Dynamic Selection

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Exploiting Neighboring-Group Interactions for the Self-Selection of a Catalytic Unit**

Giulio Gasparini, Leonard J. Prins, and Paolo Scrimin**

Dynamic combinatorial chemistry (DCC) is based on the principle that the thermodynamic composition of a dynamic library of molecules, that is, a library of which the components are held together either by noncovalent bonds or reversible covalent bonds, spontaneously changes upon the input of an external stimulus.[1] This can be either the addition of a target molecule, but also an alteration of the environment (pH, light, etc.). Ideally, the composition of the library changes in favor of the component that is the most stable under the changed conditions.[2] In the past decade, DCC has emerged as a powerful tool for the discovery of, sometimes very surprising, molecular receptors and novel materials.[3]

In principle, DCC could be applied to the selection of a catalyst by shifting the equilibrium of the library with amplification of a molecular receptor for a transition state of a given reaction.[4] By decreasing the energy of the transition state by formation of a complex with this molecular receptor (that is, a catalyst), the reaction rate is obviously accelerated. This concept was first developed by Pauling,[5] and applied to catalyst discovery with catalytic antibodies[6] and imprinted polymers.[7] As a transition state is an elusive species, a stable analogue is required possessing similar features in terms of shape and charge distribution. However, despite the success of DCC, its use for catalyst discovery is significantly lagging, as evidenced by a very limited number of publications and, generally, very modest rate accelerations.[4] This fact suggests that the endeavor is very challenging. In analogy with enzyme catalysis, an ideal catalyst should first bind to the substrate and subsequently transform it to product.[8] Accordingly, the catalyst should both recognize the substrate and the transition state, although the thermodynamic stabilization of the latter must be much higher. It is not surprising that in enzymes the substrate and transition state recognition loci are quite often different because of the different tasks they have to accomplish.[9] Herein we present the dynamic self-selection of a functional group which induces a 60-fold rate enhancement in the basic hydrolysis of a neighboring carboxylic ester; that is, the selection of a catalytic unit on the way to the selection of a fully-fledged catalyst.

Recently, the "tethering" strategy has emerged as a powerful tool for the detection of weak, noncovalent interactions between substrates and a target.[10] This approach implies that the target molecule is covalently linked to a scaffold molecule which has the additional ability to interact in a reversible manner with library members (Scheme 1). In this way, the recognition event between target and library component becomes intramolecular, which, for entropic reasons, significantly enhances the sensitivity of the screening process. In fact, Houk has recently pointed out that among the most efficient enzymes are those that covalently bind the substrate before its transformation into products.[11]

During studies on hydrazone-based libraries, we recently observed that the presence of a phosphonate group in **1** resulted in the preferential incorporation of hydrazide **B** with respect to **A**, owing to an intramolecular, electrostatic interaction between the oppositely charged phosphonate and ammonium groups.[12] The phosphonate group was chosen as a target because it is a model for the transition state of a carboxylic ester hydrolysis. Following the above concept that stabilization of the transition state should lead to an increased rate of hydrolysis, we argued that the phosphonate group in **1** could be used to self-select hydrazides that would enhance the cleavage rates of the corresponding carboxylic ester. Thus, we have screened a library of nine hydrazides, and present herein compelling data showing the existence of a correlation between thermodynamic amplification in the dynamic screening and the efficiency in assisting in intramolecular catalysis.

The nine components of the library were chosen from commercially available hydrazides, of which eight could potentially interact with a phosphonate moiety, either by electrostatic interactions (**B**, **C**) or the formation of one or more hydrogen-bonds (**D**–**I**) (Scheme 1). Hydrazide **A** was not expected to interact with the target and was used as an internal standard. We also screened aldehyde **2**, which contains a neutral methoxy group: the resulting library served as a neutral reference to determine the intrinsic stabilities of the hydrazones in the absence of the target. Any shift in the library composition using scaffold **1** with respect to that obtained using scaffold **2** can then be ascribed to an intramolecular stabilization between the hydrazide and the phosphonate target.[13]

Library equilibration studies were performed by adding either scaffold **1** or **2** (5 mM) to a mixture of hydrazides **A**–**I** (each 1.5 equivalent) in CD_3OD. The mixtures were kept at 50 °C until the thermodynamic equilibrium was reached, which was detected by the absence of any further change in

[*] G. Gasparini, Dr. L. J. Prins, Prof. Dr. P. Scrimin
Department of Chemical Sciences, University of Padova
and CNR ITM, Padova Section
Via Marzolo 1, 35131 Padova (Italy)
Fax: (+39) 049-827-5239
E-mail: leonard.prins@unipd.it
paolo.scrimin@unipd.it

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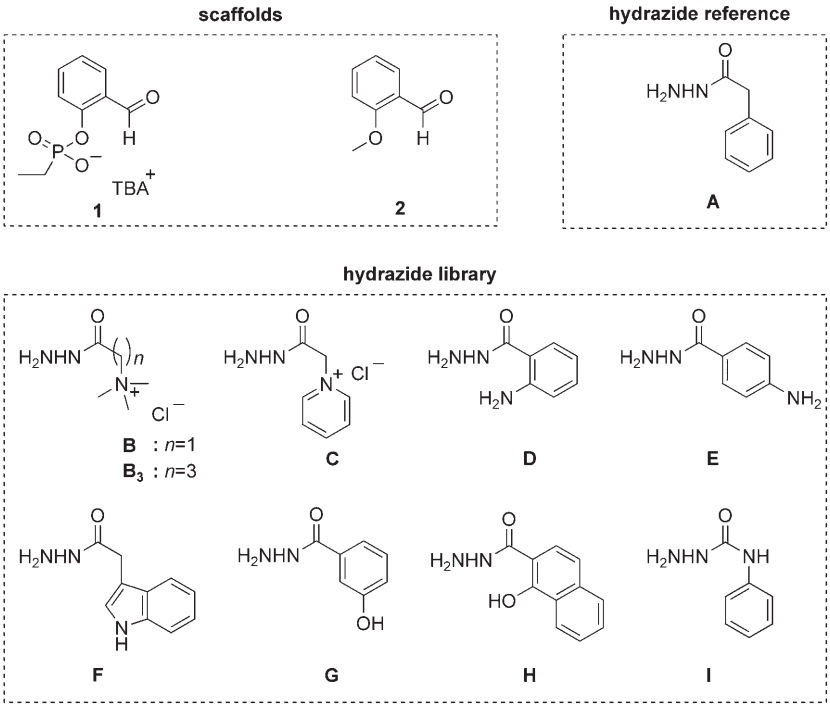


Scheme 1. Scaffolds **1**, **2**, reference hydrazide **A**, and the hydrazide library (**B–I**). TBA = tetra-*n*-butylammonium.

the ^{1}H NMR spectra of the mixtures (typically 12 hours for scaffold **1**, and 3 days for scaffold **2**). All hydrazones are characterized by the presence of a signal in the $\delta = 8$–9 ppm fingerprint region of the ^{1}H NMR spectrum originating from the hydrazone C–H proton. A direct determination of the library distribution by integration of these signals was not possible owing to a partial overlap and the presence of signals in this region from free hydrazide **C**. This problem could be resolved by measuring ^{1}H–^{13}C HSQC-spectra of both mixtures. The additional separation of signals in the ^{13}C dimension allowed the individuation and quantification of each hydrazone present.[14] Concentrations of **1B–I** and **2B–I** were determined relative to **1A** and **2A**, respectively, after which the amplification was calculated (Figure 1, light gray bars).[15] The data reveal that charged hydrazones **1B** and **1C** are amplified in the mixture.

To confirm the observed amplification factors and to maximize precision in this proof-of-concept study, all the hydrazones were also individually screened against the reference hydrazide **A** (Scheme 2). In this case, the competition experiments were performed by adding scaffold **1** or **2** (5 mM) to a solution containing hydrazide **A** (5 equiv) and one of the hydrazides **B–I** (5 equiv) in CD_3OD. The obtained amplification factors confirm the trend observed for the full library screening (Figure 1, dark gray bars).[16] Furthermore, the data now substantiate the previously uncertain amplification of hydrazone **1I**. Considering the fact that the screening was performed under conditions where amplification effects are not maximal,[17] we could confirm that three of the eight hydrazides (**B**, **C**, and **I**) were preferentially selected, and **B** (1.8) to a higher extent with respect to **C** and **I** (1.3 each).

These results clearly demonstrate that hydrazones **1B**, **1C**, and **1I** are stabilized as a consequence of an intramolecular interaction between the phosphonate and the functional group present in the hydrazide unit. This is supported by the fact that the addition of a phosphate to a hydrazone

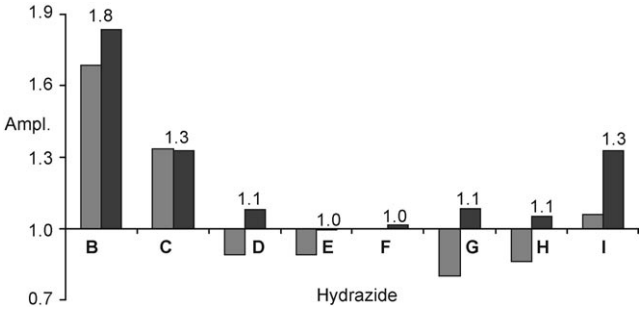
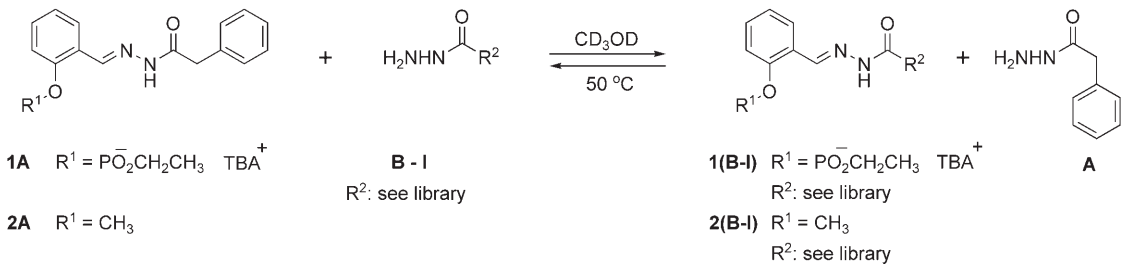


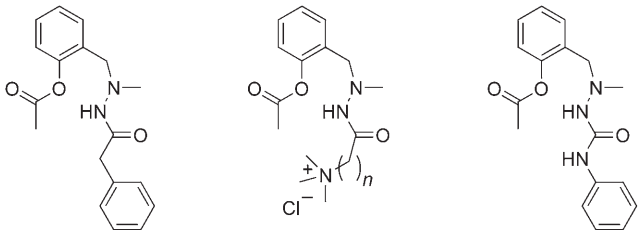
Figure 1. Observed amplification for each hydrazide in reference to hydrazide **A** obtained either from ^{1}H–^{13}C HSQC-spectra (light gray bars) or from separate mixing experiments (dark gray bars).

library obtained from hydrazides **A** and **B** and benzaldehyde did not result in any detectable change of the composition at thermodynamic equilibrium (data not shown). Following our hypothesis, the positioning of these functional groups near an



Scheme 2. Competition experiments. All experiments were performed using scaffold **1** (or **2**, 5 mM), hydrazide **A** and either one of the hydrazides **B–I** in a 1:5:5 ratio in CD_3OD at 50 °C.

ester moiety should consequently result in an enhanced cleavage rate of this ester because of transition state stabilization. In addition, the extent of such a catalytic effect should be correlated to the extent of amplification observed (**B** > **C** ≈ **I** > reference **A**). To establish such a correlation, we studied hydrazides **B** and **I** in detail, as they should express a different type and strength of interaction with the transition state. Therefore, compounds **3B**, **3I** and **3A** (which serves as a reference) were prepared in which the structural elements of hydrazides **A**, **B**, and **I**, were positioned in close proximity to a neighboring carboxylate ester (Scheme 3). Compared to the parent hydrazone structures,



Scheme 3. Functionalized phenyl acetates that were studied.

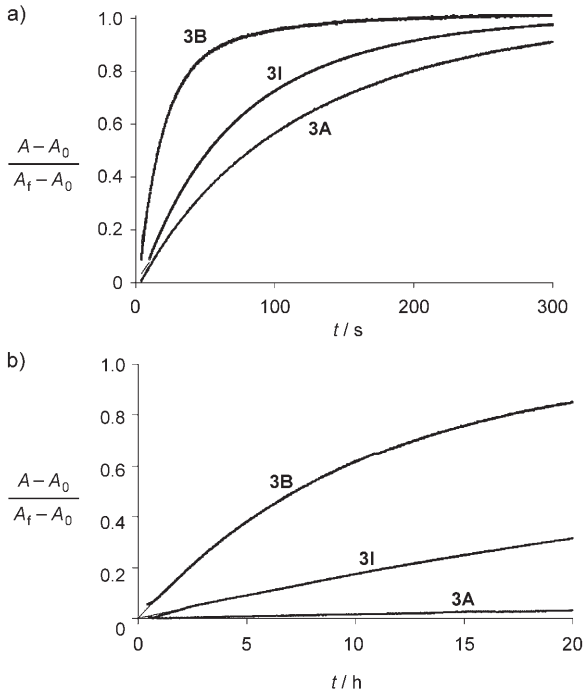


Figure 2. Changes of the absorbance at 280 nm upon a) methanolysis and b) hydrolysis of compounds **3A**, **3B**, and **3I**.

two small structural changes had to be introduced. The C=N double bond had to be reduced to render the structure stable under the basic conditions required for ester cleavage. Such a covalent post-modification is very common in imine-based dynamic combinatorial chemistry.[18] Secondly, the resulting secondary amine had to be methylated to prevent an intramolecular attack of the amine on the neighboring ester.

The effect of the presence of the ammonium and urea groups in **3B** and **3I**, respectively, on the ester cleavage was initially studied by measuring the methanolysis rates of **3A**, **3B**, and **3I** because of the similarity to the conditions under which the amplification studies were performed. Twelve equivalents of sodium methoxide were added to 0.6 mM solutions of esters **3A**, **3B**, and **3I** in methanol at room temperature, and the methanolysis was followed by measuring the increase in absorbance at 280 nm (Figure 2a). The resulting curves were fitted using a first-order exponential yielding the pseudo-first-order rate constants given in Table 1, entry 1. The resulting rates are in good agreement with the results of the amplification studies, both in terms of the order of reactivity ($k_{obs,3B} > k_{obs,3I} > k_{obs,3A}$) and the relative acceleration (4.8:1.6:1 for **3B**, **3I**, and **3A**, respectively).

Several control experiments confirm that the increased ester cleavage rate results from an intramolecular neighboring-group effect. Firstly, the presence of one equivalent of tetramethylammonium chloride did not affect the methanolysis rate of compound **3A** at all. This excludes the possibility that the higher rate observed for **3B** is simply due to a change in ionic strength in the mixture. In other words, it shows that the ammonium ion needs to be present in close proximity to the carboxylic ester to induce a catalytic effect. Secondly, measuring the methanolysis rate of compound **3B** at decreasing substrate concentrations yielded the same rate constants, which is in strong support of intramolecular catalysis. Finally, if the ester moieties in **3B** and **3I** are cleaved at a higher rate owing to a stabilization of the negative charges in the transition state by the neighboring groups (Figure 3), we should observe an enhanced catalytic effect upon decreasing the polarity of the medium, because of a lower solvation ability of the solvent. Therefore, we performed hydrolysis studies of compounds **3** both in a 1:1 and a 1:9 mixture of H_2O/CH_3CN buffered at pH 11.[19] The observed order of reactivity for compounds **3** in the 1:1 mixture is in line with the trend observed for the methanolysis reactions, although the hydrolysis rate for compound **3B** is slightly higher than expected (ca. 16-fold; Table 1, entry 2). Importantly, however, a decrease of the

Table 1: Observed pseudo-first-order rate constants for the ester cleavage of compounds **3A**, **3B**, and **3I** under different basic conditions.[a]

Entry[b]	**3A**	**3B** (k_{3B}/k_{3A})		**3I** (k_{3I}/k_{3A})	
1	0.82×10^{-2}	3.96×10^{-2}	(4.8)	1.28×10^{-2}	(1.6)
2	1.85×10^{-5}	3.02×10^{-4}	(16.4)	2.42×10^{-5}	(1.3)
3	4.41×10^{-7}	2.64×10^{-5}	(59.9)	5.23×10^{-6}	(11.8)

[a] k_{obs} [s^{-1}]. Kinetics were followed by UV/Vis spectroscopy at 280 nm. [b] Entry 1: [**3**] = 0.6 mM, [NaOMe] = 7.2 mM, MeOH, 25 °C; entry 2: [**3**] = 0.6 mM, pH 11 ([CAPS] = 60 mM), H_2O/CH_3CN = 50:50, 45 °C; entry 3: [**3**] = 0.6 mM, pH 11 ([CAPS] = 60 mM), H_2O/CH_3CN = 10:90, 45 °C.

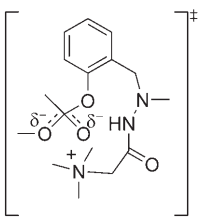


Figure 3. Stabilization of the transition state during the methanolysis of **3B**.

polarity of the medium results in a significant jump in hydrolysis rates both for compounds **3B** and **3I** relative to the reference compound **3A**. In a 1:9 mixture of H_2O/CH_3CN compounds **3B** and **3I** are now hydrolyzed approximately 60 and 16 times faster, respectively (Figure 2b and Table 1, entry 3). These results strongly support our hypothesis that the enhanced reactivity of compounds **3B** and **3I** is indeed due to transition-state stabilization.[20,21] The fact that the ammonium moiety is the best catalytic unit indicates that there is a considerable amount of charge development in the transition state and charge–charge interaction prevails over hydrogen bonding in its stabilization. Accelerations similar to those that we have found here have been obtained with imprinted polymers, where catalysis is also based only on transition state stabilization.[7a,c,22]

Classical studies on intramolecular interactions between neighboring groups have generally shown the importance of the geometry of the complex.[23] To assess the influence of the geometry in this system, we have studied the behavior of hydrazide $\mathbf{B_3}$ and phenyl acetate $\mathbf{3B_3}$,[24] in which the ammonium group is attached by a propylene rather than a methylene spacer. A competition experiment between hydrazide $\mathbf{B_3}$ and reference hydrazide **A** using both scaffolds **1** and **2** yielded an amplification factor of 1.5, which is slightly less than that obtained for hydrazide **B** under the same conditions (1.8). The same trend is observed for the methanolysis rate of $\mathbf{3B_3}$ ($k_{obs} = 3.33 \times 10^{-2}\ s^{-1}$), which is lower than that obtained for **3B**, but still represents an acceleration of 4.1 with respect to reference compound **3A**. These results show that increasing the spacer length reduces the efficiency of the ammonium group in catalyzing the ester cleavage.[25]

In summary, although the selected catalytic functionality is hardly unexpected,[21] we have demonstrated the great potential of the tethering strategy for detecting noncovalent interactions that play a role in catalysis. Using a phosphonate target as a transition-state analogue of the hydrolysis of an ester bound to the reacting aldehyde, we have provided a proof-of-principle for the self-selection of functional groups that assist in catalysis. Very simple, commercially available molecules were used, which also illustrates the scope of this approach. In terms of developing enzyme-like catalysts, the tethering strategy, in contrast to currently performed dynamic screening methods, allows for an independent optimization of the binding and catalytic events. We have reported herein on the catalytic unit selection, but research in our laboratory is currently aimed at selecting also the recognition site to fully implement the catalyst selection.

Experimental Section

The syntheses and characterization data of compounds $\mathbf{B_3}$, **3A**, **3B**, $\mathbf{3B_2}$, $\mathbf{3B_3}$, and **3I** can be found in the Supporting Information, together with characteristic parts of the ^{1}H-^{13}C HSQC and ^{1}H NMR spectra used for determination of the amplification factors. The Supporting Information also contains a plot of log k_{obs} as a function of pH obtained for the hydrolysis of compounds **3A** and **3B**, the methanolysis of compound $\mathbf{3B_3}$, and the methanolysis of **3B** at different concentrations.

Procedures for the equilibration experiments: a) Libraries: A mixture of hydrazides **A**–**I** (each 7.5 mM final concentration) were added to scaffold **1** or **2** (5 mM) in CD_3OD. The mixtures were kept at 50 °C until the thermodynamic equilibrium was reached (no further change in the ^{1}H NMR spectra of the mixtures). ^{1}H–^{13}C HSQC spectra (see Supporting Information) were used to assess library composition.

b) Couples: Freshly prepared mother solutions of the scaffold molecule (**1** or **2**, 100 mM in CD_3CN) and the hydrazides (**A**–**I**, 500 mM in CD_3OD) were used to prepare the mixtures of **1** or **2**:**A**:(**B**–**I**) in a 1:5:5 ratio with a final scaffold concentration of 5 mM. The solutions were kept at 50 °C and monitored by ^{1}H NMR spectroscopy until no additional changes were observed in time. Integration of the hydrazone signals yielded the relative concentrations of the two hydrazones.

General procedure for the kinetic experiments: A stock solution of **3** (10 mM in CH_3CN) was diluted and added to a cuvette containing either a) a NaOMe (7.2 mM) solution in MeOH at 25 °C, b) a 1:1, or c) a 9:1 mixture of CH_3CN:H_2O containing CAPS (3-(cyclohexylamino)-1-propanesulfonic acid) buffer (60 mM) at 45 °C, obtaining a final concentration of **3** of 0.6 mM. Kinetics were followed by measuring the increase of absorbance at 280 nm in time. Ester cleavage was confirmed by HPLC and ESI-MS.

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[13] In principle, the amplification may also be affected by the occurrence of repulsive or intermolecular interactions.

[14] ^{1}H–^{13}C HSQC spectroscopy can also be used to follow the kinetics of hydrazone exchange. This allows a full evaluation of both the kinetic and thermodynamic parameters of a dynamic, multicomponent library and, in addition, eliminates any problem in peak assignment. This methodology will be published in due course.

[15] The lowest concentrations are in the order of 0.25 mM. The errors in the amplification factors are estimated to be around 15 %.

[16] The amplification in this kind of systems depends on the number of equivalents of hydrazides added,[12] which might explain the slightly different amplification factors between the two methods of screening.

[17] As we have shown earlier,[12] maximum amplification is observed under very dilute conditions (0.2 mM), as under these conditions, competing intermolecular interactions are minimal. However, at such concentrations the exchange kinetics are very slow. Therefore, for practical reasons we decided to work at an intermediate 5 mM concentration of scaffold. From our previous studies, this implies a drop in amplification from the maximum value of 3.1 to the observed intermediate value of 1.8.

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[19] The pH refers to the value of the pure aqueous component, and was not corrected for the mixture.

[20] In principle, the enhanced hydrolysis rate of **3B** compared to **3A** could also be a result of an enhanced local concentration of OH^-. If this would have been the case, a plot of $\log k_{obs}$ vs pH would be nonlinear. Therefore, the hydrolysis rates of both **3A** and **3B** were determined at pH 7–11 (see Supporting Information). The analysis showed that for pH 9–11, $\log k_{obs}$ increases linearly with the pH with $\log k_{\mathbf{3B}}$ and $\log k_{3a}$ increasing linearly with pH with the same slope. Based on these results, we conclude that the higher hydrolysis rate of **3B** is indeed due to transition-state stabilization. Interestingly, for pH values below 9, the difference in hydrolysis rates between **3A** and **3B** vanishes, and the slope of the curve by plotting $\log k_{obs}$ versus pH changes dramatically for **3A**. We ascribe this to the protonation of the tertiary amine in both **3A** and **3B** which, for both structures, results in a positive charge located very close to the carboxylic ester moiety. Consequently, at more acidic pH values the "catalytic" effect of the ammonium group in **3B** is taken over by the protonated tertiairy amine.

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