

structure, the two crown moieties are nicely oriented to form a threaded complex with **1**. On the possible turn structures, the one having ⁴Leu-⁵Leu at *i*+1, *i*+2 positions gave the most stable structure for the complex.^[13]

In conclusion, we have prepared peptidic pseudorotaxanes [**A**·**1**] and [**B**·**1**] through self-assembly under thermodynamically controlled conditions. These supramolecular systems display thermoregulated optical properties that could make them useful for the development of optical devices. Future work will focus on the applications of these supramolecular systems, as well as on the preparation of receptors with different amino acids by a combinatorial approach, permitting the molecular engineering of novel materials with unique properties.

Received: September 1, 1998

Revised: October 15, 1999 [Z12364]

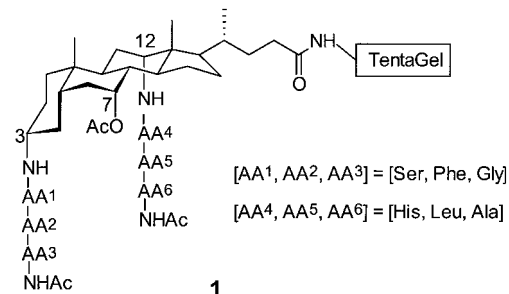
- [1] D. Philp, J. F. Stoddart, *Angew. Chem.* **1996**, *108*, 1242–1286; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1154–1196, and references therein.
- [2] a) P. R. Ashton, S. E. Boyd, C. G. Claessens, R. E. Gillard, S. Menzer, J. F. Stoddart, M. S. Tolley, A. J. P. White, D. J. Williams, *Chem. Eur. J.* **1997**, *3*, 788–798; b) R. Jäger, F. Vögtle, *Angew. Chem.* **1997**, *109*, 966–980; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 930–944; c) J.-P. Sauvage, C. O. Dietrich-Buchecker, J.-C. Chambron in *Comprehensive Supramolecular Chemistry*, Vol. 9 (Ed.: J.-M. Lehn), Pergamon, Oxford, **1996**, p. 43; d) D. G. Hamilton, J. E. Davies, L. Prodi, J. K. M. Sanders, *Chem. Eur. J.* **1998**, *4*, 608–620.
- [3] For reviews, see: a) N. Voyer, *Top. Curr. Chem.* **1996**, *184*, 1–37; b) N. Voyer, J. Lamothe, *Tetrahedron* **1995**, *34*, 9241–9284.
- [4] Recently, Leigh reported the formation of an achiral rotaxane and molecular shuttle incorporating a Gly-Gly dipeptide: a) D. A. Leigh, A. Murphy, J. P. Smart, M. Z. Slawin, *Angew. Chem.* **1997**, *109*, 752–756; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 728–732; b) S. A. Lane, D. A. Leigh, A. Murphy, *J. Am. Chem. Soc.* **1997**, *119*, 11092–11093.
- [5] a) N. Voyer, J. Roby, D. Deschênes, J. Bernier, *Supramol. Chem.* **1995**, *5*, 61–69; b) N. Voyer, B. Guérin, *Tetrahedron* **1994**, *4*, 989–1010; c) N. Voyer, *J. Am. Chem. Soc.* **1991**, *113*, 1818–1821.
- [6] a) P. R. Ashton, M. C. T. Fyfe, P. T. Glink, S. Menzer, J. F. Stoddart, A. J. P. White, D. J. Williams, *J. Am. Chem. Soc.* **1997**, *119*, 12514–12524; b) P. R. Ashton, P. T. Glink, J. F. Stoddart, P. A. Tasker, A. J. P. White, D. J. Williams, *Chem. Eur. J.* **1996**, *2*, 729–736.
- [7] P. R. Ashton, E. J. T. Chrystal, P. T. Glink, S. Menzer, C. Schiavo, N. Spencer, J. F. Stoddart, P. A. Tasker, A. J. P. White, D. J. Williams, *Chem. Eur. J.* **1996**, *2*, 709–728.
- [8] P. R. Ashton, R. Ballardini, V. Balzani, M. Gómez-López, S. E. Lawrence, M. V. Martínez-Díaz, M. Montalti, A. Piersanti, L. Prodi, J. F. Stoddart, D. J. Williams, *J. Am. Chem. Soc.* **1997**, *119*, 10641–10651.
- [9] M. C. Manning, *Biocatalyst Design for Stability and Specificity*, American Chemical Society, Washington, DC, **1993**, chap. 3.
- [10] R. W. Woody, *Peptides, Polypeptides, and Proteins*, Wiley, New York, NY, **1986**, pp. 339–350.
- [11] Measured at 45 °C and assuming the formation of 1:1 complex only. M. P. Mack, R. R. Richardson, R. A. Palmer, R. G. Ghirardelli, *J. Am. Chem. Soc.* **1976**, *98*, 7830–7832.
- [12] Modeling was performed using MOE software from the Chemical Computing Group (www.chemcomp.com) using a CVFF-91 force field.
- [13] It is likely that the most stable structure of [**A**·**1**] shown in Figure 4 is accompanied by one or more other β -turn complexes in solution. This would explain the difficulties in crystallizing [**A**·**1**] and discrepancies in the NMR integrations.

Application of Combinatorial Procedures in the Search for Serine-Protease-Like Activity with Focus on the Acyl Transfer Step**

Hilde De Muynck, Annemieke Madder, Nadia Farcy, Pierre J. De Clercq,* M. Nieves Pérez-Payán, Liselotte M. Öhberg, and Anthony P. Davis

The attainment of the selectivities and efficiencies of enzymes remains a major goal in organic chemistry.^[1] The efficiency of the serine protease α -chymotrypsin in cleaving peptide bonds, relative to the uncatalyzed hydrolysis, serves as an illustrative example.^[2] Although notable progress has been made in the development of synthetic hydrolases,^[3] the real challenge, the cleavage of nonactivated amides, still remains. Several years ago we embarked on a long-term program aimed at the development of synthetic hydrolases with the focus on gaining a better understanding of the enzymatic process. In particular we developed non-peptidic organic molecules possessing an array of functional groups in a suitable geometry for eventual hydrolytic activity.^[4] In contrast to that work, in which a carefully designed molecule was targeted, we describe herein the first results of a study in which serine-protease activity is searched by combinatorial techniques.^[5]

Our long term goal is to develop a system that is characterized by three independent peptidic chains, each containing one of the three active residues^[6] of the classic triad (Ser, His, and Asp), and possessing serine-protease-like activity. As a first step towards this goal we describe here: 1) the mix-split synthesis of the library **1** containing 729 (3⁶) members, in which the two most important catalytic residues

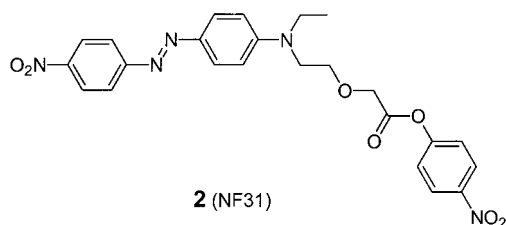


Ser and His are each incorporated into one of the two tripeptidic chains generated on a steroidal scaffold, 2) the screening of the solid-phase-bound library for reaction with the test substrate **2** (NF31) as a model for the first step of the

[*] Prof. Dr. P. J. De Clercq, H. De Muynck, Dr. A. Madder, N. Farcy
Laboratory for Organic Synthesis
Department of Organic Chemistry, University of Gent
Krijgslaan 281, 9000 Gent (Belgium)
Fax: (+32) 9-2644998
E-mail: pierre.declercq@rug.ac.be
M. N. Pérez-Payán, L. M. Öhberg, Prof. Dr. A. P. Davis
Department of Chemistry, Trinity College
Dublin 2 (Ireland)

[**] This research was supported by FWO-Vlaanderen (G.0051.98), GOA (GOA96009), and TMR (ERBFMRXCT 960011). We thank Prof. Dr. S. Hoste (Gent) for the use of the microscopic camera.

Supporting information for this article is available on the WWW under <http://www.wiley-vch.de/home/angewandte/> or from the author.

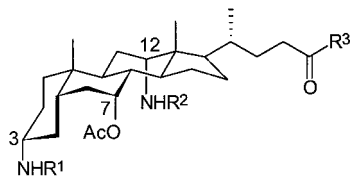


enzymatic mechanism (see below), and 3) the identification of three active sequences **1a–c** by recursive deconvolution (Table 1).

Table 1. Sequences from the hexapeptide library **1** as identified by recursive deconvolution.

Sequence	AA ¹	AA ²	AA ³	AA ⁴	AA ⁵	AA ⁶
1a	Gly	Phe	Ser	Leu	His	Ala
1b	Phe	Ser	Phe	Leu	His	Ala
1c	Ser	Ser	Phe	Leu	His	Ala
1d	Ser	Phe	Ser	Leu	His	Ala

After attachment of the cholic acid derivative **3** (Alloc = allyloxycarbonyl, Boc = *tert*-butoxycarbonyl) to TentaGel-NH₂ (TG-NH₂),^[7] the hexapeptide library was constructed following the split-mix protocol using a set of three amino acids (Ser(*OrtBu*), Phe, Gly) in the combinatorial construction of the first chain and another set of three amino acids (His(*Trt*), Ala, Leu) in the construction of the second chain (*Trt* = trityl = triphenylmethyl).^[8] All peptide couplings except one were effected in a classical way using amino acids



3: R¹ = Alloc, R² = Boc, R³ = OH

4: R¹ = Alloc, R² = Boc, R³ = NH-TG

5: R¹ = AA¹-AA²-AA³-NHAc, R² = Boc, R³ = NH-TG

6: R¹ = AA¹-AA²-AA³-NHAc, R² = H, R³ = NH-TG

protected with the 9*H*-fluoren-9-ylmethoxycarbonyl (Fmoc) group and *N,N'*-diisopropylcarbodiimide/1-hydroxy-1*H*-benzotriazole (DIC/HOBt) methodology. The two peptide strands were attached in succession. After deprotection of the Alloc group in **4**, the first strand was constructed on the free amino group at C-3 and then capped with acetylimidazole to yield **5**. Early attempts at coupling the first amino acid of the second peptide chain with the free amino group at C-12 of **6** (obtained after acid removal of the Boc group of **5**) failed because of steric encumbrance. In this particular case the coupling reagent 1-hydroxy-7-azabenzotriazole was used instead.^[9]

The choice of a suitable substrate for discovering relevant activity is critical. Indeed, not only should its structure allow for easy detection of an active member within the library, but reaction should also occur by a mechanism that is related to the enzymatic process. It occurred to us that the activated

ester **2** could be a suitable substrate for that purpose.^[10] Indeed, it is very reactive towards amines, including sterically hindered ones, and not readily cleaved by primary alcohols.^[11] In practice, when **2** was exposed to TG-NH₂, TG-Ser-NHAc, and TG-His-NHAc under standard screening conditions (exposure of 10 mg beads to 50 μ L of a 0.002 M solution of **2** in 800 μ L CH₃CN, RT, overnight, followed by thorough rinsing with DMF and CH₂Cl₂) the appearance of deep red beads was only observed in the case of TG-NH₂ (Figure 1a); Ser- and His-bound substrates normally do not react to a

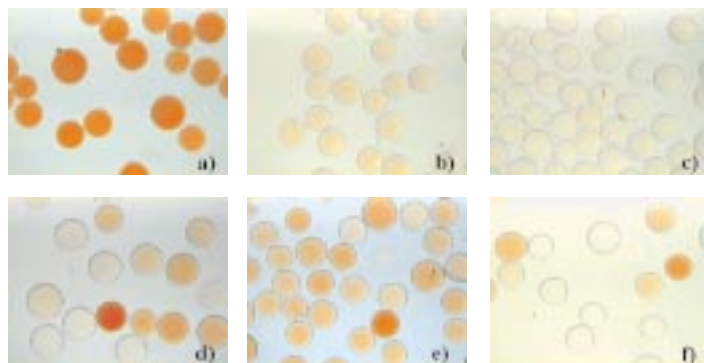


Figure 1. Top: TentaGel beads after reaction with **2**: a) TG-NH₂, b) TG-His-NHAc, c) TG-Ser-NHAc. Bottom: the three sublibraries after reaction with **2**: d) Ala-sublibrary, e) His-sublibrary, f) Leu-sublibrary.

major extent (Figure 1b, c), so any observed reactivity could reveal a mechanism in which both amino acid residues are involved. The library was screened under standard conditions at the stage of the last sublibraries in which the identity of the sixth capped amino acid is known to be Ala, His, or Leu. Each pool containing a total of 243 different members was screened separately and hits were observed in each sublibrary (Figure 1d–f). Inspection of Figure 1d reveals clearly the Ala-sublibrary to be the most promising one in view of discovering a unique sequence. As expected, the testing of the sublibraries at the protected amino acid stage (for example, Ser(*OrtBu*) and His(*Trt*)) did not lead to any hits.

The identification of the most active member(s) within the Ala-sublibrary was performed using recursive deconvolution.^[12] Figure 2 illustrates the procedure that led to the discovery of the sequence Ala-His-Leu as the preferred AA₆-AA₅-AA₄ tripeptidic chain. After selection of Ala as the sixth amino acid (Figure 1d), three new sublibrary pools, each containing 81 different sequences, were constructed in which the identity of the fifth amino acid was known. The testing of these separate pools clearly revealed His as the preferred fifth amino acid (Figure 2a). In the same way Leu was selected as the last amino acid of the first tripeptidic chain after the testing of three new sublibraries, each now containing 27 different members (Figure 2b). The same procedure was used to unravel the AA₃-AA₂-AA₁ chain. It is typical of the deconvolution procedure that choices are more difficult to make while proceeding towards the full identification of an active member. Indeed, the relative increase of the number of active members in the newly created pools leads to a more-even distribution of the color among the beads. Three active members, **1a–c**, were eventually identified (Table 1).

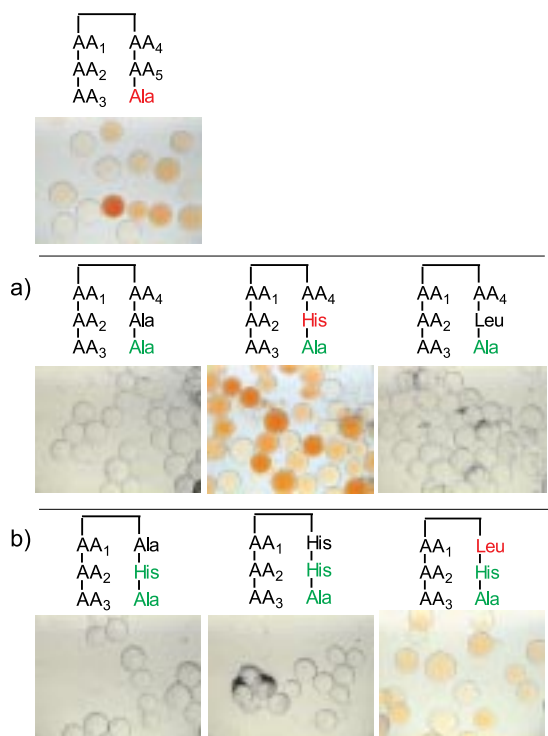


Figure 2. The deconvolution procedure for the identification of the first peptidic strand of the Ala-sublibrary; a) three newly created sublibraries, each containing 81 members that allow the identification of AA₃ as His, b) three newly created sublibraries, each containing 27 members that allows the identification of AA₄ as Leu.

A major advantage of the recursive deconvolution procedure resides in the numerous resyntheses of structurally biased libraries that need to be performed. Indeed, not only are active members within the library resynthesized so that they become available for separate testing, inactive members are also obtained that can serve as control substrates. Sequence **1d** (Table 1) is illustrative in this context: it contains both His and Ser residues, only differs from **1a** in the identity of the first amino acid, and yet is inactive under the standard screening conditions (Figure 3).

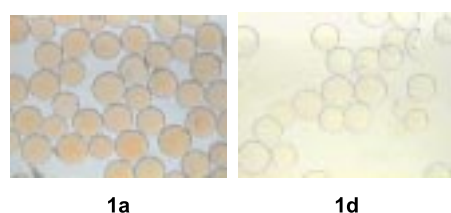


Figure 3. The active sequence **1a** and the non-active sequence **1d** after exposure to **2** (10 mg beads, 850 μ L of a 12×10^{-5} M solution in CH₃CN).

As far as the mechanism of the above process is concerned several aspects need to be clarified. In view of the thorough rinsing procedure (**2** is soluble in CH₂Cl₂ and DMF) there can be no doubt about the covalent nature of the binding of the chromogenic acyl residue to the library component. However, it is difficult at this stage to differentiate unambiguously between acylated-His or esterified-Ser substances. Therefore the three above colored pools **1a–c** were each subjected to mild hydrolysis conditions (H₂O/DMF, 45 °C, 5 days); in each case the red color was retained, which indicated the ester

nature of the covalent linkage.^[13] The well studied mechanism of peptide hydrolysis by the serine protease α -chymotrypsin proceeds by the transfer of the acyl part of the amide to the Ser residue, which is assisted by His (and by Asp although the exact role of the latter is still a matter of debate)^[14] followed by hydrolysis of the acylated enzyme by water by essentially the same but inverse mechanism. In this context the observed reactivity in our study is related to the first step of the enzymatic mechanism, namely the acyl-transfer step. The latter can still involve general base catalysis or nucleophilic catalysis, mechanisms that imply the participation of both Ser and His residues.^[15] At the present stage it is not possible to determine the origin of the difference in reactivity between the active sequences **1a–c** and the inactive sequence **1d**. An attractive explanation would consist in a geometrical arrangement involving the Ser and His residues of **1a–c** that allows for cooperative participation, a process that is not possible for **1d**, although this remains to be proven. Also at this point it is not possible to assess the role, if any, of the solid support in the process.

As far as the de-acylation step is concerned we were fortunate to observe that when the colored pools obtained from **1a–c** were subjected to more forceful hydrolytic conditions (H₂O/DMF, 65 °C, 48 h), the beads originating from **1a** and **1b** became colorless, while those derived from **1c** remained red (Figure 4). This suggests that a different

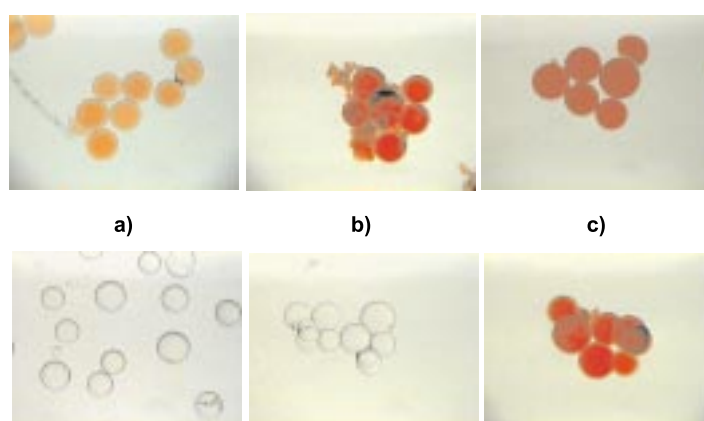


Figure 4. Top: the active sequences after exposure to **2** (10 mg beads, 1 mL of a 2×10^{-3} M solution in CH₃CN): a) **1a**, b) **1b**, c) **1c**. Bottom: the same acylated (**2**) sequences after reaction with H₂O/DMF (65 °C, 48 h).

mechanism may be operating in the de-acylation step. The possibility that the Ser-His couple is involved in both the acylation and the de-acylation stages is of special interest in view of the eventual development of a truly catalytic system.

In summary, we have described the discovery of a few peptide sequences characterized by the presence of both Ser and His that cause the unusual cleavage of an activated *p*-nitrophenyl ester. The discovery rests on the combinatorial mix-split technique for the construction of the hexapeptide library, on the visual detection of active members, and on the identification of the active sequences using recursive deconvolution.

Received: July 8, 1999 [Z13692]

- [1] Y. Murakami, J. Kikuchi, Y. Hisaeda, O. Hayashida, *Chem. Rev.* **1996**, 96, 721–758.
- [2] A. J. Kirby, *Angew. Chem.* **1996**, 108, 770–790; *Angew. Chem. Int. Ed. Engl.* **1996**, 35, 707–734.
- [3] K. W. Hahn, W. A. Klis, J. M. Stewart, *Science* **1990**, 248, 1544–1547. For recent examples of artificial hydrolases not involving Ser, see a) J. Suh, S. S. Hah, *J. Am. Chem. Soc.* **1998**, 120, 10088–10093; b) L. Baltzer, K. S. Broo, H. Nilsson, J. Nilsson, *Bioorg. Med. Chem.* **1999**, 7, 83–91.
- [4] A. Madder, P. J. De Clercq, *J. Org. Chem.* **1998**, 63, 2548–2559.
- [5] For a recent combinatorial approach in the discovery of catalysts for phosphate hydrolysis, see D. A. Héroult, A. Berkessel, *Angew. Chem.* **1999**, 111, 99–102; *Angew. Chem. Int. Ed.* **1999**, 38, 102–105.
- [6] D. M. Blow, *Acc. Chem. Res.* **1976**, 9, 145–152.
- [7] J. F. Barry, A. P. Davis, M. N. Pérez-Payan, M. R. J. Elsegood, R. F. W. Jackson, C. Gennari, U. Piarulli, M. Gude, *Tetrahedron Lett.* **1999**, 40, 2849–2852.
- [8] a) A. Furka, F. Sebestyen, M. Asgedom, G. Dibo, *Int. J. Pept. Protein Res.* **1991**, 37, 487–493; b) K. S. Lam, S. E. Salmon, E. M. Hersh, V. J. Hruby, W. M. Kazmierski, R. J. Knapp, *Nature* **1991**, 354, 82–84; c) R. A. Houghten, C. Pinila, S. E. Blondelle, J. R. Appel, C. T. Dooley, J. H. Cuervo, *Nature* **1991**, 354, 84–86.
- [9] L. A. Carpino, *J. Am. Chem. Soc.* **1993**, 115, 4397–4398.
- [10] NF31 refers to Nadia Farcy, see A. Madder, N. Farcy, N. G. C. Hosten, H. De Muynck, P. J. De Clercq, J. Barry, A. P. Davis, *Eur. J. Org. Chem.* **1999**, 11, 2787–2791.
- [11] For example, no transesterification of **2** is observed when treated with an excess of MeOH (RT, 12 h, CH₃CN).
- [12] a) E. Erb, K. D. Janda, S. Brenner, *Proc. Natl. Acad. Sci. USA* **1994**, 91, 11422–11426; b) S. M. Freier, D. A. M. Konings, J. R. Wyatt, D. J. Ecker, *J. Med. Chem.* **1995**, 38, 344–352; c) D. A. M. Konings, J. R. Wyatt, D. J. Ecker, S. M. Freier, *J. Med. Chem.* **1996**, 39, 2710–2719; d) L. Wilson-Lingardo, P. W. Davis, D. J. Ecker, N. Hébert, O. Acevedo, K. Sprinkle, T. Brennan, L. Schwarcz, S. M. Freier, J. R. Wyatt, *J. Med. Chem.* **1996**, 39, 2720–2726.
- [13] 50% cleavage of acetylimidazole is already observed in MeOH after 1 h at RT.
- [14] S. C. Zimmerman, J. S. Korthals, K. D. Cramer, *Tetrahedron* **1991**, 47, 2649–2660.
- [15] The mechanism for the hydrolysis of *p*-nitrophenyl esters by serine proteases is still under discussion, see a) D. M. Quinn, J. P. Elrod, R. Ardis, P. Friesen, R. L. Schowen, *J. Am. Chem. Soc.* **1980**, 102, 5358–5365; b) R. A. Hess, A. C. Hengge, W. W. Cleland, *J. Am. Chem. Soc.* **1998**, 120, 2703–2709.

A Self-Complexing [2]Catenane

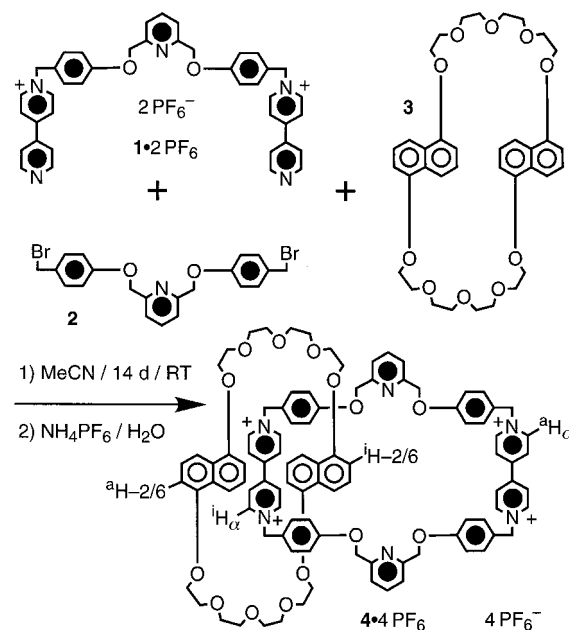
Beatriz Cabezon, Jianguo Cao, Francisco M. Raymo, J. Fraser Stoddart,* Andrew J. P. White, and David J. Williams*

Molecules capable of recognizing one or more identical copies of themselves can be employed for the noncovalent synthesis^[1] of supermolecules and/or supramolecular arrays. Indeed, self-replicating systems,^[2] self-assembling capsules,^[3]

and supramolecular polymers^[4,5] have all been developed successfully by employing appropriate self-complementary molecules as precursors. Consequently, we contemplated the possibility of synthesizing receptor molecules incorporating mechanically interlocked^[6] components by modifying the design of some [2]catenanes^[7] such that they acquire receptor characteristics. These [2]catenanes incorporate a 1,5-dioxynaphthalene-based macrocyclic polyether component interlocked with cyclobis(paraquat-4,4'-biphenylene)^[8] and are able to bind^[7] π -electron-deficient and π -electron-rich substrates. These recognition phenomena have already been exploited in the synthesis of a “rotacatenane”^[7b] composed of a 1,5-dioxynaphtho[38]crown-10 moiety interlocked with cyclobis(paraquat-4,4'-biphenylene)^[8] and with a third acyclic polyether component containing a 1,5-dioxynaphthalene ring system threaded through the cavity of the bipyridinium-based cyclophane and stoppered by bulky groups.

To generate self-complementary [2]catenanes, we have lengthened and introduced some flexibility into the spacers separating the bipyridinium units in the tetracationic cyclophane component. By inserting a 2,6-bis(oxymethyl)pyridine unit between the two phenylene rings of the 4,4'-bitolyl spacers, the size of the cavity associated with cyclobis(paraquat-4,4'-biphenylene) can be enlarged and enriched with hydrogen bonding acceptors—namely, the nitrogen and oxygen atoms of the 2,6-bis(oxymethyl)pyridine unit. Here, we report the template-directed synthesis of a self-complementary [2]catenane and the formation of a supramolecular homodimer composed of two mutually interpenetrating [2]catenanes both in solution and in the solid state.

Reaction of **1**·2PF₆ and **2**^[9] in the presence of the 1,5-dioxynaphthalene-based macrocyclic polyether **3** gives the [2]catenane **4**·4PF₆ in 18% yield after counterion exchange (Scheme 1), together with small amounts of a [3]catenane incorporating one tetracationic cyclophane and two macrocyclic polyether components.



Scheme 1. The template-directed synthesis of the [2]catenane **4**·4PF₆.

[*] Prof. J. F. Stoddart, Dr. B. Cabezon, J. Cao, Dr. F. M. Raymo
Department of Chemistry and Biochemistry
University of California, Los Angeles
405 Hilgard Avenue, Los Angeles, CA 90095-1569 (USA)
Fax: (+1) 310-206-1843
E-mail: stoddart@chem.ucla.edu
Prof. D. J. Williams, Dr. A. J. P. White
Department of Chemistry, Imperial College
South Kensington, London, SW72AY (UK)
Fax: (+44) 171-594-5835