Catenanes

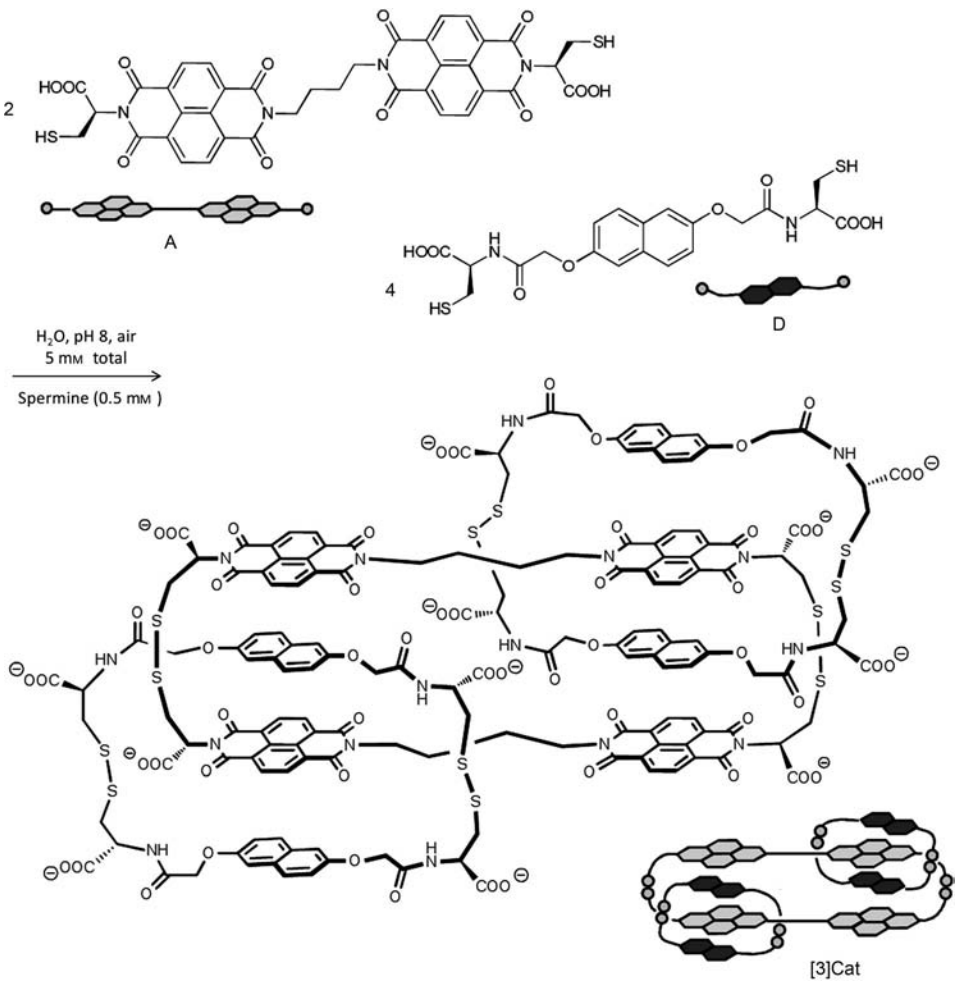
DOI: 10.1002/ange.201106885

Templated Dynamic Synthesis of a [3]Catenane**

Fabien B. L. Cougnon, Nicholas A. Jenkins, G. Dan Pantoş, and Jeremy K. M. Sanders**

We present herein the unprecedented and efficient formation of a donor–acceptor [3]catenane from an aqueous dynamic combinatorial system containing only linear building blocks and $NaNO_3$ (1M). Even more remarkably, the synthesis is improved if the salt, which raises the ionic strength and presumably encourages hydrophobic association, is replaced by a sub-millimolar concentration of spermine acting as a template (Scheme 1).

Although of potential interest in the context of nanoscience,[1] donor–acceptor [3]catenanes have remained relatively unexplored because of their challenging synthesis. Stoddart and co-workers showed that donor–acceptor interactions could be efficiently used to reversibly assemble preformed macrocycles into the thermodynamically more stable [3]catenane.[2] This approach is not trivial: careful design of the size and geometry of the rings is necessary and, while the assembly of the catenane is nearly quantitative, the synthesis of each of the rings involves a series of delicate steps, ultimately leading to an overall low yield. Apart from a few variations and improvements to the method developed by the Stoddart group, these constraints have made examples of donor–acceptor [3]catenanes rare in the literature.[2–4]



Scheme 1. Synthesis of a [3]catenane in water, templated by the presence of spermine.

We previously showed that dynamic combinatorial chemistry[5] led to an unexpected variety of [2]catenanes.[6,7] To investigate the possibility of forming higher-order interlocked structures by applying methods of dynamic combinatorial chemistry, we designed building block A (acceptor),[8] composed of two large hydrophobic electron-deficient π systems (1,4,5,8-naphthalenediimide) connected through a flexible aliphatic chain (Scheme 1). Building block A is terminated by two cysteine components, which provide both the thiol necessary for disulfide exchange and carboxylate anions for water solubility. The objective was to explore the behavior of A in the presence of the electron-rich 2,6-dialkoxynaphthalene building block D (donor), which has already been studied in detail in aqueous libraries.[6]

[*] F. B. L. Cougnon, N. A. Jenkins, Dr. G. D. Pantoş,[+]
Prof. J. K. M. Sanders
University Chemical Laboratory, University of Cambridge
Lensfield Road, CB2 1EW, Cambridge (UK)
E-mail: jkms@cam.ac.uk

[+] Present address: Department of Chemistry, University of Bath
Bath, BA2 7AY (UK)
E-mail: g.d.pantos@bath.ac.uk

[**] We are grateful to EPSRC (F.B.L.C., J.K.M.S.); Pembroke College, Cambridge and the University of Bath (G.D.P.) for financial support. We thank Dr. Artur R. Stefankiewicz for helpful discussions, Nandhini Ponnuswamy for help with NMR spectroscopy, and Dr. Ana Belenguer for maintaining the LC–MS facility.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201106885.

The first library was prepared by dissolving A and D to give a total concentration of 5 mM (1:2 molar ratio) in water at pH 8 in the presence of $NaNO_3$ (1M), because the formation of catenanes is known to be promoted upon addition of salt.[6,9] The library was stirred for four hours in air in capped vials to allow oxidation of the thiol building blocks. LC–MS analysis revealed that the fully oxidized library contains a relatively small number of significant components, which is far from the diversity expected from such flexible building blocks. The only detectable members of this library are the closed monomer D, the dimers D_2 and A_2, as well as two catenanes composed of one and two donor dimers interlocked with an acceptor dimer, [2]Cat and [3]Cat, respectively (Figure 1c).

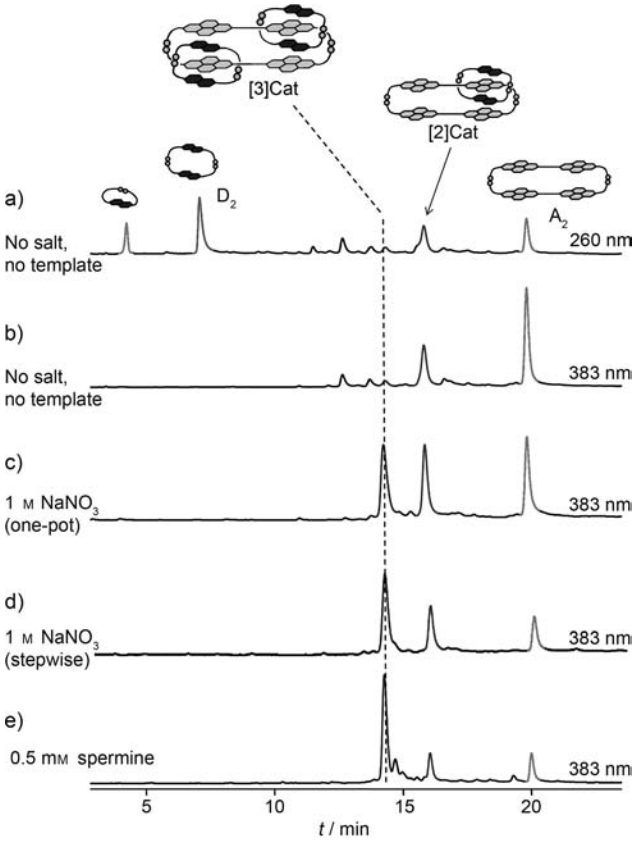


Figure 1. HPLC analysis of an aqueous library composed of A and D (1:2 molar ratio, 5 mM total concentration), prepared a, b) in the absence of $NaNO_3$, recorded at a) 260 nm and b) 383 nm. The same library was prepared in presence of 1 M of $NaNO_3$, c) in one pot (383 nm) or d) after stepwise addition of D (383 nm). e) The library was also prepared in the presence of 0.5 mM of spermine (383 nm). Note that the closed monomer D and dimer D_2 can only be detected at 260 nm.

Both catenanes were unambiguously identified by tandem ESI-MS and MS/MS. The [3]catenane displays doubly and triply charged molecular ions (*m*/*z* 1752.5 and 1167.8), corresponding to a species composed of two acceptor and four donor building blocks (Figure 2). The fragmentation pattern of [3]Cat is characteristic of that expected for a [3]catenane, with the loss of one or both rings producing the intermediate [2]Cat (*m*/*z* 1271.6, doubly charged), dimers A_2 (*m*/*z* 1583.5) and D_2 (*m*/*z* 958.9), and smaller fragments.

The presence of [2]Cat along with remaining non-interlocked dimers suggested that the library is fully oxidized before thermodynamic equilibrium is reached. This hypothesis is consistent with previous observations[6a] and was confirmed by a series of kinetic analyses. To compare the rates of oxidation of building blocks A and D, we prepared a library in a 1:1 molar ratio, which showed that the acceptor A oxidizes faster than donor D (Figure 3b), rapidly forming the acceptor dimer A_2. Although the rate of oxidation of the thiols should be similar for the two building blocks, it is perhaps not surprising that favorable hydrophobic and π–π interactions[10] make the formation of A_2 a pseudo-intramolecular process and therefore faster than the formation of the electron-rich and hence unfavorable dimer D_2. Strikingly, the two building blocks do not form any mixed macrocycles, which may be rationalized by their difference in size and the likely templation of A_2 by D and of D_2 by A through donor–acceptor interactions. The kinetic profiles of the formation of A_2, [2]Cat, and [3]Cat (Figure 3c) show that the acceptor dimer A_2 is threaded by two open donor dimers consecutively, thereby forming [2]Cat and [3]Cat successively (Figure 3). From this study, we concluded that the formation of the [3]catenane appears to be a stepwise process, and the library evolves until all the donor building block is fully oxidized, preventing thermodynamic equilibrium from being reached.[6a] If the disulfide exchange were to occur for a longer period of time to allow the system to reach thermodynamic equilibrium, the unthreaded residues should be recycled into [3]Cat, which is a kinetic trap and may also be the thermodynamic product in this library. Consequently, the formation of the [3]catenane is limited both by the fast rate of thiol oxidation, which prevents the library from reaching thermodynamic equilibrium, and by the kinetic competition between the formation of the unthreaded and the threaded macrocycles.

To test this supposition, we investigated the effect of successive additions of the fresh thiol D, which reinitiates the reversible process and favors threading by providing a high acceptor/donor ratio. Two equivalents of D were added in aliquots of 0.5 equivalents every two hours to a library containing the acceptor A. As expected, the yield of the [3]catenane increased from 33 (one-pot procedure, Figure 1c) up to 55% (stepwise additions, Figure 1d) with the final ratio between the building blocks being equal in both cases. These yields were evaluated by HPLC from the peak areas. Unfortunately, further increasing the number of successive additions (0.25 equivalents added every two hours) or changing the time between the additions did not lead to any improvement of the [3]catenane yield, most likely owing to the low overall concentration of free thiols slowing the exchange kinetics.

All the significant library members have been isolated and characterized by ^{1}H NMR spectroscopy (500 MHz, 298 K, D_2O). Whereas A_2 and [2]Cat display the very broad resonances characteristic of flexible macrocycles,[11] the [3]catenane exhibits a spectrum with sharp signals, in which

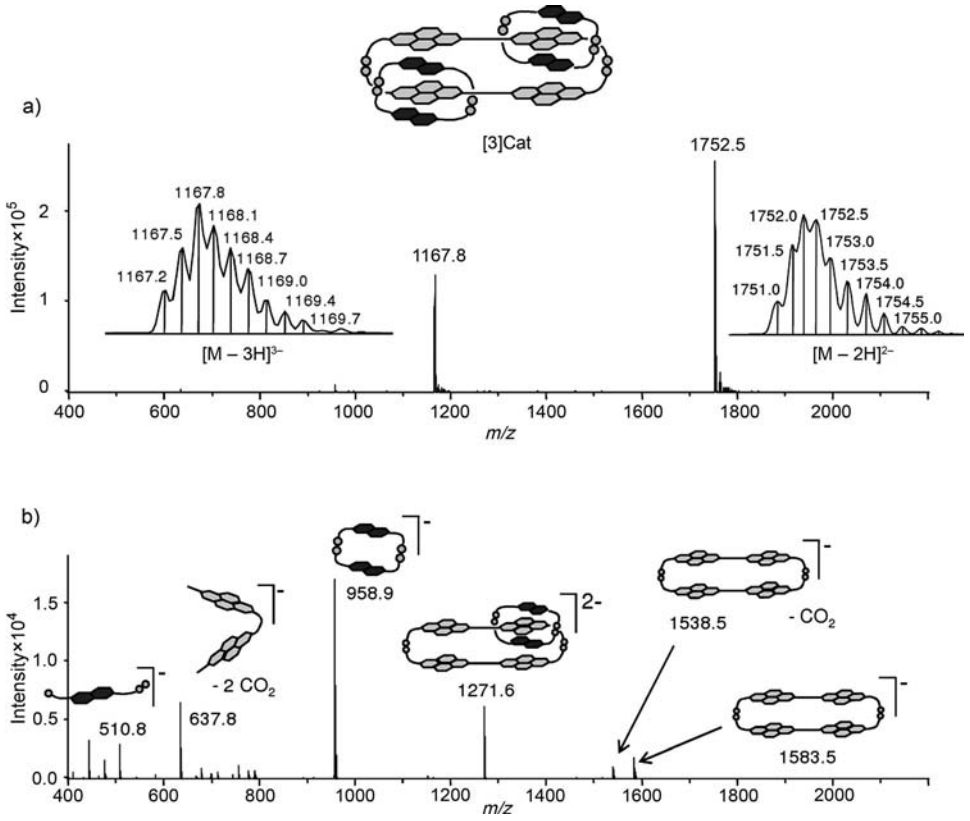


Figure 2. Tandem a) MS and b) MS/MS fragmentation of [3]Cat. For clarity, only the cyclic fragments are shown; however the MS/MS signals may also correspond to the linear species. The gray cartoon dots represent the sulfur atoms of the cysteine residues.

the resonances for a single asymmetric conformation can be clearly identified: two sets of signals were assigned to the outer ($\delta = 8.1$–8.6 ppm) and inner ($\delta = 6.9$–7.8 ppm) acceptor units and similarly, two sets of signals were observed for the outer ($\delta = 6.4$–7.15 ppm) and inner ($\delta = 4.7$–6.3 ppm) donor units (Figure 4a). NOE correlations between the outer donor protons and the protons of the aliphatic chain suggest that the most likely conformation of [3]Cat is the one drawn in Figure 4.

The catenane synthesis described above appears to rely on the increased polarity of the reaction medium (1 M $NaNO_3$ versus pure water, Figure 1b,c) to promote the burying of solvent-exposed hydrophobic surfaces.[6,9] In a salt-free reaction, [3]Cat is formed only in trace amounts, and the library is dominated by the homodimers A_2 and D_2. Of all

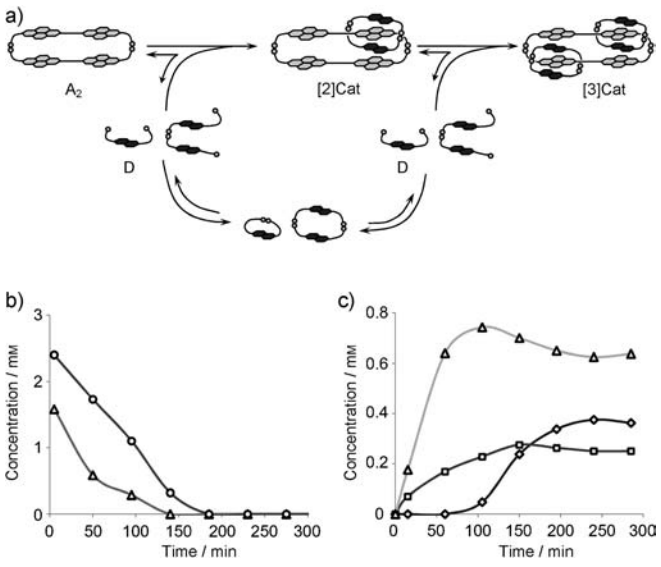


Figure 3. a) Proposed mechanism for the synthesis of [3]Cat, involving the successive formation of A_2, [2]Cat, and finally [3]Cat. Kinetic profile of b) the consumption of building blocks A (△) and D (○) and c) of the formation of A_2 (△), [2]Cat (□), and [3]Cat (◇), in a library composed of A and D (5 mM total concentration, 1:1 molar ratio) in water pH 8, in the presence of $NaNO_3$ (1 M). The concentrations were evaluated from the HPLC peak areas.

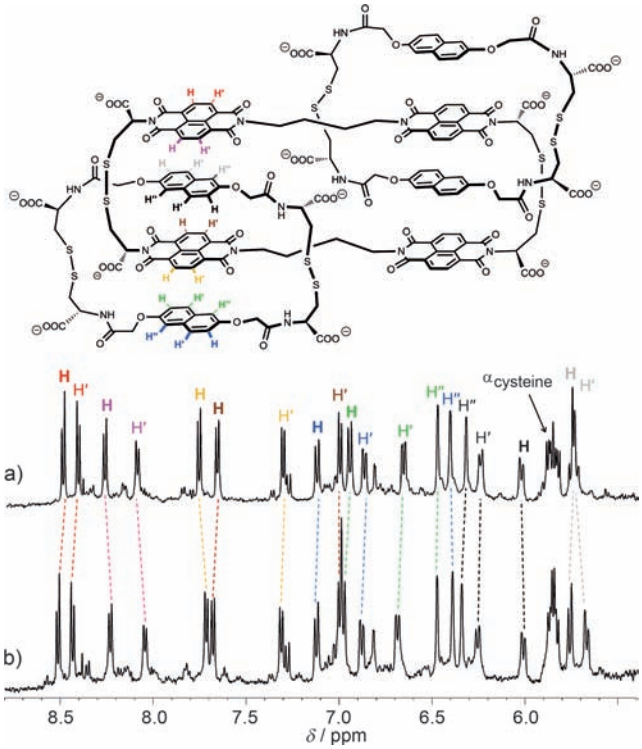


Figure 4. ^{1}H NMR spectrum (D_2O, 295 K, 500 MHz) of [3]Cat a) in the absence and b) in the presence of one equivalent of spermine.

the library members observed in this system, the [3]catenane possesses the highest density of carboxylate groups, thus raising the question of whether a suitable polycation might act as a template.

In nature, polyamines such as spermine bind to the array of negative charges of the DNA backbone and play an important role in many cellular processes,[12] thus prompting the development of artificial spermine binders.[13] To our delight, addition of merely 0.5 mM spermine promoted the amplification of [3]Cat to 60% yield (Figure 1e), suggesting a strong interaction between the [3]catenane and spermine. Increasing the concentration of template does not further increase the yield of [3]catenane. It is well-documented and understood, but counterintuitive, that adding too much template can lead to amplification of smaller, weaker binding receptors.[14] No amplification was observed in the presence of up to 1M NH_4Cl, implying 1) that the interaction between [3]Cat and spermine results from more than the sum of random carboxylate–ammonium interactions and 2) that the effect of metal salts such as $NaNO_3$ may be due to more than purely ionic-strength effects. The geometrical distribution, hydrophobicity, and number of ammonium centers present in the polyamine are crucial for an efficient recognition, as demonstrated by libraries templated by the shorter spermidine and putrescine, in which [3]Cat was amplified to lesser extent (see the Supporting Information).

The [3]Cat binds spermine with an association constant of $(1.1 \pm 0.01) \times 10^5\,M^{-1}$ as determined by ^1H NMR spectroscopy titration experiments, thus confirming the strong interaction between the two species (Figure 4b). Modeling[15] suggests that the aromatic moieties of the [3]catenane are too closely packed to accommodate a cavity large enough for spermine. Therefore we believe that the template binds to the solvent-accessible surface of the catenane, interacting with the arrayed carboxylate ions in a manner reminiscent of spermine's interactions with the phosphate groups of double helical DNA.

To conclude, we have shown for the first time that acyclic units can spontaneously self-assemble into a donor–acceptor [3]catenane, either in a high-polarity medium or in the presence of spermine. The amplification of the [3]catenane in the presence of spermine highlights the importance of small template molecules in the controlled assembly of large complex structures through a multitude of seemingly weak interactions in a highly competitive medium. This work brings a new insight into the mechanism of donor–acceptor self-assembly and provides an alternative route to the efficient synthesis of [3]catenanes. The formation of this catenane is not fully under thermodynamic control, so kinetic parameters also play a role in this process. To limit the formation of competing species, we have therefore developed a stepwise dynamic approach, moving away from pure dynamic combinatorial chemistry, in which the exchange occurs continuously. This approach involves the idea of an evolutionary process with iterated cycles of selection and amplification, which is closer than pure dynamic combinatorial systems to the phenomena observed in biological systems.[5]

We believe that the principles developed herein can be extended to access larger structures than [3]catenanes by using building blocks containing more than one acceptor and/or donor unit. This work is considered as being the first step towards the self-assembly of more complex polycatenanes.

Experimental Section

Preparation of a "one-pot" library: Stock solutions (5 mM) of A and D were prepared by dissolving the building blocks in aqueous NaOH (10 mM) and subsequent titration with NaOH (100 mM) to pH 8. The stock solutions were mixed in the relevant ratio to obtain the library. When necessary, salt ($NaNO_3$) was added directly in solid form to produce a 1M solution. The final library solutions (0.5 mL) were stirred in close-capped vials for at least four hours before LC–MS analysis.

Preparation of a "templated" library: Stock solutions (7 mM) of A and D were prepared by dissolving the building blocks in aqueous NaOH (10 mM) and subsequent titration with NaOH (100 mM) to pH 8. The stock solutions were mixed in the relevant ratio to obtain the library and diluted with a concentrated aqueous stock solution of template to reach a total concentration in building blocks of 5 mM and adequate concentration of the template. The final library solutions (0.5 mL) were stirred in close-capped vials for at least four hours before LC–MS analysis.

Preparation of a "stepwise addition" library: A stock solution of A (5 mM, 0.2 mL) was prepared by dissolving the building block in aqueous NaOH (10 mM) and subsequent titration with NaOH (100 mM) to pH 8. $NaNO_3$ was added directly in solid form to give a 1M solution. Every two hours, a freshly prepared aqueous stock solution of D (50 μL, 5 mM, 1M $NaNO_3$, pH 8) was added. The addition was repeated four times before LC–MS analysis.

Received: September 28, 2011
Revised: October 27, 2011
Published online: December 30, 2011

Keywords: catenanes · molecular recognition · self-assembly · supramolecular chemistry · template synthesis

[1] *From Non-Covalent Assemblies to Molecular Machines* (Eds.: J. P. Sauvage, P. Gaspard), Wiley-VCH, Weinheim, **2010**.

[2] a) K. Patel, O. Š. Miljanić, J. F. Stoddart, *Chem. Commun.* **2008**, 1853; b) P. R. Ashton, C. L. Brown, E. J. T. Chrystal, T. T. Goodnow, A. E. Kaifer, K. P. Parry, A. M. Z. Slawin, N. Spencer, J. F. Stoddart, D. J. Williams, *Angew. Chem.* **1991**, *103*, 1055; *Angew. Chem. Int. Ed. Engl.* **1991**, *30*, 1039; c) A. Coskun et al., *J. Am. Chem. Soc.* **2011**, *133*, 4538, see the Supporting Information; d) J. M. Spruell et al., *Nat. Chem.* **2010**, *2*, 870, see the Supporting Information; e) P. R. Ashton, S. E. Boyd, C. G. Claessens, R. E. Gillard, S. Menzer, J. F. Stoodart, M. S. Tolley, A. J. P. White, D. J. Williams, *Chem. Eur. J.* **1997**, *3*, 788.

[3] a) V. Blanco, M. Chas, D. Abella, C. Peinador, J. M. Quintela, *J. Am. Chem. Soc.* **2007**, *129*, 13978; b) M. Chas, V. Blanco, C. Peinador, J. M. Quintela, *Org. Lett.* **2007**, *9*, 675; c) A. L. Hubbard, G. J. E. Davidson, R. H. Patel, J. A. Wisner, S. J. Loeb, *Chem. Commun.* **2004**, 138; d) D. G. Hamilton, N. Feeder, S. J. Teat, J. K. M. Sanders, *New J. Chem.* **1998**, *22*, 1019; e) D. G. Hamilton, N. Feeder, L. Prodi, S. J. Teat, W. Clegg, J. K. M. Sanders, *J. Am. Chem. Soc.* **1998**, *120*, 1096.

[4] For other relevant self-assembled [3]catenanes, see: A. Hori, K. Kumazawa, T. Kusukawa, D. K. Chand, M. Fujita, S. Sakamoto, K. Yamaguchi, *Chem. Eur. J.* **2001**, *7*, 4142.

[5] For reviews of dynamic combinatorial chemistry, see: a) F. B. L. Cougnon, J. K. M. Sanders, *Acc. Chem. Res.*, **2011**, DOI:10.1021/ar200240m; b) A. Herrmann, *Org. Biomol. Chem.* **2009**, *7*, 3195; c) S. Ladame, *Org. Biomol. Chem.* **2008**, *6*, 219; d) J.-M. Lehn,

Chem. Soc. Rev. **2007**, *36*, 151; e) M. M. Rozenman, B. R. McNaughton, D. R. Liu, *Curr. Opin. Chem. Biol.* **2007**, *11*, 259; f) P. T. Corbett, J. Leclaire, L. Vial, K. R. West, J.-L. Wietor, J. K. M. Sanders, S. Otto, *Chem. Rev.* **2006**, *106*, 3652; g) B. de Bruin, P. Hauwert, J. N. H. Reek, *Angew. Chem.* **2006**, *118*, 2726; *Angew. Chem. Int. Ed.* **2006**, *45*, 2660.

[6] a) F. B. L. Cougnon, H. Y. Au-Yeung, G. D. Pantoş, J. K. M. Sanders, *J. Am. Chem. Soc.* **2011**, *133*, 3198; b) H. Y. Au-Yeung, G. D. Pantoş, J. K. M. Sanders, *J. Org. Chem.* **2011**, *76*, 1257; c) H. Y. Au-Yeung, G. D. Pantoş, J. K. M. Sanders, *Angew. Chem.* **2010**, *122*, 5459; *Angew. Chem. Int. Ed.* **2010**, *49*, 5331; d) H. Y. Au-Yeung, G. D. Pantoş, J. K. M. Sanders, *J. Am. Chem. Soc.* **2009**, *131*, 16030; e) H. Y. Au-Yeung, G. D. Pantoş, J. K. M. Sanders, *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 10466.

[7] For other examples of [2]catenane discovery from dynamic combinatorial chemistry, see: a) M.-K. Chung, P. S. White, S. J. Lee, M. R. Gagné, *Angew. Chem.* **2009**, *121*, 8839; *Angew. Chem. Int. Ed.* **2009**, *48*, 8683; b) K. R. West, R. F. Ludlow, P. T. Corbett, P. Besenius, F. M. Mansfeld, P. A. G. Cormack, D. G. Sherrington, J. M. Goodman, M. C. A. Stuart, S. Otto, *J. Am. Chem. Soc.* **2008**, *130*, 12218; c) R. T. S. Lam, A. Belenguer, S. L. Roberts, C. Naumann, T. Jarrosson, S. Otto, J. K. M. Sanders, *Science* **2005**, *308*, 667.

[8] For the synthesis of asymmetrically substituted 1,4,5,8-naphthalenediimides, see: K. Tambara, N. Ponnuswamy, G. Hennrich, G. D. Pantoş, *J. Org. Chem.* **2011**, *76*, 3338.

[9] For the use of salt to increase medium polarity, see: a) M. Fujita, F. Ibukuro, H. Hagihara, K. Ogura, *Nature* **1994**, *367*, 720; b) M. Fujita, F. Ibukuro, K. Ogura, *J. Am. Chem. Soc.* **1995**, *117*, 4175.

[10] M. S. Cubberley, B. L. Iverson, *J. Am. Chem. Soc.* **2001**, *123*, 7560.

[11] H. Y. Au-Yeung, P. Pengo, G. D. Pantoş, S. Otto, J. K. M. Sanders, *Chem. Commun.* **2009**, 419.

[12] a) C. W. Tabor, H. Tabor, *Annu. Rev. Biochem.* **1984**, *53*, 749; b) K. Igarashi, K. Kashiwagi, *Biochem. Biophys. Res. Commun.* **2000**, *271*, 559.

[13] For examples of spermine receptors, see: a) R. Pérez-Fernández, M. Pittelkow, A. M. Belenguer, L. A. Lane, C. V. Robinson, J. K. M. Sanders, *Chem. Commun.* **2009**, 3708; b) L. Vial, R. F. Ludlow, J. Leclaire, R. Pérez-Fernández, S. Otto, *J. Am. Chem. Soc.* **2006**, *128*, 10253; c) H. Isobe, N. Tomita, J. W. Lee, H. J. Kim, K. Kim, E. Nakamura, *Angew. Chem.* **2000**, *112*, 4427; *Angew. Chem. Int. Ed.* **2000**, *39*, 4257; d) Y. M. Jeon, D. Whang, J. Kim, K. Kim, *Chem. Lett.* **1996**, 503.

[14] a) K. Severin, *Chem. Eur. J.* **2004**, *10*, 2565; b) P. T. Corbett, J. K. M. Sanders, S. Otto, *J. Am. Chem. Soc.* **2005**, *127*, 9390.

[15] Molecular modeling was performed at PM6 semiempirical level with MOPAC2009 using the COSMO solvation model. MOPAC2009, James J. P. Stewart, Stewart Computational Chemistry, Colorado Springs, CO, USA, http://OpenMOPAC.net (**2008**).

