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Constitutional Dynamic Chemistry

# DOSY NMR Experiments as a Tool for the Analysis of Constitutional and Motional Dynamic Processes: Implementation for the Driven Evolution of Dynamic Combinatorial Libraries of Helical Strands**

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*Dedicated to David Reinhoudt on the occasion of his 65th birthday*

Constitutional dynamic chemistry (CDC)[1] is the chemistry of molecular or supramolecular species and libraries of species, generated from components connected either by reversible covalent bonds[1,2] or by noncovalent interactions, respectively.[1] CDC takes advantage of these dynamic linkages for the expression of (supra)molecular diversity through crossover recombination of a set of building blocks. At thermodynamic equilibrium, specific changes in the environmental parameters can lead to the amplification/selection of preferred constituents generated in the libraries. This selection is of great interest for drug discovery purposes; for example, the presence of molecular targets such as enzymes, can discriminate against the best inhibitor through an in situ dynamic screening of the equilibrating mixture.[3] CDC has also recently been shown to respond to various external chemical or physical stimuli such as protons, phase transitions, temperature, or electric field modulation.[4] These libraries can afford the tuning of various physical properties by controlling the molecular, supramolecular, or macromolecular constitution of their dynamic functional entities, thus extending CDC to the domain of materials science.[1b,5] Whatever the application domain of CDC, one of the crucial prerequisites concerns the analysis of the libraries. For example, HPLC methods often require one to freeze the component exchange before analysis because the chromatographic interactions themselves can disturb thermodynamic equilibrium and affect the ratio between the library constituents. On the other hand, NMR spectroscopy methods do not interfere with the constitutional expression of the libraries, but only a few compounds can be characterized in a mixture. Thus, the need for deconvolution methods not involving chemical modifications appears to be of special interest for the analysis of more complex dynamic combinatorial libraries (DCLs) containing constituents generated by recombination of the components.

A diffusion ordered spectroscopy (DOSY) NMR experiment represents an attractive noninvasive method for the analysis of DCLs because it allows the measurement of the diffusion coefficient of a certain molecular species that is directly related to its hydrodynamic radius according to the Stokes–Einstein equation.[6] DOSY NMR techniques provide two-dimensional maps in which one axis corresponds to the chemical shift and the other one corresponds to the diffusion coefficient. Supramolecular entities,[7] as well as mixtures of molecules,[8] have been studied by this method, but no implementation for CDC purposes has been reported.

Herein we discuss the analysis of a previously described DCL composed of helical molecular strands and its subsequent molecular evolution toward [2 × 2] gridlike arrays in the presence of $Zn^{II}$ ions by DOSY NMR methods.[9] We highlight this method as a powerful complementary tool for the analysis of equilibrated mixtures in which minimal structural changes take place.

We first characterized the spatial dimensions of individual compounds **1**–**6** in solution by DOSY NMR techniques to evaluate the potential of the diffusion methodology for the deconvolution of closely related structures in combinatorial mixtures (Figure 1).

Molecular strands derived from the linking of pyrimidine and pyridine units by hydrazone bonds are known to display a persistent helical shape in solution.[10] They were chosen as references because their structures have been well characterized by X-ray radiocrystallography, allowing a direct comparison of the solid-state structure with the calculated dimensions of the objects in solution (Figure 2 and Table 1).

Compound **1** is a bow-shaped flat structure in the crystal form,[9] and displays a diffusion coefficient ($D$) of 750 $\mu m^2 s^{-1}$ at room temperature, corresponding to a hydrodynamic radius of 5.3 Å. The calculations used to fit this value with an oblate ellipsoidal object lead to dimensions of 15 × 8.4 Å, which are close to those values found in the crystal form (15 × 8.8 Å).[9] The hydrodynamic radii for the series of helical strands **1**, **2**, and **4** increase progressively, yielding dimensions for an oblate ellipsoid which are in agreement with those calculated on the basis of solid-state structures (Table 1).[9,10] The introduction of the larger trimethoxyphenyl residues in **3**, **5**, and **6** leads to a significant increase in DOSY-derived

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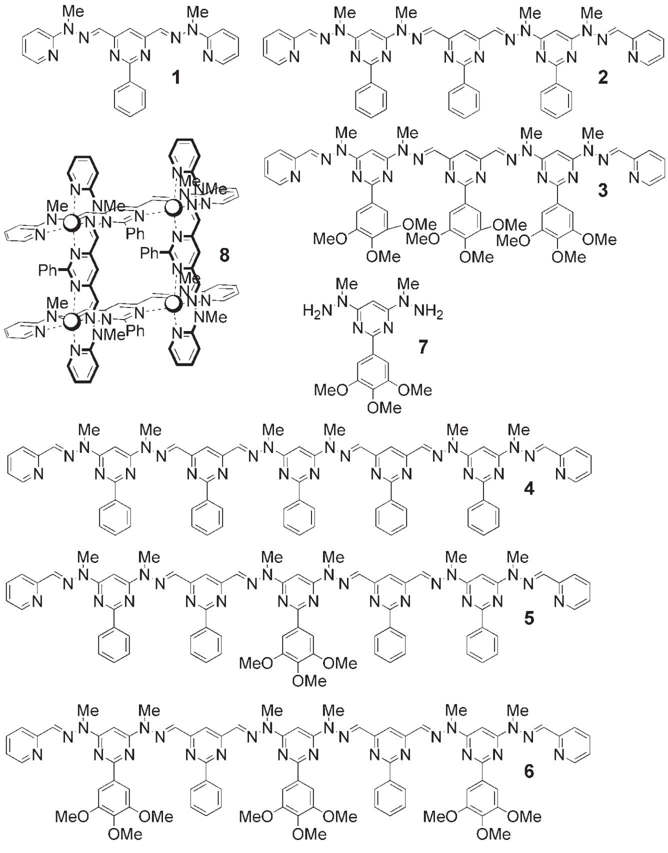

*Figure 1.* Linear representation of pyrimidine–hydrazine-type molecular strands **1**–**7**, and gridlike array **8** (Zn$_4$**1**$_4$).

*Table 1:* Comparison of the dimensions of molecules **1**–**6** and **8** in the solid state as determined by X-ray crystallography and the dimensions in solution as determined by DOSY NMR experiments at various temperatures.

| Compound | *T* [K] | *D* [$\mu$m$^2$ s$^{-1}$][a] | DOSY hydrodynamic radius [Å] and dimensions[b] (diameter×height) | Calculated dimensions [Å][c] (diameter×height) |
|---|---|---|---|---|
| **1** | 296 | 750 | 5.3 (15×8.4) | (15×8.8) |
| **2** | 298 | 660 | 6.1 (19.5×8.8) | (19.4×5.1) |
| | 209 | 167 | 5.5 (19.5×7.2) | |
| **3** | 298 | 590 | 9.0 (22.4×15.8) | (22.4×5.2) |
| | 182 | 82 | 7.9 (22.4×5.2) | |
| **4** | 296 | 550 | 7.2 (19.5×12) | (19.4×7.2) |
| | 220 | 210 | 6.5 (19.5×10) | |
| | 209 | 140 | 6.3 (19.5×9.6) | |
| **5** | 296 | 490 | 8.1 (21.6×13.8) | (21.6×7.4) |
| | 209 | 180 | 5.1 (21.6×7.6) | |
| **6** | 298 | 440 | 9.1 (22.5×16.2) | n.d. |
| | 209 | 150 | 6.1 (22.5×7.8) | |
| **8** | 298 | 550 | 11.5 (18×11) | (17.8×11) |
| | 233 | 215 | 11.1 (18×11) | |

[a] All measurements were performed in CDCl$_3$, except for compound **8**, which was dissolved in CD$_3$CN. The diffusion values in both solvents have been determined from calibration curves established with reference compounds and for a given solvent. [b] The dimensions were fitted from the hydrodynamic radius using an oblate ellipsoid shape; the absolute uncertainty is ±0.5 Å. [c] The average dimensions are reported from the crystal structures of compounds **1**–**5** and **8** as described in Figure 2 (see the Supporting Information). The crystal structure for compound **6** is not available.

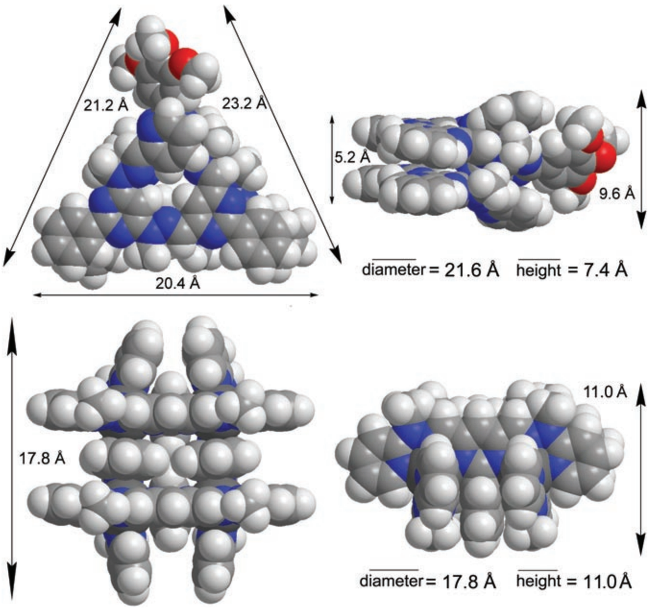

*Figure 2.* Measured dimensions and derived average dimensions (diameter and height for an oblate ellipsoid) for the solid-state molecular structures determined by X-ray crystallography for the helical compound **5** (top) and the gridlike array **8** (Zn$_4$**1**$_4$) (bottom).

dimensions, which are also in agreement with those calculated from crystal structure data (see reference [9] and Figure 2).

The dimensions of the [2×2] gridlike array **8** (Zn$_4$**1**$_4$)[12] were determined in a solution of deuterated acetonitrile at 298 and 233 K and the values agree with the dimensions (18×11 Å) found in the crystal structure (Figure 2, Table 1).

A DOSY NMR experiment was performed at 209 K on a mixture of two distinct compounds, **2** and **4**—one- and two-turn helices, respectively—which differ in their radii by about 1 Å. Considering the region between 3.6 and 4.0 ppm (Me–N resonance signals), the two helical strands were clearly separated by their diffusion coefficients (172 and 143 $\mu$m$^2$s$^{-1}$), yielding individual hydrodynamic radii of 5.3 and 6.4 Å for **2** and **4**, respectively, which are in good agreement with the values determined for the individual compounds in solution (Table 1; see the Supporting Information).

The hydrodynamic radii for helical strands **2**–**6** appear to be very sensitive to temperature. For example, compound **5**, a two-turn helix bearing one trimethoxyphenyl group on its central pyrimidine moiety, displays an increase in hydrodynamic radius (from 5.1 to 8.1 Å) of 60% between 209 and 296 K. Similar changes are observed with all other helical strands. Notably, the one-dimensional NMR spectra do not display variations in chemical shifts and do not show broadening of the resonance signals upon changing the temperature. Furthermore, changes in the hydrodynamic radius are not observed for the rigid grid structure **8** at different temperatures (Table 1 and see the Supporting Information). These data indicate that there is an increase in the average size of the helical strands as the temperature is increased. The changes in the hydrodynamic radius could correspond to an increase of the calculated height (from 7.6 to 13.8 Å assuming that the diameter remains constant) of 80 %. The corresponding motion consists of an extension along the axis of the helix and is shown in Figure 3 for compound **5**, representing a temperature-dependant reversible change in molecular size by a springlike nanomechanical extension–contraction (breathing) motion of the helical strands. The result illustrates the conformational dynamics of these structures and points to the potential usefulness of DOSY NMR experiments to study motions leading to changes in overall molecular size and volume.

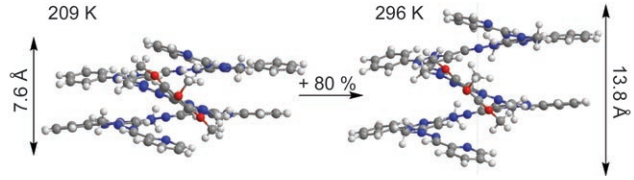

***Figure 3.*** Representation of the springlike extension of the average conformation of helical strand **5** between 209 K (7.6 Å) and 296 K (13.8 Å) in $CDCl_3$, as indicated by DOSY NMR measurements and modeled with AM1 calculations.

We then turned our attention to a library of compounds obtained by mixing compounds **1** and **7** in $CDCl_3$ with a scandium triflate catalyst under microwave activation (Figure 4, top).[9,13] This system produces a set of helices of variable lengths and composition (incorporation of both phenyl pyrimidine and trimethoxyphenyl pyrimidine groups) by crossover recombinations. On the basis of previous investigations involving mass spectroscopy, we expected this DCL to contain at least six distinct helical strands having up to 2.6 turns (eight hydrazone sites).[9] The one-dimensional $^1$H NMR spectrum (Figure 4 a) displays a complex pattern of signals that are characteristic of helical-shaped structures (triplet signals shifted below $\delta = 7$ ppm).[10] We obtained an efficient deconvolution of this DCL after 12 hours of data accumulation at 296 K and fitting the data for the extended region 8.4–5.2 ppm (Figure 4 a,b) by using the DOSY technique. Five different levels of diffusion rates were revealed

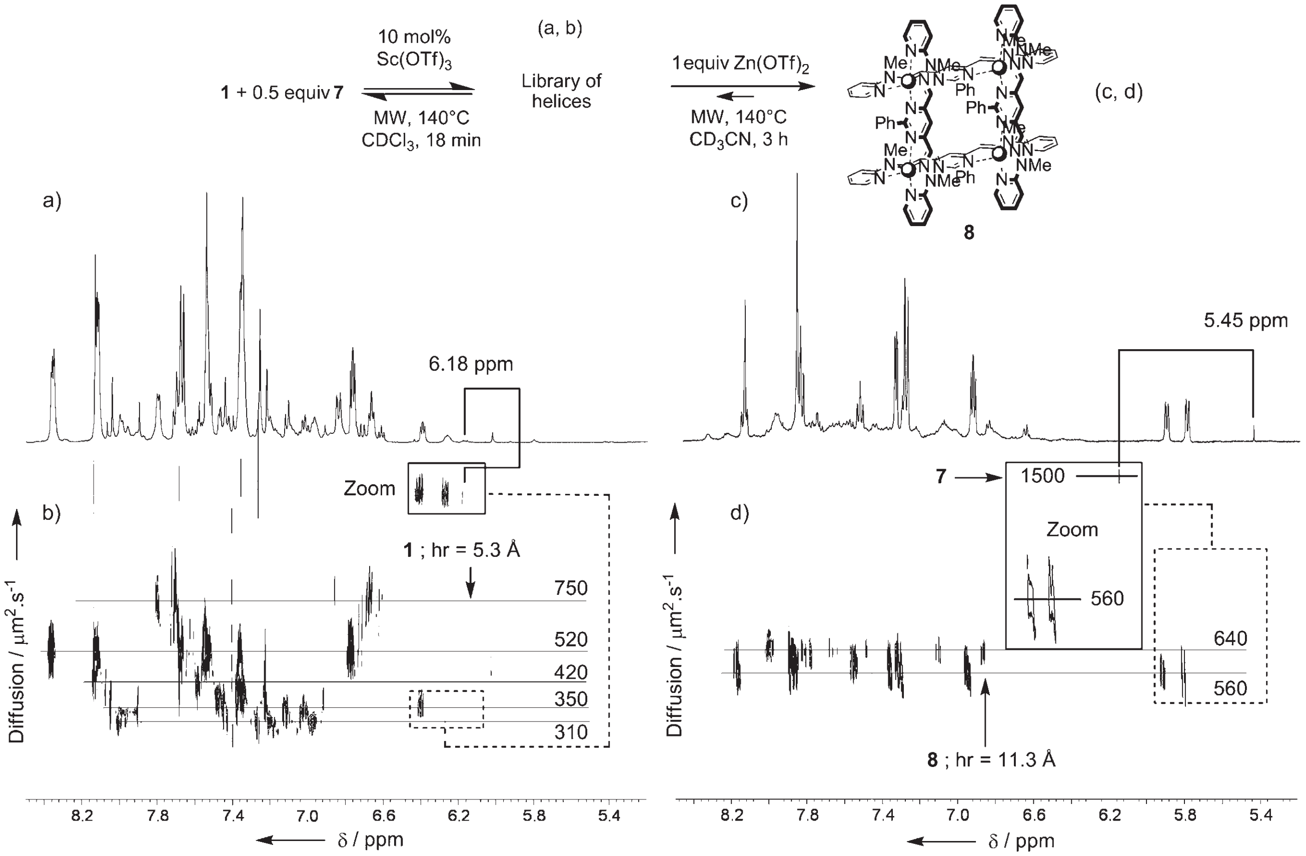

***Figure 4.*** Top: Global equation describing the generation of a dynamic library of molecular strands from compounds **1** and **7**, under $Sc^{III}$ catalysis and microwave (MW) activation. a) $^1$H NMR spectrum (expanded region: $\delta = 8.4$–$5.2$ ppm) of the library of helices at equilibrium (296 K); b) DOSY spectrum of the expanded region in (a) with horizontal lines and diffusion values corresponding to the different species detected in solution (the zoomed-in region shows a lower slice for a more defined region) (hr = hydrodynamic radius); c) $^1$H NMR spectrum of the library after addition of $Zn^{II}$ ions (296 K); d) DOSY spectrum of the expanded region of the library after addition of $Zn^{II}$ ions (the zoomed-in region shows a lower slice for a more defined region). The signals that appear at diffusion coefficients between 1000 and 2000 $\mu m^2 s^{-1}$ are the consequence of a transfer of magnetization from the molecules of the library to the solvent.

and small signals from compounds present in small amounts, such as the resonance signal at $\delta = 6.18$ ppm, were visualized in the second dimension (see zoomed-in region in Figure 4b).

The structure presenting the smallest diffusion coefficient of 310 $\mu m^2 s^{-1}$ corresponds to an eight-site helix bearing four trimethoxyphenyl groups, as expected from results of previous investigations by mass spectrometry.[9] The other diffusion signals ranging from 750 $\mu m^2 s^{-1}$ (compound **1**, Table 1) to 350 $\mu m^2 s^{-1}$ relate to helices increasing in size from 1 to 2.6 turns and incorporating an increasing number of trimethoxyphenyl groups.

Moreover, the addition of one equivalent of $Zn^{II}$ ions relative to the initial amount of compound **1** used in the previously equilibrated DCL leads to a dramatic recombination of the mixture towards the selective formation of the gridlike array **8** ($Zn_4$**1**$_4$), indicative of the enforced generation and amplification of the corresponding ligand **1** (Figure 4, top). The resulting mixture can be analyzed by a DOSY $^1H$ NMR experiment by looking at the region 8.4–5.2 ppm (Figure 4c,d). The NMR signals of the major compound, which displays a diffusion coefficient of 560 $\mu m^2 s^{-1}$, is superimposable on that of pure compound **8** at this temperature. This result is confirmed by the presence of the two characteristic doublets between 5.8 and 6 ppm which correspond to the two nonequivalent *ortho*-phenyl protons of **1** when incorporated in the gridlike structure **8**. A small amount of a short helical strand with a diffusion coefficient of 640 $\mu m^2 s^{-1}$ can be detected, as well as the expected release of compound **7** from longer strands (see zoomed-in region and the singlet at $\delta = 5.45$ ppm, Figure 4d). This observation is in agreement with the fact that two-site ligands bearing a trimethoxyphenyl group cannot be incorporated into [2×2] grids because of steric reasons and the formation of grid **8** leads to the enforced generation of ligand **1**.[9]

In conclusion, we have shown that DOSY NMR techniques are useful and reliable complementary, noninvasive spectroscopic methods for the analysis of DCLs. The method can discriminate between the differential diffusion of species having various hydrodynamic radii, even when there is only very small variations in their structures, thus potentially affording valuable information on constitutional dynamic systems. As illustrated by the variation in the hydrodynamic radii of helical strands as a function of the temperature, this technique also appears to be of interest for determining small conformational/shape changes in molecular and supramolecular systems, thus providing a new approach to the investigation of motional processes that are either difficult to observe or are unobservable by other methods.

## Experimental Section

The syntheses of compounds **1–8** were described earlier: **1**;[11] **2**, **4**, **5**;[10] **3**, **6**, **7**;[9] **8**.[12]

DOSY NMR experiments: The spectra were recorded on a Bruker Avance 500 spectrometer, at 11.7 Tesla, at the resonating frequency of 500.13 MHz for $^1H$, using a BBI Bruker 5-mm gradient probe. The temperature was regulated at 298 K and no spinning was applied to the NMR tube. The diffusion NMR experiments were performed with a pulsed-field gradient stimulated echo (PFGSTE) sequence, using bipolar gradients.[14,15] The bipolar gradient duration and the diffusion time were optimized for each sample and were in the range of 1 to 1.5 ms and 100 to 200 ms, respectively. The evolution of the pulsed-field gradient during the NMR diffusion experiments was established in 30 steps, applied linearly between 1 and 50 $G\,cm^{-1}$. The duration of each NMR diffusion experiment was adjusted to finally obtain a minimum signal-to-noise ratio of 20. DOSY spectra were generated by using the program GIFA 5.2 (DOSY module), developed by the NMRTec company, using adapted algorithms, such as the inverse Laplace transform and maximum entropy, to build the diffusion dimension.[16] Calculation of hydrodynamic radii requires knowing solvent viscosities at different temperatures. $CDCl_3$ and $CD_3CN$ viscosity calibration experiments were performed by DOSY NMR between 300 K and 193 K. A mixture of well characterized molecules (tetramethylsilane, strychnine, and *tert*-butyl alcohol) in $CDCl_3$ was used as reference species, allowing the calculation of the viscosity by the Stokes–Einstein equation.

X-ray radiocrystallography data: The X-ray crystallography data for compounds **1–4** were described earlier: **1**, **3**;[9] **2**, **4**.[10] X-ray diffraction measurements were performed on a Bruker–Nonius Kappa CCD diffractometer using graphite-monochromated $Mo_{K\alpha}$ radiation ($\lambda = 0.71073$ Å) for compound **5** and on a Bruker Smart 6500 diffractometer using synchrotron radiation ($\lambda = 0.31840$ Å) at the European Synchrotron Research Facility in Grenoble (Beamline ID11) for compound **8**. The structures were solved by direct methods and refined by full-matrix least squares techniques based on $F^2$. The non-hydrogen atoms were refined with anisotropic displacement parameters. In the case of compound **8**, the triflate anions and acetonitrile molecules were refined using geometrical restrains. The small size and the instability of the crystals (caused by the loss of solvent) together with the high disorder of the triflate anions can explain the lower resolution of this crystal structure. Calculations were performed by SHELX-97 crystallographic software package. CCDC 653203 (**5**) and 653202 (**8**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre (www.ccdc.cam.ac.uk/data_request/cif).For further experimental details, see the Supporting Information.

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[1] a) J.-M. Lehn, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 4763; b) J.-M. Lehn, *Prog. Polym. Sci.* **2005**, *30*, 814; c) J.-M. Lehn, *Chem. Soc. Rev.* **2007**, *36*, 151.

[2] For dynamic combinatorial/covalent chemistry, see for example: a) J.-M. Lehn, *Chem. Eur. J.* **1999**, *5*, 2455; b) P. T. Corbett, J. Leclaire, J. Vial, K. R. West, J.-L. Wietor, J. K. M. Sanders, S. Otto, *Chem. Rev.* **2006**, *106*, 3652.

[3] a) O. Ramström, J.-M. Lehn, *Nat. Rev. Drug Discovery* **2001**, *1*, 26; b) J. D. Cheeseman, A. D. Corbett, J. L. Gleason, R. J. Kazlanskas, *Chem. Eur. J.* **2005**, *11*, 1708.

[4] a) N. Giuseppone, J.-M. Lehn, *Chem. Eur. J.* **2006**, *12*, 1715; b) For the influence of pH and temperature on dynamic covalently bonded rotaxanes, see: H. Kawai, T. Umehara, K. Fujiwara, T. Tsuji, T. Suzuki, *Angew. Chem.* **2006**, *118*, 4387; *Angew. Chem. Int. Ed.* **2006**, *45*, 4281; c) S. Nampally, J.-M. Lehn, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 5938; d) N. Giuseppone, J.-M. Lehn, *Angew. Chem.* **2006**, *118*, 4735; *Angew. Chem. Int. Ed.* **2006**, *45*, 4619; e) N. Giuseppone, J.-L. Schmitt, J.-M. Lehn,

*Angew. Chem.* **2004**, *116*, 5010; *Angew. Chem. Int. Ed.* **2004**, *43*, 4902.

[5] a) C.-F. Chow, S. Fujii, J.-M. Lehn, *Angew. Chem.* **2007**, *119*, 5095; *Angew. Chem. Int. Ed.* **2007**, *46*, 5007; b) T. Ono, S. Fujii, T. Nobori and J.-M. Lehn, *Chem. Commun.* **2007**, 46; c) N. Giuseppone, G. Fuks, J.-M. Lehn, *Chem. Eur. J.* **2006**, *12*, 1723; d) N. Giuseppone, J.-M. Lehn, *J. Am. Chem. Soc.* **2004**, *126*, 11448.

[6] a) Y. Cohen, L. Avram, L. Frish, *Angew. Chem.* **2005**, *117*, 524; *Angew. Chem. Int. Ed.* **2005**, *44*, 520; b) E. L. Hahn, *Phys. Rev.* **1950**, *80*, 580; c) O. E. Stejskal, J. E. Tanner, *J. Chem. Phys.* **1965**, *42*, 288.

[7] a) R. Rymdén, J. Carlfors, P. Stilbs, *J. Inclusion Phenom.* **1983**, *1*, 159; b) B. Olenyuk, M. D. Levin, J. A. Whiteford, J. E. Shield, P. J. Stang, *J. Am. Chem. Soc.* **1999**, *121*, 10434; c) M. Greenwald, D. Wessely, I. Goldberg, Y. Cohen, *New J. Chem.* **1999**, *23*, 337; d) M. Shaul, Y. Cohen, *J. Org. Chem.* **1999**, *64*, 9358; e) W. H. Otto, M. H. Keefe, K. E. Splan, J. T. Hupp, C. K. Larive, *Inorg. Chem.* **2002**, *41*, 6172; f) S. Viel, L. Mannina, A. Segre, *Tetrahedron Lett.* **2002**, *43*, 2515; g) L. Allouche, A. Marquis, J.-M. Lehn, *Chem. Eur. J.* **2006**, *12*, 7520; h) T. E.-S. L. Frish, F. W. B. van Leeuwen, D. N. Reinhoudt, W. Verboom, M. S. Kaucher, J. T. Davis, Y. Cohen, *Chem. Eur. J.* **2007**, *13*, 1969.

[8] D. Wu, A. Chen, C. S. Johnson, Jr., *J. Am. Chem. Soc.* **1993**, *115*, 4291.

[9] N. Giuseppone, J.-L. Schmitt, J.-M. Lehn, *J. Am. Chem. Soc.* **2006**, *128*, 16748.

[10] J.-L. Schmitt, A.-M. Stadler, N. Kyritsakas, J.-M. Lehn, *Helv. Chim. Acta* **2003**, *86*, 1598.

[11] K. M. Gardinier, R. G. Khoury, J.-M. Lehn, *Chem. Eur. J.* **2000**, *6*, 4124.

[12] M. Barboiu, M. Ruben, G. Blasen, N. Kyritsakas, E. Chacko, M. Dutta, O. Radekovich, K. Lenton, D. J. R. Brook, J.-M. Lehn, *Eur. J. Inorg. Chem.* **2006**, 784.

[13] N. Giuseppone, J.-L. Schmitt, E. Schwartz, J.-M. Lehn, *J. Am. Chem. Soc.* **2005**, *127*, 5528.

[14] J. E. Tanner, *J. Chem. Phys.* **1970**, *52*, 2523.

[15] R. M. Cotts, M. J. R. Hoch, T. Sun, J. T. Marker, *J. Magn. Reson.* **1989**, 252; R. Johnson, Jr., *Prog. Nucl. Magn. Reson. Spectrosc.* **1999**, *34*, 203.

[16] M. A. Delsuc, T. E. Malliavin, *Anal. Chem.* **1998**, *70*, 2146.