Enantioselective Resolving Resins from a Combinatorial Library. Kinetic Resolution of Cyclic Amino Acid Derivatives

M. David Weingarten, Klara Sekanina, and W. Clark Still*

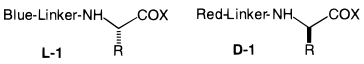
Department of Chemistry, Columbia University
New York, New York 10027

Received April 27, 1998

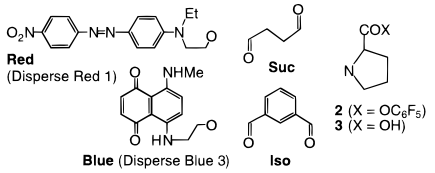
Solid materials that bind small molecules enantioselectively are valuable as resolving agents in chiral separations. Coupled with modern HPLC, even modestly enantioselective materials (e.g., with separation factors (α) as low as 1.1) are commonly used to resolve small organic compounds.[1,2] While such methods work well for analytical resolutions, large-scale chromatographic resolutions are often less practical.[3] If however a tightly binding and highly enantioselective (e.g., $\alpha \geq 10$) resin or other solid material were readily available, then it would be possible to resolve compounds by simply stirring the racemate with such a resin and filtering.[4]

The quest for highly enantioselective materials primarily for use in chiral chromatography is an active area of research.Typical methodologies involve structure-based design and empirical modification of known chiral selector molecules.[5] In this paper, we describe a new approach that involves the parallel synthesis and screening of a combinatorial library of potential chiral selectors on polystyrene synthesis beads.[6] Our screen allows picking the most enantioselective library member beads by visual inspection under a low-power microscope. We demonstrate the feasibility of this approach by making and screening a small (60-member) combinatorial library of potential resolving resins and showing that enantioselective library members can be readily distinguished and used in a heterogeneous kinetic resolution process that corresponds to resolution by filtration.

An Enantioselectivity Screen. To find the most enantioselective members of our chiral selector library, we developed a simple two-color differential binding screen that allows us to estimate the enantioselectivity of each library member visually.[6c,7] The screen employs enantiomeric probe molecules that are labeled with different colored dyes. In this work, we were seeking chiral selectors for resolving amino acid derivatives and suitable probes thus included the blue L-amino acid **L-1** and the red D-amino acid **D-1**.

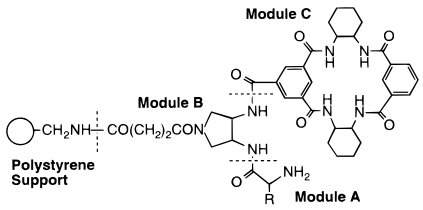


The idea was to treat an equimolar mixture of these colored probe molecules with a library of chiral selectors on synthesis beads in which each bead carried a different selector. The result of such an experiment would be that highly enantioselective binding would yield beads that were red or blue whereas unselective binding would yield beads that were brown.[8]



The particular probes we used are shown above as **2** and **3**. The commercially available Disperse Blue and Red dyes (bound to LPro and DPro derivatives respectively) we used were chosen to be visually distinct and not significantly bound by our selector library members. We also varied linkers (here succinyl and isophthaloyl) connecting the dye labels to the proline derivatives we wished to resolve.

A Chiral Selector Library. For our library of chiral selectors, we prepared a library of chiral amines whose members could react with and bind to probes **2** or **3** either by acylation or salt formation, respectively. To promote significant enantioselectivity in these reactions, we generally chose amines with an adjacent chiral center and a large conformationally restricted surface bearing additional chiral centers and polar functionality to further define the reactive amine's microenvironment. The general structure of our library is shown below.[9] Module A (15 different D- and L-amino acids) carries the nucleophilic/basic amine, module B (*RR* and *SS* stereoisomers) is a turn element that directs modules A and C toward one another, and module C (*RRRR* and *SSSS*) provides the large functionalized surface. Given the number of variants of each module, the final library had 60 (15 $\times$ 2 $\times$ 2) different members and included both enantiomers of each chemically distinct member.



The library was prepared by encoded split synthesis on 100 μm polystyrene synthesis beads; thus, different library members were segregated on different beads (i.e., one bead, one chiral selector).[10] Details are given in the Supporting Information.

Screening Results. Most of our work to date has focused on enantioselective acylation using pentafluorophenyl esters **2**. When an excess of these probes Dye-Suc-(DL)**2** or Dye-Iso-(DL)**2** was treated with the above bead-supported library for 1−5 h (25 °C, $CHCl_3$), and the library was examined under a low-power microscope, beads having distinct red and blue colorations (as well as many brown beads) were indeed observed (Figure 1A,B). From the hues of the beads, it appeared that some library members were more enantioselective than others and that the isophthaloyl-linked proline derivatives were generally best resolved (Figure

(1) Review: Ahuja, S., Ed. *Chiral Separations by Liquid Chromatography*; ACS Symposium Series 471; American Chemical Society: Washington, DC, 1991.

(2) In a chromatographic resolution, α is the ratio of retention times of enantiomeric analytes. In a kinetic resolution, *S* is the ratio of reaction rate constants of enantiomeric starting materials.[11]

(3) Review of large scale resolutions: Crosby, J. *Tetrahedron* **1991**, *47*, 4789.

(4) Previous filtration-like resolutions with an enantioselective solid: Cao, G.; Garcia, M. E.; Alcala, M.; Burgess, L. F.; Mallouk, T. E. *J. Am. Chem. Soc*. **1992**, *114*, 7574. Iwai, M.; Shoji, H.; Shimazu, S.; Uematsu, T. *Chem. Lett.* **1993**, 989.

(5) E.g.: Pirkle, H. W.; Pochapsky, T. C. *Chem. Rev.* **1989**, *89*, 347.

(6) Other combinatorial libraries of chiral selectors have been recently described: (a) Garcia, M. E.; Gavin, J. A.; Deng, N.; Andrievsky, A. A.; Mallouk, T. E. *Tetrahedron Lett.* **1996**, *37*, 8313. (b) Jung, G.; Hofstetter, H.; Feiertag, S.; Stoll, D.; Hofstetter, O.; Wiesmuller, K.-H.; Schurig, V. *Angew. Chem., Int. Ed. Engl*. **1996**, *35*, 2148. (c) Gennari, C.; Ceccarelli, S.; Piarulli, U.; Montalbetti, C. A. G. N.; Jackson, R. F. W. *J. Org. Chem.* In press.

(7) Boyce, R.; Li, G.; Nestler, H. P.; Suenaga, T.; Still, W. C. *J. Am. Chem. Soc.* **1994**, *116*, 7955.

(8) Unselective beads are brown (not purple) because light transiting such a bead is filtered by both dyes (red − blue = brown, whereas red + blue = purple).

(9) Library members are structurally related to known sequence-selective receptors for peptides: Wennemers, H.; Yoon, S. S.; Still, W. C. *J. Org. Chem.* **1995**, *60*, 1108.

(10) Ohlmeyer, M. H. J.; Swanson, R. N.; Dillard, L. W.; Reader, J. C.; Asouline, G.; Kobayashi, R.; Wigler, M.; Still, W. C. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 10922.

S0002-7863(98)01447-4 CCC: $15.00
Published on Web 08/20/1998

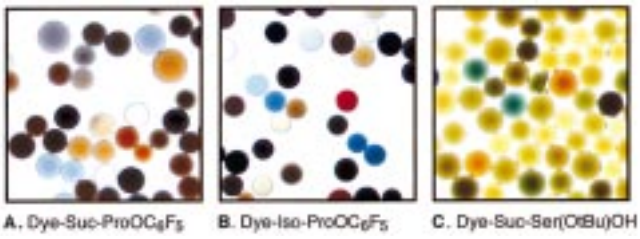


Figure 1. Chiral selector library beads screened for enantioselective binding by treatment with differentially labeled enantiomeric amino acid derivatives.

Table 1. Enantioselection of Two-Color Assay-Selected Library Members Using Proline-Based Probes **2**

library member[a]	dye-Suc-**2**[b]	dye-Iso-**2**[b]
LHis-(*SS*)B-(*RRRR*)C	49% ee for D**2**	(4% ee for D**2**)[c]
DHis-(*RR*)B-(*SSSS*)C	51% ee for L**2**	(2% ee for L**2**)[c]
LAsp-(*SS*)B-(*RRRR*)C	44% ee for D**2**	(11% ee for D**2**)[c]
DAsp-(*RR*)B-(*SSSS*)C	48% ee for L**2**	(7% ee for L**2**)[c]
LAsn-(*SS*)B-(*SSSS*)C	51% ee for L**2**	73% ee for D**2**
DAsn-(*RR*)B-(*RRRR*)C	39% ee for D**2**	81% ee for L**2**

[a] Amino acid side chains are protected (Supporting Information). [b] Enantiomeric excess (ee) and prefered configuration of bead-bound dye-linker-**2**. [c] Enantioselectivity data for comparison purposes only, 2-color screening with dye-Iso-**2** did not select these library members.

1B). Control experiments verified that the observed selectivities did not result from differential binding of the different dye moeties. The reddest and bluest beads found with each probe were picked and decoded to determine the structures of their associated chiral selectors. To better quantify the enantioselectivity of these potential resolving resins, they were individually resynthesized on a gram scale and again treated with excess red and blue **2**. Subsequent treatment with NaOMe and HPLC quantification of the released dyes provided a determination of the enantiomeric excesses (%ee) of the proline derivatives bound to the beads (Table 1).

These results indicate enantioselective acylation of chiral selectors on the color-selected library beads corresponding to 45−75% ee. For every red bead we found in our assay, we also found a blue bead carrying the enantiomeric library member. In addition, high enantioselectivity was found with proline probe analogues lacking the dyes (see below). Thus, differing dye moieties are not responsible for the observed selectivities, though they do exert a detectable effect as the extents of enantioselection found with enantiomeric receptors were not identical (see Table 1).

While the measurements of enantioselectivity reported above entailed resynthesis of color-selected library members, we have also been able to estimate (±5%) the % ee of material on the beads by quantitative color analysis of the microscopic images of individual beads obtained with a color CCD camera. Such a system could be used for the automatic scanning of very large selector libraries to locate the most enantioselective members.

Resolving Resins for Amino Acid Derivatives. Given the significant enantioselection found with some of our chiral selector resin beads, we tested their ability to resolve related proline derivatives lacking the dyes. The experiment was a kinetic resolution[11,12] that entailed stirring an excess of a chiral selector resin with a racemic proline pentafluorophenyl ester while monitoring the quantity and % ee of the proline derivative remaining in solution after filtering away the beads. Using MeO-Iso-Pro-OC_6F_5 and the chiral selector resin DAsn-(*RR*)B-(*RRRR*)C in $CHCl_3$, the kinetic resolution results shown in Figure 2A were obtained. This resolution yielding 80% ee at 60% conversion corresponds to a selectivity *S*-value of 7.8 and matches nicely with the ∼75% ee shown in Table 1 for this receptor and the corresponding dye-labeled proline derivative. While proline derivatives without dyes can thus be readily resolved with the probe-selected resolving resins, a bifunctional N-acylating moiety (here succinyl or isophthaloyl) on the proline appears to be necessary for high enantioselection (e.g., $S = 1.4$ with benzoyl-Pro-OC_6F_5).

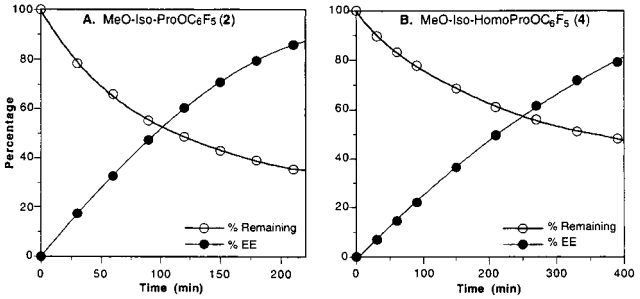
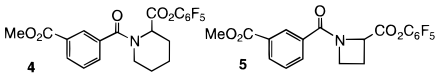


Figure 2. Kinetic resolution of methyl *N*-isophthaloyl cyclic peptides with resolving resin DAsn-(*RR*)B-(*RRRR*)C.

The DAsn-(*RR*)B-(*RRRR*)C chiral selector resin that was selected with proline probes dye-Iso-**2** was also able to resolve certain other cyclic amino acid derivatives. In particular isophthaloyl proline homologues **4** and **5** are resolved by the above kinetic resolution and filtration method. Homoproline **4** was



particularly well resolved ($S = 20.2$) as shown in Figure 2B. Azetidine **5** was resolved less efficiently ($S = 1.5$) but the enantiomer selectively bound was opposite (i.e., D) to that found with **2** and **4**.

Conclusion. This work shows how simple manual methods of parallel (split) synthesis and parallel (visual) screening can be used to find novel enantioselective binding compounds. Though the library in this model study was tiny (60 members) by contemporary standards, we were still able to find resolving compounds and resins with enantioselectivities that significantly exceeded those of typical chiral HPLC phases. These results demonstrate considerable promise for using combinatorial methods in the discovery or optimization of efficient chiral selectors for particular substrates or even classes of substrates. Even with completely manual methods, it would not be difficult to make and screen libraries thousands of times larger than that described here and almost certainly find even better resolving resins. While the particular binding chemistry (irreversible enantioselective acylation) we have used is obviously not ideal for practical resolutions because the resolving resin is not recyclable, analogous approaches involving reversible binding can be readily imagined. Indeed, preliminary experiments with the same 60-member amine library and certain dye-labeled amino acids (e.g., dye-Suc-Ser-(OtBu)OH) gives a clear indication of enantioselective salt formation as shown by the reddish and blueish beads in Figure 1C.

Acknowledgment. This work was supported by NSF grant CHE97-07870 (W.C.S.) and NIH Postdoctoral Fellowship GM18073 (M.D.W.).

Supporting Information Available: Synthetic procedures for preparation and screening of probes **2** and the chiral selector library (16 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

JA981447Z

(11) Kinetic resolution review: Kagan, H. B.; Fiaud, J. C. *Top. Stereochem.* **1988**, *18*, 249.

(12) For related solution phase stoichiometric kinetic resolutions of amines and alcohols, see: Wiesner, K.; Jay, E. W. K.; Tsay, T. Y. R.; Demerson, C.; Jay, L.; Kanno, T.; Krepinsky, J.; Vilim, A.; Wu, C. S. *Can. J. Chem.* **1972**, *50*, 1925. Hiraki, Y.; Tai, A. *Chem. Lett.* **1982**, 341. Franck, A.; Ruchardt, C. *Chem. Lett.* **1984**, 1431. Yamada, S.; Ohe, T. *Tetrahedron Lett.* **1996**, *37*, 6777; Somfai, P. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 2731.