may be large enough to allow the smallest atoms, molecules, or ions (He, H_2, Li^+, Be^{2+}) to pass through, a possibility that is currently under investigation. The cavity of **11** is essentially restrained only by the third unopened five-membered ring bearing an unfavorable endocyclic [5,6] double bond, and oxidation or other addition reactions may be able to cleave this bond. More importantly, the fragile azide functions of dienes **1a–c** can be replaced with other 1,3-dipoles to effect a reaction course similar to that of Scheme 2. Preliminary experiments with the bis-nitrile oxide system analogous to **1a** provide encouraging results.

Received: April 22, 1999 [Z13305IE]
German version: *Angew. Chem.* **1999**, *111*, 2508–2512

Keywords: azides · cycloadditions · Diels–Alder reactions · fullerenes · semiempirical calculations

[1] a) Y. Rubin, *Chem. Eur. J.* **1997**, *3*, 1009–1016; b) Y. Rubin, *Top. Curr. Chem.* **1999**, *199*, 67–91.

[2] a) J. C. Hummelen, M. Prato, F. Wudl, *J. Am. Chem. Soc.* **1995**, *117*, 7003–7004; b) L. L. Shiu, K. M. Chien, T. Y. Liu, T. I. Lin, G. R. Her, T. Y. Luh, *J. Chem. Soc. Chem. Commun.* **1995**, 1159–1160; c) C. K. F. Shen, H. Yu, C.-G. Juo, K.-M. Chien, G.-R. Her, T.-Y. Luh, *Chem. Eur. J.* **1997**, *3*, 744–748; d) G. X. Dong, J. S. Li, T. H. Chan, *J. Chem. Soc. Chem. Commun.* **1995**, 1725–1726; e) P. R. Birkett, A. G. Avent, A. D. Darwish, H. W. Kroto, R. Taylor, D. R. M. Walton, *J. Chem. Soc. Chem. Commun.* **1995**, 1869–1870; f) M. J. Arce, A. L. Viado, Y. Z. An, S. I. Khan, Y. Rubin, *J. Am. Chem. Soc.* **1996**, *118*, 3775–3776; g) G. Schick, A. Hirsch, H. Mauser, T. Clark, *Chem. Eur. J.* **1996**, *2*, 935–943.

[3] a) A. Hirsch, *The Chemistry of the Fullerenes*, Thieme, Stuttgart, **1994**; b) A. Hirsch, *Top. Curr. Chem.* **1999**, *199*, 1–65.

[4] a) M. Prato, C. Li, F. Wudl, V. Lucchini, *J. Am. Chem. Soc.* **1993**, *115*, 1148–1150; b) T. Grösser, M. Prato, V. Lucchini, A. Hirsch, F. Wudl, *Angew. Chem.* **1995**, *107*, 1462–1464; *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 1343–1345; c) B. Nuber, F. Hampel, A. Hirsch, *Chem. Commun.* **1996**, 1799–1800; d) J. Averdung, J. Mattay, *Tetrahedron* **1996**, *52*, 5407–5420; e) M. R. Banks, J. I. G. Cadogan, I. Gosney, P. K. G. Hodgson, P. R. R. Langridge-Smith, D. W. H. Rankin, *J. Chem. Soc. Chem. Commun.* **1994**, 1365–1366.

[5] a) Y. Rubin, S. Khan, D. I. Freedberg, C. Yeretzian, *J. Am. Chem. Soc.* **1993**, *115*, 344–345; b) Y. Z. An, G. A. Ellis, A. L. Viado, Y. Rubin, *J. Org. Chem.* **1995**, *60*, 6353–6361; c) Y. Z. An, A. L. Viado, M. J. Arce, Y. Rubin, *J. Org. Chem.* **1995**, *60*, 8330–8331.

[6] In deference to Prof. Emmanuel Vogel's life-long work on bridged annulenes, we propose that this nomenclature be followed over the current IUPAC and CAS systems; see a) E. Vogel, W. Pretzer, W. A. Böll, *Tetrahedron Lett.* **1965**, 3613–3617; b) F. Gerson, G. Gescheidt, U. Buser, E. Vogel, J. Lex, M. Zehnder, A. Riesen, *Angew. Chem.* **1989**, *101*, 938–940; *Angew. Chem. Int. Ed. Engl.* **1989**, *28*, 902–904; c) J. A. Marco, J. F. Sanz, E. Vogel, B. Schwartzkopff-Fischer, H. Schmickler, J. Lex, *Tetrahedron Lett.* **1990**, *31*, 999–1002; see also ref. [2c].

[7] H. Schmidbaur, R. Hager, B. Huber, G. Müller, *Angew. Chem.* **1987**, *99*, 354–356; *Angew. Chem. Int. Ed. Engl.* **1987**, *26*, 338–340.

[8] See the Supporting Information.

[9] A number of crystallization attempts for X-ray crystallographic determination were performed with the three series of derivatives described in this work. So far, only microcrystalline material has been obtained.

[10] Heating of 1,4-diphenylbutadiene, *o,o'*-dinitro-1,4-diphenylbutadiene, or *o,o'*-diamino-1,4-diphenylbutadiene with C_{60} in toluene or ODCB at temperatures up to 180 °C gave new products in neither case.

[11] Y. Rubin, P. S. Ganapathi, A. Franz, Y.-Z. An, W. Qian, R. Neier, *Chem. Eur. J.* **1999**, *5*, No. 11.

[12] a) W. Oppolzer, *Angew. Chem.* **1977**, *89*, 10–24; *Angew. Chem. Int. Ed. Engl.* **1977**, *16*, 10–23; b) G. Brieger, J. N. Bennett, *Chem. Rev.* **1980**, *80*, 63–97; c) A. G. Fallis, *Can. J. Chem.* **1984**, *62*, 183–234; d) D. Craig, *Chem. Soc. Rev.* **1987**, *16*, 187–238.

[13] For the chemistry of 1,4-diaminobutadienes, see a) R. Sustmann, K. Lücking, G. Kopp, M. Rese, *Angew. Chem.* **1989**, *101*, 1701–1704; *Angew. Chem. Int. Ed. Engl.* **1989**, *28*, 1713–1715; b) D. H. R. Barton, C. C. Dawes, G. Franceschi, M. Foglio, S. V. Ley, P. D. Magnus, W. L. Mitchell, A. Temperelli, *J. Chem. Soc. Perkin Trans. 1* **1980**, 643–647; c) R. R. Schmidt, A. Wagner, *Synthesis* **1981**, 273–275.

[14] Reaction with triplet O_2 upon workup cannot be entirely excluded. However, singlet oxygen is generated in close to quantitative yields upon excitation of most fullerene derivatives by visible light: J. L. Anderson, Y. Z. An, Y. Rubin, C. S. Foote, *J. Am. Chem. Soc.* **1994**, *116*, 9763–9764, and ref. [5c].

[15] a) C. S. Foote, S. Wexler, *J. Am. Chem. Soc.* **1964**, *86*, 3879–3880; b) C. S. Foote, S. Wexler, W. Ando, R. Higgins, *J. Am. Chem. Soc.* **1968**, *90*, 975–981; c) T. Sato, I. Moritani, M. Matsuyama, *Tetrahedron Lett.* **1969**, 5113–5116; d) Y. Nakaraida, T. Nomura, S. Kanouchi, R. Sato, C. Kabuto, H. Sakurai, *Chem. Lett.* **1983**, 209–212; e) G. Rio, J. Berthelot, *Bull. Soc. Chim. Fr.* **1970**, 1509–1511; f) G. Rio, J. Berthelot, *Bull. Soc. Chim. Fr.* **1971**, 2938–2942.

Self-Assembly of a Tetrahedral Lectin into Predesigned Diamondlike Protein Crystals**

Nir Dotan, Dorit Arad, Felix Frolow, and Amihay Freeman*

The implementation of chemical and structural principles for the construction of supramolecular one-, two-, and three-dimensional arrays was demonstrated by the self-assembly of synthetic organic molecules.[1–8] The analogous use of nucleic acids or proteins as building blocks offering uniform molecular population, larger structures, and specific intermolecular recognition is, however, far from being fully exploited. Branched DNA molecules were successfully ligated into a cube[9] and a truncated octahedron,[10] or self-assembled into a two-dimensional array.[11] Two-dimensional protein crystals were readily assembled by reconstruction of bacterial cell surface layers on a solid support,[12] two and three distinctive "unidirectionally" oriented protein layers were assembled on metallic surfaces or liposomes,[13, 14] and bispecific antibodies were employed for the construction of an oriented monolayer of bacteriorhodopsin on a metalic surface.[15]

To the best of our knowledge, self-assembly of proteins into a predesigned three-dimensional protein lattice has not been described. The development of methodologies for the for-

[*] Prof. A. Freeman, N. Dotan, Dr. D. Arad, Prof. F. Frolow
Department of Molecular Microbiology and Biotechnology
Faculty of Life Sciences, Tel Aviv University
Tel Aviv 69978 (Israel)
Fax: (+972) 3-6409407
E-mail: amihayf@post.tau.ac.il

[**] The authors are grateful to Prof. Y. Kashman, School of Chemistry, Tel Aviv University, for NMR/MS analysis and to Mr. Talmon Arad, The Department of Structural Biology, The Weizmann Institute, for electron microscopy photographs.

  1433-7851/99/3816-2363 $ 17.50+.50/0

mation of such protein lattices carries potential for the construction of protein scaffolds serving as platforms for the ordered positioning of other proteins by protein fusion or of organic molecules by specific binding.

Here we propose and demonstrate the use of a binding protein with a known detailed structure as a building block for the construction of predesigned three-dimensional protein lattice by specific cross-linking. An appropriate "biligand" may be selected to impose a predetermined relative orientation of the cross-linked protein molecules, leading to the predesigned lattice configuration. This imposed orientation will be affected by the close proximity of the surface of the cross-linked proteins due to ligand-specific binding and intermolecular interactions.

To demonstrate this approach we chose the nearly tetrahedral lectin concanavalin A (**1**, Figure 1 a) as a model building block. This lectin is a tetramer of a fully characterized

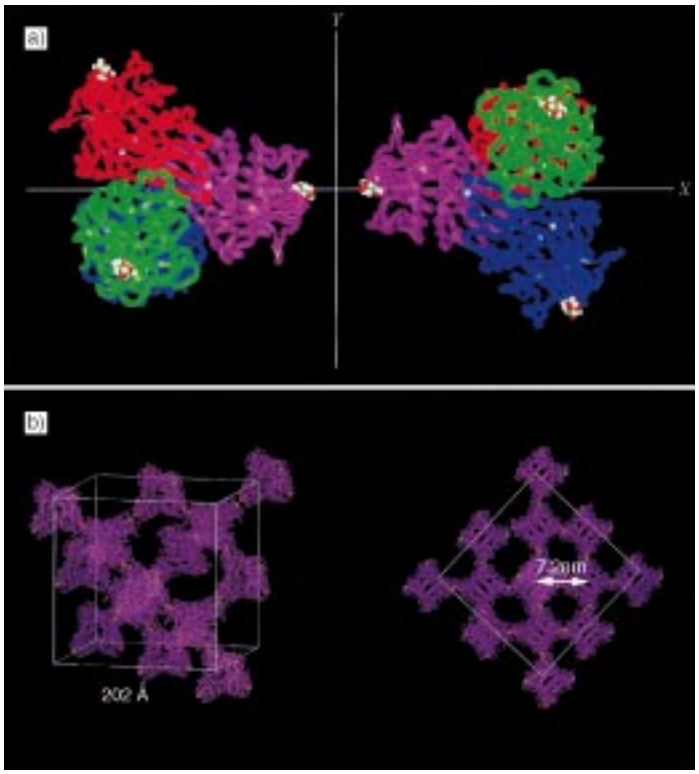


Figure 1. a) Model of two molecules of **1** approaching each other in a staggered orientation (dihedral angle $-60°$). The distance between the anomeric oxygen atoms of the bound mannoses is 10 Å. b) Two views of the unit cell of the predicted diamondlike supramolecular lattice formed by molecules of **1** cross-linked by **2**.

structure[16] and presents four binding sites for α-D-mannopyranoside or α-D-glucopyranoside whose relative locations are analogous to those around an sp^3-hybridized carbon atom. Cross-linking of **1** by a bismannoside with an appropriate spacer imposing staggered positioning (Figure 1 a) will lead to the formation of the computer-modeled diamondlike three-dimensional protein lattice shown in Figure 1 b.

The bismannoside spacer required for this purpose may be deducted from calculations of the interaction energies between two **1**–mannose complexes approaching each other on an imaginary line connecting their centers of gravity, as a function of the distance between the two anomeric oxygen atoms of the complexed mannose units and the dihedral angles. The results of these calculations indicated a minimum of interaction energy for the combination of a C_2 spacer and staggered positioning (Figure 2, black arrow).

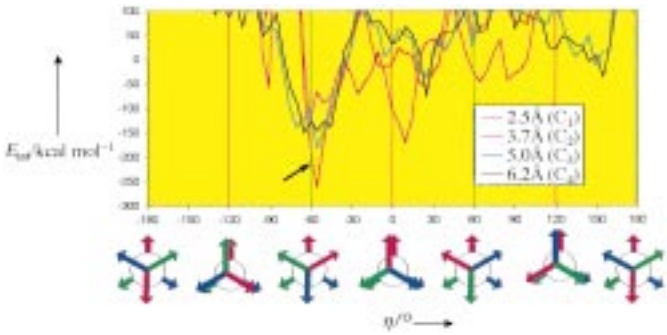
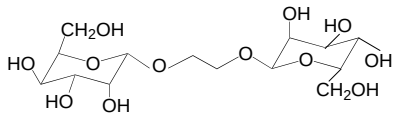


Figure 2. The effect of spacer length and dihedral angle on calculated interaction energies of cross-linked molecules of **1**. The red, blue, and green arrows in the Newman projections represent the corresponding color-coded monomer building blocks of **1** in Figure 1 a.

Bismannopyranoside **2**, presenting a bismannoside unit with a C_2 spacer, was synthesized, purified, and characterized (see Experimental Section). Addition of two molar equivalents of **2** to **1** (5 mg mL^{-1}) at pH 7.0 affected quantitative



2

precipitation of cross-linked **1** with rapid formation of protein crystals. Chemical analysis of the bismannoside content of the washed precipitate revealed a molar ratio of 2.04:1 ratio (average of three experiments), supporting the working hypothesis that each molecule of **1** was cross-linked through four molecules of **2** to its neighbors. This is in accordance with the envisaged diamondlike model described in Figure 1 b. The amount of **1** precipitated depended on the molar ratio **2**:**1**, with an optimum at 2 and a decrease above 10 (Figure 3).

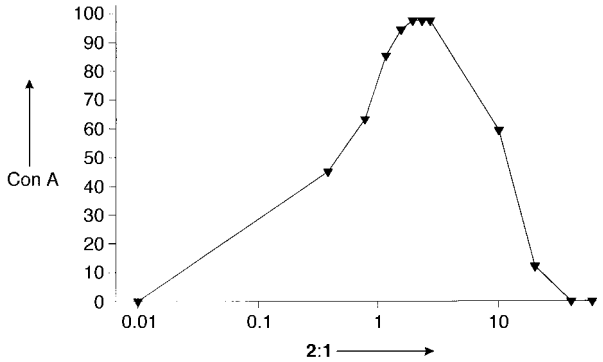


Figure 3. Effect of the molar ratio of **2**:**1** on precipitate formation of cross-linked Con A **1**. The percentage of **1** precipitated was determined spectrophotometrically as described in the Experimental Section.

  1433-7851/99/3816-2364 $ 17.50+.50/0 *Angew. Chem. Int. Ed.* **1999**, *38*, No. 16

The rate of precipitate formation and the crystal size resulting from cross-linking of **1** by **2** were dependent on pH. The highest rates were obtained in the pH range 6.5 – 8.5. The rate of cross-linking could be readily slowed by employing low (e.g. 3.5 – 4.5) or high pH values (e.g. 9.5), allowing control of crystal size from 50 – 100 μm obtained at pH 3.5 to about 100 nm obtained at pH 7. The rate of cross-linking could also be slowed by the addition of an excess of a competitive ligand (α-D-methylmannopyranoside). The cross-linked crystals were stable to environmental changes including substitution of the medium by doubly distilled water, pH changes throughout the range 4.5 – 8.0, and exposure to high concentrations of a competitive ligand (α-D-methylmannopyranoside up to 1.5 M concentration at pH 7.0).

Electron transmission micrographs of negatively stained crystalline precipitates (Figure 4) revealed a highly ordered three-dimensional crystalline array with distances of 6.9 ± 0.3 nm between centers of neighboring molecules of **1**

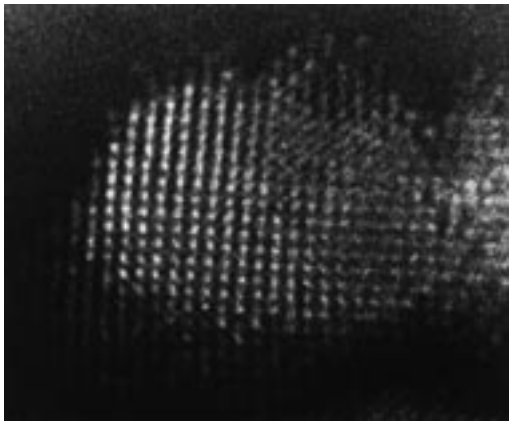


Figure 4. Electron transmission micrograph of a negatively stained crystal obtained from mixing **1** with **2** in a 1:2 molar ratio (magnification × 336 000).

(average of 105 measurements taken from several micrographs). This finding is in good agreement with the predicted distance of 7.2 nm for the diamondlike model (Figure 1 b).

X-ray diffraction experiments using small (0.1 × 0.1 × 0.1 mm) single crystals resulted in weak diffraction patterns of 6-Å resolution. Analysis of this data revealed a pseudo-cubic orthorombic unit cell (space group F_{222}) with cell dimensions $a = 200$, $b = 204$, $c = 208$ Å (matric tensor distortion from the orthorombic symetry of about 0.99 %, prior to imposing space group symmetry constraints on the cell parameters) with an average of 204 Å, similar to the expected cubic *F*-centered arrangement of the diamondlike structure with unit cell dimension $a = 202$ Å. The deviation from the true cubic symmetry is apparently due to absence of a true rotational axis in concanavalin A monomers.

Combination of the data on the cross-linking process, the chemical composition of the precipitates obtained, and the results of the structural analysis by electron microscopy and X-ray crystallography supports the feasibility of our suggested approach to the fabrication of the predesigned protein crystal: A diamondlike protein crystal could be obtained by cross-linking of **1** with **2**, and it is in good structural agreement with the simulated model.

Cross-linking of lectins by natural and synthetic di- and oligosaccharides into protein crystals was previously described for wheat germ agglutinin,[17] calcium-dependent mannose-binding protein,[18] galectin from heart muscle,[19] soy bean agglutinin,[20, 21] isolectins,[22, 23] and concanavalin A.[24, 25] To the best of our knowledge, this accumulating data and experience on lectin cross-linking by natural branched oligosaccharides or their synthetic analogues was not extended or applied for the self-assembly of lectins as building blocks into a predesigned protein lattice. We believe that this accumulating know-how on the synthesis of di- and oligosaccharides and the increasing number of resolved lectin structures may be combined with our suggested approach for the controlled fabrication of new, predesigned three-dimensional protein lattices and scaffolds.

Experimental Section

Molecular modeling and interaction energy calculations were carried out on Silicon Graphics IRIS 4D workstation, employing Insight II 2.3.5 software (Biosym Technologies, San Diego, CA, USA) and structural data from the protein data base (PDB), file 5CNA. Interaction energies were calculated employing the Insight II docking module with cffu force field parameters. The pH was set to 4.5. The oxygen atom connected at the C1 position of the mannose unit bound to **1** on the left side of Figure 1 a served as the rotation center. The distance between the two anomeric oxygen atoms was set to the appropriate spacer length. The molecule on the right was rotated at 5° intervals around the axis connecting the centers of gravity of the two molecules and the mannose anomeric oxygen atoms at the binding sites, and the interaction energy calculated. Upon completion of a whole cycle, the distance between the two anomeric oxygen atoms was extended and a new iteration conducted.

The synthesis of **2** was carried out by a modification of the method described by Mowery:[26] Ethyleneglycol (50 mL, Merck), α-D-mannose (10 g, Sigma), and preequilibrated Dowex 50WX80-200 (5 g, Aldrich) were incubated with stirring for 48 h at 65 °C. The resin was filtered off, and the filtrate added into acetone (500 mL). The precipitate thus obtained was redissolved in DMSO (40 mL), mannose (10 g) was added followed by Dowex 50WX8-200 (5 g) preequilibrated with DMSO, and the reaction mixture incubated with stirring at 65 °C for 100 h. The resin was filtered off, and an aliquot (4 mL) of the filtrate added into acetone (40 mL). The precipitate was redissolved in doubly distilled water and purified by HPLC on a Supelcosil LC-NH_2 column (250 × 10 mm, 5 μm) employing acetonitrile/water (75/25, first run; 83/17, second run) as mobile phase. The purified product was recovered by lyophilization. ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta = 4.70$ (t, $J = 6$ Hz, 2H), 4.57 (d, $J = 7$ Hz, 1H), 4.45 (t, $J = 6$ Hz, 1H), 3.69 (br d, $J = 6$ Hz, 2H), 3.63 (ddd, $J = 10$, 4, 2 Hz, 1H), 3.60 (br d, 1H), 3.53(br d, $J = 6$ Hz, 2H), 3.40 (4H); FAB-MS: *m*/*z* (%) calcd: 386; found: 409 (386+Na^+, 25), 387 (386+H^+, 28), 329 (29), 225 (386 – 163+2H, 50), 177 ($C_6H_9O_6$, 74), 163 ($C_6H_{11}O_5$, 100).

Cross-linking studies: The percentage of **1** (Sigma) precipitated by cross-linking with **2** was determined spectrophotometrically (280 nm) following incubation for 24 h at 4 °C of a solution of **1** (5 mg mL^{-1}) in 0.1 M HEPES, pH 7.0, containing 0.15 M NaCl, 1 mM $CaCl_2$, 1 mM $MnCl_2$, and 0.008 % NaN_3. The crystal for X-ray crystallography was prepared by the hanging drop method by mixing **1** (5.2 mg mL^{-1}) in 0.01 M MES, pH 6.0, containing 1 mM $CaCl_2$, 1 mM $MnCl_2$, and 0.1 M NaCl (4 μL) with 0.045 M citrate (5 μL), pH 3.5, containing 4.5 % (w/v) PEG 3300 and 0.2 % NaN_3 (same composition also served in well reservoir (700 μL)), and **2** (0.08 mg mL^{-1}, 2 μL, final pH of the drop 4.5), and incubating for one week at 20 °C. Smaller cross-linked crystals for electron microscopy were similarly obtained by the addition of 4 μL of aqueous solution of **2** (0.1 mg mL^{-1}) into 100 μL of **1** (0.5 mg mL^{-1}) in 0.05 M HEPES, pH 7.0, containing 1 mM

  1433-7851/99/3816-2365 $ 17.50+.50/0

$CaCl_2$, 1 mM $MnCl_2$, and 0.1 M NaCl. Crystals were recovered following 24 h of incubation at 4 °C.

Chemical analysis of the crystals: Crystals were dissolved in 30 mM HCl, pH 1.5, and the amount of **1** was determined spectrophotometrically. A sample from this solution was immediately mixed with a ninefold volume of acetonitrile. The precipitating protein was removed by centrifugation, and the amount of remaining soluble **2** determined by HPLC.

Electron microscopy: The electron transmission micrograph shown in Figure 4 was prepared from samples placed on a freshly discharged, 300 mesh, carbon-coated parlodion grid, stained by 1 % phosphotungstate, pH 7, and observed at 100 kV with a Philips CM-12 electron microscope.

X-ray crystallography: The imaging plate area detector Rigaku Raxis-II implemented with high-resolution blue imaging plates and equipped with a Rigaku FRC rotating anode and focusing mirrors was used for the diffraction experiments. To overcome the weak diffracting power of the crystals, the combination of the high brilliance X-ray generator and focusing mirrors was used. The detector parameters and the accurate crystal–detector distance were determined using a high-quality orthorhombic homogeneous crystal of **1**, a short time before the start of the experiment. All diffraction experiments have been performed at low temperature generated by an Oxford Cryostream device. Crystals were mounted on the loops made of a nondiffracting monofilament nylon line. Hydrocarbon oil has been used as a cryoprotectant. Small oscillation angle (0.5°) diffraction images were taken from several crystals of the approximate size of 0.1 mm in the largest dimension, using exposure time of one hour in various places of the reciprocal space. These images were indexed using HKL package of the diffraction pattern processing programs.[27]

Received: December 8, 1998
Revised version: April 23, 1999 [Z 12757 IE]
German version: *Angew. Chem.* **1999**, *111*, 2512–2515

Keywords: crystal engineering · lectins · molecular modeling · proteins · supramolecular chemistry

[1] *Comprehensive Supramolecular Chemistry*, *Vol. 9* (Ed.: J. M. Lehn), Pergamon, Oxford, **1996**.
[2] G. M. Whitesides, J. P. Mathias, C. T. Seto, *Science* **1991**, *254*, 1312–1319.
[3] G. R. Newkome, C. N. Moorefield, G. R. Baker, R. K. Behera, G. H. Escamillia, M. J. Saunders, *Angew. Chem.* **1992**, *104*, 901–903; *Angew. Chem. Int. Ed. Engl.* **1992**, *31*, 917–919.
[4] N. Kimizuka, T. Kawasaki, T. Kunitake, *J. Am. Chem. Soc.* **1993**, *115*, 4387–4388.
[5] R. Kramer, J. M. Lehn, A. deCian, J. Fischer, *Angew. Chem.* **1993**, *105*, 764–766; *Angew. Chem. Int. Ed. Engl.* **1993**, *32*, 703–705.
[6] S. I. Stupp, S. Son, H. C. Lin, L. S. Li, *Science* **1993**, *259*, 59–63.
[7] S. I. Stupp, V. LeBonheur, K. Walker, L. S. Li, K. E. Huggins, M. Kesser, A. Amstutz, *Science* **1997**, *276*, 384–389.
[8] M. Fujita, S. Y. Yu, T. Kusukawa, H. Funaki, K. Ogura, K. Yamaguchi, *Angew. Chem.* **1998**, *110*, 2192–2196; *Angew. Chem. Int. Ed.* **1998**, *37*, 2082–2085.
[9] K. Chen, N. C. Seeman, *Nature* **1991**, *350*, 631–633.
[10] Y. Zhang, N. C. Seeman, *J. Am. Chem. Soc.* **1994**, *116*, 1661–1669.
[11] E. Winfree, F. Liu, L. A. Wenzler, N. C. Seeman, *Nature* **1998**, *394*, 539–544.
[12] C. Weiner, M. Sara, U. B. Sleytr, *Biotechnol. Bioeng.* **1994**, *43*, 321–330.
[13] M. Ahlers, W. Müller, A. Reichert, H. Ringsdorf, J. Venzmer, *Angew. Chem.* **1990**, *102*, 1310–1326; *Angew. Chem. Int. Ed. Engl.* **1990**, *29*, 1269–1285.
[14] W. Müller, H. Ringsdorf, E. Rump, G. Wildburg, X. Zhang, L. Angermaier, W. Knoll, M. Liley, J. Spinke, *Science* **1993**, *262*, 1706–1708.
[15] K. Koyama, N. Yamaguchi, T. Miyasaka, *Science* **1994**, *265*, 762–765.
[16] I. J. Goldstein, R. P. Poretz in *The Lectins: Properties, Functions and Applications in Biology and Medicine* (Eds: I. E. Liener, N. Sharon, I. J. Goldstein), Oxford University Press, Oxford, **1986**, pp. 33–247.
[17] C. S. Wright, *J. Biol. Chem.* **1992**. *267*, 14345–14352.
[18] W. I. Weis, K. Drickamer, W. A. Hendrickson, *Nature* **1992**, *360*, 127–133.
[19] Y. Bourne, B. Bolgiano, D. I. Liao, G. Strecker, P. Cantau, O. Herzberg, T. Feizi, C. Cambillau, *Nat. Struct. Biol.* **1994**, *1*, 863–870.
[20] A. Dessen, D. Gupta, S. Sabesan, C. F. Brewer, J. C. Sacchettini, *Biochemistry* **1995**, *34*, 4933–4942. ■■Where is this reference mentioned in the text? ■■
[21] D. Gupta, L. Bhattacharyya, J. Fant, F. Macaluso, S. Sabesan, C. F. Brewer, *Biochemistry* **1994**, *33*, 5614–5622, and references therein.
[22] L. Bhattacharyya, M. I. Khan, J. Fant, C. F. Brewer, *J. Biol. Chem.* **1989**, *264*, 11543–11545, and references therein.
[23] L. Bhattacharyya, J. Fant, H. Lonn, C. F. Brewer, *Biochemistry* **1990**, *29*, 7523–7530.
[24] L. Bhattacharyya, M. I. Khan, C. F. Brewer, *Biochemistry* **1988**, *27*, 8762–8767.
[25] D. K. Mandal, C. F. Brewer, *Biochemistry* **1992**. *31*, 12602–12609.
[26] D. F. Mowery in *Methods in Carbohydrate Chemistry*, *Vol. II* (Eds.: R. I. Whistler, M. I. Wolfrom), Academic Press, New York, **1963**, pp. 328–331.
[27] Z. Otwinowski, W. Minor, *Methods Enzymol.* **1996**, *276*, 307–326.

N-Substituted Corroles: A Novel Class of Chiral Ligands**

Zeev Gross* and Nitsa Galili

There is a steady increase of interest in porphyrin-like macrocycles, which display many novel physical and chemical properties.[1] One class of such molecules are the corroles, whose skeleton may also be viewed as the aromatic version of corrin, the moiety ligated to cobalt in the coenzyme of vitamin B_{12}.[2] Alternatively, and more commonly, corroles are considered as analogues of porphyrins that have one less carbon atom (Scheme 1); both macrocycles are aromatic and provide an inner core containing four nitrogen atoms as an equatorial coordination template for metal ions.[3]

Indeed, corroles and porphyrins share many spectroscopic and chemical features.[2] Still, corroles act as trianionic rather than dianionic ligands, their inner core is somewhat contracted, and their symmetry (C_{2v}) is lower than that of porphyrins (D_{4h}). The first two factors were found to have a remarkable effect on the stabilization of metal ions in exceptionally high oxidation states.[4] One consequence of the different symmetries is that while substitution of one of the inner NH protons in porphyrins leads to a single product, two isomers are formed in the case of corroles (N21- and N22-substituted corroles, Scheme 2).[5] In addition, since the N-substituted

[*] Prof. Dr. Z. Gross, Dr. N. Galili
Department of Chemistry
Technion–Israel Institute of Technology
Haifa 32000 (Israel)
Fax: (+972) 4-8233735
E-mail: chr10zg@tx.technion.ac.il

[**] We acknowledge the partial support of this research by The R. and M. Rochlin Research Fund. We also thank Prof. A. Shanzer and Dr. H. Weizman, Rehovot, for the CD spectra.

  1433-7851/99/3816-2366 $ 17.50+.50/0