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Investigation of Chiral Self-Recognition of π-Acidic 3,5-Dinitrobenzoylleucine Derivatives

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Pioneering work in the design of brush-type chiral stationary phases (CSPs) using chiral recognition rationales has led to the development of a number of Pirkle-type columns.[1-4] For the separation of enantiomers, Pirkle-type CSPs use π-donor or π-acceptor moieties as attractive π-π interaction sites.[2-4] Therefore, racemic analytes, which lack π-acidic or π-basic groups, have commonly been derivatized to afford appropriate π-π donor-acceptor interactions prior to chromatography. Typically, the 3,5-dinitrobenzoyl (DNB) group is the most commonly used π-acidic moiety as a π-acceptor.[3,4] Interestingly, there were a few examples of the resolution of π-acidic DNB derivatives on π-acidic DNB α-amino acid derived CSPs.[5-9] All these reported DNB tyrosine or phenylglycine derived CSPs and/or analytes possess phenyl moieties. However, these are not real examples of resolution of π-acidic analytes on π-acidic CSPs, because the π-basic phenyl moiety on the CSP or analyte derived from tyrosine or phenylglycine could play the role of a π-donor site during chiral recognition process. Exceptionally, only one result of enantiomeric separation of DNB leucine derivatives on a DNB leucine derived CSP has been reported without any aromatic moiety as a π-basic group.[10] Since systematic studies on the resolution of real π-acidic DNB derivatized analytes on π-acidic DNB derived CSPs have not performed until now, in this study we investigated the separation of the enantiomers of π-acidic DNB leucine as esters and amides on CSP 1 and 2 derived from π-acidic DNB leucine amides (Figure 1), and to elucidate the related chiral self-recognition mechanism.

Table 1-3 shows the resolution of a homologous series of alkylesters, *N*-alkylamides and *N,N'*-dialkylamides of DNB leucine on CSP 1 and 2 derived from (*S*)-DNB *N*-propylamide and *N,N'*-dipropylamide, respectively. In general, all the separation factors obtained on CSP 1 and 2 remained constant, as the alkyl lengths increased. It is noteworthy that the (*S*)-enantiomers of the investigated analytes (n = 2, 3, 4, 10) of DNB leucine alkylesters, *N*-alkylamides and *N,N'*-dialkylamides were preferentially retained on (*S*)-DNB leucine derived CSP 1 with a secondary amide tether and on CSP 2 with a tertiary amide tether. For all resolution results, CSP 2 with a tertiary amide tether provided superior performance to CSP 1 with a secondary amide tether in terms of resolving these DNB leucine homologous analytes. Also, for the resolution of the corresponding homologous ester and amide derivatives on CSP 1 and CSP 2, the enantioselectivities of DNB leucine tertiary *N,N'*-dialkylamide derivatives (Table 3) were always greatest, while the enantioselectivities of the corresponding alkyl ester derivatives were smallest (Table 1). In view of the chiral recognition mechanism, these results suggest that the C-terminal carboxamide oxygen of the analyte is involved in a hydrogen bonding interaction.

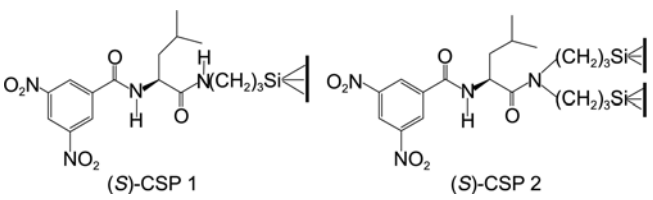
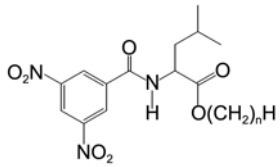


Figure 1. Structures of the (*S*)-DNB leucine amide derived CSPs used in this study.

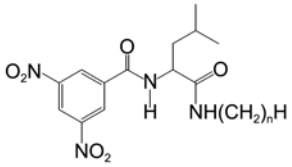
Table 1. Enantiomer resolution of a homologous series of the alkylesters of DNB leucine on (*S*)-CSP 1 and 2



	(*S*)-CSP 1			(*S*)-CSP 2		
n	α^a	$k'_1{}^b$	Conf.[c]	α^a	$k'_1{}^b$	Conf.[c]
1	1.21	1.42		2.02	1.89	
2	1.28	1.18		2.22	1.44	
3	1.29	1.05	(S)	2.19	1.20	(S)
4	1.29	0.99	(S)	2.22	1.06	(S)
5	1.29	0.98		2.19	1.05	
6	1.29	0.96		2.20	1.03	
7	1.29	0.95		2.22	1.02	

Mobile phase; 20% 2-propanol in hexane (V/V); Flow rate = 2 mL/min; UV 254 nm; [a]Separation factor. [b]Retention factor for the first eluted enantiomer. [c]Absolute configuration of the second eluted enantiomer.

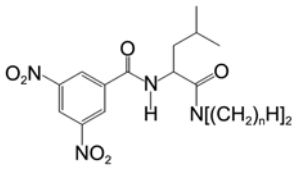
Table 2. Enantiomer resolution of a homologous series of the *N*-alkylamides of DNB leucine on (*S*)-CSP 1 and 2



	(*S*)-CSP 1			(*S*)-CSP 2		
n	α^a	$k'_1{}^b$	Conf.[c]	α^a	$k'_1{}^b$	Conf.[c]
1	1.35	1.65		3.04	2.35	
2	1.46	1.15		3.10	1.64	
3	1.50	0.96	(S)	3.04	1.37	(S)
4	1.53	0.86	(S)	2.99	1.28	(S)
5	1.52	0.73		2.96	1.08	
6	1.52	0.69		2.88	1.02	
7	1.52	0.65		2.87	0.97	
8	1.52	0.62		2.86	0.92	
10	1.51	0.56	(S)	2.85	0.83	(S)
14	1.52	0.49		2.83	0.73	
18	1.52	0.43		2.80	0.65	

Mobile phase; 20% 2-propanol in hexane (V/V); Flow rate = 2 mL/min; UV 254 nm; [a]Separation factor. [b]Retention factor for the first eluted enantiomer. [c]Absolute configuration of the second eluted enantiomer.

Table 3. Enantiomer resolution of a homologous series of the *N,N'*-dialkylamides of DNB leucine on (*S*)-CSP 1 and 2



	(*S*)-CSP 1			(*S*)-CSP 2		
n	α^a	$k'_1{}^b$	Conf.[c]	α^a	$k'_1{}^b$	Conf.[c]
1	1.60	1.99		4.14	2.27	
2	2.04	1.38	(S)	5.39	2.01	(S)
3	2.21	1.01	(S)	6.05	2.22	(S)
4	2.23	0.99	(S)	5.83	1.91	(S)
6	2.19	0.95		5.44	1.55	
8	2.15	0.91		5.37	1.41	
10	2.14	0.86		5.15	1.27	

Mobile phase; 20% 2-propanol in hexane (V/V); Flow rate = 2 mL/min; UV 254 nm; [a]Separation factor. [b]Retention factor for the first eluted enantiomer. [c]Absolute configuration of the second eluted enantiomer.

Therefore, it was proposed that the enhanced electron density of the carbonyl oxygen of the analyte is responsible for the increased enantioselectivity.[10-12] The increased basicity of the carbonyl oxygen on the secondary amide analyte in Table 2 provides a higher level of enantioselectivity relative to the corresponding ester analyte in Table 1. In addition, owing to the much enhanced electron density on the carbonyl oxygen of the tertiary amide of the analyte in Table 3, much stronger hydrogen bonding between the CSP and the tertiary amide derivative may result in increased enantioselectivity as compared with the corresponding ester and the secondary amide derivative in Table 1 and 2. In the same manner, higher enantioselectivity on CSP 2 than on CSP 1 implies that stronger hydrogen bonding by the enhanced electron density on the carbonyl oxygen of CSP 2 with a tertiary amide linkage, as compared to CSP 1 with a secondary amide linkage, enhances enantioselectivity, as shown in Table 1-3.[10,11] Consequently, these chromatographic results in the present study imply that dual hydrogen bonding interactions exist between DNB N-H of the CSP (or the analyte) and the C-terminal carbonyl oxygen of the analyte (or the CSP). We suspect that a simultaneous π-π interaction exists between the π-acidic DNB group of the CSP and the π-acidic DNB group of the analyte during chiral recognition. However, since the π-π interactions of Pirkle-type CSPs have generally been used,[2-4] it was proposed that the π-π interaction between π-acidic DNB groups may unusually exist.[10] Also, Figure 2 shows the relationship between natural logarithm of k' (retention factor) and the length of the alkyl group of a homologous series of DNB leucine *N*-alkylamides (left) and *N,N'*-dialkylamides (right) using 20% 2-propanol in hexane (V/V)

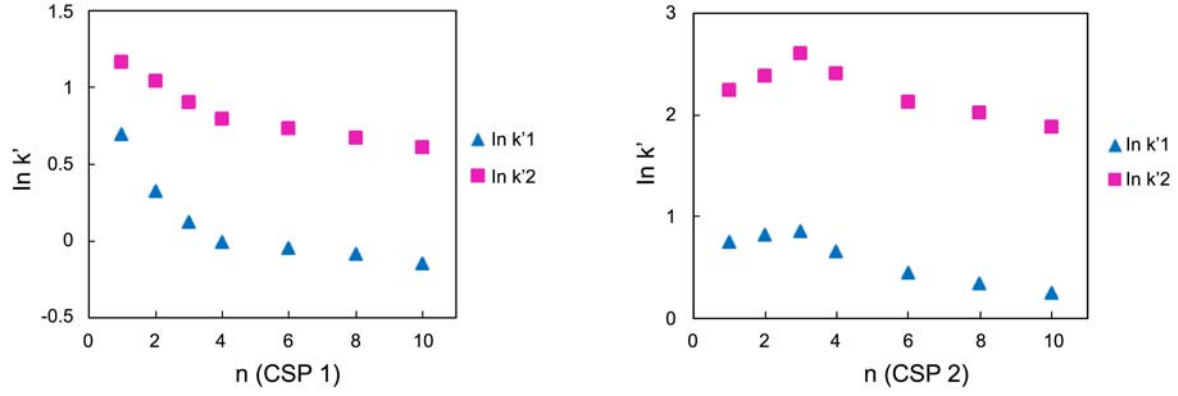


Figure 2. Relationship between natural logarithm of k′ (retention factor) and the length of the alkyl group of a homologous series of DNB leucine *N*-alkylamides (left) and *N,N'*-dialkylamides (right) using 20% 2-propanol in hexane (V/V) as the mobile phase on CSP 1 and CSP 2, respectively.

Table 4. Separation of the enantiomers of DNB leucine amide derivatives on (*S*)-CSP 1 and 2 derived from DNB leucine amides showing examples of chiral self-recognition

CSP	(*S*)-CSP **1**			(*S*)-CSP **2**		
Analyte	α^a	$k'_1{}^b$	Conf.[c]	α^a	$k'_1{}^b$	Conf.[c]
DNB leucine propyl ester	1.29	1.05	(-)(S)	2.19	1.20	(−)(S)
DNB leucine *N*-propyl amide	**1.50**	**0.96**	**(-)(S)**[d]	3.04	1.37	(−)(S)
DNB leucine *N,N′*-diethyl amide	2.04	1.38	(-)(S)	5.39	2.01	(−)(S)
DNB leucine *N,N′*-dipropyl amide	2.21	1.01	(-)(S)	**6.05**	**2.22**	**(−)(S)**[d]

Mobile phase; 20% 2-propanol in hexane (V/V); Flow rate = 2 mL/min; UV 254 nm; [a]Separation factor. [b]Retention factor for the first eluted enantiomer. [c]Absolute configuration of the second eluted enantiomer. [d]Results of enantiomer separation showing chiral self-recognition are in bold font.

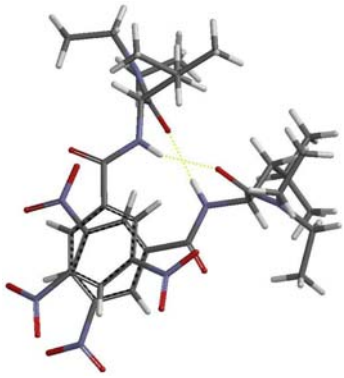


Figure 3. X-ray structure of (*S,S*)-complex viewed from the top side of the DNB rings, showing the three simultaneous interactions (a π-π interaction and two intermolecular hydrogen bonds) in the homochiral dimer of (*S*)-DNB leucine diethyl amide (**1**).[14]

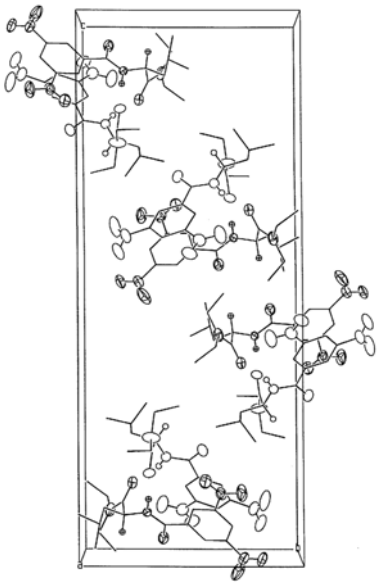


Figure 4. Crystal packing diagram showing unit cell contents; two molecules of the (*S,S*)-complex of DNB leucine diethyl amide were drawn with shaded or hollow ellipsoids, respectively. Hydrogen atoms have been removed for clarity. (*S*)-DNB leucine *N,N′*-diethyl amide behaves like the corresponding dimer.

as the mobile phase on CSP 1 and CSP 2. The results on both CSP 1 and CSP 2 shown in Figure 2 suggest that the alkyl tails of the first and second eluted enantiomers are not directed toward the silica support. Therefore, the enantiomers show either or no little differential intercalation of the alkyl groups of the first and second eluted enantiomers between strands of bonded chiral stationary phase, affording that the observed separation factors generally maintain constant in Table 2 and 3.[13]

Table 4 summarizes typical examples of enantiomer separations of DNB leucine derivatives on CSP 1 and 2. In all cases, the enantiomer forming the homochiral adsorbate is more retained on CSP 1 and 2. As marked in bold, in particular, the (*S*)-enantiomers of DNB leucine *N*-propyl and *N,N′*-dipropyl amide on CSP 1 and 2 derived from (*S*)-DNB leucine *N*-propyl and *N,N′*-dipropyl amide, respectively, are more retained. These findings reveal that chiral self-recognition occurs during the chromatographic process. Recently, we provided strong evidence to support this chiral recognition rationale based on the X-ray crystallographic structure of DNB leucine *N,N′*-diethyl amide.[14] When (*S*)-DNB leucine *N,N′*-diethyl amide (**1**) was crystallized, it was found to dimerize in the solid state. Interestingly, by chiral-self recognition, (*S*)-enantiomer (**1**) behaves like the corresponding dimer, the homochiral 1:1 complex (Figure 3 and 4). The X-ray dimeric structure of (*S*)-DNB leucine *N,N′*-diethyl amide (**1**) shows not only dual intermolecular hydrogen bonding interactions but also a π-π interaction of DNB groups for chiral recognition.[14] Also, when racemic-**1** was crystallized, it was observed that only homochiral (*S,S*)- and (*R,R*)-complex **1** were present in the unit cell of the crystal derived from racemic-**1**, as shown in Figure 5. This indicates that the homochiral complex is more stable than the heterochiral complex in solid state racemic mixture. These X-ray results along with chromatographic data show a π-π interaction between π-acidic DNB groups and chiral self-discrimination of DNB leucine *N,N′*-diethyl amide (**1**), a result of homochiral dimerization. In order to compare the X-ray results and theoretical calculations of the molecular structure of homochiral dimeric (*S*)-DNB leucine *N,N′*-diethyl amide, a study was performed using the Spartan 02' package program on the geometry of optimized structures. The calculation was optimized by the HF method using the 6-31G** basis set.[16]

Figure 6 shows that the optimized structure is consistent with the X-ray structure. In the same manner as that shown by the X-ray structure in Figure 3, the stability of dimeri-

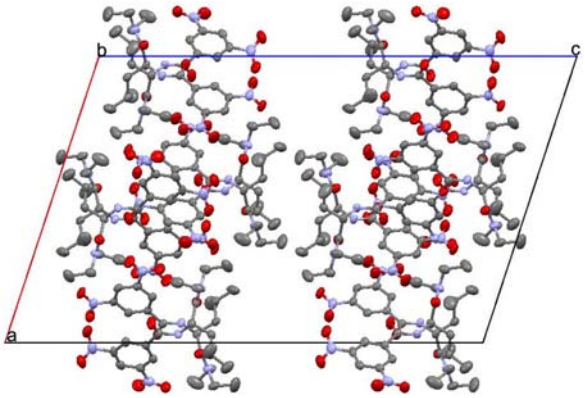


Figure 5. Crystal packing diagram showing the unit cell contents of the homochiral (*S*,*S*)- or (*R*,*R*)-complex formed from racemic DNB leucine *N*,*N*′-diethyl amide. Hydrogen atoms have been removed for clarity.[14]

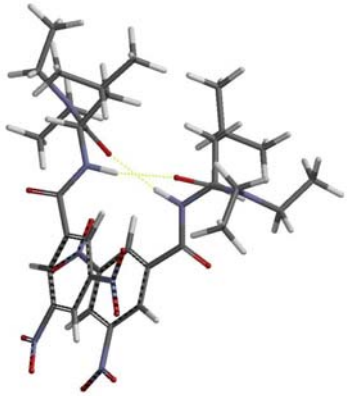


Figure 6. Optimized calculated structure of the homochiral dimerization of (*S*)-DNB leucine *N*,*N*′-diethyl amide, determined using the HF method and the 6-31G** basis set.

zation is responsible for dual intermolecular hydrogen bonds of two leucine backbones and π-π interaction between DNB groups. Both X-ray data in Figure 3 and theoretically calculated result in Figure 6 show the two aromatic DNB leucine groups for π-π interaction are partly overlapped and staggered each other. And it is proposed that the offset π-stacked geometry of two DNB leucine moieties leads a favorable π-π interaction. Consequently, these results are consistent with the HPLC results that the alkyl tails of the second eluted enantiomers are not directed toward the silica support, resulting in generally constant separation factors.

In conclusion, for systematic studies on the resolution of DNB leucine derivatives on DNB leucine derived CSPs, we performed the separation of enantiomers of π-acidic DNB leucine as esters and amides on CSP 1 and 2 derived from π-acidic DNB leucine amides. In terms of chiral recognition rationale, all chromatographic results, X-ray structures, and theoretical calculations of molecular structure show consistently not only dual intermolecular hydrogen bonding interactions with a π-π interaction between π-acidic DNB groups but also the chiral self-recognition of DNB leucine *N*,*N*′-diethyl amide.

Experimental Section

DNB leucine ester and amide derivatives were prepared using standard methods.[15] Chromatographic analyses were performed at ambient temperature using an HPLC consisting of a Waters model 510 pump, a Rheodyne model 7125 injector with a 20 μL loop, and a Dynamax UV-1 detector (Rainin, USA). Secondary amide tethered CSP 1 and tertiary amide doubled-tethered CSP 2, based on (*S*)-DNB leucine, were used as described previously.[4,11]

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