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The Internal Structure of Helical Pores Self-Assembled from Dendritic Dipeptides is Stereochemically Programmed and Allosterically Regulated**

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Pore-forming proteins, peptides, and their remodeled structures perform diverse biological and biologically inspired functions. These include the formation of viral helical coats[1a] and transmembrane channels,[1b,c] mediation of protein folding,[1d] reversible encapsulation,[1e] stochastic sensing,[1f] as well as pathogenic[1g] and antibacterial[1h] activity. Synthetic strategies to obtain porous or tubular supramolecular assemblies have been elaborated.[2] However, only several synthetic supramolecular pore structures are stable in solution and in solid state.[3] Recently, we reported a class of amphiphilic dendritic dipeptides (amphiphilic dendrons functionalized at their apex with a dipeptide) that self-assemble both in solution and in bulk into supramolecular helical pores.[4a] A *trans* conformation of the dendron is required to mediate self-assembly and to provide a new hydrogen-bonding mechanism of a parallel and partially interdigitated array of dipeptides. This conformation is selected by a solvophobic solvent[4a] or in solid state by the microsegregation of the aliphatic and aromatic parts of the dendron. It has also been suggested that the stereochemistry of the peptide determines the sense of the helical pore.[4]

Herein, we report that the structure of the helical porous structure self-assembled from dendritic dipeptides is programmed by the stereochemistry encoded in the dipeptide. A combination of solution and solid-state analysis techniques together with molecular modeling has been used to provide the sequential pathway to this self-assembly process that

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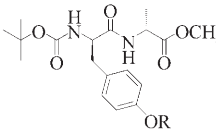
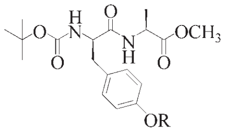
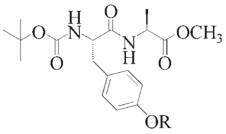
seems to operate by allosteric[5] control. The dendritic dipeptide used in these investigations is (4-3,4-3,5)12G$_2$-CH$_2$-X, in which X = Boc–L-Tyr–L-Ala–OMe, Boc–D-Tyr–D-Ala–OMe, Boc–L-Tyr–D-Ala–OMe and Boc–D-Tyr–L-Ala–OMe, and Boc = *tert*-butoxycarbonyl.



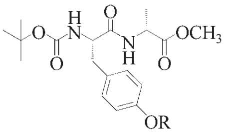
D-D



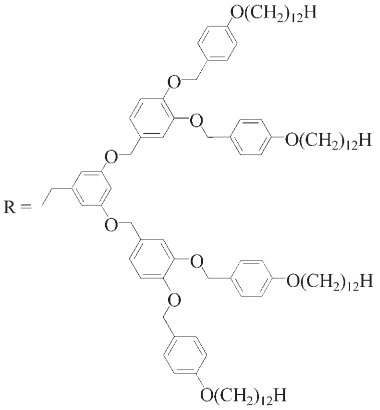
D-L



L-L



L-D



In a previous communication,[4a] we reported the self-assembly of an L-L dendritic dipeptide in solvophobic solvents[2f,6a,6b] such as cyclohexane and deuterated cyclohexane by a combination of ^{1}H NMR spectroscopy, UV/Vis and circular dichroism (CD) techniques. Small- and wide-angle X-ray diffraction (XRD) experiments were carried out on powder and oriented fibers obtained from the porous structures assembled from L-L, D-D, L-D, D-L, and from the racemic dendritic dipeptides. The pore diameter (d_{pore}) was computed for all samples from XRD data[4a,b] at temperatures between 71 and 75.4 °C in their 2D hexagonal columnar liquid-crystal phase. XRD experiments on oriented fibers concluded that the amphiphilic dendron mediates the self-assembly into a helical structure, the helical sense of which is endowed by the stereochemistry of the Tyr residue. This mechanism is related to other examples in which a stereocenter determines the twist sense of racemic helical structures,[6a–e] as suggested by the groundbreaking work of Green et al.[6e] However, the assembly of our system differs from published examples in which a stereocenter induces helicity in an achiral tubular assembly[2g] and in other supramolecular structures.[6f] It is also the dipeptide part of the dendron apex that directs the self-assembly into porous[4a] rather than closed supramolecular columnar structures.[7] However, the role of the stereochemistry of Ala, the potential cooperativity between the stereochemistry of Ala and Tyr, the structure of the dendron in the supramolecular assembly, and the stability of the porous structure at various temperatures are important mechanistic questions that were not previously addressed.[4a]

The CD data of L-L, D-D, L-D, and D-L dendritic dipeptides obtained at different temperatures during their self-assembly in cyclohexane are compared in Figure 1. In all cases, only the weak positive or negative Cotton effect associated with the molecular solution of a dendritic dipeptide[4a] is observed above 30 °C (inset in Figure 1). Below 30 °C the dendritic dipeptides self-assemble into helical porous columns, and their CD data are associated with the aromatic part of the helical supramolecular dendrimer as demonstrated by wide-angle XRD on oriented fibers[4a] (Figure 2), and by CD and

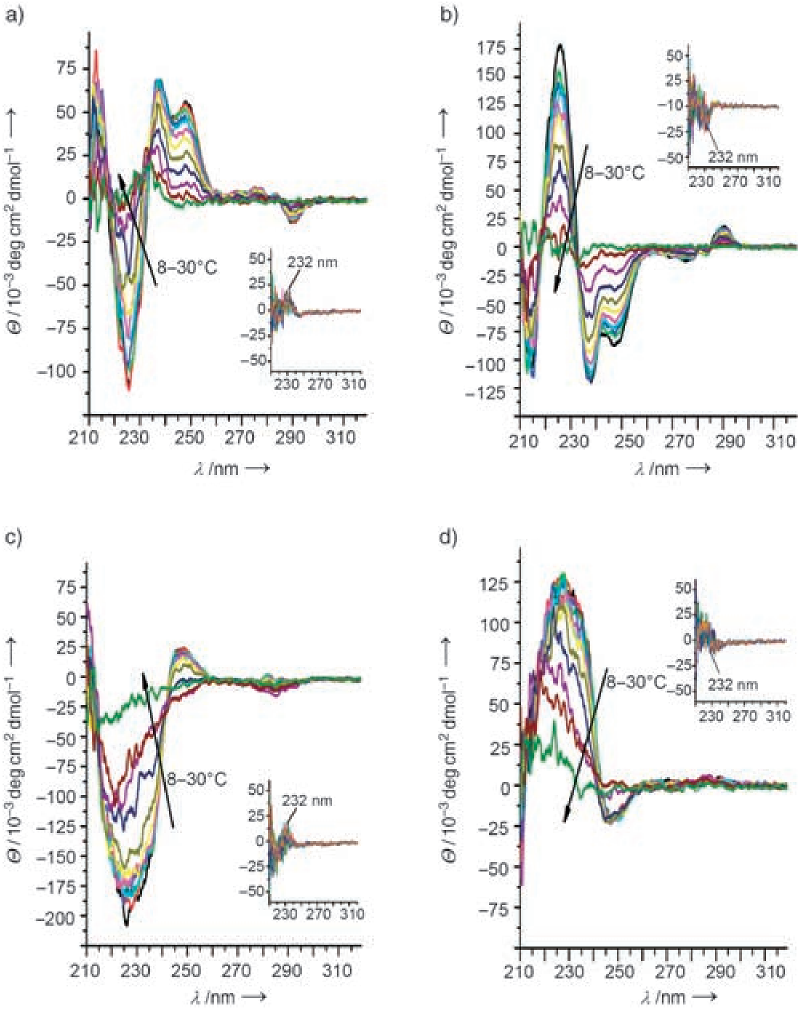


Figure 1. CD spectra (1.6×10^{-4} M in cyclohexane) of a) (4-3,4-3,5)12G$_2$–CH$_2$–Boc–L-Tyr–L-Ala–OMe; b) (4-3,4-3,5)12G$_2$–CH$_2$–Boc–D-Tyr–D-Ala–OMe; c) (4-3,4-3,5)12G$_2$–CH$_2$–Boc–L-Tyr–D-Ala–OMe; d) (4-3,4-3,5)12G$_2$–CH$_2$–Boc–D-Tyr–L-Ala–OMe. The arrows indicate temperature increases. Insets: 30–60 °C.

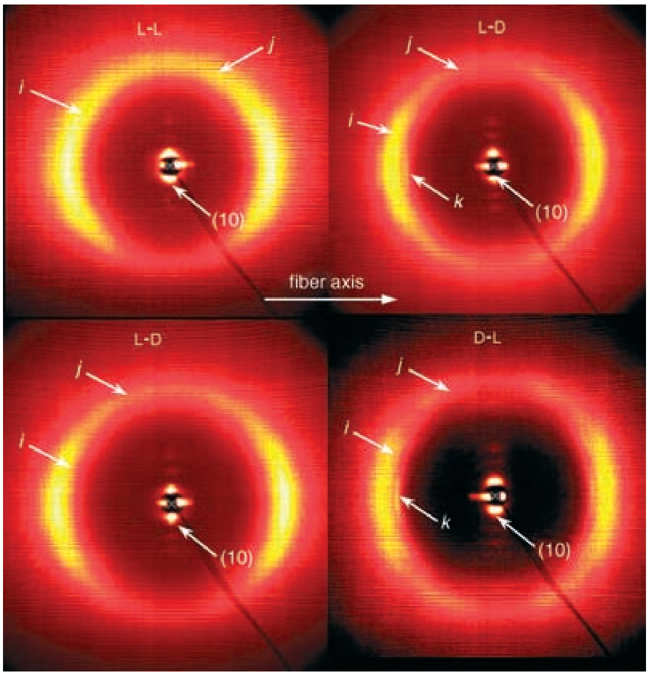


Figure 2. X-ray diffraction patterns of aligned fibers collected at 23 °C from the dendritic dipeptides L-L, D-D, L-D, and D-L. *i* = short-range helical feature of dendron at ≈4.5 Å, *j* = dendron tilt ≈60°, *k* = 5-Å registry along the column axis feature.

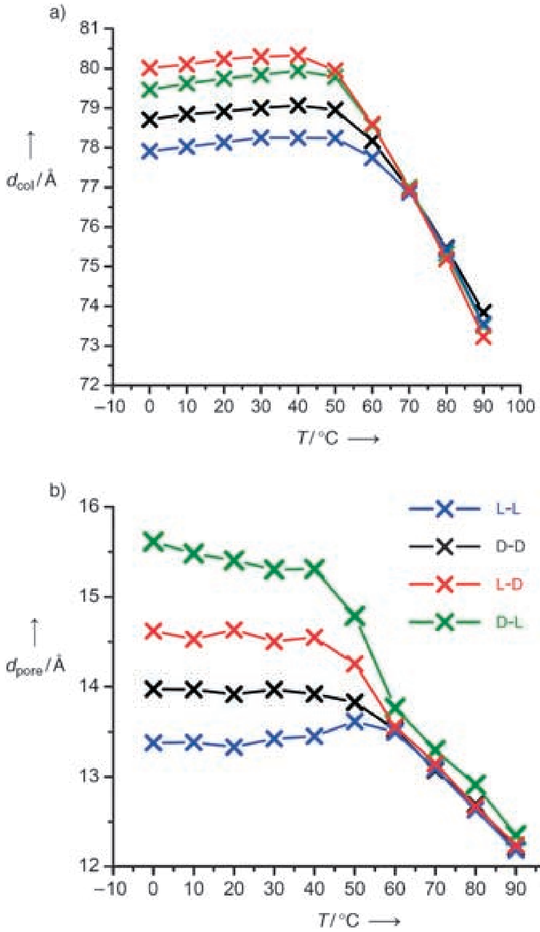


Figure 3. Influence of dipeptide stereochemistry and temperature on a) column diameter and b) pore diameter.

UV/Vis spectra of model dendrons with and without dipeptide stereocenters (Supporting Information). The CD data of L-L with D-D, and of L-D with D-L dendritic dipeptide assemblies show respective mirror-image Cotton effects. However, the CD results of L-L and D-D are different from those of L-D and D-L. This structural difference observed by CD is supported by the XRD patterns obtained from aligned samples (Figure 2). The XRD of L-L is similar with that of D-D while that of L-D to that of D-L derived porous structures. The *k* diffraction of L-D and D-L is sharper than that of L-L and D-D. In L-L and D-D, *k* is most likely overlapped with the *i* diffraction (Figure 2).

The difference between the CD of the L-L/D-D pair and those of the L-D/D-L pair indicates that the stereochemistry of Ala also plays an important role in the programming of the supramolecular pore structure. These different CD data are generated by various modes of intramolecular and intermolecular dendron arrangements in the helical porous structure that are induced by dipeptide stereochemistry.

To quantify the role of the stereochemistry of Tyr and Ala we used both small- and wide-angle XRD to investigate the structure of the supramolecular pore. The temperature-dependence of the column (d_{col}) and pore (d_{pore}) diameters[4a,b] is shown in Figure 3 for all combinations of dipeptide stereochemistry (XRD data provided in the Supporting Information). Regardless of the dipeptide stereochemistry, the supramolecular assemblies exhibit a glass transition (T_g) at 57 to 60 °C, followed by the 2D columnar hexagonal phase that becomes an isotropic liquid at 94 °C for L-D and D-L, and at 96 °C for L-L and D-D.[4a] Within experimental error, both d_{col} and d_{pore} are temperature-dependent above T_g and temperature-independent below T_g (Supporting Information). There are two messages provided in Figure 3. The large slopes of d_{col} and d_{pore} temperature-dependence above T_g and below the isotropic state introduce large errors in the estimation of the role of stereochemistry on pore structure in this temperature range. However, the constant values of d_{pore} and d_{col} below T_g provide access to a quantitative structure analysis in the temperature range of interest for practical applications. As can be observed in Figure 3b, the stereochemistry of the dipeptide influences d_{pore}. Within the experimental error of the XRD method[4a,b] and of the enantiomeric purity of the Tyr and Ala used (Supporting Information), d_{col} and d_{pore} of L-L are equal to D-D and the respective values for L-D are equal to D-L. A combination of small- and wide-angle XRD results, molecular modeling, and simulation experiments together with density data[4a] was used to create the structure of the porous columns below T_g. Figure 4 indicates that the helical sense of each column is determined by the stereochemistry of Tyr. The cross-section of the porous columns is illustrated in Figure 5. These cross-sections show that within experimental error, the internal structure obtained from L-L is identical to that obtained from

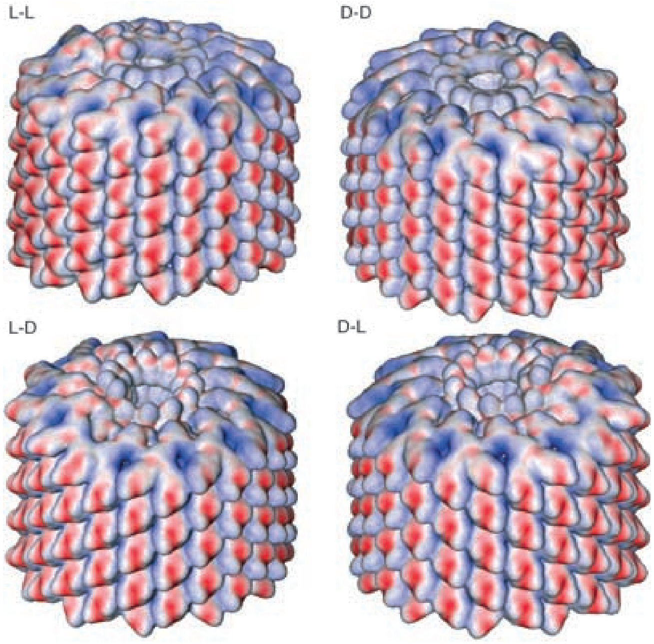


Figure 4. Molecular models of the helical porous columns constructed from XRD, molecular modeling, and density data showing the influence of dipeptide stereochemistry on the helical sense of the column. For simplicity, the alkoxy groups of the dendrons were replaced with methoxy groups.

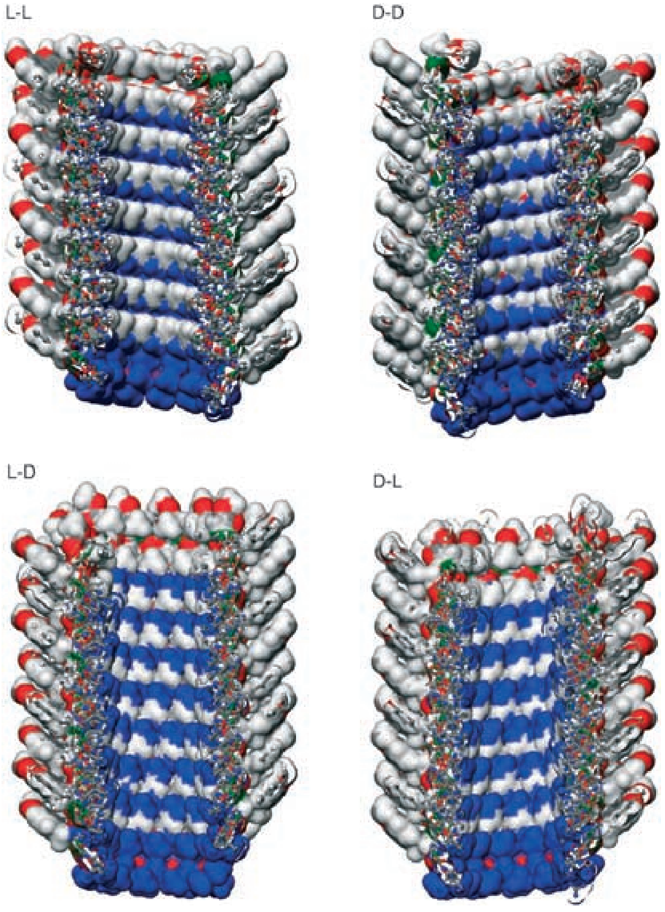


Figure 5. Influence of dipeptide stereochemistry on the cross-section structure of the hydrophobic pore and d_{pore} below T_g: L-L, $d_{\mathrm{pore}} = 13.4 \pm 1.2$ Å; D-D, $d_{\mathrm{pore}} = 14.0 \pm 1.2$ Å; L-D, $d_{\mathrm{pore}} = 14.6 \pm 1.4$ Å; D-L, $d_{\mathrm{pore}} = 15.6 \pm 1.4$ Å (white = methyl groups of Ala, blue = methyl groups of Boc, red = oxygen atoms, green = N–H of dipeptides, gray = other carbon atoms). These structures were obtained as cross-sections of the columns from Figure 4. For simplicity, the dendrons are not shown.

the D-D dendritic dipeptide, whereas that of L-D is similar to D-L. In all cases, the internal structure of the pore is hydrophobic.

The mechanism responsible for the construction of the pore can be envisioned by inspecting the dendritic dipeptide conformations from the supramolecular structures presented in Figure 4 and 5. Fragments of these dendritic dipeptide conformations containing only the benzyl ether dendritic unit attached to Tyr are compared in Figure 6 (the complete dendritic dipeptide conformations are in the Supporting Information). In all stereoisomers, the hydroxyphenyl group of Tyr is in an *anti* conformation with respect to the Boc group, whereas the dendron is *syn*. The orientation of the tetrahedral carbon atom of the Tyr stereocenter determines the dendron sense and therefore the handedness of the helix. To create a larger pore size with the stereochemistry of L-Tyr–D-Ala and D-Tyr–L-Ala, the methyl group of Ala must be located in the inner part of the pore, whereas in the case of L-Tyr–L-Ala and D-Tyr–D-Ala the methyl ester group of Ala is in the inner part of the pore. This model suggests that the stereochemistry of the dipeptide influences the conformation of the dendron, which in turn determines the conformation of the Ala part of the dipeptide. This cooperative process[4a] resembles a primitive allosteric control mechanism.[5]

The cooperativity between the stereochemistry of Tyr and Ala seems to be required to switch the overall conformation of the dendritic dipeptide and therefore, to determine the internal hydrophobic structure of the pore below T_g. The parallel and partially interdigitated arrangement of peptides in the pore[4a] facilitates the formation of the hydrogen-bonding network structure shown in Figure 7. This structure can be divided in two main types of hydrogen-bonding networks. The first is the bonding network inside one layer indicated by *j*–*k* or *l*–*m*. This is the most important for the stability and order of the supramolecular assembly. These directional in-layer hydrogen bonds are responsible for the translation along the column axis from one unit to the proximal neighbors. This translation step is controlled both by the dipeptide and by the dendron size. The in-layer network is independent on dipeptide stereochemistry and creates a *j*–*k*–*j*–*k*–*j*–*k*… or *l*–*m*–*l*–*m*–*l*–*m*… helical pattern (Supporting Information). The second hydrogen-bonding network is programmed by dipeptide stereochemistry containing the hydrogen bonds between n^{th} to $n \pm 12^{\mathrm{th}}$ units. For L-Tyr–L-Ala and D-Tyr–D-Ala, the oxygen atoms of Ala do not participate in any hydrogen bond, whereas for L-Tyr–D-Ala and D-Tyr–L-Ala, the Ala oxygen atoms create the second cross-layer network of hydrogen bonds.

L-Tyr–L-Ala and D-Tyr–D-Ala supramolecular structures have a smaller pore size than L-Tyr–D-Ala and D-Tyr–L-Ala, which indicates that the Ala stereochemistry affects the pore

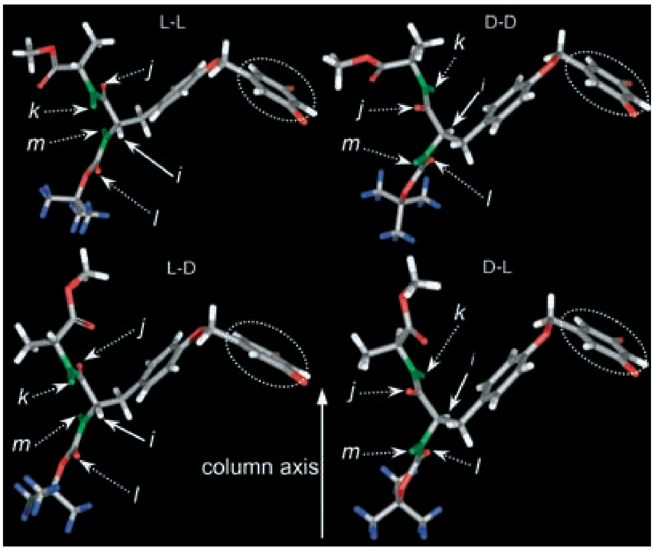


Figure 6. Influence of dipeptide stereochemistry on dipeptide conformation in the assembled supramolecular porous columns from Figure 4: *i* indicates that orientation of the L-Tyr or D-Tyr tetrahedral stereocenter determines the rotation sense, whereas the strong directional in-layer hydrogen-bonding networks *j*–*k*–*j*–*k*–*j*–*k*... or *l*–*m*–*l*–*m*–*l*–*m*... require a translation along the column axis in the supramolecular assembly. The proposed mechanism explains the cause of the helical supramolecular columnar assembly in which the stereochemistry of Tyr determines the helical sense. For simplicity, only the first benzyl ether of the dendron is shown (dotted circle).

size. At the same time, there is a slightly larger distance between layers for the latter two (see the 5-Å registry feature in Figure 2). These two results are reflected in dipeptide conformation (Figure 7), with the Ala upper region oriented perpendicular to the column axis for the L-Tyr–L-Ala or D-Tyr–D-Ala, and parallel to the column axis for L-Tyr–D-Ala or D-Tyr–L-Ala. The supramolecular helical hydrogen-bonded structure between *k*–*j* and *l*–*m* is shown in Supporting Information together with the inner and outer regions of the pore (the other cross-layer hydrogen bonds have been omitted for simplicity).

In conclusion, we report herein the first synthetic example of a helical porous supramolecular structure self-assembled from a hybrid dendritic dipeptide in which the internal structure is programmed by the stereochemistry of the dipeptide. The amphiphilic dendron is responsible for the assembly of the dipeptide into a parallel and partially interdigitated porous structure.[4a] The stereochemistry of Tyr selects the sense of the helical porous structure. However, the stereochemistry of the entire dipeptide determines the overall arrangement of the dendron in the supramolecular structure, which in turn determines the conformation of the dipeptide and the structure and diameter of the pore in a cooperative process. This supramolecular porous structure is stable over a broad range of temperatures, has a constant d_{pore} below T_g and represents the first example of a supramolecular dendronized tubular polymer[7,8] exhibiting a 3D structure[9] that is stereochemically programmed. Allosteric regulation[5] seems to be operating in this process. This self-assembly concept expands the synthetic capabilities of dendrimers and dendrons.[10]

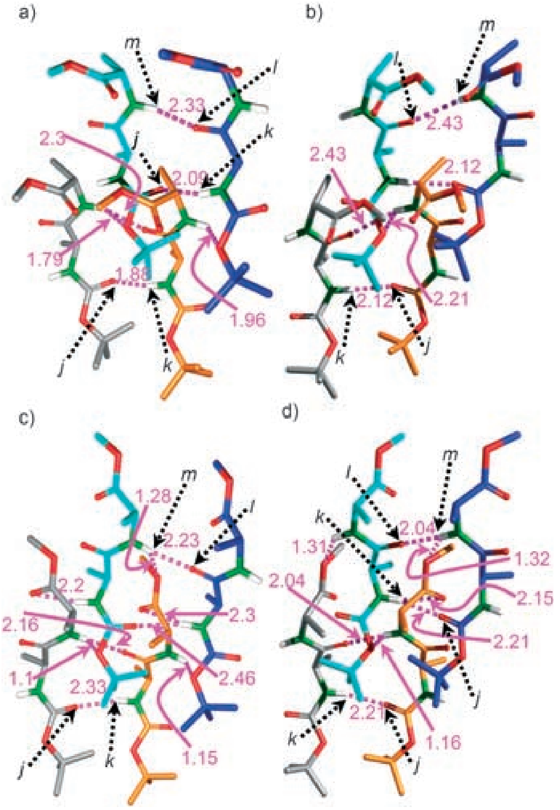


Figure 7. Hydrogen-bonding network determined by a combination of ^{1}H NMR spectroscopy, XRD analysis, and molecular modeling[4a] for a) L-L, b) D-D, c) L-D, and d) D-L dendritic dipeptides during the formation of the supramolecular pore structure (all distances in Å). The strongest in-layer directional hydrogen bonds are labeled by the same convention as in Figure 6 (*j*–*k* and *l*–*m*). The column axis is vertical; for simplicity only the N-bound hydrogen atoms are shown (white).

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[1] a) A. Klug, *Angew. Chem.* **1983**, *95*, 579–596; *Angew. Chem. Int. Ed. Engl.* **1983**, *22*, 565–636; b) R. MacKinnon, *Angew. Chem.* **2004**, *116*, 4363–4376; *Angew. Chem. Int. Ed.* **2004**, *43*, 4265–4277; c) P. Agre, *Angew. Chem.* **2004**, *116*, 4377–4390; *Angew. Chem. Int. Ed.* **2004**, *43*, 4278–4290; d) K. Braig, Z. Otwinowski, R. Hegde, D. C. Boisvert, A. Joachimiak, A. L. Horwich, P. B. Sigler, *Nature* **1994**, *371*, 578–586; e) D. Ishii, K. Kinbara, Y. Ishida, N. Ishii, M. Okochi, M. Yohda, T. Aida, *Nature* **2003**, *423*, 628–632; f) H. Bayley, P. S. Cremer, *Nature* **2001**, *413*, 226–230; g) E. Gouaux, *J. Struct. Biol.* **1998**, *121*, 110–122; h) S. Fernandez-Lopez, H.-S. Kim, E. C. Choi, M. Delgado, J. R. Granja, A. Khasanov, K. Kraehenbuehl, G. Long, D. A. Weinberger, K. M. Wilcoxen, M. R. Ghadiri, *Nature* **2001**, *412*, 452–455.

[2] a) U. F. Kragten, M. F. M. Raks, R. J. M. Nolte, *J. Chem. Soc. Chem. Commun.* **1985**, 1275–1276; b) M. J. Pregel, L. Juillien, J.-

M. Lehn, *Angew. Chem.* **1992**, *104*, 1695 – 1697; *Angew. Chem. Int. Ed. Engl.* **1992**, *31*, 1637 – 1640; c) G. W. Gokel, R. Ferdani, J. Liu, R. Pajewski, H. Shabany, R. Utrecht, *Chem. Eur. J.* **2001**, *7*, 33 – 39; d) D. T. Bong, T. D. Clark, J. R. Granja, M. R. Ghadiri, *Angew. Chem.* **2001**, *113*, 1016 – 1041; *Angew. Chem. Int. Ed.* **2001**, *40*, 988 – 1011; e) S. Rosselli, A.-D. Ramminger, T. Wagner, B. Silier, S. Wiegand, W. Häussler, G. Lieser, V. Scheumann, S. Höger, *Angew. Chem.* **2001**, *113*, 3233 – 3237; *Angew. Chem. Int. Ed.* **2001**, *40*, 3137 – 3141; f) D. J. Hill, M. J. Mio, R. B. Prince, T. S. Hughes, J. S. Moore, *Chem. Rev.* **2001**, *101*, 3893 – 4011; g) H. Fenniri, B.-L. Deng, A. E. Ribbe, *J. Am. Chem. Soc.* **2002**, *124*, 11 064 – 11 072; h) Y. Kim, M. F. Mayer, S. C. Zimmerman, *Angew. Chem.* **2003**, *115*, 1153 – 1158; *Angew. Chem. Int. Ed.* **2003**, *42*, 1121 – 1126; i) S. Hecht, A. Khan, *Angew. Chem.* **2003**, *115*, 6203 – 6206; *Angew. Chem. Int. Ed.* **2003**, *42*, 6021 – 6024; j) N. Sakai, J. Mareda, S. Matile, *Acc. Chem. Res.* **2005**, *38*, 79 – 87.

[3] a) M. R. Ghadiri, R. J. Granja, R. A. Milligan, D. E. McRee, N. Khazanovich, *Nature* **1993**, *366*, 324 – 327; b) A. Petitjean, L. A. Cuccia, J.-M. Lehn, H. Nierengarten, M. Schmutz, *Angew. Chem.* **2002**, *114*, 1243 – 1246; *Angew. Chem. Int. Ed.* **2002**, *41*, 1195 – 1198; c) M. Ohkita, J.-M. Lehn, G. Baum, D. Fenske, *Chem. Eur. J.* **1999**, *5*, 3471 – 3481; d) J.-L. Schmitt, A.-M. Stadler, N. Kyritsakas, J.-M. Lehn, *Helv. Chim. Acta* **2003**, *86*, 1598 – 1624; e) J.-L. Schmitt, J.-M. Lehn, *Helv. Chim. Acta* **2003**, *86*, 3417 – 3426.

[4] a) V. Percec, A. E. Dulcey, V. S. K. Balagurusamy, Y. Miura, J. Smidrkal, M. Peterca, S. Nummelin, U. Edlund, S. D. Hudson, P. A. Heiney, H. Duan, S. N. Maganov, S. A. Vinogradov, *Nature* **2004**, *430*, 764 – 768; b) D. C. Turner, S. M. Gruner, *Biochemistry* **1992**, *31*, 1340 – 1355.

[5] a) J. Monod, J.-P. Changeux, F. Jacob, *J. Mol. Biol.* **1963**, *6*, 306 – 329; b) M. Perutz, *Mechanisms of Cooperativity and Allosteric Regulation in Proteins*, Cambridge University Press, Cambridge, UK, **1990**; c) P. R. Evans, *Curr. Opin. Struct. Biol.* **1991**, *1*, 773 – 779.

[6] a) J. C. Nelson, J. G. Saven, J. S. Moore, P. G. Wolynes, *Science* **1997**, *277*, 1793 – 1796; b) R. B. Prince, L. Brunsveld, E. W. Meijer, J. S. Moore, *Angew. Chem.* **2000**, *112*, 234 – 236; *Angew. Chem. Int. Ed.* **2000**, *39*, 228 – 230; c) A. E. Rowan, R. J. M. Nolte, *Angew. Chem.* **1998**, *110*, 65 – 71; *Angew. Chem. Int. Ed.* **1998**, *37*, 63 – 68; d) J. J. L. M. Cornelissen, A. E. Rowan, R. J. M. Nolte, N. A. J. M. Sommerdijk, *Chem. Rev.* **2001**, *101*, 4039 – 4070; e) M. M. Green, J.-W. Park, T. Sato, A. Teramoto, S. Lifson, R. L. B. Selinger, J. V. Selinger, *Angew. Chem.* **1999**, *111*, 3328 – 3345; *Angew. Chem. Int. Ed.* **1999**, *38*, 3138 – 3154; f) J.-H. Fuhrhop, J. Köning, *Membranes in Molecular Assemblies. The Synkinetic Approach*, Cambridge University Press, Cambridge, UK, **1994**.

[7] a) S. D. Hudson, H.-T. Jung, V. Percec, W.-D. Cho, G. Johansson, G. Ungar, V. S. K. Balagurusamy, *Science* **1997**, *278*, 449 – 452; b) V. Percec, C.-H. Ahn, G. Ungar, D. J. P. Yeardley, M. Möller, S. S. Sheiko, *Nature* **1998**, *391*, 161 – 164; c) V. Percec, M. Glodde, T. K. Bera, Y. Miura, I. Shiyanovskaya, K. D. Singer, V. S. K. Balagurusamy, P. A. Heiney, I. Schnell, A. Rapp, H.-W. Spiess, S. D. Hudson, H. Duan, *Nature* **2002**, *419*, 384 – 387.

[8] a) J.-M. Lehn, *Polym. Int.* **2002**, *51*, 825 – 839; b) J. S. Moore, *Curr. Opin. Colloid Interface Sci.* **1999**, *4*, 108 – 116; c) L. Brunsveld, B. J. B. Folmer, E. W. Meijer, R. P. Sijbesma, *Chem. Rev.* **2001**, *101*, 4071 – 4097.

[9] S. Hecht, *Mater. Today* **2005**, *8*, 48 – 55.

[10] a) T. Emrick, J. M. J. Fréchet, *Curr. Opin. Colloid Interface Sci.* **1999**, *4*, 15 – 23; b) D. A. Tomalia, *Mater. Today* **2005**, *8*, 34 – 46.