A Chemosensor-Based Approach to Catalyst Discovery in Solution and on Solid Support

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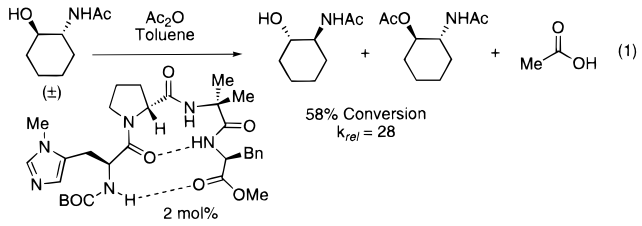
The discovery of new catalysts that mediate selective transformations is a fundamental goal in chemistry. Frequently, the discovery process involves the synthesis of many catalysts, followed by an evaluation of individual catalyst performance. More recently, however, new methods for the simultaneous evaluation of large numbers of catalysts have been reported.[1] In this regard, IR thermography,[2] colorimetric assays[3] and fluorescent substrates[4] have been utilized to select reactive catalysts from libraries of catalyst candidates. In addition, Reddington, et al. successfully applied fluorescent pH sensors to the combinatorial discovery of fuel cell anodes.[5] In the present paper, we describe a method for the simultaneous screening of multiple catalysts based on the use of a molecular sensor[6] which fluoresces upon detection of a desired reaction product. The present approach can be applied both in a spatially addressed solution-phase assay as well as in a single-bead−single-catalyst assay.[7−9]

Our interest in this area stems from our ongoing investigation of enantioselective acyl transfer catalysts. We recently reported that incorporation of nucleophilic groups (e.g., 3-alkylimidazoles) into peptides of well-defined structure produces asymmetric acylation catalysts that provided k_{rel} values of up to 28 for certain kinetic resolutions (eq 1).[10,11] Noteworthy during these studies was



(1)

the observation that the most selective catalysts typically afforded the fastest reactions; we attribute this trend to hydrogen bond-accelerated catalysis. Because these transformations produce an equivalent of acetic acid for each catalytic turnover, we surmised that a direct method of detection for this product would provide an effective and accurate means of monitoring catalytic turnover.

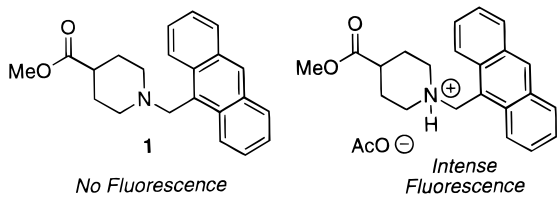


Figure 1. Acetic acid sensor in acyl transfer reactions.

To detect acetic acid evolution, we chose to employ an acid sensor based on aminomethylanthracene **1** (Figure 1). It is well-known that aminomethylanthracenes are pH sensitive fluorophores which undergo PET (photoinduced electron transfer) processes when in the free amine form, but fluoresce when protonated.[6b] Using fluorometry, we found that a toluene solution of 2-propanol (0.2 M), sensor **1** (0.25 mM), and acetic anhydride (0.2 M), upon treatment with 5 mol % of the acylation catalyst *N*-methylimidazole increases in fluorescence intensity as acyl transfer proceeds.[12] In contrast, when the same solution is allowed to stir in the absence of an acylation catalyst, the solution remains nonfluorescent, corresponding to no reaction.

An attractive feature of fluorescence-based assays is that it allows simultaneous monitoring of several reactions in a high-throughput format using a fluorescence plate reader. Thus, to demonstrate that **1** is capable of reporting relative rate information from several parallel acyl transfer experiments, we examined a variety of catalysts that are known to exhibit various degrees of catalytic activity. As shown in Figure 2, we compared seven catalysts at three different loadings (0.2, 0.6, and 1.8 mol %) for catalytic efficiency in the reaction shown in eq 1. (Only the 1.8 mol % data is shown. See the Supporting Information for the 0.2 mol % and 0.6 mol % plots.) Three replications of 21 uniform reaction mixtures containing equimolar amounts of racemic *trans*-1,2-acetamidocyclohexanol, acetic anhydride, and 0.05 mol % sensor **1** were deposited within a standard 96-well plate. Treatment of the solutions with the seven catalysts illustrated in Figure 2, followed by measurement of fluorescence intensity at various time intervals using a fluorescence plate reader, allowed for a direct readout of reaction rates (i.e., catalyst activity) as a function of acetic acid production. The results are shown graphically in Figure 2.

First, the well-known "super-acylation catalysts", PPY and DMAP,[13] emerged as the most active catalysts, while NMI, 3,4-lutidine, and pyridine gave very low reaction rates under the given conditions. More noteworthy, however, is the observation that the fluorescence assay distinguishes between NMI and the peptide-modified alkylimidazoles **2** and **3**. We have previously shown that peptide **2**, containing a D-Pro-Aib backbone, is efficient for kinetic resolutions of *trans*-1,2-acetamidocyclohexanol, exhibiting a k_{rel} of 28. Peptide **3**, containing an L-Pro-Aib backbone, is much less efficient for kinetic resolution and exhibits a k_{rel} of 3.1. Significantly, the fluorescence measurements are consistent with

(1) For reviews on the field of combinatorial catalysis, see: (a) Shimizu, K. D.; Snapper, M. L.; Hoveyda, A. H. *Chem.*−*Eur. J.* **1998**, *4*, 1885−1889. (b) Francis, M. B.; Jamison, T. F.; Jacobsen, E. N. *Curr. Op. Chem. Biol.* **1998**, *2*, 422−428.

(2) (a) Moates, F. C.; Somani, M.; Annamalai, J.; Richardson, J. T.; Luss, D.; Wilson, R. C. *Ind. Eng. Chem. Res.* **1996**, *35*, 4801−4803. (b) Taylor, S. J.; Morken, J. P. *Science* **1998**, *280*, 267−270. (c) Holzwarth, A.; Schmidt, H.-W.; Maier, W. F. *Angew. Chem., Int. Ed.* **1998**, *37*, 2644−2647 (d) Reetz, M. T.; Becker, M. H.; Kühling, K. M.; Holzwarth, A. *Angew. Chem., Int. Ed.* **1998**, *37*, 2647−2650.

(3) (a) Cooper, A. C.; McAlexander, L. H.; Lee, D.-H.; Torres, M. T.; Crabtree, R. H. *J. Am. Chem. Soc.* **1998**, *120*, 9971−9972. (b) Weingarten, M. W.; Sekanina, K.; Still, W. C. *J. Am. Chem. Soc.* **1998**, *120*, 9112−9113.

(4) Shaughnessy, K. H.; Kim, P.; Hartwig, J. F. *J. Am. Chem. Soc.* **1999**, *121*, 2123−2132.

(5) Reddington, E.; Sapienza, A.; Gurau, B.; Viswanathan, R.; Sarangapani, S.; Smotkin, E. S.; Mallouk, T. E. *Science* **1998**, *280*, 1735−1737.

(6) For comprehensive reviews of the field, see: (a) *Fluorescent Chemosensors for Ion And Molecule Recognition*; Czarnik, A. W., Ed.; American Chemical Society: Washington, DC, 1993. (b) de Silva, A. P.; Gunaratne, H. Q. N.; Gunnlaugsson, T.; Huxley, A. J. M.; McCoy, C. P.; Rademacher, J. T.; Rice, T. E. *Chem. Rev.* **1997**, *97*, 1515−1566.

(7) For a review of the "one-bead−one compound" combinatorial library method, see: Lam, K. S.; Lebl, M.; Krchnák, V. *Chem. Rev.* **1997**, *97*, 411−448.

(8) For an example of a single-bead−single-catalyst library being directly assayed for catalytic activity, see ref 2b.

(9) For an example of a fluorescence assay used to monitor enzymatic activity, see: Van Arman, S. A.; Czarnik, A. W. *J. Am. Chem. Soc.* **1990**, *112*, 5376−5377.

(10) (a) Miller, S. J.; Copeland, G. T.; Papaioannou, N.; Horstmann, T. E.; Ruel, E. M. *J. Am. Chem. Soc.* **1998**, *120*, 1629−1630. (b) Copeland, G. T.; Jarvo, E. R.; Miller, S. J. *J. Org. Chem.* **1998**, *63*, 6784−6785.

(11) For representative, nonenzymatic enantioselective acylation catalysts, see: (a) Vedejs, E.; Chen, X. *J. Am. Chem. Soc.* **1996**, *118*, 1809−1810. (b) Vedejs, E.; Daugulis, O.; Diver, S. T. *J. Org. Chem.* **1996**, *61*, 430−431. (c) Ruble, J. C.; Tweddell, J.; Fu, G. C. *J. Org. Chem.* **1998**, *63*, 2794−2795. (d) Kawabata, T.; Nagato, M.; Takasu, K.; Fuji, K. *J. Am. Chem. Soc.* **1997**, *119*, 3169−3170. (e) Oriyama, T.; Imai, K.; Hosoya, T.; Sano, T. *Tetrahedron Lett.* **1998**, *39*, 397−400.

(12) The reaction was simultaneously monitored by gas chromatography.

(13) Höfle, G.; Steglich, W.; Vorbrüggen, H. *Angew. Chem., Int. Ed. Engl.* **1978**, *17*, 569−583.

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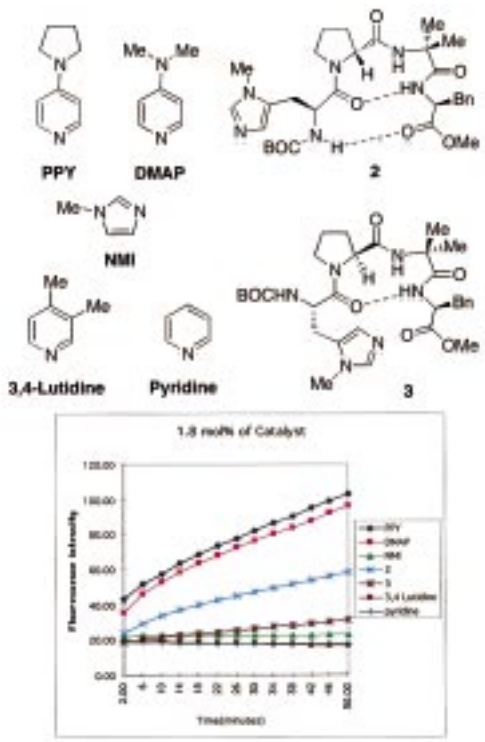


Figure 2.

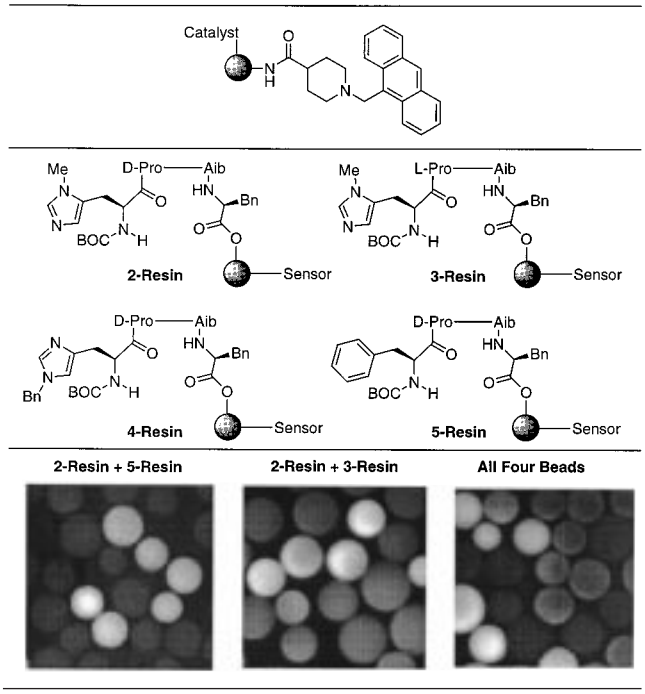
these observations; the catalytic activity of **2** was found to be substantially greater than either **3** or **NMI**. Furthermore, peptide **3** is only slightly more active than **NMI**, a catalyst which affords no opportunity for hydrogen bond-accelerated catalysis. It may be that the different stereochemical arrays afforded by **2** and **3** provide differing opportunities for the key H-bonds between substrate and catalyst. Most importantly, fluorescence provides an excellent opportunity to screen for this subtle type of rate acceleration, which may be coupled to enantioselectivity for the several peptide-based catalysts we have examined.[14]

We then sought to adapt the sensor to experiments designed to identify highly effective catalysts during a simultaneous single-bead−single-catalyst screening protocol. We covalently attached the sensor to Wang resin which was simultaneously functionalized with potential catalysts (Table 1). Covalent attachment of both sensor and catalyst to the same bead sets up a situation in which beads with the most reactive catalysts should light up to the greatest extent in the presence of alcohol and acetic anhydride. Beads with sensor alone, or with weak acyl transfer catalysts, should remain dark in the presence of alcohol and acetic anhydride. The approach is predicated on diffusion of acetic acid between beads being a slow process relative to reaction monitoring.

Employing standard solid-phase synthesis techniques, we prepared polystyrene resin beads with a uniform loading of aminomethylanthracene **1** (methoxy replaced with phenylalanine linker). As shown in Table 1, one set of such beads was functionalized with the BOC-π(Me)His-D-Pro-Aib-Phe sequence (**2-Resin**) analogous to soluble catalyst **2**. A second set was derivatized with the identical loading of BOC-π(Me)His-L-Pro-Aib-Phe (**3-Resin**) analogous to soluble catalyst **3**. A third set was prepared with a sterically deactivated histidine derivative (**4-Resin**), and a control set of beads was prepared without a histidine moiety (**5-Resin**). When each of these sets of beads was exposed to identical reaction conditions in segregated vessels, fluorescence micrographs indicated (i) that bead intensity was uniform and (ii) the relative intensity of the beads paralleled the reactivity trends that we had observed in the solution studies. In particular,

(14) The correlation between absolute rate and enantioselectivity may not be completely general. Further studies along these lines are underway.-

Table 1. Fluorescence Micrographs Showing Beads upon Exposure to Reaction Conditions[a]



[a] Fluorescence micrographs of the spatially separated bead sets are included in the Supporting Information.

beads functionalized with **2** (**2-Resin**) fluoresced more strongly than those functionalized with **3** (**3-Resin**). Fluorescence from the weakly catalytically active **4-Resin** was substantially less intense, and **5-Resin** was essentially nonfluorescent.[15,16] Importantly, pairwise mixing of beads followed by subjection to the reaction conditions indicated that highly catalytically activated beads can be distinguished from catalytically inert beads while the beads were coexisting in the same vessel (**2-Resin** + **5-Resin**). Remarkably, pairwise mixing of epimeric beads (**2-Resin** + **3-Resin**) also revealed two sets of unique beads in a single reaction vessel. Finally, completely analogous results are obtained when all four resins are mixed together in the same reaction vessel. The results suggest that this approach may be suitable for the screening of single-bead−single-catalyst libraries using either fluorescence microscopy or fluorescence-activated bead sorting.[17] Efforts along these lines are ongoing.

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Supporting Information Available: Characterization data for all compounds and experimental details for their preparation. Descriptions of kinetic runs with data and fluorescence micrographs for individual experiments (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(15) Spatially segregated samples of each set of resin beads were nonfluorescent in the absence of alcohol and acetic anhydride.

(16) Control experiments indicate that either individual or pooled samples of these beads treated with uniform amounts of acetic acid exhibit essentially uniform levels of fluorescence. See Supporting Information for details.-

(17) Needels, M. C.; Jones, D. G.; Tate, E. H.; Heinkel, G. L.; Kochersberger, L. M.; Dower, W. J.; Barrett, R. W.; Gallop, M. A. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 10700−10704.