Combinatorial 'library on bead' approach to polymeric materials with vastly enhanced chiral recognition

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Received (in Corvallis, OR, USA) 11th August 1998, Accepted 21st October 1998

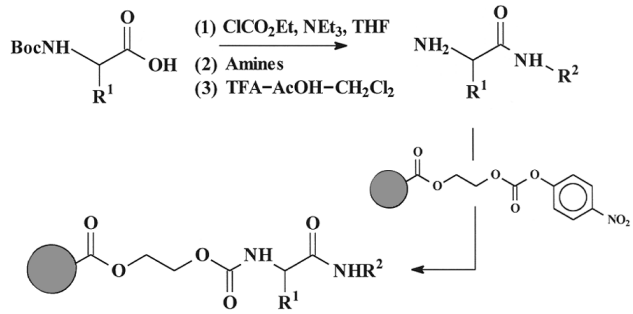
A general screening method for enantiomer recognition is introduced for the rapid preparation of novel chiral stationary phases for HPLC in which libraries of mixed chiral selectors are immobilized on polymer beads and the resulting chiral phases tested in the separation of racemic targets followed by deconvolution to afford an optimized separation medium.

Although it is well known that each enantiomer of a chiral compound may exhibit different biological activities, a number of commodity products and fine chemicals, such as drugs, agrochemicals, flavors, fragrances, and pheromones, are currently used in the form of racemic mixtures.[1] Therefore, the general trend is to replace these mixtures with single enantiomers that can be obtained directly, either by an asymmetric synthesis or by the resolution of racemates. In addition, for newly designed drugs, it may be necessary to obtain both enantiomers for pharmacological and toxicological studies. Among the separation techniques, resolution by high-performance liquid chromatography (HPLC) utilizing chiral stationary phases (CSPs) has advanced considerably in the past decade. The preparation of CSPs capable of effective enantiomer recognition is the key to this separation technique. Therefore, many CSPs for HPLC have been prepared and about 100 have been commercialized. Most are derived from various matrix-bound chiral selectors including transition metal complexes,[2] proteins,[3] antibiotics,[4] synthetic polymers and polysaccharides,[5] cyclodextrins and crown ethers,[6] or the π–π donor–acceptor complexes—'brush'-type separation media—pioneered by Pirkle.[7] Given the boundless structural and functional diversity of chiral molecules, no CSP is 'universal' and the separation of new targets may well mandate the development of new optimized complementary CSPs. Our combinatorial methods are aimed at the rapid preparation of tailor-made CSPs designed for a specific racemic solute.

Combinatorial chemistry is a very powerful tool for the preparation of large numbers of related compounds in a short period of time.[8] Today, this approach is a well-established technique used mainly to accelerate the drug discovery process. Combinatorial methods have also recently been used for the discovery of new materials.[9] Attempts to use combinatorial techniques in chromatography have focused on the field of the affinity separations employing the well-known interactions of peptides with target proteins. Typical screening methods have been used to select the most specific affinant with the highest binding constant from libraries of oligopeptides.[10]

We now report a combinatorial approach to the accelerated preparation of highly selective chiral separation media for HPLC based on chemically flexible 'brush'-type chiral selectors. The feasibility of our concept is demonstrated on model systems that involve π-basic selectors since the starting materials for these selectors are readily available in a large variety of chemistries.

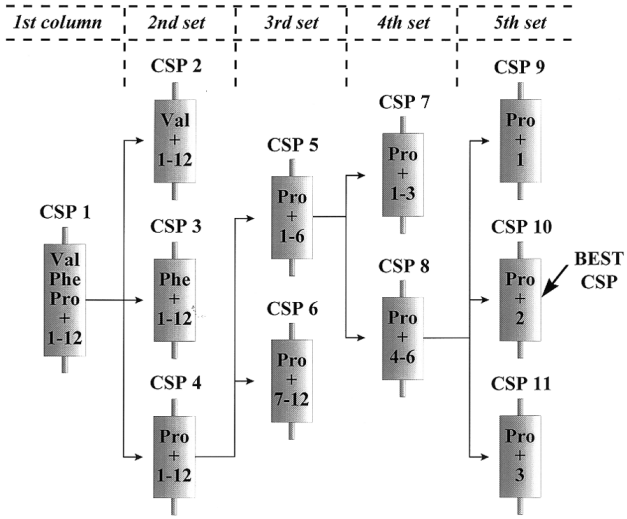
Our combinatorial approach involves the attachment of a mixed library of potential selectors to an optimized polymer support[11] followed by on-column screening for enantioselectivity, and deconvolution to identify the single best selector. A small library of amides is prepared by reaction of *N*-Boc-protected L-amino acids with a mixture of aromatic primary amines using an active ester coupling procedure (Scheme 1).† After deprotection of the amino terminus, reaction of the mixed amide library with 5 μm macroporous 4-nitrophenyl carbonate activated poly(2-hydroxyethyl methacrylate-*co*-ethylene dimethacrylate) beads[11] leads to a chiral separation medium (CSP 1) with a multiplicity of polymer-bound selectors. Synthetic polymer beads were used in this study because they provide CSPs with higher selectivities than silica as a result of the elimination of non-specific interactions.[11,12] After packing these beads into a HPLC column, selectivity is assayed by injecting various racemates of chiral targets. Although the use of columns with mixed selectors is normally not recommended for actual enantioseparations[13] it is ideally suited for our combinatorial discovery of optimized selectors.



Scheme 1

The feasibility of this 'library-on-bead' approach is demonstrated with a small model library of 36 compounds prepared by reaction of a mixture of three L-amino acids (valine, phenylalanine and proline) with 12 aromatic amines (3,4,5-trimethoxyaniline **1**, 3,5-dimethylaniline **2**, 3-benzyloxyaniline **3**, 5-aminoindane **4**, 4-*tert*-butylaniline **5**, 4-biphenylamine **6**, 1-aminonaphthalene **7**, 4-tritylaniline **8**, 2-aminoanthracene **9**, 2-aminofluorene **10**, 2-aminoanthraquinone **11** and 3-amino-1-phenyl-2-pyrazolin-5-one **12**) (Scheme 2).† Both proline and dimethylaniline are included in the library design since previous work had shown their value in the preparation of efficient CSPs.[11,12,14] Despite the presence of 36 mixed selectors within the same column, CSP 1 separates DL-(3,5-dinitrobenzoyl)leucine diallylamide‡ and other substituted amino acid amides confirming the validity of the mixed selector approach. To determine which of the 36 selectors is the most powerful, a deconvolution process involving the preparation of beads with a progressively smaller number of selectors was used. Separation factors $\alpha = (t_2 - t_0)/(t_1 - t_0)$ where t_0 is the retention time of an unretained compound (column void volume determined using 1,3,5-tri-*tert*-butylbenzene as a marker) and t_1 and t_2 are the retention times of the individual enantiomers were calculated for all the separations to demonstrate the selectivity.

In the next step, each single amino acid was coupled separately with the set of 12 amines resulting in three new polymer-based CSPs (CSP 2–CSP 4). The highest separation factor α of 13.7 was found for the proline-based column while



Scheme 2

the α values for the other two columns are close to 5. For the preparation of the third set of columns (CSP 5 and CSP 6), two proline based sub-libraries of selectors were prepared from two six-member groups of amines (**1**–**6** and **7**–**12**) and the respective columns exhibited selectivities of 13.6 and 7.3. In the next step, the six amines present in the more selective column CSP 5 were divided into two groups (**1**–**3** and **4**–**6**) and the columns CSP 7 and CSP 8 exhibited rather high α values of 17.4 and 14.9, respectively. The separation results indicate that both groups of three selectors include at least one with a very high selectivity. CSP 7 that affords somewhat higher selectivity was further deconvoluted. Three columns CSP 9–CSP 11 packed with beads containing only individual selectors were prepared. Although two of these columns (10 and 11) do not exhibit high separation factors (2.5 and 3.6, respectively), an α value of 24.7 was achieved with CSP 9 that features dimethylaniline **2** as a part of the proline selector. The rapid increase in the separation factors reflects not only the improvement in the intrinsic selectivities of the individual selectors, but also the effect of increased loading with more efficient selectors since the overall selector loading determined from nitrogen content remains virtually constant at about 0.7 mmol g^{-1} for all CSPs 1–12. NMR spectra indicate that none of the selectors binds preferentially to the support during the reaction of their mixtures.

A classical 'one column, one selector' approach would require the preparation and testing of 36 CSPs modified with each individual selector. In contrast, our combinatorial scheme documents that the parallelism advantage results in the discovery of a novel highly selective CSP from the same group of 36 selectors using only 11 columns, *i.e.* less than one third. In addition, an unlimited number of racemates may be screened through the various columns. The advantage of the mixed selector column approach becomes even more convincing with much larger sets of selectors. For example, a simple calculation reveals that the use of all 20 natural amino acids with the same 12 amines would lead to a library of 240 selectors that could be deconvoluted using only 17 columns. The question now arises as to what is the highest number of selectors that may be used simultaneously in the first column. It seems that there is no limitation from a chemical point of view. However, in a hypothetical situation in which only a single selector is active and all of the compounds are attached to the beads in equal amounts, the percentage of the active selector in the mixture decreases rapidly and despite its high specific selectivity (separation factor at a loading of 1 mmol g^{-1}), the actual selectivity of a CSP with mixed selectors may be rather small and may even vanish within the limits of experimental errors. Although the sensitivity of the chromatographic screening may somewhat limit this approach, the number of selectors that may be screened in a single column is still impressive. Obviously, the libraries of columns resulting from this approach may be used time and again for the separation of the racemates of a variety of chiral targets.

Funding of this research by the National Institute of General Medical Sciences, National Institutes of Health (GM-44885) is gratefully acknowledged. P. M. thanks the Swiss National Science Foundation for a postdoctoral fellowship.

Notes and references

† The *N-tert*-butoxycarbonyloxy-protected (Boc) amino acids (7.0 mmol) were dissolved in THF (35 ml), cooled and triethylamine (7.0 mmol) and $ClCO_2Et$ (7.0 mmol) were added slowly by syringe. After stirring at −15 °C for 1 h, a cold (−15 °C) mixture of equimolar amounts of the desired aromatic amines (total amount of amines = 7.0 mmol) in THF was admixed. Stirring continued at −15 °C for 1 h and at room temperature overnight. The organic phase was washed, dried over $MgSO_4$ and concentrated to afford the product. This product was dissolved in CH_2Cl_2 cooled to 0 °C, and treated with 1:1 mixture of TFA–AcOH for 12 h. Extraction and drying under high vacuum afforded the deprotected product mixture as a colored solid in near quantitative yield. Integration of the individual ^1H NMR signals for the amide hydrogen atoms of the compounds indicates that all expected products were formed. Et_3N (15 mmol) was added to a slurry of 4-nitrophenyl carbonate-activated poly(2-hydroxyethyl methacrylate-*co*-ethylene dimethacrylate) beads (1.6 g) (ref. 11) in THF at 0 °C. The solution of the selector mixture obtained from the deprotection step in THF was added slowly to this suspension and stirring was continued at room temperature for 3 h and then at 60 °C overnight to afford the chiral stationary phases.

‡ The chiral stationary phases were slurry packed at a constant pressure of 15 MPa into 150 × 4.6 mm i.d. stainless steel columns. Chiral separations were carried out in normal-phase mode using a 1:4 (v/v) hexane–CH_2Cl_2 mixture as the mobile phase.

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Communication 8/06397D