The Dendritic Box: Shape-Selective Liberation of Encapsulated Guests

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The possibilities for encapsulating guest molecules in dendritic hosts were proposed by Maciejewski[1] in 1982 and discussed as the main application of dendrimers ever since.[2] Despite the fact that dendrimers and hyperbranched polymers are subjects of intensive research,[3] examples of guest encapsulations are limited to an organic guest dissolved in the interior of a dendrimer. Fréchet et al. were able to solubilize pyrene in water employing a water-soluble dendrimer,[4] while Tomalia et al. have demonstrated by means of NMR relaxation measurements that organic molecules like asperin can penetrate into the interior of a polyamidoamine (PAMAM) dendrimer.[5] However, both examples are based on dynamic processes, and the guests can easily diffuse in or out of the dendrimer host, depending on the equilibrium conditions.

Recently we demonstrated that it is possible to physically lock (imprison or encapsulate) guest molecules in a monomolecular dendritic container with a diameter of approximate 5 nm, the so-called dendritic box.[6] These dendritic boxes (Scheme 1) are constructed from a flexible poly(propylene imine) dendrimer with 64 amine end groups[7] and an L-phenylalanine derivative. In solution, this 64-L-Phe box possesses a highly dense hydrogen-bonded shell with solid-state character, as has been demonstrated with ^{13}C-NMR relaxation data[6] and chiroptical studies.[8] Guest molecules are captured within the internal cavities of these boxes by constructing the dense shell in their presence.[6] In this Communication, we report on our preliminary results concerning the shape-selective liberation of guests from these dendritic boxes. The basic principle of a selective liberation is shown in Figure 1. Initially, the liberation principle has been demonstrated with the 64-L-Phe box (Scheme 1), as we have previously demonstrated that this box is the most ideal molecular container obtained so far.

In a first example, we encapsulated Bengal Rose (**1**) and *p*-nitrobenzoic acid (**2**) together in a dendritic box. Exhaustive dialysis with acetone/water (cellulose 24 Å, seven times, 5% water in acetone) is used to remove the adhered or excess **1** and **2**. Control experiments using different solvents, higher temperatures, or sonification show no release of any of the encapsulated guests from the box. The concentrations of encapsulated guests are estimated by comparison of UV spectra of guests that are in and out of the box. The relation of the number of molecules of **1** that are encapsulated in the box as a function of the concentration of **1** used in the reaction is given in Figure 2. This figure indicates that the maximum number of guests per dendritic box is directed by the architecture of the dendritic box, and obviously by the properties of the guest.[9]

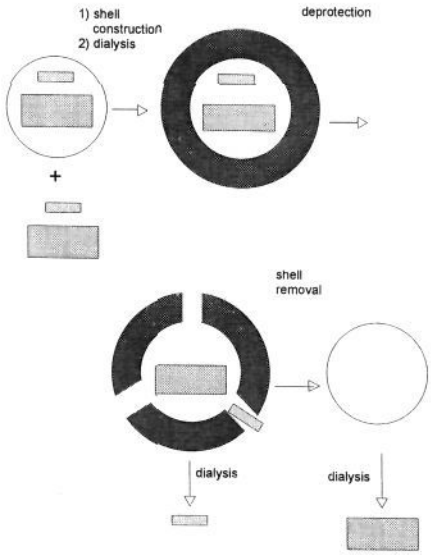
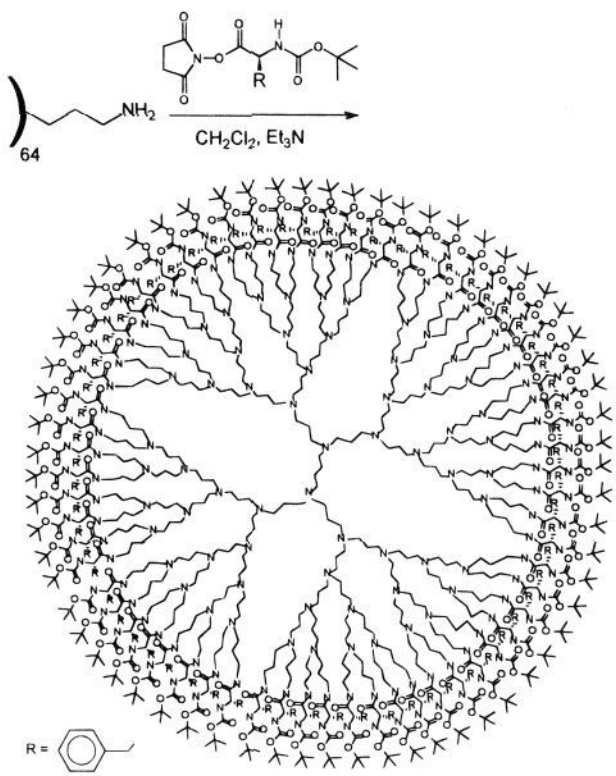


Figure 1. Schematic presentation of the principle of encapsulation and shape-selective liberation. First, two guests that differ in size are encapsulated in the box, and dialysis is used to remove adhered and excess guest. Next, the shell is partially perforated, yielding a modified dendritic box in which only the larger guest is entrapped and from which the smaller guest is liberated. Subsequent removal of the shell liberates the larger guests, and the starting poly(propylene imine) dendrimer is recovered.

Scheme 1



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(3) See, for instance: (a) Issberner, J.; Moors, R.; Vögtle, F. *Angew. Chem.* **1994**, *106*, 2507; *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 2413. (b) Newkome, G. R.; Moorefield, C. N.; Keith, J. M.; Baker, G. R.; Escamilla, G. H. *Angew. Chem.* **1994**, *106*, 701; *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 666. (c) Wooley, K. L.; Hawker, C. J.; Fréchet, J. M. J. *J. Am. Chem. Soc.* **1993**, *115*, 4375. (d) Miller, T. M.; Neenan, T. X.; Kwock, E. W.; Stein, S. M. *J. Am. Chem. Soc.* **1993**, *115*, 356.
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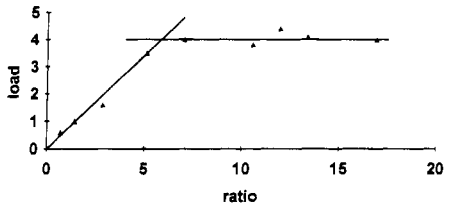
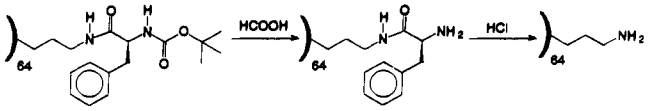


Figure 2. Number of Bengal Rose molecules encapsulated in one dendritic box as determined by UV−vis spectroscopy versus the molar ratio of Bengal Rose and dendrimer during the encapsulation reaction (using 10^{-3} M poly(propylene imine) dendrimer in CH_2Cl_2).

Table 1. Selective Liberation of Several Dyes from the 64-L-Phe Box



	dense shell		partly opened		completely opened	
	[3][a]	[dye][b]	[3][a]	[dye][b]	[3][a]	[dye][b]
Bengal Rose (**1**)	0.8	4.1	0	3.9		0.001
Rhodamide (**4**)	0.8	3.9	0	3.8		0.001
New Coccine (**5**)	0.4	2.6	0	2.6		0.001

[a] The concentration of **3** is detected by integration of the EPR signal of (perforated) box in $CHCl_3$ in comparison with a sample of **3** with a known concentration in $CHCl_3$. In the case of the completely opened system, the concentration of **3** has not been measured. [b] The concentration of the different dyes is measured using UV−vis spectroscopy of the (perforated) box in $CHCl_3$ and compared with a sample of dye with known concentration. The completely opened system has been investigated in water.

After encapsulation of four molecules of **1** and 8−10 molecules of **2** per box, hydrolysis of the tBOC groups with formic acid (95% HCOOH, 16 h) is performed. Subsequent dialysis (5% water in acetone) of the reaction mixture yields a perforated dendritic box in which only the four molecules of **1** are entrapped, whereas all the **2** is dissolved in the acetone/water mixture.[10] Bengal Rose (**1**) cannot be liberated from the perforated box, even with the addition of hydrochloric acid (12 N). However, hydrolysis of the outer shell using 12 N HCl under reflux for 2 h liberates **1** after dialysis (100% water), and starting poly(propylene imine) dendrimer is recovered in 50−70% yield.

To improve the detection of the concentration of different guests encapsulated into the box, we used an EPR probe together with a UV−vis probe in the next set of experiments. 2,2,3,4,5,5-Hexamethyl-3-imidazoliumyloxy methyl sulfate free radical **3** has been employed as the small guest, whereas the dyes Bengal Rose (**1**), Rhodamide B (**4**), and New Coccine (**5**) have been used as the large guests (Table 1). Table 1 clearly shows that after removal of the *N*-tBOC protecting group with formic acid EPR probe, **3** is liberated completely, as no EPR resonance is detected in the dendrimer after dialysis.[11] In all these cases, no significant difference in the UV−vis spectrum for the dendrimer with the dense shell and the partially perforated shell is detected.[12]

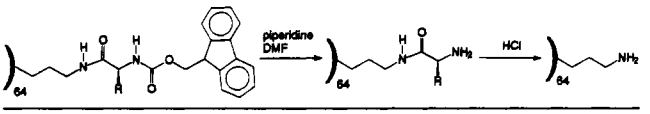
(9) Although a large number of experimental results clearly point to the presence of a supramolecular and ordered arrangement of (the larger) guests in the dentritic box, the nature of the encapsulation is not of direct importance for the shape-selective liberation presented here. Even a model in which a statistical distribution of dissolved guests is assumed can be used.

(10) The liberated guests are characterized by UV−vis spectroscopy; identical spectra are observed for virgin and liberated dyes. The perforated and recoved poly(propylene imine) dendrimers are characterized by NMR spectroscopy and HPLC.

(11) The decrease in EPR signal from the box with encapsulated EPR probe and the perforated box after dialysis is at least 3 orders of magnitude.

(12) The load differences observed are within the error of detection; if possible, clathrate formation of solvent is taken into account.

Table 2. Selective Liberation of **6** and **7** from Several Different Dendritic Boxes[a]



	dense shell		partly opened		completely opened	
	[6]	[7]	[6]	[7]	[6]	[7]
L-Alanine	8	7.8	0	0.05		0.001
L-Phenylalanine	8	7.6	0	7.3		0.002
L-tBu-serine	4	3.9	0	3.7		0.003
L-Try-cysteine	4	4.2	0	4.0		0.002
L-tBu-aspartic ester	8	8.2	0	3.9		0.001

[a] For the details of detection, see note *b* of Table 1.

For this principle to be applicable in a more general fashion, it should be valid not only for L-Phe-derived dendrimers but also at least for other amino acid-derived dendritic boxes, e.g., FMOC-protected amino acids (Table 2). In this series we have used nitrophenol (**6**) as the small guest and methylene violet 3RAX (**7**) as the large guest. Table 2 shows that, depending on the amino acid used as the shell component, the shape-selective liberation of **6** and **7** can be tuned considerably. Remarkably, we have found that the maximum concentrations of **6** and **7** encapsulated are either four or eight guests per dendritic box, which perfectly match the symmetry of these poly(propylene imine) dendrimers. Both **6** and **7** are liberated completely after removal of the FMOC group from the L-alanine-derived dendrimer with piperidine in DMF (20% piperidine in DMF, 20 min, room temperature), while in the case of the FMOC-protected L-phenylalanine-derived dendritic box, only **6** is liberated after the hydrolysis of the FMOC group, and **7** is removed after complete hydrolysis using hydrochloric acid (12 N, reflux, 2 h). The FMOC-protected tBu-aspartic ester-derived box liberates all of **6** and half of the concentration of **7** after partial perforation. The remainder of **7** is liberated only after complete hydrolysis.

Two remarkable observations are presented above. (1) The shapes of the guest and cavity determine the number of guests entrapped in the dendritic box, while the architecture of the dendrimer is important as well. (2) A shape-selective liberation can be achieved by removing the shell in two steps. Apparently from the results presented here, the size of the guest, the size of the perforation made in the dense shell, and the position of the guest (for **7**) determine the possibility of removing the guests by dialysis. The absence of diffusion of the guests from the (perforated) box by solvent-induced conformational changes or protonation is proven by control experiments. Furthermore, the exhaustive dialysis employed ensures that the thermodynamically stable guest−host systems are obtained after hydrolysis and dialysis. Problems related to kinetic effects differentiating between guests that are liberated rapidly or more slowly are thus avoided. No conclusions can be drawn with respect to the required conversion of hydrolysis before liberation sets in; however, solvent-induced conformational changes are excluded to be responsible for liberation only.

In conclusion, we have presented evidence that a shape-selective liberation of guests encapsulated in a dendritic box can be accomplished by a two-step process. The results show that a pathway for fine-tuning is available, and further research with these new guest−host systems is in progress.

Supplementary Material Available: Synthesis and chracterization of the dendritic box, and procedures for the encapsulation and stepwise liberation of guests (5 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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