TOWARD MODULAR CHEMISTRY WITH THE DENDRITIC BOX AS MODULE

J.F.G.A. JANSEN, H.W.I. PEERLINGS, J.C.M. VAN HEST, E.W. MEIJER*

Laboratory of Organic Chemistry, Eindhoven University of Technology, P.O. Box 513, 5600 MB Eindhoven, The Netherlands

E.M.M. DE BRABANDER - VAN DEN BERG

DSM Research, P.O. Box 18, 6160 MD Geleen, The Netherlands

ABSTRACT. In this paper we like to present the dendritic box as a new tool toward modular chemistry. A dendritic structure with a densely-packed shell is prepared via modification of the fifth generation poly(propylene imine) dendrimers with N-BOC-L-phenylalanine groups. This so-called dendritic box has a diameter of approximately 4.5 nm, as determined by DLS and SAXS, and has internal cavities which can be used for the encapsulation of guest molecules. Supramolecular encapsulation is achieved by performing the construction of the box in the presence of the guest molecules, followed by dialysis to remove excess and adhered guest. A number of properties of encapsulated guests is critically influenced by the host, like the observation of induced optically activity and a solvent-independent fluorescence of dyes encapsulated as well as the formation of a triplet radical pair of 3-carboxy-proxyl. A shape-selective liberation of encapsulated guests is accomplished by a two-step hydrolysis of the shell. The potentials of these boxes as new supramolecular architectures within the nanometer regime are discussed and the physical locking of guest molecules is proposed as a new binding principle useful for modular chemistry.

1. Introduction

The interest in dendrimeric macromolecules arises from the unique properties of these highly branched structures that have a defined number of generations and functional end groups [1,2]. The high degree of control over molecular weight and shape has led to the synthesis of unimolecular micelles [3], spherical and cone-shape mesostructures [4], as well as stratified dendrimers possessing generations of different structure [5]. Diameters of the spherical dendrimers range from 3-10 nanometers, enabling these structures to be building blocks of a new chemistry set [6].

After the initial reports on dendritic molecules [7], proposals have been made for the construction and applications of guest-host systems made out of dendrimers [1,2,8]. The concept of topological trapping by core-shell molecules is based on the fact that, at some stage in the synthesis of dendrimers, the space available for the new generation or end-group modification is not sufficient to accommodate all the atoms required for complete conversion (the so-called sterically induced stoichiometry) [1]. We will discuss here the synthesis of a dendritic box consisting of a flexible core with a rigid shell [9]. These boxes have internal cavities available in which guest molecules can be physically entrapped due to the rigid shell.

2. The dendritic box

The flexible core of our dendritic box is based on poly(propylene imine) dendrimers which were synthesized by the divergent approach [10]. A repetitive reaction sequence using the double Michael addition of a primary amine to acrylonitrile followed by the heterogeneously catalyzed hydrogenation of the nitriles to primary amines, yields diaminobutane-based poly(propylene imine) dendrimers DAB-dendr-$(NH_2)_n$ with n= 4, 8, 16, 32, 64, and 128 primary amine end groups. The unmodified dendrimers are very flexible and possess glass transition temperatures of approximately -40 °C and -65.°C for the CN- and NH_2-terminated dendrimers, respectively [10]. More recently a variety of end-group modifications have been reported [11]. For the construction of the rigid shell of the dendritic box a critical end-group modification of the cascade polyamine with an appropriate bulky group is performed. For instance, the N-hydroxy-succinimide ester of a tert-butyloxycarbonyl (t-BOC)-protected L-phenylalanine is allowed to react with the fifth generation poly(propylene imine) dendrimer in a CH_2Cl_2-triethylamine mixture (figure 1). Extended washing procedures were used to obtain pure dendritic box with a molecular weight of almost 24,000 [9].
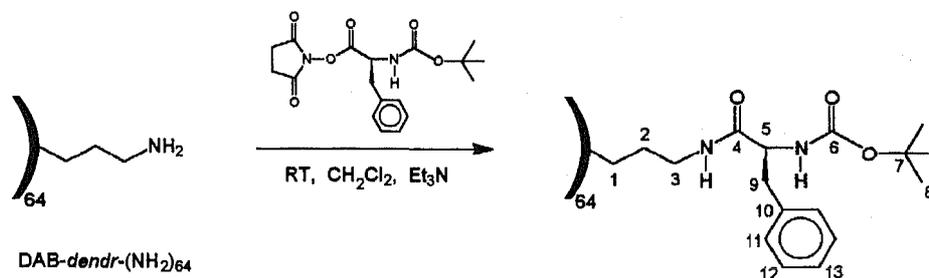
Figure 1. Schematic presentation of the synthesis of the amino acid-terminated poly(propylene imine) dendrimers, including an atomic numbering of the shell of the dendritic box.

Structure elucidation of the dendritic box was performed with a variety of characterization techniques; IR, UV, ^{1}H and ^{13}C NMR spectroscopy data are all in agreement with the structure assigned. However, the resonances in the ^{13}C NMR spectra showed a significant line broadening for the higher generations. Spin-lattice (T_1) and spin-spin (T_2) relaxation measurements were performed and the results for the shell atoms were compared with the corresponding data of the other lower generations (figure 2). The observed increase of T_1 relaxation times after the third generation is indicative of a decrease in molecular motion for the higher generations; an almost solid-phase behavior of the shell in solution is proposed. Further evidence for this close packing of the shell is found from chiroptical studies (see later). Presumably, intramolecular hydrogen bonding between several L-Phe residues in the shell is contributing to this solid-phase character. Unfortunately, MALDI-TOF and electrospray mass spectrometry studies have not been successful yet.
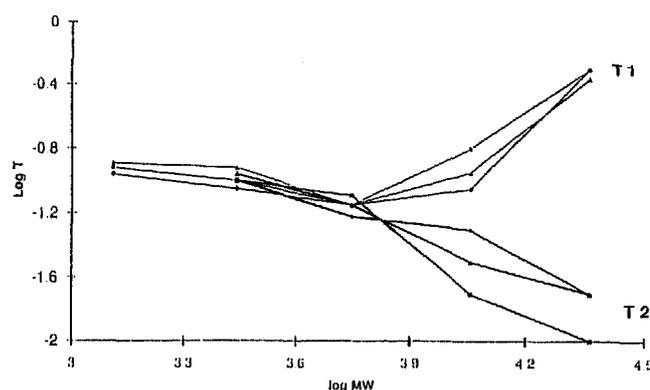

Figure 2. Double logarithmic graph of relaxation data (carbon T_1 and T_2) versus molecular weight (generation) for the carbon atoms 1, 2, and 3 (see figure 1) as recorded at 75MHz in chloroform.

CHARMm molecular mechanics calculations of the DAB-dendr-(N-t-BOC-L-Phe)$_{64}$ are performed to get insight into the three-dimensional structure. A globular architecture is found with an estimated radius of 23 ± 3 Å. Dynamic light scattering studies of the dendritic box in solution showed single particle behavior with a radius of gyration of 17 ± 4 Å (which resembles a radius of the box of 22 Å). Finally, SAXS measurements gave a radius of gyration of 18 Å for the dendritic box.

The choice of L-Phe as the amino acid component of the shell has been made from a study in which we compared a variety of amino acids of different size. By using larger amino acids like L-Trp it is not possible anymore to modify all the end groups due to the restricted space available verifying the sterically induced stoichiometry principle [1,6]. On the other hand by performing the modification reaction with smaller amino acids, like L-Ala and L-Leu such a dense packing is not achieved, as concluded from NMR and modeling studies, as well as the encapsulation experiments described in the next paragraph. L-Tyr in the t-BOC protected form is comparable with L-Phe as shell component with respect to dense packing, but lacks the good solubility in most organic solvents. Therefore, we selected the DAB-dendr-(NH-t-BOC-L-Phe)$_{64}$ as the dendritic box, being a nanometer-sized host system for a variety of guest molecules [9].

3. Encapsulation of guest molecules into the dendritic box

The experimental and modeling results prompted us to propose that we prepared molecules with a solid shell and a flexible core that will have internal cavities available for guest molecules. As the shell is constructed in the last step, it is possible to perform this coupling reaction in the presence of guest molecules. In fact, we encapsulated molecules with some affinity for tertiary amines within the dendritic box. Excess of guest and/or traces of guests adhering to the surface are removed by extensive washing and/or dialysis. When a dendrimer of lower generation was used, the shell is not dense enough to capture the guests and they were removed by extraction. A large variety of guest molecules have been encapsulated and this opens a plethora of interesting chemical and biochemical applications. We will discuss some of these nanometer-sized guest-host systems here as well as the properties of the guest molecules that are so critically influenced by the dendritic box.
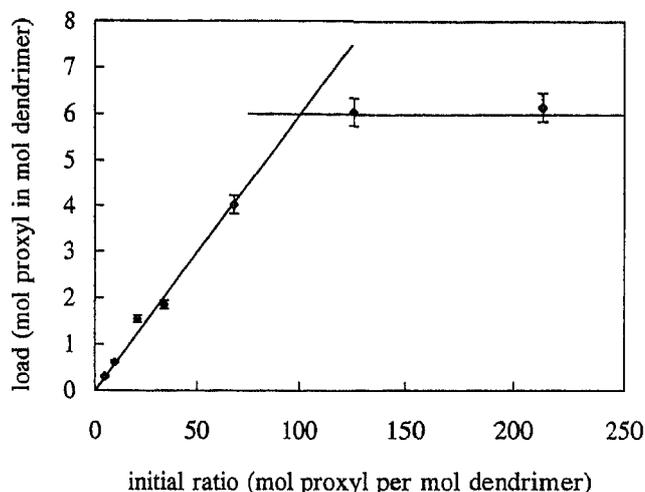
Figure 3. Number of 3-carboxy-proxyl radicals trapped in the dendritic box as determined by
ESR spectroscopy versus the molar ratio of radical and dendrimer in the initial
solution prior to the encapsulation reaction.

By carring out the encapsulation reaction in the presence of a varying
concentration of 3-carboxy-proxyl, the number of entrapped radicals could be
varied from 0.3 to 6.0 molecules per dendritic box as determined by ESR
spectroscopy [12]. The number of 3-carboxy-proxyl radicals in the dendritic
box does not increase above six (figure 3), clearly demonstrating that the
maximum attainable number of radicals is restricted by the shape of the cavities
in the box. The ESR spectra of 3-carboxy-proxyl@DAB-dendr-(NH-t-BOC-
LPhe)$_{64}$ dissolved in 2-methyltetrahydrofuran are strongly temperature depend-
ent. At 305 K an essentially isotropic ^{14}N-coupled ESR spectrum is observed,
characteristic for a rapid rotational diffusion of the radical spin probes.
Lowering the temperature results in a decreasing intensity of the isotropic
spectrum and the appearance of an anisotropic ESR spectrum, consistent with a
more restricted motion of the spin probe. In the temperature range from 150 to
250 K a superposition of the motionally narrowed (isotropic with $A_{iso}(N)$=1.40-
1.42 mT) and the slow-motion (anisotropic with A_{zz} (N)=3.38 mT) spectrum is
observed. This superposition indicates that the micro-environment of the
encapsulated 3-carboxyl-proxyl molecules is not uniform over the interior of the
dendritic box. A solid sample of 3-carboxy-proxyl@DAB-dendr-(NH-t-BOC-
LPhe)$_{64}$ with more than 1.6 molecules per box shows at lower temperatures a
(partial) ferromagnetic alignment of the radicals. The observation of a A_{ms} = 2
ESR transition exhibiting a partially resolved 1:2:3:2:1 hyperfine coupling
pattern due to two ^{14}N nuclei with A(pair) =1/2A(3-carboxy-proxyl) showed

376

unambiguous spectral evidence of the presence of a triplet-state radical pair (figure 4). The intensity of the $\Delta_{ms} = 2$ signal follows Curie law ($I = C/T$) between 4.2 and 100 K, consistent with a triplet ground state. To the best of our knowledge this is the first observation of an intermolecular ferromagnetic exchange interaction in a non-crystalline guest-host assembly. Since these types of interactions are often observed intramolecularly or in organic crystals, we are prompted to conclude that the dendritic box possesses some peculiar ordering properties apparently dictated by the architecture of the dendritic skeleton.
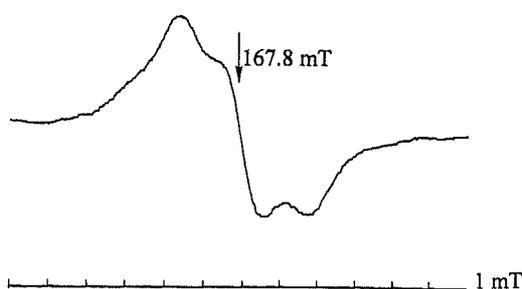

167.8 mT

1 mT

Figure 4. $\Delta_{ms} = 2$ ESR spectrum of a solid sample of 3-carboxy-proxyl@DAB-dendr-(NH-t-BOC-LPhe)$_{64}$ at 4.2 K.

As another example we have encapsulated a variety of organic dye molecules into the dendritic box [9]. Rose Bengal is encapsulated in a similar fashion as the spin probe described above. The number of Rose Bengal molecules encapsulated could be estimated after prolonged dialysis by comparison of the UV spectra of guests that are inside or outside of the box. The relation between the number of encapsulated molecules of Rose Bengal as a function of the concentration of Rose Bengal used in the shell-forming reaction is depicted in figure 5. Also in this case the maximum number of guest molecules attainable is limited, in this case to four. It is tempting to propose that each of the four guest molecules is occupying one large cavity present in the dendritic box. Although the absorption spectra of Rose Bengal and Rose Bengal@DAB-dendr-(NH-t-BOC-LPhe)$_{64}$ are identical, there is a large difference in the fluorescence spectra as recorded in CHCl$_3$.The strong fluorescence at $\lambda_{max} = 600$ nm for Rose Bengal@DAB-dendr-(NH-t-BOC-LPhe)$_{64}$ is completely absent in the case of the supramolecular isomer of Rose Bengal outside the box. In the latter the fluorescence is quenched effectively. The emission of the guest-host system is relatively insensitive to solvent effects, hence, we believe that we

have prepared a fluorescent sphere with an environmental-independent emission profile.
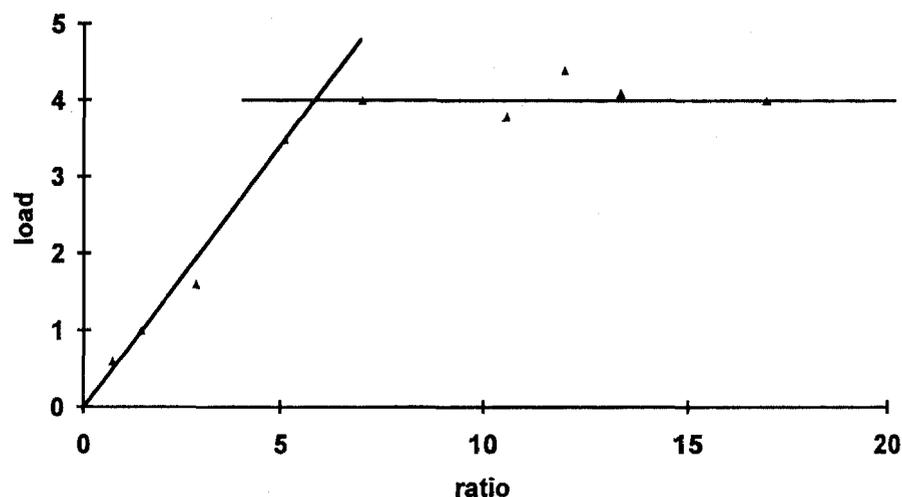

Figure 5. Number of Rose Bengal molecules encapsulated (load) in one dendritic box as determined by UV-vis spectroscopy versus the molar ratio of Rose Bengal and dendrimer during the encapsulation reaction.

Eriochrome Black T is a pH-dependent dye that is very soluble in polar solvents and can be encapsulated in the box. Due to the many (62) tertiary amines present in the interior of the box, Eriochrome Black T shifts its absorption spectrum from λ_{max} = 280 nm for free dye in CH_2Cl_2 to λ_{max} = 360 and 570 nm for dye in the box and in CH_2Cl_2. As soon as the absorption spectrum of free dye and encapsulated dye are different it is not possible to determine accurately the number of molecules encapsulated in a simple way. Since Eriochrome Black T is very soluble in water or acetonitrile, while the dye@box is insoluble in these solvents, we used this system to study the diffusion of the dye out of the box. Even after prolonged heating, dialysis or sonification the aqueous phase of the dispersion did not become colored due to diffusion. Therefore, it was concluded that the diffusion of dye out of the box is unmeasurably slow.

By comparing the encapsulation results of a large variety of dye molecules, it became apparent that many coplanar dye molecules with an ionic group can be encapsulated into the dendritic box. For concentrated solutions of

378

large dyes in the encapsulation reaction the maximum number of dye molecules entrapped is four, which is related to the architecture of the dendritic box. Large three-dimensional dyes or coplanar dyes without ionic or polar groups are hard to encapsulate and only small numbers of the ratio guest per host are observed, typically around 0.1 as the average number of guests per box. Smaller polar guests can be encapsulated with maximum numbers beyond four, but in almost all cases an integer number of six or ten is found.

These results suggest that the procedure employed here produces a unimolecular compartmented structure in which guest molecules can be encapsulated and for which the diffusion out of the box is unmeasurably slow.

4. Optical activity of the dendritic box

Since the modification reaction of the poly(propylene imine) dendrimers is performed with enantiomerically pure amino acids, it is of interest to study the chiroptical properties of the box and the guest@box systems [13,14]. Much to our surprise, we noticed that the optical activity of the DAB-dendr-(NH-t-BOC-L-Phe)n decreases drastically on going from dendrimers of the first generation with four end groups ($[\alpha]_D$ = -11; c=1, CHCl$_3$) to the dendritic box of the fifth generation ($[\alpha]_D$ = -0.1; c=1, CHCl$_3$) with 64 end groups. This decrease in optical activity is not due to (partial) racemization of the amino acids employed, as was demonstrated with HPLC using a chiral stationary phase. The specific optical rotations as a function of generation are given in figure 6. A more thorough investigation employing a variety of different amino acid derivatives revealed that this decrease of optical rotation with increasing generation is a general phenomenon for all of the t-BOC-protected amino acids used [13]. Circular dichroism and optical rotatory dispersion measurements confirmed the results of the specific optical rotations. Using model systems we investigated the solvent dependence of the lower generations and found that for the L-Phe derivative the optical rotation is strongly influenced by the solvent. The optical rotations varied from $[\alpha]_D$ = 7.3 (c=1, toluene) to $[\alpha]_D$ = -6.4 (c=1, acetonitrile).
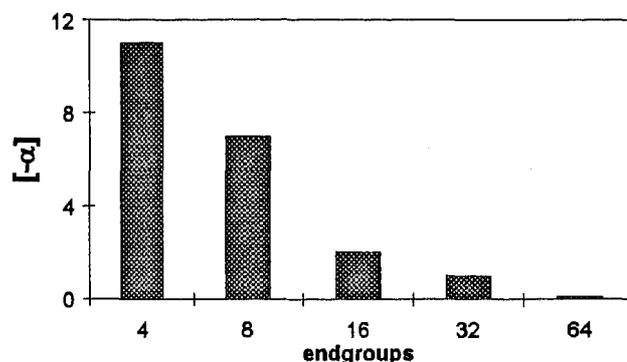
Figure 6. Specific optical rotations at the sodium D line for the t-BOC-L-Phe modified
poly(propylene imine) dendrimers as measured in CHCl₃.

In order to investigate the importance of both the amide and the carbamate functionality in the end groups, a system is synthesized in which the carbamate group is replaced by an acetal moiety, while the shape is almost constant (figure 7). When the model compound of the propylamine and the end group was submitted to optical rotation measurements in various solvents, it was shown that this model compound showed only a marginal solvent dependence as the $[\alpha]_D$ value varied at values between 40 and 60. The specific optical rotations of the various generations of acetal-modified poly(propylene imine) dendrimers were measured and proved to be independent of the generation [15].
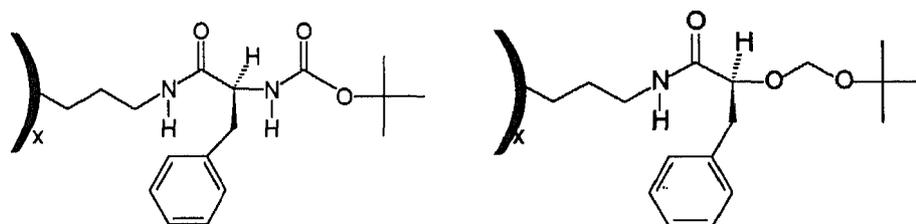

Figure 7. The dendritic box versus a modification in which the carbamate group is replaced by an acetal moiety.

In order to explain the peculiar optical behavior of the dendritic box, with its solid-like shell, it is first necessary to explain the difference in solvent dependence of the model compounds on the optical activity. A strong solvent, concentration, or temperature dependence of the optical rotation of organic

compounds has been observed already about a century ago [16], but a detailed rational explanation is still lacking [17]. It is assumed that different solvents give rise to different distributions of conformations, which sometimes (but not necessarily) leads to large differences in optical rotations. If in the dendritic shell of the box several conformations are frozen in, an average optical activity will be observed. In the case of the dendritic box this will apparently tend to a vanishing optical rotation and for the acetal-modified dendrimers to a nearly constant value of about 42. Hence, the highly dense packing of end groups in the multiple-hydrogen bonded shell of the dendritic box gives rise to different frozen-in conformations, leading to an internal compensation of optical activity.

Stimulated by the observation of induced chirality of dyes dissolved into chiral bilayers and micelles [18], the circular dichroism (CD) spectra of a variety of dyes encapsulated in the dendritic box have been recorded. Induced circular dichroism spectroscopy is based on the transfer of chirality from the environment to an achiral dye and could therefore be applicable to these boxes. The vanishing optical activity is caused by a compensation effect and local optical activity is still thought to be present. In figure 8 the results are given for two samples of Rose Bengal@DAB-dendr-(NH-t-BOC-L-Phe)$_{64}$ with one and with four molecules of Rose Bengal per dendritic box on the average. Although both samples show identical UV spectra, a dramatic difference is observed in their induced CD spectra. The dendritic box with one molecule of Rose Bengal encapsulated exhibits an induced CD spectrum related to the UV spectrum, in which all bands possess a negative Cotton effect. However, an exciton-coupled spectrum is observed when four molecules of Bengal Rose are encapsulated on the average in a single dendritic box. This exciton coupling indicates the close proximity of chromophores with a certain fixed orientation [14]. All explanations for the induced CD observed are speculative, however, it is reasonable to assume that some chirality is present in the cavities of the dendritic box, despite the vanishing optical activity of the shell.
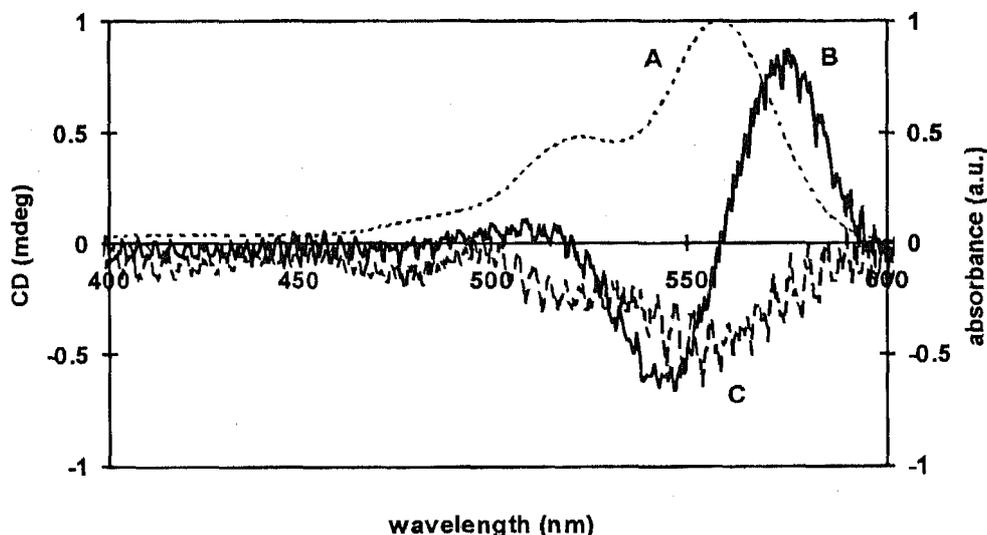
Figure 8. UV (A) and CD spectra of Rose Bengal@DAB-dendr-(NH-t-BOC-L-Phe)$_{64}$
containing one (C) and four (B) molecules of Rose Bengal.

5. Shape-selective liberation of encapsulated guests

So far, the dendritic box has been used to encapsulate guest molecules into the internal cavities present. The rigid, densely packed shell of the DAB-dendr-(NH-t-BOC-L-Phe)$_{64}$ limits the diffusion out of the box of almost all guest molecules studied up to now. Obviously, it is difficult to determine the diffusion of solvent molecules accurately, but all experimental data available so far show that small molecules like CH_2Cl_2 can penetrate through the rigid shell. If a dendritic box is made from the t-BOC protected glycine amino acid, a semi-permeable box is made [19]. This idea of tuning the density of the shell by decreasing the size of the end groups has been used to obtain a shape-selective liberation of guests form the dendritic box made from L-Phe [20].

After encapsulation of four molecules of Rose Bengal and 8-10 molecules of para-nitrobenzoic acid together in a dendritic box, hydrolysis of the t-BOC groups with formic acid (95% HCOOH, 16h) was performed. Subsequent dialysis of the reaction mixture (5% water in acetone) yielded a perforated dendritic box in which only the four molecules of Rose Bengal are entrapped, whereas all para-nitrobenzoic acid was dissolved in the acetone/water mixture. Rose Bengal cannot be liberated from the perforated box, not even after

the addition of 12 N hydrochloric acid. However, hydrolysis of the outer shell using 12 N HCl under reflux for 2 h liberated Rose Bengal after dialysis (100% water) and the starting poly(propylene imine) dendrimer was recovered in 50-70% yield. By applying this two-step hydrolysis procedure to a variety of different mixtures of guest molecules it was shown that this shape-selective liberation is a general principle [20]. Furthermore by changing the amino acids in the shell and the protecting group of the amino acid it proved to be possible to fine-tune this pathway of liberation completely.

6. Toward modular chemistry and conclusions

We have discussed the synthesis of dendritic boxes possessing a unimolecular compartmented structure in which guest molecules are physically locked. Evidence is presented that the encapsulation is dominated by the architecture of the dendrimer and that some supramolecular ordering is present. Furthermore, a shape-selective liberation of guests can be accomplished by a two-step process.

It is envisaged that the binding between guests and dendritic box can be used as a new tool in modular chemistry. Therefore, we are working on large guest molecules that are partly inside and partly outside the dendritic box. The inside part is fuctionalized with an anchoring group and a spacer is used to make these large guests compatible with the shell [21]. When the large part that is outside the box becomes functional or possesses functional groups, it should be possible to build larger structures, comparable with the key-and-lock principle, recently published by Newkome et al. [22]. It is very appealing to us to exploit the functionality of four as is found for the maximum number of many encapsulated guests. The approach described here based on the dendritic box will lead to modular chemistry at the nanoscopic level and by using mechanical bonding between modules.

7. Acknowledgement

8. References

1. Tomalia, D.A., Naylor, A.M., Goddard ,W.A. III, Starburst-Dendrimere: Kontrolle von Grösse, Gestalt, Oberflächenchemie, Topologie und Flexibilität beim Übergang von Atomen zu makroscopischer Materie, (1990) *Angew. Chem.,* **102,** 119.

2. Fréchet , J.M.J., Functional Polymers and Dendrimers: Reactivity, Molecular Architecture, and Interfacial Energy, (1994) *Science,* **263,** 1710.

3. Newkome, G.R., Moorefield, C.N., Baker, G.R., Saunders, M.J., Grossman, S.H., Unimoleculare Micellen, (1991) *Angew. Chem.,* **103,** 1207.

4. Tomalia, D.A., Baker, H., Dewald, J., Hall, M., Kallos, G., Martin, S., Roeck, J., Ryder, J., Smith, P., A New Class of Polymers: Starburst-Dendritic Macromolecules, (1985) *Polymer Journal,* **17,** 117.

5. Wooley, K.L., Hawker, G.J., Fréchet, J.M.J., Unsymmetrical Three-Dimensional Macromolecules: Preparation and Characterization of Strongly Dipolar Dendritic Macromolecules, (1993) *J. Am. Chem. Soc.,* **115,** 11496.

6. Tomalia, D.A., Durst, D. (1993) *Topics in Current Chemistry,* Weber E. (ed), Springer-Verlag, Berlin, **165,** 93.

7. Buhleier, E., Wehner, W., Vögtle, F., "Cascade"- and "Nonskid-Chain-like" Syntheses of Molecular Cavity Topologies, (1978) *Synthesis,* **155.**

8. Maciejewski, M., Concepts of Trapping Topologically by Shell Molecules, (1982) *J. Macromol. Sci. - Chem.,* **A17,** 689.

9. Jansen, J.F.G.A., de Brabander - van den Berg, E.M.M., Meijer, E.W., Guest molecules encapsulated into a Dendritic Box, (1994) *Science,* **266,** 1226.

10. De Brabander - van den Berg, E.M.M., Meijer, E.W., Poly(propylenimin)-Dendrimere: Synthese in grösserem Massstab durch heterogen katalysierte Hiedrierungen, (1993) *Angew. Chem.,* **105,** 1370.

11. Jansen, J.F.G.A., Meijer, E.W., manuscript in preparation.

12. Jansen, J.F.G.A., Janssen, R.A.J., de Brabander - van den Berg, E.M.M., Meijer, E.W., Triplet Radical Pairs of 3-Carboxyproxyl Encapsulated in a Dendritic Box, (1995) *Adv. Mat.,* **7,** 561.

13. Jansen, J.F.G.A., Peerlings, H.W.I., de Brabander - van den Berg, E.M.M., Meijer, E.W., Optische Aktivität chiraler dendritischer Oberflächen, (1995) *Angew. Chem.,* **107,** 1321.

14. Jansen, J.F.G.A., de Brabander - van den Berg, E.M.M., Meijer, E.W., Induced Chirality of Guest Molecules encapsulated into a Dendritic Box, (1995) *Recl. Trav. Chim. Pays-Bas,* **114,** 225.

15. Peerlings, H.W.I., Jansen, J.F.G.A., de Brabander - van den Berg, E.M.M., Meijer, E.W., Optical activity of dendrimers with chiral end groups, (1995) *ACS, Polym. Sci. & Eng. ,* **73,** 342.

16. Winther, C., Zur Theorie der optischen Drehung III, (1907) *Z. Phys. Chem.,* **60,** 590.

17. Eliel, E.L., Wilen, S.H. (1994) *Stereochemistry of Organic Compounds,* J. Wiley & Sons Inc., New York, **1076** (1994).

18. Kurzitake, T., Nakashima, N., Moritsu, K., Enhanced circular dichroism and fluidity of disk-like aggregates of a chiral, single-chain amphiphile, (1980) *Chem. Lett.,* **1347.**

19. Bosman, A.W., Janssen, R.A.J., Meijer, E.W., unpublished results.

20. Jansen, J.F.G.A., de Brabander - van den Berg, E.M.M., Meijer, E.W., The Dendritic Box: Shape-Selective Liberation of Encapsulated Guests, (1995) *J. Am. Chem. Soc.,* **117,** 4417.

384

21. Van Hest, J.C.M., Jansen, J.F.G.A., Meijer, E.W., unpublished results.
22. Newkome, G.R., Güther, R., Moorefield, C.N., Cardullo, F., Echegoyen, L., Pérez-Cordero, E., Luftmann, H., Wege zu dendritischen Netzwerken: Bis-Dendrimere durch Verknüpfung von Kaskadenmolekülen über Metallzentren (1995) *Angew. Chem.*, **107**, 2159.