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High-Throughput Screening of Enantioselective Catalysts by Immunoassay

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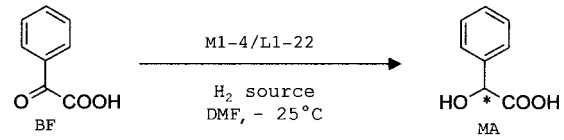
Interest is continuously growing in the application of powerful combinatorial approaches to the discovery of new catalysts by combining synthesis and screening of large libraries.[1] Although this area is still in its infancy, several catalysts have already been identified by parallel synthesis and high-throughput-screening (HTS) techniques.[2] However, the factor limiting an efficient extension of this research to asymmetric catalysis remains the lack of efficient methods for rapid screening of enantioselective reactions.[3] To overcome this major obstacle, a first approach consists in screening the catalyst library for activity using an HTS procedure and then testing each lead for enantioselectivity by conventional methods,[4] but this is only amenable if few catalysts from the broad library are active. Clearly, a high-throughput technique allowing quantification of both activity and enantioselectivity would accelerate the rate at which asymmetric catalysts are discovered. Recently, IR thermography,[5] capillary array electrophoresis,[6] CD-HPLC,[2c] and electrospray ionization based on the use of isotopically labeled substrates[7] have emerged as promising but equipment-intensive techniques for the screening of enantioselective catalysts.[8]

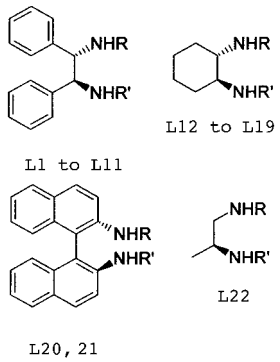
Herein, we describe a new HTS method for the simultaneous screening of multiple catalysts for the enantioselective reduction of ketones. This procedure, allows quantification of both yields and enatiomeric excess (*ee*) in an HTS format using inexpensive and commonly available equipment.

Our interest in this field was focused on the enantioselective reduction of α-keto acids by hydrogen transfer using chiral metal complexes. Although considerable effort was expended to develop applicable methods for the enantioselective preparation of α-hydroxy acids,[9] only a few papers[10] are devoted to the development of straightforward procedures involving the direct conversion of α-keto acids to chiral α-hydroxy acids. As a model reaction, we chose the enantioselective reduction of benzoyl formic acid (BF) into mandelic acid (MA). The catalyst library was prepared by combining a set of 22 chiral diamine-based ligands and four different metal species (Scheme 1).



Metal library:
M1 : $[RuCl_2(p\text{-cym})]_2$
M2 : $[RuCl_2(benz)]_2$
M3 : $[RhCl_2(Cp)]_2$
M4 : $[IrCl_2(Cp)]_2$

Ligand library:



L	R	R'
1	H	H
2	CF_3SO_2	H
3	$C_4F_9SO_2$	H
4	$4\text{-}CF_3C_6H_4SO_2$	H
5	$2,4,6\text{-}CH_3C_6H_2SO_2$	H
6	$4\text{-}CH_3C_6H_4SO_2$	H
7	$C_6F_5SO_2$	H
8	CF_3CO	H
9	C_6H_5CO	H
10	$4\text{-}CH_3C_6H_4SO_2$	$4\text{-}CH_3C_6H_4SO_2$
11	$C_6H_5CH_2CO_2$	$C_6H_5CH_2CO_2$
12	H	H
13	CF_3SO_2	H
14	$C_4F_9SO_2$	H
15	$4\text{-}CH_3C_6H_4SO_2$	H
16	$2,4,6\text{-}CH_3C_6H_2SO_2$	H
17	$4\text{-}CF_3C_6H_4SO_2$	H
18	CF_3SO_2	CF_3SO_2
19	CH_3SO_2	CH_3SO_2
20	H	H
21	CF_3SO_2	H
22	H	H

Scheme 1. Target reaction and catalyst library.

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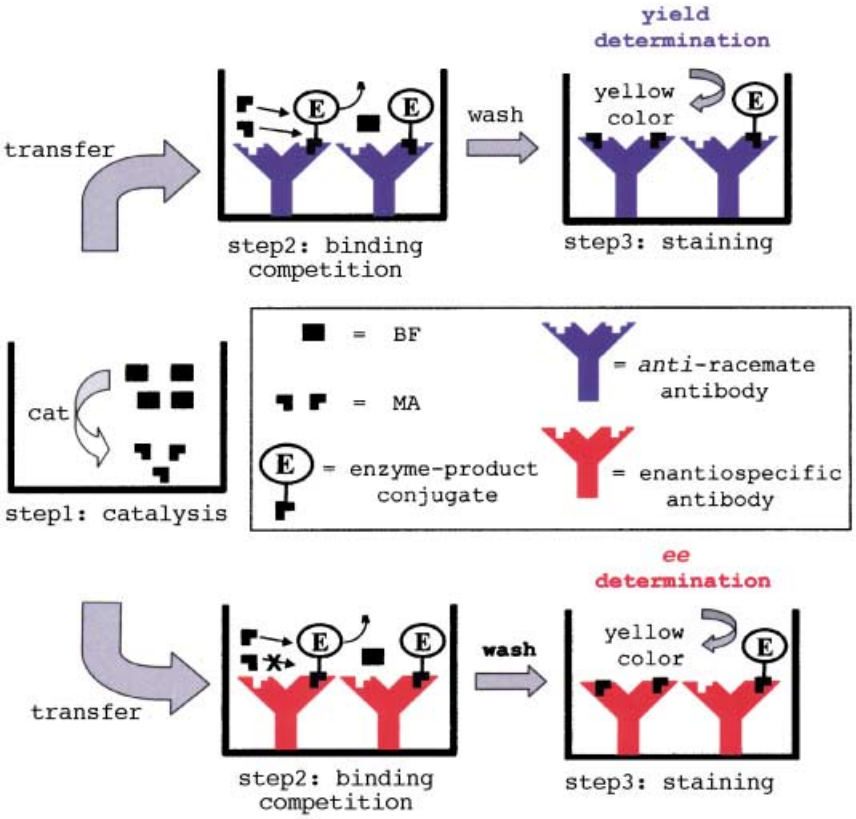

Figure 1. Schematic depiction of the HTS procedure using EIA.

For the screening of this library, we decided to use competitive enzyme immunoassays (EIA).[11] This powerful analytical method is based on the highly specific binding properties of antibodies which allow the direct measurement of the concentration of a given product in complex mixtures. Although EIA is widely used in the area of biology and diagnosis in particular, its application in the field of chemistry is not advanced. We envisioned that this analytical tool would be well adapted to measurement in an HTS format of both yield and enantiomeric excess of an enantioselective chemical transformation. By using an antibody that binds indiscriminately the two enantiomers of the product (MA for our model reaction) it should be possible to measure the concentration of the reaction product in the crude mixtures and thus to calculate the yield of the reactions. Determination of the enantiomeric excess would then be achieved by measuring the concentration of one enantiomer with an enantiospecific antibody. Thus, evaluation of one enantioselective catalyst will involve a three-phase procedure, the reaction itself and two jointly run EIA (Figure 1).

The crude catalyzed reaction mixture and a product–enzyme conjugate are added to a 96-well microtiter plate containing a specific *anti*-product antibody immobilized in the solid phase. Catalytic activity results in the formation of product that competes with the enzyme–product conjugate for antibody-binding sites. This leads to a decrease in absorbance (signal related to the solid-phase-bound enzyme activity), which is related to the concentration of product in the reaction mixture. With the use of simple and cheap automated equipment (plate washer and plate absorbance reader), thousands of catalysts can be screened easily per day.

Antibodies with appropriate binding specificity are of critical importance for the elaboration of our HTS technique. Stereoselective interaction between enantiomers and monoclonal or polyclonal antibodies is well known[12] and has recently been used in various applications.[13] For this purpose, we used monoclonal antibodies (mAbs) raised against hapten H3 (a vanillin mandelic acid analogue) that tightly bind a broad range of molecules with an MA moiety, as we previously reported.[14] We found that among these antibodies, two mAbs have the properties mentioned above. mAb-15 is an antibody that binds the two enantiomers of MA (relative affinity for (*S*)-MA = 6.4 mM; (*R*)-MA = 7 mM as determined by EIA experiments) equally well, whereas mAb-8 exhibits high stereoselectivity toward (*S*)-MA (relative affinity for (*S*)-MA = 55 μM, cross reactivity for (*R*)-MA < 0.1 %). Typical EIA calibration curves are presented in Figure 2.

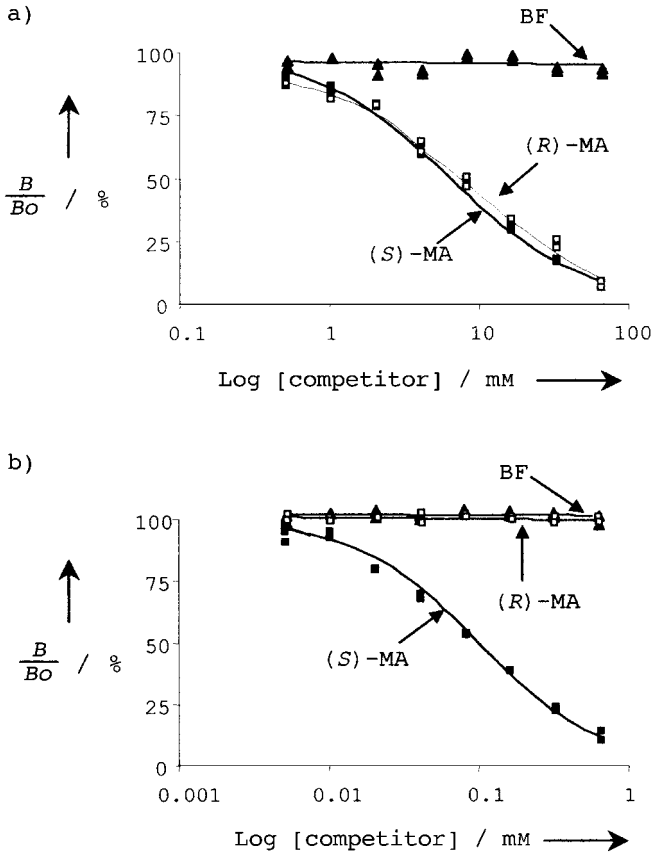

Figure 2. Antibody specificities. a) mAb-15; b) mAb-8. *B* and *Bo* represent the bound enzyme activity measured in the presence and absence of competitor, respectively.

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The ability of these mAbs to bind MA selectively allowed us to cover a large array of reaction conditions without any interference due to BF or to the catalyst library (cross-reactivities were less than 0.1 %). Having these analytical tools in hand, we performed the HTS on our catalyst library. The latter was prepared by heating the metal precursors and the ligands in DMF. Two classical hydrogen sources (HCOOH/tetraethylammonium (TEA) and *i*PrOH/KOH) were used to achieve the catalyses in DMF. Each distinct combination was run in one well of a 96-well microtiter plate (see Supporting Information). This leads to 176 different reactions which were screened in a few hours using our HTS method. Representative results are shown in Figure 3.

To validate our technique, we compared EIA and HPLC values on 42 representative samples from the crude catalyzed reactions. Good correlation was obtained for EIA and HPLC analysis (Figure 4) although a fairly broad variation in enantiomeric excess was observed (the precision of the *ee* determination was evaluated at $\pm 9\%$, see Supporting Information). Linear regression analyses gave the following equations: $EIA = 0.984\,HPLC - 0.569$, $r^2 = 0.971$, $n = 42$ and $EIA = 1.031\,HPLC - 0.464$, $r^2 = 0.932$, $n = 42$ for the yield and *ee* determination, respectively.

From a chemical point of view, some general trends can be drawn from this screening: the best results were obtained with HCOOH/TEA as hydrogen source, NaOH/iPrOH gave less than 30 % yields and poor *ee* values regardless of the catalyst used (results not shown). Thus, using HCOOH/TEA as hydrogen source, more than 50 % of the catalysts were very efficient in terms of yield but only 5 % of them gave an enatiomeric excess of over 60 % *ee*, underlining the need for a screening method that allows determination of both yield and *ee*. The best catalyst identified by our HTS technique is a combination of M1 and L2 in DMF/HCOOH/TEA. Under these conditions, the lead catalyst allows quantitative reduction of BF to MA with an enantiomeric excess of 81 % *ee* (MA(*S*). This result was confirmed by HPLC (yield = 98 %; 79 % *ee*) and reproduced on the mmol scale without significant decrease in yield or *ee*.

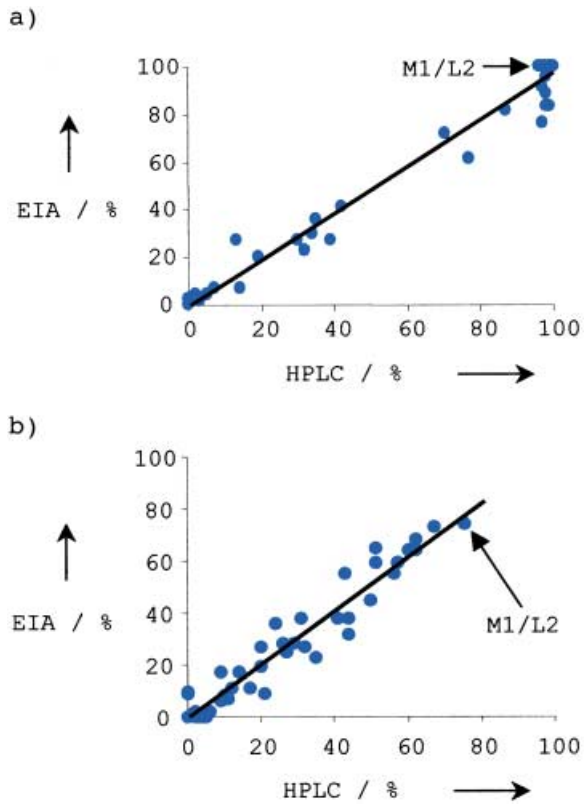

Figure 4. HPLC/EIA correlation. a) yields ; b) *ee* (*S*) values.

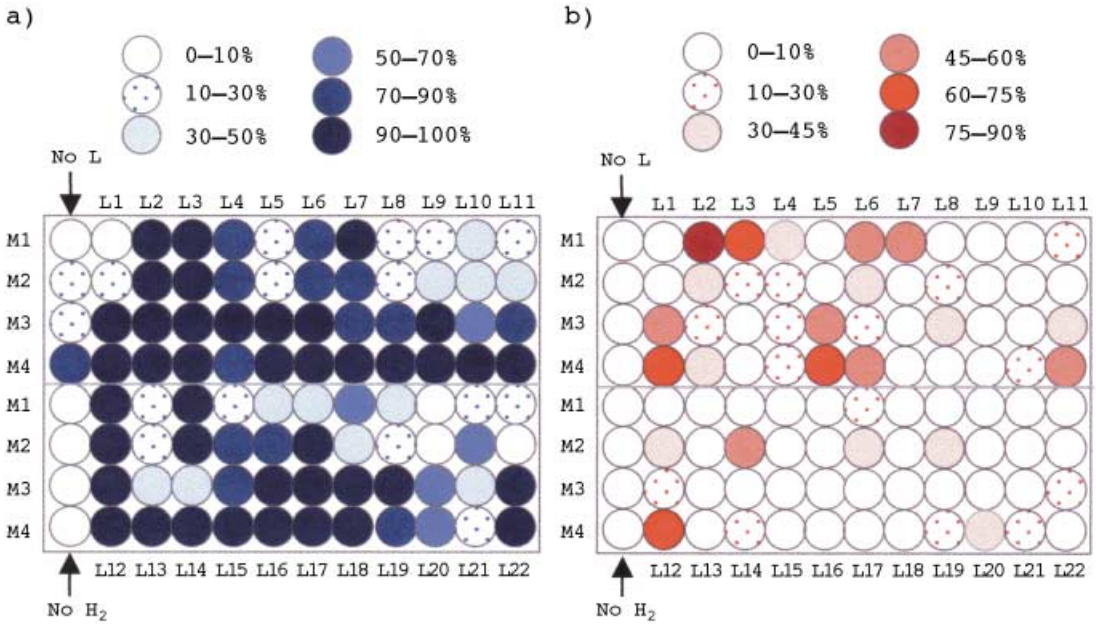

Figure 3. Screening of the catalyst library for the reduction of BF to (*S*)-MA. a) yields ; b) *ee* (*S*). Yields and *ee* values are presented in a color-coded format (blue for yields and red for *ee*). Catalyses were performed in DMF using HCOOH/TEA as hydrogen source and 1.6 % of chiral catalyst (metal/ligand ratio 1:1.2 in DMF) at room temperature for 14 h. The control experiments without hydrogen source or without ligand are given in the left rows.

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In conclusion, we have demonstrated the value of immunoassays for the development of highly efficient methods to screen enantioselective catalysts. The underlying principle of the procedure is general and should be successfully applied to any type of catalysis if one has antibodies with the required binding specificity. In fact, the immune system itself is a huge combinatorial machine providing a diverse set of specific binding molecules, and it is generally accepted that antibodies can be raised against virtually any compound of interest according to one's need. Furthermore, the use of antibodies for automated assays is routine and involves simple and time-saving experimental procedures. For these reasons, immunoassays appear to be effective ways of developing new high-throughput screening for the discovery of catalysts.

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Ordered Langmuir – Blodgett Films of Amphiphilic β-Hairpin Peptides Imaged by Atomic Force Microscopy**

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Langmuir and Langmuir – Blodgett (LB) films of peptides have a number of potential applications.[1–7] The fulfillment of this potential will depend on developing the molecular level understanding needed to control both the order within these films and their structures. Self-assembly in thin peptide films can be characterized by circular dichroism[8–10] and IR[11, 12] spectroscopies, fluorescence microscopy,[13–15] and at high resolution by grazing incidence X-ray diffraction (GIXD),[3, 14] and atomic force microscopy (AFM).[5, 16] Previous fluorescence microscopy results demonstrate that peptide **1** self-assembles into liquid- and solid-phase domains at the air – water (A – W) interface.[13] Here, we have used AFM with carbon nanotube tips to image LB films of **1** and its longer relative, **2**. The images show crystalline, two-dimensional order in films of **1** and **2** and demonstrate that the assembly parameters can be varied through well-defined changes in peptide structure.

The 14- and 18-residue peptides **1** and **2** both have a DPro-Gly sequence in the middle of alternating hydrophilic (Glu)

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