Selection of Enantioselective Acyl Transfer Catalysts from a Pooled Peptide Library through a Fluorescence-Based Activity Assay: An Approach to Kinetic Resolution of Secondary Alcohols of Broad Structural Scope

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Abstract: An assay employing a fluorescently labeled split and pool peptide library has been applied to the discovery of a new class of octapeptide catalysts for the kinetic resolution of secondary alcohols. A highly diverse library of peptide-based catalysts was synthesized on solid-phase synthesis beads such that each individual bead was co-functionalized with (i) a uniform loading of a pH-sensitive fluorophore and (ii) a unique peptide-based catalyst. The library was then screened for activity in acylation reactions employing (±)−*sec*-phenylethanol as the substrate and acetic anhydride as the acylation agent. From the most active catalysts, a lead peptide (**4**) was identified that provides a selectivity-factor (k_{rel}) of 8.2 upon resynthesis and evaluation under homogeneous conditions. A "directed" second-generation split and pool peptide library was synthesized such that the new peptide sequences in the library were biased toward the lead structure. Random samples of the second generation library were screened in single bead assays that revealed several new peptide-based catalysts that afford improved selectivities in kinetic resolutions. Peptide catalyst **13** proves effective for the kinetic resolution of *sec*-phenylethanol ($k_{rel} = 20$), as well as eight other secondary alcohols of a broad substrate scope ($k_{rel} = 4$ to >50).

Introduction

The discovery of small molecules that function as efficient catalysts for synthetically important reactions stands as a major challenge for chemists. Techniques that allow for the simultaneous screening of numerous catalysts hold promise for the field since they may allow for accelerated catalyst discovery.[1,2] Such approaches also have the potential to mimic selection processes evolved by natural systems, where evolution of complex chemical systems proceeds by iterative screening and selection protocols. Two problems among many to be addressed if the full potential of diversity-oriented catalyst discovery is to be realized are the following: (a) the synthesis of genuinely diverse catalyst libraries and (b) the identification of the most promising catalysts from pooled mixtures containing many less interesting ones.

We have been focusing on peptide-based catalyst libraries for several reasons, including a general interest in the minimal structural basis of a polypeptide that is required to achieve efficient enantioselective catalysis.[3,4] In addition, peptides offer the practical advantage that both the theoretical and actual diversity of catalyst libraries is massive using the split-and-pool synthesis strategy.[5] For catalyst selection, we have been developing fluorescence-based activity assays that allow localization of a fluorescent signal on a synthesis bead that carries upon it an active, unique acyl transfer catalyst.[6,7] Selection of the brightest beads from a pooled mixture subjected to reaction conditions thus allows the identification of the most active catalysts in a library.[8]

We had previously found that peptides containing the nucleophilic *N*-methylimidazole (**NMI**) moiety are effective catalysts for the kinetic resolution of certain secondary alcohols.[9]

(1) Jandeleit, B.; Schaefer, D. J.; Powers, T. S.; Turner, H. W.; Weinberg, W. H. *Angew. Chem., Int. Ed.* **1999**, *38*, 2494−2532.

(2) For several recent reviews of combinatorial catalysis, see: (a) Reetz, M. T. *Angew. Chem., Int. Ed.* **2001**, *40*, 284−310. (b) Crabtree, R. H. *Chem. Commun.* **1999**, *17*, 1611−1616. (c) Kuntz, K. W.; Snapper, M. L.; Hoveyda, A. H. *Curr. Opin. Chem. Biol.* **1999**, *3*, 313−319. (d) Francis, M. B.; Jamison, T. F.; Jacobsen, E. N. *Curr. Opin. Chem. Biol.* **1998**, *2*, 422−428.

(3) For an overview of enzymatic catalysts and mechanisms, see: (a) Wong, C.-H.; Whitesides, G. M. *Enzymes in Synthetic Organic Chemistry*; Elsevier Science Ltd.: Oxford, 1994; Chapter 2. (b) Lamzin, V. S.; Dauter, Z.; Wilson, K. S. *Curr. Opin. Struct. Biol.* **1995**, *5*, 830−836.

(4) Jarvo, E. R.; Copeland, G. T.; Papaioannou, N.; Bonitatebus, P. J.; Miller, S. J. *J. Am. Chem. Soc.* **1999**, *121*, 11638−11643.

(5) (a) Furka, A.; Sebestyen, F.; Asgedom, M.; Dibo, G. *Int. J. Pept. Protein Res.* **1991**, *37*, 487−493. (b) Lam, K. S.; Salmon, S. E.; Hersh, E. M.; Hruby, V. J.; Kazmierski, W. M.; Knapp, R. J. *Nature* **1991**, *354*, 82−84. (c) 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.

(6) (a) Copeland, G. T.; Miller, S. J. *J. Am. Chem. Soc.* **1999**, *121*, 4306−4307. (b) Harris, R. F.; Nation, A. J.; Copeland, G. T.; Miller, S. J. *J. Am. Chem. Soc.* **2000**, *122*, 11270−11271.

(7) For other fluorescence-based assays of catalyst libraries, see: (a) Reddington, E.; Sapienza, A.; Gurau, B.; Viswanathan, R.; Sarangapani, S.; Smotkin, E. S.; Mallouk, T. E. *Science* **1998**, *280*, 1735−1737. (b) Shaughnessy, K. H.; Kim, P.; Hartwig, J. F. *J. Am. Chem. Soc.* **1999**, *121*, 2123−2132. (c) Yeung, E. S.; Su, H. *J. Am. Chem. Soc.* **2000**, *122*, 7422−7423.

(8) For several examples of other studies where catalysts of interest have been identified from pooled mixtures of catalysts, see: (a) Francis, M. B.; Jacobsen, E. N. *Angew. Chem., Int. Ed.* **1999**, *38*, 937−941. (b) Berkessel, A.; Herault, D. A. *Angew. Chem., Int. Ed.* **1999**, *38*, 102−105. (c) Taylor, S. J.; Morken, J. M. *Science* **1998**, *280*, 267−270.

(9) For reviews of catalytic kinetic resolution, see: (a) Keith, J. M.; Larrow, J. F.; Jacobsen, E. N. *Adv. Synth. Catal.* **2001**, *343*. 5−26. (b) Hoveyda, A. H.; Didiuk, M. T. *Curr. Org. Chem.* **1998**, *2*, 537−574.

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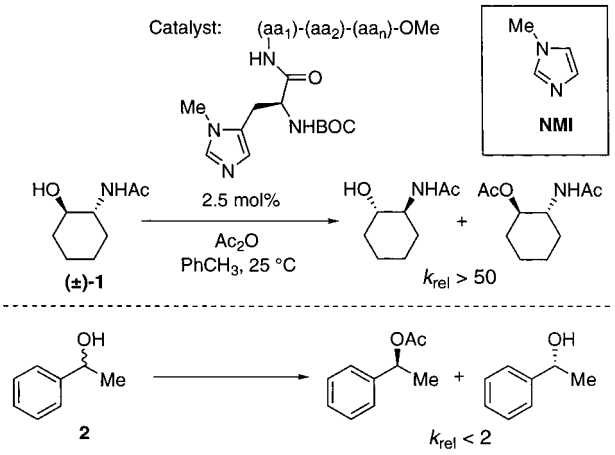
Scheme 1

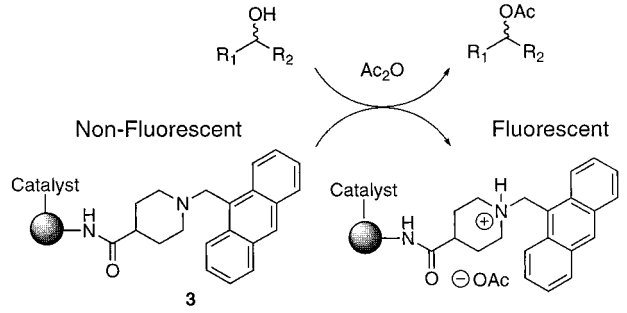


Kinetic resolutions of functionalized substrates such as (±)-**1** (Scheme 1) can be achieved with $k_{rel} > 50$ (recovered starting material, 96% ee at 52% conversion).[10−13] In particular, amide-functionalized alcohols that have the potential to form amide-based hydrogen bonds to the catalyst have been among the best substrates we have studied. To expand the scope of the catalysts to include resolutions of *unfunctionalized* substrates (i.e., substrates lacking additional hydrogen bond donors and acceptors), we set out to screen a highly diverse library of potential catalysts. Notably, at the outset of the study, we were unclear as to what peptide sequences (if any) would be optimal for the kinetic resolution of this class of substrates—none that we had prepared previously exhibited appreciable selectivity in attempted resolutions of alcohols such as **2** (Scheme 1). This lack of selectivity was a particular concern since these new substrates would lack functional groups that could lead to obvious multipoint contacts with the catalyst during the stereodifferentiating step of the reaction. Herein, we disclose the results of this study, which include the discovery of a new set of octapeptide catalysts that are effective for the kinetic resolution of a number of unfunctionalized substrates.

Results and Discussion

Assay, Library Design, and Screening. The assay that we have described previously relies upon proton-activated fluorescence within a bead such as that illustrated by structure **3** (Scheme 2).[6,14] Thus, as an acylation reaction between an alcohol

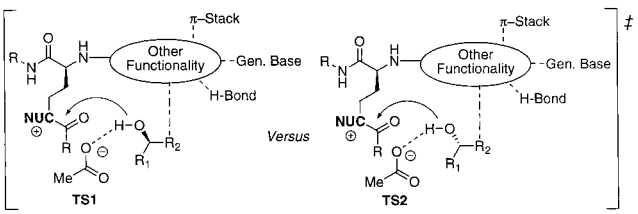
Scheme 2



and acetic anhydride proceeds, generation of product (ester + acetic acid) is signaled by an increase in fluorescence intensity. A key feature of this assay, within the context of enantioselective acylation reactions, is that the fluorescence signal is coupled to catalyst *activity*, but not the degree of *enantioselectivity* that a particular catalyst may afford. Nevertheless, we formulated a hypothesis that selection of catalysts based on activity alone might lead to catalysts that also exhibited good degrees of selectivity.

Our hypothesis was predicated on the fact that each of the active catalysts will exhibit a level of activity associated with the core alkylimidazole resident in each peptide. (Alkylimidazoles are fully competent catalysts in the absence of the peptide.[15]) Then, the most active of the catalysts would exhibit an additional increment of rate acceleration that could arise from a secondary effect in the rate-determining transition state (TS1 versus TS2, Scheme 3). The increment could come from a well-placed hydrogen bond, a π-stacking interaction, favorable ion-pairing geometries, etc. The essential feature of the fastest catalysts therefore could be a multi-point contact between catalyst and substrate in the rate-determining TS. Therefore, we speculated that since the peptide-based catalysts are chiral and optically pure, and the substrates are racemic, on average the rate accelerations would be accompanied by enantioselectivity. Screening catalysts *en masse* could allow a means of evaluating the hypothesis: that selection of catalysts that exhibit substantial rate acceleration would lead to catalysts that also exhibited appreciable enantioselectivities. Of course, this approach could select for catalysts that accelerate both enantiomers of a racemate equally, leading to low k_{rel} values for some catalysts that also turn out to be highly active. However, such a screening approach substantially narrows the field of catalyst candidates, focusing our investigation exclusively on those that are highly active.

Scheme 3



Our library design is illustrated below (Figure 1). Specifically, an octapeptide format was selected wherein the first and last amino acids were held constant with alanine and π-(Me)-histidine (Pmh), respectively. Fourteen unique amino acid

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

(11) For reports of techniques that allow high throughput assays for enantioselectivity of each catalyst within a library, see: (a) Korbel, G. A.; Lalic, G.; Shair, M. D. *J. Am. Chem. Soc.* **2001**, *123*, 361−362. (b) Guo, J.; Wu, J.; Siuzdak, G.; Finn, M. G. *Angew. Chem., Int. Ed.* **1999**, *38*, 1755−1758. (c) Reetz, M. T.; Becker, M. H.; Klein, H.-W.; Stockigt, D. *Angew. Chem., Int. Ed.* **1999**, *38*, 1758−1761.

(12) For alternative representative nonenzymatic catalysts that effect kinetic resolution of racemic alcohols, see: (a) Fu, G. C. *Acc. Chem. Res.* **2000**, *33*, 412−420. (b) Vedejs, E.; Daugulis, O. *J. Am. Chem. Soc.* **1999**, *121*, 5813−5814. (c) Spivey, A. C.; Fekner, T.; Spey, S. E. *J. Org. Chem.* **2000**, *65*, 3154−3159. (d) Sano, T.; Miyata, H.; Oriyama, T. *Enantiomer* **2000**, *5*, 119−123. (e) Kawabata, T.; Nagato, M.; Takasu, K.; Fuji, K. *J. Am. Chem. Soc.* **1997**, *119*, 3169−3170.

(13) *S*-values (=k_{rel}) were calculated according to the method of Kagan. See: Kagan, H. B.; Fiaud, J. C. *Top. Stereochem.* **1988**, *18*, 249−330.

(14) For pioneering studies on the use of aminomethylanthracenes as pH and metal-ion sensors, 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.

(15) Nucleophilic versus general base catalysis with alkylimidazoles has been a subject of some debate. We have adopted the nucleophilic paradigm for this analysis. (a) Guibe-Jampel, E.; Bram, G.; Vilkas, M. *Bull. Soc. Chim. Fr.* **1973**, 1021−1027. (b) Höfle, G.; Steglich, W.; Vorbrüggen, H. *Angew. Chem., Int. Ed. Engl.* **1978**, *17*, 569−583. (c) See: Pandit, N. K.; Connors, K. A. *J. Pharm. Sci.* **1982**, *71*, 485−491.

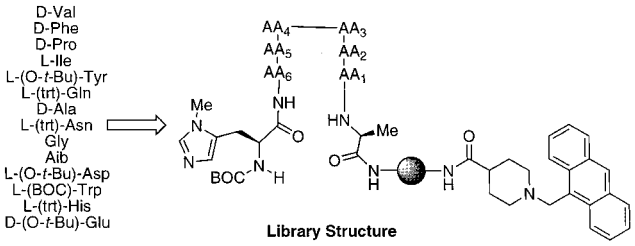


Figure 1. Library design and composition.

monomers were then incorporated in the remaining six positions using the split-and-pool method. The theoretical diversity of such a library (14^6) is 7.5 million unique catalysts.[16] We employed 5 g of 400−500 μm beads in our synthesis; therefore, we estimate that our library contained approximately 100,000 unique peptide catalysts on beads containing a uniform loading of fluorophore.[17]

A sample of the library was selected at random and screened for the reaction of racemic *sec*-phenylethanol (**2**). Specifically, about 1000 catalysts were screened conveniently over the course of a 60 min period. From the assay (Figure 2a), 27 of the brightest beads were manually selected, and 16 of the least bright beads were selected for secondary screening. The beads were then washed and subjected to single bead kinetic resolutions, with encouraging results. In the single-bead, on-resin assay, the k_{rel} values of the brightest beads were, on average, higher than those observed when the darker beads were employed as catalysts (Figure 2b). Since, in our experience, the enantioselectivities exhibited by the peptide-based catalysts we have been studying tend to be lower when the catalysts are immobilized,[18] and in particular in single bead assays, we sought to determine the structures of the most interesting catalysts, and then to screen them under more favorable homogeneous conditions.

Catalyst Identification and Validation. The structures of the most selective catalysts were determined by submitting the beads to single bead Edman degradation analysis.[19] The sequences that were obtained for two of the peptides are shown in Figure 3a. Resynthesis and rescreening of these peptide catalysts (2.5 mol %) were then carried out under homogeneous conditions. Kinetic resolutions were performed that verified that selective catalysts had indeed been obtained. In particular, peptide **4** exhibits a $k_{rel} = 8.2$ for the resolution of *sec*-phenylethanol **2** (−65 °C, 40% conversion). Peptide **5** affords a $k_{rel} = 5.9$ in the same resolution, but preferentially acylates the opposite enantiomer of the racemic substrate, despite possessing the same absolute configuation of the key nucleophilic amino acid. Furthermore, these results stand in contrast to the best previously disclosed peptide-based asymmetric acylation catalysts from our laboratory; none was found to be selective ($k_{rel} < 1.8$) with substrate **2** under optimized conditions.

In addition to the appreciable enantioselection exhibited by catalysts **4** and **5**, their exceptional catalytic *activity* is of note. In particular, the reaction rate exhibited by peptide **4** (toward the faster reacting enantiomer) is substantially greater than that of *N*-methylimidazole (NMI), highlighting the fact that the enantioselectivity observed is due to accelerative effects. Indeed, for the fast reacting enantiomer, peptide **4** exhibits activity greater than *N*,*N*-(dimethylamino)pyridine (DMAP), which is known to be substantially more active than NMI (Figure 3b).[15]

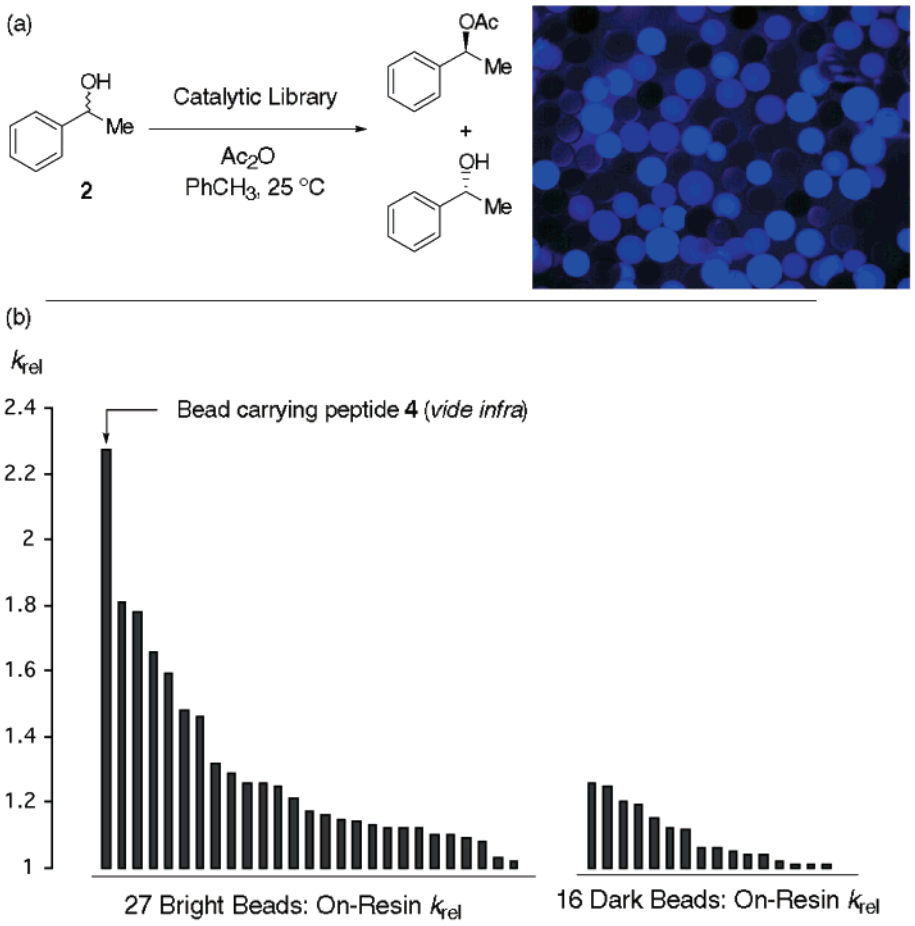


Figure 2. (a) Fluorescence micrograph showing distribution of bead intensities during catalytic reaction. (b) On-resin k_{rel} data for single bead assays. Data for the "bright" beads are to the left; data for the "dark" beads are to the right.

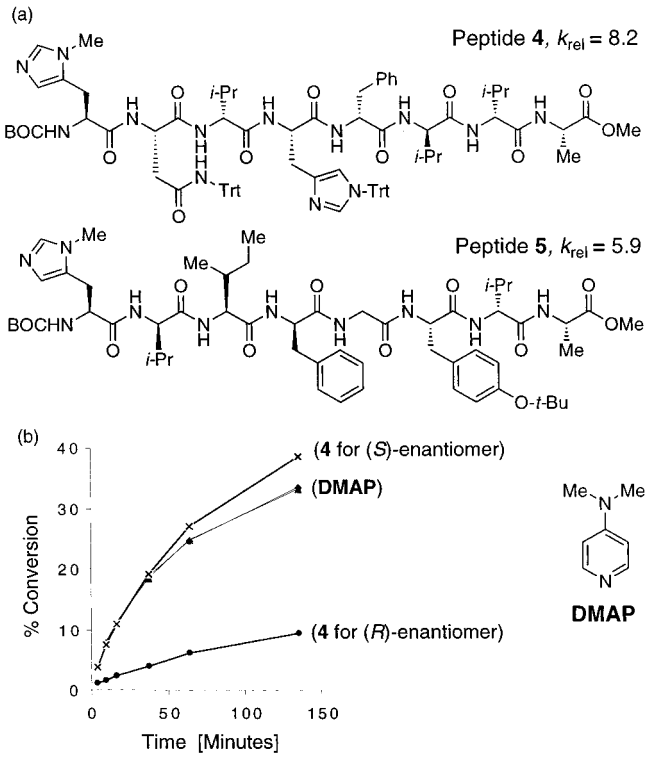


Figure 3. (a) New peptide catalysts that exhibit appreciable k_{rel} values for unfunctionalized substrate **2**. (b) Plot verifying the high activity of peptide **4** relative to **DMAP**.

These observations underscore the fact that the fluorescence-based activity screen is effective in identifying highly active catalysts.

Directed Library Synthesis and Screening. With catalyst **4** identified from the fluorescence-based activity screen, we sought to identify catalysts that would provide enhanced degrees of selectivity for the kinetic resolution of the unfunctionalized substrates such as *sec*-phenylethanol. For this goal, we synthesized a second generation split and pool peptide library, such that the members were *biased* toward the structure of the best "hit" structure (**4**). The new library was sufficiently different, however, to allow for the identification of improved catalysts.[20] Experimentally, a split and pool library was synthesized wherein the beads were partitioned unevenly at each split step: 65% of the beads were functionalized with the residue corresponding to that of parent peptide **4** at each position. The remaining 35% of the beads were divided into 14 equal portions, and coupled with 14 unique amino acid monomers.[21] Accordingly, a new

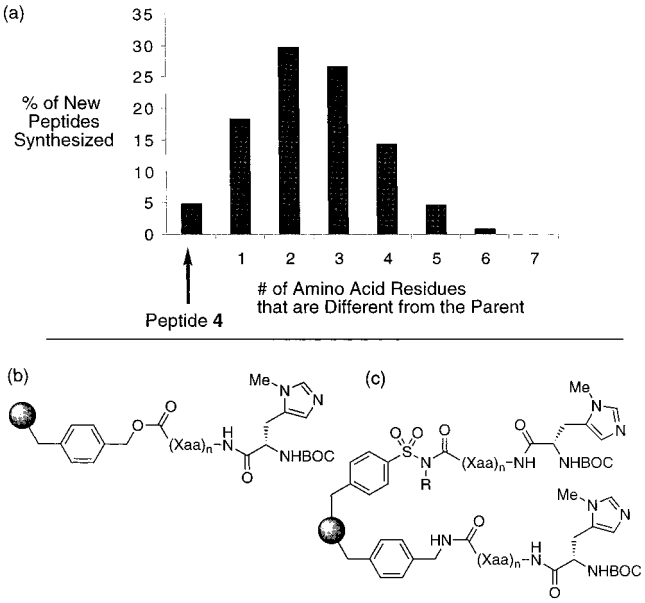


Figure 4. (a) Theoretical composition of the second-generation split/pool library. (b) Structure of the noncleavable library to be screened "on-resin." (c) Format of the co-functionalized library. The strand with the safety catch linker is cleaved into the multi-well plate for screening. The other strand is retained on the bead for single bead Edman degradation.

library was obtained that possessed the theoretical composition shown in Figure 4a. Of the approximately 6,000 unique structures that would be represented,[22] 5% were likely to be identical to the parent structure, providing an important internal control. Eighteen percent of the library would differ from the parent by only one amino acid, but that residue might be inserted anywhere in the sequence (except the final residue which was always BOC-Pmh). Thirty percent would differ from the parent by two residues, and so on, as indicated in Figure 4a.

The second generation library was independently synthesized twice so that single bead screening experiments could be conducted with two different formats. In both cases, a random sample of the biased library was screened in a multi-well format. In the first synthesis, the Wang linker was used to prepare the second generation library. Single bead assays were then conducted in each well of a multi-well plate with the catalyst immobilized on the bead (Figure 4b). In the second case, the library was synthesized with beads that were co-functionalized with both a permanent, noncleavable linker and also the safety catch linker,[23] such that catalyst samples could be cleaved directly into a multi-well plate (Figure 4c). This latter approach would allow for "single bead" screening of peptides, but under homogeneous conditions. It was hoped that this latter approach might provide a more realistic prediction of how the catalysts derived from a single bead might perform once they were resynthesized, purified, and rescreened under homogeneous conditions. For this latter format of the second generation library, cleavage was accomplished such that the *C*-terminal *N*-propyl amide was obtained.

The single bead assays were carried out under kinetic resolution conditions where *sec*-phenylethanol (**2**) was used as the substrate. Reactions were quenched after a uniform period of time to allow for a comparison of the relative activity and selectivity afforded by each catalyst. A conventional chiral GC

(16) This value is achieved by the randomization of 14 monomers in 6 unique positions ($=14^6$).

(17) The 400−500 μm beads contain ~20−25 beads per mg. 5 g of beads therefore contains 100 000 to 125 000 beads.

(18) For representative examples of resin-bound catalysts that afford similar selectivities on-resin and in solution, see: (a) Clapham, B.; Cho, C. W.; Janda, K. D. *J. Org. Chem.* **2001**, *66*, 868−873. (b) Shimizu, K. D.; Cole, B. M.; Krueger, C. A.; Kuntz, K. W.; Snapper, M. L.; Hoveyda, A. H. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 1703−1707.

(19) Single bead Edman degradations were carried out by Midwest Analytical (St. Louis, MO).

(20) (a) This technique is related to the "Directed Evolution" approach to enzyme optimization. See: Arnold, F. H. *Acc. Chem. Res.* **1998**, *31*, 125−131 and references therein. (b) Liebeton, K.; Zonta, A.; Schimossek, K.; Nardini, M.; Lang, D.; Dijkstra, B. W.; Reetz, M. T.; Jaeger, K.-E. *Chem. Biol*. **2000**, *7*, 709−718.

(21) Figure 4a specifically relates to the version of the library that was synthesized with the safety catch linker. The version of the library employing the Wang linker involved a *C*-terminus that was fixed as alanine, and was consequently variable at only six positions. See Supporting Information for details.

(22) The library size is limited by the number of beads used in the split and pool synthesis. See Supporting Information for details.

(23) Backes, B. J.; Virgilio, A. A.; Ellman, J. A. *J. Am. Chem. Soc.* **1996**, *118*, 3055−3056.

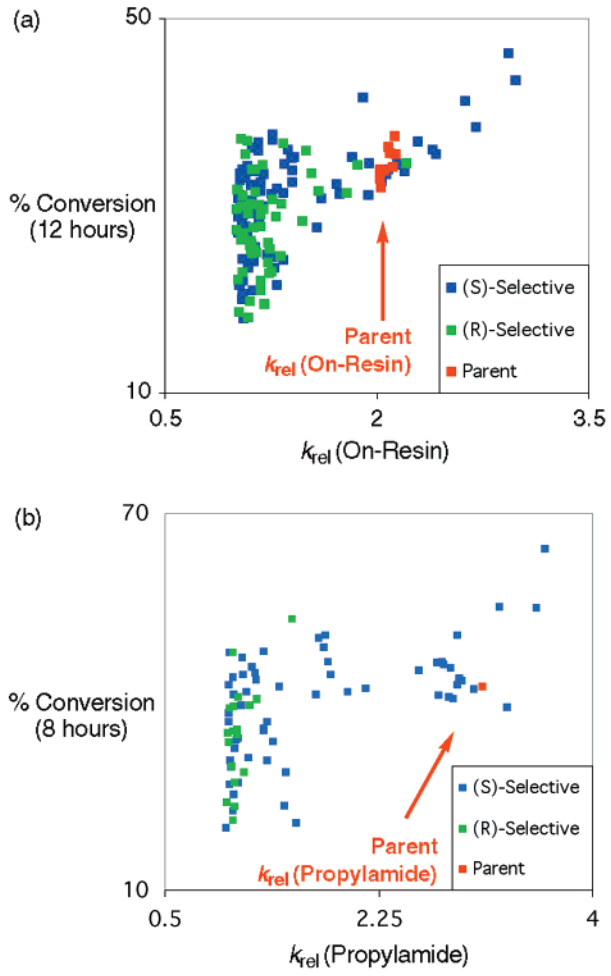
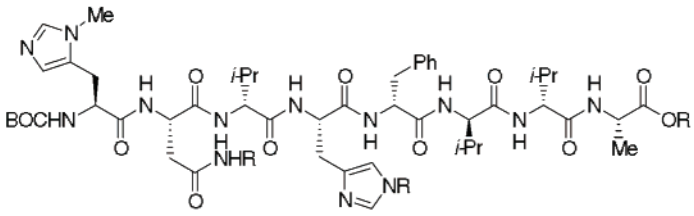


Figure 5. Plots of "activity vs selectivity" for kinetic resolutions of *sec*-phenylethanol employing the second generation split and pool peptide library. The two plots were obtained from two directed libraries independently synthesized and independently screened.

assay was employed for this purpose. The results obtained with the second generation library are displayed in Figure 5. Figure 5a depicts the activity and selectivity afforded by the catalysts that were screened while still immobilized on the beads (i.e., the library shown in Figure 4b). Figure 5b shows the data for the library that was subjected to cleavage such that the *C*-terminal *N*-propyl amides were deposited into wells, and screened in solution (i.e., the library depicted in Figure 4c). A number of observations are worthy of note: (i) These data unambiguously reveal new catalysts of both improved absolute activity and improved enantioselectivity relative to the parent peptide (**4**). (ii) The majority of the library consists of catalysts which afford k_{rel} values of near unity for the kinetic resolution of *sec*-phenylethanol. This is perhaps expected since the majority of peptide-based catalysts in this library differ substantially from the parent structure upon which the library was based (cf., Figure 4a). (iii) In both formats (Figure 5a,b), the second generation library reveals a modest trend among those catalysts that are approximately as selective, *or more so*, than the parent: there appears a loose correlation between the more active and the more selective of the catalysts. (iv) As a control, shown in red in Figure 5a is a cluster of catalysts that are identical in structure to the parent peptide sequence (**4**). Notably, these exhibit on-resin k_{rel} values of ~2.0 for the kinetic resolution, providing an important point of calibration. (v) The second generation directed library also contained a number of catalysts that preferentially acylate the opposite enantiomer of the substrate, but with lower selectivity. (Catalysts selective for the (*S*)-enantiomer are shown with blue dots; catalysts selective for the (*R*)-enantiomer are denoted with green dots.)

Directed Library Results. Because the single bead assays afforded catalysts exhibiting enhanced enantioselectivity relative to the parent (**4**), it became critical to validate the data shown in Figure 5 through catalyst identification and rescreening. Four of the most selective beads from each format of the directed library were submitted to single bead Edman degradation to determine the structures of the catalysts. The sequences of the eight new peptides are shown in Table 1. Each sequence was then independently synthesized as the *C*-terminal methyl ester

Table 1. Sequences for Eight New Peptides Selected from the Second Generation Libraries and Kinetic Resolution Data for *sec*-Phenylethanol



									Single Bead Screen k_{rel}[a]	Solution k_{rel}[b]
Parent Sequence (**4**):	Pmh	L-(trt)Asn	D-Val	L-(trt)His	D-Phe	D-Val	D-Val	L-Ala	2.1 (On-Resin) 3.1 (Propyl amide)	8.2
6	Pmh	L-(trt)Asn	D-Val	L-(trt)His	D-Pro	D-Val	L-Leu	L-Ala	3.0	7.9
7	Pmh	L-(tBu)Asp	D-Val	L-(trt)His	D-Ala	D-Val	L-(trt)His	L-Ala	2.6	8.1
8	Pmh	L-(trt)Asn	D-Val	L-(trt)His	D-Phe	D-Val	L-(tBu)Asp	L-Ala	2.7	8.4
9	Pmh	L-(tBu)Thr	D-Val	L-(trt)His	D-Phe	D-Val	L-(trt)His	L-Ala	2.9	9.6
10	Pmh	L-(trt)Asn	D-Val	L-(trt)His	D-Phe	D-Val	L-Ile	L-Ala	3.6	8.1
11	Pmh	L-Ile	D-Val	L-(trt)His	D-Phe	D-Val	L-(trt)Gln	L-Ala	3.6	8.7
12	Pmh	L-(trt)Asn	D-Val	L-(trt)His	D-Phe	D-Val	L-(trt)Gln	L-Leu	3.3	12
13	Pmh	L-(tBu)Thr	D-Val	L-(trt)His	D-Phe	D-Val	L-(tBu)Thr	L-Ile	3.2	20

[a] Single bead screens were conducted either on resin (catalysts **6**−**9**) or following single-bead cleavage to the *N*-propyl amide (catalysts **10**−**13**). Kinetic resolutions were conducted at 25 °C. [b] Solution k_{rel} values were obtained with HPLC purified peptides under homogeneous conditions at −65 °C. See Experimental Section for details.

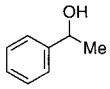
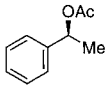
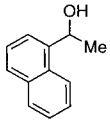
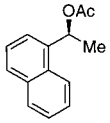
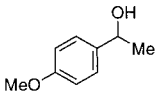
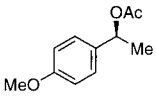
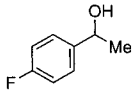
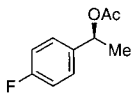
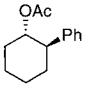
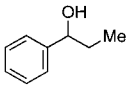
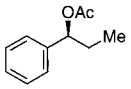
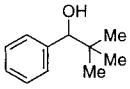
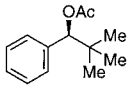
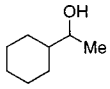
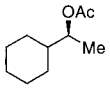
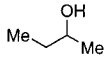
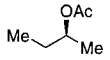
and purified by reverse-phase HPLC. Also shown in Table 1 are the k_{rel} values obtained with each catalyst in (i) the single bead assay and (ii) the kinetic resolution following resynthesis and purification. Peptides **6**−**9** were identified from the "on-resin" single bead assays, and upon rescreening in solution produced k_{rel} values that were comparable to peptide **4**. Peptide **9** actually affords a k_{rel} value of 9.6, which is perhaps an experimentally significant improvement over peptide **4** (cf., k_{rel} = 8.2). Peptides **10**−**13** were identified from the screens of peptides cleaved to the *C*-terminal *N*-propyl amide. Each of these catalysts also performs similarly, or better than parent peptide catalyst **4**. Among these peptides, catalysts **12** and **13** afford k_{rel} values that are 12 and 20, respectively. These results reflect unambiguous improvements for enantioselectivity with respect to catalyst **4**. These results stand in stark contrast to our originally reported peptide-based catalysts that afforded essentially no enantioselectivity for unfunctionalized substrates.

It is important to note that the single bead assays (either with resin-bound peptides or those that had been cleaved from single beads to the *N*-propyl amide) do not provide an exact prediction of which will be the most enantioselective upon rescreening under homogeneous conditions as the methyl ester. For example, catalyst **10** affords a k_{rel} value of 3.6 in the single bead assay, and a k_{rel} value of 8.1 under optimized homogeneous conditions; catalyst **13** affords a k_{rel} value of 3.2 in the single bead assay, but a k_{rel} value of 20 under homogeneous conditions. While the reasons for this are currently under study, from a pragmatic point of view, the selection process meets its principle objective: identification of individual catalysts that are most interesting for further study. It is the case that the *rescreening* experiments (homogeneous conditions with purified peptides) are conducted under different conditions than the *selection* experiments (single bead assays), which may account for differences that we observe. Nevertheless, while screens of the type we have employed may miss certain selective catalysts, the approach effectively narrows the field of catalyst candidates to a manageable number for further study by conventional means.

The structures of the new catalysts are of interest with respect to the selectivities that each affords; in particular, the improvements afforded by **12** and **13** are noteworthy. All eight catalysts share sequence homology with respect to the parent (**4**) in the *i*+2 position (D-Val), the *i*+3 position (L-(trt)His), and the *i*+5 position (D-Val). But, the two most selective catalysts lack the L-Ala of the parent in the *C*-terminal position and have L-Leu (**12**) or L-Ile (**13**) in its place. Perhaps this change in a remote position relative to the Pmh residue reflects conformational proximity of the *N*-terminal and *C*-terminal residues of the octapeptide during the stereochemistry-determining transition state. Conformational studies of peptides **4**, **12**, and **13** will investigate this possibility. Preliminary studies reveal that peptide **13** exhibits a very sharp ^{1}H NMR spectrum ($CDCl_3$, 25 °C) that may reflect a unique, monomeric conformation in solution.

Generalization to Other Substrates. The finding that catalyst **13** affords appreciable enantioselectivity in the kinetic resolution of an unfunctionalized substrate is one that could have implications for the application of peptide-based enantioselective catalysts across a broader substrate scope than had been previously possible. To examine this notion, we investigated octapeptide **13** as a catalyst for kinetic resolution of a range of substrates (Table 2). Reactions were conducted with a uniform set of conditions (2.5 mol % catalyst **13**, −65 °C, 40−50% conversion). This decision was made with the goal of rapidly evaluating the scope of a given catalyst without respect to optimization on a per substrate basis. Notably, catalyst **13** provides a $k_{rel} > 50$ for α-naphthyl-substituted alcohol **14** (entry 2; cf. $k_{rel} = 20$ for compound **2**, entry 1). Para substitution results in a modest drop in selectivity with *p*-methoxy-bearing substrate **15** affording $k_{rel} = 16$ (entry 3; *p*-fluoro-substituted substrate **16** affords $k_{rel} = 11$, entry 4). *trans*-2-Phenylcyclohexanol (**17**) is an excellent substrate for kinetic resolution, undergoing resolution with a $k_{rel} > 50$ under these conditions (entry 5). Higher degrees of substitution on aryl−alkyl secondary alcohols also modulate the observed selectivity. For example, *sec*-phenylpropanol (**18**) exhibits $k_{rel} = 8.2$ with catalyst **13** (entry 6) and *tert*-butyl-substituted alcohol **19** is resolved with k_{rel} = 30 (entry 7). Finally, we examined whether secondary alcohols that do not possess an aromatic substituent might be substrates for kinetic resolution with catalyst **13**. Indeed, cyclohexyl ethanol **20** is resolved with a moderate, but significant k_{rel} = 9.0 (entry 8). Even *sec*-butanol **21** is resolved with a $k_{rel} = 4.0$ under these conditions (entry 9), indicating that the catalyst can discriminate to some degree between a methyl group and an ethyl group. These latter two substrates are rarely reported in studies on nonenzymatic kinetic resolution. The appreciable kinetic resolution of these substrates suggests that catalyst **13**

Table 2. Kinetic Resolution Results Employing Peptide **13** (2.5 mol %) as a Catalyst[a]

Entry		Racemic Substrate	Preferred Product Enantiomer	k_{rel}[b]
1	**2**:			20
2	**14**:			>50
3	**15**:			16
4	**16**:			11
5	**17**:			>50
6	**18**:			8.2
7	**19**:			30
8	**20**:			9.0
9	**21**:			4.0

[a] Reactions were carried out at −65 °C in PhMe (2.5 mol % **13**). Reactions were run to 40−50% conversion. [b] k_{rel} values were determined according to the method of Kagan (ref 13) employing chiral GC or HPLC analyses. See Supporting Information for details.

may well be a reasonable lead catalyst for further optimization with respect to these types of substrates.[24]

Conclusions

Collectively, the results in Table 2 reveal catalyst **13** to be somewhat general in scope. In addition, these data indicate that peptide-based catalysts can be found for asymmetric acylation of a wide variety of alcohol types. Such catalysts are not limited to substrates that bear acetamide functional groups. In addition, it is worth noting that catalyst **13** was identified from combinatorial screening techniques that were designed for evaluation of *sec*-phenylethanol. Yet, catalyst **13** provides enantioselection with a range of different alcohols. Presumably, screening similar catalyst libraries against a particular substrate would result in the identification of different catalysts that would afford different and perhaps improved selectivities for these and other alcohols. That one such set of screens resulted in the identification of an effective catalyst for the targeted substrate (as well as others) bodes well for the application of this approach to a wide variety of structural types.

The mechanistic basis for the enantioselectivities achieved with the peptides reported in this article is unclear at this time. It is our contention that the sequences of peptides **4** and **13** are sufficiently nonobvious that they would not have been identified by rational design methods. Nevertheless, from diversity-oriented screens we have found catalysts that are effective for kinetic resolutions of unfunctionalized substrates. These same octapeptides represent reasonable lead structures for the development of new catalysts that could benefit from further diversity-based screening techniques, and also mechanistic studies. The application of screens for rate acceleration with catalyst libraries thus offers a potentially powerful method for the rapid identification of new chiral catalyst motifs that are both mechanistically interesting and of potential utility to the synthetic community.

(24) Reactions may be run with reduced catalyst loading (0.5 mol %) without erosion of k_{rel} or conversion. In these experiments, Hünig's base (0.4 equiv) is employed as an additive. See Supporting Information for details.

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Supporting Information Available: Experimental details for all experimental aspects of the study, including compound characterization (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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