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Supramolecular Autoregulation

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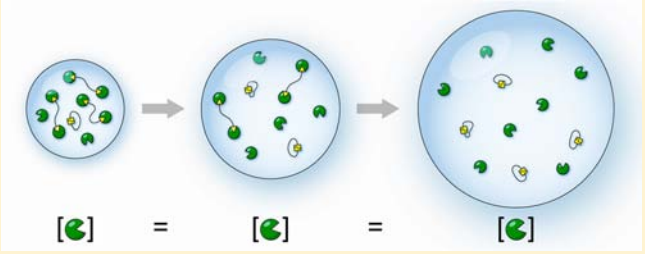
Supporting Information

ABSTRACT: Enzyme activity in biological systems is often governed by control mechanisms in which the catalytic properties are made sensitive or insensitive to differences in enzyme or substrate concentration. Here, we report the first supramolecular system where the catalytic activity is made concentration independent through the use of newly designed inhibitor molecules. The precise concentration dependence of coupled supramolecular equilibriums between free catalyst, inhibited catalyst, active inhibitor, and inactive inhibitor allows to keep the concentration of free catalyst at 1 mM in a broad concentration range, yielding an autoregulated catalytic system.



1. INTRODUCTION

Autoregulation is a fascinating adaptive process found within many different biological systems and is used to adjust or mitigate the response a system has to a stimulus. A large variety of sophisticated regulation mechanism is found in natural systems that permits this internal control upon stimuli. For example, catalytic activities in biochemical pathways are often regulated through complex feedback loops, in which they are, e.g., made insensitive to differences in the enzyme concentration.[1] The importance of understanding these phenomena in biology is gaining full attention in the field of systems biology, and recently some initial approaches have been undertaken to mimic processes in artificial systems.[2−4] One of the chemical processes that potentially can benefit from an autoregulation mechanism is found in the field of organocatalysis.[5−8]

Organocatalysis is an intriguing way to synthesize complex molecules. Such reactions are catalyzed using specific interactions between the catalysts and substrates without using (transition) metals. This type of catalysis often leads to excellent yields with high stereoselectivity and is mimicking in many aspects its natural counterpart. The efficiency and the selectivity of these organocatalysts are in general concentration-dependent; i.e., the turnover numbers, chemical selectivity, and stereoselectivity are dependent on the concentration of catalyst used in the reaction.[9−13] This phenomenon is mainly thought to be related to (i) the catalytic mechanism that involves more than one molecule of catalyst in the catalytic cycle (reaction order with respect the catalyst is higher than 1) and (ii) catalyst aggregation, which is obviously concentration-dependent. To remove the concentration-dependent catalytic properties has been described as a profound challenge for organocatalysis and requires the autoregulation of the active catalyst concentration.[14] Therefore, the introduction of autoregulation in organocatalysis is a challenging next step to provide robustness in the process, and it involves mastering of the complexity in supramolecular systems. We will use organocatalysis here to illustrate the concept of supramolecular autoregulation, also because this concept is often found in controlling enzymatic conversions in nature.

In biological systems, supramolecular interactions are often used to control the enzyme concentration and, thus, the catalytic activity. A simple but highly effective biological strategy for controlling catalytic activity is the use of complexation, yielding supramolecular complexes that are catalytically inactive or less active.[15] These can be formed by individual enzymes (oligomeric complexes) or by the combination of enzymes and specific scaffold proteins (heterogenic complexes). The final complex entity acts as a reservoir for single enzymes and allows their release after dilution, keeping the active enzyme concentration constant and thereby having the catalytic activity autoregulated.

Research in our group over the past 15 years has focused on the design and study of well-defined supramolecular systems and their application in different fields such as materials science,[16] biomedicine,[17] electronics,[18] etc. A well-defined multicomponent system developed and used by us and others is the concentration-dependent dynamic equilibrium as shown in Figure 1.[19] The 2-ureido-4[1*H*]-pyrimidinone (UPy) unit shows self-complementarity in forming highly stable dimers ($K_{dim} = 6 \times 10^7$ M^{-1} in $CDCl_3$). After tautomerization to the 2-ureido-6[1*H*]-pyrimidinone, it becomes complementary to the 2,7-diamido-1,8-naphthyridine (NaPy) unit, yielding a UPy-NaPy complex ($K_a = 5 \times 10^6$ M^{-1} in $CDCl_3$). This equilibrium is regulated by Le Chatelier's principle, and as a consequence, the distribution of species can be controlled as a function of the total concentration. Selectivity toward the formation of UPy-

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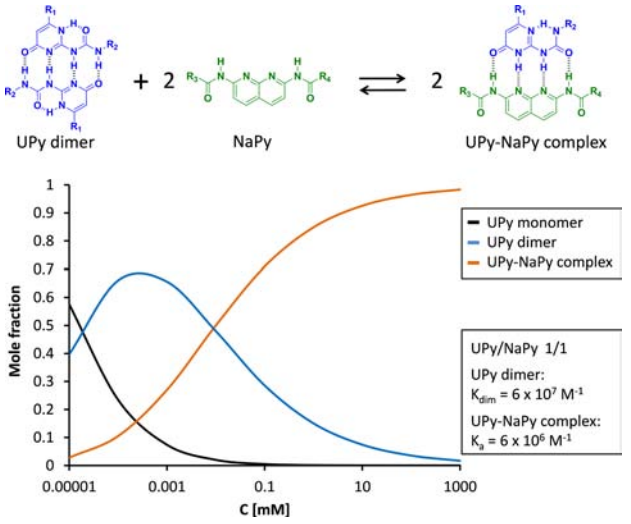

Figure 1. Top: Dynamic supramolecular equilibrium between UPy dimers, NaPy, and UPy-NaPy complex. Bottom: Calculated fractions of UPy in UPy dimers, UPy monomers, and UPy-NaPy complex.[19]

NaPy complexes has been applied, for example, in the selective synthesis of well-defined supramolecular block copolymers.[20]

Here, we introduce such a supramolecular system based on UPy-NaPy motifs capable of keeping the concentration of free NaPy constant upon several dilutions, thereby engineering the first supramolecularly autoregulated system. Remarkably, we found in preliminary studies that the basic character of the 1,8-naphthyridine unit in the NaPy motif makes it an efficient catalyst for different mild base-catalyzed reactions. Thus, the catalytic site of NaPy can be *a priori* supramolecularly deactivated after UPy-NaPy complexation. In this Article, we report the preliminary catalytic results of this autoregulated system in a model Michael reaction, showing the clear benefits of bringing together complex supramolecular systems and organocatalysis.

2. RESULTS AND DISCUSSION

2.1. Design of the Cyclic Supramolecular Scaffolds.

Mathematical models suggest that the use of cyclic scaffolds, formed upon UPy intramolecular dimerization of bifunctional molecules, can increase the concentration dependence of the equilibrium shown in Figure 1. Where normally these bifunctional molecules form supramolecular polymers, some design rules exists to make them preferentially exist in cyclic forms.[21−24] In order to achieve the synthesis of stable cyclic monomers that only polymerize at very high concentrations, we decided to prepare different scaffolds of the optimized length functionalized with the UPy motif at both ends. Earlier molecular studies showed that bifunctional UPy derivates with short spacers (shorter than 14) are not able to dimerize in an intramolecular way due to the antiparallel fashion of the UPy association.[24] One unexplored and easy synthetic route to access scaffolds with long, flexible linkers between the UPy units is by direct reaction of a mono-UPy isocyanate[25] and the corresponding aliphatic diol in the presence of dibutyltin dilaurate. Scaffolds **1a−e** were prepared using this strategy. Compound **2** was prepared as a model system in which the UPy dimerization takes place in an intermolecular way, compared to the scaffolds **1** where dimerization takes place intramolecularly. NaPy derivative **3** was made by a standard route to act as the organocatalyst.

While the bifunctional UPy scaffolds with short spacers (**1a,b**) were insoluble in chloroform—probably due to the formation of supramolecular linear polymers—scaffolds **1c−e** presented a remarkable solubility. Analysis of the ^{1}H NMR spectra confirmed that the tautomeric behavior of UPy units present in the scaffold structures **1c−e** is in agreement with previously reported results, having 4[1*H*]-pyrimidinone as the major tautomer present in $CDCl_3$ and the 6[1*H*]-pyrimidinone as the major tautomer in DMSO-d_6.[26]

Initial evidence for the intramolecular dimerization came from comparing the ^{1}H NMR spectra ($CDCl_3$, 298 K) of **1c−e** with the spectrum of the model compound **2**. In the spectrum of **2** the signal corresponding to the resonance from the urethane NH proton (g in Figure 2) appeared at 4.87 ppm and

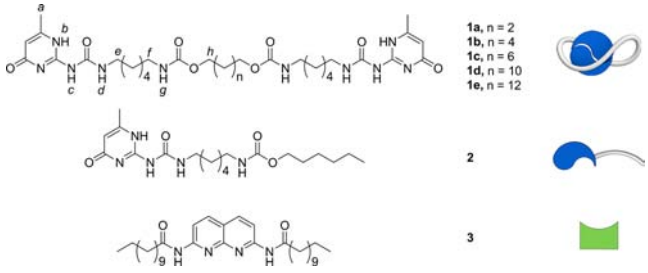

Figure 2. Chemical structures for scaffolds **1a−e**, model compound **2**, and organocatalyst **3**.

was found to be quite insensitive to concentration (Figure S1, Supporting Information). In dilute samples of the bifunctional UPy scaffolds **1** (0.5 mM), the signals for the urethane NH protons (labeled "g") were found to be downfield shifted (**1c**, 5.04 ppm; **1d**, 5.21 ppm; and **1e**, 5.17 ppm) relative to that of model compound **2** (Figure S3). These results indicated that urethane protons g in the compounds **1c−e** are most likely involved in intramolecular H-bonding and the cyclic monomer arrangement presents a different local chemical environment. In addition, NOE studies were carried out in order to evaluate the size of the species of **1c−e** detected in $CDCl_3$ under dilute conditions. Small, positive NOEs were obtained in all three cases, suggesting a low molecular weight for the folded species (Figure S4).[21] At low concentrations (0.5−4 mM), the ^{1}H NMR signals of compounds **1c−e** were concentration independent, suggesting that mainly cyclic monomers are present. At higher concentrations, new signals related to the formation of larger supramolecular complexes were detected in the ^{1}H NMR spectra (Figure 3; also see Figures S5−S7). The main differences between resonances of the monomeric species and the larger aggregates are found in the signals corresponding to the UPy motif (protons c and d in Figure 2), NH of the urethane moiety (proton g), and the alkyl chain (mainly proton e). The fact that nonviscous solutions are obtained in the concentration range from 10^{-2} to 10^{-1} M suggests a low concentration of linear polymers even at these high concentrations.

Using these ^{1}H NMR spectroscopy data (*vide supra*), we were able to determine the maximum concentration of monomeric cycles (MCMC) of the different scaffolds **1c−e**. Figure 3 shows plots of the high concentration of monomers within monomeric cycles as a function of overall concentration for scaffolds **1c−e**. The higher value of MCMC obtained for scaffold **1c** (10 ± 1 mM) with respect to those obtained with scaffolds **1d** (8 ± 1 mM) and **1e** (4 ± 1 mM) suggests that the

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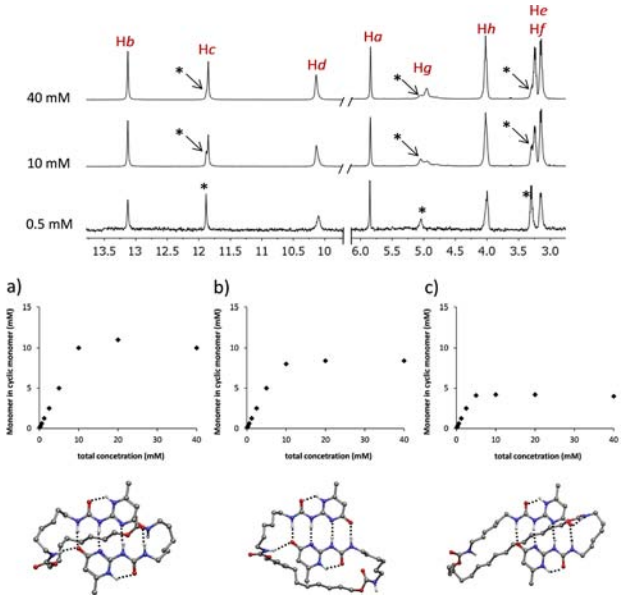

Figure 3. Top: ^{1}H NMR spectra (400 MHz, $CDCl_3$, 298 K) of scaffold **1c** at different concentrations. Asterisks indicate the distinguishable signals originating from the cyclic monomer. Bottom: MCMC values as a function of total concentration in $CDCl_3$ at 298 K for scaffolds **1c**–**e** (panels a–c, respectively) and molecular models proposed in solution. The models correspond to energy minima obtained with molecular mechanics calculations using the AMBER* force field in $CHCl_3$ (MACROMODEL 9.9). Nonpolar hydrogen atoms have been omitted for clarity.

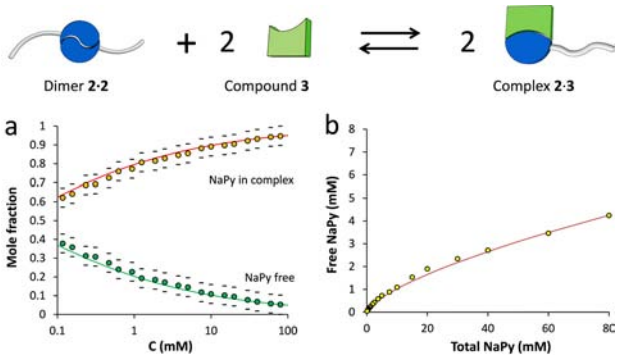

Figure 4. Top: Schematic representation of the equilibrium between the dimeric form of compound **2**, compound **3**, and complex **2·3**. Bottom: (a) Measured fractions determined by ^{1}H NMR spectroscopy in $CDCl_3$ (filled points) and calculated (lines) for free **3** and the complex **2·3** as a function of concentration in an equimolar mixture of **2** and **3**. (b) Measured concentration of free **3** in solution (filled points) and calculated (red line) as a function of total concentration. K_{dim}(UPy) = 6 × 10^7 M^{-1}, and K_a(UPy-NaPy) = 3 × 10^6 M^{-1}.

monomeric cyclic species becomes less stable as the length of the linker increases. These results are in full agreement with those previously reported in similar systems and can be directly related to a decrease in the effective concentration.[27] After the synthesis and assembly studies of the scaffolds **1c**–**e** and the model compound **2**, we studied how the concentration-dependent equilibrium between the free NaPy and UPy-NaPy complex is affected.

2.2. Concentration-Dependent Studies. The position of the equilibrium between UPy dimers, NaPy, and the UPy-NaPy complex and hence the selectivity of the heterocomplexation are regulated by Le Chatelier's principle. Therefore, at high concentrations the formation of UPy-NaPy heterodimer is favored, while upon dilution the formation of UPy dimers becomes more important, with the consequent release of free NaPy molecules.[19] As an example, the observed concentration dependence of equimolar mixtures of the model compound **2** with the NaPy-based molecule **3** in $CDCl_3$ is shown in Figure 4a.

Upon dilution of a concentrated sample containing the **2·3** complex from 80 to 0.12 mM (Figure 4a), the selectivity for formation of the heterodimer complex was found to be decreased from 95% to 62%, respectively. By plotting the concentration of free **3** as a function of the total concentration of **3**, it can be appreciated that dilution (from 80 to 10 mM) results in a decrease in concentration of free **3** (from 4.2 to 1.1 mM).

Therefore, the internal supramolecular mechanism mitigates the effect that dilution has on the concentration of free **3**. The results obtained from the concentration-dependent ^{1}H NMR experiment on **2** and **3** were fitted to a mathematical model assuming the reported[28] K_{dim}(UPy) = 6 × 10^7 M^{-1}. The calculated selectivity for the UPy-NaPy complex (K_a = 3 × 10^6 M^{-1}, Figure 4a and Figure S9) is in good agreement with previously reported results in $CDCl_3$. Simulation of the equilibrium using different dimerization and association constants predicts that a change in distribution of the species at a given concentration (selectivity) is possible; however, no relevant effect on the concentration dependence is observed (Figures S10 and S11). In a second study, changing the molar ratio between the UPy and NaPy units showed an effect on the equilibrium upon dilution; however, total autoregulation of the NaPy concentration was not achieved (Figure S12).

Employing the cyclic monomeric scaffolds **1c**–**e**, it is expected that the competition between intra- and intermolecular interactions will yield a higher concentration dependence associated with Le Chatelier's principle (Figure 5). Indeed, when we performed a ^{1}H NMR dilution experiment in $CDCl_3$ employing scaffolds **1c**–**e**, the concentration dependence was remarkably increased in all cases (Figure 5a; also see Figure S13). For example, it can be seen in Figure 5a that, in the case of scaffold **1d**, the system is composed almost exclusively of the complex **1d·3** at high concentrations (C = 80 mM) and that after dilution to 0.12 mM, all NaPy **3** is released in its free form. Fine adjustment of the effective molarity in the scaffolds **1** by changing the length of the spacer between the two UPy units allows for complete autoregulation of free catalyst **3**. Indeed, in the presence of scaffold **1c** or **1d**, the concentration of free **3** is buffered at 1.0 ± 0.2 mM in a broad concentration range of 4–80 mM of total **3** (Figure 5b). Interestingly, scaffold **1e** is not able to promote the total autoregulation of free **3**. This different behavior is most likely related to the lower stability of its monomeric cycle observed in the conformational studies (Figure 3). The high concentration dependence observed for scaffolds **1c**–**e** can be simulated in good agreement by employing a simplified mathematic model (Figures S14 and S15) and assuming that only the cyclic monomer of the scaffolds is formed (Figure 5c). It is remarkable that the autoregulation of free **3** found experimentally with **1c**,**d** was even better than predicted by the model (Figure 5d).

2.3. Organocatalysis. After the design of the scaffold and the catalyst, we studied the effect that the presence of **1d** has on the catalytic properties of **3** in order to test the internal mechanism of autoregulation. The basic character of the NaPy unit in **3** makes it an efficient organocatalyst for the well-

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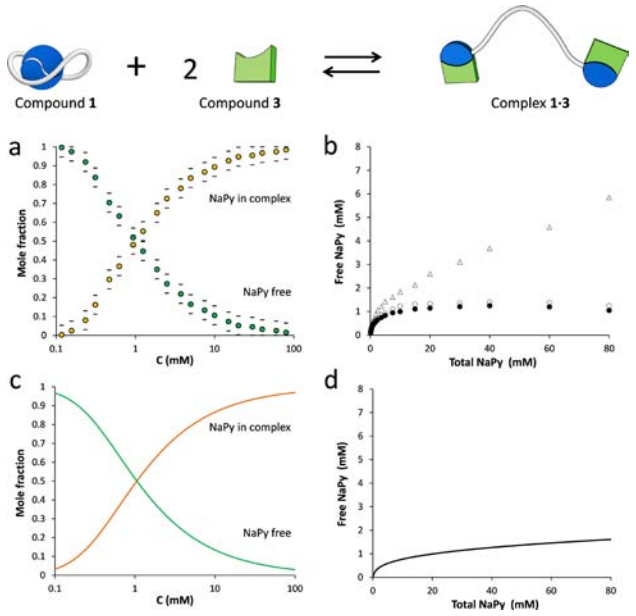

Figure 5. Top: Schematic representation of the equilibrium between cyclic monomers of **1**, compound **3**, and complex **1·3**. Bottom: (a) Measured fractions determined by ^{1}H NMR in $CDCl_3$ of free **3** and the complex **1d·3** as a function of the total concentration. (b) Measured concentrations of free **3** as a function of the total concentration of **3** in a 1:2 mixture of either scaffold **1c** (filled circles), **1d** (circles), or **1e** (triangles) and **3**, respectively. (c) Calculated fractions of free **3** and the complex **1·3** as a function of total concentration of **3**. (d) Calculated concentration of free **3** as a function of total concentration. The UPy:NaPy ratio employed in the calculations was 1:1 (K_I(UPy) = 1 × 10^7 and K_a(UPy-NaPy) = 3 × 10^6 M^{-1}).

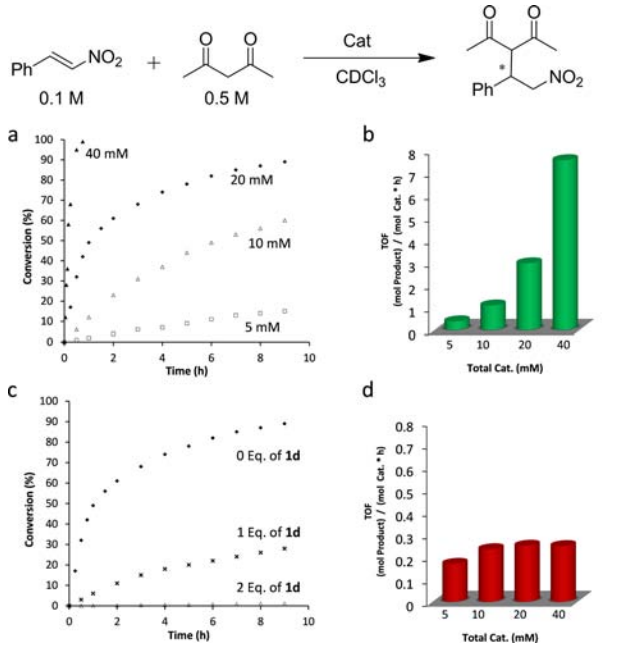

Figure 6. Top: Michael addition of 2,4-pentanedione to *trans*-*β*-nitrostyrene. Bottom: (a) Conversion as a function of time employing different concentrations of **3**. (b) Catalytic activity of **3** in the Michael addition at different concentration of catalyst. (c) Conversion as a function of time in a catalytic system formed by compound **3** (20 mM) with different equivalents of scaffold **1d**. (d) Catalytic activity of a system formed by **3** and **1d** in a equimolar ratio of UPy:NaPy units at different concentrations.

studied Michael addition of 2,4-pentanedione to *trans*-*β*-nitrostyrene. The catalytic activity of **3** is expressed in turnover frequency (TOF = [Product]/[Catalyst]·h^{-1}) and is strongly related to the specific concentration of **3** (Figure 6a,b). Such concentration dependence is proposed to be related to the hydrogen-bonding donor and acceptor properties of **3**, which are also involved in the activation of both reagents via hydrogen bonding.[29] When the reaction was carried out in the presence of 1 equiv of **1d**, a clear decrease of reactivity was found (Figure 6c). After the addition of **1d**, the starting reaction rate was decreased by a factor of 12 (Figures S17 and S18). Remarkably, the reaction can be stopped after addition of another equivalent of **1d**; showing the selectivity and efficiency of this scaffold for the supramolecular inhibition of the catalytic properties of **3**.

Under the catalytic conditions employed, the system composed of an equimolar mixture of the UPy and NaPy units presented 95% of compound **3** in the form of the complex **1d·3** and 5% of free **3**. This selectivity is in agreement with the data obtained in the absence of reagents, suggesting that the equilibrium is not affected under the catalytic conditions. It is worth mentioning that although the ^{1}H NMR data under these catalytic conditions suggest a concentration of free **3** of around 1 mM, the reactivity observed was similar to that of a system with a 7 mM concentration of free **3**. This discrepancy is proposed to be related to the inherent complexity and dynamic nature of this supramolecular system. As a consequence, scaffold **1d** nonlinearly deactivates **3** in a way that resembles the results observed in recent studies carried out with enzymes, where the lifetime of the active and inactive configuration regulates the final catalytic activity.[30] However, in this system the TOF has become concentration independent and strikingly different from that of the non-autoregulated system (Figure 6d). Cyclic scaffold **1d** is able to keep constant the concentration of the free catalyst **3** and to mitigate the effect that a dilution has on the catalytic properties, yielding the first example of supramolecular autoregulation in organocatalysis.

3. CONCLUSIONS

In summary, we report a unique supramolecular system that is able to keep the concentration of an organic catalyst constant over a broad overall concentration regime, due to the presence of a scaffold. This autoregulation is based on two distinct competitive molecular interactions in a multicomponent system. These molecular interactions have different concentration-dependent profiles, based on Le Chatelier's principle. This difference in concentration-dependent behavior is able to compensate the decreased concentration of a given species after dilution due its lower association to the scaffold. The high concentration dependence achieved with the cyclic scaffolds **1c,d** in the equilibrium of free and complexed **3** mitigates the effect that dilution has on the final catalytic properties. The combination of supramolecular chemistry with organocatalysis has created a complex catalytic behavior that mimics one of the enzymatic autoregulation mechanisms found in nature. This is a starting point to develop new complex systems with positive and negative feedback loops. It will open new ways to study well-defined models or to mimic biological systems.

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■ ASSOCIATED CONTENT

Supporting Information

Additional spectroscopic data, concentration-dependent studies, details on the equilibrium model and the curve-fitting procedure, catalytic methods, and catalytic results. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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