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On-Bead Combinatorial Approach to the Design of Chiral Stationary Phases for HPLC

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A library of 36 L-amino acid anilides, which are potential selectors for chiral HPLC, was synthesized in solution and attached to functionalized macroporous polymer beads. The best selector from the library was identified by a deconvolution process using the HPLC separation of several racemic *N*-(3,5-dinitrobenzoyl)-α-amino acid alkylamides as a probe. In each deconvolution step, a series of chiral stationary phases (CSPs) containing a subset of the amino acid anilide selector library was screened for enantioselectivity. After the best CSP was chosen, the library was further deconvoluted until the single best selector was found. The highest selectivity was obtained with a L-proline-1-indananilide that exhibited α values up to 23 under normal-phase HPLC conditions. In addition, six CSPs were prepared using individual selectors from the library, and screening results indicate that the deconvolution process indeed led to the most selective receptor.

The pharmaceutical field offers a rapidly expanding range of applications for the resolution of racemates by chromatography.[1] High-performance liquid chromatography (HPLC) with chiral stationary phases (CSPs) for the determination of the composition of enantiomeric mixtures in biological and pharmacological studies is now a well-established analytical tool. Moreover, the application of the method on a preparative scale for the production of enantiomerically pure compounds in amounts suitable for biological testing, toxicological studies, and even, at a later stage, clinical testing is gaining increasingly wide acceptance. During the preliminary test phase of new chiral drugs, chromatography allows rapid access to both of the pure enantiomers and can replace advantageously the often lengthy elaboration of an enantioselective synthesis.

A wide variety of CSPs for HPLC applications have been developed, enabling enantiomer separation for a broad range of compounds of different types with a variety of combinations of functionalities. Proteins,[2] polysaccharides,[3] synthetic polymers,[4] cyclodextrins,[5] macrocyclic antibiotics,[6] and low-molecular-weight synthetic selectors[7] have been linked to, or adsorbed onto, solid supports, typically macroporous silica beads, affording effective CSPs. The small, synthetically accessible selectors used in Pirkle's remarkable "brush-type" CSPs offer some distinct advantages over high-molecular-weight biopolymers. They are amenable to extensive chemical manipulations, allowing a rational optimization of both their structure and their immobilization mode. In this context, we recently reported the design of brush-type CSPs with improved performance based on our size monodisperse macroporous beads. In particular, greatly enhanced enantioselectivities were observed when compared to analogous silica-based CSPs as a result of decreased nonspecific interactions.[8] Similarly, a thorough study of linkers led to the design of a binding chemistry that minimizes its effects on enantiomer recognition.[9]

If a new racemic solute has to be resolved, commercially available CSPs are first screened and separation conditions such as mobile-phase composition, flow rate, and temperature optimized. Since this whole process may not afford adequate enantioselectivities, an alternative method is to develop a new CSP with a tailored selector capable of efficient enantiomer recognition.

The synthesis and screening of combinatorial libraries[10,11] is a validated strategy for the identification and study of ligand/

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receptor interactions.[12] It has been shown that the screening of libraries of molecules tethered to solid supports in binding assays with soluble molecules can greatly facilitate the identification of key structural elements responsible for host/guest interactions and molecular recognition.[10,13] To our knowledge, the only reported attempts to use combinatorial techniques in chromatography have only focused on affinity chromatography[14] and capillary electrophoresis.[15] Our combinatorial methods are aimed at the rapid preparation of tailor-made CSPs for HPLC designed for a specific racemic solute. Recently, we described an application of Pirkle's principle of reciprocity[16,17] in a combinatorial scheme that led to the design of novel highly selective substituted dihydropyrimidine-based CSPs.[18] A single enantiomer of the target racemate was immobilized on a macroporous polymeric support and used for HPLC screening of a library of racemic compounds. The best separated compound was prepared in enantiomerically pure form and coupled to a support providing a CSP for the efficient separation of the target racemate. Obviously, the CSP prepared by this approach is theoretically optimized for the resolution of one racemate only, although in practice, this CSP may have a somewhat broader selectivity.

In this report, we demonstrate an alternative combinatorial approach in which a CSP carrying a library of enantiomerically pure potential selectors is used directly to screen for enantioselectivity in the HPLC separation of target analytes. The best of the bound selectors for the desired separation is then identified in a few deconvolution steps. As a result of the "parallelism advantage", the number of columns that have to be screened in this deconvolution process to identify the most selective single selector CSP is much smaller than the number of actual selectors in the library.

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EXPERIMENTAL SECTION

All reactions were carried out in standard oven-dried (120 °C) glassware under an argon atmosphere blanket. Reagent-grade chemicals were purchased from Aldrich or Sigma and used without further purification. Tetrahydrofuran (THF) was freshly distilled from sodium benzophenone ketyl radical under nitrogen. Analytical thin-layer chromatography (TLC) was performed on Merck silica gel 60 F_{254}-coated plates. Compounds were visualized by dipping the plates in a basic potassium permanganate solution followed by heating. IR spectra were obtained on a Nicolet Mattson Genesis II FT-IR spectrophotometer (KBr). ^{1}H and ^{13}C NMR spectra were measured on Bruker AMX-300 or AMX-400 spectrometers in $CDCl_3$.

General Procedure for the Preparation of Amino Acid-Based Libraries. The following amino acids and aromatic amines were used to prepare libraries: *N-t*-Boc-L-valine (Val), *N-t*-Boc-L-phenylalanine (Phe), *N-t*-Boc-L-proline (Pro); 3,4,5-trimethoxyaniline, 3,5-dimethylaniline, 3-benyloxyaniline, 1-aminonaphthalene, 4-tritylaniline, 2-aminoanthracene, 5-aminoindane, 4-*tert*-butylaniline, 4-aminobiphenyl, 2-aminofluorene, 2-aminoanthraquinone, and 3-amino-1-phenyl-2-pyrazolin-5-one.

The *N-tert*-butyloxycarbonyloxy-protected (Boc) amino acids were activated for coupling with amines. First, 7.0 mmol of an amino acid was dissolved in THF (35 mL), cooled to −15 °C, and treated dropwise using a syringe with triethylamine (0.97 mL, 7.0 mmol) and $ClCO_2Et$ (0.67 mL, 7.0 mmol). After stirring at −15 °C for 1 h, a cold (−15 °C) mixture of aromatic amines composed of an equimolar mixture of the desired amines (total amount of amines 7.0 mmol) in THF (25−30 mL) was added dropwise. Stirring was continued at −15 °C for 1 h and at room temperature overnight. The resulting suspension was concentrated in vacuo, diluted with ethyl acetate (200 mL), and extracted with 1 mol/L HCl (3 × 100 mL), saturated aqueous $NaHCO_3$ (2 × 100 mL), and saturated aqueous NaCl (2 × 100 mL). The organic phase was separated, dried over $MgSO_4$, and concentrated in vacuo to afford quantitatively the product mixture as a colored solid or a foam.

General Procedure for the Deprotection of Libraries. The product obtained from the procedure described above (7.0 mmol) was dissolved in CH_2Cl_2 (10 mL), cooled to 0 °C, and treated with trifluoroacetic acid/acetic acid 1:1 (30 mL). After deprotection, the resulting solution was stirred at room temperature overnight. After TLC (hexane/ethyl acetate 2:1) confirmed the disappearance of starting materials, the reaction mixture was concentrated in vacuo and diluted with H_2O (30 mL) and CH_2Cl_2 (20 mL). A solution of 2 mol/L KOH was then added at 0 °C until the pH was 9−10. The aqueous phase was further extracted with CH_2Cl_2 (3 × 100 mL), and the combined organic phases were washed with H_2O (100 mL) and saturated aqueous NaCl (100 mL), dried over $MgSO_4$, and concentrated in vacuo. Drying under high vacuum provided a quantitative yield of the deprotected product mixture as a colored solid. Integration of the individual ^{1}H NMR signals for the amide hydrogens of the compounds clearly showed that all expected products were formed.

General Procedure for the Preparation of Single Selectors. *N*-*t*-Boc-L-proline (1.5 g, 7.0 mmol) was dissolved in CH_2Cl_2 (30 mL), cooled to 0 °C, and treated with 2-ethoxy-1-(ethoxycarbonyl)-1,2-dihydroquinoline (EEDQ; 2.0 g, 8.0 mmol) in one portion. After dropwise addition of an aromatic amine (7.0 mmol), the resulting reaction mixture was stirred at 0 °C for 2 h and at room temperature overnight. The reaction mixture was then diluted with CH_2Cl_2 (100 mL) and washed successively with 1 mol/L HCl (3 × 50 mL), saturated aqueous $NaHCO_3$ (2 × 50 mL), H_2O (50 mL), and saturated aqueous NaCl (2 × 50 mL). The organic phase was dried ($MgSO_4$) and concentrated in vacuo to afford the product quantitatively as a colored foam or solid. The following amines were used in this procedure: 5-aminoindane (**4**) to afford **13**, 4-*tert*-butylaniline (**5**) (→**14**), 4-aminobiphenyl (**6**) (→**15**), 3,5-dimethylaniline (**2**) (→**16**) 1-aminonaphthalene (**7**) (→**17**), and 4-tritylaniline (**8**) (→**18**).

Cleavage of the *t*-Boc groups was performed with trifluoroacetic acid/acetic acid as described above, giving 80−90% of pure product according to ^{1}H NMR.

General Procedure for the Preparation of Chiral Stationary Phases. To a slurry of 4-nitrophenyl carbonate-activated poly(2-hydroxyethyl methacrylate-*co*-ethylene dimethacrylate) beads prepared according to the method described previously[8,19] (1.6 g) in THF (15 mL) was added triethylamine (2.09 mL, 15 mmol) at 0 °C. The suspension was then treated dropwise with a solution of the product mixture obtained from the deprotection step (maximum 7.0 mmol), in THF (20 mL) and stirring was continued at room temperature for 3 h and at 60 °C overnight, providing a brownish reaction mixture. The modified beads were then filtered and washed repeatedly with THF, H_2O, 1 mol/L KOH, CH_3OH, acetone, and Et_2O and then dried under high vacuum. The selector content was determined by elemental analysis for nitrogen.

Chromatography. The chiral stationary phases were slurry packed at a constant pressure of 150 bar into 150 × 4.6 mm i.d. stainless steel columns. A Waters HPLC system consisting of two 510 HPLC pumps, a 717 plus autosampler, and a 486 UV detector, and controlled by Millennium 2010 software, was used for all of the chromatography.

Chiral separations were carried out using a 20% hexane/dichloromethane mixture as a mobile phase. The separation factors α (selectivity) were calculated using the equation $\alpha = k'_2/k'_1$ where k'_1 and k'_2 are the retention factors of the enantiomers defined as $k'_i = (t_R - t_0)/t_0$. t_R and t_0 represent the retention times of the compound and 1,3,5-tri-*tert*-butylbenzene (void volume marker), respectively. The racemic analytes *N*-(3,5-dinitrobenzoyl)-α-amino acid alkyl amides were prepared by methods similar to those reported previously.[20]

RESULTS AND DISCUSSION

Preparation of Library of Chiral Selectors. As outlined schematically in Figure 1, our strategy consists of the following steps. A mixture of chiral compounds (from A* to S*) is immobilized on a solid support and packed to afford a "library column" which is tested in the resolution of targeted racemic compounds. If some separation is achieved, the column may be "deconvoluted" to identify the selector possessing the highest selectivity. Deconvolution consist in the stepwise preparation of a series of "sublibrary columns" of lower diversity, each of which contains a CSP with a reduced number of library components (see, for example, three columns with subgroups of selectors A*−F*, G*−M*, and N*−S* in Figure 1).

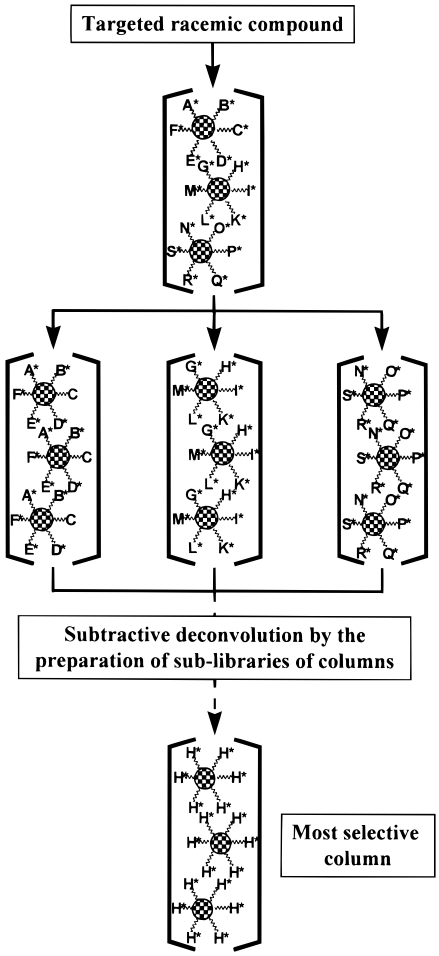

Figure 1. Illustration of the concept applied in an on-bead combinatorial deconvolution scheme to identify an effective selector H* from a multicomponent chiral stationary phase (selector library A*−S*).

The feasibility of this approach is demonstrated with a model library of 36 compounds constructed from a combination of three L-amino acids (valine, phenylalanine, proline) and 12 aromatic amines (3,4,5-trimethoxyaniline (**1**), 3,5-dimethylaniline (**2**), 3-benyloxyaniline (**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**)) (Figure 2). The libraries were prepared by a two-step procedure that includes the coupling of amino acids with a mixture of amines followed by deprotection of the resulting Boc-protected amides (Scheme 1). Both steps were performed in solution to allow for fast analysis of the homogeneity of the mixtures. The amine mixtures used in the synthesis contained equimolar quantities of building blocks **1**−**12** and were selected for their similar reactivities toward the activated amino acids.

Activation of the *N*-Boc-protected α-amino acids and acylation of the various amines was accomplished by conversion of the carboxylic acids to mixed anhydrides with $NEt_3/ClCO_2Et$ followed

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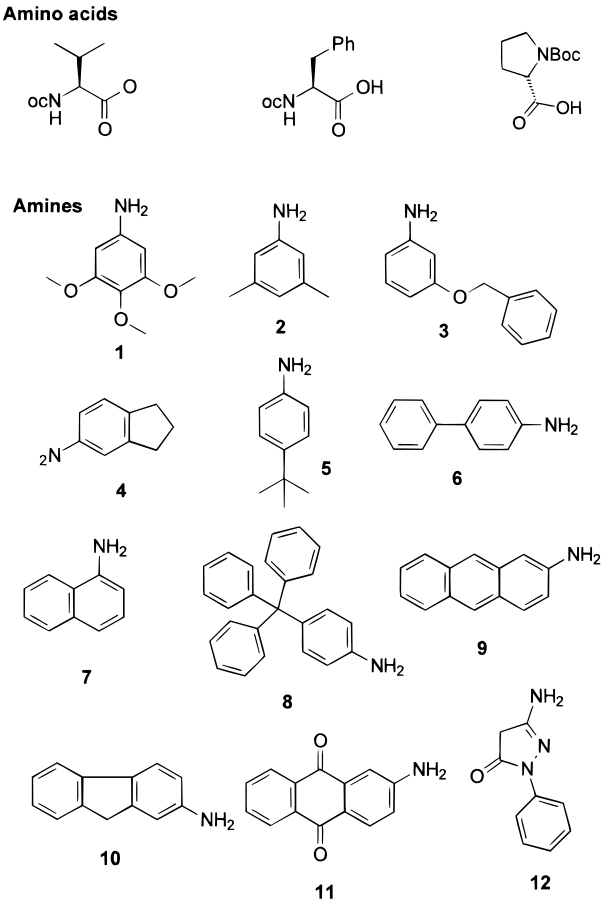
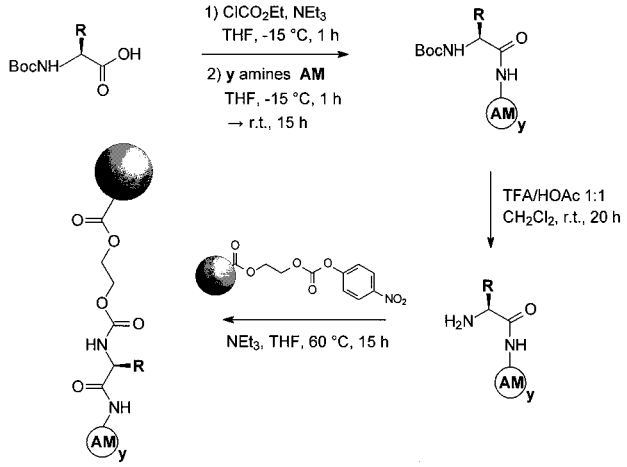


Figure 2. Building blocks used for the preparation of π-basic-substituted amino acid-based selector mixtures.

by reaction with the amine mixture to afford a mixture of chiral amides. In all cases, the yields of the last step were essentially quantitative with no side products detected by NMR analysis. Other activation methods such as 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC)/1-hydroxy-1*H*-benzotriazole (HOBt),[21] *N*-hydroxysuccinimide (HOSu)/dicyclohexylcarbodiimid (DCC),[22] or benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) [23] gave less reactive activated forms of the acid that did not fully acylate the aromatic amines. The hydrophobic nature of the libraries produced with the protected building blocks allows their separation from traces of unreacted amines or amino acids by simple extraction. After the coupling step was carried out, the Boc-protecting group was readily removed by treatment with a trifluoroacetic acid/acetic acid/CH_2Cl_2 (3:3:2) mixture. Surprisingly, only partial cleavage of the Boc group was obtained even after extended reaction times if the cleavage was carried out with a standard 5% solution of trifluoroacetic acid in CH_2Cl_2. The resulting product mixtures were obtained as colored foams or powders. Characterization by NMR, MS, and IR analyses indicated that all members of the library were present in the mixtures.

Preparation of Chiral Stationary Phases with Mixed Selectors. The homogeneous mixtures of amino acid derivatives

Scheme 1



in solution were then immobilized onto a polymeric solid support in order to test them for the resolution of racemates. Coupling of the amide mixtures with 5-μm macroporous 4-nitrophenyl carbonate activated poly(hydroxyethyl methacrylate-*co*-ethylene dimethacrylate) beads (HEMA−EDMA)[24] afforded the chiral stationary phases with a multiplicity of selectors (Scheme 1). The individual library members are assumed to show similar reactivities toward the carbonate groups of the activated support beads since they all contain a primary amine reactive group derived from the amino acid component. Activation of size monodisperse hydroxyl-functionalized HEMA−EDMA beads was accomplished by reaction with 4-nitrophenyl chloroformate as published elsewhere[8] and yielded 0.94 mmol/g carbonate functionalities. A slurry of these beads in THF was treated with an excess of the respective amide mixtures. In this way, the heterogeneous population of all selectors present in the mixture should be attached to each bead. The use of columns with mixed dissimilar selectors has not been recommended for actual enantioseparation[25] since they may exhibit opposite signs of enantioselectivity, interact with each other, and/or interact simultaneously with an analyte molecule and thus negatively affect the overall selectivity. However, the majority of mixed selectors in our combinatorial approach is not completely dissimilar. They are based on identical chiral core and differ only in the structure of the auxiliary substituents. Systematic studies of the effects of substituents attached to chiral selectors on their preferences to interact stronger with the specific enantiomer that would support this claim are scarce. For example, Pirkle's separation of 54 5-arylhydantoins has demonstrated systematically higher retention of (+)-enantiomers.[26] Our study of the separation of over 150 compounds with identical dihydropyrimidine scaffold and various substituents also did not indicate any changes in retention order.[18] This suggests that the use of an identical scaffold decreases the probability of inversion in selectivity. Therefore, the mixed selector method appears to constitute a worthwhile approach to optimized CSPs.

Screening for Enantioselectivity. As expected from the design of the experiment, the HPLC column packed with CSP **1** containing all 36 members of the library with π-basic substituents

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Table 1. Separation of Derivatized Amino Acids on CSP 2−CSP 14[a]

series		amino acid	amine [b]	loading (mmol/g)	selectivity factor α			
					I[c]	**II**[d]	**III**[e]	**IV**[f]
1	CSP **2**	Val	1−12	0.68	4.9	5.1	4.6	6.7
	CSP **3**	Phe	1−12	0.60	5.8	6.3	4.5	4.5
	CSP **4**	Pro	1−12	0.74	13.7	14.4	6.6	8.5
2	CSP **5**	Pro	1−6	0.78	13.6	19.8	10.5	9.7
	CSP **6**	Pro	7−12	0.77	7.3	7.7	5.4	5.8
3	CSP **7**	Pro	1−3	0.75	17.4	19.8	9.0	8.6
	CSP **8**	Pro	4−6	0.74	14.9	15.3	12.2	9.0
4	CSP **9**	Pro	4	0.75	23.1	22.1	13.2	12.8
	CSP **10**	Pro	5	0.74	11.5	10.7	10.6	9.6
	CSP **11**	Pro	6	0.74	12.4	13.5	8.1	7.7
control	CSP **12**	Pro	1	0.63	24.7	25.2	13.5	12.8
	CSP **13**	Pro	2	0.74	2.5	2.9	1.8	1.9
	CSP **14**	Pro	3	0.70	3.6	4.6	3.5	2.7

[a] Conditions: column 150 × 4.6 mm i.d.; mobile phase, 20% hexane in dichloromethane; flow rate, 1 mL/min; UV detection at 254 nm. [b] For structures of amines, see Figure 2. [c] (3,5-Dinitrobenzoyl)leucine diallylamide. [d] (3,5-Dinitrobenzoyl)leucine diethylamide. [e] (3,5-Dinitrobenzoyl)alanine diallylamide. [f] (3,5-Dinitrobenzoyl)alanine diethylamide.

separates π-acid-substituted amino acid amides. Although encouraging since it suggests the presence of at least one useful selector, this result does not reveal which of the numerous selectors on CSP **1** is the most powerful. Therefore, a deconvolution process that involves the preparation of series of beads with smaller numbers of attached selectors is used. Thus, in the next step, each single amino acid is coupled separately with the set of 12 amines resulting in 3 new mixed-ligand CSPs (CSP **2**−CSP **4**). Once packed into HPLC columns these CSPs were evaluated. Table 1 summarizes the separation results for the enantiomers of various *N*-(3,5-dinitrobenzoyl)-α-amino acid derivatives **I**−**IV**. For all these CSPs, the *S*-enantiomers are always retained longer than are the *R*-enantiomers. The use of a hexane/dichloromethane mobile phase affords separations of these derivatives as exemplified in the collection of chromatograms obtained on various CSPs for analyte **I** (Figure 3). Although the peaks for some of the columns are somewhat broader due to less perfect packing and lower efficiency, the separation factors α can safely be calculated to select the best performing column in each series.

The highest selectivity of 13.7 in the first series of columns, CSPs **2**−**4**, was found for the proline-based CSP **4** while the α values of the two other columns are close to 5 (see analyte **I**, Table 1). The increased selectivity for proline-based selectors attached to silica beads has also been observed by others.[27] To further determine which of the 12 compounds in the proline-based column affords the highest selectivity, a third set of columns is prepared (CSP **5**, CSP **6**) by splitting the 12 members of the amine building blocks **1**−**12** (Figure 2) in two subgroups. Thus, the first proline-based sublibrary column (CSP **5**) is prepared using six amines with smaller aromatic substituents (amines **1**−**6**), and the second column (CSP **6**) with amines characterized with larger substituents (**7**−**12**). CSP **5** and CSP **6** exhibit selectivities of 13.6 and 7.3, respectively. In the next step, the six amine group present in the more selective column CSP **5** is divided again into two groups (**1**−**3**, **4**−**6**), containing three amines with mainly meta-substituted aromatic amines and another three with para-substituted amines. Quite unexpectedly, segregation of selectors with meta- and para-substituted amines in two CSPs increases selectivities of both new columns indicating that synergetic effects occur in the mixtures of selectors [25] (vide infra). The respective columns CSP **7** and CSP **8** exhibit rather high α values of 17.4 and 14.9 for analyte **I** and indicate that both groups involve at least one selector with a very high selectivity. This is not surprising for CSP **7**, since a selector substituted with **2** that is also present in this column was previously shown to be quite successful.[8] Since the performance of these two columns was similar (CSP **7** separates better analytes **I** and **II** while CSP **8** performs better with analytes **III** and **IV**), we decided to further deconvolute CSP **8**, which involves entirely new selectors. Three columns, CSPs

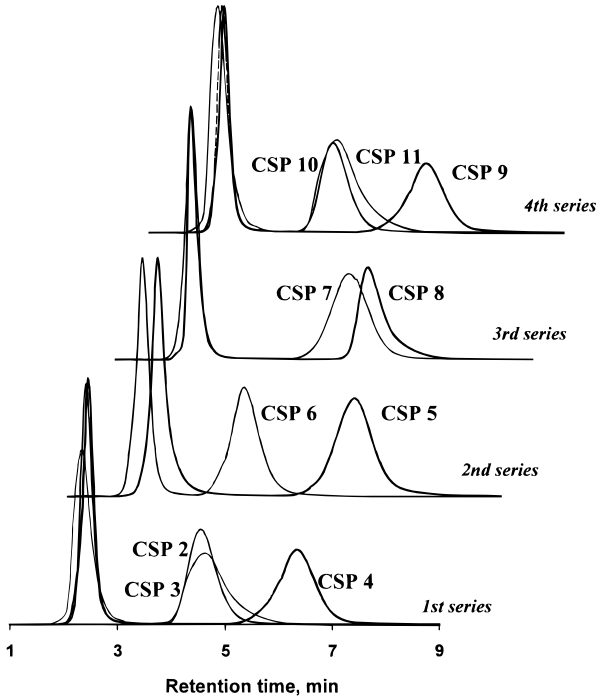

Figure 3. Overlay of chromatograms obtained with CSPs **2**−**11** prepared in four deconvolution series. Conditions: analyte, (3,5-dinitrobenzoyl)leucine diallylamide; column, 150 × 4.6 mm i.d.; mobile phase, 20% hexane in dichloromethane; flow rate, 1 mL/min, UV detection 254 nm.

(27) Pirkle, W. H.; Murray, P. G. *J. Chromatogr.* **1993**, *641*, 11. Ihara, T.; Sugimoto, Y.; Asada, M.; Nakagama, T.; Hobo, T. *J. Chromatogr., A* **1995**, *694*, 49.

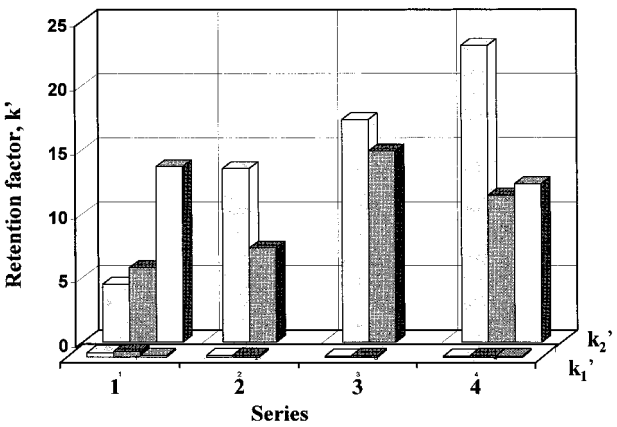

Figure 4. Retention factors *k′* determined for (3,5-dinitrobenzoyl)-leucine diallylamide on CSPs **2**−**11**. For conditions, see Figure 3.

9−**11**, packed with beads containing only individual selectors were prepared (CSPs **9**−**11** with amines **4**−**6**, respectively). Although all these columns exhibit rather high selectivities, an α value of 23.1 is achieved with CSP **9** featuring **4** as a part of the proline selector. Figure 4 shows the changes in separation factors *k′* determined for (3,5-dinitrobenzoyl)leucine diallylamide on CSPs **2**−**11**.

Since our method of screening initially operates by selecting groups of molecules rather than individual compounds and since the difference between both CSP **7** and CSP **8** was small, it is possible that our best CSP **9** is not the most efficient selector of the original mixture. To confirm this as well as to satisfy our curiosity to uncover which other selector is powerful, we prepared three additional columns CSPs **12**−**14** containing single proline-based selectors with amines **1**−**3** as a control**.** As expected from our previous research,[8] CSP **12** prepared with amine **2** also exhibits very high selectivity (α = 24.7 for analyte **I**) similar to that of CSP **9**. Surprisingly, CSP **13** and CSP **14**, prepared with amines **7** and **8**, respectively, afford only modest α values of less than 4.

Once again, the *S*-enantiomers are retained longer than the *R*-enantiomers for all CSPs **9**−**14** prepared from individual selectors. This indicates that there is no inversion of selectivity in this group as a result of the use of different substituents.

The rapid increase in the separation factors observed for the individual series of columns reflects not only the improvement in the intrinsic selectivities of the individual selectors but also the effect of increased loading with the most potent selector. Although the overall loading determined from nitrogen content remains virtually constant at about 0.7 mmol/g for all CSPs **1**−**12**, the fractional loading of each selector increases as the number of selectors in the mixture decreases, provided the reactivity of all selector molecules in the immobilization step is essentially equal. Thus, the whole method of building block selection and sublibrary synthesis can be also viewed as an amplification process.

In the classical one-column/one-selector approach, the number of columns that have to be tested equals the number of selectors. Using the chemistry described above, this would require the preparation, packing, and testing of 36 CSPs. In contrast, our combinatorial scheme allows us to obtain a very highly selective CSP from the same group of 36 selectors using only 11 columns or less than one-third. A simple theoretical calculation reveals that the use of all 20 natural amino acids with 12 amines would lead to a library of 240 selectors. While the preparation and testing of 240 columns would be time-consuming, the mixture of these selectors could be deconvoluted using our approach in 15 columns representing only $^1/_{16}$ of the total number of columns that would be otherwise required. The parallelism advantage of the "library-on-bead" approach with mixed-selector column would be even more impressive with much larger libraries of selectors for which the deconvolution by splitting the library in each step to two or three sublibraries would rapidly lead to the most selective CSP. Obviously, this approach can dramatically decrease the time required for the development of a novel CSP.

Although the power of the combinatorial approach is clearly demonstrated, this method has also some limitations. For example, in a hypothetical situation in which only a single selector is active and all members of a much larger library are attached to the beads in equal amounts, the percentage of the active selector in the mixture is low, and despite its possibly high specific selectivity (selectivity per unit of loading), the actual selectivity of a mixed-selector CSP may be rather small because the loading of the specific selector is very low. Accordingly, the peaks for both enantiomers may elute close to each other and the actual separation may become impossible to discern within the limits of experimental errors. Thus the sensitivity of the chromatographic screening may somewhat limit this approach. However, the number of selectors that may be screened in a single column is still impressive.

Another limitation of our approach is common to many combinatorial techniques. The rapid screening of mixtures of compounds can fail to discover some hits. This penalty is counterbalanced by the advantage of considerably increased speed of screening and large number of tested selectors. However, the danger of missing good selectors should not be overestimated. In a hypothetical case, simple averaging would imply that an equimolar mixture that contains one very potent selector (α = 15) together with two completely inactive compounds would exhibit an overall selectivity factor of 5 while another mixture of three selectors each with α = 6 affords a separation medium with selectivity factor of 6. Following the procedure outlined above, the latter mixture would be further deconvoluted to find a mediocre selector with α = 6 and miss the best one. However, this may not be the typical case because this assumes (i) a linear proportionality of selectivity to the amount of attached selector, (ii) the perfect additivity of selectivities, and (iii) the lack of synergistic effects of selectors within the beads. A comparison of the data shown in Table 1 for mixed selectors CSP 7 and CSP 8 with averaged selectivities for individual CSPs 9−11 and CSPs 12−14, respectively, clearly documents that the mixed-selector column has a selectivity for the critical analyte higher than the average value. For example, mixed CSP 7 is characterized by an α value of 19.8 for (3,5-dinitrobenzoyl)leucine diethylamide while the average selectivity of CSPs 9−11 is only 15.4. Obviously, the most potent selector prevails in the mixture, thus decreasing the danger of missing a real hit. These data also indicate again that the mixtures are unlikely to contain selectors with opposite signs of enantioselectivity.

In contrast, there are less limitations from the chemical point of view. The preparation of large, well-defined, libraries that involve

amino acid building blocks has been demonstrated many times.[10] Carefully optimized reaction conditions for the preparation of other mixed libraries can also ensure that each desired compound is present in sufficient amount. However, the reaction rates of some individual selectors with the activated solid support may be lower than others and those that react more readily would then occupy a majority of the sites within the beads. Since the most reactive selectors may not be the most selective, testing of a slightly larger number of specifically designed CSPs may be required to avoid false negative results.

Although high levels of enantioselectivity in the range of α values well over 20 may be unnecessary or even undesired for the analytical separations of enantiomers since they lead to rapid increase in the overall analysis time, they are desirable for preparative applications in columns operating under overload conditions.

CONCLUSION

Combinatorial chemistry, a powerful tool in many areas such as drug discovery, materials research, and catalysis, can also be used in the area of molecular recognition to discover new selectors for chiral HPLC. The strategy involving simultaneous attachment of a library of selector candidates on each bead and packing a single column followed by screening of the separation ability for various racemates has now been validated. The limits of this general method are set by the sensitivity of the detection that is required for a system characterized by low loading of the best selector at the early stage of deconvolution and by the remote possibility of simultaneous attachment of selectors with opposite signs of enantioselectivity. Although we have chosen simple π-basic-substituted amino acids for our model library of selectors used in the separation of π-acidic analytes to demonstrate the concept, many existing libraries of organic compounds could also be attached to reactive beads. These media would then allow the screening for chiral recognition and accelerate the design of novel chiral stationary phases with high selectivity for the desired target racemate. As an additional benefit, such a study carried out with structurally related families of selectors can further improve the general understanding of chiral recognition.

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