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Single Molecule Solvation and Its Effects on Tautomeric Equilibria in a Self-Assembled Capsule

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Abstract: A self-assembled cylindrical capsule provides a nanoscale environment that affects keto−enol equilibria. The equilibrium constants for encapsulated β-ketoesters show values that differ by an order of magnitude from that of the free tautomers in solution. For complexes with a single, large encapsulated guest, the inner surfaces of the capsule and the seam of the hydrogen bonds influence the equilibrium between the encapsulated keto and enol forms. For complexes of smaller β-ketoesters, the coencapsulated solvent influences the equilibria. The solvent reduces the space available and affects the positioning of the ester in the capsule.

Introduction

Keto−enol tautomerism is one of the most thoroughly studied equilibrium processes in chemistry.[1] For the present study, the earliest relevant observation is more than 70 years old, when Sobotka[2,3] reported that enolization of ketones is favored in complexes with choleic acid. A discussion of choleic acid complexes is provided by the Fiesers,[4] and is all the more relevant as it raises the very idea of encapsulation phenomena in which a "(guest) is not covalently bonded to the enclosing molecules (host)". Since many choleic acid complexes show integral stoichiometries, we examined them by NMR for evidence of capsule formation but found only undefined aggregates in solution. The most recent relevant observation is by Fujita,[5] who encapsulated a β-diketone exclusively in its enol form. In general, the position of the equilibrium (K = [enol]/[keto]) is determined by the chemical and electronic structure of the molecule and its surroundings. More specifically, conjugation with other double bonds stabilizes the enol form,[6] and, for β-ketoesters, solvent plays a role: polar protic solvents that compete strongly for the hydrogen sites bond disrupt the intramolecular hydrogen bond of the enol form and shift the equilibrium toward the keto isomer.[7] We report here a study of how keto−enol equilibria respond to reversible encapsulation, and explore the effects of single solvent molecules on the system.

Methods

Synthetic molecular capsules held together by hydrogen-bonding,[8] metal−ligand coordination,[9] and, more recently, ion pairs[10] have been extensively investigated as nanosized chambers. In these, it is possible to stabilize otherwise reactive enol and siloxane intermediates,[11] to demonstrate kinetic profiles reminiscent of autocatalysis,[12] to accelerate bimolecular reactions with regioselectivity,[13] and to create asymmetric spaces by coencapsulated chiral molecules.[14] This research also takes advantage of the ability of some hosts to coencapsulate two different guests. It allows the direct observation, by conventional ^{1}H NMR spectroscopy, of solutes in contact with a single molecule of typical organic solvents.[15] The solute/solvent interactions within the capsule are amplified in the confined space and the interaction persists for many orders of magnitude longer than the nanoseconds that characterize diffusion complexes.

A reversibly formed cylindrical host such as capsule $\mathbf{1_2}$ in Figure 1 surrounds up to three guest species[16] and the lifetimes of the encapsulation complexes are in the order of milliseconds to hours depending on what is inside.[17] The coencapsulation complexes form at ambient temperatures in the liquid phase through the process of self-

(1) For leading references see: Kresge, A. J. *Acc. Chem. Res.* **1990**, *23*, 43−48.
(2) Sobotka, H.; Kahn, J. *Biochem. J.* **1932**, *26*, 898.
(3) Sobotka, H.; Kahn, J. *Berichte*, **1932**, *65*, 227.
(4) Fieser, L. F.; Fieser, M. *Steroids*; Reinhold: New York, 1959; p 58.
(5) Kumazawa, K.; Biradha, K.; Kusukawa, T.; Okano, T.; Fujita, M. *Angew. Chem., Int. Ed. Engl.* **2003**, *42*, 3909−3913.
(6) Carey, F. A.; Sundberg, R. J. *Advanced Organic Chemistry*, fourth edition; Kluwer Academic: New York, 2000.
(7) Mills, S. G.; Beak, P. *J. Org. Chem.* **1985**, *50*, 1216−1224; Gajewski, J. J. *J. Org. Chem.* **1992**, *57*, 5500−5506.
(8) Conn, M.; Rebek, Jr., J. *Chem. Rev.* **1997**, *97*, 1647−1668; Sherman, J. C. *Tetrahedron* **1995**, *51*, 3395−3422; Boehmer, V.; Vysotzky, M. O. *Austral. J. Chem.* **2001**, *54*, 671−677; Hof, F.; Craig, S. L.; Nuckolls, C.; Rebek, Jr., J. *Angew. Chem., Int. Ed. Engl.* **2002**, *41*, 1488−1508.
(9) Fujita, M.; Umemoto, K.; Yoshizawa, M.; Fujita, N.; Kusukawa, T.; Biradha, K. *Chem. Commun.* **2001**, 509−518; Hirakoa, S.; Fujita, M.; *J. Am. Chem. Soc.* **1999**, *121*, 10239−10240; Kusukawa, T.; Fujita, M.; *J. Am. Chem. Soc.* **2002**, *124*, 13576−13582.
(10) Lee, S. B.; Hong, J. J. *Tetrahedron Lett.* **1996**, *37*, 8501−8504; Corbellini, F.; Fiammengo, R.; Timmerman, P.; Crego-Calama, M.; Verslius, K.; Heck, A. J. R.; Luyten, I.; Reinhoudt, D. N. *J. Am. Chem. Soc.* **2003**, *124*, 6569−6575.
(11) Kusukawa, T.; Yoshizawa, M.; Fujita, M. *Angew. Chem., Int. Ed. Engl.* **2001**, *40*, 1879−1884; Makeiff, D. A.; Vishnumurthy, K.; Sherman, J. C. *J. Am. Chem. Soc.* **2003**, *125*, 9558−9559.
(12) Chen, J.; Körner, S.; Craig, S. L.; Lin, S.; Rudkevich, D. M.; Rebek, Jr., J. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 2593−2596.
(13) Chen, J.; Rebek, Jr., J. *Org. Lett.* **2002**, *4*, 327−329.
(14) Scarso, A.; Shivanyuk, A.; Hayashida, O.; Rebek, Jr., J. *J. Am. Chem. Soc.* **2003**, *125*, 6239−6243.
(15) Scarso, A.; Shivanyuk, A.; Rebek, Jr., J. *J. Am. Chem. Soc.* **2003**, *125*, 13981−13983.
(16) Shivanyuk, A.; Rebek, Jr., J. *Angew. Chem., Int. Ed. Engl.* **2003**, *42*, 684−686; Shivanyuk, A.; Rebek, Jr., J. *Chem. Commun.* **2002**, 2326−2327.
(17) Craig, S. L.; Lin, S.; Chen, J.; Rebek, Jr., J. *J. Am. Chem. Soc.* **2002**, *124*, 8780−8781.

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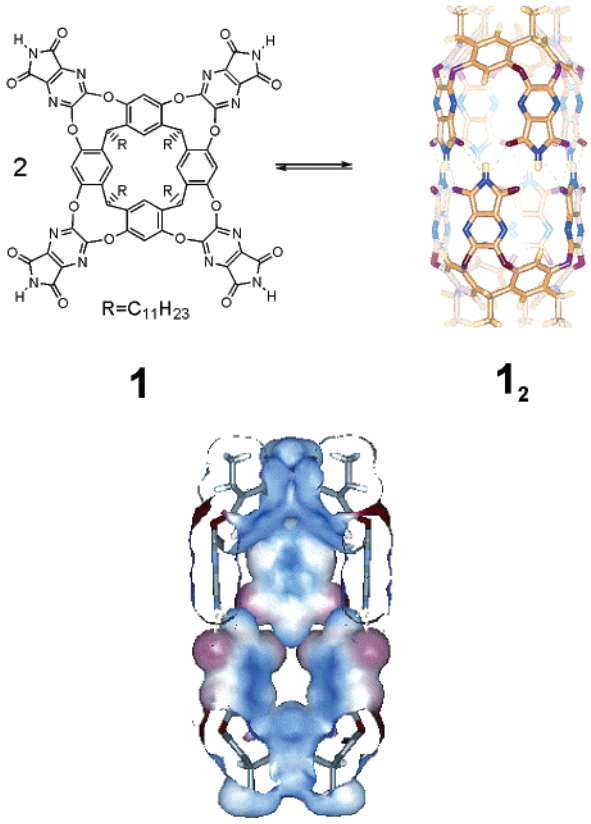


Figure 1. (Top) Line drawing of the synthetic receptor **1** and energy-minimized dimeric structure of the capsule $\mathbf{1_2}$; the $-C_{11}H_{23}$ alkyl chains have been removed for clarity. (Bottom) Front view of the capsule with the electrostatic potential surfaces. Some atoms have been removed to show the inner surface of the cavity.

assembly. The assembly is further instructed by the appropriate filling of space; capsules do not form in the absence of guests, but guests that fill about half of the available interior volume induce the assembly of the encapsulation complex. This is comparable to what is usually observed in organic liquids where the average packing coefficients are in the range 0.50−0.55.[18]

Because of its particular cylindrical shape, $\mathbf{1_2}$ restricts tumbling of elongated guests, and gives rise to a new form of isomerism, social isomerism.[19] The seam of eight bifurcated hydrogen bonds at the middle of the capsule attracts polar functions of the guests and the aromatic subunits at the ends of the capsule preferentially accommodate less-polar groups. For example, anions are encapsulated in the middle with $CHCl_3$ at either end[20] and mandelic acid/alcohol combinations present their hydroxyl groups toward each other in the center.[14] In fact, a gradient of polarity exists in the capsule. These attributes were applied to examine the single molecule solvation effect on keto/enol equilibria. The β-ketoesters used are shown in Scheme 1.

Results and Discussion

(A) Encapsulation of Single Tautomeric Species. The cavity volume of $\mathbf{1_2}$ is ~425 Å^3, and only one molecule of **2k**/**2e** (~170 Å^3) can be accommodated within. This was confirmed at 265 K by NMR integration of the encapsulated guest compared to the capsule's NH resonances. The two halves of the host show different resonances for the imide NH as shown in Figure 2 by the presence of two singlets for each complex. The signals for

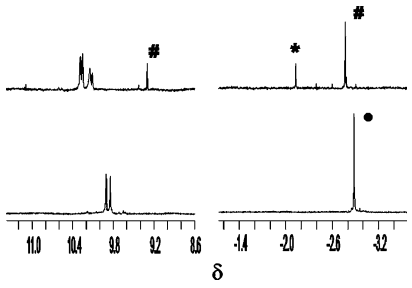
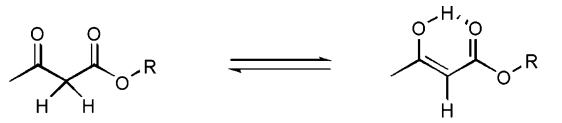


Figure 2. ^{1}H NMR spectra (600 MHz) in mesitylene-d_{12} at 265 K for the encapsulation complexes of **2k**/**2e** (top) and **3k**/**3e** + CH_2Cl_2 (bottom) at 265 K in $\mathbf{1_2}$. [**2k** + **2e**] = 100 mM, [**3k** + **3e**] = 100 mM, CH_2Cl_2 = 100 mM, $\mathbf{1_2}$ = 2 mM. **2k** *; **2e** #; **3k** •.

Scheme 1. Tautomeric Equilibria between β-Ketoesters **2k**, **3k**, and **4k** and the Corresponding Enolesters **2e**, **3e**, and **4e**



R= $-CH_2C_6H_5$ **2k** R= $-CH_2C_6H_5$ **2e**
R= $-C(CH_3)_3$ **3k** R= $-C(CH_3)_3$ **3e**
R= $-CH_2CH_3$ **4k** R= $-CH_2CH_3$ **4e**

Table 1. Guest Volumes, Equilibrium Constants, and Packing Coefficients for Different Combinations of β-Ketoesters and Guests G Coencapsulated in $\mathbf{1_2}$ at 265 K. [**keto** + **enol**] = 100 mM, G = 100 mM, $\mathbf{1_2}$ = 2 mM[a]

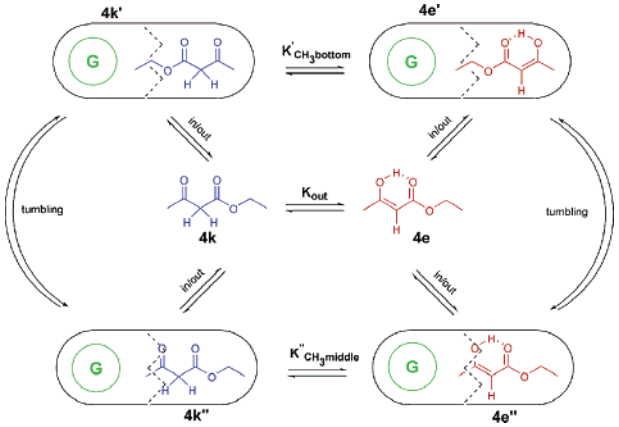
β-ketoester	guest G	guest volume Å^3	K out	K′ CH_3 bottom	K″ CH_3 middle	PC
2k	–	–	0.43	3.57	–	0.40
3k	CH_2Cl_2	60	0.22	–	< 0.05	0.50
4k	CH_3CH_3	42	0.37	0.30	0.66	0.37
4k	CH_3CH_2OH	52	0.33	2.54	0.47	0.40
4k	CH_2Cl_2	60	0.25	1.63	0.50	0.41
4k	$BrCH_2Cl$	67	0.22	0.89	0.25	0.43
4k	$CHCl_3$	75	0.24	0.61	0.10	0.45
4k	$(CH_3)_2CHCl$	76	0.25	0.63	0.09	0.45
4k	$(CH3)_2CHBr$	84	0.39	0.62	<0.05	0.47
4k	CCl_4	91	0.24	0.31	0.12	0.49
4k	CCl_3Br	114	0.31	0.28	0.10	0.54
4k	C_6H_6[b]	77	0.38	<0.05	<0.05	0.45
4k	C_6H_{12}[b]	97	0.36	<0.05	<0.05	0.50

[a] Errors on K, K′, and K″ arise from NMR integration and are estimated at ±10%. [b] For C_6H_6 and C_6H_{12} at 265 K, the spectra of the encapsulated **4k** and **4e** were not sharp due to residual tumbling of the encapsulated guests and a lower temperature (250 K) was therefore employed.

the encapsulated isomeric guests **2k** and **2e** are shifted characteristically upfield: the methyl groups appear at −2.13 and −2.77 ppm ($\Delta\delta$ = −3.71 and −4.27 ppm, respectively) and the enol OH is at 9.3 ppm ($\Delta\delta$ = −3.20 ppm). Integration of the methyl groups gave the relative amounts of keto and enol forms; the equilibrium constant K_{out} = [**2e**]/[**2k**] outside the capsule in the bulk solvent was 0.4 but inside the capsule the value was $K'_{CH_3\ bottom}$ = 3.6. In the complex for **2k** the β-carbon is placed deep in the cavity and is surrounded by aromatic surfaces and this apolar nanoenvironment favors the equilibrium toward the enol form compared to the free guest in solution. Nevertheless, the 9-fold increase in the equilibrium constant due to encapsulation appears too large to arise only from solvation effects: there are no known solvents that show such a large K value for β-ketoesters.[7] It is also known that for these tautomers the enol isomer is favored enthalpically, while the keto is favored entropically, both in the gas and liquid phases.[21]

(18) Mecozzi, S.; Rebek, Jr., J. *Chem.—Eur. J.* **1998**, *4*, 1016−1022.
(19) Shivanyuk, A.; Rebek, Jr., J. *J. Am. Chem. Soc.* **2002**, *124*, 12074−12075.
(20) Hayashida, O.; Shivanyuk, A.; Rebek, Jr., J. *Angew. Chem., Intl. Ed. Engl*, **2002**, *41*, 3423−3426.

Scheme 2. Equilibrium Processes Involved in the System Composed by **4k**/**4e** and a Second Coencapsulated Guest G[a]



[a] At 265 K, the tumbling of the guests becomes restricted on the ^{1}H NMR time scale (600 MHz).

The low packing coefficient for **2k**/**2e** ($PC = 0.40$) leaves room for atmospheric gases and may explain why there is such a preference for the enol.

(B) Coencapsulation. With a smaller ketone such as **3k** no clear encapsulation is observed: a single molecule of **3k** is too small to fill the appropriate amount of space but two ketones fill too much space. Accordingly, the coencapsulation occurs when solvents such as CH_2Cl_2 are present in solution, and a reasonable packing coefficient is achieved (Table 1).

Figure 2 shows the ^{1}H NMR spectrum for the complexes of **3k**/**3e** + CH_2Cl_2 in $\mathbf{1}_2$: only one species is present as only two singlets appear for the capsule NH resonances. This is typically observed for an unsymmetrically filled host.

The apolar *tert*-butyl group is anchored at the end of the capsule and experiences maximum shielding (t-Bu = −2.88 ppm, $\Delta\delta = -4.17$ ppm). As a consequence, the β-carbon of **3k**/**3e** is placed near the polar middle of the cavity that features the imides. These functions can compete with the intramolecular hydrogen bond in the enol form, and may shift the equilibrium toward the keto form ($K''_{CH_3\ middle} < 0.05$). The K value in solution is 0.22, similar to **2k**/**2e**. Comparing **2** and **3**, we sense how the inner gradient of polarity—the functional groups of the capsule's cavity—interacts with the guests to increase or decrease the equilibrium constant for the species that in solution have comparable concentrations.

An even greater effect on the equilibrium appears in complexes of an even shorter β-ketoester **4k** with different guests G. The equilibria involved are depicted in Scheme 2.

At 300 K broad signals for the encapsulated **4k**/**4e** are observed. The tumbling rate of the tautomers that causes the interconversion of the social isomeric complexes **4k′**/**4k″** and **4e′**/**4e″** is intermediate on the ^{1}H NMR time scale (600 MHz). At 265 K this process becomes slow and separate signals for the two isomeric complexes of each tautomeric species are observed (Figure 3). At this temperature a 2D-EXSY experiment (mixing time 0.3 s) for **4k**/**4e** coencapsulated with CH_2Cl_2 showed in/out exchange cross-peaks for each tautomer (**4k**/**4k′**, **4k**/**4k″**, **4e**/**4e′**, **4e**/**4e″**) as well as exchange between the two social isomers of each tautomer (**4k′**/**4k″**, **4e′**/**4e″**). No exchange cross-peaks were detected between keto and enol forms in

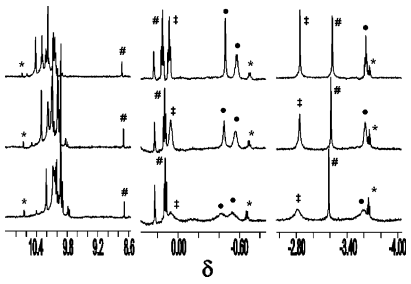


Figure 3. ^{1}H NMR spectra (600 MHz) in mesitylene-d_{12} for the encapsulation complexes of **4k**/**4e** + $CHCl_3$ at 265 K (top), 285 K (middle), and 300 K (bottom) in $\mathbf{1}_2$. [**4k** + **4e**] = 100 mM, $CHCl_3$ = 100 mM, $\mathbf{1}_2$ = 2 mM. **4k′** •; **4k″** ‡; **4e′** #; **4e″** *.

solution or within each pair of social isomers (**4k**/ **4e**, **4k′**/**4e′**, **4k″**/**4e″**). Therefore the chemical equilibrium takes place in solution and in the capsule at a much slower rate than the guest uptake and release.

The various equilibrium constants K, $K'_{CH_3bottom}$ and $K''_{CH_3middle}$ for **4k**/**4e** coencapsulated with a series of guests are reported in Table 1, along with values for both social isomeric orientations. The K' values are always larger than K'' due to the different polarity of the inner surfaces of the cavity that faces the β-carbon. As was the case for **2k** and **3k**, the equilibrium constant increases when the β-carbon of **4** is deep in the aromatic cavity. When this carbon is near the polar middle of the cavity the value of K'' decreases.

The change of G has a negligible effect on the K_{out} values in solution, at least in the concentration range employed for these studies. Dilution experiments from 200 to 0.5 mM also showed approximately constant K values for **2**, **3**, and **4** outside the cavity. But for the encapsulated species **4k**/**4e**, two distinct trends are observed for the two social isomers. Increasing the size of G affects the K' and K'' values by influencing the average position of the β-carbon. When this atom is pushed toward the ends of the capsule, the shielding effect due to the aromatic surfaces results in a larger $\Delta\delta$. The values of $\Delta\delta$ of the enol OH residue follow that trend. For both social isomers a larger G is associated with an increase in the $\Delta\delta$ as reported in Figure 4.

The behavior of the complexes of **4** with ethane is peculiar. Gases inside $\mathbf{1}_2$ have been shown[22] to require more space; the packing coefficients are about 70% of those for liquid guests.[18] If this is also true in the case at hand, a correction by this value gives the "effective" volume of **4k**/**4e** with ethane. The K' and K'' values in Figure 5 fit the trend of guest effect on the equilibrium constants and the same is also true for the trend of $\Delta\delta$ in Figure 4. Even so, it is not known whether bulk properties such as liquid or gas can be assigned to single molecules inside capsules.

Voluminous guests such as C_6H_6 and C_6H_{12} strongly favor **4k**. In these cases, even if their molecular volumes are comparable to other guests G employed, the lower K values observed are likely to be dependent on the less spherical shape of these guests.

The $\Delta\delta$ reflects the shielding effect due to positioning over aromatic surfaces and is therefore a rough indication of the inner polarity of the cavity. (Figure 6) The trends allow a qualitative correlation of this effect with K' and K'' values. The size increase

(21) Folkendt, M. M.; Weiss-Lopez, B. E.; Chauvel, J. P.; True, N. S. *J. Phys. Chem.* **1985**, *89*, 3347−3352.

(22) Shivanyuk, A.; Scarso, A.; Rebek, Jr., J. *Chem. Commun.* **2003**, 1230−1231.

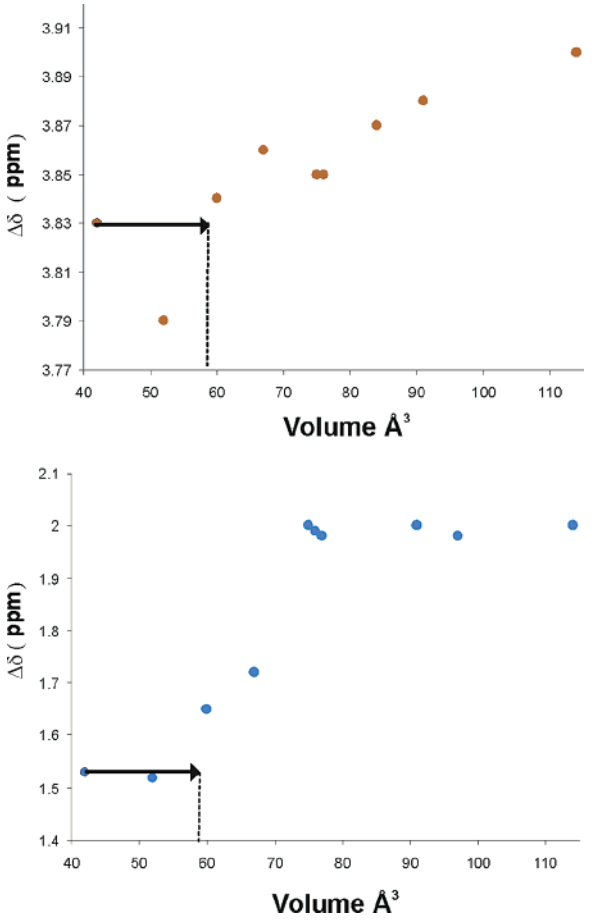


Figure 4. Plots of the shielding effect $\Delta\delta$ (ppm) of the enol OH hydrogen with the solvent G volume (Å^3) for the two social isomers **4e′** (top) and **4e″** (bottom). The arrows and the dotted line represent the correction applied to the volume of ethane.

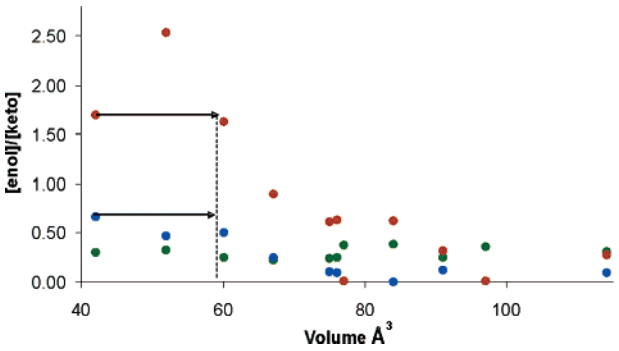


Figure 5. Plot of K in solution (green) and for the two social isomers (K′, orange; K″, blue) of **4k**/**4e** at 265 K determined by ^{1}H NMR (600 MHz) in $\mathbf{1}_2$ in mesitylene-d_{12} with the overall packing coefficient.[**4k** + **4e**] = 100 mM; [G] = 100 mM; [$\mathbf{1}_2$] = 2 mM. The arrows and the dotted line represent the correction applied to the volume of ethane.

of G due to repulsive interactions with **4** favors the keto isomer for both social isomers. Although **4k** is negligibly larger than the enol **4e** (118.5 Å^3 vs 116.0 Å^3),[23] the ketone is more flexible than the compact, intramolecularly hydrogen bonded enol. Indeed, the enol isomer is favored enthalpically, while the keto is favored entropically, both in the gas and liquid phases.[21] An increase in the size of the solvent molecule G reduces the residual space of the cavity and favors the tautomeric species

(23) Nicholls, A.; Sharp, K.; Honig, B. *Proteins* **1991**, *11*, 281.

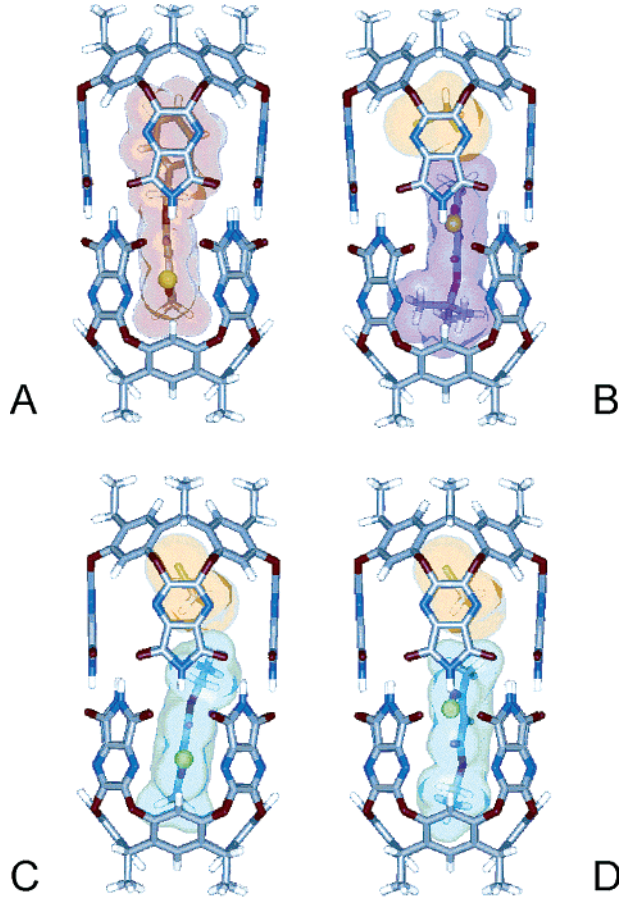


Figure 6. Minimized structures (MM$^+$)[24] for the encapsulation complexes of the enols **2e** (A), **3e** + CH_2Cl_2 (B), and both social isomers for **4e** + CH_2Cl_2 (C) and (D). The enol OH protons are marked in green in order to visualize their position within the capsule $\mathbf{1}_2$.

that can adopt conformations that minimize interguest steric clashes. Accordingly, the *K′* and *K″* values are very small for both social isomers when G is large. The capsule acts as an environment with a gradient of polarity, while the solvent coguest modulates the available space.

Conclusions

In conclusion, here we reported an example of supramolecular assembly that allows the manipulation of the equilibrium between tautomers. For keto−enol equilibria the *K* values are in a range of 1 order of magnitude, not large in terms of Gibbs free energy and arising solely from solvation effects. Subnanomolar positioning of the keto−enol partners due to host−guest and guest−guest interactions, in combination with the polarity of the environment that surrounds the molecules, are the sources of the effects. In the future it may be possible to regulate the present effects of coencapsulation with chiral environments[14] and manipulate the selectivity of reactions.[13]

Experimental Section

1. General. Deuterated solvents were used as purchased from Cambridge Isotope Laboratories. The β-ketoesters were obtained from Sigma-Aldrich or Acros Chemicals and used without further purification. Capsule $\mathbf{1}_2$ was prepared and purified according to procedures previously described.[24]

(24) Hayashida, O.; Sebo, L.; Rebek, J., Jr. *J. Org. Chem.* **2002**, *67*, 8291−8298.

2. NMR Spectroscopy Studies. 1D and 2D NMR data were recorded on a Bruker 600-DRX (600 MHz) spectrometer. Chemical shifts (δ) are expressed as parts per million (ppm) relative to the peak for $SiMe_4$ (TMS; $\delta = 0$ ppm), and referenced internally with respect to that for the protio solvent impurity. 2D-NOESY (EXSY) experiments were acquired with a spectrum width of 18 ppm, a relaxation delay d_1 of 2.5 s, mixing time d_8 of 0.3 s, using 1 K data points in the t_2 dimension and 400 data points in the t_1 dimension, with subsequent weighting with the sine-bell function using 64 scans for each t_1 increment.

The temperature of the probe was calibrated prior of each experiment using a solution of 4% methanol in methanol-d_4 and applying the empirical equation that correlates the $\Delta\delta$ difference between the CH_3 and the OH resonance to the real temperature.[25] The spectra were processed and analyzed with the program XWIN NMR.[26]

3. Preparation of the Samples. Solutions of the complexes were prepared by mixing mesitylene-d_{12} solutions of capsule $\mathbf{1_2}$ with mesitylene-d_{12} solutions of the β-ketoesters and coguests, G. Typically, 400 μL of guests solution at 125 mM was added to 100 μL of a solution of $\mathbf{1_2}$ at 10 mM concentration to give 500 μL of a solution of the capsule $\mathbf{1_2}$ (2 mM) and the guest (100 mM). NMR data were recorded directly after allowing the samples to equilibrate at the desired temperature for 1 h until the thermodynamic equilibrium was reached. The equilibrium constants were determined by direct integration of the corresponding peaks of the encapsulated and free tautomers.

4. Molecular Modeling. Structures of the cylindrical capsule $\mathbf{1_2}$ and the hydrocarbons were built and minimized (AMBER* force field with a dielectric constant of 2.3 for benzene) using the program Macromodel or Maestro (Schroedinger, Inc.) on a Silicon Graphics Octane workstation. The volumes of the molecules and the inner space of the capsule were determined using the program GRASP[23] (probe radius of 1.5 Å).

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(25) Braun, S.; Kalinowski, H. O.; Berger, S. *150 and More Basic NMR Experiments*; Wiley-WCH: 1998.
(26) *Bruker BioSpin*, GmbH.