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## Communications

### Chromatographic Separation of the Enantiomers of Amino Acid Esters as Benzophenone Imine Derivatives

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In the previous study, a convenient way of enantiomer separation of amino acid ethyl esters derivatives on chiral stationary phases (CSPs) derived from 3,5-dinitrobenzoyl (DNB) (S)-leucine, (R)-phenylglycine and  $\beta$ -amino acid was demonstrated.<sup>1</sup> For the derivatization of amino acid ethyl esters, a  $\pi$ -basic benzophenone imine moiety was introduced to utilize for  $\pi$ - $\pi$  interaction with a  $\pi$ -acidic DNB group of CSP. Benzophenone Schiff base derivatives have been used for the synthesis of amino acids by phase-transfer alkylations.<sup>2,3</sup> The benzophenone imine derivatives of amino acid esters were readily prepared by stirring of benzophenone imine<sup>4</sup> and amino acid ethyl esters hydrochloride salts in methylene chloride at room temperature (Figure 1).<sup>3</sup> Although the enantiomers of these compounds provided generally good resolution on the previous CSPs, some analytes such as asparagine derivative showed no enantioseparation.<sup>1</sup> In this paper, the study of the resolution of these analytes is extended to utilize Whelk-O 1 CSP in Figure 2.<sup>5</sup> Whelk-O 1 CSP was originally designed to separate the enantiomers of naproxen and other non-steroidal anti-inflammatory drugs and has been found to be applicable in resolving broad spectrum of racemates.<sup>6-13</sup> From X-ray data, Whelk-O 1 CSP incorporates a molecular cleft which consists of a  $\pi$ -acidic DNB group perpendicular to a  $\pi$ -basic aromatic substituent.<sup>14</sup> And the DNB amide N-H is placed in the cleft formed by the two aromatic systems. When the mechanistic studies of the enantioseparation of several types of analytes on Whelk-O 1 are considered,<sup>6-12</sup> the enantiomers of benzophenone imine derivatives of amino acid esters are expected to resolve on Whelk-O 1 CSP.

Tables 1 and 2 show chromatographic data for the separation of the enantiomers of various amino acid ethyl and methyl esters as benzophenone Schiff base derivatives on

Whelk-O 1 CSP. Whelk-O 1 affords good enantioselectivities for all analytes studied, providing their base-line separations. The separation factors for ethyl esters are comparable to those for the corresponding methyl esters. The enantiomers of benzophenone imine derivatives of asparagine, aspartic acid, glutamic acid and homoserine ethyl ester which are not separable on the previous CSPs are well resolved on Whelk-O 1 CSP.<sup>1</sup> Other analytes show much greater separation factors on Whelk-O 1 CSP than on the previous CSPs, because Whelk-O 1 CSP is attributed to a highly preorganized chiral cleft which allows two simultaneous face-to-face and face-to-edge  $\pi$ - $\pi$  interactions to occur.<sup>8,14</sup> As shown in Tables 1 and 2, the (-)-(S)- or (-)-(2S, 3S)-enantiomers for the examined analytes are eluted before the (+)-(R)- or (+)-(2R,3R)-enantiomers. The correspond-

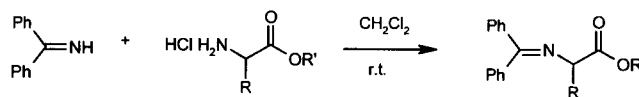


Figure 1. Preparation of benzophenone imine derivatives of amino acid esters.

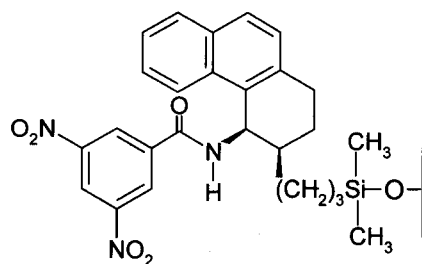


Figure 2. Structure of (R,R)-Whelk-O 1 CSP used in this study.

**Table 1.** Separation of the enantiomers of benzophenone imine derivatives of amino acid ethyl esters on (R,R)-Whelk-O 1

Analyte	$\alpha$	$k'_1$	Retained*
alanine	1.35	3.04	(+) (R)
valine	4.18	1.62	(+) (R)
leucine	1.61	2.03	(+) (R)
isoleucine	2.57	1.55	(+) (2R,3R)
methionine	1.57	3.49	(+) (R)
asparagine	1.16	5.38	(+) (R)
aspartic acid#	1.16	5.35	(+) (R)
glutamic acid#	1.44	8.00	(+) (R)
homoserine	1.40	15.42	(+)
phenylglycine	1.36	3.80	(+) (R)
phenylalanine	2.92	2.90	(+) (R)

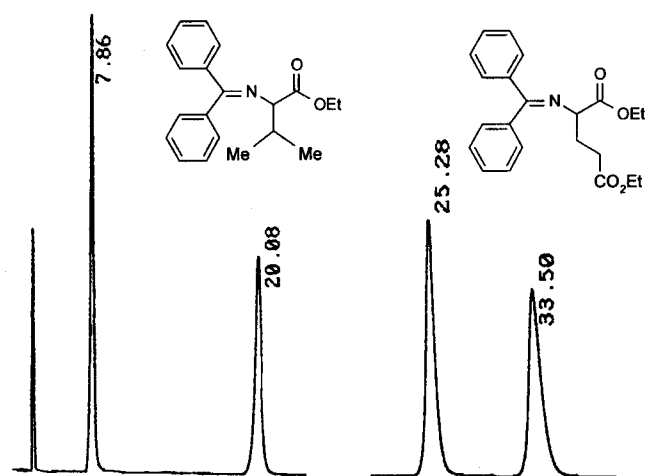
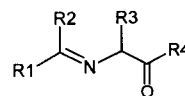
Mobile phase=2% 2-propanol in hexane (V/V); Flow rate=1 mL/min; UV 254 nm; \*indicates absolute configuration and/or the sign of optical rotation of the second eluted enantiomer determined by an in-line polarimetric detector (Shodex OR-1M) set at 589 nm; #Diethyl ester.

**Table 2.** Separation of the enantiomers of benzophenone imine derivatives of amino acid methyl esters on (R,R)-Whelk-O 1

Analyte	$\alpha$	$k'_1$	Retained*
alanine	1.40	2.45	(+) (R)
valine	4.02	1.41	(+) (R)
leucine	1.59	1.86	(+) (R)
phenylglycine	1.48	3.45	(+) (R)
phenylalanine	3.13	2.60	(+) (R)

Mobile phase=2% 2-propanol in hexane (V/V); Flow rate=1 mL/min; UV 254 nm; \*indicates absolute configuration and the sign of optical rotation of the second eluted enantiomer determined by an in-line polarimetric detector (Shodex OR-1M) set at 589 nm.

ence of the elution orders of the enantiomers of these analytes on Whelk-O 1 CSP suggests a similarity of their chiral recognition mechanisms. Typical chromatograms are

**Figure 3.** Separation of the enantiomers of valine ethyl ester and glutamic acid diethyl ester as benzophenone imine derivatives; Mobile phase=2% 2-propanol in hexane (V/V); Flow rate=1 mL/min; UV 254 nm; Temperature ambient; Injection amount 10 nmol.**Table 3.** Separation of the enantiomers of leucine or valine ester and amides as benzophenone imine or benzaldimine derivatives on (R,R)-Whelk-O 1

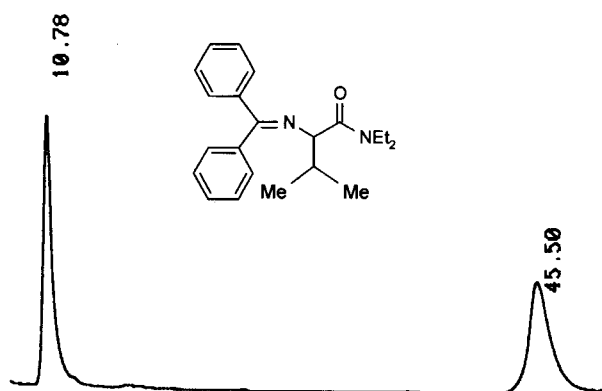
Entry	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	$\alpha$	$k'_1$	Retained*
1	Ph	Ph	<i>i</i> -Bu	OE <sub>t</sub>	1.61	2.03	(+) (R)
2	Ph	H	<i>i</i> -Bu	OE <sub>t</sub>	1.00	3.06	
3	Ph	Ph	<i>i</i> -Bu	NHn-Bu	1.05	4.34 <sup>#</sup>	(+) (R)
4	Ph	Ph	<i>i</i> -Bu	NE <sub>t</sub> <sub>2</sub>	2.30	7.20 <sup>#</sup>	(-) (R)
5	Ph	Ph	<i>i</i> -Pr	OE <sub>t</sub>	4.18	1.62	(+) (R)
6	Ph	H	<i>i</i> -Pr	OE <sub>t</sub>	1.08	3.46	(+) (R)
7	Ph	Ph	<i>i</i> -Pr	NHn-Bu	1.29	4.38 <sup>#</sup>	(+) (R)
8	Ph	Ph	<i>i</i> -Pr	NE <sub>t</sub> <sub>2</sub>	6.02	2.70 <sup>##</sup>	(-) (R)

Mobile phase=2% 2-propanol in hexane (V/V); Flow rate=1 mL/min; UV 254 nm; \*indicates absolute configuration and the sign of optical rotation of the second eluted enantiomer determined by an in-line polarimetric detector (Shodex OR-1M) set at 589 nm; <sup>#</sup>5% 2-propanol in hexane (V/V); <sup>##</sup>10% 2-propanol in hexane (V/V).

presented in Figure 3.

Table 3 shows the chromatographic data of leucine or valine ester and amides as benzophenone imine or benzaldimine derivatives. It is observed that the elution orders of leucine and valine amides as benzophenone imine derivatives are all consistent with those of their ester derivatives. When the benzaldimine moiety is used for the derivatization of leucine or valine ethyl ester instead of the benzophenone imine group, either no or marginal enantioselectivity is observed (entries 2 and 6). For example, the enantiomers of benzophenone imine derivative of valine ethyl ester are greatly resolved ( $\alpha=4.18$ ), whereas the separation factor for the corresponding benzaldimine derivative is 1.08. Due to the steric hindrance of adjacent phenyl groups, each phenyl group of benzophenone imine derivative has a restricted freedom of rotation. Thus benzophenone imine derivatives may afford conformational preference to benzaldimine derivatives for chiral discrimination. It is also observed that the capacity factor ( $k'_1$ ) for the first eluted enantiomer of the benzophenone imine derivatives is smaller than that of the benzaldimine derivatives (entries 1 and 2, entries 5 and 6). From the study of CPK molecular models, it implies that one phenyl group of benzophenone imine moiety plays a role of blocking an approach of CSP from one face, whereas the benzaldimine analyte may not have such a facial selectivity to restrict access to the interaction site of the CSP. Therefore, the molecular cleft of CSP is considered to approach toward the opposite side of one phenyl group of benzophenone imine on the preferentially retained enantiomer, while the other phenyl group of its benzophenone moiety is involved in  $\pi$ - $\pi$  interaction with DNB group of CSP.

The enantioselectivity of leucine or valine *n*-butyl amide as benzophenone imine derivative is significantly less than that of the corresponding ethyl ester whereas retention is greatly increased (entries 3 and 7). However, the enantiomers of their *N,N*-diethylamide analytes show greater separation factors and retentions than do the ethyl ester deri-



**Figure 4.** Separation of the enantiomers of valine N,N-diethylamide as benzophenone imine derivative; Mobile phase=10% 2-propanol in hexane (V/V); Flow rate=1 mL/min; UV 254 nm; Temperature ambient; Injection amount 10 nmol.

vatives (entries 4 and 8). It implies that a hydrogen bonding interaction between the carbonyl oxygen of the analyte and DNB N-H amide of CSP is essential for chiral recognition. On the other hand, the N-H of secondary amide derivative is considered to be detrimental to the chiral recognition, providing unnecessary achiral retention. The chromatogram illustrating the separation of the enantiomers of valine N,N-diethylamide as benzophenone imine derivative is presented in Figure 4. The chromatographic data with the study of CPK molecular models suggest a chiral recognition rationale which leads 1) simultaneous face-to-face and face-to-edge  $\pi$ - $\pi$  interaction between the molecular cleft of the CSP and one phenyl group of benzophenone imine of the analyte and 2) a hydrogen bonding interaction between DNB N-H of the CSP and the carbonyl oxygen of the analyte.

In conclusion, a simple and convenient way of the enantioseparation of the benzophenone Schiff base derivatives of various amino acid ethyl and methyl esters on Whelk-O 1 CSP was described. The enantiomers of all examined amino

acid esters as their benzophenone imine derivatives were well resolved on Whelk-O 1 CSP. The (+)-(R)- or (+)-(2R, 3R)-enantiomers of the examined analytes are selectively retained on (R,R)-Whelk-O 1 CSP. Based on the observed chromatographic results and the study of CPK molecular models, a chiral recognition rationale consistent with observed elution orders was proposed. It is expected that Whelk-O 1 CSP will be useful for the resolution of other amino acid esters and amides as benzophenone imine derivatives.

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## New Amino Acid Derivatives for the Synthesis of Pharmaceutical Peptides by Liquid Phase Method: Boc-Asp(OPse)-OH · DCHA and Boc-Glu(OPse)-OH · DCHA

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The side carboxyls of aspartic acid (Asp) and glutamic acid (Glu) should be protected semipermanently and orthogonally to  $\alpha$ -groups during peptide synthesis, since they have the possibility of branching a peptide chain *via* intramolecular or intermolecular reactions. Among various protecting

groups, benzyl group which can be removed by catalytic hydrogenolysis has been the most widely used in conjunction with *t*-butoxycarbonyl (Boc) group. This group, however, has some drawbacks: instability under acidic condition for removal of the Boc group and difficulty of facile detach-