Highly selective chiral recognition on polymer supports: preparation of a combinatorial library of dihydropyrimidines and its screening for novel chiral HPLC ligands

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A library of more than 140 racemic 4-aryl-1,4-dihydropyrimidines has been synthesized using the single step Biginelli multicomponent condensation; the individual compounds were screened for enantiomer resolution, and the best candidate attached to a solid macroporous polymer support to afford a highly selective separation medium for chiral HPLC.

Combinatorial chemistry is a powerful tool for the rapid preparation of large numbers of different compounds that may be used in the development of new drug candidates and drugs,[1] metal complexing ligands,[2] polymers,[3] materials for electronics,[4] sensors,[5] peptidic ligands for affinity chromatography,[6] and in supramolecular chemistry.[7] The increasing awareness that individual enantiomers of drugs, agrochemicals, pheromones, flavors, fragrances and some other compounds have different interactions with biological systems drive both industry and academia to find means to replace racemates with their single enantiomers. Although the liquid chromatographic separation of enantiomers using chiral stationary phases (CSPs) has emerged only recently, a large number of chiral stationary phases (CSPs) are now commercially available.[8] Despite the variety of CSPs, none of these is universal and new chiral separation media must often be developed for the separation of specific compounds. The well-defined small molecule selectors pioneered by Pirkle[9] using chiral blocks derived from natural sources (amino acids) and porous silica as a support afford the most flexibility in the development of many different chiral stationary phases.

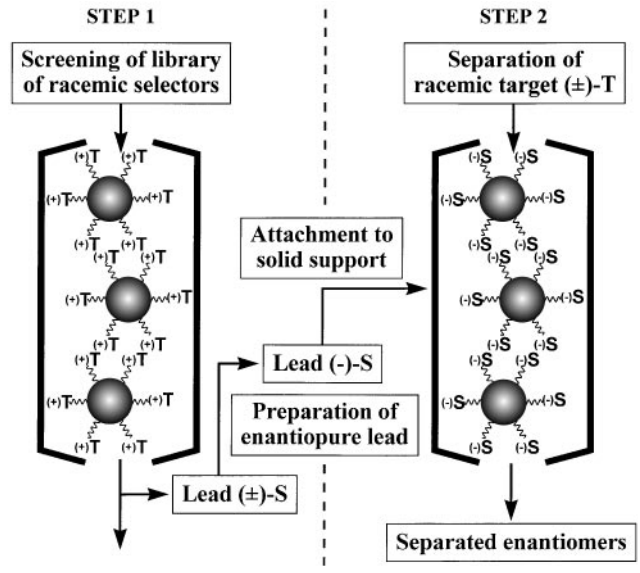
We have now designed an approach that uses combinatorial chemistry as a tool for the *rapid* development of novel, highly selective, chiral stationary phases for HPLC. Our approach applies the so-called principle of reciprocity, which has been introduced by Pirkle for the development of CSPs capable of separating important classes of pharmaceuticals. This principle states that if a single molecule of a chiral selector has different affinities for the enantiomers of another substance, then a single enantiomer of the latter will have different affinities for the enantiomers of the initial selector molecule.[10]

The reciprocal method is especially suitable for situations in which the target enantiomer is known and its separation from a racemate is required. Since chromatographic techniques are readily automated, a broad variety of novel chiral ligands may be considered. Scheme 1 illustrates the general concept of our approach. In the first step, a single enantiomer (+)-T of the target racemate is immobilized onto a suitable polymeric support[11] and the resulting CSP is used for the HPLC screening of a library of racemic compounds (potential selectors) that have been prepared using parallel combinatorial synthesis. The best separated member of this library (±)-S is then prepared as the single enantiomer [*e.g.* (−)-S], coupled to an optimized solid support,[11,12] and used in the second step for the required separation of the racemic target. While only two columns are required in this technique, the tradeoff for the simplicity of this approach is that the developed CSP is optimized for the enantioseparation of only one compound. In reality, this column may well separate other racemates as a result of cross selectivity, however, the α values may be lower.

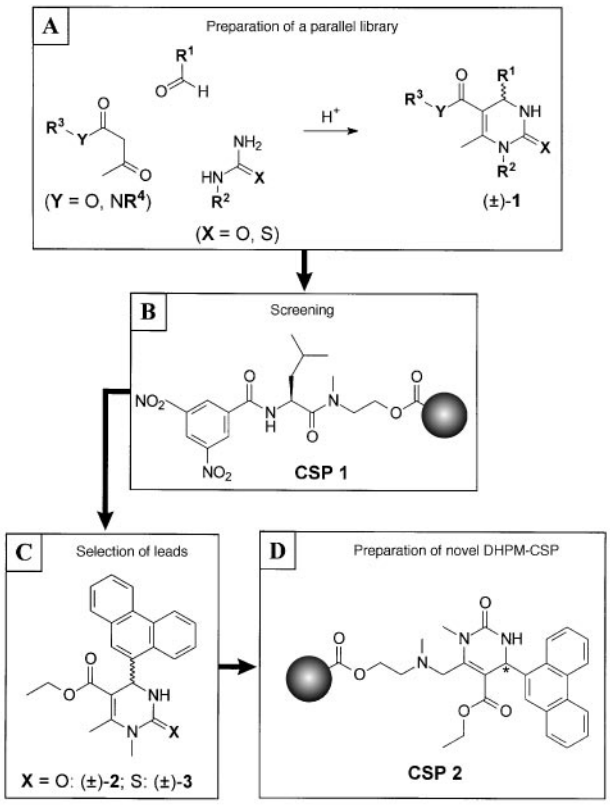
We have demonstrated the feasibility of this concept with a parallel library of racemic 4-aryl-1,4-dihydropyrimidines (DHPMs) (±)-**1** [Scheme 2(A)]. The DHPM scaffold resulted from our search for novel chiral selectors that do not rely on chiral starting materials from natural sources. DHPMs are easily accessible by the Biginelli multicomponent cyclocondensation developed more than 100 years ago.[13] This simple one-pot reaction combines a β-keto ester or β-keto amide, with an aldehyde and a urea or thiourea. Since a large number of components for the condensation are commercially available, over 140 various DHPMs have been prepared using combinations of 25 aldehydes with six ureas or thioureas, and seven acetoacetates or acetoamides and tested in this initial study.

The library was screened with a chiral stationary phase that contains (*S*)-(3,5-dinitrobenzoyl)leucine (model target) attached to poly[(*N*-methyl)aminoethyl methacrylate-*co*-methyl methacrylate-*co*-ethylene dimethacrylate] beads[12] [Scheme 2(B)] using normal-phase mode HPLC conditions. The separation factor $\alpha = (t_2 - t_0)/(t_1 - t_0)$, where t_0 is the retention time of an unretained compound or 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, was calculated to measure the selectivity of the separation.

While some racemic DHPMs in the library are not resolved at all ($\alpha = 1.0$), α values of 5.2 and 11.7 were achieved for the top candidates 4-(9-phenanthryl)-2-oxo-DHPM (±)-**2** and its thioxo analogue (±)-**3** [Scheme 2(C)], respectively. A single crystal X-ray structure analysis of the 2-oxo compound reveals a cleft-like conformation of the aromatic and heterocyclic ring systems



Scheme 1



Scheme 2

that appears particularly well suited for sterically controlled interactions.

For the next step, the preparation of the actual CSP requires the preparation of one of the lead compounds in enantiomerically pure form, followed by attachment to a support. Although different routes to the desired single enantiomer can be designed, we simply separated it from the racemate using a semi-preparative column packed with CSP 1 used previously for the screening process. Attachment of the enantiopure selector to the amino functionalized macroporous polymethacrylate support requires its modification to include a suitable coupling site. Although hydrolysis of the ester group to a free carboxy group seems to be attractive, rather harsh conditions have to be used resulting in decomposition of the DHPM. Therefore, a more convenient route—bromination of the methyl group at the C6 position—was applied instead. Since the bromination step is not well suited for the 2-thioxo-DHPM, we used the best 2-oxo-DHPM compound from the library: 4-(9-phenanthryl)-2-oxo-DHPM (−)-**2**. Coupling of the brominated DHPM to the amino functionalized support affords CSP 2 [Scheme 2(D)] which contains 0.20 mmol g^{-1} of the selector. A variety of racemates including amino acid and non-steroid anti-inflammatory drug (profens) derivatives, and dihydropyrimidines were separated on CSP 2 with α values up to 8. These α values are rather high, especially if one considers that the unoptimized methodology used in this demonstration leads to a CSP with a loading of only 0.20 mmol g^{-1}. Although the selectivity may not always be a linear function of loading, the specific selectivity of DHMP selectors is actually higher than that of amino acid based selectors. Since dihydropyrimidines possess well-established pharmacological potential,[8] the ability of CSPs with DHPM selectors to separate these drugs is a very promising tool for the development of these and similar drugs in their single enantiomeric form.

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Notes and References

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