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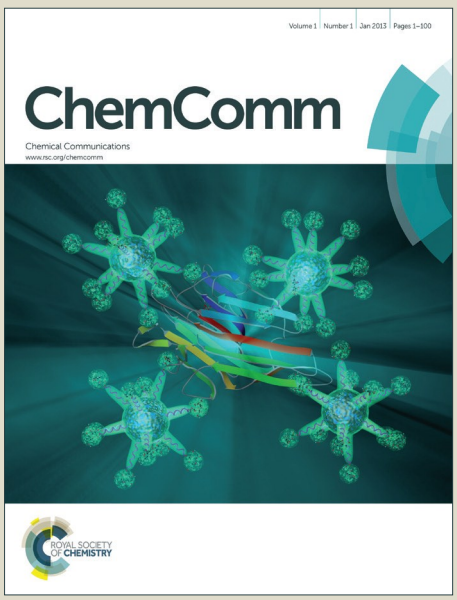

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FEATURE ARTICLE

From Simplicity to Complex Systems with Bioinspired Pseudopeptides

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The pseudopeptidic compounds are chemical species with attractive applications in many fields of chemistry. The increasing of complexity in pseudopeptidic molecules has allowed an improvement of their properties, very often after following an initial bio-inspiration. Two main types of complexity can be proposed: structural and interactional. These operational processes have been recently used for the generation of new elaborated pseudopetidic molecules and complex molecular systems. This Feature Article presents a brief discussion on the recent advances done following this rational and with these privileged molecules.

Introduction

The study of complex systems has attracted the interest of the research community in the last years.[1] Complexity is a ubiquitous characteristic in natural life. If we take Biology as an example, we realize that the life into a cell occurs through the complex interaction between elaborated organic molecules that work synchronized. However, chemical research has traditionally escaped from facing complex systems.[2]

We, as chemistry researchers, usually tend to the reductionism of working with simplified models, of easier design, preparation, control and understanding. However, during the simplification process, we can lose important information and we probably overrule the intrinsic importance of complexity for the performance of successful molecular systems.[3]

For chemical researches, especially those working in the field of Supramolecular Chemistry and Chemical Biology, Nature has been always a source of inspiration and also a target for our projects. However, real natural systems are still difficult to handle in the chemistry lab for full understanding of the biological process at the molecular level. During the last decade our research group has been working on different fields related to the supramolecular chemistry using Biology as an inspiration for the design of minimalistic simplified model systems.[4] However, the conventional chemical research would be clearly located at a lower level than Biology in a complexity frame (Fig. 1). This different complexity can be expressed as the chemical or structural complexity of the implicated molecular entities (horizontal axis) or as an interactional complexity (vertical axis), coming from the presence of the interacting chemical networks of molecules in bio-systems. Regarding that, we have been trying to approach our research in the chemical field closer to our initial inspiration, by increasing the complexity of the studied systems. To that, we envisioned two different and complementary operational pathways.

The first one would consist of simply increasing the structural and chemical elaboration of the newly designed structures (path a in Fig. 1). The second one (path b) is more difficult and less exploited to date, and would operate by setting up systems able to combine and to mutually interact in a network

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fashion. Both approaches can converge and connect for finally rendering more elaborated and efficient chemical entities. We have recently progressed in both directions and a brief discussion of our advances in this field is described in this Featured Article, also highlighting selected examples from other researchers in the field.

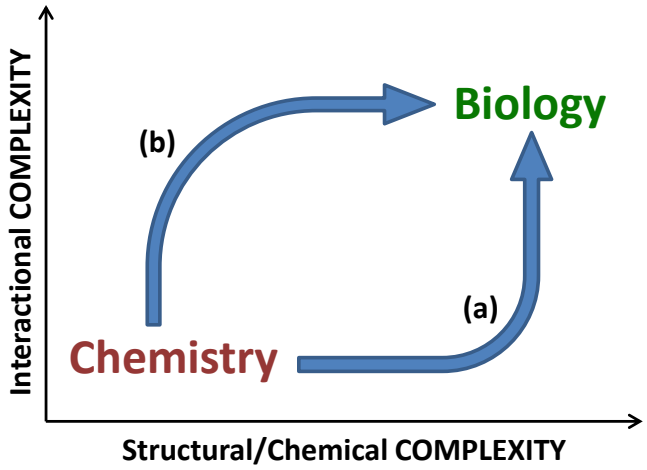


Fig. 1. Plot of the Chemistry and Biology location on a hypothetical complexity frame.

Pseudopeptidic molecules as building blocks

Developing a research program on new chemical structures is a fascinating enterprise. The inspirational process for designing the intended molecular entities can start from different perspectives from the gaining of basic knowledge on physicochemical properties to the implementation of specific tailor-made functions for a given application. Related to that, Nature provides precious data. In biological processes, peptides and proteins are the main *workers*, acting as the main catalytic, communication, signalling and structural elements. These molecules are made by the connection of amino acids. Probably, the amino acid structure represents one of the most concentrated expressions of chemical functionality in an organic molecule.[5] Besides, the many different chemical functionalities of the side chains allows modulating different physicochemical properties like polarity, H-bond abilities, hydrophobicity, acid-base behaviour, charge at different pH, coordination properties, aromatic nature, etc… Therefore, one can conclude that structures made of amino acid moieties should be good candidates as a starting point, allowing a large range of diversity and modularity.[6] Besides, through the combination of natural amino acids with non-natural organic scaffolds, it is possible to modulate additional properties such as flexibility and solubility of the final compounds with a minimal synthetic effort. With this design, pseudopeptidic molecules are excellent candidates.[7]

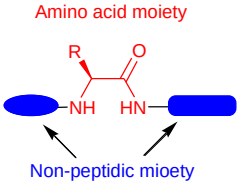


Fig. 2 General structural design of a pseudopeptidic molecule

Synthetic pseudopeptidic receptors

From the supramolecular chemistry perspective, the use of pseudopeptidic molecules as synthetic receptors for ions and small molecules represents an appealing alternative. The pseudopeptidic receptors have potential interaction sites coming from the peptide bond functionality, as well as from the amino acid side chain. Besides, additional functionalities can be introduced in the non-peptidic part of the hosts. For a better defined conformation in solution, the macrocyclic structure for the host is usually preferred.[8] According, macrocycles of small-medium sizes showed to bind small cations (**1**) like Ag(I)[9] or anions (**2**) in non-polar and polar organic solvents. Besides, a detailed NMR structural analysis showed interesting conformational properties that were fundamental to understand the binding phenomena.[10] The inclusion of a fluorescent naphthalene moiety (**3**) also allowed using these systems as ratiometric sensors for *N*-protected aromatic amino acids.[11] However, the strength of the host-guest interaction is modest, thus limiting the use of the receptors to non-polar organic media, typically chloroform.

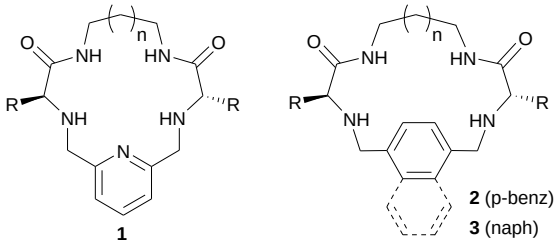


Fig. 3 Small pseudopeptidic macrocycles used as molecular receptors

With the aim of improving the binding performance of the molecules and also to be able to recognize larger biologically interesting species,[12] two complementary approaches can be done: to increase either the size or the tridimensional nature of the host (Fig.4). Initially, we decided to increase the size of the macrocycles, which would allow the pseudopeptides to host larger guests within their cavity.

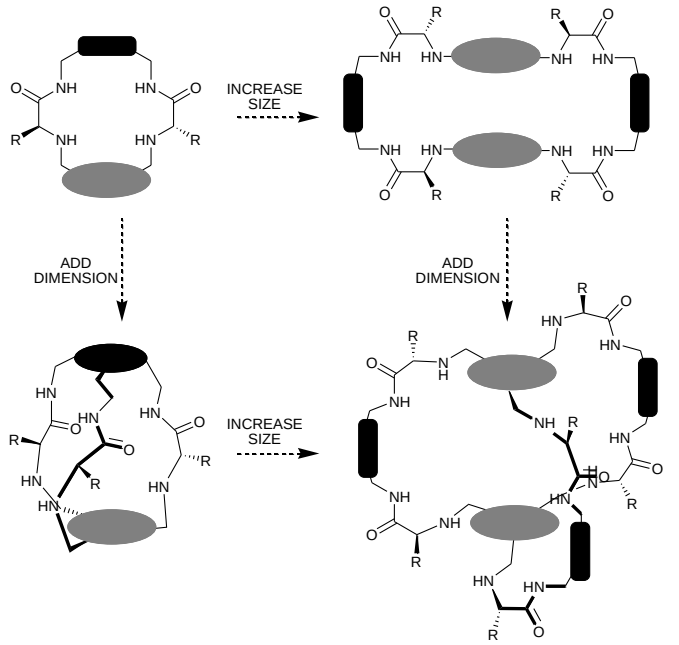
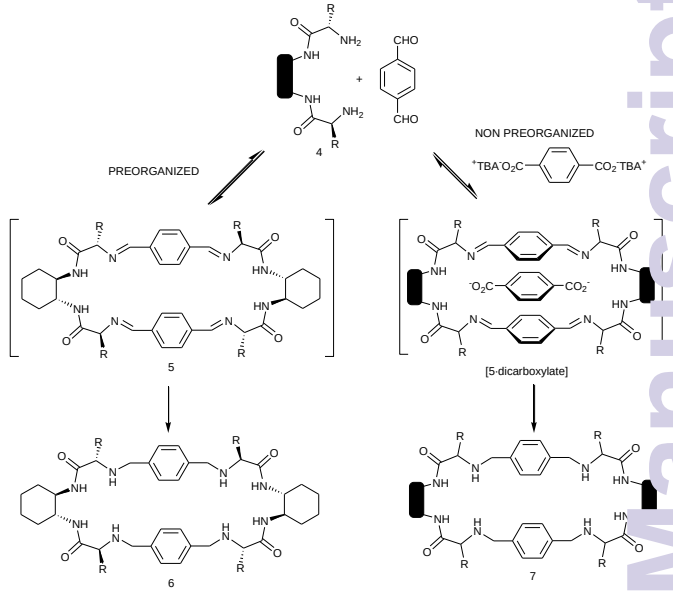


Fig. 4. The process of evolving the structural complexity of the pseudopeptidic macrocycles by enlarging their size or by adding a dimension



Scheme 1. Synthesis of large pseudopeptidic macrocycles by a [2+2] reductive amination reaction

The synthesis of larger pseudopeptidic macrocycles is not an easy task and it is usually limited to highly pre-organized precursors.[13] Otherwise, side products complicate the key macrocyclization reaction, and tedious purification steps are needed to separate different oligomers.[14] Several approaches have been used to overcome this problem, such as the use of protecting groups or templates to facilitate the formation of the intended macrocyclic size.[15] In this regard, we proposed the synthesis of large pseudopeptidic macrocycles by a one-pot two-steps [2+2] reductive amination reaction of bis(amino amide) **4** and an aromatic dialdehyde, where the key step is the initial formation of a macrocyclic tetraimine (Scheme 1). This species is dynamic thanks to the reversible nature of the imine bond, allowing error correction and modulation of the species toward the most stable one. This was accomplished by using a configurationally driven preorganization, defined by a *match/mismatch* relationship of the stereogenic centres of the amino acids and the chiral *trans*-cyclohexane-1,2-diamine central spacer.[16] Interestingly the compounds bearing (*R*,*R*) diamine and (*S*) amino acids led to the efficient formation of the macrocycle (*match* combination) while the (*R*,*R*) diamine with the (*R*) amino acid rendered a complex mixture (*mismatch* combination).[17] For the systems not being preorganized (those bearing a flexible spacer[18] or a *mismatch* combination[19]) the presence of an anionic template led to the formation of the correct macrocycle within the dynamic covalent mixture of imines. In all cases, the in situ reduction of the imine bond produced the final macrocycles in good yields with synthetic utility.[20]

The obtained macrocycles showed interesting supramolecular properties both in solution or onto surfaces and in the solid state.[22] For instance, the Val derivative of macrocycle **6** (R = iPr) recognized *N*-protected dipeptides with a moderate selectivity for the dipeptides bearing Phe at the C terminus.[23] However, once again, the molecular recognition proceeded in mainly non-polar organic media.

An alternative way to increase the complexity of the receptors is to add an additional dimension by preparing closed cages as shown in Fig. 4. This strategy has been successfully done by different researchers in the field Fig. 4. For instance, Kubik designed the preparation of peptidic cages (**8**) for the encapsulation of anions in competitive media.[24] These cages can be obtained by linking peptide-macrocycles through triazole click-chemistry or by using dynamic covalent chemistry of disulfides (see below). Both approaches led to improved hosts with excellent binding abilities even in very polar media. The Jolliffe group has also used the cage framework to design receptors for inorganic anions, with different selectivity depending on the macrobicyclic architecture.[25] In recent studies, the Haberhauer group showed that cryptand-like receptors, some of them derived from peptides, show unexpectedly high binding constants for simple small guests, such as chloroform.[26]

Following that rational, we also considered the possibility of constructing small pseudopeptidic cages for the encapsulation of small guests by adding a third dimension (Fig. 4). The cages thus prepared, when protonated, were excellent hosts for small anions, with a very good selectivity for chloride versus other halides (Fig. 6). The corresponding binding constants toward Cl^- (measured by ^1H NMR titration in 5%H_2O acetonitrile, at 303 K) were more than two orders of magnitude higher than for Br^- or F^-.[27]

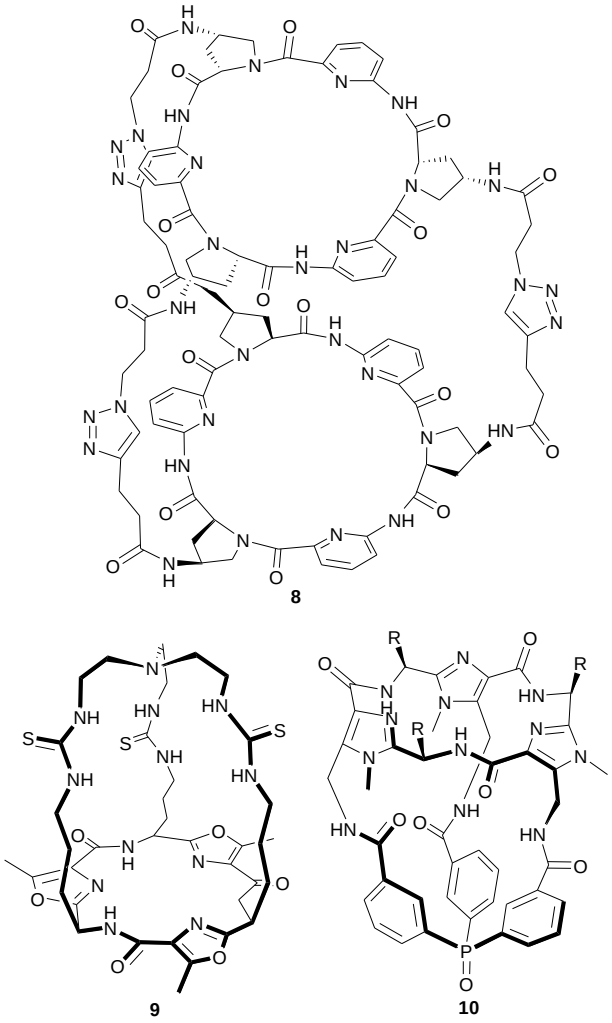


Fig. 5. Selected examples of pseudopeptidic cages as synthetic receptors form the groups of Kubik (**8**), Jolliffe (**9**) and Haberhauer (**10**).

The solid state structures for the corresponding complexes served to give a reasonable explanation of the observed selectivity: the chloride anion perfectly fits within the cage cavity while bromide is too large (producing a host distortion) and fluoride is too small (being water molecule an efficient competitor for the binding site). The binding constants for chloride can be further tuned by the external substitution of the cages through the different substituents of the molecules. More interestingly, some of the prepared hosts are able to transport chloride through lipid bilayers as models of cell membranes.[28] These anionophoric properties foresee the potential biological applications of these molecules.[29]

Finally, we also combined the increase of the size with an added dimension for the design of new pseudopeptidic large cages (Fig. 4). By using the general rationale described in Scheme 1, the replacement of the dialdehyde by 1,3,5-triformylbenzene allowed us to prepare the corresponding cages with good yields, in both the pre-organized or the anion-templated version of a [3+2] reductive amination reaction.[30] Some of these cages were able to bind *N*-protected dipeptides in competitive media, showing a good degree of selectivity for the Ac-EY-OH dipeptide,[31] a model for a biologically important target sequence of tyrosine kinases.

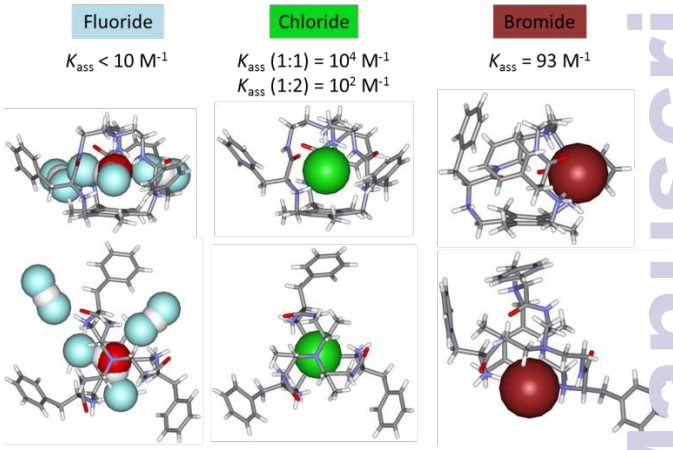


Fig. 6. Small pseudopeptidic cages as receptors for inorganic anions: solution binding constants and upper and side views of the corresponding pseudopeptidic cage complexes for fluoride (light green), chloride (green) and bromide (red-brown) as observed in solid state. Outer anions and solvent molecules have been omitted for clarity.

Other remarkable approaches to complex peptide-like structures have been described. For instance, Huc designed helical aromatic oligoamides able to encapsulate sugars within the inner cavity defined by the helix.[32] The combination of peptide-like molecules with transition metals[33] also offers interesting opportunities for preparing peptide-like structures with potential applications. Despite these approaches are highly appealing, the deep discussion on these examples is beyond the scope of this *feature article*.

Overall, the rational and sequential increase of the structural complexity of the pseudopeptidic molecules improved their abilities in molecular recognition processes, allowing studying bio-relevant guests in more competing media. This evolution will hopefully approach these systems to be used in more challenging biological applications.

Peptide-like molecular networks

An alternative way to increase the complexity in a chemical system should be by creating a mixture of interconnected components with high structural diversity (path b in Fig. 1). If the members of this system can exchange, the corresponding dynamic chemical system could be adaptive. This rationale has been recently exploited by dynamic covalent chemistry using pseudopeptidic molecules. Thus, Dynamic Combinatorial Libraries (DCL) have been initially developed by different pioneering researchers in the field.[34] This methodology proposes the generation of a dynamic mixture of components that are able to rapidly exchange and interconvert. This property makes the DCLs adaptive and able to express changes in the stability of the whole system as a response to external stimuli (Fig. 7). The stimuli can be a chemical species as a template or a change in the conditions (temperature, pressure, pH, ionic strength, electrical field, light, etc…). Thus, the best adapted member of the library amplifies at the expense of the other congeners (Fig. 7). The key issue for the generation of a

DCL is the implementation of reversible covalent bonds able to form and dissociate under smooth reaction conditions. For the dynamic bonds, mainly C=N and S-S bonds have been exploited, among others.[34] The presence of amino and thiol functionalities in some amino acids (i.e. cysteine) facilitates the synthesis of amino acid-based building blocks. Besides, the abilities of the peptide-like molecules to establish non-covalent interactions, even in highly competitive media also suggested that dynamic covalent systems based on pesudopeptides could be appealing for the emergence of new and interesting properties. Moreover, the building blocks made of amino acids contain interesting structural and chemical information closely related to the one present in larger peptides and proteins, thus being a very good benchmark model to study non-covalent interactions in peptides and peptide-related molecules.

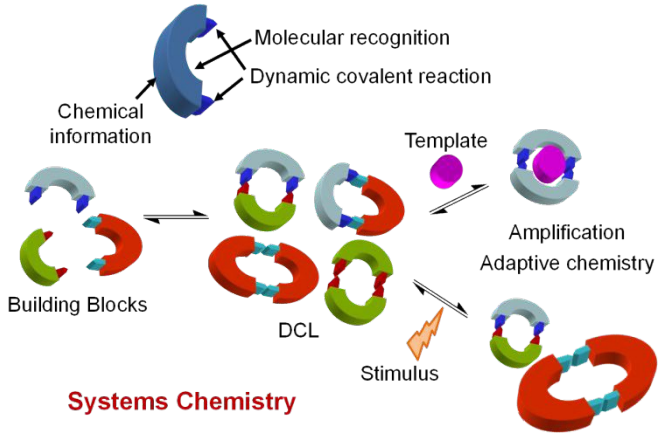
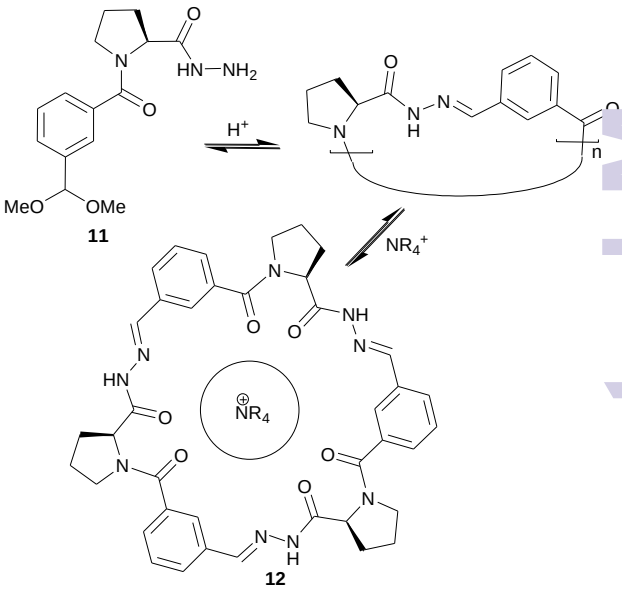


Fig. 7. Cartoon representation of the DCC approach for the preparation of different macrocycles (in this case).

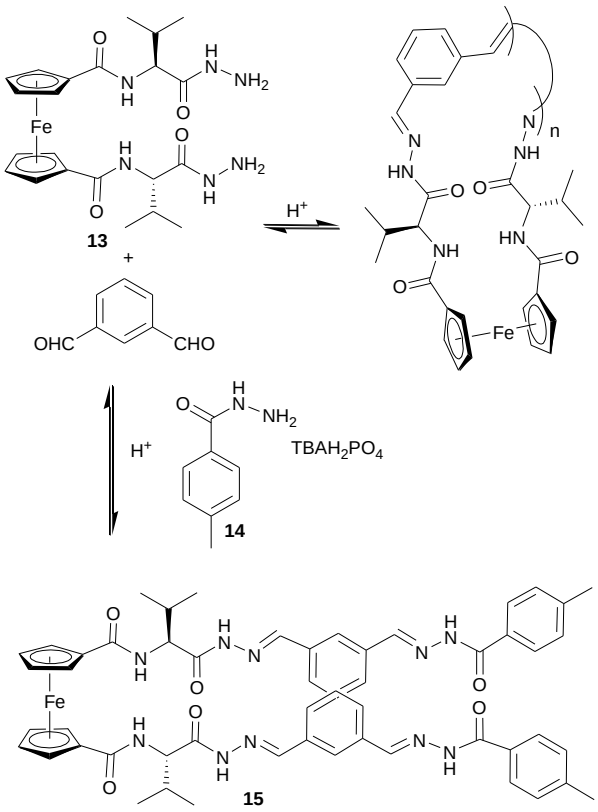
Dynamic pseudopeptides based on C=N bonds

The carbon-nitrogen double bond is a paradigmatic functional group for the generation of dynamic chemical systems. The reversible nature of this linkage makes it able to self-correct and to adapt to external stimuli (Fig. 7). Thus, the simplest C=N grouping would correspond to the imine bond, which is highly dynamic but suffers an inherent instability depending on the medium. Examples of imine-based dynamic libraries built with pseudopeptidic building blocks have been already discussed previously, since our anion-templated synthesis of macro(bi)cycles can be considered as obtained from a small DCL of imines (Scheme 1). The isolation of the member amplified by the presence of the anion template was possible by the in situ reduction of the imines. Other, more elaborated DCLs have been also prepared exploiting other N=C moieties, like hydrazones. These are dynamic at acidic pH values but the exchange can be frozen by increasing the pH. A pioneering work in the field was reported by Sanders and co-workers as depicted in Scheme 2.[35] The authors prepared a simple BB (**11**) by attaching hydrazine at the carboxylic end and an aromatic acyl at the amino terminus of proline. The molecule had a dimethylacetal group in *meta* position of the aromatic ring, which can be considered as a masked aldehyde. In acidic pH, the acetal is quickly hydrolyzed and the generated aldehyde reacts with the acylhydrazine ends to render a hydrazone bond. Since this process was reversible at acidic conditions, the system produced a mixture (library) of dynamic pseudopeptidic macrocycles (Scheme 2). The addition of organic cations like a quaternary ammonium salt produced the rearrangement of the species, thus altering the composition of the mixture in solution. The macrocyclic ring that interacted more efficiently with the added template was highly amplified, being in this case the corresponding trimer (**12**). The isolation and characterization of the amplified species was carried out by increasing the pH, which switched off the dynamic process.



Scheme 2. DCL of pseudopeptidic macrocyclic acylhydrazones able to respond to the presence of quaternary ammonium cations

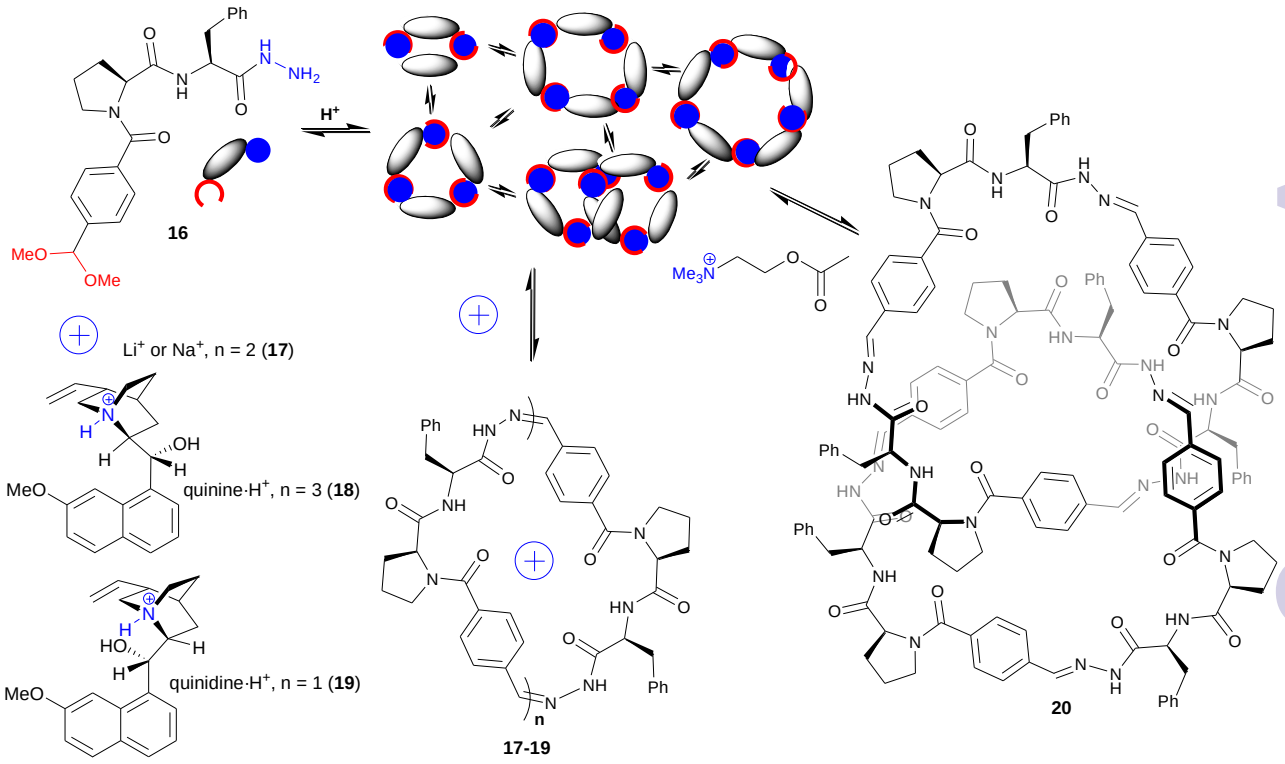
The beauty of the structures based on amino acids is the versatility and the diversity of potential binding sites for different substrates. Thus, the same group reported the design of the building block formed by the connection of two valine residues through their amino end to a ferrocenedicarbonyl spacer, and with the corresponding *C*-termini transformed into acylhydrazines (**13**). Acidic condensation of **13** with isophthaldehyde produced a complex mixture of macrocyclic hydrazones of different sizes (Scheme 3).[36] These macrocycles were isolated and displayed interesting structural features by NMR and CD experiments, such as a helical conformation in solution. Besides, UV titration data showed the ability of these systems to recognize different anions in organic solvents, and the results were further confirmed by NMR spectroscopy. However, the macrocycles were able to exchange in solution in the presence of traces of acid. Therefore, the authors decided to use the same building block to generate a DCL of acylhydrazones, by combining **13** with isophthandehyde and an additional modopodal acylhydrazine (**14**), which would allow the formation of open-chain, acyclic species within the library (Scheme 3). Rather surprisingly, the presence of dihydrogenophosphate anion amplified the formation of the



Scheme 3. DCL of open chain and macrocyclic pseudopeptidic acylhydrazones designed to interact with inorganic anions.

open species (**15**). This compound showed strong binding toward that anion, and detailed spectroscopic analysis rendered also a helical conformation of the host, possibly wrapping the dihydrogenophosphate anion by setting a rather complex H-bonding pattern.[37]

A slightly more elaborated design was conceived by the introduction of a Pro-Phe dipeptide and shifting the acetyl group to the *para* position of the aromatic ring (**16**). In this case the acidic equilibration rendered a much more complex dynamic library of pseudopeptidic hydrazones (Scheme 4). This DCL adapted the composition of members to the presence of several cations through the amplification of different macrocyclic sizes. Thus Na^+ and specially Li^+ led to the practically exclusive formation of the corresponding macrocyclic trimer (**17**).[38] The observed amplification was due to the cation inclusion in the pseudopeptidic macrocycle as proposed by the observed changes in the FT-IR, ^1H and ^{13}C NMR spectra of **17** in the presence of the LiI salt. The binding mode was further supported by ^7Li NMR and [illegible] experiments.[39] The authors proposed the combined action of intramolecular H-bonds and metal coordination through the carbonyls and the π cloud of the aromatic rings of the pseudopeptide. Remarkably, the structurally related BB with a *meta* disposition in the aromatic backbone formed the corresponding dimer as the major macrocycle in the presence of the same metal salts, underscoring the importance of the structure of the BBs on the final library composition.



Scheme 4. DCL of pseudopeptidic hydrazones that respond to the presence of different cationic species, such as inorganic alkali metals, alkaloids and a neurotransmitter, leading in each case to the amplification of different macrocyclic rings or a [2]-catenane.

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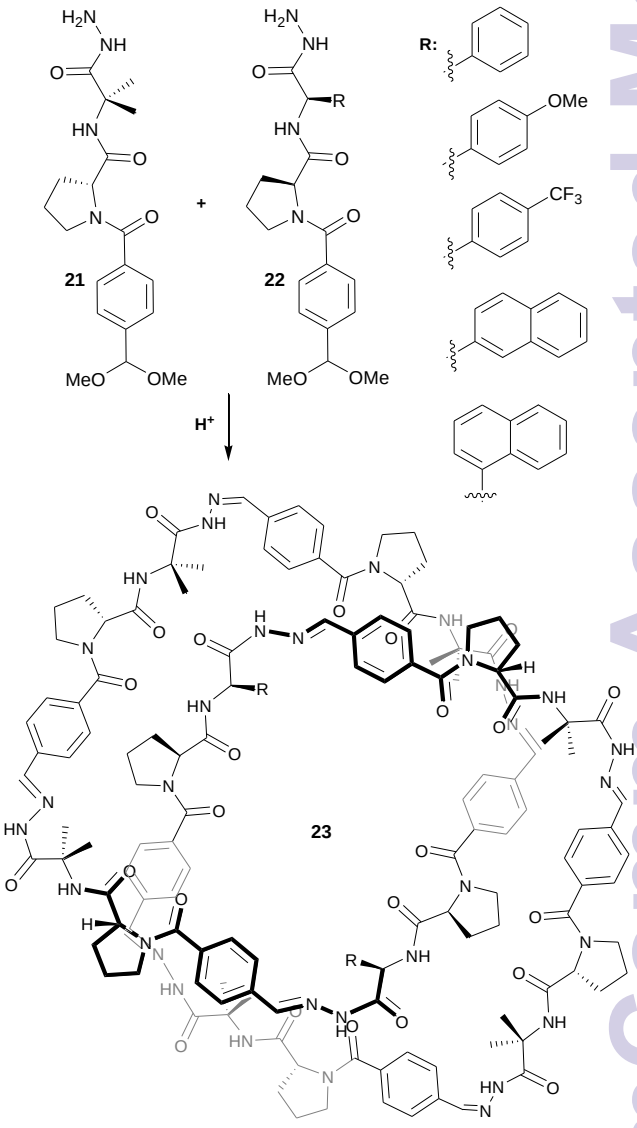
The versatility of the methodology was exemplified when different organic cationic templates were added to the same library (Scheme 4). Thus, the addition of diastereomeric alkaloids amplified completely different species from the mixture: quinine promoted the tetramer (n = 3, **18**) while quinidine led to the formation of the dimer (n = 1, **19**).[40]

One of the most appealing features of the dynamic covalent chemistry approach is its excellent capability for the discovery of unexpected species. In many cases, completely new species can be formed due to the specific and strong interactions with a given target substrate. Thus, even the members initially absent or present in very low concentration in the DCL can be amplified and identified after de addition of a template. For these reasons, Lehn termed the DCLs as virtual combinatorial libraries.[41] A specially shocking and seminal example was described by Sanders and Otto[42] also starting from the same BB **16** (Scheme 4). They added acetylcholine, a neurotransmitter with biological relevance, and observed the amplification of a much larger new species, with a molecular weight corresponding to a cyclic hexamer. The new species was synthesized and isolated in a considerably high yield (67%) from a template-induced preparative-scale synthesis. After the suitable and detailed structural characterization, they concluded that the hexamer was actually a [2]-catenane (**20**) formed by the mutual threading of two macrocyclic trimers. Thermodynamic studies showed that this catenane was an extraordinarily efficient receptor for acetylcholine, with an affinity in the nanomolar range. Moreover NMR experiments led the authors to confirm that the acetylcholine was deeply included within the interior of the interlocked structure. The results from this pioneering work demonstrated the power of the DCC procedure and that interlocked molecules can be spontaneously and efficiently formed under controlled experimental conditions.

Another highly remarkable example of interlocked pseudopeptides from a DCL of hydrazones was reported by Gagné and co-workers.[43] They used a combination of pseudopeptidic building blocks (**21** and **22** in Scheme 5) with a central dipeptide scaffold, containing in one case L-prolyl-α,α-dimethyl glycine (**21**) or L-prolyl and different aromatic L-amino acids (**22**). Also in this case, the C-termini were capped with hydrazine and the N-termini were modified with the same acylaryl group bearing a dimethyl acetal in *para*. After equilibration at acidic conditions, they observed the spontaneous main formation of octamers containing a combination of the given BBs ($\mathbf{21}_6$-$\mathbf{22}_2$). These octamers were actually [2]-catenanes (**23**) formed by the threading of two identical macrocyclic tetramers, as confirmed by NMR spectroscopy in solution, as well as X-Ray diffraction of crystal structures in the solid state. The data suggested that the R residue in one of the BB was very important for the formation of the interlocked pseudopeptides. A further deep structural study of a much larger variety of examples[44] with different aromatic R groups in **22** allowed the identification of the non-covalent interactions responsible for the formation of the catenanes (Figure 8). The authors were able to grow crystals suitable for X-ray diffraction studies with several examples. They observed many H-bonding contacts within and between interlocked rings and, additionally, several π-π and CH-π interactions that are crucial for the formation of the observed species. More importantly, NMR studies demonstrated that these important contacts were retained in solution, allowing the authors to propose those contacts as the driving forces for the outcome of the library.



Scheme 5. DCL of acylhydrazone-linked catenanes based on pseudopeptides

The same group studied the stereoselective amplification of pseudopeptidic receptors for different nucleosides. A DCL formed by the racemic BB **21** rendered a mixture of cyclic oligomers of different sizes[45] and bearing all the possible

stereochemical combinations of chiral centres. The addition of (-)-adenosine induced the amplification of the corresponding dimer with a preferred configuration. The HPLC analysis coupled with a laser polarimeter detection, complemented with assays using isotopically labelled pseudoenantiomers confirmed that the amplified dimer had (*S*,*S*) configuration.[46]

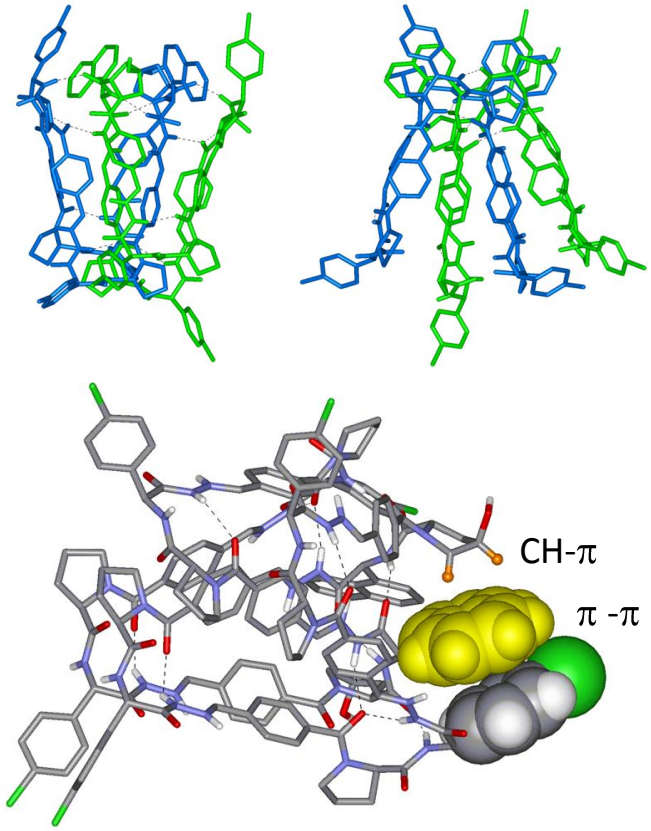
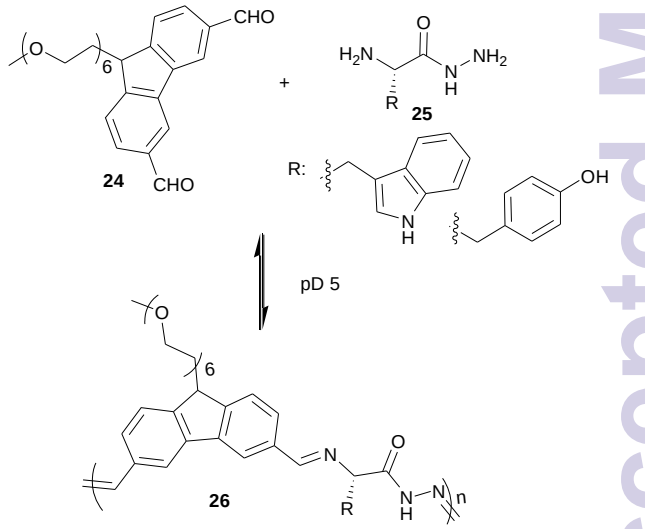


Fig. 8. Single crystal structure of a selected catenated pseudopeptide obtained from the DCL of pseudopeptidic hydrazones reported by Gagné and co-workers, re-built from the corresponding cif file. Non-polar H-atoms were omitted for clarity and possible H-bonds are shown as dashed lines. In the upper view two different views of the [2]-catenane are shown with each of the ring in a different colour (green/blue). In the bottom view the inter-chain non-covalent interactions have been highlighted: CH···π and π-π contacts established by the large naphthyl residue (CPK in yellow) with either hydroxyproline residue (CH in orange) or *p*-chlorophenyl (CPK), respectively

The utility of the DCC procedure was further shown by adding different templates to the same racemic DCL. Thus, the corresponding deracemization of the mixture amplified two different macrocyclic pseudopeptides: the (*S*,*S*)-dimer from (-)-cytidine while the (*R*,*R*,*R*,*R*)-tetramer from (-)-2-thiocytidine.[47] Therefore, the DCC methodology has also demonstrated to be useful for the generation of receptors able to exert chiral molecular recognition, which is a specially challenging issue in supramolecular chemistry.

The preparation of pseudopeptidic polymeric materials is also an exciting topic where DCC procedures have found potential applications. This was illustrated by studies with peptide-like dynamers, which are essentially DCLs of oligomeric pseudopeptides. Thus, Lehn and co-workers[48] described the generation of these species from a central aromatic dialdehyde (**24**) and aminoacylhidrazines (**25**) in aqueous acetate buffer (pD 5). Under these conditions both imine and hydrazone bonds are dynamic, leading to the formation of polymeric species with relatively good solubility in aqueous medium thanks to the polyether chains attached to the structure **24**. The polymers thus formed (**26**) tend to self-organize leading to globular particles folded by hydrophobic interactions (as studied by cryo-TEM, LS, DOSY NMR, and SANS studies). These polymers displayed surprisingly low polydispersity and, most remarkably, they retained the behaviour of the two dynamic covalent bonds allowing aminoacydic building block exchange. The particles thus formed are reminiscent of globular proteins with nucleation-elongation behaviour driven by hydrophobic effects. These fascinating systems were proposed by the authors as simple models for the evolution processes in complex matter.

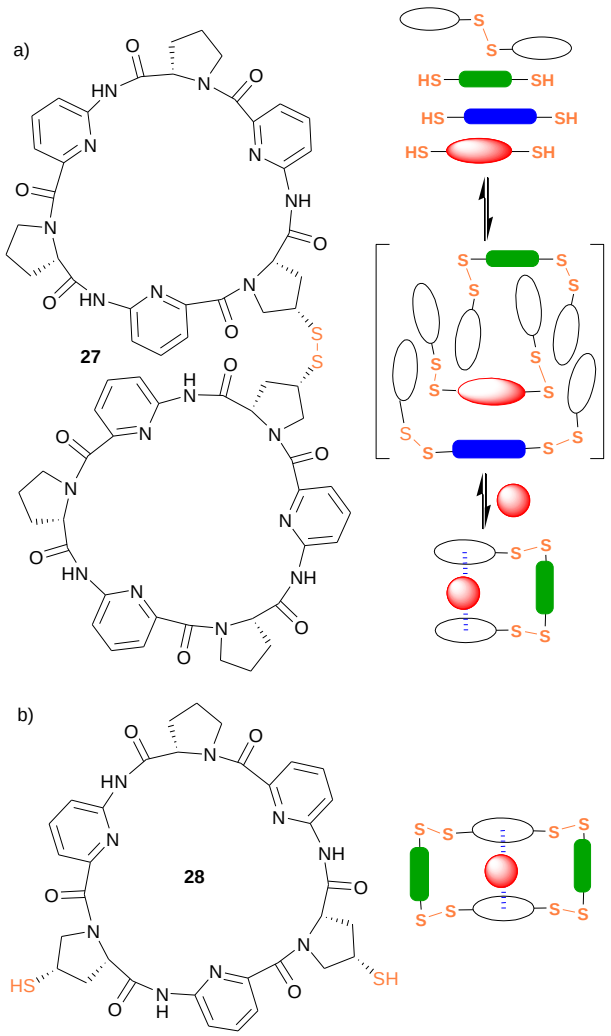


Scheme 6. Peptide-like dynamers able to form globular folded particles with biomimetic properties.

Dynamic pseudopeptides based on disulphide bonds

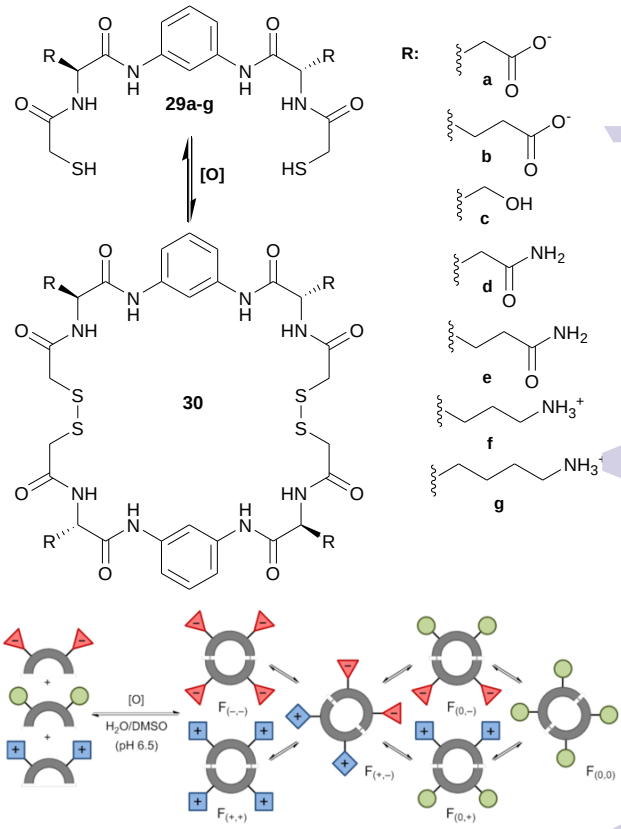
Another reversible covalent bond widely used in the DCC field has been the disulphide group. Disulphides can be formed in aqueous solution in the presence of oxygen and at slightly basic pH. Besides, the reversible disulphide exchange is also compatible with these reaction conditions and many other functional groups. On the other hand, thiols can be easily implemented in amino acid-derived structures using relatively simple chemistry. This fact has made the disulphide exchange one of the most popular reactions for the generation of DCLs of pseudopeptides in competitive media, including aqueous solvents. Otto and Kubik have jointly used this approach for the design and identification of a pseudoeptidic receptor for anions in aqueous medium.[49] Considering the binding abilities of macrocyclic pseudopeptides from Kubik's lab and their trend to form dimeric supramolecular complexes (both in solution and in the solid state)[50] they decided to prepare a DCL of dimers joined by disulphide bonds (Scheme 7). The library was designed to generate different receptors by setting different dithiols as potential spacers between the corresponding macrocycles with an appended thiol (**27**). The

addition of either iodide or sulphate amplified two different species, where the nature of the spacer maximized the binding: This trend was further confirmed by ITC titration with the isolated receptors from templated preparative-scale syntheses. The same groups reported an improved second-generation DCL system by attaching two thiols in each macrocyclic pseudopeptide (**28**). In this case, the mixture mainly produced macrobicycles by the connection of two BBs with two dithiol arms, thus forming the corresponding closed cage structures (see cartoon representation in Scheme 7b). Interestingly, from this library, a receptor with a nanomolar affinity for sulphate anion in aqueous acetonitrile was identified.[51] This result is very remarkable for a receptor based on neutral H-bond donors to bind the sulphate anion in aqueous medium, since this is a very challenging and elusive recognition motif.



Scheme 7. DCL of disulphide-dimers of macrpcyclic pseudopeptides leading to the formation of bivalent binders for anions joined by either one (a) of two (b) linkers.

The non-covalent interactions in disulphide-based DCLs can be also established between the building blocks themselves within each member of the mixture. A recent example from our group is depicted in Scheme 8. We prepared a bioinspired DCL of simple C_2 symmetrical pseudopeptidic dithiols (**29a-g**). They had a common central *meta*-phenylene spacer [illegible] favouring macrocyclization and two terminal mercaptoacet[illegible] residues for the disulphide formation. The implementation [illegible] an amino acid moiety in each arm provided the BBs [illegible] peptide-like chemical and structural information. This desig[illegible] and the addition of small amounts of DMSO as organic co-solvent[52] allowed the generation of DCLs of macrocycl[illegible] pseudopeptides (mainly dimers like **30**, see Scheme 8) with residues of different polarity and molecular charges at p[illegible] values close to neutrality. From these BBs, a library for mimicking the evolutionary patterns of halophilic proteins was designed.[53] The members of the library concentrating acidic residues (from Asp and Glu, **30a,b**) were amplified by th[illegible] increase of salt concentration at the expense of those le[illegible] charged or neutral at the working pH. Interestingly, making parallelism with the Nature, the halophilic organisms hav[illegible] modified their proteins (after biological evolution) [illegible] increasing the acidic residues on the surface. This evolutionary adaptation allowed them to retain the structure and func[illegible] of their biological machinery at high salt concentration.

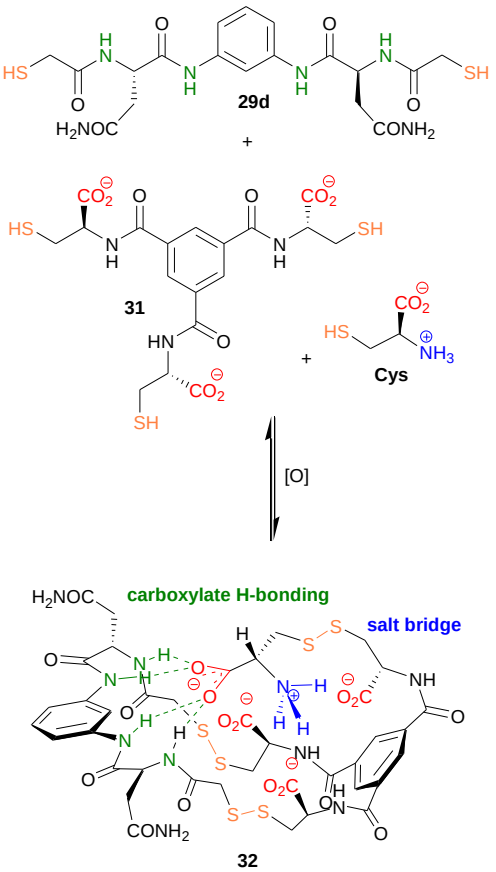


Scheme 8. DCL of pseudopeptidic macrocycles bearing differently charged side cha[illegible]s.

Despite the differences between biological evolution and chemical adaptation, the similarities found in our study ar[illegible] quite remarkable and underline the utility of simple molecula[illegible] networks for mimicking biological processes. Following this study, a more diverse and complex library was prepare[illegible] containing positive, negative and neutral residues within the same backbone pseudopeptide.[54] The adaptation ability of th[illegible] complex molecular network to the increase of salt gave

important information about the electrostatic interactions in aqueous medium. Thus, for instance, we demonstrated that the anionic residues produced library members more sensitive to the changes of the ionic strength. Complementary NMR and molecular dynamics simulations suggested that these differences are due to a more efficient folding of the anionic species by intramolecular H-bonding. The measurement of all the possible exchange equilibrium constants (cartoon in Scheme 8) allowed the full understanding of the complete molecular network in depth. Accordingly, with that information, we were able to design and set up dynamic libraries expressing either cooperative or competitive relationships[55] within the mixture upon the action of a simple meaningful stimulus: the increase of the ionic strength.[54] Overall, this study showed the DCL utility for the new systems chemistry topic.

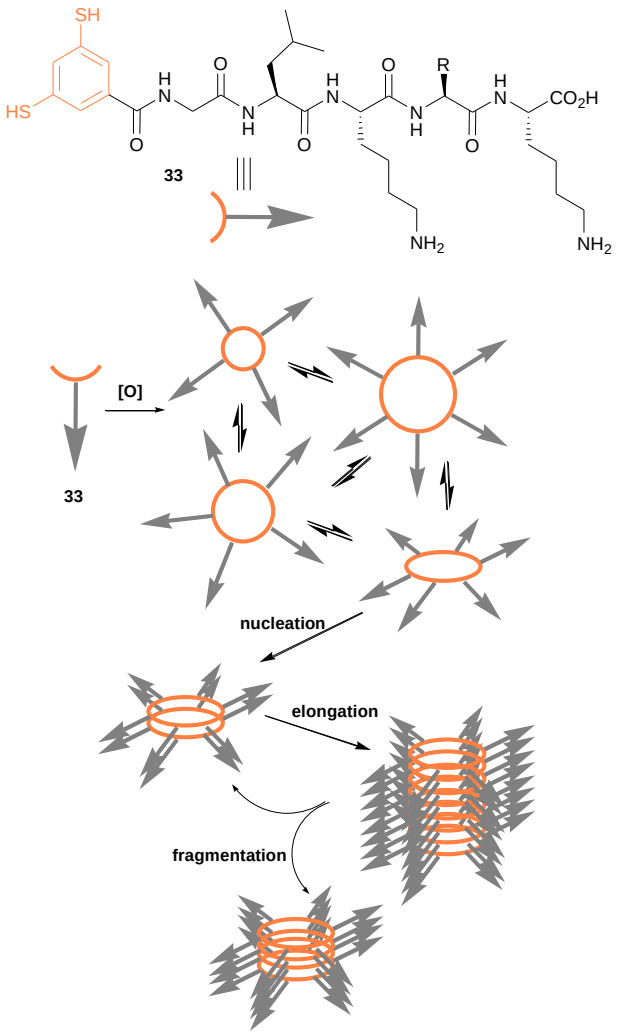
Another interesting example of molecular selection through non-covalent interactions was also recently reported by our group (Scheme 9).[56] In this work a DCL formed by mixing BBs with a different valence number was generated. To that, the bipodal BB **29d** was reacted with the tripodal BB **31**, formed by attaching three Cys amino acids to a central trimesic acid. The library of the corresponding disulphides rendered a complex mixture of homo- and hetero-oligomers. However, when performing the reaction in the presence of Cys as a monopodal thiol, a practically single species was formed from the virtually very diverse possibilities of different topologies.



Scheme 9. Spontaneous formation of a heterotrimer from a topologically diverse DCL of pseudopeptidic disulphides through intramolecular interactions and folding.

The species was isolated at preparative scale and fully characterized to be the corresponding heterotrimer **32** obtained by the closed macrocyclic disulphide between **29d** and **31**, and the additional S-S bond attachment of the cysteine to the third free thiol of **31**. The NMR and molecular modelling data suggested that this species was obtained due to the establishment of several non-covalent interactions in a cooperative fashion (see Scheme 9). Thus, the pending zwitterionic Cys arm would fold into the macrocyclic cavity, setting amide-carboxylate H-bonds and ammonium-carboxylates salt-bridges, as depicted in Scheme 9. The presence of the folded structure in solution was confirmed by key ROESY experiments on the isolated heterotrimer. The formation of **32** occurred through error-correction processes and required the zwitterionic form of the Cys to set the polar and electrostatic interactions. Control experiments at different environmental conditions and with other BBs strongly supported this interactional model.[57]

A final example of a dynamic molecular network within the pseudopeptidic field has been the discovery of self-replicating species. Self-replication is a very important property of living organisms that is also present at the molecular level. When a given molecular entity promotes its own formation, this species can be classified as a self-replicator. Otto and co-workers[58] described simple BBs (**33**) for displaying self-assembling properties in a DCL. They were prepared by connecting a short amphiphilic peptide sequence (Scheme 10) designed to form a β-sheet secondary structure by intermolecular non-covalent H-bonding and hydrophobic interactions. The 3,5-dimercaptobenzoic acid attached at the *N*-terminus was added for establishing dynamic disulphide bonds leading to oligomeric macrocycles (Scheme 10). Accordingly, when the DCL was generated, the system formed macrocycles of different sizes in dynamic equilibrium in aqueous buffer. These species formed self-assembled aggregates by stacking the macrocyclic rings assisted by the β-sheet secondary structure between the peptidic arms (see Scheme 10). These aggregates elongated toward nanometer-sized fibrils, where the fibrils ends act as templates for the formation of additional macrocycles (self-replication). The authors discovered that DCLs seeded with a given macrocycle size (hexamer or heptamer) experienced an exponential growth of that species from the DCL of interconverting macrocycles. Moreover, they observed that the type of mechanical agitation also affected the outcome of the system. Thus, stirring the reaction mainly produced heptamers while shaking promoted hexamers, with a convincing explanation for this difference. More in-depth mechanistic studies showed that the mechanical energy promotes the liberation of the replicator from an inactive self-assembled state, thus overcoming the common self-inhibition observed in self-assembled replicators.[59] In further studies, the same group described the behaviour of structurally different replicators depending on the peptide sequence (R in Scheme 9)[60] or the effect of the presence of fluorinated alcohols (trifluoroethanol) as organic co-solvents.[61]



Scheme 10. Replication process from a DCL of pseudopeptidic macrocycles

Conclusions and outlook

The pseudopeptides are attractive molecules with a high potential in many fields of chemistry, especially in molecular recognition and chemical biology. Their relatively easy synthesis and large structural and functional diversity have made them very appealing for preparing tailor made structures and for biomimetic processes. However, the intrinsic complexity of the biological systems is still far away from the current research in the field of pseudopeptides. In the recent years, several research groups including ourselves have tried to increase the complexity of the pseudopeptides following conceptually different approaches. The obvious increment of the structural elaboration of the pseudopeptides has shown to render more efficient systems in molecular recognition. An alternative to the structural elaboration is the preparation of dynamic chemical networks able to adapt and respond to external stimuli. The complexity in these dynamic networks arises from the molecular diversity of the different species, as well as from the adaptive properties of the systems. This last approach has found fundamental applications in the discovery and preparation of very efficient receptors or even unexpected species, like the interlocked catenanes. These results open the possibility of using dynamic pseudopeptidic networks for the discovery of new drugs[62] or materials.[63] Therefore, the increase of complexity can be used as a benefit in the pseudopeptidic research. At the conceptual extreme of complex systems, one can imagine the natural molecular networks as the final goal. To reach that level of complexity and sophistication, an additional parameter must be considered. Most of the examples here described worked under thermodynamic control and thus, there is a limit for the systems to evolve, marked by the thermodynamics. However, life is a far from the equilibrium process, where kinetic stability allows living beings to exist. Recently, some researchers have claimed that the preparation of dynamic chemical networks operating away from the thermodynamic equilibrium would be a very good benchmark scenario to create functional chemical networks, to mimic biological processes or even to make important advances in the understanding of the origin of life.[64] Within this field, the role of pseudopeptidic or peptide-like molecules seems to be capital and research on that direction is clearly envisioned.[65] Surely we will experience the appearance of new and exciting avenues in those directions, where the complexity of the pseudopeptidic molecules might be considered as an important parameter.

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