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Evolution of a Constitutional Dynamic Library Driven by Self-Organisation of a Helically Folded Molecular Strand

Luciana Lisa Lao, Jean-Louis Schmitt, and Jean-Marie Lehn*[a]

Abstract: Conversion of macrocyclic imine entities into helical strands was achieved through three- and four-component exchange reactions within constitutionally dynamic libraries. The generation of sequences of the intrinsic helicity codon, based on the hydrazone–pyrimidine fragment obtained by condensation of pyrimidine dialdehyde **A** with pyrimidine bis-hydrazine **B**, shifted the equilibrium between all the possible macrocycles and strands towards the full expression (>98%) of helical product [**A**/**B**]. Furthermore, it was shown that chain folding accelerated the dynamic exchange reactions among the library members. Lastly, in four-component experiments (involving **A**, **B**, **E** and either **C** or **D**), even though the macrocyclic entities ([**A**/**C**], [**B**/**E**]; [**A**/**D**], [**B**/**E**]) were the kinetically preferred products, over time dialdehyde **A** relinquished its initial diamine partners **C** or **D** to opt for bis-hydrazine **B**, which allowed the preferential formation of the helically folded strand. The present results indicate that self-organisation pressure was able to drive the dynamic system towards the selective generation of the strand undergoing helical folding.

Keywords: constitutionally dynamic chemistry · helical structures · imines · macrocycles · self-assembly

Introduction

Constitutional dynamic chemistry (CDC)[1] aims at studying chemical systems composed of entities that are able to undergo continuous variations in their constitution both on the molecular as well as supramolecular levels through dissociation into, exchange and recombination of their components through reversible breaking and formation of covalent or non-covalent connections, thus generating a whole set of interconverting entities. Such constitutional dynamic libraries (CDLs) are able to respond to physical stimuli or chemical effectors by constitutional variations. On the molecular level, a particularly rich set of reversible covalent connections is the family of C=N double bonds, which result from the condensation of an amine with a carbonyl group and include imine, hydrazone and oxime functions.[2]

On the other hand, the characteristic geometrical features of helices have been the subject of research investigations in both biology and chemistry. Numerous reviews and in-depth analyses on the design and control of the folding of molecular strands have been well documented.[3] Diverse building blocks as well as synthetic avenues for the formation of helical polymers or foldamers have been reported.[4] Of particular interest is a special class of dynamic foldamers, that is, helical molecular strands that posses reversible covalent connections.[5–8] In the context of dynamic systems, it has been shown that 1) helical folding can serve as the driving force of imine metathesis polymerisation, which elongates the starting oligomers,[5] 2) dynamic foldamers can respond to external effectors such as ligand templation by amplifying the most suitable foldamer,[6a] and participate in cooperative self-assembly of supramolecular coordination polymers[6b] and 3) reversible formation of helical foldamers can be controlled by external chemical effectors such as Lewis acids and metal ions.[7] Such studies have shown the response and adaptability of dynamic foldamers towards external factors. The present work is devoted to the investigation of the intrinsic ability of a dynamic system capable of generating a helical molecular strand to select those components in a dynamic library that favour the formation of the helical product. Such a process represents a component selection driven by helical folding propensity.

In our earlier work, we have demonstrated that it is possible to generate well-defined molecular architectures, such as helically folded strands, by self-assembly from suitable heterocyclic components connected through reversible hydra-

[a] Dr. L. L. Lao, Dr. J.-L. Schmitt, Prof. Dr. J.-M. Lehn
ISIS, Université de Strasbourg
8 allée Gaspard Monge
67083 Strasbourg (France)
Fax: (+33) 3-6885-5140
E-mail: lehn@isis.u-strasbg.fr

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zone functions.[7,8] In analogy to 2,2′-bipyridine, the hydrazone–pyrimidine (hyz-pym) unit shows marked conformational preference for a *transoid* orientation around the connecting bond. Consequently, a sequence of such units undergoes helical self-wrapping and enforces helicity of the molecular strands.[8a] It forms the basic helicity codon and three such units constitute one helical turn. Molecular strands built from hyz-pym sequences may be generated by polycondensation of difunctional monomers, such as **A** and **B**, yielding helically folded polymers [**A**/**B**], as shown in Scheme 1.[8c]

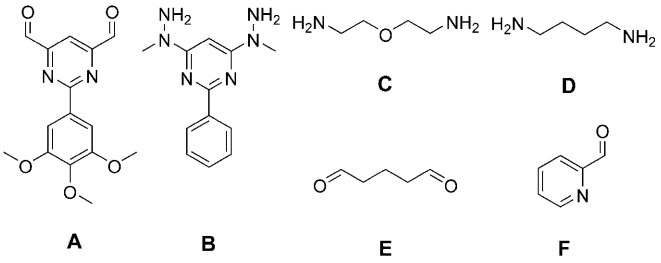
The non-covalent interactions of π–π stacking of the aromatic rings stabilise the helical conformation of polymer [**A**/**B**]. Similar aromatic stacking, coupled with solvophobic and hydrogen-bonding interactions, operates in the formation of other foldamers described in the literature.[3b]

The CDC concept presents a unique avenue for generating dynamic molecular systems and adaptive materials that are able to respond to external stimuli/driving forces. In our laboratory, several examples developed include temperature,[9] pH,[9] gelation,[10] metal ions[7,11] and electric field[12] responsive materials.

Herein, we aim to investigate if helical folding through the formation of a helicity codon can act as a driving force for component exchange and selection between different imine entities and hence favour the formation and amplification of a helical polymer [**A**/**B**] in a library of dynamic constituents.

Results and Discussion

Dynamic libraries of imine and hydrazone compounds formed from components A–E: Sets of dynamic compounds were generated through reversible condensation reactions between the dialdehydes **A** and **E** with bis-hydrazine and diamines **B**, **C** and **D** in chloroform at 25 °C for 24 h. Table 1 summarises the products obtained. The composition of the CDLs formed was analysed by high-resolution mass spectrometry (see the Supporting Information). Only macrocyclic structures of various sizes were identified in the mixtures formed from the [**A**/**C**], [**A**/**D**] and [**B**/**E**] combinations, but isolation was unsuccessful. Linear polymers were obtained for the [**C**/**E**] and [**D**/**E**] combinations (see Scheme 2).

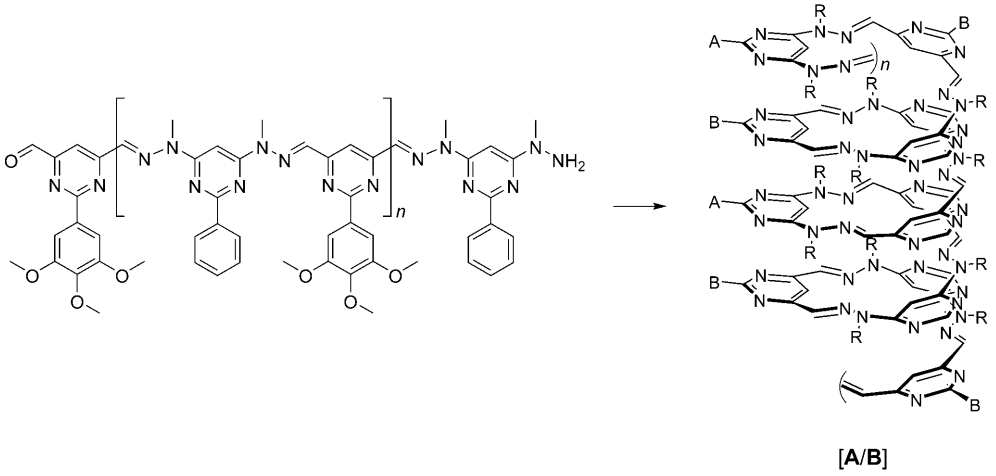


Folding-driven formation of a helical polymer through three-component exchange reactions: Three-component exchange reactions were set up according to the equilibria shown in Scheme 3 to study the evolution of macrocycles [**A**/**C**] and [**A**/**D**] towards the formation of the helical polymer [**A**/**B**].

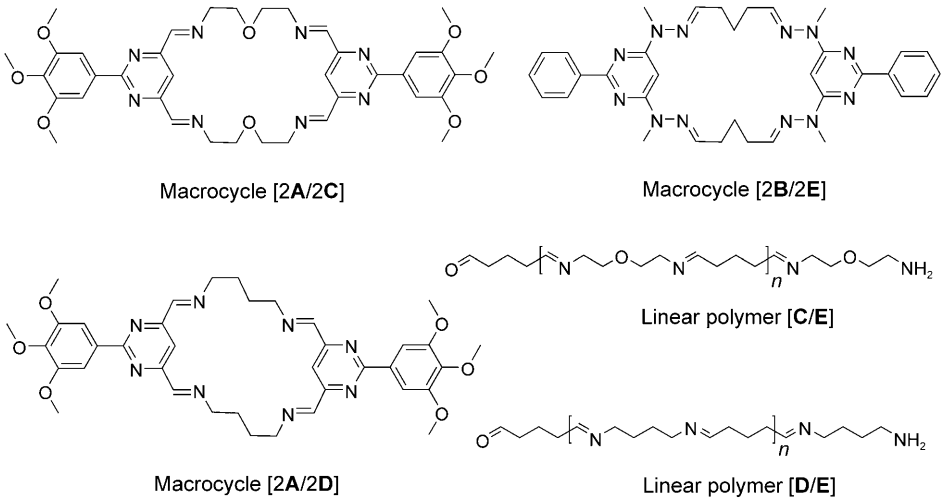
Table 1. Imine and hydrazone products from condensation of carbonyl (**A**,**E**) and amino-containing (**B**,**C**,**D**) components.[a]

	Pyrimidine dialdehyde **A**	Glutaraldehyde **E**
B	helical polymer [**A**/**B**]	macrocycles [**B**/**E**] (2**B**+2**E**, 3**B**+3**E**, 4**B**+4**E** 5**B**+5**E**, and 6**B**+6**E**)
C	macrocycles [**A**/**C**] (2**A**+2**C** and 4**A**+4**C**)	linear polymer [**C**/**E**]
D	macrocycles [**A**/**D**] (2**A**+2**D**, 3**A**+3**D** and 4**A**+4**D**)	linear polymer [**D**/**E**]

[a] Condensation reactions performed in chloroform at 25 °C for 24 h.



Scheme 1. Helical polymer [**A**/**B**] (right) generated by polycondensation of pyrimidine dialdehyde **A** with pyrimidine bis-hydrazine **B**; linear representation (left). Lateral groups (R = Me, A = Ph, B = 3,4,5-$(MeO)_3C_6H_2$) are omitted for clearer helical representation (right).



Scheme 2. Schematic representation of imine and hydrazone products listed in Table 1.

a) [A/C] + B ⇌ [A/B] + C

b) [A/D] + B ⇌ [A/B] + D

Scheme 3. Dynamic component exchange reactions leading to the formation of helical polymer [**A**/**B**] from the sets of macrocycles resulting from the combinations a) [**A**/**C**] and b) [**A**/**D**] (in $CDCl_3$, at 60 °C for 24 h).

When the sets of macrocycles [**A**/**C**] were subjected to an equimolar amount of pyrimidine bis-hydrazine **B** in equilibrating conditions (60 °C, 24 h in $CDCl_3$), the macrocycles [**A**/**C**] were destroyed, diamine **C** was liberated and replaced by the bis-hydrazine **B** in favour of the formation of helical polymer [**A**/**B**]. The ^{1}H NMR spectra in Figure 1 clearly demonstrate this exchange reaction. Initially macrocycles [**A**/**C**] showed sharp, well-defined signals. However, after the exchange reaction occurred, these distinct signals disappeared and were replaced by a set of small, more or less broad signals characteristic of the helical polymer [**A**/**B**]. At the same time, sharp signals for diamine **C** emerged—a sign of release (>98%, Figure 1) of free diamine **C** into the solution mixture (for assignment of ^{1}H NMR spectra, see Figure S7 in the Supporting Information).

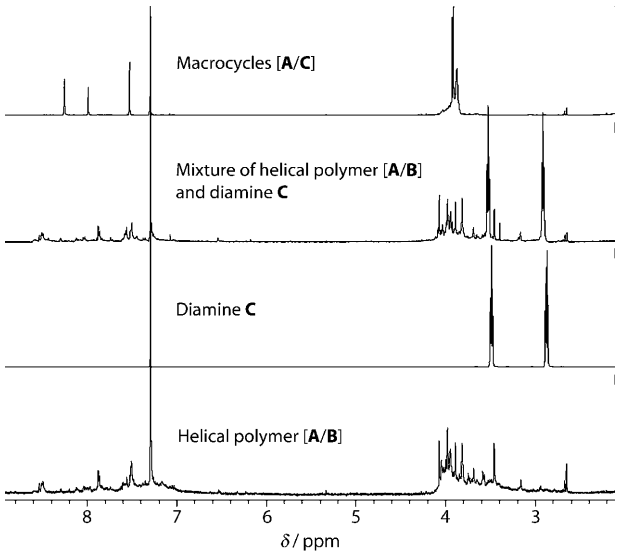


Figure 1. Dynamic exchange reaction between macrocycles [**A**/**C**] and bis-hydrazine **B** according to Scheme 3a, monitored by ^{1}H NMR spectroscopy (24 h, 60 °C, $CDCl_3$). The amounts of macrocycles (top) and of released diamine (second from top) were obtained by integration with respect to the signal of internal reference TMS.

The formation of the helical polymer [**A**/**B**] was also confirmed by ESI-TOF mass spectrometry, as shown by Figure 2. No macrocycles [**A**/**C**] were detected, while helical strands [**A**/**B**] of up to seven repeating units were formed after 24 h of exchange reaction in chloroform at 60 °C.

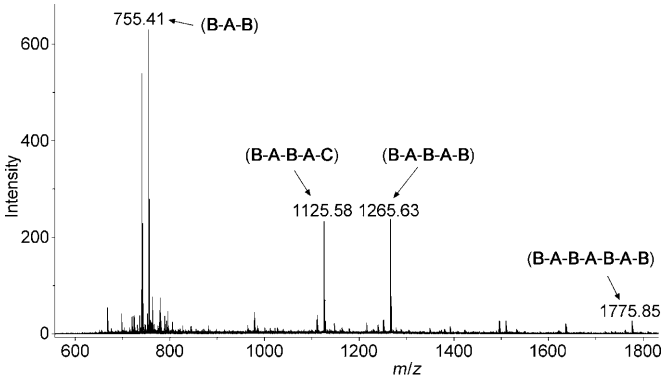


Figure 2. Formation of helical polymer [**A**/**B**] from macrocycles [**A**/**C**] according to Scheme 3a, detected by mass spectrometry ESI-TOF analysis of the mixture obtained (24 h, 60 °C, $CDCl_3$).

The reaction mixture was also tested by LC–MS. However, analysis of the results was difficult because polymer [**A**/**B**] was destroyed by the acidic aqueous environment of the LC–MS apparatus. As a result, only smaller fragments of the polymers could be detected (Figure S4 in the Supporting Information). Therefore, ESI-TOF mass spectrometry was chosen for subsequent analyses of the exchange reactions mixtures.

When macrocycles [**A**/**D**] were similarly subjected to an equimolar amount of pyrimidine bis-hydrazine **B**, the helical polymer [**A**/**B**] was formed at the expense of diamine **D**, which was eventually released into the solution. The reaction was again monitored by ^{1}H NMR spectroscopy and mass spectrometry (see the Supporting Information).

Comparison of the equilibrations of foldable and non-foldable molecular strands: All the observations in the previous section indicate that the library constituents underwent an exchange of their components, which resulted in the final generation of the helical polymer [**A**/**B**], via the hyz-pym fragment formed by condensation of the **A** and **B** components. To further investigate if folding-driven selection of the "correct" components that allow the formation of a helical strand is indeed a driving force of this process, two parallel, equivalent studies utilising foldable and non-foldable compounds were conducted.

Pyridine-2-aldehyde **F**, the structure of which is similar to pyrimidine dialdehyde **A**, but cannot polymerise into a folded strand, was selected to represent **A** in the competitive condensation reactions outlined in Table 2.

Table 2. Relative amount of hydrazone and imine entities at equilibrium for the competitive condensation formation of hydrazone [**F**/**B**] against imine [**F**/**C**] and imine [**F**/**D**] in $CDCl_3$.[a]

a) 2**F** + **B** + **C** ⇌ *x*[**F**/**B**] + *y*[**F**/**C**]

b) 2**F** + **B** + **D** ⇌ *x*[**F**/**B**] + *y*[**F**/**D**]

Reaction	Hydrazone, *x* [%] (±1.5%)	Imine, *y* [%] (±1.5%)
a	80 [**F**/**B**]	20 [**F**/**C**]
b	70 [**F**/**B**]	30 [**F**/**D**]

[a] Mixture of **F**/**B**/**C** in $CDCl_3$ reached equilibrium after 288 h at 25 °C. Mixture of **F**/**B**/**D** in $CDCl_3$ reached equilibrium after 240 h at 25 °C followed by 68 h at 60 °C. At extended times, the amounts of hydrazone and imine did not change.

Following this reaction, aldehyde **F**, bis-hydrazine **B** and diamine **C** or **D** in a 2/1/1 ratio were dissolved in $CDCl_3$ and the mixture was equilibrated. The ratio of the products, *x* and *y* ($x+y=100\,\%$), was determined by comparing the area of the NMR signals belonging to each hydrazone and imine. Each reaction was followed until equilibrium was reached and no change in product distribution was observed afterwards. The results at equilibrium are listed in Table 2.

Since reaction b (Table 2) proceeded very slowly at 25 °C, the mixture was heated to 60 °C to accelerate the equilibration. To confirm that the equilibrium had been reached, it was also approached by mixing and equilibrating solutions in which hydrazone [**F**/**B**] and imine [**F**/**C**] or [**F**/**D**] had been pre-formed. The same product distributions were obtained at equilibrium: hydrazone [**F**/**B**] reached 80 and 70%, respectively. These results point to a moderate bias in favour of hydrazone over imine formation.

Next, another three-component equilibration study involving components that can lead to a folded strand was set up according to the equilibria shown in Table 3. It is important to note that these reactions were designed to be equivalent, differing only in the absence and presence of chain self-folding in the former and latter, respectively.

Dynamic libraries were generated by mixing equimolar amounts of **A**, **B** and **C** or **D**. The changes in the relative amounts of each constituent, *x* and *y* ($x+y=100\,\%$), were monitored over time and the results are given in Table 3.

Table 3. Kinetic evolution of three-component dynamic library towards the amplification of helical polymer [**A**/**B**] constituents.[a]

a) **A** + **B** + **C** ⇌ *x*[**A**/**B**] + *y*[**A**/**C**]

b) **A** + **B** + **D** ⇌ *x*[**A**/**B**] + *y*[**A**/**D**]

Reaction	*t* [h]	[**A**/**B**], *x* [%] (±1.5%)	[**A**/**C**], *y* [%] (±1.5%)	[**A**/**D**], *y* [%] (±1.5%)
a	1	33	67	
	19	50	50	
	237	>98	<2	
b	1	23		77
	4	50		50
	240	>98		<2

[a] Equilibration performed in $CDCl_3$, at 25 °C for up to 250 h. At equilibrium, helical polymer [**A**/**B**] was formed quantitatively (>98%) and the free diamines **C** and **D** were obtained. Due to the complex NMR spectrum of helical polymer [**A**/**B**], its amount was determined by comparing the sharp, unique peaks of unreacted **B** (H5 proton, see Figure S8 in the Supporting Information) and unreacted **C** (CH_2O protons, see Figure 1) or **D** (CH_2N protons, see Figure S5 in the Supporting Information). Over time, the peak of unreacted **B** decreased while that of unreacted **C** or **D** increased.

The data in Table 3 show that the major kinetic products obtained at early times were the sets of macrocycles [**A**/**C**] and [**A**/**D**], respectively. Over time, these macrocycles evolved towards formation of the helical polymer [**A**/**B**], incorporating bis-hydrazine **B** and liberating diamine **C** or **D** in the process. At equilibrium, helical polymer [**A**/**B**] reached full conversion (>98%) and no macrocycles [**A**/**C**] or [**A**/**D**] were present.

In Figure 3 (top), open triangles and squares represent the evolution of library constituents of a dynamic system possessing no folding capability as in reaction a described in Table 2, whereas filled triangles and squares represent reaction a described in Table 3 in which self-folding capability is present through the (**A**+**B**) combination. In a similar fashion, Figure 3 (bottom) compares reaction b in Tables 2 and 3. Thus, Figure 3 displays parallel comparisons of the evolution of dynamic libraries in the presence and absence of hyz-pym helicity codon formation from condensations of **A** and **B**.

The intrinsic folding capability of the [**A**/**B**] molecular strand[8] has at least two profound effects: 1) Thermodynamically, it shifted the equilibrium towards the full expression (>98%, corresponding to >2.3 kcal mol^{-1} free energy difference) of helical polymer constituent [**A**/**B**]. As described in Table 2, the amount of non-helical hydrazone [**F**/**B**] at equilibrium is 80 or 70% (corresponding to 0.8 or 0.5 kcal mol^{-1} free energy difference) when the competing diamine present in the library is **C** or **D**, respectively, indicating a moderate bias in favour of hydrazone over imine formation. However, upon the replacement of **F** by **A**, driven by more than three-fold increase in stability, all non-helical entities of macrocycles [**A**/**C**] and [**A**/**D**] were destroyed and fully replaced by the self-folding helical polymer [**A**/**B**], as shown in Table 3 and Figure 3. 2) Kinetically, it accelerated the dynamic exchange reactions among the library members. In Figure 3 (top), helical polymer [**A**/**B**]

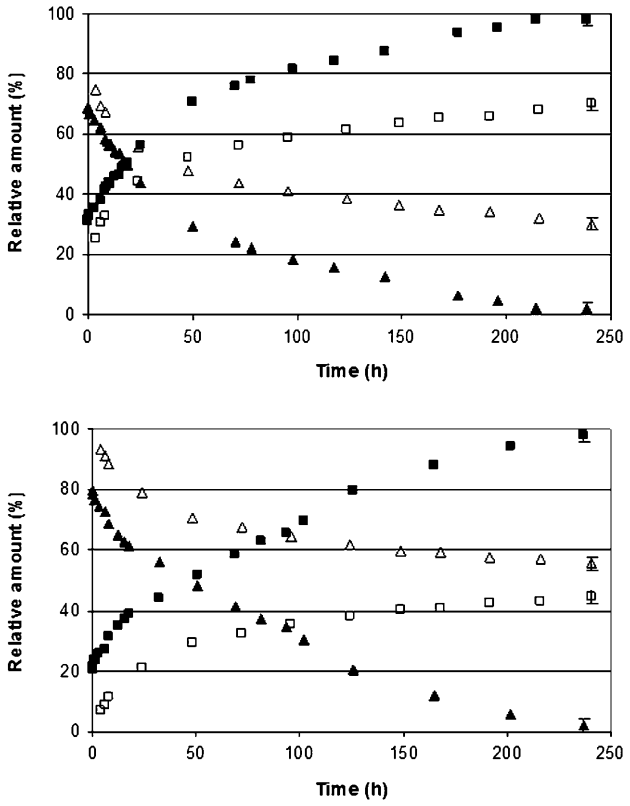


Figure 3. Comparison of dynamic library evolution in the presence and absence of hyz-pym helicity codon formation in **A**/**B**/**C** versus **F**/**B**/**C** systems (top) and **A**/**B**/**D** versus **F**/**B**/**D** systems (bottom) at 25 °C in $CDCl_3$. The release of free **C** follows the same curve as that of formation of [**A**/**B**] (top) and similarly the release of free **D** follows the same curve as that of the formation of [**A**/**B**] (bottom). ▲: [**A**/**C**], ■: [**A**/**B**], △: [**F**/**C**] and □: [**F**/**B**].

reached 50 % in 20 h, while its non-helical counterpart [**F**/**B**] required 50 h to reach the same level. A more pronounced acceleration is demonstrated in Figure 3 (bottom) through the comparison of **F**/**B**/**D** and **A**/**B**/**D** dynamic library systems. It is important to note that **F**+**D** imine connection is slightly favoured over the **F**+**C** one and thus the transformation of the [**F**/**D**] towards the [**F**/**B**] condensation product proceeded at a slower pace. From Figure 3 (bottom), it is shown that to reach 50 % [**F**/**B**] and 50 % [**F**/**D**], more than 250 h were required. In comparison, on replacement of **F** by **A**, which makes the generation of the hyz-pym helicity codon possible, 50 % helical polymer [**A**/**B**] was readily obtained in 50 h.

Folding-driven formation of helical polymer through four-component exchange reaction: In the next phase, we investigated the behaviour of mixtures of macrocyclic compounds (see Table 1) not containing the helicity codon and therefore not expected to be folded. The component exchange and the resulting products were analysed according to the equilibria shown in Scheme 4.

When macrocycles [**A**/**C**] and [**B**/**E**] were mixed and heated to 60 °C for 72 h in the presence of a catalytic

a) [**A**/**C**] + [**B**/**E**] ⇌ [**A**/**B**] + [**C**/**E**]

b) [**A**/**D**] + [**B**/**E**] ⇌ [**A**/**B**] + [**D**/**E**]

Scheme 4. Dynamic exchange reaction between mixtures of non-folded entities (see Table 1) leading to the formation of the helical polymer [**A**/**B**] (in $CDCl_3$, at 60 °C for 72 h).

amount of bis-hydrazine **B** (10 % of the total imine bonds present), the two macrocycles exchanged their components, generating two new polymers, namely, the helical polymer [**A**/**B**] and the linear polymers [**C**/**E**] or [**D**/**E**], not expected to adopt a helical form. The exchange reactions were monitored by ^{1}H NMR spectroscopy through the disappearance of the characteristic signals of the existing macrocycles and appearance of the signals of the new polymers. Figure 4 illustrates the exchange reaction of Scheme 4a.

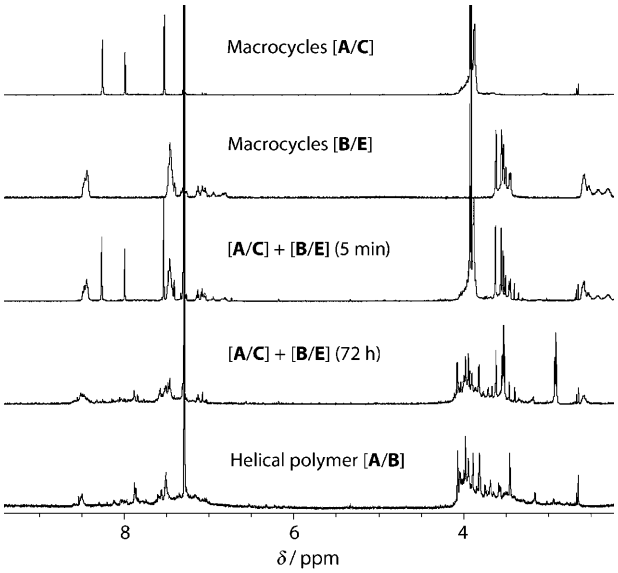


Figure 4. Dynamic exchange reaction according to Scheme 4a, recorded by ^{1}H NMR spectroscopy after 5 min and 72 h equilibration in $CDCl_3$ at 60 °C; bottom trace: helical polymer formed from **A**+**B** (see text for description).

At early times (5 min), the NMR spectrum showed the combined signals of macrocycles [**A**/**C**] and [**B**/**E**]. Thereafter, component exchange occurred and the initially sharp, characteristic signals of macrocycles [**A**/**C**] and [**B**/**E**] disappeared, indicating that these entities were broken down. At the same time, the release of diamine **C** and dialdehyde **E** and their reversible recombination into polymer [**C**/**E**] were observed along with the formation of helical polymer [**A**/**B**], shown by the appearance of many small, broad polymer peaks. The total amount of diamine **C**, present in polymer [**C**/**E**] and free in solution, was quantified with respect to an internal standard (TMS) and was found to be >98 %. The same attempt to quantify dialdehyde **E** was unsuccessful as its peak overlaps with that of helical polymer [**A**/**B**] (see also Figure S9 in the Supporting Information). At the end

(about 72 h), the solution mixture contained only the helical polymer [**A**/**B**] and linear polymer [**C**/**E**].

This result was further confirmed by mass spectrometry analysis, as shown in Figure 5. Both macrocycles [**A**/**C**] and [**B**/**E**] were no longer present. Instead, helical polymers [**A**/**B**] of different numbers of repeating units as well as a linear chain [**C**/**E**] of up to 18 repeating units were detected.

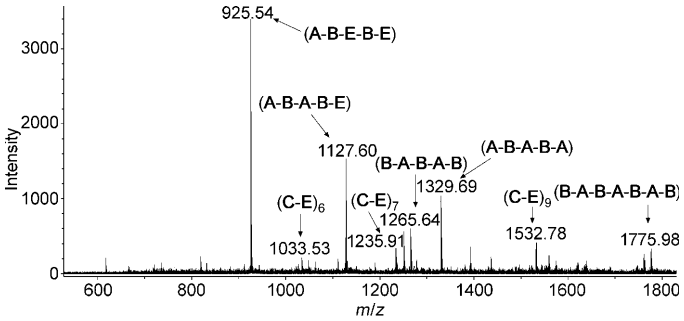


Figure 5. Formation of helical polymer [**A**/**B**] and linear polymer [**C**/**E**] from macrocycles [**A**/**C**] and [**B**/**E**] according to Scheme 4a, detected by mass spectrometry ESI-TOF analysis of the mixture obtained after equilibration (72 h, 60 °C, $CDCl_3$). In a related study, NMR analysis shows that [**B**/**E**] combination decreased over time and was eventually replaced by [**A**/**B**] (see Figure S9 in the Supporting Information).

The same study performed following the equilibrium shown in Scheme 4b gave similar results. Macrocycles [**A**/**D**] and [**B**/**E**] exchanged their components to yield helical polymer [**A**/**B**] and linear polymer [**D**/**E**]. In both scenarios, it is important to note that the driving force for the helical polymer formation is strong enough to achieve 1) breaking of macrocycles [**A**/**C**] or [**A**/**D**] and subsequent exchange of diamine **C** or **D** for the bis-hydrazine **B** and 2) breaking of macrocycles [**B**/**E**] to supply bis-hydrazine **B** to the system and hence allow for the formation of helical polymer [**A**/**B**].

Next, four-component kinetic equilibration was performed to find out if the system would directly select the suitable pair of dialdehyde and bis-hydrazine/diamine that gives the most stable constituent or if some intermediate constituents were formed prior to the final products. This experiment was carried out as outlined in Table 4.

Table 4. Kinetic evolution of four-component dynamic library towards the amplification of helical polymer [**A**/**B**] constituent and its agonists, linear polymers [**C**/**E**] and [**D**/**E**].[a]

a) **A** + **B** + **C** + **E** ⇌ [**A**/**B**] + [**A**/**C**] + [**B**/**E**] + [**C**/**E**]

b) **A** + **B** + **D** + **E** ⇌ [**A**/**B**] + [**A**/**D**] + [**B**/**E**] + [**D**/**E**]

Reaction	T [°C]	t [h]	[**A**/**C**]	[**A**/**D**]	[**B**/**E**]	[**A**/**B**]	[**C**/**E**]	[**D**/**E**]
a	25	3	high		high	low	low	
	25	70	high		high	low	low	
	60	64	low		low	high	high	
b	25	3		high	high	low		low
	25	77		high	high	low		low
	60	74		low	low	high		high

[a] Equilibration performed in $CDCl_3$.

The four-component dynamic library formed according to the equilibration reactions shown in Table 4 gave rise to the four possible constituents, the relative amounts of which changed with time. The absence of distinct peaks for both helical and linear polymers made the quantification of the product constituents difficult. Therefore, changes in the relative composition of the products could only be monitored qualitatively by using ^{1}H NMR spectroscopy and identified by mass spectrometry. The results are summarised in Table 4.

At the beginning, when the four components were free to choose their condensation partners, diamines **C** and **D** were quick to combine with dialdehyde **A** to form macrocycles [**A**/**C**] and [**A**/**D**], respectively, while dialdehyde **E** combined with bis-hydrazine **B** to form macrocycles [**B**/**E**] (see Table 1). For both reactions (Table 4), the change in the amounts of the library constituents was slow at 25 °C. However, upon heating to 60 °C for about 70 h, the amounts of the constituents were reversed, displaying a decrease/disappearance of macrocycles [**A**/**C**], [**A**/**D**] and [**B**/**E**] and a prominent increase in the formation of helical polymers [**A**/**B**] and linear polymers [**C**/**E**] and [**D**/**E**].

In summary, the four-component dynamic libraries above would first generate the kinetic products comprising macrocycles [**A**/**C**] or [**A**/**D**] and [**B**/**E**] as the intermediates. After some time, the constitutional dynamic library evolved and rearranged its constituents through component exchange to yield the sets of final products, the helical [**A**/**B**] and the linear [**C**/**E**] or [**D**/**E**] polymers.

The relationship among the library members described by reaction a in Table 4 can be described by the network diagram, shown in Figure 6. A similar diagram can also be constructed for the library members of reaction b in Table 4. The four constituents of the dynamic library occupy the corners of a square graph. Related constituents, sharing one of their components, share an edge and are antagonists, that is, an increase in one leads to a decrease in the other. On the other hand, unrelated constituents, sharing no common components, are located on the diagonals and behave as agonists, that is, they both increase together. In Figure 6, it is an

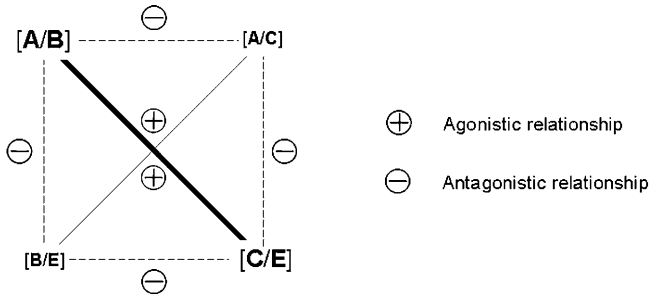


Figure 6. Connectivities between the constituents in the square network of the four-membered dynamic library described in Table 4, representing agonistically (diagonals) and antagonistically (edges) related constituents. The thicker diagonal indicates the preferential expression/upregulation of the agonistic constituents [**A**/**B**] and [**C**/**E**].

internal stimulus, the stability of the helically folded structure, that drives the constitutional dynamic library towards the expression/upregulation of the helical polymer [**A**/**B**] and its agonistic partner [**C**/**E**].

Conclusion

Three- and four-component constitutionally dynamic systems were generated through reversible imine and hydrazone bond formation with the aim of investigating the contribution provided by the helical folding ability of a molecular strand to the selection of its components. Based on the results and the discussions above, the following conclusions can be drawn:

1) The condensation reactions in Table 2 show that in competition with imines [**F**/**C**] and [**F**/**D**], the amount of non-foldable hydrazone [**F**/**B**] ranged from 70 to 80% at equilibrium—thermodynamic distribution in the absence of chain folding.
2) On replacement of **F** by **A**, which allows the generation of the hyz-pym helicity codon, imine macrocycles formed by [**A**+**C**] and [**A**+**D**] converted fully to helical strand [**A**/**B**] (Table 3). In other words, the amount of foldable hydrazone [**A**/**B**] reached completion (>98%) at equilibrium. This finding indicates that the generation of a helically folded strand by helicity codon formation drove the $-NH_2$ (amine or hydrazine) selection and finally shifted the equilibrium towards full expression of helical polymer [**A**/**B**]—thermodynamic shift due to chain folding.
3) The capability to form a helical molecular strand [**A**/**B**] accelerated the dynamic exchange reactions among the library members. Comparing reaction b in Table 3 with Table 2, expressing 50% helical constituent product [**A**/**B**] took approximately 50 h, whereas expressing 50% non-helical product [**F**/**B**] under the same conditions required more than 250 h—exchange rate acceleration due to chain folding.
4) Helical polymer [**A**/**B**] is not the preferred kinetic product. When **A**, **B** and **D** were freely mixed, pyrimidine dialdehyde **A** first reacted preferentially with diamine **D**, forming macrocycles [**A**/**D**] as kinetic products—kinetic selection. Thereafter, the dynamic library evolved towards the expression of helix [**A**/**B**]. A similar situation was encountered with other combinations and also in four-component systems.
5) In more general terms, it is the intrinsic property of the system, the formation of a helically folded strand, that provides a driving force for the adaptation/evolution of the CDL network, leading to the selection and amplification/upregulation of the most stable library member of a constitutionally dynamic system.
6) As an effect of an internal factor, point 5) complements the numerous cases in which a constitutional dynamic system is driven by external factors, either physical stimuli (such as temperature[9]) or chemical effectors.[1,2,9,11]
7) Most significantly, the present results may in principle be extendable to biopolymers (biodynamers), whereby for instance the formation of a particular folded strand would induce the selection of the appropriate components. Such effects may be considered as potentially operative in prebiotic evolution, driving the system by either internal (present case) or external[10a] self-organisation.

Experimental Section

General: All reagents and solvents were purchased at the highest commercial quality and used without further purification unless stated otherwise. Pyrimidine dialdehyde **A** and pyrimidine bis-hydrazine **B** were synthesised according to a literature procedure.[8b] Diamine (N_2O) **C** was obtained from TCI Europe, whereas diamine (N_2C_4) **D**, glutaraldehyde **E** (50% in H_2O) and pyridine-2-aldehyde **F** were obtained from Sigma–Aldrich. Deuterated chloroform used for all reaction schemes was flashed through basic alumina immediately prior to use to remove any trace of acid. ^1H NMR spectra were recorded on a Bruker Avance 400 spectrometer at 400 MHz. The spectra were internally referenced to the residual proton solvent signal. Electrospray (ESI-TOF) analyses were performed by Service de Spetrométrie de Masse de l'Institut de Chimie, Université de Strasbourg, on a Bruker MicroTOF mass spectrometer. LC–MS was performed by coupling a Thermo Finnigan Accela chromatography apparatus and a Thermo Finnigan Surveyor MSQ+ mass spectrometer.

General procedure for imine/hydrazone condensation reactions: Equimolar amounts of dialdehydes (**A** or **E**) and diamines or bis-hydrazine (**B**, **C** or **D**) were dissolved in fresh $CDCl_3$ at a concentration of 10 mM. The solution was stirred overnight at room temperature prior to use in other experiments.

Procedure for three-component exchange reactions: Macrocycles [**A**/**C**] or [**A**/**D**] were prepared according to the above procedure in an NMR tube (0.6 mL, 10 mM). An equimolar amount of pyrimidine bis-hydrazine **B** was added to the mixture and the solution was heated to 60°C for 24 h. The NMR tubes were topped with Teflon caps to keep a constant concentration upon heating. Exchange reactions were monitored by NMR spectroscopy and LC–MS or ESI-TOF.

Procedure for four-component exchange reactions: Macrocycles [**A**/**C**], [**A**/**D**] and [**B**/**E**] were individually prepared. Equimolar amounts of two different macrocycles were mixed and a catalytic amount of pyrimidine bis-hydrazine **B** (equivalent to 10% of the total imine bonds present) was added. An aliquot of the mixture was transferred to an NMR tube (topped with a Teflon cap) and heated to 60°C for 72 h. Exchange reactions were monitored by NMR spectroscopy and LC–MS or ESI-TOF.

Procedure for equilibration experiments and determination of kinetic data: A typical protocol is realised by the preparation of a fresh solution of the desired compounds in $CDCl_3$, allowed to equilibrate in an NMR tube (0.6 mL, 10 mM). The first NMR spectrum was usually recorded 3 min after mixing all of the compounds with spinning the tube at room temperature. Subsequently, NMR spectra were recorded at pre-determined time points. All NMR tubes were topped with Teflon caps to keep a constant concentration throughout measurement.

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