Enantioselective Recognition by a New Chiral Stationary Phase at Receptorial Level

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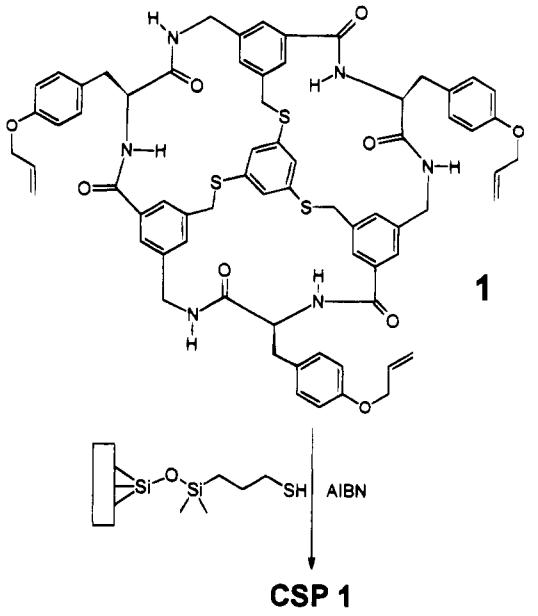
A wide variety of chiral stationary phases (CSPs) for HPLC applications have been so far developed, enabling enantiomer separations of a broad range of compounds with different types and combination of functionalities and stereogenic elements.

Proteins,[1] carbohydrates,[2] synthetic polymers,[3] cyclodextrins,[4] macrocyclic antibiotics,[5] and low molecular weight synthetic selectors[6] have been linked to or adsorbed on solid supports yielding effective sorbents for analytical and/or preparative separations. Small, synthetically accessible selectors offer some distinct advantages over high molecular weight biopolymers as they are amenable to extensive chemical manipulations allowing a rational optimization of both structure and grafting mode; moreover, soluble models mimicking the CSP modes of action can be used to spectroscopically elucidate the recognition process.

Here we describe a new HPLC chiral phase (**CSP 1**), obtained by covalent attachment of the synthetic C_3-symmetric receptor **1**[7] to silica gel microparticles, and its ability to resolve neutral substrates having diverse structural features. Receptor **1** was covalently bound to γ-mercaptopropyl silica gel by free radical addition of thiol groups to the double bonds of the *O*-allyl protecting groups (Scheme 1); the reaction proceeded cleanly under mild conditions to give the modified silica with a receptor loading density of 0.12 mmol/g.[8] Among the vast assortment of CSP so far described, **CSP 1** is unique in that it has a concave, rigid binding site and an array of alternating hydrogen bond donors and acceptors located around the macrocycle periphery;[9] unlike other CSPs having cyclic binding cavities (e.g. those containing cyclodextrins or macrocyclic antibiotics) our phase is totally synthetic and thus available in both the enantiomeric forms. Chromatographic data collected under isocratic elution with organic solvents (Table 1) testify the exceptional enantioselectivity for simple amino acid derivatives; **CSP 1** shows strong affinities for the L enantiomers of *N*-Boc-amino acid methylamides, while the D enantiomers are scarcely and unselectively retained. The observed shape and function selectivity, favoring amino acids with branched or hydroxylated side chains, is consistent with previous results obtained by NMR in $CDCl_3$ with **1** or its phenylalanine analog macrocycle.[10] For example, a separation factor (α) of 43 is obtained for the enantiomers of threonine (**7**), corresponding to a difference in binding affinities ($\Delta\Delta G$) for **CSP 1** of −2.2 kcal/mol[11] (Figure 1). Spectroscopic and computational evidences suggest a binding mode of **1** in which the small C-terminus of the guests is located inside the receptor cavity while engaging three hydrogen bond interactions with the exposed amide functions of the guest; little variations in this binding pattern produce drastic changes in enantioselection: with *N*-Boc-valine, replacement of the terminal *N*-methylamide with hydroxymethylene reduces α from 35.33 to 1 (i.e. no resolution). The strong affinity of **1** for *N*-cyclopropanoyl L-amino acid derivatives[10] is also found with **CSP 1** (entries 9, 10, 12): with Ala-*tert*-butyl amides a 14-fold increase in enantioselectivity is observed passing from the *N*-acetyl to *N*-cyclopropanoyl derivative. Binding motives other than inclusion are available to *N*-3,5-dinitrobenzoylated amino acid *n*-hexyl amides (entries

Scheme 1



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(8) A slurry of γ-mercaptopropyl silica gel (900 mg, %C 5.32; %H 1.07), tyrosyl macrocycle **1** (160 mg, 0.13 mmol), and AIBN (7 mg, 0.04 mmol) in 5 mL of freshly distilled CH_2Cl_2 was heated at 55 °C in a closed vessel for 8 days; two additional portions of AIBN (7 mg each) were added after 3 and 6 days. After cooling to rt, modified silica was collected by filtration, washed sequentially with 200 mL portions of CH_2Cl_2, MeOH, and CH_2Cl_2, and dried at 40 °C under reduced pressure. Anal. Found: %C 13.50; % H 1.25; % N 1.00. Loading density of macrocycle, based on N: 0.12 mmol/g. FT-IR (KBr): 2948, 2858, 1653, 1558, 1511 cm^{-1}. TG and DSC analysis showed no weight loss and chemical stability up to 280 °C. **CSP 1** was slurry packed into a 250 × 2 mm column using conventional methods. Column efficiency, measured on achiral solutes (benzene, nitrobenzene) eluted with hexane/$CHCl_3$ 90/10 was found to be >7000 theoretical plates. 1,3,5-Tri-*tert*-butylbenzene (normal phase mode) and $NaNO_3$ (reversed phase mode) were used as void volume markers.

(9) Similar totally synthetic macrocyclic CSP based on a chiral crown-ether bound to polystyrene resin has been previously prepared; see: (a) Sogah, G. D. Y.; Cram, D. J. *J. Am. Chem. Soc.* **1976**, *98*, 3038. (b) Sogah, G. D. Y.; Cram, D. J. *J. Am. Chem. Soc.* **1979**, *101*, 3035.

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(11) Calculated from $\Delta\Delta G = -RT \ln \alpha$.

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Table 1. Chromatographic Resolution of Amino Acid Derivatives on CSP 1[a]

entry	compound	k'_D[b]	k'_L[b]	α[c]
1	*N*-Boc-Ala-NHMe	0.51	4.64	9.10
2	*N*-Boc-Val-NHMe	0.27	9.54	35.33
3	*N*-Boc-Leu-NHMe	0.23	3.07	13.35
4	*N*-Boc-Ile-NHMe	0.57	8.44	14.80
5	*N*-Boc-Phe-NHMe	0.23	0.89	3.87
6	*N*-Boc-Ser-NHMe	2.34	58.10	24.83
7	*N*-Boc-Thr-NHMe	1.09	46.88	43.01
8	*N*-Ac-Ala-NH-*t*-Bu	0.97	1.47	1.52
9	*N*-cPr-Ala-NH-*t*-Bu	1.20	25.19	20.99
10	*N*-cPr-Phe-NH_2	4.09	22.51	5.50
11	*N*-Boc-valinol	0.44	0.44	1.00
12	*N*-cPr-valinol	7.75	20.61	2.66
13	*N*-3,5-Dnb-Ala-NHC_6H_{13}	1.17	0.56	2.09
14	*N*-3,5-Dnb-Val-NHMe	2.47	2.03	1.22
15	*N*-3,5-Dnb-Val-NHC_6H_{13}	1.11	0.38	2.92
16	*N*-3,5-Dnb-Leu-NHC_6H_{13}	0.71	0.39	1.82
17	*N*-3,5-Dnb-Ile-NHC_6H_{13}	0.90	0.32	2.81
18	*N*-3,5-Dnb-Met-NHC_6H_{13}	1.19	0.63	1.89
19	*N*-3,5-Dnb-Phe-NHC_6H_{13}	0.84	0.46	1.83

[a] Eluents: 0.5% MeOH in CH_2Cl_2 (1% MeOH, entries 6 and 7). Flow rate: 0.5 mL/min. Temperature: 25 °C. Detectors: evaporative light scatter, *T*: 30 °C, $P_{(air)}$: 2 atm (entries 1–12) and UV at 254 nm (entries 13–19). Abbreviations: cPr = cyclopropanoyl, 3,5-Dnb = 3,5-dinitrobenzoyl. [b] Retention factor. [c] Enantioselectivity factor.

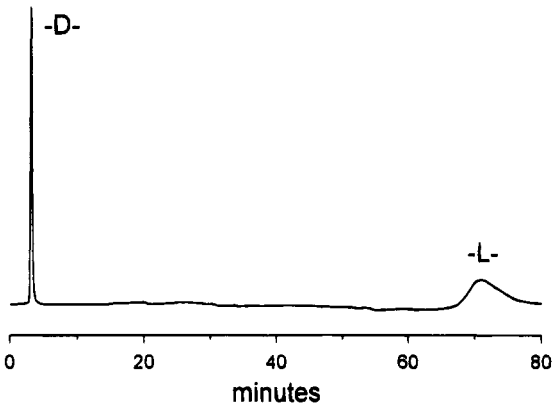

Figure 1. Chromatographic resolution of racemic *N*-Boc-Thr-NHMe on **CSP 1**; experimental conditions as in Table 1.

13–19): here the D enantiomers are preferentially retained, although with smaller enantioselectivity than that with Boc protected methylamides; the π-acidic aromatic rings of the analytes presumably undergo stacking interactions with the electron rich tyrosyl rings or with the aromatic rings at the outer walls of the macrocycle. Interestingly, even in the presence of a small C-terminus (entry 14) the D enantiomer is more strongly retained; comparison of the α values for *N*-methyl and *N*-hexyl amides (entries 14 and 15) clearly shows the presence of two contrasting mechanisms (inclusion and π-stacking), with a preference for the second one.

The chemical stability of **CSP 1** allowed us to evaluate its binding properties in aqueous media;[12] thus, under typical reversed-phase conditions (10% CH_3CN in water), Boc protected amino acid methylamides are well resolved with enantioselectivities (α) in favor of L enantiomers in the range 1.5–2.5; for the more lipophilic *N*-cyclopropanoyl-Ala-*tert*-butyl amide an α value of 4.95 is recorded, corresponding to a $\Delta\Delta G$ of −0.9 kcal/mol (see supplementary material). As expected, the relative affinities of the various amino acids for the chiral phase are inverted respect to the normal-phase elution.

Additional chromatographic data are collected in Table 2. Neutral tripeptides having L configured *N*-terminal

Table 2. Chromatographic Resolutions on CSP 1[a]

entry	compound	k'	α[b]	eluent[c]
20	cPr-D-Gln(γ-trityl)-L-Pro-Gly-NHC_6H_{13}	0.68	3.34[d]	B
21	cPr-L-Gln(γ-trityl)-L-Pro-Gly-NHC_6H_{13}	2.27		B
22	cPr-D-Ala-L-Pro-L-Ala-$NHC_{12}H_{25}$	0.23	21.04[d]	B
23	cPr-L-Ala-L-Pro-L-Ala-$NHC_{12}H_{25}$	4.84		B
24	*Z*-α-phosphonoglycine trimethyl ester	0.19[e]	14.40	A
25	1,1′-bi-2-naphthol	1.33(*R*)[e]	1.19	C
26	*trans*-9,10-dihydro-9,10-dihydroxy-phenanthrene	2.5(*R,R*)[e]	1.50	A
27	α-methyl-α-phenylsuccinimide	1.56[e]	1.30	D
28	*N*-cPr-1-phenylethylamine	1.73(*S*)[e]	1.34	E
29	*N*-3,5-Dnb-1-phenylethylamine	2.53(*R*)[e]	1.10	E
30	2,4-dinitrophenyl ethyl sulfoxide	0.97[e]	2.19	E

[a] Flow rate: 0.5 mL/min. Temperature: 25 °C. Detectors: evaporative light scatter, *T*: 30 °C, $P_{(air)}$: 2 atm (entries 20–24) and UV at 254 nm (entries 25–30). [b] Enantioselectivity factor. [c] Eluents: 0.5% (A) and 1% (B) MeOH in CH_2Cl_2, 0.5% 2-propanol in CH_2Cl_2 (C), CH_2Cl_2 (D), hexane/CH_2Cl_2/2-propanol 50/50/3 (E). [d] Diastereoselectivity factor. [e] Retention factor (and configuration) of the first eluted enantiomer.

residues are preferentially retained, with separation factors[13] up to 21; considering the high loading capacity of our narrow-bore column (e.g. 3.0 mg of *N*-Boc-D,L-Ile-NHMe per run, baseline separation) these large selectivities could be exploited for large scale isolations, on preparative columns, of diastereomerically pure peptides.

Although highly selective for simple amino acids and peptide derivatives, **CSP 1** is also capable to separate the enantiomers of nonpeptidic substrates, with various types, numbers, and combinations of H-bond donor and/or acceptor sites (entries 24–30); compounds with polar groups other than amides, e.g. sulfinyl or phosphinoyl, are also well resolved, indicating a broad applicability of this new CSP. Simple monofunctional compounds like acylated amines are resolved as well; in analogy with *N*-protected amino acids, the enantioselectivity direction changes with the nature of the acylating agent for the enantiomers of 1-phenylethylamine, indicating a switching between two possible recognition mechanisms involving either the functionalized opening of the macrocycle or its external surface.

In conclusion we have shown that covalent binding of the synthetic C_3-symmetric receptor **1** to silica gel provides an effective CSP which can be used to rapidly evaluate binding interactions of several substrates under similar conditions; low levels of enantioselection are easily and quantitatively measured, due to the inherently high efficiency of modern chromatographic techniques. Moreover, investigation of the enantioselective binding in an aqueous environment showed an unchanged, although reduced in respect to organic solvents, preference of **1** for L amino acid derivatives: we expect that similar receptors with larger, hydrophobic binding cavities will show extended scope in the investigation of host interactions with neutral and charged guests in water-rich environment.

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Supporting Information Available: FT-IR of **1** and CSP **1**; plots of k'_D,k'_L and α versus eluent composition for Ala-, Val-, Leu-, Ser-, Thr- (Boc-methylamides/normal-phase), and *N*-cyclopropanoyl-Ala-*tert*-butylamide (reversed-phase); chromatographic data for Ala-, Val-, Leu-, Ile-, Phe-, Ser-, Thr- (Boc-methylamides) under reversed phase conditions (6 pages).

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(13) Control experiments have been carried out on a column packed with the starting γ-mercaptopropyl silica: no retention was observed for the examined tripeptides under the same elution conditions.