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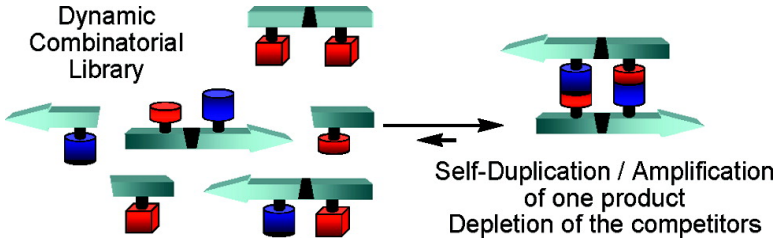

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Self-Duplicating Amplification in a Dynamic Combinatorial Library

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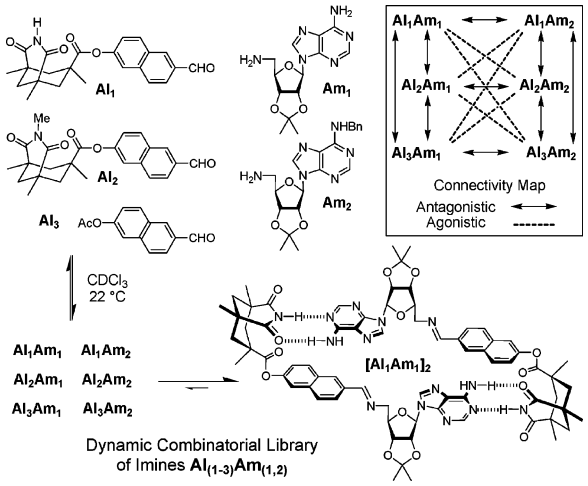
Dynamic combinatorial chemistry (DCC) rests on the design and the study of libraries of species connected by reversible (supra)-molecular bonds.[1,2] At equilibrium, the expression of the products in a dynamic combinatorial library (DCL) is governed by thermodynamics and as a consequence, the additional presence of molecular targets can lead to the in situ screening of the "best-fitted" constituents. Investigations on DCC by several research groups have highlighted the wide potentialities of the main concept in various fields of chemical research such as drug discovery,[3] self-assembly of inorganic architectures, receptor generation, substrate binding, and catalyst screening.[2c,4] DCC has also been shown to respond to external chemical or physical stimuli such as protons, phase transition, temperature, or electric field modulations, thus opening interesting potentialities for material science.[5,6] Recently, we have envisaged the possibility to couple DCC with self-replicating systems.[7] Indeed, the overexpression of a compound by making copies of itself from a pool of reshuffling constituents in a series of competing equilibria would be of significance for biologists and chemists who are interested in various minimal "self-amplifying" systems in their quest to bridge the gap with a possible origin of life. Moving in this direction, we describe here a preliminary investigation of a DCL which amplifies a product that constitutes its own target, that is, the one capable of self-duplication.

To design such a system, we envisioned several building blocks capable of (i) reversible covalent associations and (ii) displaying, or not displaying, complementary supramolecular units in order to produce, or not produce, a template with self-recognition properties (Scheme 1). The key molecule was chosen as imine $\mathbf{Al_1Am_1}$, synthesized by the condensation of aldehyde $\mathbf{Al_1}$ (derived from a Kemp's imide) and adenosine amine $\mathbf{Am_1}$. This self-complementary dynamic compound, inspired by a related "non-dynamic" isosteric system described by Julius Rebek,[8,9] is able to strongly associate with itself into complex $\mathbf{[Al_1Am_1]_2}$. Its dimerization in a chloroform solution through hydrogen bonding was confirmed by ^{1}H NMR (see Supporting Information (SI)). It is observed that the N−H imide resonance signal of compound $\mathbf{Al_1}$ is shifted from 7.6 to 8.8 ppm when $\mathbf{Al_1}$ is mixed with $\mathbf{Am_1}$ (c = 15 mM, equimolar ratio), and to 11.2 ppm for the condensed imine $\mathbf{Al_1Am_1}$, which is in agreement with the formation of complex $\mathbf{[Al_1Am_1]_2}$.[8b,10] The presence of homodimer $\mathbf{[Al_1Am_1]_2}$ in solution was also confirmed by exact mass spectrometry (ESI-TOF). While $\mathbf{Al_1}$ contains a free imide bond on the Kemp recognition group, $\mathbf{Al_2}$ is protected by a methyl group on the nitrogen which prevents the formation of hydrogen bonds with the adenine moiety. $\mathbf{Al_3}$ is also an analogue of $\mathbf{Al_1}$ but without a Kemp's recognition group and with an acetyl group instead, in order to display similar activation energy as $\mathbf{Al_1}$ for the condensation reaction with amines. $\mathbf{Am_1}$ and $\mathbf{Am_2}$ also present close structures, but in the latter, the hydrogen bonds with the Kemp's imide are sterically restricted by the protection of the adenine with a benzyl group.

Scheme 1. Representation of the 11 Library Members Obtained by Mixing the Three Aldehydes ($\mathbf{Al_1}$-$\mathbf{Al_3}$) and the Two Amines ($\mathbf{Am_1}$, $\mathbf{Am_2}$) in Deuterated Chloroform[a]



[a] In this combinatorial set, one imine, namely $\mathbf{Al_1Am_1}$, can self-assemble through hydrogen bonds and produce homodimer $\mathbf{[Al_1Am_1]_2}$. In the frame (middle right), the connectivity map describes the antagonistic and the agonistic constitutional relationships between the six imines of the library.[11]

We then turned to the thermodynamic study of the DCL described in Scheme 1. We set up a first experiment (**DCL1**) by mixing $\mathbf{Al_2}$, $\mathbf{Al_3}$, $\mathbf{Am_1}$, and $\mathbf{Am_2}$ (15 mM each at 22 °C in $CDCl_3$), to determine the distribution of the eight library members at equilibrium (Figure 1, black bars). The isoenergetic nature[2a] of **DCL1** is here clearly demonstrated by the statistical distribution of the products (equal expression of the two amines and two aldehydes (3.4 mM each) and equal expression of the four imines $\mathbf{Al_{(2,3)}Am_{(1,2)}}$ (5.8 mM each)). This reflects, as expected, the absence of specific supramolecular interactions between the library members. In a second experiment (**DCL2**), we mixed together $\mathbf{Al_1}$, $\mathbf{Al_2}$, $\mathbf{Al_3}$, $\mathbf{Am_1}$, and $\mathbf{Am_2}$ (15 mM each at 22 °C in $CDCl_3$). In this library, the production of homodimer $\mathbf{[Al_1Am_1]_2}$ leads to a strong bias in the expression of the constituents at equilibrium, far from the statistical distribution (Figure 1, gray bars). In a third experiment (**DCL3**), we divided the process in two steps: (i) set up of a pre-equilibrated eight member library without $\mathbf{Al_1}$ (**DCL1**, Figure 1, black bars), and then (ii) addition of $\mathbf{Al_1}$ (15 mM) to this library. In **DCL3**, it takes five times longer to reach equilibrium (22 days) compared to **DCL2** (t = 109 h), but the competition produces an identical distribution of constituents in both **DCL2** and **DCL3** (Figure 1, gray bars for both conditions). These last two experiments confirmed the good thermodynamic control of the overall system in these conditions, that is, without local pseudominima on the hypersurface of energy/constitution. By comparing the "non-self-duplicating" **DCL1** (black bars) and the "self-duplicating" **DCL3** (gray bars), we can conclude on the three following features. First, the expression of the self-duplicator $\mathbf{Al_1Am_1}$ (9.03 mM) is increased by +83% compared to the theoretical statistical distribution (4.94

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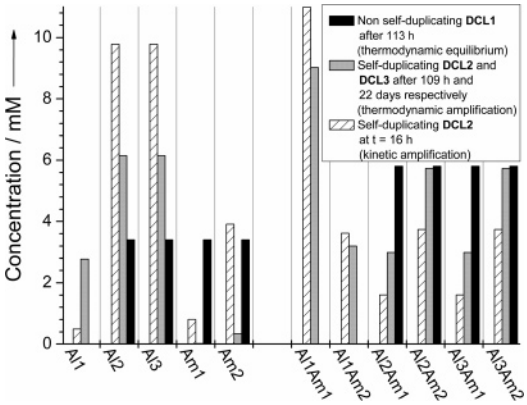

Figure 1. Representation of the products' concentration in various dynamic combinatorial libraries (**DCL1**−**3**), determined by ^{1}H NMR at 22 °C in $CDCl_3$ (c = 15 mM for each of the starting materials.

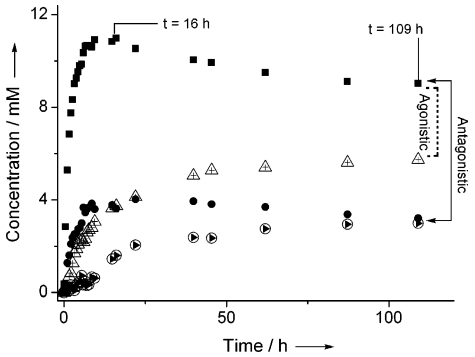

Figure 2. Evolution as a function of time of the concentration of the 6 products $\mathbf{Al_{(1-3)}Am_{(1,2)}}$ in the 11-member dynamic combinatorial library (**DCL2**) described in Scheme 1. Concentrations were determined by ^{1}H NMR and products are represented as follows: (■) $\mathbf{Al_1Am_1}$, (●) $\mathbf{Al_1Am_2}$, (○) $\mathbf{Al_2Am_1}$, (+) $\mathbf{Al_2Am_2}$, (right solid triangle) $\mathbf{Al_3Am_1}$, and (Δ) $\mathbf{Al_3Am_2}$.

mM). Second, this amplification of the self-duplicator is realized by the takeover of the resources of its direct competitors, that is, the imines having antagonistic connectivity[11] with $\mathbf{Al_1Am_1}$ (namely $\mathbf{Al_1Am_2}$, $\mathbf{Al_2Am_1}$, and $\mathbf{Al_3Am_1}$, (3.0 mM each), see middle right frame in Scheme 1). Thus, the amplification of the self-duplicator $\mathbf{Al_1Am_1}$ compared to its direct competitors reaches a value of +200%. And last, the agonistic connectivity[11] between the self-duplicator $\mathbf{Al_1Am_1}$ together with $\mathbf{Al_2Am_2}$ and $\mathbf{Al_3Am_2}$ leads to a small increase of the last two products compared to the statistical distribution (5.7 mM; +14%).

We also studied the kinetic behavior of the 11-member library **DCL2** by plotting the concentration of the constituents as a function of time (Figure 2). This kinetic evolution is biased compared to the "non-self-duplicating" **DCL1** involving $\mathbf{Al_2}$, $\mathbf{Al_3}$, $\mathbf{Am_1}$, and $\mathbf{Am_2}$, in which the 4 imines display very similar initial rates in the competition experiment ($\approx 4.5 \times 10^{-1}$ mM·h^{-1}, see SI). In **DCL2**, the self-duplicator $\mathbf{Al_1Am_1}$ is produced with a V_0 of 59×10^{-1} mM·h^{-1}, which is about 60 times faster than the condensation of $\mathbf{Al_2Am_1}$, and $\mathbf{Al_3Am_1}$ (0.96×10^{-1} mM·h^{-1}); 13 times faster than $\mathbf{Al_2Am_2}$ and $\mathbf{Al_3Am_2}$ (4.6×10^{-1} mM·h^{-1}); and 6 times faster than $\mathbf{Al_1Am_2}$ (9.6×10^{-1} mM·h^{-1}). These differential rates lead to a maximum (+200%) of amplification (kinetic amplification) of the self-duplicator (10.98 mM) compared to its immediate competitor $\mathbf{Al_1Am_2}$—and of +160% compared to the average concentration of the six imines—at t = 16 h (Figure 1, striped bars). We assume that the kinetic amplification is mainly the result of a pre-association complex between $\mathbf{Al_1}$ and $\mathbf{Am_1}$ as indicated by the kinetic studies of the individual reactions (SI).

In conclusion, we have demonstrated that it is possible to self-amplify one product in a DCL, namely the one that can self-complementarily direct its own formation. The expression of the components in the library evolves along both kinetic and thermodynamic biases that both lead to the amplification of the best duplicator. Because of the double reversibility of the system (supramolecular H-bonds and molecular imine condensation), the competition is not only ruled by the differential rates of formation of the components, but also by the possible takeover of the building blocks of the antagonistic competitors,[11] thus leading to the decrease of their absolute concentration. From a "Darwinian" point of view, such a system illustrates the selection of the most efficient self-duplicator by the destruction of the entities which are not (or less, such as $\mathbf{Al_1Am_2}$) able to duplicate themselves. We are currently studying the possibility to couple a dynamic combinatorial library with a "self-replicating loop",[7d,12] thus leading to an auto-catalytic behavior, that is, displaying a sigmoid concentration−time profile for the self-replicated member of the library.

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Supporting Information Available: Characteristic NMR spectra as well as a discussion of kinetics and thermodynamics data. This material is available free of charge via the Internet at http://pubs.acs.org.

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(12) We use the term "self-replication" for auto-catalytic systems displaying a sigmoid concentration−time profile and the term "self-duplication" for a system displaying the general property to thermodynamically or kinetically (or both) favor its own formation (see also SI).

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