Dynamic Combinatorial Libraries

Selection and Amplification of a Catalyst from a Dynamic Combinatorial Library**

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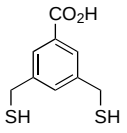
The development of catalysts that rival the efficiency and selectivity of enzymes is one of the holy grails in chemistry. To date, success in the design and synthesis of such systems is limited by an imperfect understanding of the host–guest interactions underlying efficient activity. As an alternative, several selection-based approaches to the discovery of new catalytic systems are being developed.[1] Selection can be based on activity such as in the in vitro evolutionary screening and selection of ribozymes,[2] while another approach involves selection of receptors on the basis of the affinity for the transition state of a given reaction. In practice, screening is based on the affinity for a stable transition-state analogue (TSA). In molecular imprinting, a polymer is formed around a TSA that is subsequently removed to generate catalytically active molecular-sized cavities.[3] The catalytic potential of antibodies raised against a TSA has also been explored.[4] Although catalysts have been produced using these methods, success has been relatively rare, whereas the experimental difficulties, time, and expense associated with these methods are considerable. Herein we introduce an alternative approach based on dynamic combinatorial chemistry.[5]

In dynamic combinatorial chemistry, a mixture of compounds is generated by linking small building blocks together through a reversible reaction. Reversibility ensures that the dynamic combinatorial library (DCL) is in thermodynamic equilibrium and responsive to external influences. Upon exposure of a DCL to a molecular target, those library members that bind to the target are stabilized; the equilibrium shifts, and strong binders are amplified at the expense of the poor binders in the library.[6] After the exchange of building blocks has been turned off, the amplified compound(s) can be isolated directly from the frozen library.[7] Thus dynamic combinatorial chemistry is not only a method of selection but also provides a synthetic route towards the selected compounds.

We have demonstrated that disulfide chemistry is well-suited for the generation of DCLs of macrocycles in water.[8] Disulfide formation occurs readily by oxidation of thiols upon exposure to air. Exchange takes place under mild conditions (pH 7–9) in the presence of thiolate, and is switched off either under acidic conditions or after removal of the thiolate. Thiol oxidation is sufficiently slow under the conditions we use to allow equilibration to occur.[8] We recently reported a DCL made by mixing dithiol building blocks **1, 2**, and **3** and showed how exposure of this library to two guest molecules led to the amplification of two receptors for hydrophobic ammonium ions.[6j] Given the high affinity of the selected library members for cationic hydrophobic molecules, we have now used the same library to search for catalysts for the Diels–Alder reaction between acridizinium bromide (**4**) and cyclopentadiene (**5**; Scheme 1).[9] As the transition state of the Diels–Alder reaction is similar to the product **6**, we reasoned that **6** would be a good TSA. Thus we stirred equimolar amounts of



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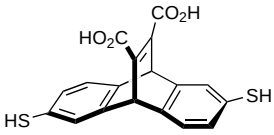

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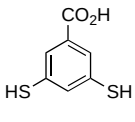

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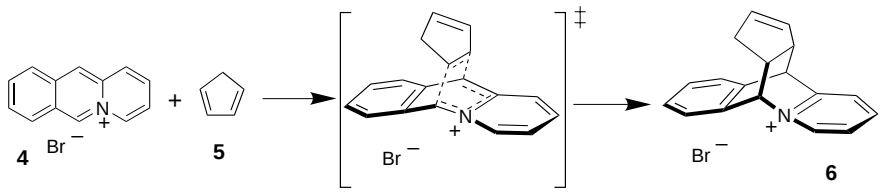

Scheme 1. Diels–Alder reaction demonstrating the similarity between transition state and product.

1, **2**, and **3** in water at pH 8.5 in the presence of atmospheric oxygen for 3–5 days. The product distributions of the resulting DCL in the absence of any template (Figure 1 A) and after addition of **6** (Figure 1 B) were analyzed by HPLC and mass spectrometry. Addition of the TSA results in the selection and amplification of two macrocycles, which were identified by mass spectrometry as **7** and **8** (Figure 1), the same receptors selected in our previous study.[6j] Both macrocycles are formed as mixtures of stereoisomers since racemic **2** was used as a building block. Macrocycles **7** and **8** can be obtained from a biased DCL made from **2** and **3** in the ratio in which they are incorporated in the selected macrocycles. Together, the two macrocycles of interest make up 79% of the library material in the presence of TSA **6** (Figure 2 B), whereas they constitute only 12% in the absence of template (Figure 2 A).

We have studied the binding of diene **4** and Diels–Alder product **6** to hosts **7** and **8** by means of isothermal titration microcalorimetry (ITC; Table 1). Heterotrimer **8** was found to bind the diene starting material more strongly than it binds the Diels–Alder product. In contrast, homotrimer **7** binds the product more strongly than it does the starting material. This suggests that heterotrimer **8** will be catalytically inactive, as the incorporation of cyclopentadiene (**5**), and the subsequent Diels–Alder addition, would lead to a less stable complex. In contrast, incorporation of **5** into the **7**·**4** complex would lead to a more stable species, indicating that **7** could be catalytically active.

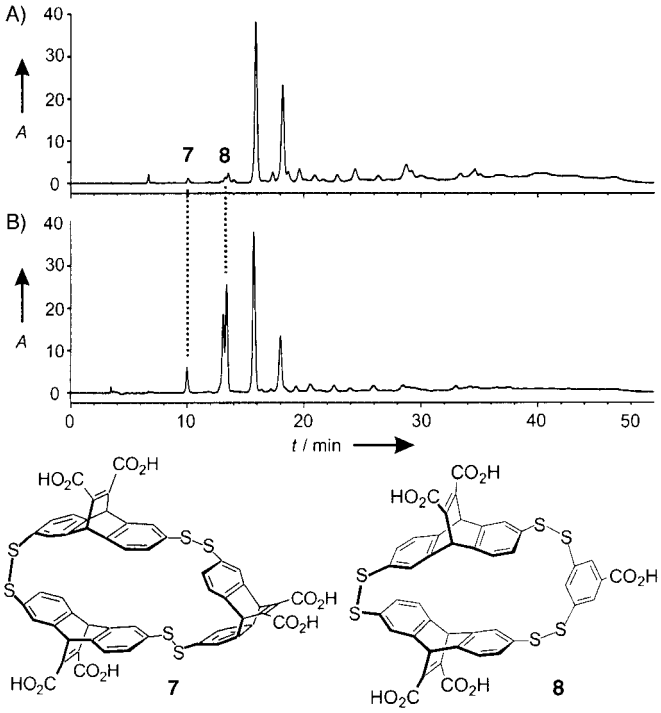

Figure 1. HPLC analyses of the DCL made from dithiols **1, 2**, and **3** (3.3 mM each): A) in the absence of any template, and B) in the presence of TSA **6**, which induced the amplification of macrocycles **7** and **8** (as mixtures of stereoisomers). HPLC analyses were carried out on a 250×4.6 mm 5-μm Hypersil SAX anion-exchange column with a gradient of 0.025–0.5 M ammonium formate in 2-propanol/acetonitrile/water (55:35:10).

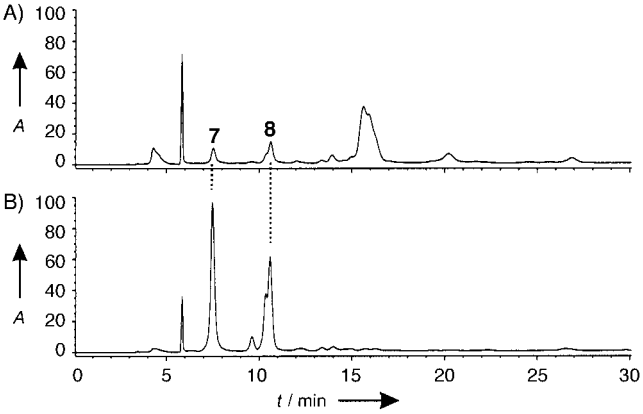

Figure 2. HPLC analyses of a DCL made from dithiols **2** (8.1 mM) and **3** (1.9 mM): A) in the absence, and B) in the presence of TSA **6** (5 mM). HPLC conditions as described in Figure 1.

Table 1: Thermodynamic data for the binding of **4** and **6** with **7** and **8**.[a]

		7	**8**
4	K_1 [M^{-1}]	1.3×10^5	6.4×10^5
	$\Delta G°$ [kJ mol^{-1}]	−29.1	−33.1
	$\Delta H°$ [kJ mol^{-1}]	−23.7	−40.6
	$T\Delta S°$ [kJ mol^{-1}]	5.4	−7.5
6	K_1 [M^{-1}]	2.4×10^5	3.9×10^5
	$\Delta G°$ [kJ mol^{-1}]	−30.7	−31.9
	$\Delta H°$ [kJ mol^{-1}]	−25.8	−38.5
	$T\Delta S°$ [kJ mol^{-1}]	4.9	−6.6

[a] Equilibrium constants (K_1), Gibbs energies ($\Delta G°$), enthalpies ($\Delta H°$), and entropies $T\Delta S°$ of the binding of **4** and **6** to hosts **7** (mixture of stereoisomers) and **8** (major diastereomer) at 298 K were determined by using isothermal titration microcalorimetry. Guest solutions (0.85 mM) were titrated into host solutions (0.075 mM), all prepared in 10 mM borate buffer (pH 9.0).

We have measured the rates of the Diels–Alder reaction of **4** with excess **5** in the absence and the presence of 1.2 equivalents of **7** and **8**. Indeed, **7** induced a modest acceleration of the Diels–Alder reaction, whereas **8** turned out to be catalytically inactive. Figure 3 shows the observed (pseudo) first-order rate constants for the uncatalyzed reaction and the cycloaddition catalyzed by **7** as a function of the concentration of dienophile **5**.

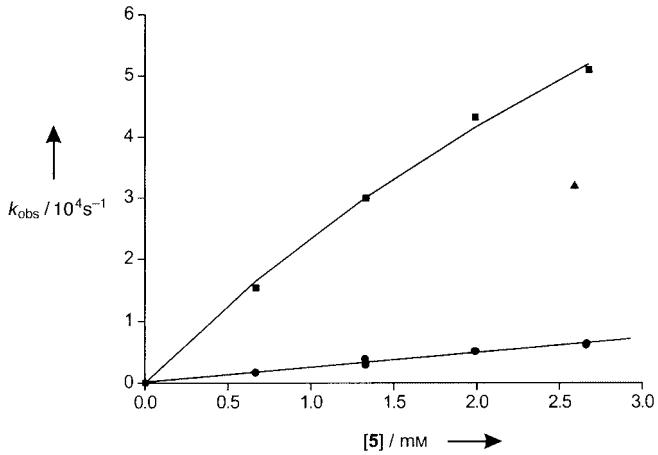

Figure 3. Observed rate constants (k_{obs}) for the Diels–Alder reaction between **4** and **5** in the absence (●) and presence (■) of macrocycle **7** as a function of the concentration of **5**. The solid lines represent fits of the data to a linear function (uncatalyzed reaction) or to Equation (1) (catalyzed reaction). The observed rate constant for the Diels–Alder reaction in the presence of **7** and product **6** (1 equiv) is also shown (▲).

The binding constant data suggest a catalytic mechanism in which the macrocycle strongly binds diene **4**, leaving enough space to simultaneously bind dienophile **5** in the same cavity. Thus the Diels–Alder reactants are in proximity, increasing the rate of their reaction. It can be shown (see Supporting Information) that when using excess **5** and assuming complete binding of **4** to **7**:

$$k_{obs} = \frac{K_2[\mathbf{5}]_t}{1 + K_2[\mathbf{5}]_t} k_{cat} \quad (1)$$

In [Eq. (1)], k_{obs} is the observed pseudo-first-order rate constant at a given concentration of **5**, k_{cat} is the first-order rate constant for the Diels–Alder reaction of the ternary **4**·**5**·**7** complex, $[\mathbf{5}]_t$ is the total concentration of **5** in solution, and K_2 is the equilibrium constant for the binding of **5** to the binary **4**·**7** complex. Using this equation to fit the kinetic data, we obtained values for K_2 and k_{cat} of $1.4(\pm 0.5) \times 10^2\,\text{M}^{-1}$ and $1.9(\pm 0.5) \times 10^{-3}\,\text{s}^{-1}$, respectively. Comparing the values of k_{cat} with the second-order rate constant of the uncatalyzed reaction yields an effective molarity of 0.08 M for **5** at the site of reaction.

We did not expect **7** to exhibit efficient turnover because we have used the reaction product as the TSA. Any catalyst selected this way is bound to be inhibited by the product. However, the binding constants of starting material **4** and product **6** to catalyst **7** (Table 1) are of the same order of magnitude, suggesting that **4** is able to compete with **6** for binding to the catalyst, so that some turnover should be possible. Indeed, when we added 1 equivalent of **6** to the reaction mixture, **7** still accelerated the Diels–Alder reaction (Figure 3).

Although the efficiency of **7** is modest when compared with other noncovalent catalysts for Diels–Alder reactions,[10] we have established that dynamic combinatorial chemistry has potential as a tool for catalyst development. The method is more controllable and experimentally less demanding than the catalytic antibody approach and generates better-defined systems than imprinted polymers.[11] Whereas our current building blocks only provide nonspecific hydrophobic aromatic surfaces, incorporating functional groups capable of specific recognition and activation of the substrate should improve catalytic efficiency.

Experimental Section

Acridizinium bromide (**4**) and its cyclopentadiene adduct **6** were prepared as described in the literature.[9] Cyclopentadiene (**5**) was prepared by thermal cracking of dicyclopentadiene immediately before use.

In a typical experiment the dithiols (10 mM overall) were suspended in water (1–10 mL) and a NaOH solution (1.0 M; 1 equiv with respect to the number of carboxylic acid groups) was added. After all the dithiol had dissolved, the pH value was adjusted to 8.5. Where appropriate, the TSA **6** (5 mM) was added and the mixtures were allowed to oxidize and equilibrate for 3–5 days by stirring in an open vial. Evaporated water was replenished every day.

Rate constants for the cycloaddition between **4** and **5** were determined by UV/Vis spectroscopy: the decrease of the absorbance of diene **4** was monitored at 396 nm using initial rate kinetics.[12] Measurements were performed in cuvettes (1 mm path length) initially containing the appropriate concentrations of **4, 6**, and **7** in 10 mM borate buffer at pH 9.0 at $25 \pm 0.1\,^{\circ}\text{C}$. After thermal equilibration, a solution of **5** (48–143 mM, 5 μL) in 1-propanol was injected and the solution was homogenized. The cuvettes were stoppered carefully to prevent evaporation of **5**. Four reactions were followed simultaneously in each run. Observed rate constants (k_{obs}) were obtained by using the following expression:[12] $k_{obs} = \mathrm{d}(Abs)/\mathrm{d}t\,(d(\varepsilon_4 - \varepsilon_6)[\mathbf{4}]_0)^{-1}$. In this equation, $\mathrm{d}(Abs)/\mathrm{d}t$ is the change in absorbance during the first 10 % of the reaction, d is the path length of the cuvette, and ε_4 and ε_6 are the extinction coefficients of **4** and **6**, respectively. These extinction coefficients were determined separately under the same conditions as those used in the kinetic runs.

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