelle-Bound 1 ($\mathbf{1}_{SDS}$)



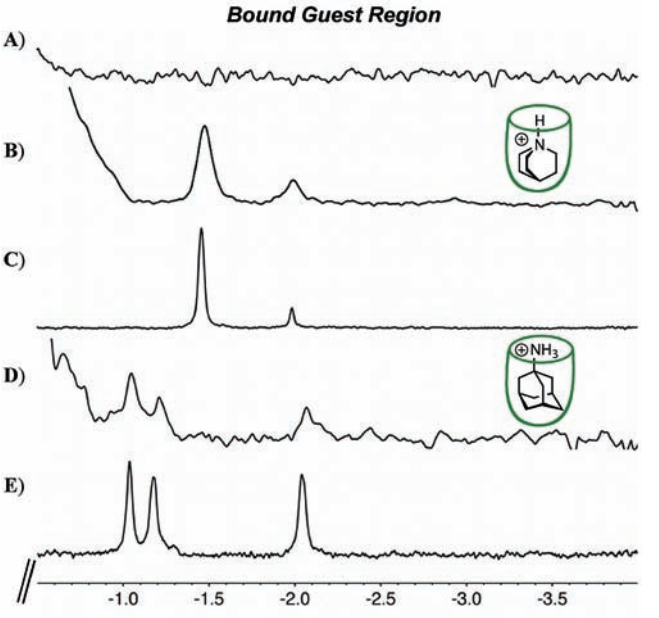


Figure 3. ^{1}H NMR detection of caviplexes in human serum with 40% (v/v) D_2O at 37 °C: (A) serum; (B) **1**·**4** in serum; (C) **1**·**4** in D_2O; (D) **1**·**5** in serum; (E) **1**·**5** in D_2O. Serum was saturated with **1** (1.2 mg/mL), and $C(\text{guest})_{total}$ = 20 mM.

the ^{1}H NMR signals of the micelle-associated cavitand were broadened, the downfield chemical shift of the resorcinarene methine proton (to 5.7 from 4.1 ppm; Figure 2) is an indicator of a vase conformation because of reduced shielding by the aromatic walls. The surfactant was not a good guest; some small organic molecules (**4**−**9**) were bound instead (Scheme 2). In contrast, guests **6**−**9** were not bound in water, even at relatively high concentrations (40 mM). Presumably, the effects of SDS to dissociate the cavitand dimer $\mathbf{1}_2$ and preorganize a vase conformation, as well as to increase the caviplex solubility (>1 mM), resulted in a broader range of guests.[22] Guests also dissociated more slowly from the micelle-associated caviplex, with a barrier of 14.4 kcal/mol for **4** at 37 °C (EXSY).

Cavitand **1** and an excess of guest (20 mM) were dissolved in human serum, a bicarbonate-buffered pH 7.4 medium, and then diluted to 40% (v/v) deuterium oxide and incubated at 37 °C. The distinct NMR signatures in the upfield region (<0 ppm) associated with slow guest exchange allowed direct observation of the caviplexes **1**·**4** and **1**·**5** (Figure 3), albeit at near the detection limit. In short, cavitand **1** binds guests with hydrophobic bulk of appropriate size and shape in this com-

dx.doi.org/10.1021/ja2055676 |J. Am. Chem. Soc. 2011, 133, 19653–19655

plex environment. Moreover, caviplex **1**·**4** persisted intact for more than 24 h, apparently resistant to degradation by serum enzymes.[23]

In summary, a carbohydrate conjugate of a deep cavitand that is soluble in buffered aqueous solutions, SDS micelles, and human serum was synthesized and characterized. The structural and recognition properties of this synthetic receptor were context-dependent. Either in either dilute aqueous buffers or in association with SDS micelles, the binding of hydrophobic small organic molecules was demonstrated with slow guest exchange on the NMR time scale (600 MHz). The potential dissolution of the receptor in either a cell membrane or serum provides a basis for exploring cell delivery applications. Also, the cavitand was not observed to bind the components of normal human serum, and it was resistant to degradation, which are features that suggest future applications in biofluid component analysis.

■ ASSOCIATED CONTENT

Supporting Information. Experimental protocols and characterization data, including synthesis of **1**, solubility determination, complexation thermodynamics, model of velcraplex $\mathbf{1}_2$, and NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ACKNOWLEDGMENT

We are grateful for support from the Defense Threat Reduction Agency—Joint Science and Technology Office, Translational Medical S&T Division. This research was also supported by the NSF (grant CHE 1037590). We thank Dr. Laura Pasternack for helpful discussions regarding NMR experiments.

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(15) At low concentration (0.25 mM) of guests **4** and **5**, additional resonances could be observed in the upfield NMR region. The signals were tentatively assigned to an encapsulation isomer of the guest; the low concentration hindered analysis.

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