LETTER

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A catalyst for an acetal hydrolysis reaction from a dynamic combinatorial library

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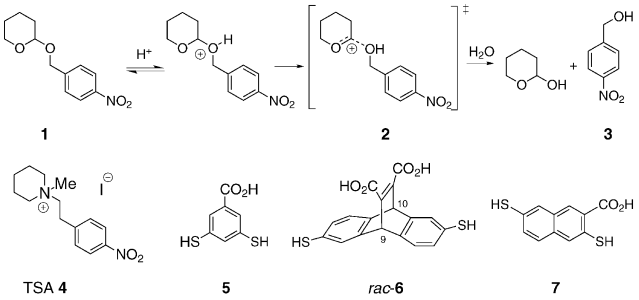
A transition-state analogue (TSA) for an acetal hydrolysis reaction was found to select and amplify a macrocycle from a dynamic combinatorial library (DCL) of disulfides in water. This host was able to accelerate the reaction by a factor of two; a similar value was progressively reached when the macrocycle was gradually produced in the course of the reaction.

Dynamic combinatorial chemistry[1] is gaining ground as an attractive selection-based approach[2] for the discovery of new catalytic systems.[3] In a DCL, all library members are connected through a set of reversible reactions, which creates a mixture that is under thermodynamic control. Introducing a template that mimics the transition state of a chemical transformation (a transition-state analogue or TSA) will shift the equilibria in the direction of those receptors that bind the TSA and, as a result, should have the potential of catalyzing the corresponding reaction.

We recently became interested in the challenging idea of using this strategy to produce an abiotic catalyst that mimics glycosidase function, focusing on the hydrolysis of model acetal **1**. The rate determining step for this reaction involves the cleavage of the heterocyclic carbon–oxygen bond of the protonated acetal *via* transition state **2**.[4]



We reasoned that ammonium salt **4** should be a suitable TSA as it resembles transition state **2** in terms of shape and charge distribution[5] and it is also a promising template for the amplification of macrocyclic hosts from DCLs made from dithiols **5–7** in water. Our previous studies have demonstrated that such building blocks can engage in hydrophobic and cation–π interactions with non-polar ammonium salts.[6] Moreover, the disulfide exchange chemistry in which these building blocks can participate is complementary to the acetal hydrolysis conditions: disulfide bonds undergo rapid exchange in the presence of a catalytic amount of thiolate anion in water at pH 7–9 (DCLs conditions), whereas the bonds—and therefore the library members—are kinetically stable under acidic conditions (pH < 5, hydrolysis conditions).[7]

When equimolar amounts of building blocks **5–7**[8] were submitted to (irreversible) air oxidation and simultaneous (reversible) disulfide exchange at pH 8.5 for 3–5 days, a complex DCL was obtained (Fig. 1a). The same library prepared in the presence of TSA **4** showed amplification of two species (Fig. 1b), which were identified by mass spectrometry as two diastereomeric trimers of building block **6**;[9] both of them being macrocycles that exhibit an unfunctionalized aromatic cavity of similar shape and size (Fig. 2). Starting from a biased library made of only dithiol **6** and TSA **4**, the trimers **8** constitute now more than 80% of the library material (Figs. 1c and d) and, after scale-up, we were able to isolate 40 mg of the potential catalyst by preparative HPLC (Fig. 1e).

The binding of TSA **4** with hosts **8** (as a mixture of stereoisomers) was studied by isothermal titration microcalorimetry and was found to be enthalpy driven ($K = 2.6 \times 10^5$ M^{-1}, $\Delta G^\circ = -30.9$ kJ mol^{-1} $\Delta H^\circ = -24.6$ kJ mol^{-1} and $T\Delta S^\circ = +6.3$ kJ mol^{-1}), which is consistent with cation–π interactions being the major contributor to binding.

The kinetics of the hydrolysis of **1** were studied in the presence of 1.3 equivalents of hosts **8** in H_2O–CH_3CN 97 : 3 (v/v) under acidic conditions (pH 4.2) at 25 °C. The addition of the small amount of acetonitrile was required to ensure solubility of the starting material. The disappearance of **1** and the formation of product **3** were monitored by HPLC at 300 nm. The rate of hydrolysis obeyed first-order kinetics with $k_{cat} = 9.94 \times 10^{-4}$ min^{-1} (△ in Fig. 3). We have also monitored the

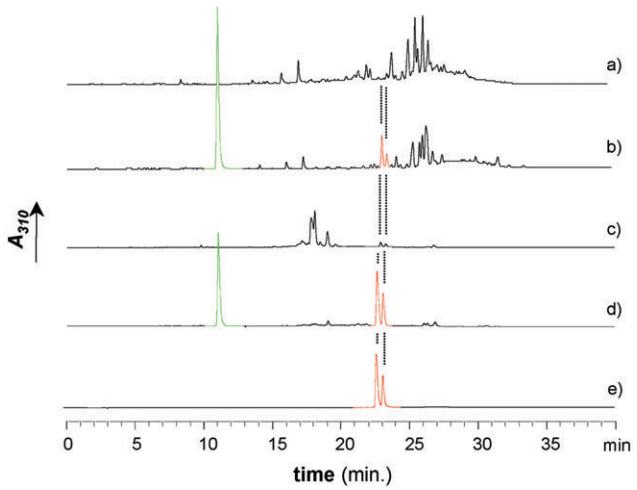


Fig. 1 HPLC analyses (310 nm) of the DCLs made from a) dithiols **5–7** in the absence of template; b) dithiols **5–7** in the presence of TSA **4**; c) dithiol **6** in the absence of template; d) dithiol **6** in the presence of TSA **4**. e) HPLC analysis (310 nm) after scale-up and purification of **8** from trace d). HPLC analyses were carried out using a 250 × 4.6 mm Waters Symmetry C_{18} column with a gradient of 5–95% CH_3CN (0.1% TFA) in H_2O (0.1% TFA). Peaks due to TSA **4** and receptors **8** (as a mixture of stereoisomers) are shown in green and red, respectively.

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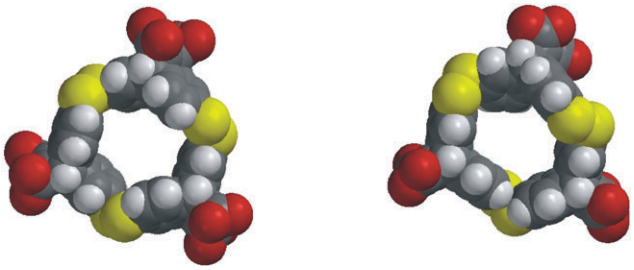

Fig. 2 Energy-minimized structures (PM3 level, Spartan′04[10]) of trimeric hosts **8**. Left: minor diastereoisomer and right: major diastereoisomer. Colour code used for labelling atoms: C = grey; H = white; O = red; S = yellow.

kinetics of hydrolysis of **1** at pH 4.2 in the absence of hosts **8**, giving k_0 of 4.70×10^{-4} min^{-1} (○ in Fig 3). Comparison of the values of k_{cat} and k_0 indicates that hosts **8** accelerate the hydrolysis of **1** by a factor of 2.1.

In order to establish whether this acceleration is due to the reaction taking place in the hydrophobic cavity of receptors **8**, we studied the hydrolysis of **1** in the presence of 3.9 equivalents of **6**. Unexpectedly, the rate of the reaction in the presence of **6** turned out to increase progressively with time (◇ in Fig. 3). The study of the related HPLC traces revealed that, in the course of the hydrolysis of acetal **1**, dithiol **6** is completely converted to the corresponding disulfide trimers **8** (Fig. 3, traces a–f).[11] Trimer formation most likely occurs under kinetic control, since under the acidic conditions of the hydrolysis experiments disulfide exchange is extremely slow.[7,12] During the first 15% conversion, when the amount of hosts **8** is still insignificant, the rate constant for the reaction (obtained from the slope of the dashed line in Fig. 3) is 4.82×10^{-4} min^{-1}, which is—within experimental error—the same as the value for k_0. Control experiments on a solution of **6** revealed that the production of receptor **8** is independent of the presence of **1** or its hydrolysis products, indicating that this process is not templated.

The absence of any catalytic activity due to **6**, as well as the acceleration of the reaction following the formation of the macrocyclic species, demonstrate that the cavities of receptors **8** are responsible for acceleration of the hydrolysis of acetal **1**, probably through stabilization of the transient positive charge that develops during the reaction. We have been unable to ascertain whether hosts **8** accelerate the reaction by shifting the

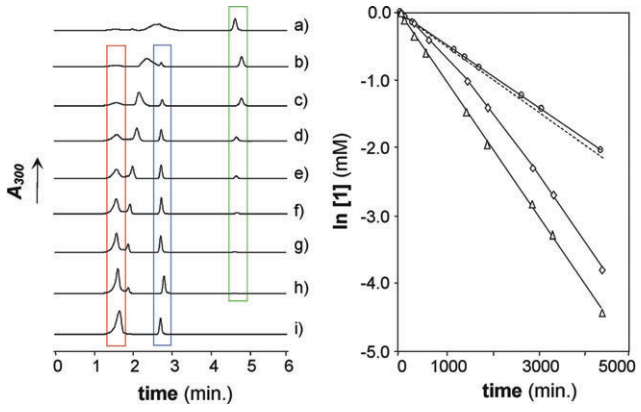

Fig. 3 Left: HPLC analyses (300 nm) of the hydrolysis experiments of **1** in the presence of **6** (3.9 equiv.) after a) 1.4; b) 4.9; c) 10.1; d) 24.0; e) 31.3; f) 47.6; g) 55.2; h) 76.6 and i) 97.3 hours. HPLC analyses were carried out using a 250 × 4.6 mm Waters Symmetry C_{18} column with a mixture of CH_3CN–H_2O (80 : 20). Acetal **1**, product **3** and hosts **8** are highlighted in green, blue and red, respectively. Right: semi-logarithmic plots of the concentration of **1** *versus* time, *t*, at pH 4.2 in the absence of any catalyst (○); in presence of 3.9 equiv. of **6** (◇); and 1.3 equiv. of **8** (△). The dashed line represents the tangent of the curve for the reaction in the presence of **6** at $t = 0$.

protonation pre-equilibrium more towards $\mathbf{1} \cdot H^+$, whether they actually stabilize the transition state **2**, or whether they act through a combination of these effects. The only modest acceleration is almost certainly a consequence of the lack of functional groups that can participate in the catalytic process. Our research efforts are now directed towards the development of a new generation of building blocks that contain such functional groups. Given that we have met with promising degrees of success in our two first attempts to use dynamic combinatorial chemistry for catalyst development (this study and ref. 3), we are optimistic that dynamic libraries can be developed into a practical method for catalyst discovery in the near future.

Experimental

Acetal **1** and TSA **4** were obtained using the procedures described in refs. 13 and 14, respectively.

In a typical DCL 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 the dithiols had dissolved, the pH value was adjusted to 8.5. Where appropriate, the TSA **4** (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).

The equilibrium constant (K), enthalpy (ΔH°) and entropy ($T\Delta S^\circ$) of binding of TSA **4** in hosts **8** at 298 K were determined using isothermal titration microcalorimetry. Guest solutions (0.91 mM) were titrated into host solutions (0.10 mM, mixture of stereoisomers), all prepared in 10 mM borate buffer (pH 9.0).

In a typical kinetic experiment, the hydrolysis reaction was initiated by the injection of 30 μL of **1** (33.3 mM in CH_3CN) in a thermostatted cell initially containing 970 μL of a solution of either **8** (as a mixture of stereoisomers; 1.34 mM; 1.3 equiv.) or **6** (4.02 mM; 3.9 equiv.) in citrate buffer (10 mM, 970 μL, pH = 4.2) at 25 °C. Reactions were monitored by analysing 5 μL aliquots by HPLC. The pseudo-first-order rate constants were determined from the slopes of semi-logarithmic plots of [**1**] against time.

Acknowledgements

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8 Building blocks **5** and **6** were obtained as reported in ref. 6. Dithiol **7** has been prepared from 3,7-dihydroxy-2-naphthoic acid using analogous procedures.
9 As **6** was synthesized as a racemic mixture of two enantiomers (see ref. 6), the ^{1}H-NMR spectrum of **8** revealed 16 signals: 4 belonging to a diastereoisomer of D_3-symmetry (where every monomer has the same configuration [*i.e.*, (9*R*,10*R*) or (9*S*,10*S*)] and 12 belonging to a diastereoisomer of C_2-symmetry (where one monomer has an opposite configuration compared to the other two). From the relative integration of the ^{1}H-NMR signals it is possible to assign the C_2-symmetry to the major signal in HPLC (ratio of $\sim$2 : 1 in agreement with a statistical distribution).
10 *Spartan'04*, Wavefunction, Inc., Irvine, CA, USA.
11 The two diastereomers overlap in the HPLC conditions used for the kinetic experiments.
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