# Enantioselective and Diastereoselective Binding Study of Silica Bound Macrobicyclic Receptors by HPLC

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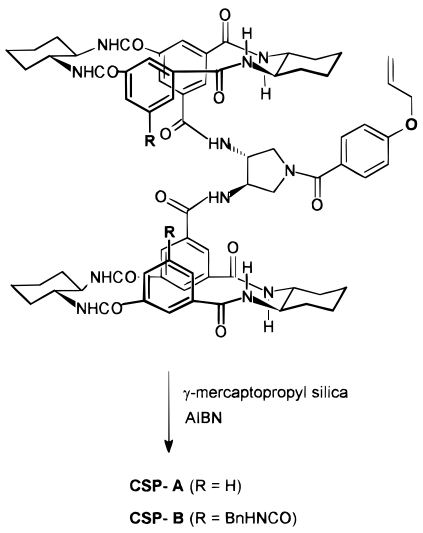
Systematic investigation of enantioselective binding processes can be conveniently carried out by direct chromatographic methods after immobilization of a given chiral host on a suitable inert matrix. In a previous paper,[1] we found close agreement between HPLC retention data gathered with a silica-bound, macrotricyclic chiral stationary phase (CSP) and association constants measured in free solution by NMR for a range of small molecule peptidic guests. CSP's with highly preorganized, medium-sized hosts are attractive since they often afford high levels of enantioselectivity and yet have relatively low molecular complexity, thus facilitating the understanding of operative chiral recognition mechanisms from easily collected chromatographic data.

Here we describe the properties of two novel, chemically bonded chiral stationary phases (**CSP-A** and **CSP-B**) prepared from synthetic receptor systems that combine a semirigid framework with multipoint hydrogen-bonding sites (Scheme 1). Both phases incorporate previously described $C_2$-symmetric, two-armed receptors[2] covalently linked to LiChrosorb 5$\mu$ silica. These receptors are readily prepared from two identical macrocyclic armlike substructures connected by a *trans*-diaminopyrrolidine linker, the ring nitrogen of which carries an allyl-terminated chain for grafting to the silica support. The macrocyclic arms are tetraamides prepared from chiral 1,2-diaminocyclohexane and isophthalic acid derivatives. They serve as conformationally restricted surfaces that carry an array of spatially defined hydrogen bond donors and acceptors. In conjunction with the linker, the macrocyclic arms fold to form a concave binding site made up of aromatic rings and amide functional groups that can make associative interactions with guests having complementary shapes and functionalities. These chiral hosts were grafted to $\gamma$-mercaptopropyl silica microparticles by AIBN free radical addition and produced CSP's with receptor loading between 0.07 and 0.09 mmol/g of silica.

Enantioselectivity and retention data collected from a diverse set of simple racemic guests on **CSP-A** and **CSP-B** (Table 1)[3] were used to probe the binding interactions involved in the chiral recognition process. Among the peptidic guests, the largest enantioselectivities were found for amino acid derivatives that were *N*-acylated by 3,5-dinitrobenzoyl (DNB) groups. *N*-Acylation by other aromatic acids was also effective, with the enantioselectivities varying as DNB > pentafluorobenzoyl > naphthoyl ∼ benzoyl. Aliphatic *N*-acylation (e.g. by Boc, Ac) led to no significant enantioselection.

**Scheme 1**




Among DNB-substituted amino acid derivatives, both phases showed a preference for *S*-configured guests and a dependence of the degree of enantioselectivity on the nature of the side chain and the *C*-terminal functionality. Among the various amino acids studied, the best enantioselection was found with Leu, Ile, and Met. Interestingly, all of these amino acids have the same number of heavy atoms in their side chains and are probably being selected because their side chains are of similar size and make a good fit to the concave binding site of the receptor. The best *C*-terminal functionality for high enantioselection was a secondary amide, and the extent of enantioselection did not depend on the bulk of the amide substituent (entries 2, 12−14). Amino acid esters generally resolved poorly (entries 3 vs 11) while the tertiary amides we studied did not resolve at all (entry 2 vs 15).

Comparison of retention data for the more retentive *S* enantiomers of different ArCO-Leu-hexylamides (entries 3, 8, 9, and 10) revealed that the DNB derivatives were always the most strongly retained. The increased affinity of the DNB derivatives for the CSP's contributed ∼0.5 kcal/mol in enantioselectivity over the other aroyl Leu derivatives, suggesting that aromatic−aromatic interactions are playing a role in stabilizing the host−(*S*)-guest complexes. Although the exact nature of these interactions is unclear at this time, it could be interpreted according to the electrostatic model proposed for $\pi$-stacked porphyrin[4] and $\pi$-stacked phenyls in 1,8-diarylnaphthalenes.[5]

Other 3,5-dinitrobenzoylated mono- or difunctional amines, alcohols, and amino alcohols were also effectively

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(1) Gasparrini, F.; Misiti, D.; Villani, C.; Borchardt, A.; Burger, M. T.; Still, W. C. *J. Org. Chem.* **1995**, *60*, 4314.

(2) For a description of related dye-labeled receptors, see: Wennemers, H.; Yoon, S. S.; Still, W. C. *J. Org. Chem.* **1995**, *60*, 1108.

(3) Chromatographic data were collected with chlorinated solvents for comparison purposes; other eluting systems (acetone, AcOEt, $CH_3CN$, MBTE, or water-based eluents) can be used as well, although with reduced enantioselectivities.

(4) Hunter, C. A., Sanders, J. K. M. *J. Am. Chem. Soc.* **1990**, *112*, 5525.

(5) Cozzi, F.; Cinquini, M.; Annunziata, R.; Siegel, J. S. *J. Am. Chem. Soc.* **1993**, *115*, 5330. Cozzi, F.; Ponzini, F.; Annunziata, R.; Cinquini, M.; Siegel, J. S. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1019.

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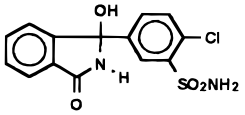
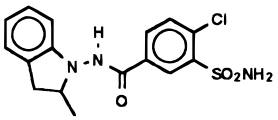
**Table 1. Chromatographic Data for the Resolution of Racemates on CSP-A and CSP-B**

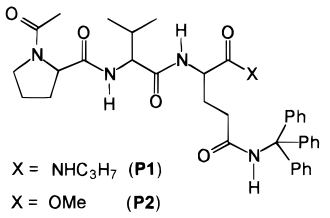
| | | CSP-A | | | CSP-B | | | |
|---|---|---|---|---|---|---|---|---|
| entry | compd | $K'_1$[a] | $\alpha$[b] | conf[c] | $K'_1$[a] | $\alpha$[b] | conf[c] | eluent[d] |
| 1 | *N*-DNB-Ala-CONH-$C_6H_{13}$[e] | 1.70 | 2.30 | *R* | 1.70 | 1.86 | *R* | a |
| 2 | *N*-DNB-Val-CONH-$C_6H_{13}$ | 0.78 | 3.37 | *R* | 0.96 | 2.27 | *R* | a |
| 3 | *N*-DNB-Leu-CONH-$C_6H_{13}$ | 1.41 | 6.49 | *R* | 1.63 | 4.97 | *R* | a |
| 4 | *N*-DNB-Ile-CONH-$C_6H_{13}$ | 0.72 | 5.07 | *R* | 0.92 | 2.33 | *R* | a |
| 5 | *N*-DNB-Met-CONH-$C_6H_{13}$ | 1.30 | 5.06 | *R* | 1.40 | 4.25 | *R* | a |
| 6 | *N*-DNB-PhGly-CONH-$C_6H_{13}$ | 1.09 | 2.17 | *R* | 1.21 | 2.79 | *R* | a |
| 7 | *N*-DNB-Phe-CONH-$C_6H_{13}$ | 1.17 | 1.73 | *R* | 1.37 | 1.93 | *R* | a |
| 8 | *N*-PFB-Leu-CONH-$C_6H_{13}$[e] | 0.85 | 2.38 | *R* | 0.88 | 1.86 | *R* | a |
| 9 | *N*-benzoyl-Leu-CONH-$C_6H_{13}$ | 0.61 | 2.09 | *R* | 0.79 | 1.49 | *R* | a |
| 10 | *N*-naphthoyl-Leu-CONH-$C_6H_{13}$ | 0.54 | 1.72 | *R* | 0.87 | 1.27 | *R* | a |
| 11 | *N*-DNB-Leu-$COOCH_3$ | 0.44 | 1.34 | *R* | 0.59 | 1.25 | *R* | a |
| 12 | *N*-DNB-Val-CONH-$CH_3$ | 2.59 | 2.66 | *R* | 4.23 | 1.71 | *R* | a |
| 13 | *N*-DNB-Val-CONH-$^tC_4H_9$ | 0.57 | 2.87 | *R* | 0.60 | 1.77 | *R* | a |
| 14 | *N*-DNB-Val-CONH-1-adamantyl | 0.56 | 2.64 | *R* | 0.57 | 1.55 | *R* | a |
| 15 | *N*-DNB-Val-$CONEt_2$ | 0.36 | 1.00 | | 0.37 | 1.00 | | a |
| 16 | *trans*-1,2-diaminocyclohexane[f] | 1.28 | 4.40 | *R,R* | 1.14 | 3.56 | *R,R* | b |
| 17 | *trans*-2-aminocyclohexanol[g] | 5.27 | 2.46 | | 5.40 | 1.45 | | a |
| 18 | 2-aminocyclohexanone[g] | 0.35 | 1.34 | | 0.49 | 1.25 | | a |
| 19 | 1,2-diphenylethylenediamine[f] | 0.84 | 1.97 | *S,S* | 1.08 | 1.00 | | b |
| 20 | 1-phenylethylamine[g] | 0.68 | 1.15 | *S* | 0.68 | 1.13 | *S* | a |
| 21 | norephedrine[g] | 2.94 | 1.17 | 1*S*,2*R* | 1.84 | 1.40 | 1*S*,2*R* | a |
| 22 | *trans*-1,2-cyclohexanediol[h] | 3.76 | 1.63 | *S,S* | 2.75 | 1.41 | *S,S* | c |
| 23 | *trans*-1,2-cycloheptanediol[h] | 4.91 | 1.54 | *S,S* | 2.94 | 1.70 | *S,S* | c |
| 24 | *trans*-1,2-cyclooctanediol[h] | 3.72 | 1.83 | *S,S* | 2.39 | 2.05 | *S,S* | c |
| 25 | *trans*-9,10-dihydro-9,10-dihydroxyphenanthrene | 3.17 | 1.13 | *S,S* | 3.49 | 1.13 | *S,S* | a |
| 26 | $\beta$-binaphthol | 1.37 | 1.17 | *R* | 1.34 | 1.00 | | e |
| 27 | 6,6'-dibromo-$\beta$-binaphthol | 5.93 | 1.17 | *R* | 3.61 | 1.20 | *R* | e |
| 28 | 7,7'-dibromo-$\beta$-binaphthol | 4.09 | 1.39 | *R* | 3.20 | 1.00 | | e |
| 29 |  | 12.38 | 1.32 | | 10.15 | 1.00 | | d |
| 30 |  | 18.65 | 1.34 | | 16.95 | 1.14 | | a |

[a] Retention factor defined as $(t_i - t_0/t_i)$, where $t_i$ and $t_0$ are the retention times of a solute and of an unretained compound, respectively. [b] Enantioselectivity factor defined as $k'_2/k'_1$. [c] Configuration of the first eluted enantiomer. [d] Eluents: a, 0.5% MeOH in $CH_2Cl_2$; b, 2% MeOH in $CH_2Cl_2$; c, 1% MeOH in $CHCl_3$; d, 5% MeOH in $CH_2Cl_2$; e, 1% EtOH in $CHCl_3$. [e] DNB, 3,5-dinitrobenzoyl; PFB, pentafluorobenzoyl. [f] As *N,N'*-3,5-dinitrobenzoylamide (DNB). [g] As *N*-3,5-dinitrobenzoylamide. [h] As 3,5-dinitrophenyl carbamate.

resolved. However, the presence of an electron deficient aromatic group was not essential for successful resolution, as many guests lacking such a group (e.g. binaphthols, entries 26−28) also separated well. These results suggest that enantioselection relies on a combination of hydrogen bonding and aromatic−aromatic interactions. We generally found that the two hosts on **CSP-A** and **CSP-B** behave similarly with small guests, in terms of the magnitude and sense of enantioselectivity.

Since interaction of small guests is confined to a limited portion of the relatively large host molecules, larger polyfunctional guests might be expected to have additional associative interactions with the hosts and therefore show enhanced affinity (and possibly selectivity) for our stationary phases. To test this hypothesis, we individually prepared all eight stereoisomers of the tripeptide Ac-Pro-Val-Gln(*N*-trityl) as their propyl amide (**P1**) and the methyl esters (**P2**).

This peptide was chosen because one of its stereoisomers (D,L,D) had been previously reported to bind the host on **CSP-A,** and related dipeptidic L-Val-D-Gln(*N*-trityl) derivatives were reported to bind the **CSP-B** host.[2] In this previously reported work, the peptides were attached to a solid support (polystyrene) and the receptors were in free solution, whereas here the peptides are in free solution while the receptors are on the solid support (silica).




(**P1**) Ac-Pro-Val-Gln(γ-trityl)-$NHC_3H_7$

(**P2**) Ac-Pro-Val-Gln(γ-trityl)-OMe

A chromatogram of the eight stereoisomeric tripeptidic amides **P1** on the **CSP-A** column is shown in Figure 1. Although the peptide mixture was not completely resolved, the D,L,D stereoisomer of **P1** was found to have a much higher affinity for **CSP-A** than any of the other stereoisomers. Thus **CSP-A** selected the same tripeptide amide stereoisomer found in previous work by an assay having the guest and host on opposite phases. The enantioselectivity of **CSP-A** for the D,L,D/L,D,L enantiomeric pair of **P1** was remarkably high ($\alpha = 16$). Under the same experimental conditions, **CSP-B** gave an almost identical chromatographic pattern for tripeptide amide mixture **P1**. However, **CSP-B** had different stereochemical preference in that the most tightly bound stereoisomer now had the L,L,D configuration. This high affinity

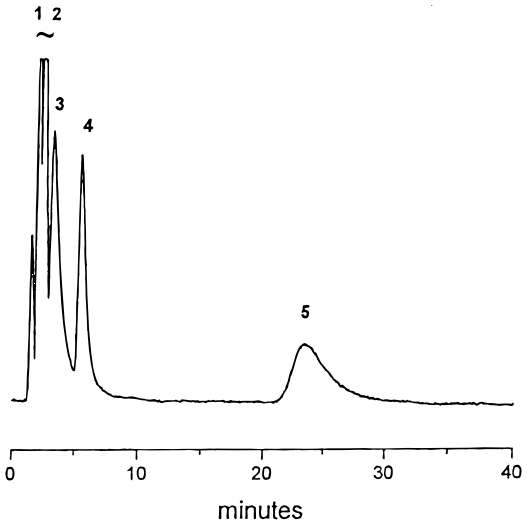



**Figure 1.** Chromatogram of **P1** on **CSP-A**. Eluent, 2% MeOH in $CH_2Cl_2$; flow rate, 0.5 mL/min; *T*, 25 °C; UV detection, 260 nm. Peak assignment (obtained by coinjection of individual diastereomers) as follows: 1 (D,D,L + D,D,D + L,L,L); 2 (L,D,D + D,L,L); 3 (L,D,L); 4 (L,L,D); 5 (D,L,D).

was again accompanied by a large enantioselectivity for the L,L,D/D,D,L pair ($\alpha = 17$). This result is also in line with the previous solid phase assays in which a soluble, dye-labeled analogue of **CSP-B** was found to selectively bind tripeptides containing L-Val-D-Gln subsequences.

In contrast, both **CSP-A** and **CSP-B** showed low affinities and low diastereo- and enantioselectivity for the tripeptide esters (**P2**). Though the **P2** affinities were low, the most retained stereoisomers had the same D,L,D (**CSP-A**) and L,L,D (**CSP-B**) stereochemistries found with amides **P1**. These results clearly show that small variations in the structure of either hosts or guests incorporating multiple binding sites can induce large energetic variations in binding and even switch the stereochemical preference of the recognition process.

In an attempt to better characterize the interaction of **P1** with our CSP's, enthalpic and entropic contributions to analyte retention and enantioselectivity were evaluated. These measurements were made from variable temperature chromatographic runs using expressions 1 and 2 respectively, where *R* is the gas constant, *T* the absolute temperature of the column, and Φ is the column phase ratio.[6]

$$\ln k' = -\Delta H/RT + \Delta S/R + \ln \Phi \qquad (1)$$

$$\ln \alpha = -\Delta\Delta H/RT + \Delta\Delta S/R \qquad (2)$$

For adsorption of analytes on brush type phases, $\Delta H$ and $\Delta S$ are usually both negative. Thus the process is exothermic and accompanied by a reduction in entropy, as expected for a simple bimolecular association process. Under such conditions, analyte retention decreases with increasing temperature.

Surprisingly, we found on **CSP-A** (see Figure 2) and **CSP-B** that retention of the loosely bound isomeric peptides increased on raising the column temperature

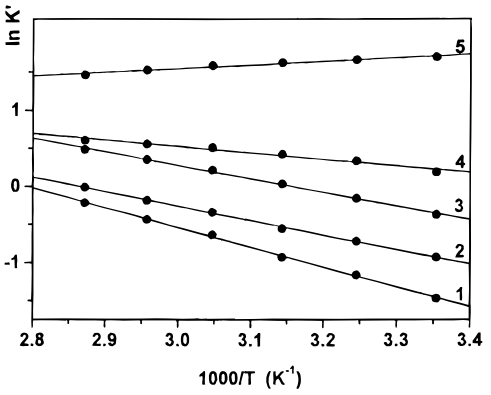



**Figure 2.** Van't Hoff plots for the separation of **P1** on **CSP-A**. Eluent, 3% MeOH in $CH_2Cl_2$; flow rate, 0.25 mL/min. Other conditions as in Figure 1.

**Table 2. Selected Thermodynamic Parameters for the Resolution of P1 on CSP-A and CSP-B**

| peptide | $\Delta H^a$ | $\Delta S^b + \ln \Phi$ |
|---|---|---|
| | CSP-A | |
| L,D,L (**P1**) | 3.5 ± 0.3 | 11.0 ± 0.5 |
| D,L,D (**P1**) | −1.0 ± 0.3 | 0.2 ± 0.5 |
| | CSP-B | |
| D,D,L (**P1**) | 2.4 ± 0.3 | 6.0 ± 0.5 |
| L,L,D (**P1**) | −1.2 ± 0.3 | 0.0 ± 0.5 |

[a] In kcal/mol. [b] In cal/mol K.

(entropy-driven retention), while retention for the last eluted isomer showed the usual temperature dependence (enthalpy-driven retention). Thermodynamic results from Van't Hoff analysis of retention data collected over a 25−75 °C temperature range for the L,D,L/D,L,D and D,D,L/L,L,D enantiomeric pairs are given in Table 2. Both host and guests are expected to be strongly solvated by a large number of methanol molecules (the polar component of our eluting system) and adsorption of guests should be accompanied by desolvation and release of adsorbed methanol to the bulk mobile phase. Thus the observed thermodynamic quantities likely include significant contributions from these processes. The different temperature behavior observed for the isomers of **P1** on our CSP's may be explained by the simultaneous occurrence of multiple recognition mechanisms, most likely involving different complexation sites located in the interior or on the external surface of the hosts. Positive enthalpy and entropy of adsorption, as encountered in our system for the less retained isomers of **P1**, can be explained by extensive reorganization of solvent structure. Enthalpy and entropy of adsorption are dominated by desolvation processes with overall unfavorable $\Delta H$ and favorable $\Delta S$ terms. For the last eluting isomer (optimal fit with the CSP) $\Delta H$ of complex formation overcomes $\Delta H$ of desolvation while the opposing contributions to $\Delta S$ cancel each other. However, other processes (e.g. changes in host or guest conformational flexibility) whose enthalpy−entropy balance is not easily estimated may also contribute to the observed chromatographic behavior. Although we are unaware of similar phenomena in chiral chromatographic systems occurring in organic solvents, several examples of association processes in aqueous solution showing favorable $\Delta S$ and unfavorable $\Delta H$ have been described previously.[7]

In summary, we have shown that silica-bound receptors can be used effectively to investigate binding interactions in chiral systems. For the peptidic model com-

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pounds we examined, chromatographic affinity data are in agreement with results previously reported for a solid-phase binding assay and are here complemented with enantioselectivity values. A practical advantage of the HPLC technique over traditional ones for obtaining relative binding energies is that only small amounts of host are required to prepare a column with which hundreds of guests can be readily analyzed. We are currently investigating the application of our CSP's to the screening of larger peptide libraries, and results of these studies will be reported in due course.

## Experimental Section

Bonding to silica, column packing, chromatographic efficiency evaluation, and column void volume determination were performed as described in ref 1. **CSP-A** Anal.: C, 12.45; H, 1.38; N, 1.42. Loading density: 0.09 mmol/g, based on N. FT-IR (KBr): 1653, 1540 $cm^{-1}$. Theoretical number of plates: >6000 for a 250 × 1.8 mm i.d. column. **CSP−B** Anal.: C, 11.71; H, 1.12; N, 1.29. Loading density: 0.07 mmol/g, based on N. FT-IR (KBr): 1653, 1540 $cm^{-1}$. Theoretical number of plates: >6000 for a 250 × 1.8 mm i.d. column.

Variable temperature runs were performed by placing the columns inside a temperature-controlled oven and collecting the data at six temperatures from 25 to 75 °C ($\Delta T \pm 0.5$ °C). Linear regression analysis of the data (Origin 4.1 software, Microcal Inc.) was used to extract $\Delta H$, $\Delta S$, and the correlation coefficients for the ln $k'$ vs $1/T$ plots. Uncertainties were determined from the standard deviation of slope and intercept for $\Delta H$ and $\Delta S$, respectively, at the 95% confidence level.

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