Aqueous Solubilization of a Synthetic Charge-Transfer Receptor via Cycloamylose Inclusion

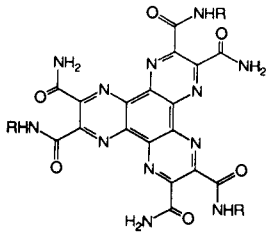
Jude T. Rademacher and Anthony W. Czarnik*

Department of Chemistry
The Ohio State University
Columbus, Ohio 43210

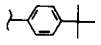
Received November 3, 1992

While all biochemistry takes place in an aqueous milieu, not all biochemicals are water soluble, and various solubilization strategies have evolved in response. For example, nucleic acid bases (e.g., guanine) are water *in*soluble; glycosylation yields soluble conjugates without the loss of desirable base-pairing properties. Similarly, the organic components used to construct many synthetic receptors do not inherently impart water solubility to the receptor. Such solubility problems can be ameliorated by the covalent introduction of highly polar solubilizing functional groups (e.g., carboxylate or ammonium)[1] remote from the binding site. However, nature does not always employ covalent methods to bring about water solubility; important examples include the encapsulation of in vivo generated indole by tryptophan synthetase and the solubilization of fatty acids and acylglycerols by bile salts. We now report on a noncovalent approach to the solubilization of an organic π-acid, suggested by the work of Lawrence,[2] which permits charge-transfer associations of this hydrophobic receptor to be observed in aqueous solution.

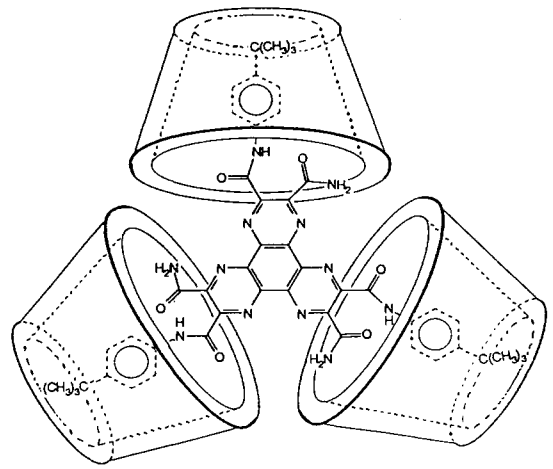
Derivatives of the electron-deficient 1,4,5,8,9,12-hexaazatriphenylene (HAT), including hexacarboxamide **1**,[3] form charge-transfer (CT) complexes with π-bases in organic solvents. However, the exceedingly low water solubility of **1** precludes similar studies in aqueous solution. Rather than constructing a



1, R = H

2, R = 

more water-soluble hexaamide derivative that could have led to aggregation, we synthesized the tris(*tert*-butylphenyl)hexaamide **2**.[4] Predictably, **2** is also highly water insoluble; however, the *p-tert*-butylphenyl group is complexed strongly in water by β-cycloamylose (βCD) and its derivatives.[5] We thus observe that a 20 mM aqueous solution of heptakis(2,6-di-*O*-methyl)-β-cycloamylose (DM-βCD)[6] stirred with excess **2** yields an aqueous solution of **2**; the same experiment using **1** shows no increased solubilization. Filtration and filtrate lyophilization affords a yellow solid that consists of DM-βCD:**2** in a 7.5:1 ratio as determined by NMR integration.[7] Homonuclear nuclear Overhauser effect measurements in D_2O solution demonstrate magnetization transfer between phenyl and several cyclodextrin protons but not with the anomeric protons, which point away from the cavity. While we predict that up to three βCDs might complex to one molecule of **2**, nonstoichiometric sample ratios as we observe are the rule rather than the exception in other CD-solubilized materials.[8] The exact solution stoichiometry cannot be determined readily, but use of the K_d for the βCD·*p-tert*-butylphenol complex[5] permits estimation that 91% of **2** will exist in the tricomplexed form **4**.[9] The noncovalent HAT conjugate remains stable (<5% change) both in aqueous solution (pH 8) and in the solid form for at least 4 months, as established by its unchanged UV and ^{1}H NMR spectra.[10]



4

An aqueous solution (0.2 M borate buffer, pH 8) of the HAT conjugate ([**2**] = 0.2 mM) yields colored CT bands upon addition of various water-soluble π-bases (e.g., indole [red], anthracene-9-carboxylic acid [red], and dianthrol [green]). The solution forms a green CT complex (λ_{max} 530 nm) with 9,10-dimethyl-2,3,6,7-tetrahydroxyanthracene (THA; **3**). As shown in Figure 1, the CT band increases with increasing [THA] with ultimate saturation occurring by 10 mM. Using a nonlinear least-squares

(1) See, for example: (a) Stauffer, D. A.; Barrans, R. E.; Dougherty, D. A. *J. Org. Chem.* **1990**, *55*, 2762; (b) Diederich, F. J.; Dick, K. *J. Am. Chem. Soc.* **1984**, *106*, 8024.

(2) Dick, D. L.; Rao, T. V.; Skumaran, D.; Lawrence, D. S. *J. Am. Chem. Soc.* **1992**, *114*, 2664.

(3) Kanakarajan, K.; Czarnik, A. W. *J. Org. Chem.* **1986**, *51*, 5241.

(4) Hexaamide **2** was prepared by reaction of HAT trianhydride with *p-tert*-butylaniline (see Kanakarajan, K.; Czarnik, A. W. *J. Heterocycl. Chem.* **1988**, *25*, 1869 for related reactions), closure of the amic acid with trifluoroacetic anhydride, and reaction of the resulting triimide with ammonia. NMR confirms the formation of both possible isomers, D_{3h} symmetrical and unsymmetrical; only the symmetrical isomer **2** is depicted. Full synthetic and characterization details are available as supplementary material.

(5) The K_d for aqueous βCD complexation to *p-tert*-butylphenol is 2.75 × 10^{-5} M (Matsui, Y.; Nishoika, T.; Fujita, T. *Top. Curr. Chem.* **1985**, *128*, 61).

(6) We thank the Dr. Allan Hedges of American Maize Products Co., Hammond, IN, for supplying research samples of the cycloamyloses used.

(7) The ratio is determined by comparison of integrations at 4.9 ppm (DM-βCD, 7 H per molecule) and 1.3 ppm (**3**, 27 H per molecule) in DMSO-d_6/D_2O. We have found ratios determined by integration to concur with those determined by C/N microanalytical data.

(8) A wide variety of CD-solubilized pharmaceuticals are described in the brochure by Pharmatec, Inc., Alachua, FL 32615.

(9) To make an estimation of speciation, we must make two simplifying assumption: (i) that the intrinsic binding of one DM-βCD to one arm of **2** is similar to that of *p-tert*-butylphenol to βCD (K_{11} = 3.64 × 10^4 M^{-1}) and (ii) that there is no cooperativity (positive or negative) in the three binding events (for an example of CD speciation with negative cooperativity, see: Connors, K. A.; Paulson, A.; Toledo-Velasquez, D. *J. Org. Chem.* **1988**, *53*, 2023). The first assumption is reasonable, and the second seems reasonable given the rigid nature of all entities and the fact that models show the CDs need not touch one another to complex fully. With these assumptions, it follows that K_{11} = 1.09 × 10^5 M^{-1}, K_{12} = 3.64 × 10^4 M^{-1}, and K_{13} = 1.21 × 10^4 M^{-1}. We used the program GENDIS (D. Leussing) to calculate that, under the initial concentrations used ([**2**]$_0$ = 0.2 mM and [DM-βCD]$_0$ = 7.5 × [**2**]$_0$ = 1.5 mM), **2** with speciate as follows: 3:1 complex, 91.5%; 2:1 complex, 8.3%; 1:1 complex, 0.2%; free **2**, 0.002%. Within these simplifying assumptions, the major species of **2** in solution is predicted to be that depicted by structure **4**.

(10) The amide groups of **2** are bonded to a strongly electron-withdrawing framework, and such amides hydrolyze much more rapidly than, e.g., phenyl amides. Furthermore, one of the most notable properties of cyclodextrins is their ability to effect acyl-transfer reactions on many bound esters and amides. For these reasons, it was important to confirm that the complex is hydrolytically stable.

0002-7863/93/1515-3018$04.00/0

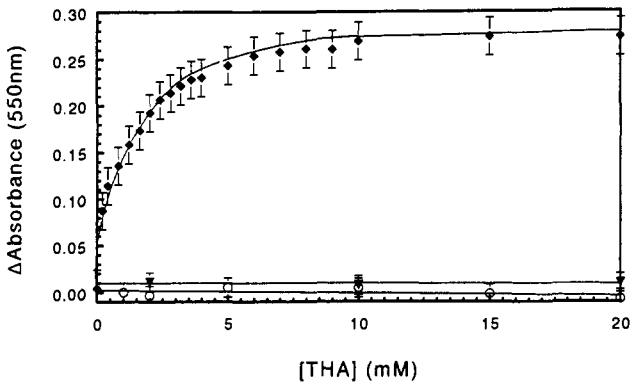
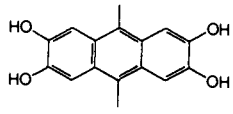


Figure 1. Charge-transfer complexations of HAT/DMβ-CD in H_2O. (◆) HAT/DMβ-CD; (○) DMβ-CD; (▼) HAT.



3

method,[11] we calculate an observed equilibrium binding constant of 1010 ± 70 M^{-1}; the association constant is rather large, but of similar magnitude than that reported previously[12] for TCNE/tetramethyl-*p*-phenylenediamine in ether. This CT band is not formed in solutions of DM-βCD/**3** or in an aqueous suspension of **2** and **3**; addition of DM-βCD to the latter rapidly gives rise to the green CT complex. As expected for a CT band, the new absorption decreases (by 50%) upon warming the solution to 50 °C; because the thermochromic effect is reversible over at least four heating–cooling cycles, a stoichiometric redox reaction between **3** and **4** is excluded.[13]

By outfitting synthetic receptors with hydrophobic rather than hydrophilic substituents, molecule recognition studies may be done in aqueous media via cycloamylose inclusion. In addition to providing water solubility, such conjugation also has the potential to suppress aggregation and to provide an additional tool for obtaining size selectivity.

Acknowledgment. This work was supported by The Petroleum Research Fund of the American Chemical Society. FT-NMR spectra were obtained using equipment funded in part by NIH Grant No. 1 S10 RR01458-01A1. We are grateful to Professor Daniel Leussing (The Ohio State University) for valuable discussions regarding the multiple equilibria of **2** with DM-βCD. A.W.C. thanks the A. P. Sloan and the Camille and Henry Dreyfus Foundations for support in the form of fellowships and Eli Lilly and Company for support in the form of a granteeship.

Supplementary Material Available: Experimental and spectral data for the synthesis of the compounds used in this study; Scheme depicting the synthesis of **2** (3 pages). Ordering information is given on any current masthead page.

(11) Binding constants were determined by using the computer program ENZFITTER, available from Elsevier-BIOSOFT 68 Hills Road, Cambridge CB2 1LA, U.K.

(12) Liptay, W.; Briegleb, G.; Schindler, K. *Z. Electrochem.* **1962**, *66*, 331.

(13) Foster, R. *Organic Charge-Transfer Complexes*; Academic Press: New York, 1969; p 189.