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Dyn[n]arenes: Versatile Platforms To Study the Interplay between Covalent and Noncovalent Bonds

Laurent Vial*, Florent Perret* and Julien Leclaire*

Dedicated to Professor Jeremy K. M. Sanders on the occasion of his 73rd birthday"

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Abstract: Dyn[n]arenes are the strict polydisulfide-based analogues of pillar[n]arenes. Although less famous than their static homologues, these dynamic cavitands possess unique (stereo)chemical features. The disulfide bridge between monomeric units not only provides these architectures with constitutional lability but also an additional stereogenic element. As a result, the covalent assembling process can be guided by supramolecular interactions with or without a targeted partner, enabling to decipher supramolecular at work from the synthetic stage. Not only can the size and functions be chosen on demand, but the stereochemical features can be precisely controlled by careful choice on the assembling conditions. Eventually, deciphering the interactions at work during self-assembly opens the way toward innovative applications such as biomolecules sensing. The account describes the synergistic interplay between covalent and non-covalent bounds found in dyn[n]arenes.

1. Introduction

With the discovery of crown ethers in 1967, Charles Pedersen introduced to the world the very first synthetic host molecules dedicated to molecular recognition of guests (Figure 1).^[1] Since then, macrocyclic containers of various shapes and sizes have been prepared by supramolecular chemists. Throughout decades, cyclodextrins, calixarenes and more recently cucurbiturils and pillararenes have emerged as powerful binders for a variety of molecular partners, as well as versatile building blocks to elaborate complex architectures (Figure 1, for recent reviews about cucurbit[n]urils, pillar[n]arenes, cyclodextrins, calix[n]arenes, see ref²⁻⁵). If these blockbuster cavitands are synthetically very accessible and robust, they suffer from several limitations. First, their harsh assembling conditions generally rules out the use of the famous template effect (or more generally of supramolecular interactions to guide the synthetic procedure). Secondly, these procedures generally deliver unfunctionalized architectures, which are poorly water-soluble. To further obtain architectures which can selectively and efficiently interact with biomolecules in physiological media, post-functionalization is required. At the very end of the 20th century, an innovative synthetic strategy using non-covalent interactions as driving forces to reversible covalent bond formation was proposed by Jean-Marie Lehn and Jeremy K. M. Sanders: dynamic combinatorial chemistry (DCC) was born.^[6] With this concept, it seemed possible to marry the best of both cyclophane and dynamic covalent chemistries. In this perspective, we have explored and developed a new family of disulfide-based cyclophanes named dyn[n]arenes. Dyn[n]arenes are defined as p-cyclophanes based on mono- to tetra-functionalized 1,4-dithiophenol units linked by disulfide bridges. In this respect, they encompass the strict dynamic covalent analogues of pillar[n]arenes, made from 2,5-disubstituted p-dithiophenols. Although widely used in DCC, *m*-substituted dithiophenol generally deliver flatter macrocycles with remarkable self-association property but less well-defined cavity. This paper proposes an account on this family based on p-dithiophenols, whose first member was born at the University of Cambridge and was further enlarged throughout several studies in Lyon.



Julien Leclaire was educated at the Ecole Normale Supérieure de Lyon. After a PhD on dendrimer chemistry at the University of Toulouse, he joined the group of J. K. M. Sanders in Cambridge wherein he worked on disulfide-based dynamic covalent chemistry. He started his academic career as an assistant then associate Professor at Aix-Marseille Université, studying non-covalent interactions within Lipases. In 2013, he was appointed Full Professor and Applied Supramolecular Chemistry group leader at the University of Lyon. In 2020, he was awarded the Jean-Marie Lehn Prize for his work on molecular recognition within complex mixtures by the French Chemical Society. In 2021, he funded MeCaWaRe corporation which recycles strategic metals from end-of-use batteries using CO₂-based supramolecular chemistry.



Florent Perret received his PhD from the University of Clermont-Ferrand, under the joint supervision of N. Morel-Desrosiers and A. W. Coleman, working on the thermodynamic and structural studies of the complexation of sulfonatocalix[4]arenes with amino-acids and peptides. After two years in S. Matile's group in Geneva where he carried out research on synthetic ion channels, he was nominated in 2008 as assistant professor in Lyon where he joined the group of H. Parrot-Lopez, working on the synthesis of amphiphilic cyclodextrins. Since 2014, his research activities mainly focus on

Dynamic Combinatorial Chemistry.



During his PhD and Marie Curie fellowship, Laurent Vial had the chance to work under the guidance of two awesome supervisors: Prof. Jérôme Lacour (University of Geneva, Switzerland) and Prof. Jeremy K. M. Sanders (University of Cambridge, UK), respectively. He is currently a CNRS research scientist in the Applied Supramolecular Chemistry group (University of Lyon, France), working on artificial receptors for biologically relevant molecules with applications as biosensors and therapeutics.

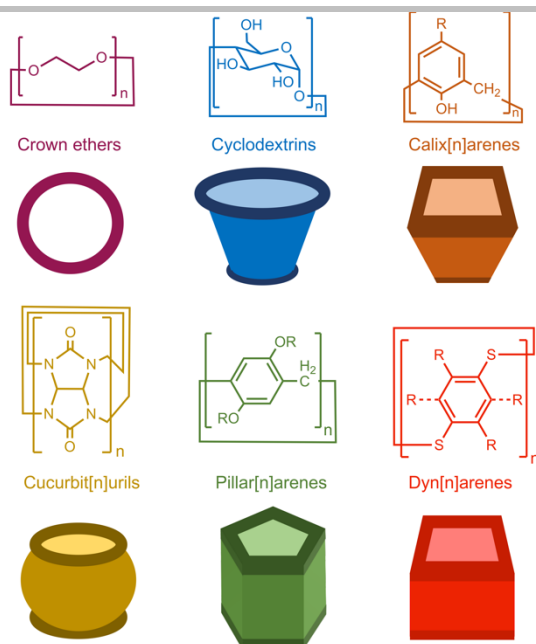


Figure 1. Examples of molecular containers reported in the literature.

2. Synthesis and Self-Assembly

2.1. Building blocks: synthesis and information implementation

2.1.1. Building block synthesis

The prerequisite to the formation of dyn[n]arene is the obtention of functionalized *p*-dithiophenols. A key step in the synthetic sequence is therefore the introduction of two thiol moieties directly on the aromatic ring.

2.1.1.1 Generic preparation of (di)thiophenols. The first example of thiophenol synthesis dates back to 1890, when Rudolph Leuckart described the reaction between a benzene diazonium salt and an aryl xanthate derivative.^[7] Thiophenols can also be formed from strong nucleophiles such as aromatic Grignard reagents, by coupling with tetramethylthiuram disulfide. The intermediate *N,N*-dimethyldithiocarbamate, obtained can then be reduced to thiophenols by hydrides.^[8,9] Since these early examples, many strategies involving catalytic amounts of transition metals have been developed to enable the conversion of aryl halides or triflates to thiophenols in greener and milder conditions.^[10–13] Among metal-free approaches, those involving the Newman-Kwart rearrangement (NKR) are amongst the most popular. They indeed enable the formation of ortho-functionalized aryl thiols from the corresponding *o*-phenol derivatives.^[14] The NKR process consists in the conversion of an *O*-aryldithiocarbamate into a *S*-aryldithiocarbamate, usually in a strongly polar solvent and at high temperatures. *S*-aryldithiocarbamates are subsequently hydrolyzed in basic conditions, affording the corresponding thiophenols. More than 40 years after its first report by Newman^[15] and Kwart,^[16] catalyzed versions of the rearrangement emerged. Lloyd-Jones *et al.* used catalytic amounts of palladium bis(tri-*tert*-butylphosphine) to successfully rearrange a variety of para-substituted substrates at a much lower temperature (100°C) than the thermally activated version, thus limiting degradation reactions.^[17] In 2015, Nicewicz *et al.* also proposed a photocatalyzed process involving pyrylium-like bases.^[18] This photochemical redox reaction requires electron-rich substrates to proceed and can be performed at room temperature. Two radical-based reactions involving electron-rich substrates were recently reported. The first, developed by Pittelkow *et al.* uses cerium (IV) ammonium nitrate in DMSO at room temperature.^[19] In such conditions, the activation barrier is much lower (*ca.* by 110 kJ·mol⁻¹) than for the conventional thermally activated process. More recently, Roesel *et al.* performed NKRs by applying catalytic charge inputs at room temperature electrolysis in galvanostatic set-up.^[20]

2.1.1.2. Functionalized *p*-dithiophenols. To afford a molecular toolbox of dyn[n]arene precursors of contrasted stereoelectronic features, three bis- or tetra-functionalized 1,4-dithiophenols **A**, **C** and **D** (Figure 2ii and iii) were synthesized by performing the traditional NKR reaction, either before or after ring functionalization. The 2,5-diamino-1,4-benzenedithiol building block **B** is commercially available. Synthesis of 2,5-dimercaptoterephthalic acid (building block **A**) was described by some of us and consists in a three steps sequence from diethyl 2,5-dihydroxyterephthalate.^[21] The *S*-thiocarbamate intermediate could be obtained by thermal NKR in neat conditions and, after basic hydrolysis, building block **A** was obtained with 89% overall yield. 2,3,4,5-tetrafluorobenzene-1,4-dithiol **C** was prepared according to the synthetic route described by Raasch *et al.*^[22] The *S*-thiocarbamate intermediate was produced from the commercial tetrafluorohydroquinone by a solvent-free carbamation reaction at high temperature, followed by a thermal NKR in diphenylether. After hydrolysis, building block **C** was obtained quantitatively. A rigid and sterically crowded building block (**D**), including a polycyclic spacer between the carboxylic acid end groups and the thiol moieties was also synthesized from benzoquinone. The phenyl ring extension into an octahydro-anthracene aromatic core incorporating two methylene bridges was conducted by successive metal-free and metal-catalyzed cycloadditions. The second cycloaddition led to a mixture of diastereomers (*cis* and *trans*), which were separated by chiral preparative chromatography. After NKR on *O*-thiocarbamate derivative, the last step consisted in a ruthenium catalyzed [2 + 2] cycloaddition with dimethylacetylene-dicarboxylate, yielding the tetracarboxylic acid derivative **D** (figure 2iii).^[23]

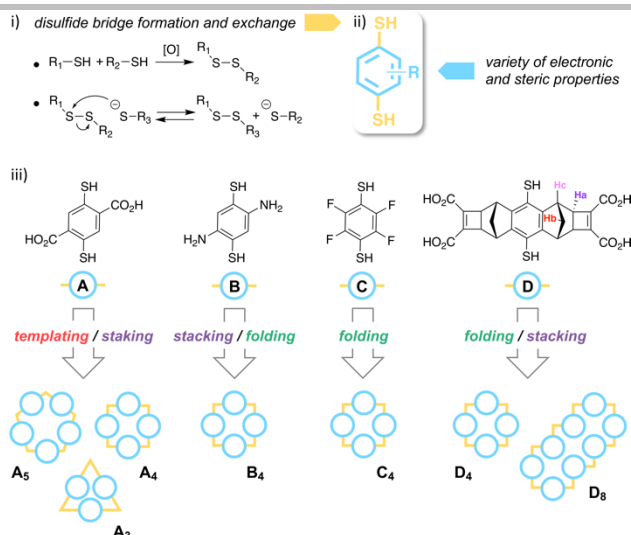


Figure 2. Size selective formation of dyn[n]arenes: i) thiol aerobic oxidation and disulfide exchange on ii) generic p-dithiophenol exemplified on iii) building blocks **A-D** which selectively self-assemble into dyn[n]arenes of various sizes depending on the driving force exploited. H_a (purple), H_b (red) and H_c (pink)-bearing groups of **D** display decreasing bulkiness.

2.1.2. Unsuccessful building blocks post-functionalization

In order to expand our family of dyn[n]arene precursors, the post-functionalization of building block **A** and **B** was envisioned. To derivatize building block **A**, a N,N-diethylthiocarbamate protecting group was first introduced to avoid unwanted S-acylation reactions. While subsequent couplings with various primary amines was successful, unexpected tricyclic benzothiazine products **A'** were mainly formed at the final the S-deprotection step (Figure 3).^[24] Thus, the trityl protecting group was tested as an alternative and introduced on **A**, following to the procedures reported by Otto *et al.*^[25] and Waters *et al.*^[26] It enabled the conversion of **A** into 2,5-amidated-1,4-dithiophenols **E** bearing various end groups and a dozen of new derivatives were synthesized with good yields.^[27] For building block **B**, although amide couplings could be conducted with satisfying yields on 2-cyanoethyl protected derivative of **B**, side reactions were also encountered during the deprotection step. In fact, bicyclic adducts such as 2-phenylbenzothiazole **B'** were observed as major products. It is worth to notice that a similar reactivity has been described by Patel *et al.*^[28] Unfortunately, when amide building blocks **E** were submitted to aerobic oxidation into disulfides, an unexpected reactivity was again encountered. Thiol groups preferentially reacted intramolecularly by oxidative coupling with the nitrogen moiety of the neighboring amide rather than intermolecularly with another thiol group. This interfering process delivered sulfenyl amides **E'** as major products rather than amide-bearing dyn[n]arenes **E_n** (Figure 3).

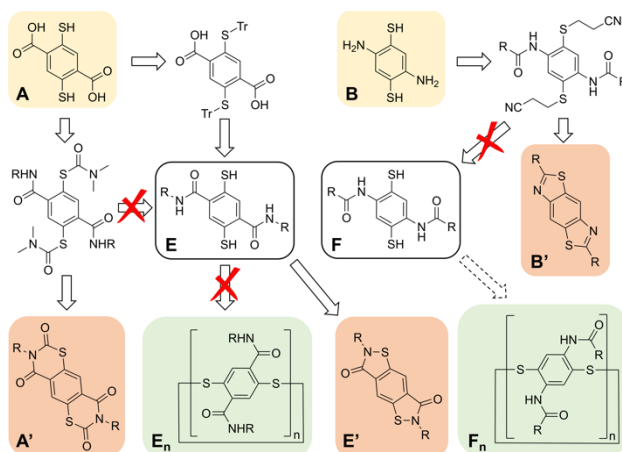


Figure 3. Unexpected reactivity of ortho-functionalized thiophenol derivatives during deprotection or aerobic oxidation. Carboxylate to amide conversion can be conducted from **A** to **E** via trityl protection/deprotection (avoiding **A'** formation) but aerobic oxidation leads to **E'** rather than **E_n**. Similarly, cyanoethyl deprotection is accompanied by N,S oxidative coupling from **B** to **B'**.

2.2. Oligomerization

2.2.1. By irreversible means

Disulfide bridged paracyclophanes can be synthesized *via* irreversible pathways, starting from 1,4-dialkoxybenzenes.^[29] Macroscopic bistetrasulfides were obtained by Dehaen and coll. after the reaction of the 1,4-dialkoxybenzenes with sulfur monochloride in the presence of AlCl₃ as a catalyst. Subsequent reduction with sodium borohydride and re-oxidation in the presence of a base eventually afforded dyn[4]arenes bearing methoxy or ethoxy groups. As for static cyclophanes, such harsh assembling conditions are tolerated by quite a narrow range of functional groups.

2.2.2. By reversible means

Disulfide bond formation and exchange occur spontaneously in aqueous solution at pH between 7 and 9, and may be quenched by lowering the pH below the pK_a of the thiols involved. This thermodynamic lability was exploited by Sanders *et al.*^[30–32] and others^[21,30,33–35] to obtain topologically complex architectures and receptors in aqueous media. During self-assembling in aqueous solutions, the slow oxidation of the thiols competes with the disulfide exchange (Figure 2i). The latter, must be (and is generally) faster than the former, which can require up to several days to be complete^[36,37]. Various strategies have been explored to accelerate one or both of these reaction with the help of additives such as DMSO,^[38] selenols,^[39] and diselenides,^[40] or glutathione redox buffer.^[41]

All dynamic combinatorial libraries (i.e., the products of dynamic combinatorial chemistry) reported in this account were generated under thermodynamic control by aerobic oxidation in pH-neutral buffers and without any additives.

2.3. Dial a size using supramolecular interactions

In DCC, stabilizing interactions involving some library members may induce their amplification at the expense of others. In this context, three classes of selection mode or molecular scenario can be distinguished, namely *templating*, *folding* and *stacking* (Figure 4).

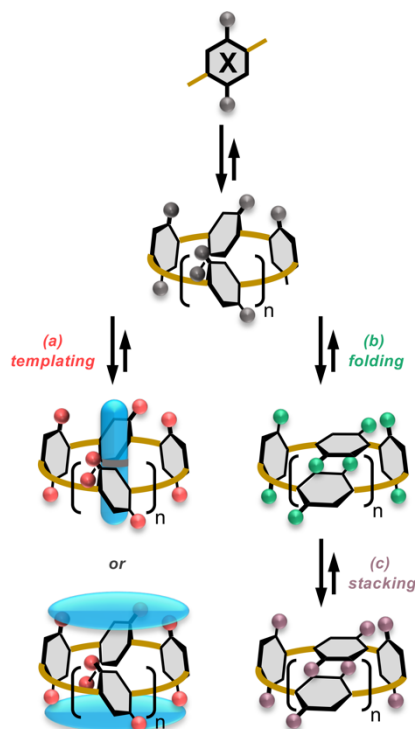


Figure 4. Driving forces behind the disulfide-based self-assembly of dyn[n]arenes from functionalized 1,4-bisthiophenols **X** = **A**, **B**, **C**, and **D**. (a) templating : **X** = **A**, $n = 0$ (rim), $n = 1$ and 2 (cavity); (b) folding : **X** = **C**, $n = 1$ or **X** = **D**, $n = 1$; (c) stacking upon folding: **X** = **B**, $n = 1$ or **X** = **D**, $n = 5$.

The first study focusing on the aerobic oxidation of substituent-free *p*-dithiophenol reported the exclusive formation of the cyclo-trimeric adduct as an insoluble species.^[42] If the selection of such a small-ring size could be imputed at first sight to the absence of bulky ortho-substituents, modelling revealed that the mean distance between these sites does not vary significantly with the cyclophane's size.^[33] In this seminal example, the packing energy in the solid lattice was the driving force at work, which did select and amplified this first dyn[3]arene ever reported.

Aggregation-induced selection was similarly observed from the parent *p*-phenylenediamine building block **B**. In this case, the stereoelectronic features of the ortho amino group dramatically impacts the intra- and intermolecular interactions, which may take place in the intermediate linear and final cyclic oligomers. As the NH_2 group can both act as a powerful hydrogen bond donor and acceptor,^[43] the phenylene units of dyn[4]arene **B**₄ fold to optimize both intramolecular and intermolecular self-pairing (Figure 4, (b) and (c)). In other words, folding and aggregation are the two concomitant driving forces, which lead to the exclusive formation of the cyclotetrameric adduct regardless the solvent used for assembling.

Whether in organic or in a neutral aqueous medium, libraries made from the perfluorinated building block **C** quantitatively yielded the tetrameric structure **C**₄, parent compound of the cyclotrimer described by Raasch *et al.*^[22] The identity and purity of these species were confirmed by HPLC-MS and the crystal structure additionally revealed that this cyclophane adopts a flattened conformation in the solid state. ¹⁹F NMR experiments showed that this structural feature survives the dissolution in an organic medium, and confirmed that folding, induced by intramolecular C-F / pi interactions, is the driving force behind the quantitative formation of **C**₄ (Figure 4, (b)).^[33]

In the absence of template and in neutral aqueous solution, libraries generated from building block **A** led to a mixture of tri- tetra- and penta-meric cyclo-oligomers, as HPLC-UV/MS analyses revealed it. Our approach consisted in making the most out of the dynamic features of the system, i.e. to screen for the conditions enabling to quantitatively amplify one species at the expense of all other library members. In theory, this should yield a sample that would only require template and buffer removal, to deliver the targeted dyn[n]arene. Intriguingly, HPLC-UV/MS indicated that the nature of the buffer was having an impact on the amount of cyclotetramer. Yet, with biogenic polyamine templates, such as spermine, spermidine, lysine or cadaverine, quantitative formation of cyclic tetramer **A**₄ was observed. Post-assembling acidification delivered a precipitate, which appeared to be pure dyn[4]arene when re-dissolved and analyzed with HPLC-MS.^[21,33] All the aforementioned linear guests templated the formation of the cyclophane cavity (molding), as their terminal ammonium groups formed multiple salt bridges with both polycarboxylate rims (Figure 4, (a) top). Within this polyamine family, switching from a linear to a C3-symmetrical species such as 2,4,6-triethyl-1,3,5-benzenetrimethanamine led to the quantitative

formation of an insoluble white solid adduct in neutral aqueous medium. This material could be identified upon solid state ^{13}C and ^{15}N NMR analyses as a barrel-type complex made from a self-assembled cyclic trimer **A**₃ capped by two triammonium templates (Figure 4, (a) bottom).^[44] Disassembling this complex upon acidic treatment delivered the dyn[3]arene **A**₃ as a pure species, a procedure which could also be scale-up to afford multigram amounts of this dyn[3]arene. Surprisingly, HPLC-UV analyses of the purified material, which was fully characterized in solution by multi-nuclear NMR, did not lead to any signal. Bulky diammonium guest, such as 2,5-dimethyl-1,4-phenylenediamine and 2,3,5,6-dimethyl-1,4-phenylenediamine, which may again deliver inclusion complexes rather than bind the polycarboxylate rims (figure 4, (a) top), proved to be the most efficient to amplify the dyn[5]arene **A**₅ up to 80 % of the libraries analyzed by HPLC-UV/MS.^[45] Yet, in the absence of quantitative amplification, this species was never isolated and further investigated. Libraries made from sterically hindered building block **D** were set in slightly more acidic conditions than with **A**, and first studied qualitatively by ESI-MS then by UPLC-UV/MS. Whereas both cis and trans diastereoisomers of **D** underwent thiol to disulfide chain elongation during the first 24h, linear oligomers made from the former overoxidized, while those made from the latter quantitatively converted into a cyclotetramer **D**₄. Thorough examination of mass spectra and especially of isotopic patterns indicated that the dyn[8]arene was coeluting with **D**₄. MS-/MS, multinuclear NMR and computational modelling revealed that maximization of internal dispersion (London) forces and minimization of steric repulsion were the intramolecular driving forces during the formation of **D**₄ which was obtained with 80% conversion (Figure 4, (b)). The remaining 20% corresponded to the octamer **D**₈, whose selection was induced by self-aggregation in the solid phase as an additional driving force (figure 4, (c)).^[23] The formation of mixed chemical libraries composed of different molecular building blocks **A**, **B**, **C** and **D** has also been studied, in order to determine whether it was possible to generate, amplify and isolate hetero-dyn[n]arenes, either as intrinsically stable species (self-selection) or by the template effect. Whatever the system studied, formation of mixtures of dyn[n]arenes, from three to six units, incorporating various building blocks was indeed obtained. It is worth noticing that their repartition within the libraries differ from the statistical ones. Formation of hetero-dyn[4]arenes ((**AB**)₂ or (**BC**)₂ for example) could be imputed to the strong intramolecular donor-acceptor interactions between electron-rich and electron-poor dithiophenol units. Until now, these hetero-dynarenes species were never isolated and further studied in depth.^[24,27,45-47]

3. Synthesis-Structure-Function Relationships

3.1. The stereochemical landscape

As depicted in Figure 5, the stereochemical landscape – *i.e.* the different accessible conformational and configurational states – of dyn[n]arenes is particularly vast. As with pillar[n]arenes, *pR* or *pS* configuration arises from the positioning of each prochiral monomer face in- or out-ward with respect to the cavity upon ring closure. These configurational stereoisomers may theoretically interconvert by flipping of the phenyl ring through the macrocyclic annulus. With monomer **C**, there is no such configurational diversity as its faces are identical and non-prochiral. Yet, we discovered that the disulfide bridge itself may be a stereogenic element providing that the dihedral rotation is slowed down. At first sight, a disulfide bridge between phenylene units may seem to provide them with increased flexibility and spaced them further from one another in comparison to the methylene linkage found in conventional cyclophanes. Therefore, one can expect it to be conformationally dynamic and even to favor the flipping of the aromatic units. In fact, the most stable conformation of a disulfide bond corresponds to a dihedral angle around $\pm 90^\circ$,^[48] which brings the phenyl units in close proximity and slows down most conformational equilibria. This close proximity between facial and dihedral stereogenic units mutually slows down their racemization. What is even more interesting is that their configuration can be interrelated or dependent, which theoretically enables chirality to propagate along the macrocyclic ring. In the field of disulfide based cyclophanes, stereoselective self-assembly is rarely addressed. This is mainly due to the fact that disulfide-based architectures can rarely be isolated (from their libraries) and be further analyzed structurally.^[49] To date, there are only three examples of stereoselective covalent self-assembly directed by supramolecular interactions. Sanders reported the stereoselective formation of a homochiral cyclotetramer of an ethenoanthracene building block templated by the inclusion of tetramethylammonium.^[50] Pantos *et al.* described the stereodivergent formation of diastereoisomeric catenanes which is dramatically governed by the configuration of the cysteine pending groups.^[51] While both studies involve chiral building blocks, the only example involving an achiral building block was provided by Otto *et al.* who reported that casting or molding could switch the selectivity of the assembling event toward the preferential formation of one configurational stereoisomer vs another.^[52] The authors could come to this conclusion by determining whether head-to-head or head-to-tail fragments were generated upon MS/MS fragmentation of library members within the libraries.

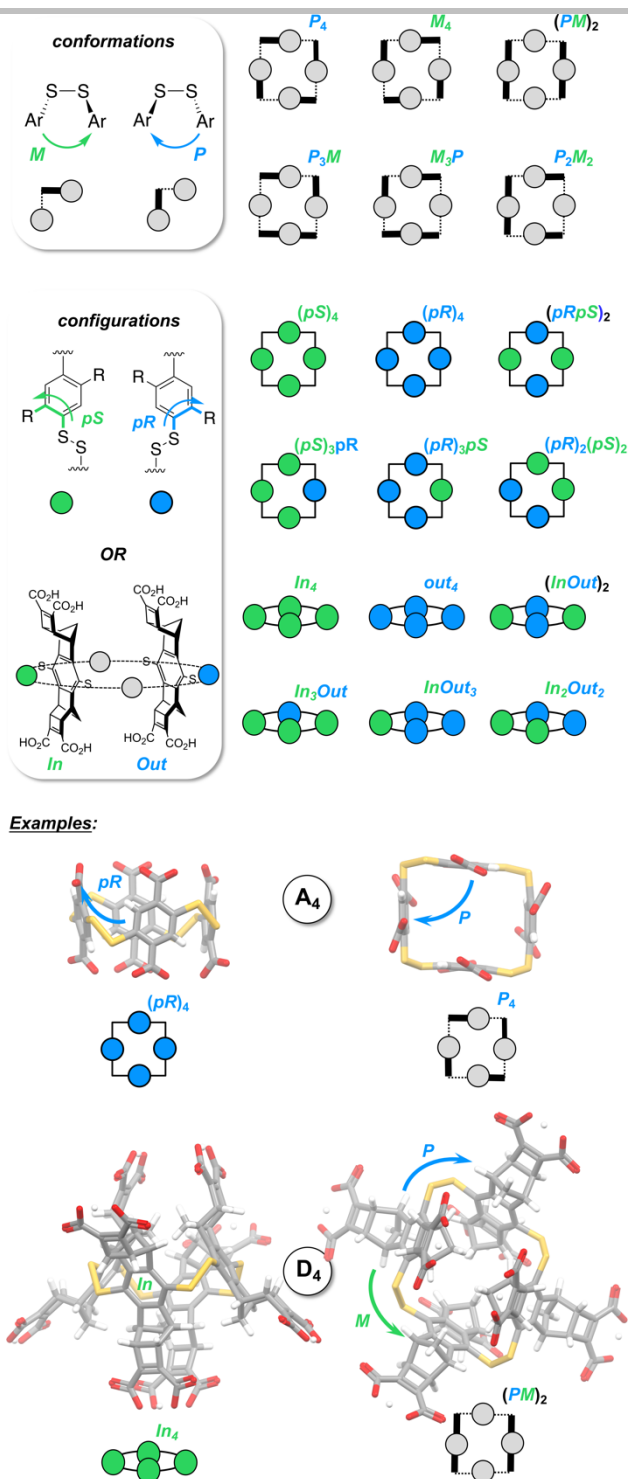


Figure 5. Conformational and configurational landscape of a dyn[4]arene, along with the three-dimensional structures of dyn[4]arenes ($(P-pR)_4$ -**A**₄ (crystallographic) and $(M-In-P-In)_2$ -**D**₄ (computed).

As mentioned before, the limiting factor in all aforementioned studies was clearly the availability of the final architectures as isolated species, which may explain while no structural investigation was conducted on the configuration of their disulfide bridges. In supramolecular chemistry, the stereochemical identity of cyclophanes (for example in calixarenes) is known to dramatically impact on their molecular recognition properties. In biochemistry, it is well known that the conformational features of the S-S bridge impacts the structure, hence on the function of biomacromolecules as well as the reactivity/sensitivity of S-S bridge itself toward degradation/derivatization processes.^[53] It has even been suggested that the handedness of the disulfide bond within prion protein may be correlated with the (mis)folding state, i.e. with the pathogenic forms.^[54] Therefore, mastering each conformation of the disulfide bridges formed during the stereoselective self-assembly of a macrocycle, by carefully choosing the stereoelectronic features of both the building blocks and templates represent a true breakthrough in stereoselective synthesis.

3.2. From function to structure

3.2.1. **B** and **C** building blocks: intra- and inter-molecular forces enable to encode conformation and configuration in the absence of template

The intriguing quantitative self-assembly of building blocks **B** and **C** into discrete cyclotetramers prompted us to investigate the underpinning selecting forces behind the phenomenon. Experimental and theoretical structural investigations confirmed that intramolecular dispersion forces and H-bonding respectively induced the folding of monomer units within each species. It also revealed that disulfide bridges actively participate to this global structural optimization, by adjusting their dihedral conformation. Dyn[4]arene **B**₄ adopts a tridimensional structure wherein aromatic rings are flipped by 90° which respect to their nearest neighbor (Figure 6). This reduces the size of the cavity but optimizes both intra- and inter-molecular hydrogen bonds. The latter indeed induces the formation of aggregates, whose mean size and amount varies with the solvent. Eventually, in depth structural investigations, which were enabled by quantitative amplification and straightforward synthetic procedures, revealed that the common stereochemical feature (a thiol reactive groups borne in para position) and the individual stereochemical features (the electronic depletion of the aromatic ring induced by four fluorine substituents or its enrichment two amino groups) of the building block pre-determine the non-covalent interactions that will be at work during the covalent oligomerization process and somehow, its outcome. These interactions stabilize or destabilize potential intermediates and products and thereby not only select the optimal size, but also the optimal conformation and configuration. While the folding of the faces is mainly induced by attractive forces, the disulfide bridges participate to this structural optimization by minimizing the steric hindrance between nearest neighboring phenylene units. Within the conformational and configuration landscape which is theoretically accessible to a dyn[4]arene, **B**₄ exclusively adopts the racemic *pRpRpSpRIMMMM* and *pSpSpRpSIPPPP* configuration. In comparison, **C**₄ folds into a diamond-shaped structure and splits into a mixture of *MPMP*, *MMPP* and *PPPP/MMM* conformers in relatively fast interconversion (the former being dominant in solution and isolated as a pure species in the solid phase, see, Figure 6).^[33]

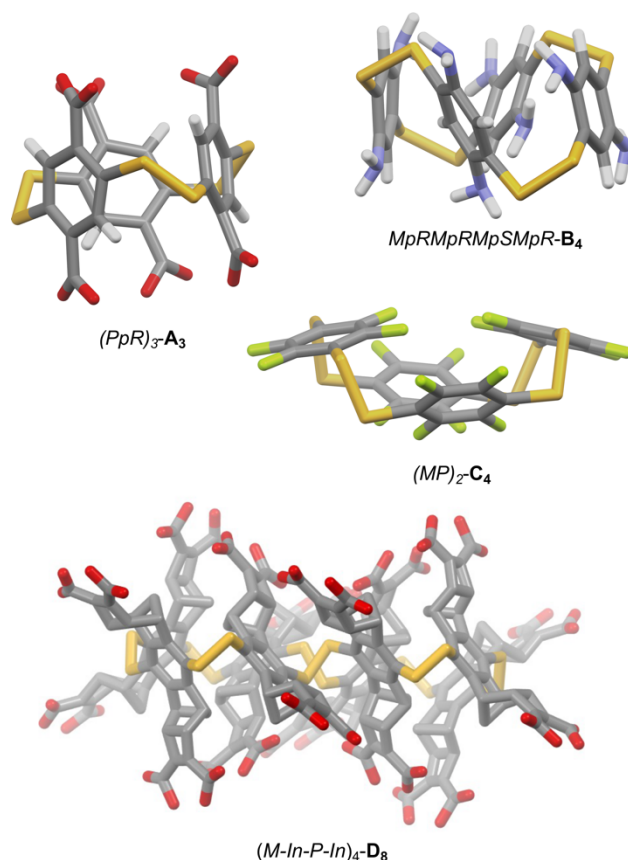


Figure 6. Computed (**A**₃, **B**₄) and crystallographic (**C**₄, **D**₈) structures of isolated dyn[n]arenes. Macrocycles were isolated as racemates when chiral. For crystallographic and computed structures of **A**₄ and **D**₄ respectively, see figure 5.

3.2.2. Templated formation of the homochiral members of the **A**-family.

As mentioned in the previous section, HPLC-UV/MS monitoring of the dynamic covalent library formed from **A** revealed the formation of dyn[3]- to dyn[5]arenes, the trimer being a kinetic intermediate which could represent up to 60 % of the material during the assembling process. As mentioned in section 2.3., the hexacarboxylate dyn[3]arene could be isolated with a 63% yield upon self-assembling with 2,4,6-triethyl-1,3,5-benzenetrimethanamine and removal of this rim-binding template. Solid-state NMR and DFT-modelling confirmed that the C₃-symmetry of triammonium template was effectively imprinted into the dyn[3]arene obtained (**A**₃): not only was it guiding the layout of the carboxylate groups into an equilateral pattern, thereby assembling the faces into a homochiral *pS*₃/*pR*₃ configuration, but it was also instructing the conformation of the disulfide bridges (Figure 6). To accommodate the hindrance of the groups borne in ortho position, disulfide linkages are forced to adopt a *MpR* or *PpS* sequence. Eventually, the C₃-symmetrical rim-binding template encodes 6-bits of (*MpR*)₃ or (*PpS*)₃ stereochemical information into dyn[3]arene. The absence of a corresponding peak in HPLC-UV/MS for this homochiral species led us suspect that this architecture was prone to self-aggregation, in proportion which could increase dramatically with the acidity of the aqueous medium. This was confirmed by DOSY-NMR in neutral aqueous conditions, which delivered diffusion

somehow cooperate, as the tilt of monomeric units around the central macrocyclic poly-disulfide ring enables octamers pairing in the three directions of space through directional weak interactions. This reveals that stereochemistry can be encoded through intramolecular interaction and the large ring size, intrinsically thermodynamically unfavored for entropic reason can be stabilized by intermolecular means (herein the packing energy). Eventually, templating, folding and packing can be used simultaneously and can even be synergistically combined to encode stereochemical information on members of the dyn[n]arene family.^[23]

3.3. From structure to properties/binding

Dyn[n]arenes proved to be a fantastic platform to explore how non-covalent interactions can affect and guide reversible covalent processes, especially their stereochemical features. Once thiols are fully oxidized, the cyclophanes are constitutionally static, which means that they no longer exchange building blocks. In this context, dyn[4]arene **A**₄ was used to revisit and challenge the lock and key paradigm. In particular we enquire whether the α,ω -diammonium guest which displays perfect steric and electronic complementarity also behaves as the best binder toward this octa-anionic cyclophane in water. As mentioned previously, (PpS)₄/(MpR)₄-**A**₄ can be produced as an imprint of aliphatic polyamines. Therefore, by construction, it should display a perfect complementarity with such guest. DFT modelling and X-ray diffraction studies confirmed that this cyclophane adopts a parallelepipedal structure in water, wherein carboxylate groups are located at the vertices. In this architecture of fixed stereochemistry, the conformational freedom is limited to the possibility of tilting one or several phenyl units around the annulus of the polydisulfide ring. As a consequence, the cavity can only accommodate aliphatic guests which can adopt an all-anti conformation. This eventually means that induced fit is rather limited with such a pair of binder partners and in particular, the carbon chain of the aliphatic guest can only be fully extended within the polyaromatic cavity.

Expectedly, combination of experimental and theoretical investigations (ITC titration and DFT calculation) revealed that butanediammonium is the best stereochemical match with **A**₄ but that hexanediammonium displays the highest binding constant within the series. Moreover, the strongest binding enthalpy is obtained with the longer octanediammonium. In fact, when the guest's chain length increases, complexation enthalpy and entropy evolve in opposite direction: the smallest diammonium displays a loose (high ΔS°) and weak (low ΔH°) binding, while octanediammonium is tightly (low ΔS°) and strongly (high ΔH°) bound. DFT calculation revealed that in the corresponding complex, the two terminal methylene units adopt a gauche conformation in order to bend their polar ammonium neighboring groups toward the tetracarboxylate rims (Figure 8, middle bottom). At first sight, this geometry appears far less optimal than for smaller diamines, wherein the ammonium end groups are each in close contact with all carboxylates of the rim (Figure 8, center).

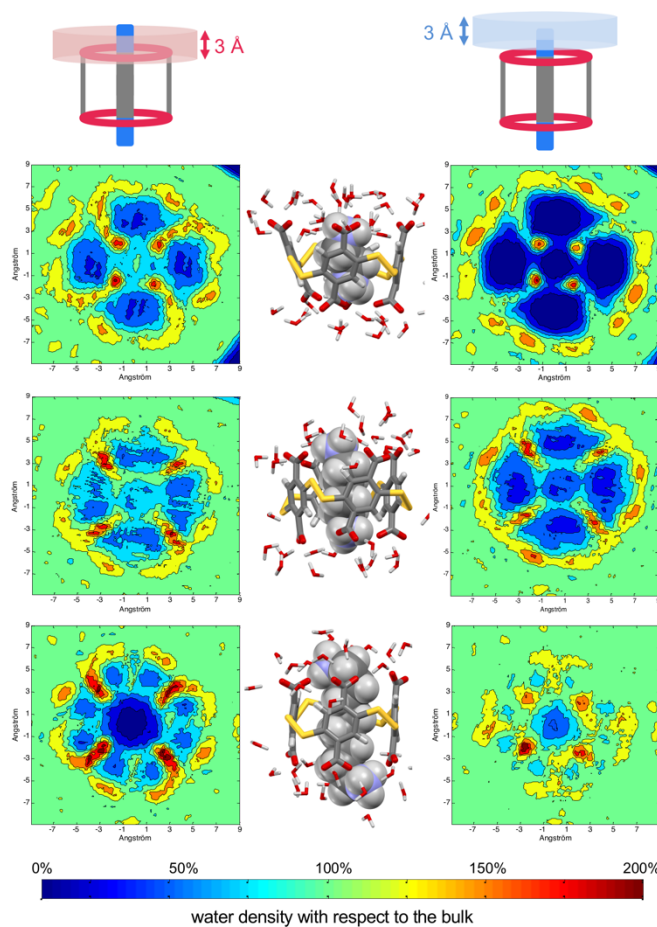


Figure 8. Time-averaged density of water molecules around the complexes formed between the homochiral dyn[4]arene **A**₄ and ethylenediamine (top), butanediamine (middle), and octanediamine (bottom) from molecular dynamics trajectories. Left and right maps correspond to the first solvation layers of the carboxylate rims and the ammonium heads, respectively. Adapted with permission from ref. ^[65], Copyright (2019), with permission from the Royal Society of Chemistry.

Counter-intuitively, formation of such a pseudo-rotaxane complex wherein the cationic end groups of the guest protrude from polyanionic rims is enthalpically more favorable than a complex with perfect geometrical complementarity between host and guest. In fact, formation of the latter requires enthalpically costly desolvation of most polar surfaces (host and guest) in order to intimately contact them. In the former formed with longer diamines, less intimate contact between charged groups enables to preserve a monolayer of water between the polar regions of the binding partners, which eventually is enthalpically more favorable. This deviation from the lock and key paradigm, well known in molecular biology and especially in protein binding^[59] was termed by us “the hydrophilic effect”.^[60] It shed some light on how binding enthalpy can be optimized in the framework of molecular recognition in water. We are currently exploring the subtle features of host-guest binding entropy in aqueous solution with an aim of providing the community with similar guidelines. The templated synthesis of water soluble dyn[n]arenes, not only offers an easy access to large amounts of analytically pure material, but it also enables thorough structural studies. By construction, as the outcome of the synthetic procedure is guided by supramolecular interactions, it reveals at the earliest possible stage the non-covalent phenomena at work during molecular recognition.

4. Applications: From Physical Chemistry to Biomolecular Recognition

Because dyn[n]arenes are youngsters in the field of supramolecular chemistry, only few applications for these molecular containers have been reported to date. The mostly rely on the ability of dyn[n]arenes to sequester molecules of biological interest.

In particular, the homochiral dyn[4]arene bearing eight carboxylic acid functions **A₄** was demonstrated to bind – through electrostatic, hydrophobic and hydrophilic interactions – biogenic polyamines (i.e. spermine, spermidine, and cadaverine) with dissociation constants in the nanomolar range in water at physiological pH.^[33] Biogenic polyamines – in particular spermine – are believed to play a key role in many cellular processes by strongly binding to DNA and RNA. This affinity for genetic material may underlie spermine’s ability to regulate cell proliferation and influence tumor growth and apoptosis.^[61] At low concentrations, spermine binding can switch the conformation of DNA from a right-handed (B-form) to a left-handed (Z-form) helix (Figure 9). Upon addition of the dyn[4]arene, the transition reversed back to the original B-DNA conformation, indicating that the receptor was indeed able to unbind spermine from DNA and thereby control the helicity of DNA.^[21]

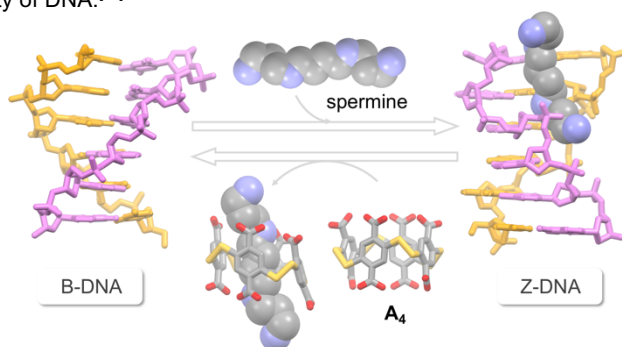


Figure 9. Control of DNA conformational helicity with a dyn[4]arene.

The homochiral poly-anionic dyn[4]arene **A₄** was able to efficiently bind more sophisticated cationic biomolecules such as chiral lysine derivatives (Figure 10, A). It was demonstrated that the remaining conformational lability of the dyn[4]arene (i.e., the possibility of a slight tilting of the phenyl rings around the -C-S- bonds) could yield induced circular dichroism (ICD) outputs upon binding with lysine derivatives in buffered water (Figure 10, B).^[62] Various mutations around the lysine moiety each displayed a unique optical readout that was exploited not only to determine the enantiomeric purity of the guest molecule with great accuracy, but also to discriminate many guests from a linear discriminant analysis (LDA) procedure (Figure 10, C).

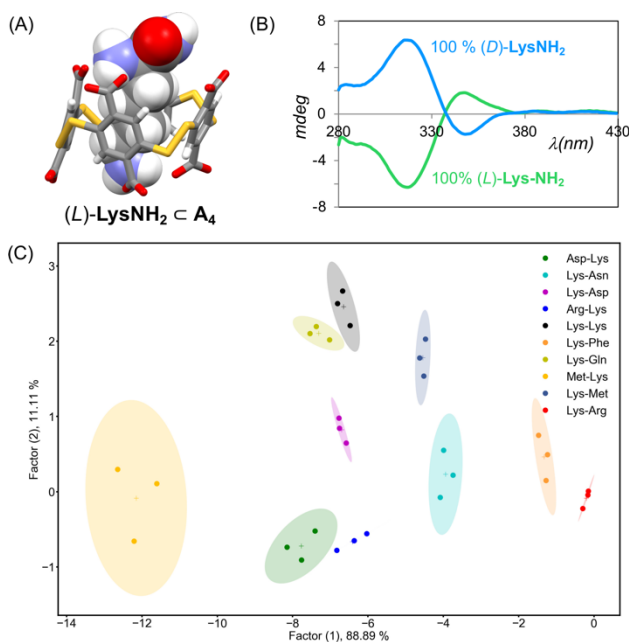


Figure 10. Chiral sensing and discrimination of Lysine derivatives with a dyn[4]arene: (A) inclusion complex between dyn[4]arene **A4** and (L)-Lys-NH₂ and the corresponding ICD responses (B), LDA canonical score plot for the ICD response at 318 nm of dyn[4]arene **A4** to 1 equivalent and 5 equivalents of (L)-Lysine-based dipeptides. Adapted with permission from ref. ^[62], Copyright (2016), with permission from the Royal Society of Chemistry.

This guest-dependent asymmetric induced fit, which can be easily detected by ECD appeared to us as a complementary method to the sensing strategy wherein the amplitude of a signal is generally correlated to the strength of the binding. In collaboration with the pharmaceutical industry, we explored the ability of dyn[n]arene to act as sensor for the detection of post-translation modification occurring in the vicinity of lysine moieties on therapeutic antibodies^[63] If this application has not reached the market yet, it illustrates the potential of dyn[n]arenes, not only as biomolecule binders but also as sensors, transporters or metabolic modulators.

5. Conclusion

In this review, we report on our studies of a new family of disulfide-based cyclophanes called dyn[n]arenes. We hope to have demonstrated that, beyond being simple host molecules, dyn[n]arenes are unique nano-objects whose size and functions can not only be chosen on demand, but whose stereochemical characteristics can also be programmed. Moreover, the deciphering of the interactions at work during the assembly processes allows the discovery of new physicochemical phenomena such as the hydrophilic effect, but also paves the way for innovative applications such as the chiroptical sensing of biomolecules. Overall, this account describes the synergistic interplay between covalent and non-covalent bonds found in dyn[n]arenes. We strongly believe that there is still plenty of room for further exciting developments that could result from a better exposure of these cavitands, which is the ambition of this account.

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Keywords: Cavitands • Dynamic combinatorial chemistry • Host-guest binding • Sensing • Stereochemistry

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