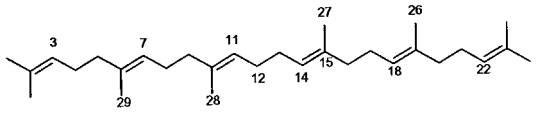
accompanied by regiospecific hydrolysis of the C26 ester group. This found parallels in the facile hydrolysis of **1** to **2** in basic methanol (route e) and in the clean reduction of **2** to **7** in $NaBH_4$/MeOH (route d).

No evidence could be obtained for microbial symbionts of *E. vannus* that could explain the formation of **1** or **2**, which are not produced by the organism food, *Dunaliella salina*. Therefore, both **1** and **2** must be products of the ciliate.

Disconnections at C3–C29, C7–C11, and C21–C25 of both vannusal A (**1**) and vannusal B (**2**) reveal the C1–C9 and C20–C25 portions of the squalene (**8**) backbone, which, however, cannot be obtained in its entirety by further bond disconnections. A route to **1** and **2** may be envisaged to occur



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from a hypothetical squalene-type precursor[3] which involves unprecedented C15–C28 and C12–14 bond formations followed by C13 extrusion. More appealing, particularly in the light that abzymes may contravene chemical rules,[4] is an unorthodox abstraction of the disfavored primary allylic proton in the squalene pathway[5] to close the central ring of **1** or **2** through bond C28–C14. Whether these are real possibilities is left to challenging biosynthetic experiments.

In summary, the molecular diversity described here suggests that prolific ciliates such as strains of *E. vannus* are border-zone species[6, 7] that deserve particular care in the planning of biodiversity.[6]

Experimental Section

Stocks Sil21 and BUN3 of *E. vannus* (Müller, 1786) were collected in August 1996 along the coasts of the Siladen and Bunaken Islands, respectively, northwest of Menado, Indonesia, in the Celebes Sea. Their membership to *E. vannus*, comprising the cluster of the marine single dargyrome cirrotype 10 forms, *E. vannus-crassus-minuta*, was defined according to the classical (morphological) taxonomic practice.[8] To obtain mass cultures, stocks Sil21 and BUN3 were grown separately in large, shallow 30 liter tanks and fed the green microalga *Dunaliella salina*. Cultures were maintained at two fissions per day by feeding excess food and were kept at $23 \pm 1\,^{\circ}C$ while exposed to alternate light and darkness with a rhythm of 12 h. Centrifuged, closely-packed cells of the Sil21 strain (14 mL; about 2×10^8 individuals) were suspended in ethanol which was then evaporated. The residue was subjected to high-performance liquid chromatography (HPLC) on a Merck Lichrosphere RP18 25×1 cm column, eluant CH_3CN/H_2O (7/3, 5 mL min^{-1}) to yield vannusal A (**1**) and vannusal B (**2**). **1**: $t_R = 8.7$ min, 9.3 mg; m.p. (water): 95 °C; $[\alpha]_D^{20} = -17$, $[\alpha]_{365}^{20} = -287$ ($c = 0.3$, MeOH); CD (MeOH): λ[nm] ($\Delta\varepsilon$ [deg L mol^{-1} cm]) = 229 (+6.2), 304 (−5.4); UV (MeOH): λ_{max} [nm] (ε [M^{-1} cm^{-1}]) = 229 sh (6600), 304 (1300); **2**: $t_R = 6.2$ min, 1.4 mg; amorphous powder; $[\alpha]_D^{20} \approx 0$, $[\alpha]_{365}^{20} = -85$ ($c = 0.05$, MeOH); CD (MeOH): λ[nm] ($\Delta\varepsilon$ [deg L mol^{-1} cm]) = 228 (+5.4), 308 (−3.1); UV/Vis (MeOH): λ_{max} [nm] (ε [M^{-1} cm^{-1}]) = 229 (5100), 301 (1100). NMR spectra were recorded with Bruker AMX-600 and Varian XL-300 spectrometers equipped for inverse detection; chemical shifts are reported relative to residual solvent signal (for CD_3OD $\delta(^1H) = 3.310$ and $\delta(^{13}C) = 49.00$; for C_6D_6 $\delta(^1H) = 7.150$ and $\delta(^{13}C) = 128.50$) and J in Hz at probe temperature 20 °C. A conformational space search was carried out with the program GMMX (steric energy minimization program based on the MMX force field by Serena Software, Bloomington, IN) and minima were refined with the MM3(96) program (by N.L. Allinger et al., distributed by QCPE, Indiana University, 17/1, February 1997) by the block diagonal Newton-Raphson energy minimization method with default parameters.

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Molecular Recognition within a Self-Assembled Cylindrical Host**

Thomas Heinz, Dmitry M. Rudkevich, and Julius Rebek, Jr.*

We recently reported the synthesis and characterization of the capsular assembly **1** (Figure 1).[1] It is a cylinder-shaped dimer held together by eight bifurcated hydrogen bonds in apolar organic solvents. The dimer has the capacity to select one large molecule or two small molecules from the bulk solution and encapsulate them reversibly. Further expressions of the capsule's unique behavior have emerged from the use of rigid, flexible and complex guests, and we relate them here.

Rigid structures such as stilbenes **2**–**5** offer a set of "rulers" for measuring the interior dimensions of the capsule (Figure 2). The (*E*)-4,4′-dimethylstilbene **2** is indeed readily-

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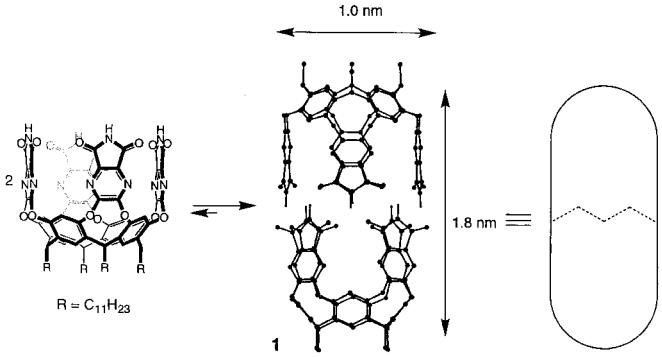

Figure 1. Self-assembly and structure of capsule **1**. In the center is the energy-minimized[4] (MacroModel 5.5, Amber* force field) structure; the long alkyl chains and CH hydrogen atoms are omitted for clarity. Right: The cartoon representation[1] used in Figures 2–6.

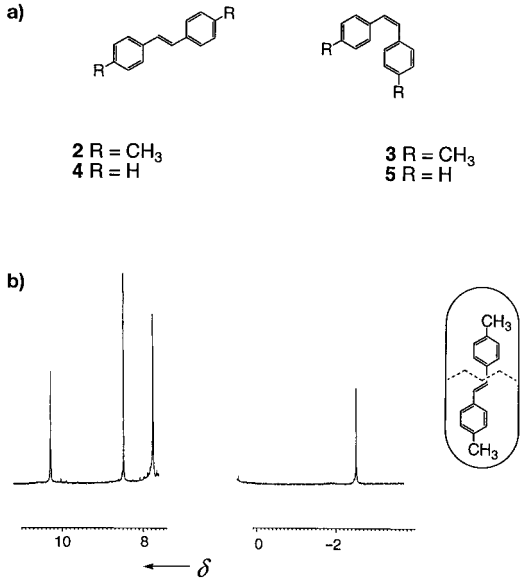

Figure 2. a) Stilbenes **2**–**5**. b) Downfield and upfield regions of the ^{1}H NMR spectrum[2] upon encapsulation of (*E*)-stilbene **2**.

encapsulated by **1** in [D_{12}]mesitylene, and relevant regions of the NMR spectrum are shown in Figure 2b.[2] The large upfield shifts of the guest methyl groups ($\delta \approx -2.8$, $\Delta\delta \approx 5$) place them near the ends of the capsule. Initial experiments with an >50-fold excess *Z* isomer **3** gave the same spectra as were seen with **2**, but this was traced to contamination of the sample with small amounts (some 1 to 2%) of **2**. Direct competition experiments using **2** and **3** gave no evidence for the encapsulation of **3**; only the spectra for **2** emerged. When a smaller excess (ca 16-fold) of **3** was employed, no encapsulation was observed. Instead, the characteristic (and complex) pattern of signals observed for mesitylene occupancy persisted.[1] The shorter unsubstituted (*E*)-stilbene **4** was also readily encapsulated, but again the *Z* isomer **5** was not. Accordingly, the selectivity **2**/**3** is at least 50:1 and is beyond the ^{1}H NMR detection limits.

Typical secondary amides such as anilide **6** and benzylamide **7** (Figures 3 and 4) are nearly as rigid as the stilbenes in that

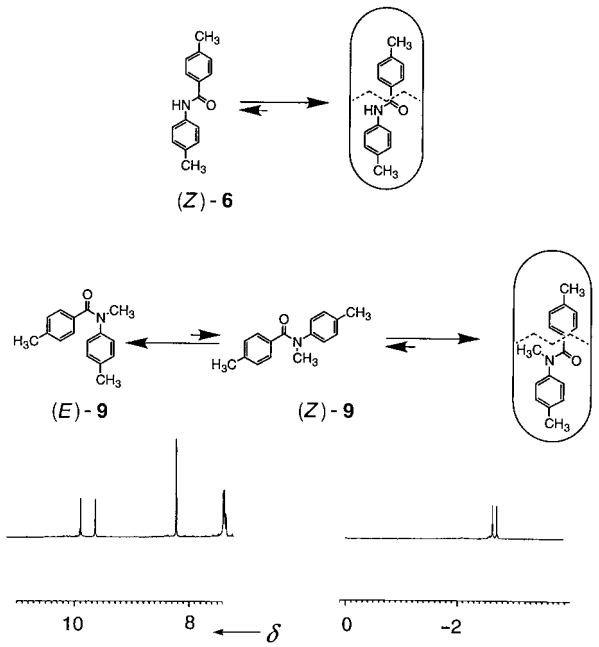

Figure 3. Anilides **6** and **9**, and portions of the ^{1}H NMR spectra[2] of encapsulated (*Z*)-**9**. Downfield region: imide-NH and arene-CH signals of **1**; upfield region: methyl signals of the guest molecule.

they exist primarily in the *Z* conformation shown. Either amide is readily encapsulated by **1** in [D_{12}]mesitylene and the NMR spectra (not shown) indicate that the two ends of the complex are now different: these guests can spin rapidly about the long axis of the capsule, but can not "tumble" within, at least not on the NMR timescale. In contrast, tertiary amides are flexible structures insofar as they exist as interconverting *Z* and *E* conformations. For example, amide **8** exists as a roughly equal mixture of *E* and *Z* isomers in $CDCl_3$ or [D_{12}]mesitylene. Upon encapsulation, a set of upfield shifted signals emerge and these appear at nearly the same chemical shifts seen for the *Z* amide **7** inside (Figure 4).

The effect of encapsulating the tertiary anilide **9** is profound. Shudo and co-workers[3] have shown that such tertiary anilides greatly favor the *E* conformation in CD_2Cl_2 and $CDCl_3$. Yet inside the capsule the signals are shifted in the just the manner observed for the *Z* secondary anilide **6** (Figure 3). Unquestionably, tertiary amides **8** and **9** are fixed in the *Z* conformation within the complex. Molecular modeling[4] indicated that the corresponding *E* conformers cannot fit inside the capsule without severe steric problems with the walls. Whatever forces favor the *E* conformation in solution are overcome by these steric clashes and the CH-π, van der Waals and dipolar interactions offered by the interior surface of the capsule to the *Z* conformation.[5] Since the ^{1}H NMR spectrum (Figure 4) did not change even when only 1.5 equivalents of **8** was present, encapsulation of this flexible molecule imposes a conformation unfavored in the bulk solution.

Hydrogen bonding stabilizes the capsule and causes dimerization of small self-complementary molecules as well. The dimers of 2-pyridone/2-hydroxypyridine and carboxylic acid are particularly well-studied cases.[6] These molecules, specifically pyridone **10** and benzoic acid **11** were encapsulated as dimers within **1** (Figure 5a); the ^{1}H NMR spectra showed the

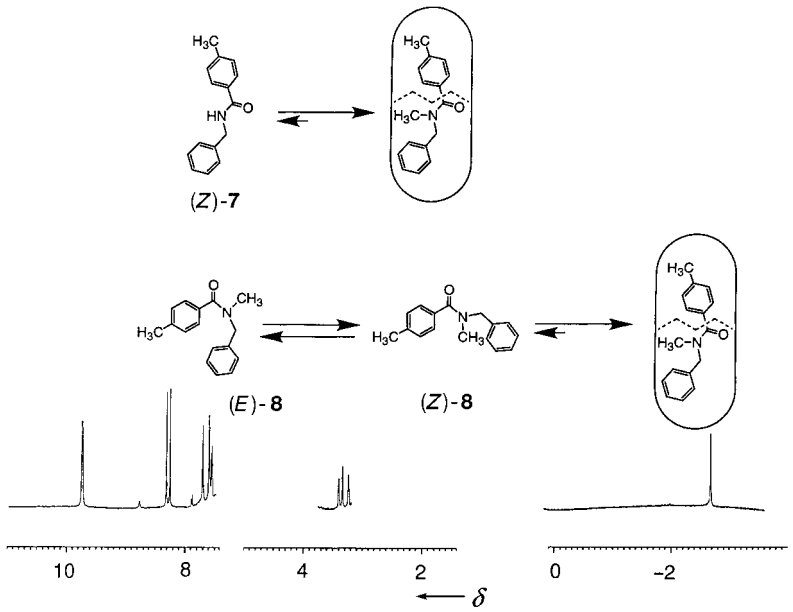

Figure 4. Amides **7** and **8**, and portions of the ^{1}H NMR spectra[2] of encapsulated (*Z*)-**8**. Downfield region: imide-NH and arene-CH signals of **1**; upfield region: methyl signals of the guest molecule. The arene-CH signals of the encapsulated guest ($\delta = 4-2$) are also shown.

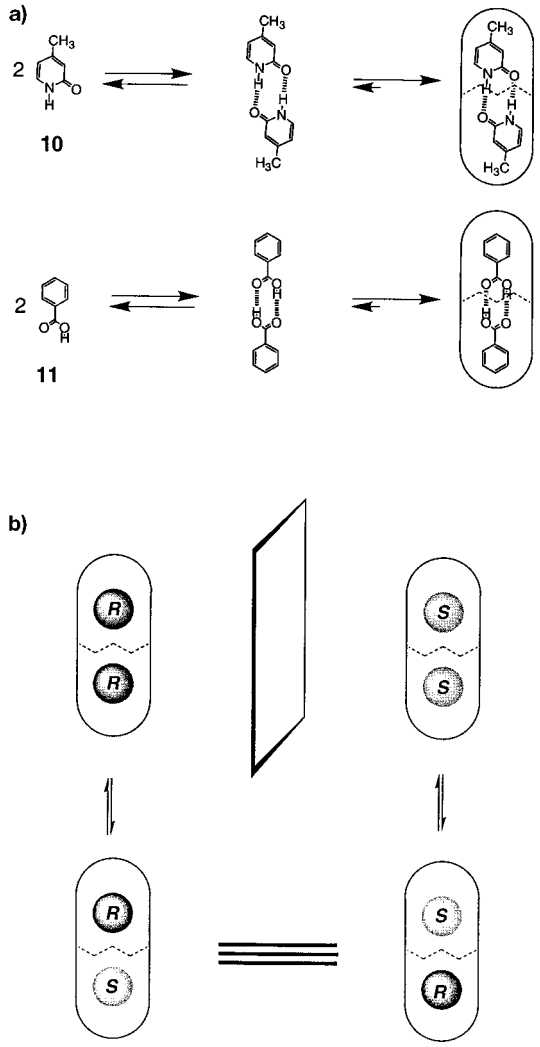

Figure 5. a) Hydrogen-bonded dimers of 4-methylpyridine-2(1*H*)-one (**10**) and benzoic acid (**11**) inside **1**. b) Cartoon representation of diastereomers formed upon encapsulation of racemic molecules.

characteristic features for the encapsulation and the integration confirmed the stoichiometry. Under the same conditions, the *p*-toluic acid dimer was not encapsulated; its length exceeds the limits imposed by the hydrogen bonds that hold the assembly together.

Encapsulation of chiral guests gives rise to diastereomeric complexes. Many cases of selective intermolecular interactions in racemic systems exist;[7] and these attractions appear to be intensified within the confines of the capsule (Figure 5b). Two different species are observed in the presence of the racemic *trans*-1,2-cyclohexanediol **12**,[8] while only one appears if only the single enantiomer **13** is available (Figure 6). In either case, integration indicates there are two guests inside each capsule, but the intensity of the signals for the enantiopure versus the racemic guests indicates more of the latter—the capsule prefers to be filled with a guest and its mirror image rather than two identical

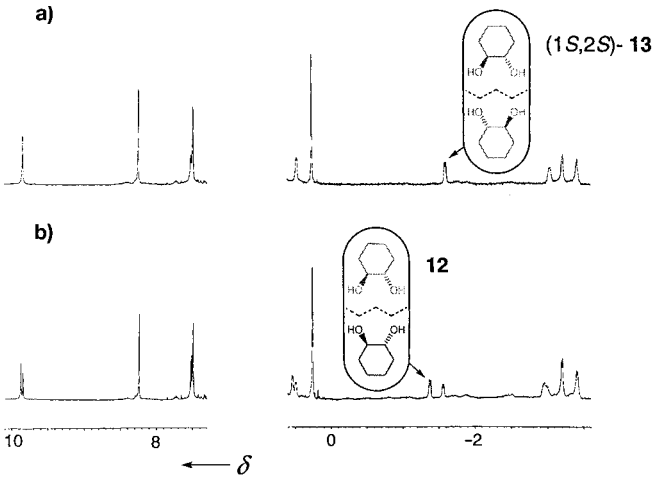

Figure 6. Portions of the ^{1}H NMR spectra[2] a) encapsulated (1*S*,2*S*)-*trans*-(1,2)-cyclohexanediol (**13**) and b) encapsulated racemic *trans*-1,2-cyclohexanediol (**12**). Downfield region: imide-NH and arene-CH signals of **1**; upfield region: cyclohexane-CH signals of the guest compounds **12**/**13** .

molecules. The systems shown in Figure 5 and Figure 6 represent complexes within complexes, or second-order supramolecules.

Finally, the encapsulation process shows a range of rates. The uptake, release, and exchange of small guests is rapid: benzene, toluene, and xylene equilibrate with the capsule in [D_{12}]mesitylene in less than one minute at ambient temperatures and NMR (millimolar) concentrations. The appropriate complexes containing two guests in each host are obtained. Likewise, a large guest (e.g. anilide **6** or stilbene **2**) replaces the poorly accommodated solvent mesitylene, on the same timescale. In contrast, the exchange rates between large guests are slow: displacement of encapsulated **2** by **6** (or vice versa) takes days to reach equilibrium. The sequence of events—hydrogen bond rupture, conformational changes, and

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guest substitution—must occur for either large or small guests. It seems likely that flaps[9] can open in the capsule to permit the exchange of small guests. The exchange of large guests may require the complete dissociation of the superstructure.

In summary, the encapsulation behavior of self-assembled capsule **1** derives from its considerable size and elongated shape. These features guarantee a selectivity for congruent molecules as guests. Even hydrogen-bonded systems—assemblies within assemblies—are temporarily frozen in space and time. The formation and dissipation of the systems ranges from seconds to days, and encapsulated species enjoy an environment insulated from the intrusions of the bulk solution where weakly bound complexes are forced to change of partners frequently. It should be possible to observe reactive intermediates whose lifetimes are on the appropriate timescales within these chambers.

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Is the Bis(*μ*-oxo)dicopper Core Capable of Hydroxylating an Arene?**

Patrick L. Holland, Kenton R. Rodgers, and William B. Tolman*

A critical mechanistic issue in C–H bond activations by metal – dioxygen species in catalytic and biological systems concerns the sequence of the O–O and C–H bond-breaking events.[1] In the context of tyrosinase, a metalloenzyme that performs aromatic hydroxylations[2] with O_2 via a spectroscopically characterized (μ-η^2:η^2-peroxo)dicopper(II) intermediate,[3] a key question is whether this intermediate attacks the arene substrate directly (**A**), or whether the O–O bond first breaks to yield a bis(μ-oxo)dicopper unit that then performs the hydroxylation (**B**, Scheme 1). Studies of synthetic systems that model the protein active site often have used dinucleating ligands with *meta*-xylyl spacers that undergo hydroxylation upon oxygenation of their dicopper(I)

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