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[15] A mixture containing $[Re(CO)_3(bpy)Cl]$ (300 mg, 0.650 mmol) and $AgPF_6$ (176 mg, 0.696 mmol) in dichloromethane (20 mL) and methanol (5 mL) was stirred for 18 h at room temperature. After filtration over celite and removal of the solvents the yellow residue was redissolved in CH_2Cl_2 (20 mL) and treated with a solution of TCNQ (33 mg, 0.163 mmol) in CH_2Cl_2 (10 mL). To the blue solution formed after 8 h (IR monitoring) *n*-pentane was added (10 mL). Cooling to $-30\,^{\circ}C$ produced a dark blue precipitate which was collected, redissolved in dichloromethane, and reprecipitated with *n*-pentane. Drying under vacuum yielded **1**-$(PF_6)_4$ (150 mg; 37 %). Elemental analysis calcd (%) for $C_{64}H_{36}F_{24}N_{12}O_{12}P_4Re_4$: C 30.87, H 1.46, N 6.75; found: C 30.65, H 1.46, N 6.40; ^{1}H NMR (CD_2Cl_2): $\delta = 7.32$ (s, 4H; TCNQ-H), 7.71 (dd, 8H; $H^{5,5'}$(bpy)), 8.26 (dd, 8H; $H^{4,4'}$(bpy)), 8.43 (d, 8H; $H^{3,3'}$(bpy)), 8.99 (d, 8H; $H^{6,6'}$(bpy)); IR (nujol): $\tilde{\nu} = 2241$ (ν(CN)), 2031, 1967, 1950 (ν(CO)), 840 cm^{-1} (δ(CH)); IR spectroelectrochemistry (CH_2Cl_2/0.1 M Bu_4NPF_6): tetracation: $\tilde{\nu} = 2235$ (ν(CN)), 2039, 1956, 1945 cm^{-1} (ν(CO)); trication: $\tilde{\nu} = 2223$, 2182 (ν(CN)), 2038, 1945, 1940sh cm^{-1} (ν(CO)); dication: 2210(br), 2149 (ν(CN)), 2036, 1934(br) cm^{-1} (ν(CO)); UV/Vis (CH_2Cl_2): λ_{max} (ε) = 680 (13100), 382 (12400), 321 (26700) nm ($M^{-1}cm^{-1}$); UV/Vis spectroelectrochemistry (CH_2Cl_2/0.1 M Bu_4NPF_6): trication: $\lambda_{max}(\varepsilon) = 1020$ (12100), 905 (9000), 425(sh), 405 (11800), 305 (23600) nm ($M^{-1}cm^{-1}$).
[16] Single crystals were obtained from a saturated solution in CD_2Cl_2. $C_{64}H_{36}F_{24}N_{12}O_{12}P_4Re_4$: Dark blue rods, $0.8 \times 0.1 \times 0.05$ mm, monoclinic, space group $P2_1/n$, $a = 10.3137(4)$, $b = 22.0360(5)$, $c = 20.6476(6)$ Å, $\beta = 97.2520(1)^{\circ}$, $V = 4655.1(2)$ Å^3, $T = 293$ K, $Z = 2$, $\rho_{calcd} = 1.776$ g cm^{-3}, $\mu(Mo_{K\alpha}) = 5.356$ cm^{-1}, 17485 reflections measured, 6048 observed with $I > 2\sigma(I)$. $R_1 = 0.0603$, $wR_2 = 0.1564$, GOF = 1.026, min/max electron density $-0.938/0.949$ e Å^{-3}. Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-160093. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).
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Targeting Molecular Recognition: Exploring the Dual Role of Functional Pseudoprolines in the Design of SH3 Ligands**

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Protein–protein interactions that are mediated by the binding of proline-rich sequences are involved in a large variety of cellular processes, for example, signal transduction, motility, membrane trafficking, and cell division.[1–5] Such proline-rich ligands adopt a left-handed polyproline II (PPII) helical conformation and bind to a highly conserved patch of aromatic amino acids. These residues are positioned to accommodate the unique geometric properties of a PPII helix on the recognition surface of, for example, SH3 domains, and to participate in van der Waals contacts and specific hydrogen bonds between carbonyl oxygen atoms of the ligand backbone and functionalities on the aromatic residues of the protein.[6] SH3 domains are enticing conceptual targets for pharmacological intervention in a number of pathologies, for example, AIDS, cancer, and inflammatory diseases, since they occur in several critical, intracellular signaling proteins.[5b, 7] The essential feature of most SH3 binding ligands is the consensus sequence Pro-Xaa-Xaa-Pro (where Xaa represents various amino acids).[8] Synthetic[5, 9] and phage-displayed[7, 10] combinatorial peptide libraries have been generated to understand the ligand properties in more detail, and these studies have yielded compounds that in general bind to SH3 domains with equal or slightly lower affinity than natural Pro-

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[**] This work was supported by the Swiss National Science Foundation.

Xaa-Xaa-Pro peptides. A step further in the design of SH3 inhibitors with improved affinity and selectivity was taken by introducing non-natural, N-substituted residues at key positions in a mSoS-derived ("son of sevenless", a guanine nucleotide exchange factor, m = mouse) dodecapeptide. It was argued that N-substituted peptoids with various side chains can be used for optimizing complementarity, and hence affinity, by increasing the number of contacts between the ligand and the domain.[5c,d]

However, a left-handed PPII conformation of the peptide remains a structural requirement for the effective binding of proline-rich ligands to SH3 domains. On the basis of these considerations the recently introduced concept of pseudoprolines (ΨPro), that is, Ser-, Thr-, or Cys-derived proline-like structures (Figure 1) with enhanced inherent properties of natural L-Pro,[11] offers a new path for further design strategies.[12] In particular, the nature of the substituents (R^1 and R^2) at the C2 position of ΨPro permits the *cis*/*trans* isomerization equilibrium to occur and thus the polyproline helix transitions (PPI/PPII) to be tailored.[11a,b]

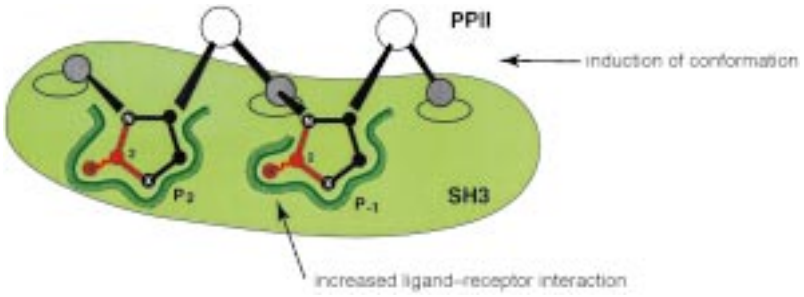

Figure 1. The dual role of functional pseudoprolines (ΨPro) in ligand recognition by the SH3 domain (gray and white spheres resemble amino acids): The formation of the schematically represented PPII helical conformation is a prerequisite for the binding of Pro-rich regions to SH3 domains. Favorable contributions for high affinity binding—complementarity in shape and thus close packing of the side chains—are not provided because of the lack of functional groups in the proline ring structure. The dual functionality of ΨPro, that is, induction of the PPII helical conformation and optimization of van der Waals contacts through the C2 substituents R^1 and R^2, leads to optimized packing and thus to higher affinity and potential specificity.

Besides its potential for adopting a PPII helical conformation, the incorporation of substituents of variable size and polarity at the C2 position of ΨPro offers a powerful tool to modulate the ligand–receptor interactions. As depicted in Figure 1, ΨPro building blocks exert a dual functionality in 1) inducing and stabilizing the relevant PPII conformation and 2) increasing and optimizing the van der Waals contacts and the formation of hydrogen bonds to the receptor. Most notably, the generation of a library of different substituents at C2 allows the factors contributing to affinity and specificity in protein–protein interactions to be explored, and to further elucidate ligand recognition by SH3 domains at a molecular level.

To exploit this principle for the design of novel SH3 ligands, the two proline residues at positions P_{-1} and P_2 in the SoS-derived peptide Val-Pro-Xaa-Pro-Val-Ser-Xaa-Pro-Lys-Lys-Lys (**1**) were replaced by ΨPro moieties (Xaa = Ser($\Psi pro^{Ph,H}$)).[13] Molecular modeling studies based on existing crystal structures revealed that C2-monosubstituted ΨPro systems with an *S* configuration results in energetically more favorable receptor interactions relative to the corresponding *R* epimers. The slightly tilted phenyl group at the *S*-configured center can be readily accommodated at sites P_{-1} and P_2 (Figure 2) with only minor structural changes in the SH3 binding pocket (for example, torsions around side chains χ_1 of Tyr 7 in P_2). The hydrogen bond between Tyr 52 and the backbone of the ligand is retained. Substituents with an *R* configuration at C2 point directly towards the SH3 domain, and result in steric clashes, particularly with the intimately packed Tyr 52 residue at P_2 or the Trp 36 residue at P_{-1}. These

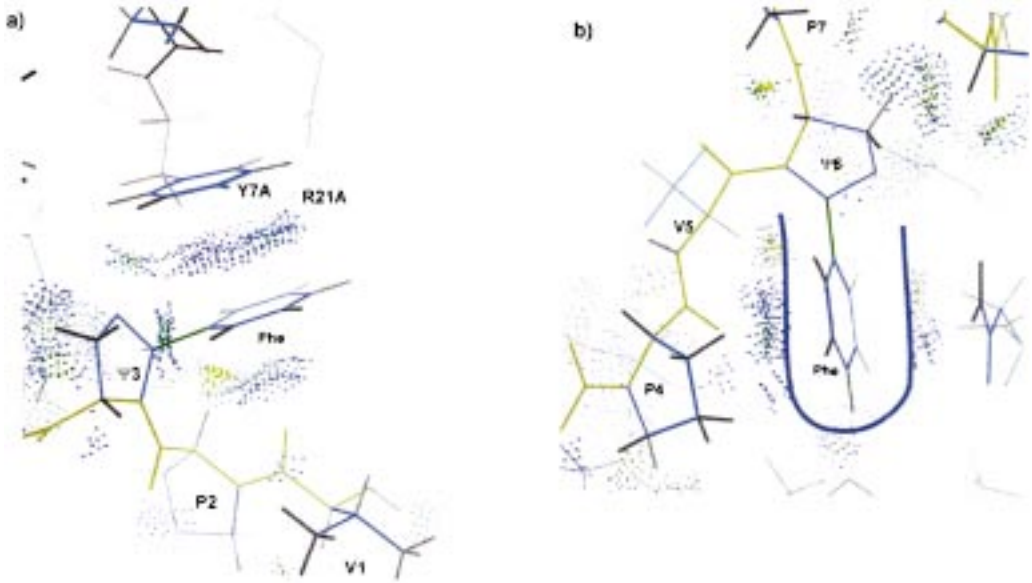

Figure 2. Close-up view of the SH3 binding pockets P_2 and P_{-1} depicting the contact surface dots[19] of the MD-averaged structure of peptide **1**: a) one possible binding mode at P_2 characterized by dominant van der Waals interactions between Tyr 7 and the phenyl substituent at C2; b) close packing in the P_{-1} binding pocket.

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steric clashes can be compensated for only by major conformational shifts in the protein or the ligand.

The resulting structures obtained from molecular dynamics (MD) simulations[14] are very similar (Figure 3), but have a slightly increased overall mobility than the native proline-rich ligand sequence (average root mean square deviation (rmsd) from the X-ray structure < 0.7 Å). This increased mobility is possibly the result of two different binding modes for the phenyl ring in position P_2.

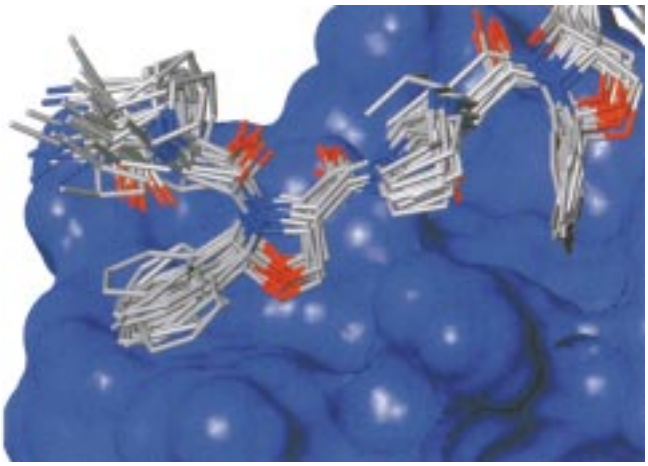

Figure 3. Superposition of 40 snapshots resulting from MD simulations of **1** (stick representation, hydrogen atoms are omitted for clarity) in a complex with the N-terminal Grb2-SH3 domain (Connolly surface).

The *S* configuration at the C2 position of the ring enables ligands to be designed with an optimized complementarity to the topography of the SH3 domain. Starting from an initial PPII helix conformation of about 40% of the investigated Pro- or ΨPro model peptides, ΨPro-containing ligands preferentially adopt a PPII conformation as a result of a markedly increased rate of isomerization about the tertiary Xaa-ΨPro–amide bond compared to oligo-L-Pro peptides.[11b]

The *S* conformers of the oxazolidine derivative Pro-Ser($\Psi^{Ph,H}$pro) and Val-Ser(Ψpro^{Ph,H}) were stereoselectively synthesized by using the post-insertion strategy.[11] The corresponding dipeptides Fmoc-Xaa-Ser-OH (Xaa = Pro, Val; Fmoc = 9-fluorenylmethoxycarbonyl) were treated with benzaldehyde dimethylacetal to yield the kinetically preferred *S*-configurated pseudoprolines.[15] These building blocks were used for the stepwise synthesis of the model peptide **1** on a solid support by Fmoc strategies.[16] The HPLC chromatogram of the peptides after cleavage from the resin shows a relatively broad peak of molecules of identical mass which indicates the existence of different, but distinct, conformations arising from the presence of the ΨPro moieties. Treatment of the peptide with trifluoroacetic acid (TFA) resulted in a sharpening of the peak shape as a consequence of the opening of the ΨPro ring restoring the parent Ser residue (Figure 4).

Circular dichroism (CD) studies of the model peptide Pro-Pro-Pro-Ser(ΨPro^{Ph,H})-Pro-Val-Ser(ΨPro^{Ph,H})-Pro-Pro-Pro-Pro (**2**) in H_2O shows the characteristic bands of a PPII conformation, that is, a strong negative Cotton effect at

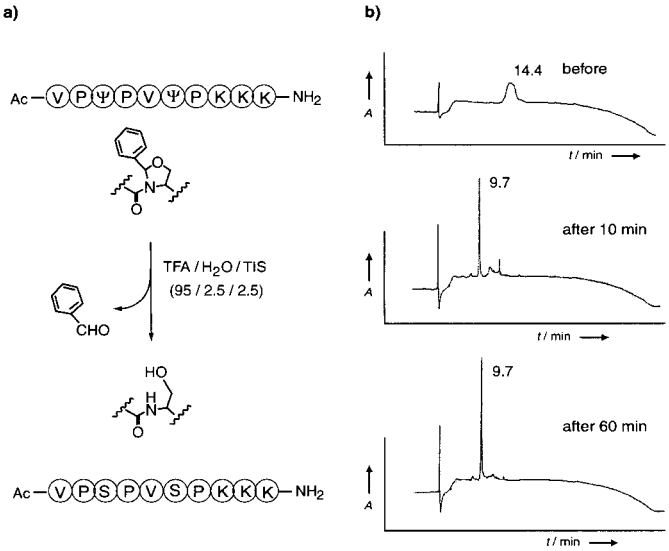

Figure 4. a) Acid treatment of peptides containing pseudoproline (Ψpro) leads to ring opening and thus to restoration of the parent amino acid (Ser); b) opening of the Ψpro-ring structure as monitored by HPLC (see text). TIS = triisopropylsilane.

$\lambda = 206$ nm and a slightly positive value at $\lambda = 228$ nm (Figure 5a). Interestingly, peptide **2** displays a transition to an unordered conformation rather than to a PPI helix in

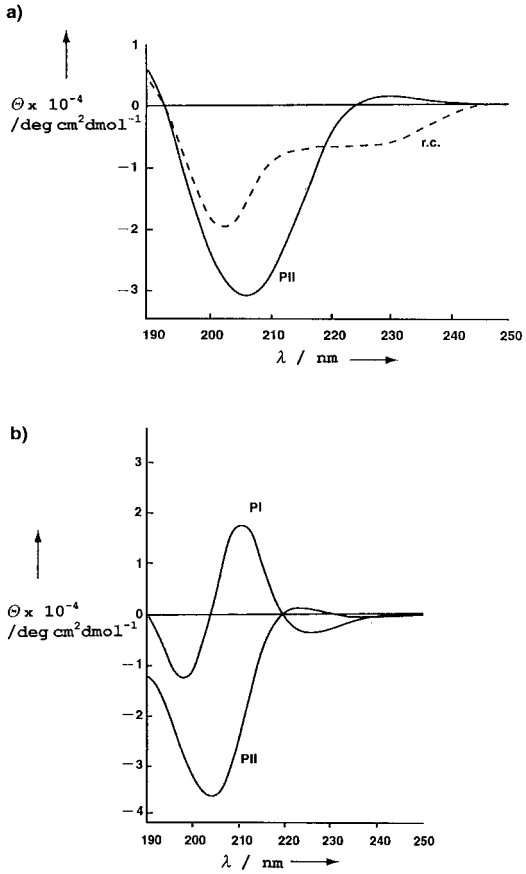

Figure 5. a) CD spectra of the ΨPro-containing peptide **2**: the spectrum measured in water shows the characteristics of a PPII helix (——) while that in propanol shows a random coil (r.c.) conformation (- - - -); $c = 1$ mg mL^{-1}. b) CD spectra of the solvent-induced PPI (propanol)/PPII (water) interconversion of poly-L-proline.

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n-propanol/water (99.5/0.5, v/v), as observed in homooligo-Pro peptides (Figure 5b). These results are in harmony with previous results which show that two ΨPro units that have a slight preference for forming *cis* amide bonds are not sufficient to induce and stabilize a PPI helix.[11, 15]

Initial in vitro assessments of the binding activity of the model monoarylated pseudoproline-containing peptide **1** to the Crk, Crkl, and Grb-2 SH3 domains by measurement of the fluorescence of Trp residues revealed dissociation constants in the lower micromolecular range, which is typical for protein–protein interactions mediated by SH3 domains. Most notably, relative to the native L-Pro-rich sequence, the ΨPro peptides exhibit an enhanced binding specificity without loss of affinity to Crk- and Crkl-SH3 domains. In competition assays using whole cell lysates[17] from the human chronic myelogenous leukemia (CML) K562 cell line,[18] ΨPro peptides have been shown to inhibit formation of the Grb2-SH3(N)/SoS complex (Figure 6). This observation indicates that C2-substituted ΨPro moieties indeed exert the postulated dual functionality in enhancing the proline effect in the recognition process.

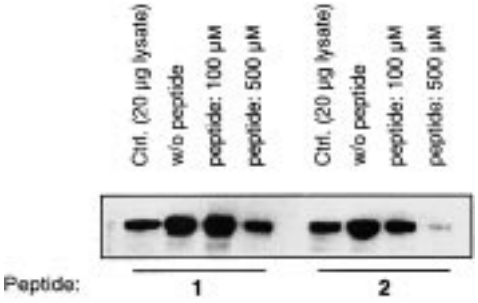

Figure 6. Western blot of the inhibition of the Grb2-SH3(N)/SoS complex formation by the native SoS sequence and a peptide containing a Cys-derived monoarylated pseudoproline. Peptides: **1** = Ac-VPPPVPPKKK-NH_2; **2** = Ac-VPPPVC($\Psi Pro^{Ph,H}$)PKKK-NH_2.

In conclusion, pseudoprolines have proven to be highly versatile for studying ligand recognition that is mediated by SH3 domains—an essential facet of cellular regulation, and thus for protein–protein interactions, in general. The results support the concept that functional pseudoprolines have a dual role, as exemplified by the successful design of novel SH3 ligands of remarkable bioactivity. A unique feature of ΨPro building blocks is that the C2 substituents facilitate optimal complementarity of the SH3 topography upon ligand recognition, and thus modulate the binding affinity and specificity, as well as promote the induction of the required helical PPII conformation in accelerating *cis*–*trans* isomerizations. Further optimizations of the ligand–receptor interactions by generating C2-substituted ΨPro libraries through applying post-insertion strategies are currently in progress.

Experimental Section

The peptides were synthesized according to standard procedures for solid-phase peptide synthesis (SPPS)[16] by applying the Fmoc strategy on Sieber amide resin.[20] The pseudoproline residue, a serine-derived 2-phenyl-2*H*-1,3-oxazolidine-4-carboxylic acid (Ser($\Psi pro^{Ph,H}$) was introduced during SPPS as a preformed dipeptide building block.[11] Briefly, the corresponding dipeptide, Fmoc-Pro-Ser-OH or Fmoc-Val-Ser-OH, was dissolved in THF and refluxed with 10 equivalents of benzaldehyde dimethylacetal and 0.2 equivalents of pyridinium *p*-toluenesulfonate (PTTS) for 1.5 h. After work-up, the two epimers were separated by flash chromatography on silica gel with CH_2Cl_2/MeOH (100/5, v/v), and characterized by electrospray-ionization mass spectrometry (ESI-MS) and ^1H NMR spectroscopy (Finnigan APCI/ESI and a Brucker DPX-400 spectrometer, respectively).

After the synthesis of the sequences using a twofold excess of amino acids or the ΨPro-dipeptides, the peptides were cleaved from the resin with TFA (1% in dichloromethane), purified by reversed-phase HPLC, and characterized by ESI-MS (**1**: *m*/*z* 1189; **2**: *m*/*z* 1287). Removal of the (4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl (Dde) protecting groups on the lysine residues in **1** was achieved with 2% H_2NNH_2 in DMF prior to cleavage of the peptide from the resin.

The CD spectra were recorded on a Jobin Yvon Mark VI circular dichrometer in quartz cells (path length 0.1 cm) with a peptide concentration of 0.1 mg mL^{-1} in H_2O and *n*-propanol/water (99.5/0.5, v/v).

Inhibition of the formation of the Grb2-SH3(N)/SoS complex by the peptides was tested with the human chronic myelogenous leukemia (CML) cell line K562 cultured in RPMI 1640 with 5% fetal bovine serum (FBS; GibcoBRL) and penicillin/streptomycin (GibcoBRL). Cell lysis was performed as described in ref. [17] and the in vitro inhibition carried out with the total cell lysate. 20 µg of the GST-tagged Grb2-SH3(N) fusion protein was preincubated for 2 h at 4 °C in 250 µL of IP buffer (20 mM TrisHCl pH 7.5, 1 mM Na_2EDTA, 100 mM NaCl, 5 vol. % glycerol, 0.1% Tween 20 (Roth) containing protease inhibitors (0.2 mg mL^{-1} phenylmethylsulfonyl fluoride (Sigma), 10 µg mL^{-1} aprotinin (Roth), 0.5 µg mL^{-1} leupeptin (Serva), 5 µg mL^{-1} antipain · HCl (Sigma), 0.7 µg mL^{-1} pepstatin (Roche)), and glutathione-sepharose beads. The K562 protein (250 µg) and the peptides (at the concentrations indicated in Figure 6) were pre-mixed in 250 µL of IP buffer, added to the SH3 domain, and incubated overnight. Precipitates were washed three times in 0.5% TX buffer (20 mM TrisHCl (Tris = tris(hydroxmethyl)aminoethane) pH 7.5, 100 mM NaCl, 5% (v/v) glycerol, 1 mM Na_2EDTA (EDTA = ethylenediaminetetraacetate), 0.5% Triton X-100 (Sigma)). After sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and semi-dryblotting, the SoS bound to GST-Grb2 SH3(N) was detected with anti-SoS antiserum (Upstate Biotechnology Inc.).

Received: January 22, 2001 [Z 16480]

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[14] The molecular graphics package INSIGHT II (version 97, Molecular Simulations, **1997**) was used. Energy minimizations and MD simulations were performed on a Silicon Graphics OCTANE by using the program CVFF/FDISCOVER (version 97, Molecular Simulations, **1997**). The simulations were performed with a cut-off distance of 12 Å, a time step of 1 fs, and a distance-dependent dielectricity constant ε of $4.00\,r$ (r = distance) The backbone coordinates of the N-terminal SH3 domain were restrained to their initial positions. Implicit treatment of solvation and conformational restraints permitted long run times of MD simulations within an appropriate time scale. After 5000 steps of steepest descent minimization, the system was heated to 298 K by applying the standard FDISCOVER protocol. After an initialization time of 10 ps, the simulation run was continued for 1 ns. Averaged structures were generated for the last 50 ps of the simulation and further minimized for 500 steps of steepest descent, followed by a conjugate gradient minimization until the rms gradient was < 0.01 kcal mol^{-1} Å^{-1}. After this procedure, the peptide ligand Val-Pro-Ser(Ψpro^{Ph,H})-Pro-Val-Ser(Ψpro^{Ph,H})-Pro-Arg-Arg-Arg was compared with Val-Pro-Pro-Pro-Val-Pro-Pro-Arg-Arg-Arg, the decameric native SoS peptide sequence 3gbq.[21] The fluctuation was analyzed for the last 500 ps of the simulation.

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Designed Molecules for Self-Assembly: The Controlled Formation of Two Chiral Self-Assembled Polynuclear Species with Predetermined Configuration**

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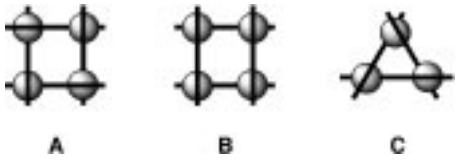
Self-assembly reactions are not yet as predictable to the same degree as classical reaction sequences. Often, highly interesting structures are obtained through a combination of intuition, conjecture, and serendipity.[1] Herein, we report the formation of two closely related supramolecular structures that were obtained in a programmed way. Our intention is to fabricate supramolecular complexes of the type **A** from octahedrally coordinating metal ions.



Complexes of this type are chiral (D_4 symmetry) as a consequence of the special way the ligand strands enfold the cations. Chirality is the main feature that distinguishes them from the related grid-type complexes **B** investigated by J.-M. Lehn and co-workers,[2a,b] and other recently reported molecular squares.[2c,d]

To achieve such structures we had to design a ligand that fulfils the following demands: 1) it must offer two terpyridine-type binding sites to cover each half of the coordination sphere of an OC-6 cation in a *mer* configuration; 2) it must be geometrically rigid, and define the side of a square in a tetranuclear self-assembled species; and 3) the orientation of the binding vectors of the two terpyridine (terpy) units must be antiparallel, in order to make the ligand coordinate to the metal ions once from "above" and once from "below" the plane defined by the four metal ions. These requirements, and especially the relative orientation of the binding sites, are fulfilled in ligand L^1, in which two 2,2′-bipyridin-6-yl groups are attached to a central pyrazine ring at positions 2 and 5.[3]

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[**] This work was financially supported by the Swiss National Science Foundation. We thank F. Nydegger for ESI-MS measurements and F. Fehr for conducting NOE experiments. Prof. C. W. Schläpfer and Prof. C. A. Daul are thanked for fruitful discussions.

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