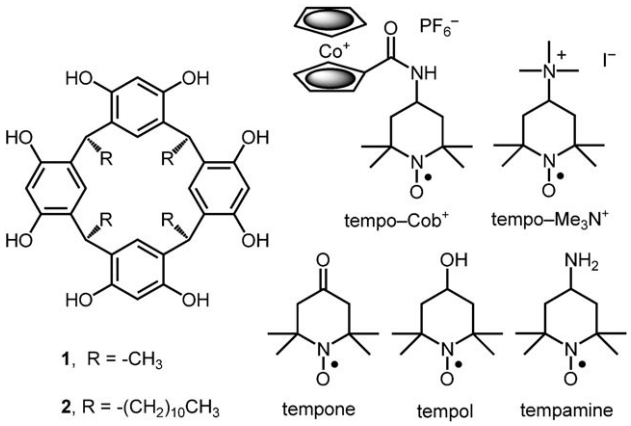
Supramolecular Chemistry

DOI: 10.1002/anie.200901272

Probing the Inner Space of Resorcinarene Molecular Capsules with Nitroxide Guests**

*Elisabetta Mileo, Song Yi, Papri Bhattacharya, and Angel E. Kaifer**

The application of electron paramagnetic resonance (EPR) spectroscopy to the investigation of supramolecular structures has received considerable attention.[1] This body of work, which is usually based on nitroxide spin labeling, is largely driven by the sensitivity of the nitroxide label to its surroundings,[2] as the magnitude of the ^{14}N hyperfine splitting constants (a_N) and g factors depend on the polarity of the environment, while the lineshapes of the EPR spectra reflect the probe's motional dynamics. Recently, Chechik and co-workers used spin-labeling techniques to study inclusion complexation by cyclodextrins,[3] while the inclusion of nitroxide spin probes by the host cucurbit[7]uril has been the subject of a recent report by Lucarini et al.[4] Bardelang et al. investigated the binding behavior of a persistent free radical covalently attached to β-cyclodextrin.[5] The Turro and Ramamurthy research groups have shown that spin probes yield very interesting data on the properties of dimeric molecular capsules formed by a deep-cavity cavitand in aqueous solution.[6] Rebek and co-workers have also attached spin labels to resorcinarene host molecules.[7] We are particularly interested in the application of EPR techniques to investigate the formation and properties of large molecular capsules that result from the self-assembly of resorcinarenes[8] in nonpolar media, such as chloroform and dichloromethane solutions. The resorcinarenes are bowl-shaped, tetrameric macrocycles formed by the condensation of resorcinol derivatives with aldehydes in an acidic medium. McGillivray and Atwood first demonstrated the formation of hexameric molecular capsules of resorcinarene **1** (Scheme 1) in the solid state.[9] Several groups have investigated this interesting self-assembly process in the solution phase by using NMR spectroscopy,[10] mass spectrometry,[11] fluorescence,[12] fluorescence resonance energy transfer,[13] and electrochemical techniques.[14] Herein, we report the continuation of our previous work on the self-assembly of resorcinarene molecular capsules in dichloromethane solution.[14] We used nitroxide spin probes (2,2,6,6-tetramethylpiperidine-1-oxyl (tempo) or derivatives) as potential guests for encapsulation by resorcinarene **2** (Scheme 1) in water-saturated dichloromethane solutions. The obtained experimental results add to our limited knowledge of the internal environment in these large capsular assemblies, and the interactions between the included guests and the internal walls of the capsule.



Scheme 1. Structures of the resorcinarene hosts and nitroxide guests used in this work.

The EPR spectra were recorded in water-saturated CH_2Cl_2 solutions in order to provide the water molecules that are necessary to complete the hydrogen-bond network required for capsule formation. The spectrum of 0.10 mM tempamine (4-amino-2,2,6,6- tetramethylpiperidine-1-oxyl) in this medium consists of three sharp peaks, which are characteristic of free nitroxide probe molecules that undergo fast motion[2] (Figure 1a). The ^{14}N hyperfine splitting and the g factor are given in Table 1. The addition of increasing amounts of resorcinarene **2** to the tempamine/CH_2Cl_2 solution leads to significant changes in the EPR spectra. In the

Table 1: Hyperfine splitting constants a_N, g factors, and rotational correlation times τ_r obtained from the EPR spectra of various nitroxide spin probes in water-saturated CH_2Cl_2 solution, before (free) and after encapsulation (bound) inside $\mathbf{2}_6$.[a]

Guest	Tempamine	Tempo–Me_3N^+	Tempo–Cob^+
$a_{N\,free}$ [G]	15.83	15.51	15.82
$a_{N\,bound}$ [G]	16.16	15.73	16.1 ± 0.5
g_{free}	2.0060	2.0062	2.0060
g_{bound}	2.0058	2.0058	2.0058
$\tau_{r\,free}$ [s][b]	1.2×10^{-11}	4.2×10^{-11}	3.4×10^{-11}
$\tau_{r\,bound}$ [s][b]	1.3×10^{-9}	2.0×10^{-9}	$(4\pm1)\times10^{-9}$

[a] Unless otherwise specified, the error margins were ±0.01 for the a_N values, ±0.0001 for the g factors, and ±10% for the τ_r values. [b] Calculated using the equation in Ref. [15].

[*] S. Yi, P. Bhattacharya, Prof. Dr. A. E. Kaifer
Center for Supramolecular Science and Department of Chemistry
University of Miami, Coral Gables, FL 33124-0431 (USA)
Fax: (+1) 305-284-4571
E-mail: akaifer@miami.edu

E. Mileo
Dipartimento di Chimica Organica "A. Mangini"
via S. Giacomo 11, 40126 Bologna (Italy)

[**] The authors gratefully acknowledge the NSF (to A.E.K., CHE-0600795 and CHE-848637) for generous support of this work. E.M. acknowledges funding from the University of Bologna for her work at the University of Miami.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.200901272.

region that corresponds to the high-field line, two different, superimposed signals are clearly visible: a sharp signal, which is characteristic of a free nitroxide probe that undergoes fast motion, and a much broader line (Figure 1b). The formation

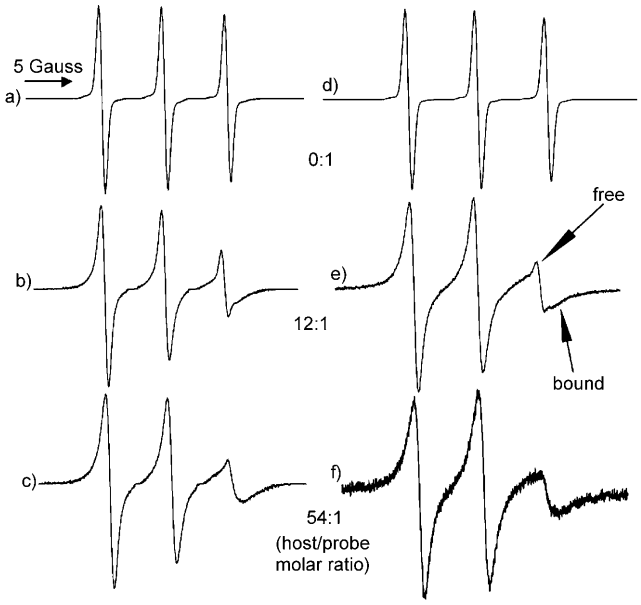


Figure 1. Experimental EPR spectra in water-saturated CH_2Cl_2 at 293 K. a) 0.10 mM tempamine, b) 0.10 mM tempamine + 1.20 mM **2**, c) 0.05 mM tempamine + 2.70 mM **2**, d) 0.10 mM tempo–Me_3N^+, e) 0.10 mM tempo–Me_3N^+ + 1.20 mM **2**, f) 0.05 mM tempo–Me_3N^+ + 2.70 mM **2**.

of a complex with tempamine as the included guest is supported by the observation that an increase in the resorcinarene concentration led to gradually higher intensities for the broader signal, while the intensity of the sharp signal that arises from the free-spin probe decreased. When the concentration of resorcinarene was increased to 2.7 mM and the [resorcinarene]/[spin probe] ratio was 54:1, the spectrum of the complexed radical became dominant (Figure 1c), and the spectroscopic parameters for the included nitroxide guest could be measured. The addition of excess resorcinarene led to an EPR spectrum in which the increased linewidth of all the lines (and especially the high-field line) reflects the reduction of the probe's tumbling rate. The observed increase of the tempamine rotational correlation time[15] τ_r from 1.2×10^{-11} s (in the absence of **2**) to 1.3×10^{-9} s (in the presence of excess **2**, see Table 1) is consistent with the encapsulation of the spin probe.

The a_N value measured for encapsulated tempamine is slightly larger than that of the free nitroxide probe (Table 1), thus indicating a small increase in the polarity of the microenvironment around the spin probe upon encapsulation. A similar conclusion can be derived from the small decrease observed in the *g* factor. These results are consistent with the expected behavior, as the large internal volume (ca. 1.375 Å^3) of the hexameric resorcinarene capsule ($\mathbf{2}_6$) guarantees that a large number of solvent molecules can be included together with the nitroxide guest, but the resorcinarene OH groups and water molecules involved in the hydrogen bonds that keep the capsule together exert a measurable effect on the polarity of the inner space of the hexameric capsule.

In light of the reported selectivity of $\mathbf{2}_6$ capsules for cationic species,[10] we decided to use cationic nitroxide derivatives as guests. Initially, we selected tempo–Me_3N^+ (4-trimethylammonium-2,2,6,6-tetramethylpiperidine-1-oxyl) and examined its interactions with resorcinarene **2** in water-saturated CH_2Cl_2 solution. In analogy with the results obtained with tempamine, the evolution of the EPR spectral patterns (Figure 1d–f), as well as the a_N and the *g* factor values (Table 1) obtained as the concentration of host **2** increased show that tempo–Me_3N^+ is sequestered inside a molecular capsule formed by the resorcinarene. Interestingly, we found that the τ_r value for tempo–Me_3N^+ included in the capsule is 2.0×10^{-9} s, that is, its tumbling rate is slightly slower than that of encapsulated tempamine. However, the free cationic nitroxide derivative in solution also exhibits a slower tumbling rate than that of neutral tempamine. Therefore, the possible presence of cation–π interactions between the positively charged trimethylammonium residue in tempo–Me_3N^+ and the aromatic inner walls of the capsule is not unequivocally substantiated by these data. Although a considerable excess of host **2** is necessary to fully encapsulate tempamine and tempo–Me_3N^+, it is clear from Figure 1 that the encapsulation of the latter probe is easier to achieve. This result is consistent with the known selectivity of hexameric capsules of host **2** for cationic compounds.

Pulse gradient stimulated echo (PGSE) NMR measurements[16] of the diffusion coefficient (D_o) of resorcinarene **2** (3 mM) in water-saturated CD_2Cl_2 yield a value of $(3.05\pm0.11)\times10^{-6}$ cm^{2}s^{-1}. In the presence of 0.5 mM tempamine, the D_o value was determined as $(3.07\pm0.05)\times10^{-6}$ cm^{2}s^{-1}. This value is in excellent agreement with our previously reported D_o values for these $\mathbf{2}_6$ molecular capsules in the same medium,[14] thus indicating that the presence of nitroxide probes does not disrupt the self-assembly of the resorcinarene host. While the NMR measurements require concentrations above 1 mM, EPR measurements are best performed at nitroxide probe concentrations around 0.1 mM (to minimize spin-exchange broadening effects). The lower overall resorcinarene concentrations in the EPR experiments may explain why the addition of a substantial host excess is required to complete the encapsulation of the nitroxide probe. At the beginning of the titration of tempamine (or tempo–Me_3N^+) with host **2**, its concentration is so low that capsule formation might not be quantitative. In addition to this possibility, lower absolute concentrations of probes and molecular capsules are expected to shift the complexation equilibrium away from encapsulation (see Figure S1 in the Supporting Information) for any given value of the corresponding equilibrium association constant. Nonetheless, at the end of the nitroxide probe titrations with resorcinarene, the probe is undoubtedly encapsulated inside a large capsular assembly, as the measured rotational correlation times indicate a considerable deceleration of the probe's tumbling rate (ca. 100-fold for tempamine and 50-fold for tempo–Me_3N^+, Table 1). These substantial changes in τ_r values can only be explained by the

incorporation of the nitroxide probe inside a large supramolecular assembly, such as $\mathbf{2}_6$.

Previous voltammetric experiments have shown that the encapsulation of the organometallic cation cobaltocenium is highly favored by $\mathbf{2}_6$ capsules. In fact, cobaltocenium is selectively complexed by the capsules, even in the presence of a large excess of tetrabutylammonium ions.[14] We exposed $\mathbf{2}_6$ capsules filled with either tempo–Me_3N^+ or tempamine to cobaltocenium, and observed the release of the nitroxide probe from the molecular capsules. The EPR experiments therefore show that the $\mathbf{2}_6$ capsules show selectivity for cobaltocenium in competition with either tempamine or tempo–Me_3N^+. As a result, we decided to prepare a novel cobaltocenium derivative tempo–Cob^+, which combines the organometallic cationic residue with a nitroxide probe in the same molecule (see the Experimental Section for synthetic details). The EPR spectrum of 0.10 mM tempo–Cob^+ in water-saturated CH_2Cl_2 shows the expected three sharp lines that correspond to the fast tumbling nitroxide residue (Figure 2a).

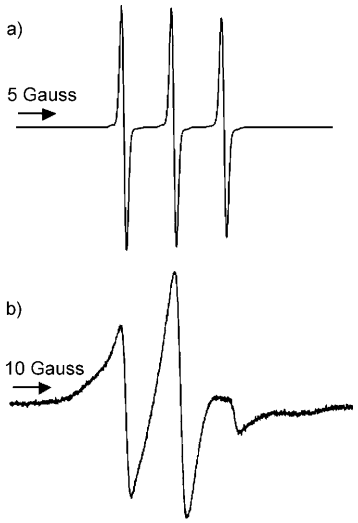


Figure 2. Experimental EPR spectra in water-saturated CH_2Cl_2 at 293 K. a) 0.10 mM tempo–Cob^+, b) 0.10 mM tempo-Cob^+ + 4.0 mM **2**.

However, the addition of excess resorcinarene host led to a considerably broadened spectrum that clearly suggests the incorporation of the tempo–Cob^+ guest into the $\mathbf{2}_6$ capsular assembly (Figure 2b). This spectrum is severely broadened and its shape indicates that the probe's tumbling motion falls in an intermediate region between the fast and slow motional regimes.[2]

We also investigated tempone (4-oxo-2,2,6,6-tetramethylpiperidine-1-oxyl) and tempol (4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl) as spin probe guests. The EPR spectrum of tempone was essentially unaffected by the presence of resocinarene, and shows only the typical signals that arise from the free nitroxide probe in solution ($a_N = 14.76$ Gauss, $g = 2.0059$, and $\tau_r = 1.3 \times 10^{-11}$ s). This finding suggests that this nitroxide probe is not included at all into the supramolecular assembly. Experiments with tempol showed that, although this probe is partially encapsulated inside the $\mathbf{2}_6$ assembly, its complexation is much less favorable than that of tempamine.

The EPR spectroscopic data collected in this work clearly support the hypothesis that encapsulation of guests with $\mathbf{2}_6$ is affected by the electrostatic nature of the guests involved. Electrostatic surface potential plots of the nitroxide probes surveyed here (see Figure S2 in the Supporting Information) reveal that their encapsulation is enhanced by surfaces with positive charge density, such as those of tempamine, tempo–MeN^+, and tempo–Cob^+. In contrast, a probe such as tempone, whose surface is predominantly laced with negative charge density, fails to undergo encapsulation. This failure is probably due to the electrostatic complementarity between positively charged guests and the mostly aromatic, inner walls of the resorcinarene capsules.

It is instructive to calculate the approximate molecular volumes from the rotational correlation times for the encapsulated nitroxide probes, in order to assess to what extent the motion of the trapped probes may reflect the overall motion of the entire assembly. By using standard equations for this purpose,[17,18] we estimated molecular volumes of 4700, 19000, and 39000 Å^3 for encapsulated tempamine, tempo–Me_3N^+, and tempo–Cob^+, respectively. Since the overall volume of the hexameric molecular capsule is expected to remain constant[19] regardless of the sequestered guest, these values reflect the relative levels of probe motion inside the capsule. In other words, while tempamine appears to have a fair degree of motion that is uncoupled to the overall motion of the supramolecular assembly, the motion of tempo–Cob^+ seems to reflect the motion of the assembly much more closely, with tempo–Me_3N^+ as an intermediate case. It can be argued that this effect may result from cation–π interactions between the guest and the inner walls of the capsule. However, as the molecular volume of the guest increases, its motional freedom inside the capsule will be reduced because of steric hindrance.

In conclusion, we have shown here that nitroxide spin probes can be used as guests for hexameric resorcinarene molecular capsules. EPR spectroscopic data show that the spin probe is more easily encapsulated as positive charge density accumulates on its surface. The motional regime of the spin probe upon encapsulation is partially decoupled from the slower motions of the supramolecular capsule for relatively small probes such as tempamine. However, as the molecular volume of the probe increases and fills a larger fraction of the capsule's cavity, the tumbling rate of the probe more closely reflects the overall tumbling rate of the entire supramolecular assembly.

Experimental Section

Commercially available nitroxide probes were used without further purification. Resorcinarene **2** was synthesized by following a reported procedure.[8a] Carboxycobaltocenium hexafluorophosphate ($CobCOOH(PF_6)$) was prepared as reported by Sheats and Rausch.[20] Pure compound was obtained by repeated washing with hot acetone and recrystallization from a mixture of acetone/hexane. 1-Chlorocarbonylcobaltocenium hexafluorophosphate ($CobCOCl(PF_6)$) was prepared by dissolving carboxycobaltocenium (50 mg, 0.13 mmol) in anhydrous CH_3CN (10 mL). Sulfonyl chloride (40 μL)

was then added and the resulting solution was heated at reflux for 24 h. After completion of the reaction, the solvents were removed under vacuum. Approximately 50 mg of $CobCOCl(PF_6)$ was obtained as a yellow solid and used in the next step without further purification.

Tempo–$Cob(PF_6)$: A solution containing tempamine (22.5 mg, 0.13 mmol) and triethylamine (15.9 mg, 0.16 mmol) in anhydrous CH_3CN (2 mL) was added dropwise to a solution of $CobCOCl(PF_6)$ (ca. 50 mg) in CH_3CN (8 mL) under N_2. The reaction mixture was stirred for 2 days at room temperature and then concentrated and cooled to −25 °C in order to precipitate the triethylammonium salt that occurred as a by-product. After filtration, the solvent was removed under vacuum, and the resulting residue was subjected to column chromatography on Sephadex LH-20, using CH_3CN as eluent. The appropriate fraction was collected and dried under vacuum to afford a mixture of tempo–Cob^+ salts (Cl^-/PF_6^-) as a highly viscous yellow liquid. This liquid was then dissolved in a small amount of water and a solution of saturated NH_4PF_6 was added dropwise. Tempo–$Cob(PF_6)$ (ca. 25 mg) precipitated as an orange solid. The solid was further purified by recrystallization from hot water/methanol, and the final product was obtained as red, needle-shaped crystals (13 mg, 18 % yield). MS (FAB): m/z 387 [M^+], 388; elemental analysis calcd (%) for $C_{20}H_{27}N_2O_2CoPF_6$: C 45.19, H 5.12, N 5.27; found: C 44.98, H 5.12, N 5.32.

EPR spectra were recorded on a Bruker EMX 200D spectrometer. The instrument settings were as follows: microwave power 0.63 mW, modulation amplitude 0.9 G, modulation frequency 100 kHz, scan time 180 s. Determination of g factors was done by using polycrystalline DPPH ($g = 2.0036$) as the reference.

Received: March 6, 2009
Published online: June 12, 2009

Keywords: encapsulation · host–guest systems · nitrogen oxides · self-assembly · spin probes

[1] For reviews, see: a) N. J. Turro, M. H. Kleinman, E. Karatekim, *Angew. Chem.* **2000**, *112*, 4608 – 4634; *Angew. Chem. Int. Ed.* **2000**, *39*, 4436 – 4461; b) P. Franchi, M. Lucarini, G. F. Pedulli, *Curr. Org. Chem.* **2004**, *8*, 1831 – 1849.

[2] a) J. H. Freed in *Spin Labeling: Theory and Applications, Vol. 1* (Ed.: L. Berliner) Academic Press, New York, **1976**, pp. 53 – 132; b) G. I. Likhtenstein in *Nitroxides: Applications in Chemistry, Biomedicine and Materials Science* (Eds.: G. I. Likhtenshtein, J. Yamauchi, S. Nakatsuji, A. I. Smirnov, R. Tamura), Wiley-VCH, Weinheim, **2008**, chap. 6.

[3] a) G. Ionita, V. Chechik, *Org. Biomol. Chem.* **2005**, *3*, 3096 – 3098; b) V. Chechik, G. Ionita, *Org. Biomol. Chem.* **2006**, *4*, 3505 – 3510.

[4] E. Mezzina, F. Cruciani, G. F. Pedulli, M. Lucarini, *Chem. Eur. J.* **2007**, *13*, 7223 – 7233.

[5] D. Bardelang, A. Rockenbauer, L. Jicsinszky, J.-P. Finet, H. Karoui, S. Lambert, S. R. A. Marque, P. Tordo, *J. Org. Chem.* **2006**, *71*, 7657 – 7667.

[6] J. Y.-C. Chen, N. Jayaraj, S. Jockusch, M. F. Ottaviani, V. Ramamurthy, N. J. Turro, *J. Am. Chem. Soc.* **2008**, *130*, 7206 – 7207.

[7] L. Kröck, A. Shivanyuk, D. B. Goodin, J. Rebek, Jr., *Chem. Commun.* **2004**, 272 – 273.

[8] a) L. M. Tunstad, J. A. Tucker, E. Dalcanale, J. Weiser, J. A. Bryant, J. C. Sherman, R. C. Helgeson, C. B. Knobler, D. J. Cram, *J. Org. Chem.* **1989**, *54*, 1305 – 1312; b) P. Timmerman, W. Verboom, D. N. Reinhoudt, *Tetrahedron* **1996**, *52*, 2663 – 2704.

[9] L. R. MacGillivray, J. L. Atwood, *Nature* **1997**, *389*, 469 – 472.

[10] a) A. Shivanyuk, J. Rebek, Jr., *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 7662 – 7665; b) L. Avram, Y. Cohen, *Org. Lett.* **2003**, *5*, 1099 – 1102; c) T. Evan-Salem, I. Baruch, L. Avram, Y. Cohen, L. C. Palmer, J. Rebek, Jr., *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 12296 – 12300.

[11] N. K. Beyeh, M. Kogej, A. Aahman, K. Rissanen, C. A. Schalley, *Angew. Chem.* **2006**, *118*, 5339 – 5342; *Angew. Chem. Int. Ed.* **2006**, *45*, 5214 – 5218.

[12] a) S. J. Dalgarno, S. A. Tucker, D. B. Bassil, J. L. Atwood, *Science* **2005**, *309*, 2037 – 2039; b) S. J. Dalgarno, D. B. Bassil, S. A. Tucker, J. L. Atwood, *Angew. Chem.* **2006**, *118*, 7177 – 7180; *Angew. Chem. Int. Ed.* **2006**, *45*, 7019 – 7022.

[13] E. Barrett, T. J. Dale, J. Rebek, Jr., *J. Am. Chem. Soc.* **2008**, *130*, 2344 – 2350.

[14] I. Philip, A. E. Kaifer, *J. Org. Chem.* **2005**, *70*, 1558 – 1564.

[15] The rotational correlation time τ_r (s) was determined using the following equation:

$$\tau_r = 6.5 \times 10^{-10} x \Delta H \left[\sqrt{\frac{h_0}{h_{-1}}} + \sqrt{\frac{h_0}{h_{+1}}} - 2 \right]$$

where ΔH is the peak-to-peak width (in Gauss) of the central line; h_{-1}, h_0, and h_{+1} are the intensities of the low-, center- and high-field lines, respectively.

[16] Y. Cohen, L. Avram, L. Frish, *Angew. Chem.* **2005**, *117*, 524 – 560; *Angew. Chem. Int. Ed.* **2005**, *44*, 520 – 554.

[17] P. C. Griffiths, C. C. Rowlands, P. Goyffon, A. M. Howe, B. L. Bales, *J. Chem. Soc. Perkin Trans. 2* **1997**, 2473 – 2477.

[18] P. P. Borbat, A. J. Costa-Filho, K. A. Earle, J. K. Moscicki, J. H. Freed, *Science* **2001**, *291*, 266 – 269.

[19] The volume of the $\mathbf{2}_6$ supramolecular assembly was calculated by molecular modeling studies to be in the range 42 000–51 000 Å^3.

[20] J. E. Sheats, M. D. J. Rausch, *J. Org. Chem.* **1970**, *35*, 3245.

