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Report

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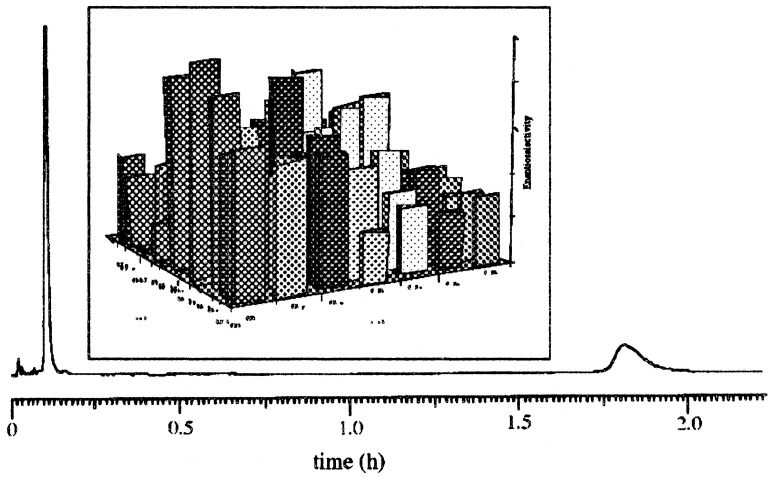

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Selection of an Optimized Adsorbent for Preparative Chromatographic Enantioseparation by Microscale Screening of a Second-Generation Chiral Stationary Phase Library

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Introduction

We recently reported a new method for preparing and evaluating microscale libraries of chromatographic adsorbents.[1,2] In this approach, libraries of silica-based adsorbents are first prepared on milligram scale using a solid phase parallel synthesis strategy. Each member of the library is then rapidly evaluated for its ability to carry out a given separation without the requirement of packing the adsorbent into a column. One of the greatest advantages of the method over other combinatorial chemistry approaches to the development of chromatographic adsorbents[3] is that the adsorbent−analyte interaction is probed directly, rather than with a tethered analogue of the analyte. Consequently, false leads for adsorbents which selectively bind tethered analytes are eliminated.

As part of our initial demonstration of this approach we prepared and evaluated a library of 50 silica-supported *N*-3,5-dinitrobenzoyl-dipeptide chiral stationary phases (CSPs) for enantioselective recognition of a test racemate (Figure 1).[2] Evaluation of this library suggested that, for this analyte, enantioselectivity strongly depends on the presence of an amino acid with a sterically bulky side chain (e.g. valine) in the aa 2 position of the CSP.[4] A minor dependence of enantioselectivity on the presence of an amino acid with a hydrogen-bonding side chain (e.g. glutamine) in the aa 1 position was also noted.

These results suggested that further optimization of adsorbent enantioselectivity could emerge from a focused library of tethered DNB dipeptides having sterically bulky groups in the aa 2 position and hydrogen-bonding groups in the aa 1 position. We now report the preparation and evaluation of such a library, as well as the selection, scale-up, and evaluation of an optimized preparative adsorbent.

Results and Discussion

Previous studies had suggested that tethered DNB dipeptide adsorbents with increased enantioselectivity for the test racemate could be discovered by preparing and evaluating a focused library with sterically bulky groups in the aa 2 position and hydrogen-bonding groups in the aa 1 position. A new library was prepared using the amino acids leucine, isoleucine, *tert*-leucine, valine, phenylalanine, tryptophan, and tyrosine in the aa 2 position and the amino acids glutamine, asparagine, serine, histidine, arginine, aspartic acid, and glutamic acid in the aa 1 position. A previously reported procedure[1] utilizing Boc amino acids was used to prepare a sublibrary of 39 DNB-dipeptide CSPs from amino acids requiring no side chain protecting groups (Figure 2). An additional sublibrary of 32 DNB-dipeptide CSPs was prepared using Fmoc amino acids for those amino acids which required side chain protecting groups (Figure 3). In all, 71 of the 98 possible structures were prepared.

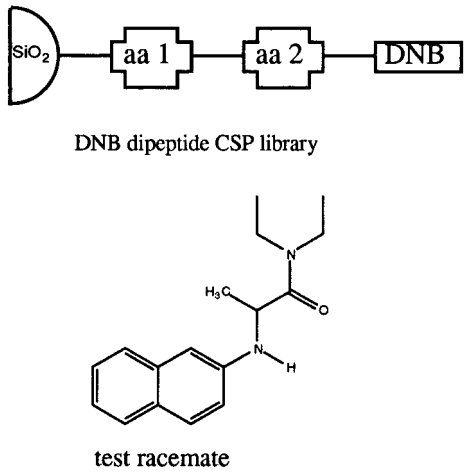

Figure 1. In a previous study, libraries of DNB-dipeptide CSPs were shown to resolve the enantiomers of the test racemate.[2] A preference for sterically bulky groups in the aa 2 position and for hydrogen-bonding groups in the aa 1 position was noted.

Evaluation of the CSP libraries, each consisting of ca. 50 mg of adsorbent in an autosampler vial, was performed using a previously reported method.[2] A dilute solution of the racemate (1 mL; 10^{-5} M) was added to each vial. The vials were allowed to equilibrate on a rotary platform shaker. Upon equilibration, the individual enantiomers become partitioned between the solid and liquid phases. Analysis of the supernatant solution in each vial shows a 1:1 ratio of enantiomers for poorly enantioselective adsorbents with greater enantiomer ratios being observed with highly enantioselective adsorbents.

Evaluation of the combined libraries shown in Figures 2 and 3 reveals many adsorbents which perform better than the best member (APS-(*S*)-Gln-(*S*)-Val-DNB) of the initial library (Figure 4). The amino acids leucine, isoleucine, and phenylalanine were generally found to be superior to valine in the aa 2 position, while glutamic acid, aspartic acid, and histidine were generally found to be superior to glutamine in the aa 1 position.

Some of the most selective adsorbents identified in the screening assay are pictured in Figure 5. To fully illustrate the potential of this approach we decided to prepare one of these materials on full scale, pack it into a column, and

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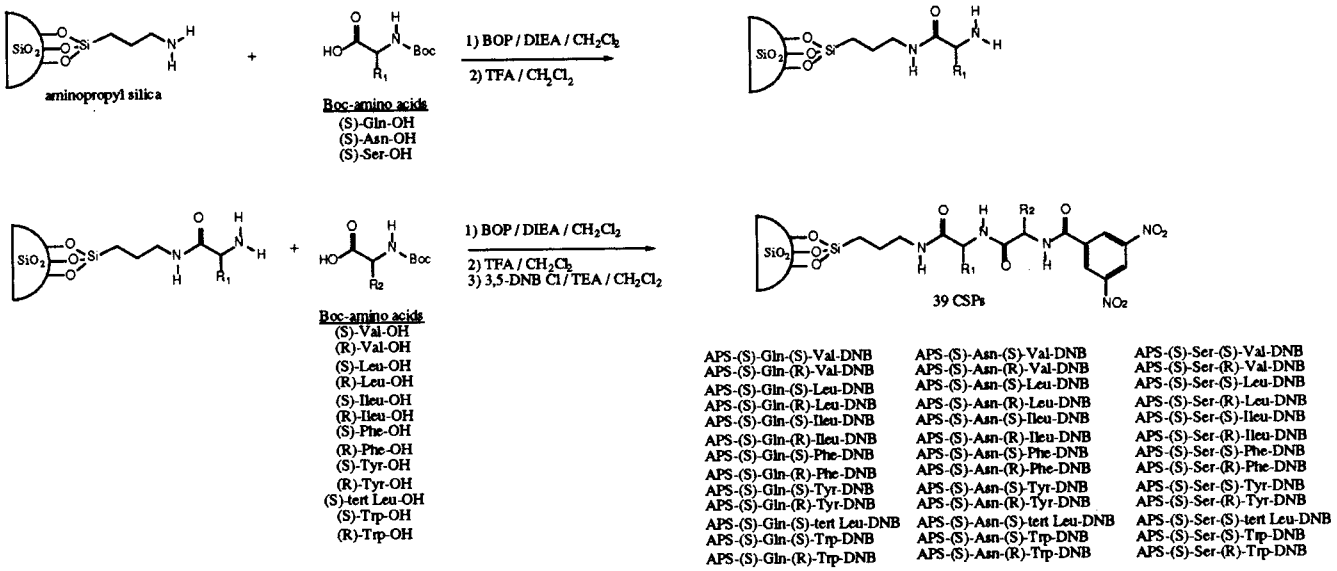

Figure 2. Approach used for preparation of the library of 36 DNB-dipeptide CSPs employing Boc protecting group strategy.

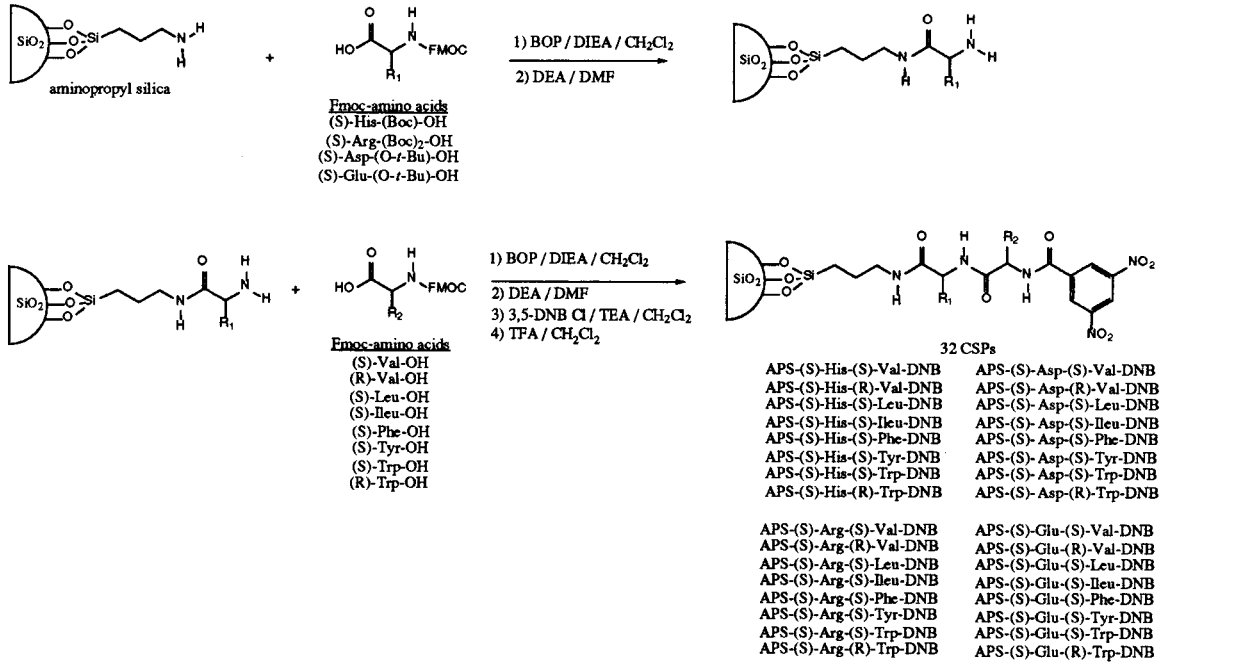

Figure 3. Approach used for preparation of the library of 32 DNB-dipeptide CSPs employing Fmoc protecting group strategy.

evaluate its utility for preparative separation of the enantiomers of the test racemate. One could imagine making such a selection among the best candidate adsorbents on the basis of manufacturing cost as well as considerations of processing economy.

We selected APS-(*S*)-Glu-(*S*)-Leu-DNB for evaluation at larger scale. This material was synthesized on 5 g scale using the solid phase synthesis protocol illustrated in Figure 3. The resulting material was packed into a 4.6 × 250 mm HPLC column and evaluated chromatographically. It has long been recognized that synthesis of CSPs by solid phase synthesis can be problematic.[5] Nevertheless, this approach allows rapid proof of principle. A CSP with further improved performance could probably be prepared by first synthesizing and purifying the chiral selector, then immobilizing it on silica.[6]

Evaluation of a 4.6 × 250 mm analytical HPLC column containing the APS-(*S*)-Glu-(*S*)-Leu-DNB CSP shows an enantioselectivity of greater than 20 for the test racemate using a 2-propanol/hexane mobile phase (Figure 6). An enantioselectivity of greater than 18 is obtained with a mobile phase of pure ethyl acetate. The test racemate has excellent solubility in ethyl acetate, an important criterion for high productivity in preparative HPLC. In addition, single-component mobile phases are preferred for large scale separations, owing to the ease of recycling.

Preparative evaluation of the analytical column (4.6 × 250 mm) containing the APS-(*S*)-Glu-(*S*)-Leu-DNB CSP was performed by injecting progressively larger amounts of a 50 mg/mL ethyl acetate solution of the racemate onto the column. The column proved capable of baseline resolution of 100 mg of the test racemate in a single injection of 2 mL.

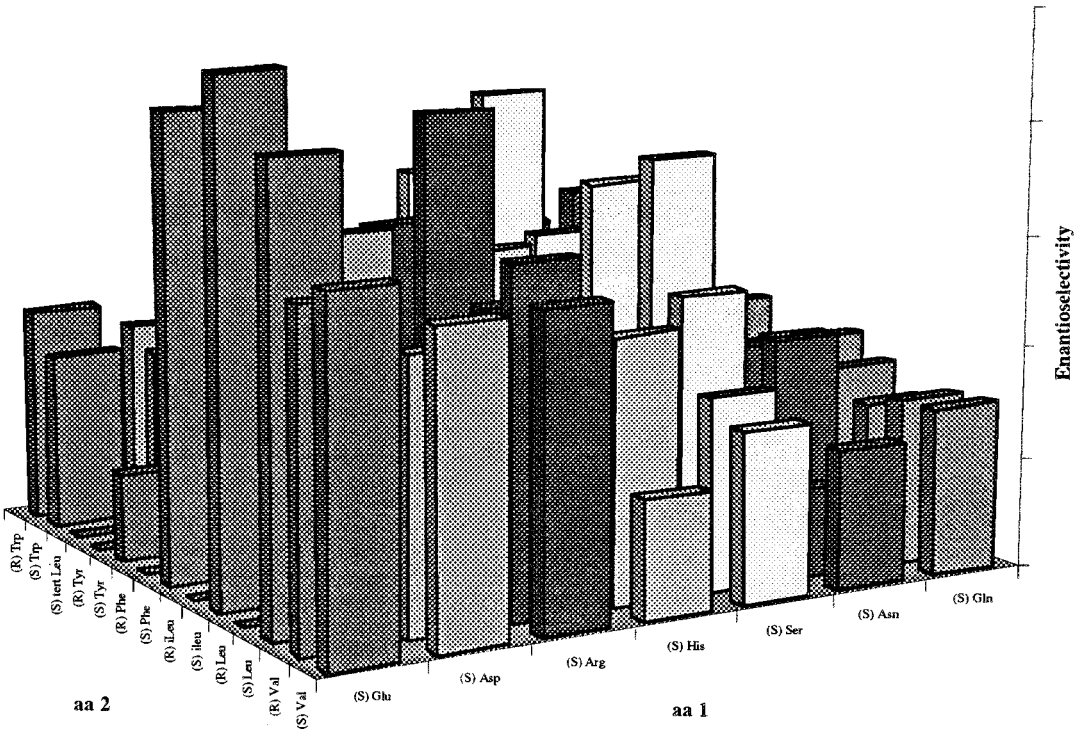

Figure 4. Evaluation of a focused library of 71 DNB-dipeptide CSPs for enantioseparation of the test racemate.

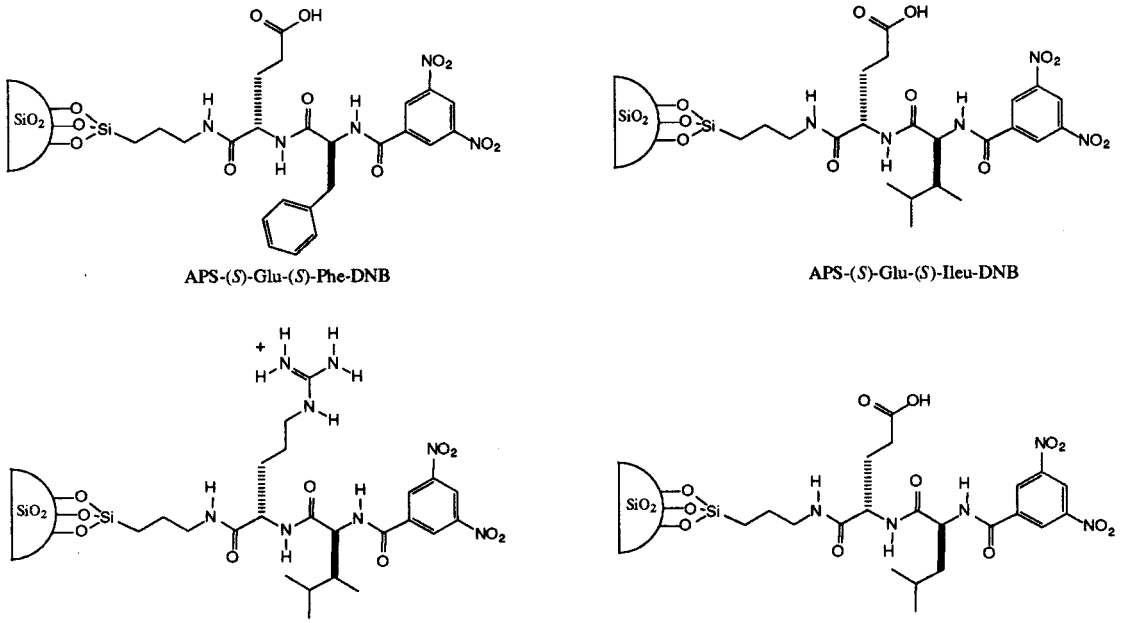

Figure 5. "Optimal" adsorbents for enantioseparation of the test racemate.

(Figure 7) Fractions collected before and after the indicated cut point were 98.4% ee and 97% ee, respectively. Further increases in productivity could almost certainly be obtained by adjusting parameters such as temperature or flow rate, and a sizable increase in productivity could be expected for the use of this CSP in a simulated moving bed (SMB) process.[7] Nevertheless, even based on this unoptimized HPLC separation, a single kilogram of this CSP could be used to separate the enantiomers of nearly a ton of racemate in a year, a clear illustration of the economic potential of this approach.

Conclusions

We have demonstrated the utility of a new combinatorial synthesis and screening approach for developing highly selective adsorbents for use in preparative chromatographic purifications. Previous evaluation of a microscale adsorbent library suggested a more focused library, which was prepared and evaluated. Scale-up of a selected adsorbent from this library afforded a material with extremely high productivity. This approach is expected to prove useful in the discovery and development of highly selective adsorbents for use in

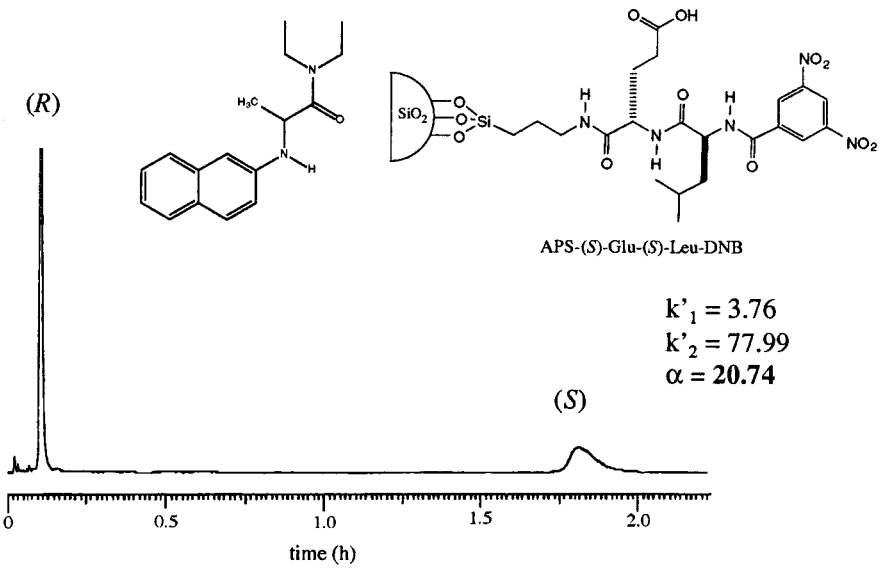

Figure 6. HPLC evaluation of a 4.6 × 250 mm analytical column containing the selected APS-(*S*)-Glu-(*S*)-Leu-DNB CSP. Mobile phase = 20% IPA/hexane; flow rate = 2.0 mL/min; detection = UV 280 nm.

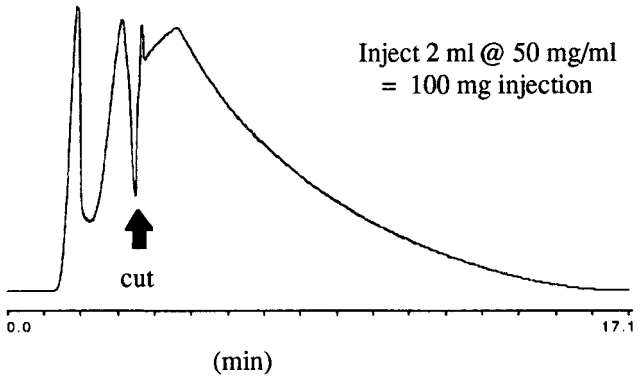

Figure 7. Preparative HPLC using a 4.6 × 250 mm analytical column containing the selected APS-(*S*)-Glu-(*S*)-Leu-DNB shows a baseline resolution of 100 mg of the test racemate in a single injection of 2 mL. Mobile phase = ethyl acetate; flow rate = 2.0 mL/min; detection = UV 380 nm. Fractions collected before and after the indicated cut point were 98.4% ee and 97% ee, respectively.

large scale separation processes for manufacturing pharmaceuticals and other products, where it is well known that increased adsorbent selectivity generally results in more economical separation processes. Although the present example concerns the separation of enantiomers, the general utility of the approach for discovery of adsorbents for other types of separations is evident. Furthermore, the approach shows promise for developing specialty adsorbents with such high selectivity that they could be used for economically attractive nonchromatographic separation and manufacturing processes such as batch filtration, membrane separation, deracemization, and incorporation into resolving machines.

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Supporting Information Available. Experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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