An Example of Exception to the Successful Use of Reciprocity of Chiral Recognition in Designing Pirkle-type Chiral Stationary Phases

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Liquid chromatographic separation of enantiomers on HPLC chiral stationary phases (CSPs) has been known as one of the most accurate and convenient means in determining the enantiomeric composition of chiral compounds. As results of significant efforts devoted to the development of effective CSPs, various CSPs are now available.[1]

Pirkle-type CSPs have been known to separate the two enantiomers of racemic compounds through a minimum of three simultaneous interactions between the CSP and the analyte with at least one interaction being enantioselective.[2] Interaction between the CSP and the analyte can be either attractive or repulsive. Especially π-π donor-acceptor interaction is essential for the chiral recognition on Pirkle-type CSPs.

In designing Pirkle-type CSPs, the reciprocity conception of chiral recognition has been successfully utilized.[3] Reciprocity of chiral recognition is simply described as following: a CSP derived from (+)-A can distinguish between (+)-B and (−)-B, then a CSP derived from (+)-B or (−)-B can distinguish between (+)-A and (−)-A. Consequently, the enantiomer of a racemate resolvable best on a certain CSP can be a most promising candidate as a chiral selector of a reciprocal CSP intended to resolve the racemates related to the chiral selector of the original CSP. While the selection of effective chiral selectors have been done in most cases on the basis of the trial-and-error method, the reciprocity conception of chiral recognition has been successfully utilized as the only rational guide in selecting most effective chiral selectors of Pirkle-type CSPs.

However, recently we reported that application of the reciprocity conception of chiral recognition in developing the most effective reciprocal CSPs is not always valid.[4] In this study, we wish to report another example of the exception to the successful application of the reciprocity conception of chiral recognition observed during the process of checking the enantioselectivities exerted by two CSPs based on (*S*)-*N*-(2,2-dimethyl-4-pentenoyl)proline-3,5-dimethylanilide **1** (Figure 1) and (*S*)-*N*-(2,2-dimethyl-4-pentenoyl)proline-3,5-dimethoxyanilide **2** (Figure 1). Previously, a CSP (CSP **3**, Figure 1) based on (*S*)-*N*-(2,2-dimethyl-4-pentenoyl)proline-3,5-dimethylanilide **1** was reported excellent in the separation of the enantiomers of *N*-(3,5-dinitrobenzoyl)-α-amino amides and esters.[5] Thereafter, a CSP (CSP **4**, Figure 1) based on (*S*)-*N*-(2,2-dimethyl-4-pentenoyl)proline-3,5-dimethoxyanilide **2** was also developed based on the reciprocity conception of chiral recognition in order to utilize in the preparative chromatographic separation of the enantiomers of the chiral selectors used in commercial CSPs.[6] However, the exact comparison of the two CSPs on the basis of the reciprocity conception of chiral recognition has not been reported.

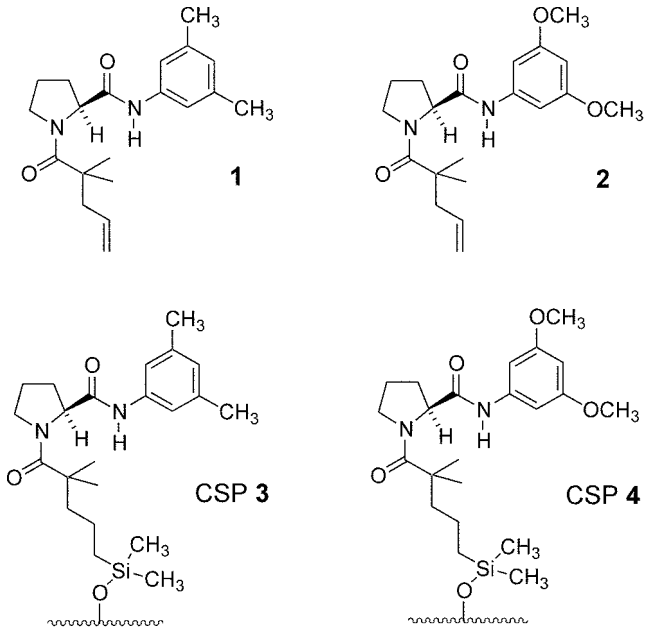

Figure 1. Structures of *N*-(2,2-dimethyl-4-pentenoyl)proline-3,5-dimethylanilide **1**, *N*-(2,2-dimethyl-4-pentenoyl)proline-3,5-dimethoxyanilide **2**, CSP **3** and CSP **4**.

In our own study, we found that racemic *N*-(2,2-dimethyl-4-pentenoyl)proline-3,5-dimethoxyanilide **2** was resolved better (k_1 = 5.41, k_2 = 45.88, α = 8.48) than racemic *N*-(2,2-dimethyl-4-pentenoyl)proline-3,5-dimethylanilide **1** (k_1 = 2.00, k_2 = 14.00, α = 7.00) on a CSP based on *N*-(3,5-dinitrobenzoyl)leucine *N*-allyl amide. The stronger π-π interaction between the relatively more π-basic 3,5-dimethoxyphenyl group of analyte **2** and the π-acidic 3,5-ninitrobenzoyl group of the CSP compared to that between the relatively less π-basic 3,5-dimethylphenyl group of analyte **1** and the π-acidic 3,5-ninitrobenzoyl group of the CSP is believed to be responsible for the longer retention and the greater enantioselectivity of analyte **2**. Consequently, we expected that CSP

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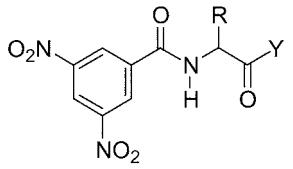
Table 1. Resolution of *N*-(3,5-dinitrobenzoyl)-α-amino amides and esters **5** on CSP **3** and CSP **4**[a]



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Analyte	R	Y	CSP **3**			CSP **4**		
			k_1[b]	k_2[c]	α[d]	k_1[b]	k_2[c]	α[d]
5a	CH_3 (alanine)	$NHCH_2CH_2CH_3$	1.05	53.63	51.08	1.23	28.91	23.50
5b		$N(CH_2CH_3)_2$	0.90	35.01	38.90	1.48	13.86	24.23
5c		OCH_2CH_3	2.29	13.63	5.95	2.26	11.35	5.02
5d	$CH(CH_3)_2$ (valine)	$NHCH_2CH_2CH_3$	0.60	28.08	46.80	0.63	17.03	27.03
5e		$N(CH_2CH_3)_2$	0.64	16.23	25.36	0.82	13.42	16.37
5f		OCH_2CH_3	1.64	11.36	6.93	1.61	8.90	5.53
5g	$CH_2CH(CH_3)_2$ (leucine)	$NHCH_2CH_2CH_3$	0.68	31.06	45.68	0.66	17.05	25.83
5h		$N(CH_2CH_3)_2$	0.69	23.09	33.46	0.83	19.37	23.34
5i		OCH_2CH_3	1.72	13.81	8.03	1.54	10.47	6.80
5j	C_6H_5 (phenylglycine)	$NHCH_2CH_2CH_3$	1.06	29.34	27.68	1.25	17.33	13.86
5k		$N(CH_2CH_3)_2$	0.94	23.97	25.50	1.30	17.72	13.63
5l		OCH_2CH_3	2.51	10.46	4.17	2.47	8.23	3.33
5m	$CH_2C_6H_5$ (phenylalanine)	$NHCH_2CH_2CH_3$	0.96	23.21	24.18	1.08	15.10	13.98
5n		$N(CH_2CH_3)_2$	0.87	16.45	18.91	1.11	14.03	12.64
5o		OCH_2CH_3	2.75	10.23	3.72	2.70	8.45	3.13
5p	$CH_2(C_6H_5OH)$ (tyrosine)	$NHCH_2CH_2CH_3$	2.50	63.96	25.58	2.62	35.84	13.68
5q		$N(CH_2CH_3)_2$	2.17	44.88	20.68	2.50	32.00	12.80
5r		OCH_2CH_3	6.65	27.62	4.15	5.95	18.80	3.16

[a]Mobile phase: 20% isopropyl alcohol in hexane. Flow rate: 2.0 mL/min. Detection: 254 nm UV. Temperature: 20 °C. In every case, (*S*)-enantiomer was eluted second. [b]Retention factor of the first eluted enantiomer. [c]Retention factor of the second eluted enantiomer. [d]Separation factor.

4 should be better than CSP **3** in the resolution of *N*-(3,5-dinitrobenzoyl)amino amides and esters because of the reciprocity of chiral recognition. However, on the contrary, CSP **3** was found to show greater enantioselectivity than CSP **4**.

The chromatographic results for the resolution of *N*-(3,5-dinitrobenzoyl)-α-amino amides and esters **5** on CSP **3** and CSP **4** with the mobile phase of 20% isopropyl alcohol in hexane are summarized in Table 1. The representative chromatograms for the resolution of *N*-(3,5-dinitrobenzoyl)lecine *N*-propyl amide **5g** on CSP **3** and CSP **4** are illustrated in Figure 2. As shown in Table 1 and Figure 2, the resolutions of *N*-(3,5-dinitrobenzoyl)-α-amino amides and esters are very excellent on both CSP **3** and CSP **4**. Especially, the resolutions of *N*-(3,5-dinitrobenzoyl)-α-amino amides are greater than the resolutions of the corresponding *N*-(3,5-dinitrobenzoyl)-α-amino esters. According to the chiral recognition mechanism proposed previously from the ^{1}H NMR study for the resolution of *N*-(3,5-dinitrobenzoyl)-α-amino amides on CSP **3**, the carbonyl oxygen of the C-terminal amide group of the analyte plays an important role as a hydrogen bonding acceptor site.[7] In this instance, the electron density at the carbonyl oxygen of the C-terminal amide group of the analyte is expected to be important for the chiral recognition. In the case of the resolution of *N*-(3,5-dinitrobenzoyl)-α-amino esters on CSP **3** or CSP **4**, the electron density at the carbonyl group of the C-terminal ester

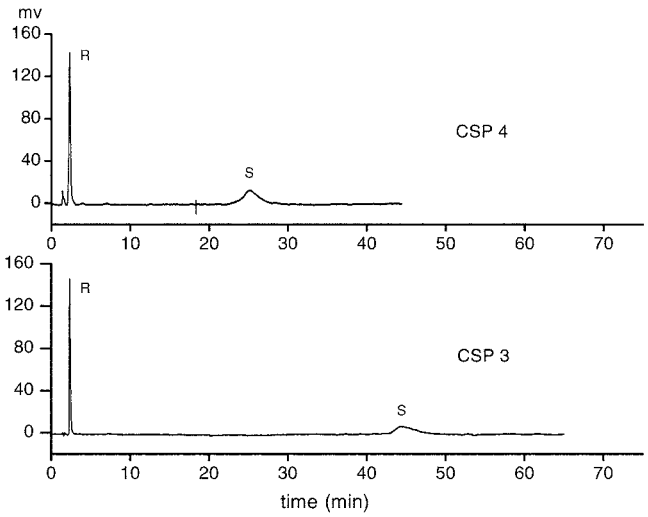

Figure 2. Chromatograms for the resolution of *N*-(3,5-dinitrobenzoyl)leucine *N*-propyl amide **5g** on CSP **3** and CSP **4**. For chromatographic conditions, see the footnote to Table 1.

group of the analyte is expected to be relatively low compared to that at the carbonyl group of the C-terminal amide group of *N*-(3,5-dinitrobenzoyl)-α-amino amides because of the electron attracting ability of the ester ethoxy group and consequently *N*-(3,5-dinitrobenzoyl)-α-amino esters should be resolved worse on CSP **3** or CSP **4** than *N*-(3,5-dinitrobenzoyl)-α-amino amides. Between the second-

ary and tertiary amides, the secondary amides are observed to be resolved generally better than the tertiary amides on CSP **3** and CSP **4**. However, we do not have any reasonable rationale for these resolution behaviors.

The most surprising and unexpected observation to note in Table 1 is the enantioselectivity for the resolution of *N*-(3,5-dinitrobenzoyl)-α-amino amides and esters **5** on CSP **3** and CSP **4**. As shown in Table 1, the enantioselectivity of CSP **3** denoted by the separation factors, α, is always greater than that of CSP **4**. These results are exactly opposite to what we expected from the reciprocity conception of chiral recognition. However, the reason is not clear yet.

Reciprocal systems of chiral recognition are not intrinsically mirror images of one another and consequently the success of one of reciprocal resolutions does not absolutely exclude the failure of the other.[8] In addition, the manner of immobilizing a chiral selector to solid support can influence the energetics of the resolution process and consequently may result in nonreciprocal behavior.[3d] Simultaneous interaction of the analyte with more than one strand of bonded phase may also result in nonreciprocal behavior.[3d] Nevertheless, the reciprocity conception of chiral recognition has been successfully utilized in designing effective CSPs. The manner of immobilizing the chiral selector to silica gel is exactly identical in CSP **3** and CSP **4** and the modes of analyte interactions with the strands of bonded phases might be equivalent. Consequently, there is no reason to suspect the successful utilization of the reciprocity conception of chiral recognition in designing more effective CSPs. However, the chromatographic resolution results on CSP **3** and CSP **4** are not consistent with what we expected from the reciprocity conception of chiral recognition. From these results, it should be noted that the use of the reciprocity conception of chiral recognition in designing effective CSPs are not always successful and consequently needs to take some degree of care.

In summary, in this study, CSP **3** and CSP **4** were applied in the resolution of various *N*-(3,5-dinitrobenzoyl)-α-amino amides and esters. In every case, CSP **3** was found to show greater enantioselectivity than CSP **4**. Based on the reciprocity conception of chiral recognition, we expected that CSP **4** exerts greater enantioselectivity than CSP **3** does. However, the chromatographic resolution results on CSP **3** and CSP **4** are exactly opposite to what we expected from the reciprocity conception of chiral recognition. From these results, we conclude that the use of reciprocity conception of chiral recognition in designing effective Pirkle-type CSPs should be done with some degree of care.

Experimental Section

Chromatography was performed with an HPLC system consisting of a Waters model 510 pump, a Rheodyne model 7125i injector with a 20 μL sample loop, a YoungLin M720 absorbance detector with a 254 nm UV filter and a YoungLin Autochro Data Module (Software: YoungLin Autochro-WIN 2.0 plus). Each of CSP **3** and CSP **4** was prepared *via* the method reported previously[5,6] and then packed into a 250 mm × 4.6 mm I.D. stainless steel HPLC column using conventional slurry packing method with an Alltech slurry packer. Based on the elemental analysis of CSP **3** (C, 5.19%; H, 0.66%; N, 0.54%) and CSP **4** (C, 5.02%; H, 0.65%; N, 0.50%), the loading level of chiral selector of CSP **3** and CSP **4** on silica gel was calculated to be 0.20 mmole and 0.19 mmole per gram of stationary phase (based on C) respectively. All chromatographic experiments were carried out at a flow-rate of 2.0 mL/min at 20 °C. The void volume was determined by the injection of 1,3,5-tri-*tert*-butylbenzene. The elution orders denoted in the footnote of Table 1 were determined by injecting configurationally known samples. Racemic and optically active analytes used in this study were available from previous study.[4]

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