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Unusual High Enantioselectivity by a New HPLC Chiral Stationary Phase

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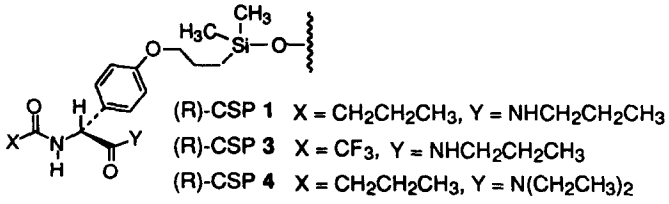
Abstract: A new chiral stationary phase prepared from (R)-*p*-hydroxyphenylglycine has been found to show unusually high enantioselectivity for the enantiomers of N-(3,5-dinitrobenzoyl)-α-amino amides. © 1997 Elsevier Science Ltd. All rights reserved.

During the last decade, chiral stationary phases (CSPs) based on helical polymers,[1] proteins,[2] cellulose derivatives,[3] cyclodextrines,[4] macrocyclic antibiotics,[5] and low molecular weight optically active chiral molecules[6] have been developed and successfully employed for the direct liquid chromatographic separation of enantiomers. Among various low molecular weight optically active chiral molecules, derivatives of α-amino acids bound to solid supports have been most widely utilized as CSPs largely because optically active α-amino acids are readily available. For example, N-(3,5-dinitrobenzoyl)-α-amino acids and N-(1- or 2-naphthyl)-α-amino acids bound to silica are commercially successful CSPs developed by Pirkle.[7] Pirkle-type CSPs are known to separate enantiomers through the enantioselective π-donor-acceptor interactions between the CSP and the analytes.[8] The N-(3,5-dinitrobenzoyl) or the N-(1- or 2-naphthyl) groups covalently bonded to an amino acid are actually used as a π-acidic or π-basic sites for the enantioselective π-donor-acceptor interactions with π-basic and π-acidic groups present in the analytes respectively.

In this study we describe a new CSP (CSP **1**) based on an α-amino acid which does not require derivatization with an extra π-acidic or π-basic reagent. CSP **1**, prepared from (R)-4-hydroxyphenylglycine, shows very high enantioselectivity for the enantiomers of N-(3,5-dinitrobenzoyl)-α-amino acid derivatives, **2**.[9] Table 1 summarizes the data for the separation of the enantiomers of various N-(3,5-dinitrobenzoyl)-α-amino acid derivatives, **2**, on CSP **1**. The enantioselectivities afforded by CSP **1** for the enantiomers of N-(3,5-dinitrobenzoyl)-α-amino amides are quite large as shown in Table 1. For example, the enantiomers of the propyl amide of N-(3,5-dinitrobenzoyl)leucine, **2g**, are resolved on CSP **1** with large separation factor



(R)-CSP **1** X = $CH_2CH_2CH_3$, Y = $NHCH_2CH_2CH_3$
(R)-CSP **3** X = CF_3, Y = $NHCH_2CH_2CH_3$
(R)-CSP **4** X = $CH_2CH_2CH_3$, Y = $N(CH_2CH_3)_2$

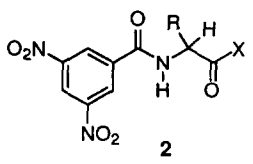


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Table 1. Resolution of Derivatives of N-(3,5-Dinitrobenzoyl)-α-amino Acids **2** on CSP **1**[a]

Anal[b]	R	X	k_1'[c]	k_2'[c]	α[d]	Conf[e]
2a	CH_3	$NH(CH_2)_2CH_3$	1.35	13.00	9.63	S
b	CH_3	$N(CH_2CH_3)_2$	1.67	9.19	5.50	S
c	CH_3	OCH_2CH_3	2.12	4.24	2.00	S
d	$CH(CH_3)_2$	$NH(CH_2)_2CH_3$	0.86	16.29	18.94	S
e	$CH(CH_3)_2$	$N(CH_2CH_3)_2$	0.87	7.26	8.35	S
f	$CH(CH_3)_2$	OCH_2CH_3	1.54	6.28	4.08	S
g	$CH_2CH(CH_3)_2$	$NH(CH_2)_2CH_3$	0.95	25.26	26.59	S
h	$CH_2CH(CH_3)_2$	$N(CH_2CH_3)_2$	1.15	12.15	10.57	S
i	$CH_2CH(CH_3)_2$	OCH_2CH_3	1.51	6.10	4.04	S
j	Phenyl	$NH(CH_2)_2CH_3$	1.71	17.82	10.42	S
k	Benzyl	$NH(CH_2)_2CH_3$	1.78	36.01	20.23	S
l	CH_2-C_6H_5-4-OH	$NH(CH_2)_2CH_3$	4.47	66.85	14.96	S
m	$CH(OH)CH_3$	$NH(CH_2)_2CH_3$	4.96	23.26	4.69	S
n	$CH_2CH_2SCH_3$	$NH(CH_2)_2CH_3$	1.86	24.79	13.33	S

[a] Chromatography was performed with a system consisting of a Waters Model 510 pump, a Rheodyne Model 7125 Injector with a 20 μl sample loop, a Youngin Model 710 Absorbance Detector and a Youngin D520B Computing Integrator. All data were obtained using 20 % isopropyl alcohol in *n*-hexane as a mobile phase with a flow rate of 2 ml/min at 254 nm UV. [b] Racemic analytes resolved on CSP **1**. [c] Capacity factors. [d] Separation factors. [e] Absolute configuration of the second eluted enantiomer.

(α = 26.59). This large separation factor is quite unusual considering that CSP **1** does not contain any extra π-basic derivatizing functional group.[10]

According to the data shown in Table 1, the (S)-enantiomers of N-(3,5-dinitrobenzoyl)-α-amino amides and esters are always retained longer on CSP **1** than are the (R)-enantiomers. The propyl amides of N-(3,5-dinitrobenzoyl)-α-amino acids are resolved much better on CSP **1** than the corresponding N,N-diethyl amides or the corresponding ethyl esters, indicating that the amide N-H hydrogen of the analytes plays an important role in the chiral recognition.

In an effort to elucidate the role of the two amide functionalities of the CSP in the chiral recognition process and to possibly improve chiral discrimination ability, we prepared the modified CSPs, CSP **3** and **4**. Because of its electron withdrawing nature, the CF_3 functionality of CSP **3** is expected to increase the acidity of the neighboring amide N-H and to decrease the basicity of the neighboring amide carbonyl oxygen. If the trifluoroacetamide N-H serves as a hydrogen bonding donor during the chiral recognition, one might expect CSP **3** to show higher chiral recognition ability than CSP **1**. However, the separation factors of the enantiomers of N-(3,5-dinitrobenzoyl)-α-amino acid amides on CSP **3** are found to be smaller than those on CSP **1**. For example, the separation factor for the propyl amide of N-(3,5-dinitrobenzoyl)leucine on CSP **3** is 4.04. Consequently, it might be supposed that the trifluoroacetamide carbonyl oxygen serves as a hydrogen bond acceptor during the chiral recognition process. Similarly, the separation factors of the enantiomers of N-(3,5-dinitrobenzoyl)-α-amino amides are found to be smaller on CSP **4** than on CSP **1**. For example, the

separation factor of the propyl amide of N-(3,5-dinitrobenzoyl)leucine on CSP **4** is 2.56. This result indicates that the N-H hydrogen of the propyl amide functionality of CSP **1** is participating in chiral recognition as a hydrogen bond donor. In addition, when the π-acidity of the N-benzoyl functional group of the analytes was reduced by replacing the N-(3,5-dinitrobenzoyl) group of analyte **2** with, for example, an N-(3-nitrobenzoyl) group or a simple benzoyl group, the separation factors on the CSP were found to be greatly reduced. The separation factors of the enantiomers of the propyl amide of N-(3-nitrobenzoyl)leucine and N-benzoylleucine on CSP **1** are 4.74 and 2.00 respectively. From these results, we believe that the 4-alkoxyphenyl group of the CSP plays an important role in the chiral recognition as an effective π-basic group for enantioselective π-π donor-acceptor interaction with the π-acidic N-(3,5-dinitrobenzoyl) groups of the analytes.

Based on these experimental observations and from the study of CPK molecular models, we propose the chiral recognition model shown in Figure 1. As shown in Figure 1, CSP **1** is suggested to interact with amide derivatives of (S)-N-(3,5-dinitrobenzoyl)-α-amino acids through two hydrogen-bonding interactions and through the π-π-interaction of the 4-alkoxyphenyl group of the CSP with the N-(3,5-dinitrophenyl) group of the analyte. The proposed chiral recognition model shown in Figure 1 is well consistent with the experimental results observed so far. However, further studies, such as intermolecular nuclear Overhauser NMR experiments, might be needed to improve or modify the proposed chiral recognition mechanism and these are underway in our laboratotry.

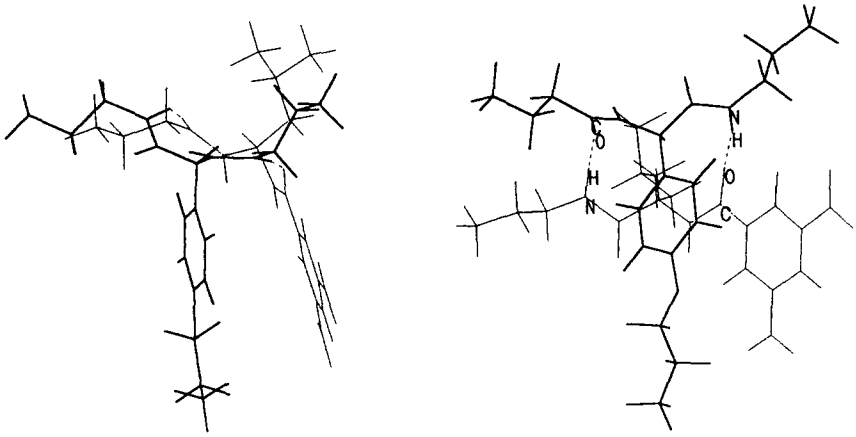

Fig. 1. Proposed chiral recognition model viewed from two different angles for the more stable (R,S)-complex between the model compound of (R)-CSP **1**, the propyl amide of (R)-N-butanoyl-*p*-propoxyphenylglycine and the propyl amide of (S)-N-(3,5-dinitrobenzoyl)leucine, **2g**. Left view is showing the occurrence of the face to face π-π interaction between the *p*-propoxyphenyl group of the CSP model compound and the 3,5-dinitrophenyl group of **2i**. Right view is showing the two hydrogen-bonding interactions between the model compound of CSP **1** and **2g**.

In summary, we have shown that CSP **1** prepared by grafting the propyl amide of (R)-N-butanoyl-4-hydroxyphenylglycine to silica shows very high enantioselectivity for the enantiomers of N-(3,5-dinitrobenzoyl)-α-amino amides. Based on the chromatographic behaviors of racemic **2** on CSP **1** and from comparison of the chromatographic data obtained from CSP **1** with those obtained from CSP **3** and **4**, we have

proposed a chiral recognition mechanism which utilizes two hydrogen bonding interactions between the CSP and the analytes and a π-π donor-acceptor interaction between the π-basic 4-alkoxyphenyl group of the CSP and the π-acidic N-(3,5-dinitrophenyl) group of the analyte. Finally, one thing to note is that the retention time of the less retained (R)-enantiomer is quite short, as shown in Table 1. Consequently, it might be expected that the chiral selector of CSP **1** can be utilized in other chiral discriminating techniques such as the separating enantiomers through chiral liquid membranes or as an NMR chiral solvating agent. Application of the chiral selector of CSP **1** to other chiral discriminating techniques is underway in our laboratory.

Acknowledgment.

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9. The detailed synthetic procedure will be discussed elsewhere. CSP **1** was also useful in separating another kind of π-acidic racemates such as 3,5-dinitroanilide derivatives of α-arylpropionic acids. These will be reported elsewhere. Elemental analysis of CSP **1** (Found % C, 6.55; % H, 0.75; % N, 0.72) showed a loading of 0.27 mmole (based on C) or 0.26 mmole (based on N) of chiral selector per gram of stationary phase. The bonded phase was slurried in methanol and packed into a 250 x 4.6 mm I.D. stainless steel column using a conventional slurry packing method and then the unreacted residual silanol groups of the bonded phase were protected by eluting a solution of 2 mL of hexamethyldisilazane in 50 mL of dichloromethane through the column.
10. Two CSPs derived from proline and leucine which are derivatized with strong π-basic aromatic functional groups have been reported to resolve under certain conditions racemic amides of N-(3,5-dinitrobenzoyl)-leucine with even greater enantioselectivity. (a) Pirkle, W. H.; Murray, P.G. *J. Chromatogr.* **1993**, 6, 11-19. (b) Pirkle, W. H.; Bowen, W. E. *J. High Resoln Chromatogr.* **1994**, 17, 629-633.

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