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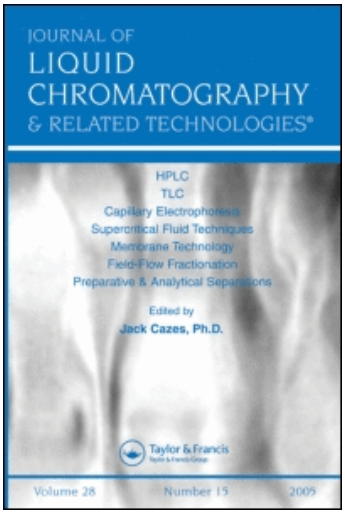

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Preparation and Application of a New Ion-Pairing Chiral Stationary Phase for the Liquid Chromatographic Resolution of *N*-(3,5-Dinitrobenzoyl)-α-amino Acids

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Abstract: A liquid chromatographic chiral stationary phase (CSP) was prepared starting from (1*S*, 2*S*)-1,2-diaminocyclohexane. The CSP containing two simple functional groups, such as a primary amino group and an ureide tethering linkage on a cyclohexane ring, was quite successful with the use of 60% 2-propanol in hexane containing 0.5% acetic acid as a mobile phase for the resolution of various *N*-(3,5-dinitrobenzoyl)-α-amino acids, the separation (α) and the resolution factors (R_S) being in the range of 1.12–1.23 and 1.33–2.99, respectively. In contrast, *N*-(3,5-dinitrobenzoyl)-α-amino acid ethyl esters and *N*-(3,5-dinitrobenzoyl)-α-amino acid amides were not resolved at all, or resolved very poorly on the CSP. From these results, it was concluded that the ionic or ion-paring interaction between the CSP and analytes plays a very important role for the chiral recognition. The chromatographic behaviors for the resolution of *N*-(3,5-dinitrobenzoyl)-α-amino acids on the CSP were found to be controlled with the variation of the type and the content of alcohol and/or acid in hexane.

Keywords: Chiral separation, Ion pairing chiral stationary phase, Liquid chromatography, *N*-(3,5-Dinitrobenzoyl)-α-amino acids

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INTRODUCTION

Liquid chromatographic chiral separations on chiral columns packed with chiral stationary phases (CSPs) have been known to be very effective for the separation of the two enantiomers of chiral compounds for both analytical and preparative purposes.[1–3] Success of this technique is dependent mostly on the availability of effective CSPs. As results of efforts devoted to the development of effective CSPs, various CSPs based on proteins,[4] polysaccharide derivatives,[5] cyclodextrins,[6] macrocyclic antibiotics,[7] amino acid derivatives,[8] chiral crown ethers,[9,10] and other synthetic low molecular weight optically active chiral molecules[11] have been developed.

Chiral recognition by CSPs has been known to require multiple molecular interactions including hydrogen bonding interactions, π–π donor–acceptor interactions, dipole–dipole interactions. and/or steric interactions; at least one interaction being enantioselective.[12] However, ionic or ion-pairing interactions have been only sparingly utilized for the chiral recognition. For example, CSPs based on cinchona alkaloids, especially quinine and quinidine, covalently immobilized onto silica have been proposed by Lindner and coworkers to employ ionic or ion-pairing interactions in addition to π–π interaction and hydrogen bonding interaction in the chiral recognition of *N*-(3,5-dinitrobenzoyl)-α-amino acids.[13,14] We also demonstrated, theoretically and experimentally, that ionic or ion-pairing interaction plays an important role in the chiral recognition for the resolution of *N*-(3,5-dinitrobenzoyl)-α-amino acids on CSPs derived from phenylglycine or hydroxyphenylglycine.[15,16] In either case, π–π donor acceptor interaction between the 3,5-dinitrobenzoyl group of analytes and the π-basic aromatic group of CSPs was considered to be an another important molecular interaction. However, ionic or ion-pairing interaction has not been fully elucidated to be useful as an attractive interaction for the chiral recognition without the aid of π–π donor–acceptor interaction.

In this study, we wish to prepare a new CSP (CSP 1, Figure 1) by bonding (1*S*, 2*S*)-1,2-diaminocyclohexane to silica gel through one amino group and apply the CSP to the resolution of *N*-(3,5-dinitrobenzoyl)-α-amino acids. The remaining free amino group of CSP 1 might provide an ionic or ion-pairing interaction site. In addition, CSP 1 does not contain any π-acidic or π-basic aromatic group. Consequently, CSP 1 is expected to show the usefulness of ionic or ion-pairing interaction for the chiral recognition without the aid of π–π donor–acceptor interaction.

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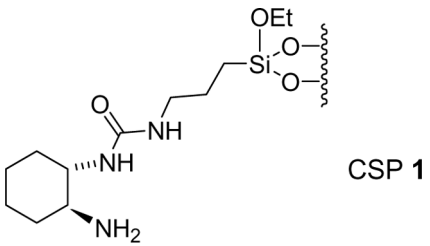

Figure 1. Structure of CSP 1.

EXPERIMENTAL

General

^{1}H NMR spectra were recorded on a Varian Mercury 300 spectrometer. IR spectra were measured with a Jasco FT/IR-300E. Melting points were taken on an Electrothermal Capillary Melting Point Apparatus (Weiss Gallenkamp, UK) and reported without correction. Optical rotations were taken on a Rudolph Research Analytical AUTOPOL IV Polarimeter (Flanders, NJ, USA).

Chromatography was performed with an HPLC system consisting of a Waters model 515 HPLC pump, a Rheodyne model 7725i injector with a 20 μL sample loop, a Waters 2487 Absorbance Detector, and a YoungLin Autochro Data Module (Software: YoungLin Autochro-2000 1.0). Chiral column temperature was controlled with a Jeiotech VTRC-620 cooling circulator (Seoul, Korea).

Analytes including optically active and racemic *N*-benzoyl- or *N*-(substituted benzoyl)-α-amino acids, esters, and amides were available from previous studies or prepared according to the procedures reported.[17,18] Each of the racemic and optically active samples was dissolved in tetrahydrofuran (usually 1.0 mg/mL) and used for the resolution on CSP 1. The usual injection volume was 1.0 μL.

Preparation of CSP 1

CSP 1 was prepared starting from (1*S*, 2*S*)-diaminocyclohexane according to the procedure shown in Figure 2 as follows:

N-tert-Butoxycarbonyl-(1S,2S)-diaminocyclohexane 2

(1*S*, 2*S*)-1,2-Diaminocyclohexane (1.14 g, 10.0 mmol) dissolved in dry dioxane (100 mL) was treated with 2-(*tert*-butoxycarbonyloxyimino)-2-

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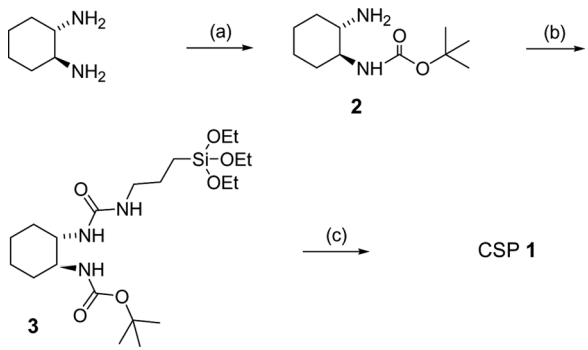

Figure 2. Scheme for the preparation of CSP 1. (a) 2-(*tert*-butoxycarbonyloxyimino)-2-phenylacetonitrile, dioxane, room temperature. (b) 3-(triethoxysilyl)propyl isocyanate, triethylamine, methylene chloride, room temperature. (c) (1) 5 μm Rexchrom silica gel, toluene, reflux. (2) trifluoroacetic acid, methylene chloride, room temperature.

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phenylacetonitrile (Boc-ON) (2.47 g, 10.0 mmol) at room temperature for 18 hr, according to the known procedure to afford *N-tert*-butoxycarbonyl-(1*S*, 2*S*)-diaminocyclohexane, 2 (0.86 g, 40% yield) as a yellow-tan solid.[19] All spectroscopic data were consistent with those reported.[19]

N-(3-Triethoxysilylpropylaminocarbonyl)-(1S,2S)-1, 2-diaminocyclohexane, **3**

N-tert-Butoxycarbonyl-(1*S*, 2*S*)-diaminocyclohexane, 2 (0.86 g, 4.0 mmol) was dissolved in methylene chloride (30 mL). To the solution was added triethylamine (0.55 ml, 0.51 mmol). The whole mixture was stirred at room temperature for 30 min, and then 3-(triethoxysilyl)propyl isocyanate (0.94 g, 4.0 mmol) was added to the reaction mixture. The whole reaction mixture was stirred again at room temperature for 12 hr, and then solvent and triethylamine were removed by rotary evaporator. The residue was purified by silica gel chromatography (tetrahydrofuran:chloroform = 1:1, v/v) to afford *N*-(3-triethoxysilylpropylaminocarbonyl)-(1*S*, 2*S*)-1,2-diaminocyclohexane, 3 (1.38 g, 75% yield) as a white solid. mp 105–107°C. $[\alpha]_D^{19.0}$5.93 ($c = 0.39$ $CHCl_3$). IR (KBr) 3332, 2931, 1684, 1636, 1579, 1531 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ(ppm) 0.60 (*t*, 2H), 1.19 (*t*, 9H), 1.38 (broad *s*, 9H), 1.50–1.60 (*m*, 2H), 1.62–1.72 (*m*, 2H), 1.90–2.08 (*m*, 2H), 2.20 (broad *m*, 1H), 3.00–3.12 (*m*, 2H), 3.38–3.48 (broad *m*, 1H), 3.77 (*q*, 6H), 4.60–4.70 (broad *s*, 1H), 4.97 (*t*, 2H).

Preparation of CSP 1 and Column Packing

A two-neck round bottom flask equipped with a Dean-Stark trap, a condenser, and a magnetic stirring bar was charged with Rexchrom silica gel (3.5 g, 5 μm, 100 Å available from Regis Technologies, Morton Grove, IL, USA) and toluene (100 mL). The mixture was heated to reflux until the complete azeotropic removal of water. To the heterogeneous solution was added *N*-(3-triethoxysilylpropylaminocarbonyl)-(1*S*, 2*S*)-1,2-diaminocyclohexane, 3 (0.90 g, 1.95 mmol) dissolved in 10 mL of toluene. The whole mixture was heated to reflux for 72 hr and then cooled to room temperature. The modified silica gel was collected with filtration and then washed successively with toluene, methanol, acetone, ethyl acetate, methylene chloride, hexane, and diethyl ether. The bonded phase was suspended in methylene chloride (30 mL) and then treated with trifluoroacetic acid (3 mL) at room temperature for 24 hr. The modified silica gel (CSP 1) was collected again with filtration and then washed successively with toluene, methanol, acetone, ethyl acetate, methylene chloride, hexane, and diethyl ether. Finally, CSP 1 was dried under high vacuum. Elemental analysis of CSP 1 (Found: C, 6.55%; H, 1.07%; N, 1.88%) showed a loading of 0.45 mmol selector (based on *C* or *N*) per gram of stationary phase. The modified silica gel was slurried in methanol and packed into a 250 mm × 4.6 mm I.D. stainless steel HPLC column using a conventional slurry packing method with an Alltech slurry packer.

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RESULTS AND DISCUSSION

CSPs based on cinchona alkaloids,[13,14] phenylglycine,[16] or hydroxyphenylglycine,[16] which have been proposed to utilize the ionic interaction for the chiral recognition, contain quaternary or primary ammonium ion as an ionic interaction site. On these CSPs, *N*-(3,5-dinitrobenzoyl)-α-amino acids were resolved, and the ionic interaction between the ammonium cation of the CSP and the carboxylate anion of analytes has been proposed to play an important role for the chiral recognition. CSP 1 contains a primary amino group, which can be easily converted to the primary ammonium ion under slightly acidic condition, and consequently, CSP 1 is expected to be useful for the resolution of chiral carboxylic acids.

Resolution of various types of chiral carboxylic acids were tried on CSP 1 under various mobile phase conditions, but only *N*-benzoyl- or *N*-(substituted benzoyl)-α-amino acids were resolved with the use of 60% 2-propanol in hexane containing 0.5% CH_3COOH as a mobile phase. The chromatographic results for the resolution of various *N*-benzoyl

or *N*-(substituted benzoyl)-α-amino acids on CSP 1 are summarized in Table 1. Even though the separation factors (α) are not so great, the clean baseline resolution was observed in every case, as shown in Table 1. Considering that CSP 1 does not contain any aromatic group, and consequently, π–π donor acceptor interaction between the CSP and analytes is not expected, the chromatographic resolution results shown in Table 1 are quite surprising. Under the mobile phase condition (pH 3.33), the carboxylic acid group of analytes is expected to present as the carboxylate group, and consequently, the ionic interaction between the primary ammonium ion of the CSP and the carboxylate group of analytes seems to play an very important role for the chiral recognition.

The importance of the ionic interaction between the primary ammonium ion of the CSP and the carboxylate group of analytes

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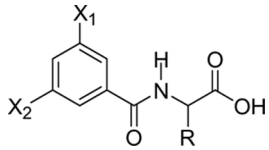
Table 1. Resolution of *N*-(substituted benzoyl)-α-amino acids on CSP 1[a]



	R	X_1	X_2	k_1	k_2	α	R_S
a	CH_3	NO_2	NO_2	17.52 (L)	21.55 (D)	1.23	2.99
b	$(CH_3)_2CH$	NO_2	NO_2	11.22 (L)	13.58 (D)	1.21	2.99
c	$(CH_3)_2CHCH_2$	NO_2	NO_2	8.41 (L)	10.34 (D)	1.23	2.47
d	C_6H_5	NO_2	NO_2	54.68 (L)	63.43 (D)	1.16	2.16
e	$C_6H_5CH_2$	NO_2	NO_2	25.44 (L)	30.53 (D)	1.20	2.12
f	$HO(CH_3)CH$	NO_2	NO_2	40.35 (L)	48.42 (D)	1.20	1.61
g	$HOCH_2$	NO_2	NO_2	83.31 (L)	93.31 (D)	1.12	1.33
h	$(4\text{-}HOC_6H_4)CH_2$	NO_2	NO_2	19.55 (L)	23.26 (D)	1.19	1.83
i	CH_3	NO_2	H	18.90 (L)	22.49 (D)	1.19	2.24
j	$(CH_3)_2CH$	NO_2	H	12.19 (L)	14.51 (D)	1.19	1.80
k	$(CH_3)_2CHCH_2$	NO_2	H	9.05 (L)	10.86 (D)	1.20	1.96
l	C_6H_5	NO_2	H	78.77 (L)	89.01 (D)	1.13	1.49
m	CH_3	H	H	15.42 (L)	18.04 (D)	1.17	1.71
n	$(CH_3)_2CH$	H	H	8.16 (L)	9.38 (D)	1.15	1.62
o	$(CH_3)_2CHCH_2$	H	H	7.52 (L)	8.87 (D)	1.18	2.00
p	C_6H_5	H	H	64.00 (L)	69.76 (D)	1.09	1.12

[a]Mobile phase: 60% 2-propanol in hexane + 0.5% CH_3COOH. Flow rate: 0.5 mL/min. Detection: 254 nm UV. Temperature: 20°C. k_1: Retention factor of the first eluted enantiomer (the absolute configuration of the first eluted enantiomer is presented in the parenthesis). k_2: Retention factor of the second eluted enantiomer (the absolute configuration of the second eluted enantiomer is presented in the parenthesis). α: Separation factor. R_S: Resolution factor.

might be evidenced by the chromatographic results for the resolution of *N*-(3,5-dinitrobenzoyl)-α-amino acid ethyl esters (Table 2) and *N*-(3,5-dinitrobenzoyl)-α-amino acid amides (Table 3). As shown in Tables 2 and 3, *N*-(3,5-dinitrobenzoyl)-α-amino acid ethyl esters are not resolved at all and *N*-(3,5-dinitrobenzoyl)-α-amino acid amides are resolved quite poorly. These results indicate that a free carboxylic acid group of analytes is absolutely required for the good resolution on CSP 1.

The nitro group on the benzoyl ring of analytes seems to play some role for the chiral recognition. As shown in Table 1, the number of nitro groups on the benzoyl ring of analytes affects the chiral recognition. By adding nitro groups on the benzoyl ring, the free carboxylic acid group of analytes seems to be deprotonated more easily, and consequently, the ionic interaction between the CSP and analytes might become more significant, the chiral recognition being improved. However, the exact chiral recognition mechanism including the exact role of the nitro group on the benzoyl ring of analytes is not clear yet.

The retention times of the two enantiomers for the resolution of *N*-benzoyl- or *N*-(substituted benzoyl)-α-amino acids on CSP 1 are quite long, as shown in Table 1. The long retention times are not desirable for the analytical purpose. As an effort to find the condition

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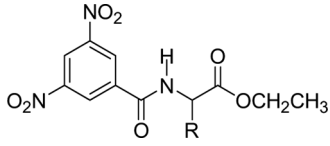
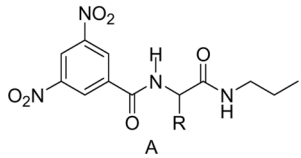
Table 2. Resolution of *N*-(3,5-dinitrobenzoyl)-α-amino acid ethyl esters on CSP 1[a]



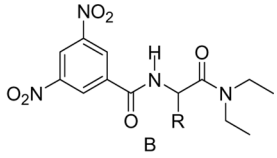
R	k_1	k_2	α	R_S
CH_3	0.31	0.31	1.00	–
$(CH_3)_2CH$	0.19	0.19	1.00	–
$(CH_3)_2CHCH_2$	0.20	0.20	1.00	–
C_6H_5	0.37	0.37	1.00	–
$C_6H_5CH_2$	0.29	0.29	1.00	–
$HO(CH_3)CH$	0.41	0.41	1.00	–
Proline	0.35	0.35	1.00	–
Tryptophan	0.41	0.41	1.00	–
Lysine	0.26	0.26	1.00	–

[a]Mobile phase: 60% 2-propanol in hexane +0.5% CH_3COOH. Flow rate: 0.5 mL/min. Detection: 254 nm UV. Temperature: 20°C. k_1: Retention factor of the first eluted enantiomer. k_2: Retention factor of the second eluted enantiomer. α: Separation factor. R_S: Resolution factor.

Table 3. Resolution of *N*-(3,5-dinitrobenzoyl)-α-amino acid *N*-propyl amides (A) and *N*-(3,5-dinitrobenzoyl)-α-amino acid *N*, *N*-diethyl amides (B) on CSP 1[a]



A



B

	A			B		
R	k_1	α	R_S	k_1	α	R_S
CH_3	0.38	1.10	0.44	0.30	1.17	0.80
$(CH_3)_2CH$	0.21	1.27	1.12	–	–	–
$(CH_3)_2CHCH_2$	0.24	1.11	0.31	0.19	1.21	0.69
C_6H_5	0.30	1.12	0.41	0.23	1.00	–
$C_6H_5CH_2$	0.29	1.21	1.08	0.21	1.26	1.03
$HO(CH_3)CH$	0.09	1.00	–	0.42	1.12	0.69
$(4\text{-}HOC_6H_4)CH_2$	0.34	1.17	0.69	0.25	1.21	0.65
Proline	0.43	1.00	–	0.40	1.00	–
Tryptophan	0.35	1.00	–	0.38	1.00	–
Lysine	0.26	1.00	–	0.37	1.00	–

[a]Mobile phase: 60% 2-propanol in hexane +0.5% CH_3COOH. Flow rate: 0.5 mL/min. Detection: 254 nm UV. Temperature: 20°C. k_1: Retention factor of the first eluted enantiomer. α: Separation factor. R_S: Resolution factor.

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of reducing retention times, *N*-(3,5-dinitrobenzoyl)valine and *N*-(3,5-dinitrobenzoyl)leucine were selected and resolved on CSP 1 with the variation of the type and the content of alcohol and acid in hexane. The resolution results are summarized in Table 4. Retention factors are quite dependent on the content of 2-propanol in hexane (see entry *a*, *b*, and *c* in Table 4). As an example, comparison of the chromatograms for the resolution of *N*-(3,5-dinitrobenzoyl)leucine with the use of 10%, 30% and 60% 2-propanol in hexane containing 0.5% acetic acid as a mobile phase is presented in Figure 3. As the content of 2-propanol in hexane is increased, the retention factors are greatly decreased, but the separation and the retention factors are somewhat sacrificed. Consequently, 2-propanol content can not be increased further over 60% to reduce the retention times. Increasing the acetic acid content from 0.5% to 1.0% decreases the retention times significantly with some sacrifice of the separation and the resolution factors (compare entry a with entry *d* in Table 4). Using 0.5% trifluoroacetic acid instead of 0.5% acetic acid decreases the retention times significantly (compare entry a with entry *e*

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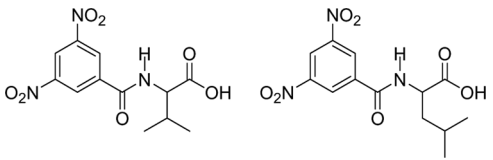
Table 4. Resolution of *N*-(3,5-dinitrobenzoyl)valine (A) and *N*-(3,5-dinitrobenzoyl)leucine (B) on CSP 1 with the variation of the type and the content of alcohol and acid in hexane[a]



A B

	Mobile phase	A				B			
		k_1	k_2	α	R_S	k_1	k_2	α	R_S
a	10% 2-PrOH in Hexane + 0.5% CH_3COOH	40.62	47.53	1.17	3.12	31.59	37.59	1.19	3.45
b	30% 2-PrOH in Hexane + 0.5% CH_3COOH	18.01	21.79	1.21	2.65	14.51	19.85	1.23	3.04
c	60% 2-PrOH in Hexane + 0.5% CH_3COOH	11.22	13.58	1.21	2.29	8.41	10.34	1.23	2.37
d	10% 2-PrOH in Hexane + 1.0 % CH_3COOH	20.32	23.16	1.14	2.96	18.99	21.46	1.13	3.22
e	10% 2-PrOH in Hexane + 0.5 % CF_3COOH	4.56	5.11	1.12	2.21	4.34	4.77	1.10	1.73
f	10% EtOH in Hexane + 0.5 % CF_3COOH	3.76	3.99	1.06	1.24	3.60	3.78	1.05	0.97

[a]Flow rate: 0.5 mL/min. Detection: 254 nm UV. Temperature: 20°C. k_1: Retention factor of the first eluted enantiomer. k_2: Retention factor of the second eluted enantiomer. α: Separation factor. R_S: Resolution factor.

in Table 4). The separation and the resolution factors decrease with the use of 0.5% trifluoroacetic acid, but still baseline resolutions are observed. Using ethanol instead of 2-propanol also reduces the retention times (compare entry *e* and entry *f* in Table 4). However, the separation and the resolution factors are much diminished with the use of ethanol, and consequently, non-baseline resolution was observed for the resolution of *N*-(3,5-dinitrobenzoyl)leucine. Overall, the chromatographic behaviors for the resolution of *N*-(3,5-dinitrobenzoyl)-α-amino acids on CSP 1 were concluded to be controlled with the variation of the type and the content of alcohol and/or acid in hexane.

In conclusion, in this study, we elucidated the importance of ionic or ion-pairing interaction for the chiral recognition without the aid of π–π donor acceptor interaction by preparing CSP 1, which contains only simple two functional groups such as a primary amino group and an ureide tethering linkage on a cyclohexane ring, and by comparing the chromatographic results for the resolution of *N*-(3,5-dinitrobenzoyl)-α-amino acids, *N*-(3,5-dinitrobenzoyl)-α-amino acid ethyl esters and *N*-(3,5-dinitrobenzoyl)-α-amino acid amides on the CSP. We also elucidated that the chromatographic behaviors can be controlled by varying the type and the content of alcohol and acid in hexane as a mobile phase. However, the exact chiral recognition mechanism is not elucidated yet and needs further study.

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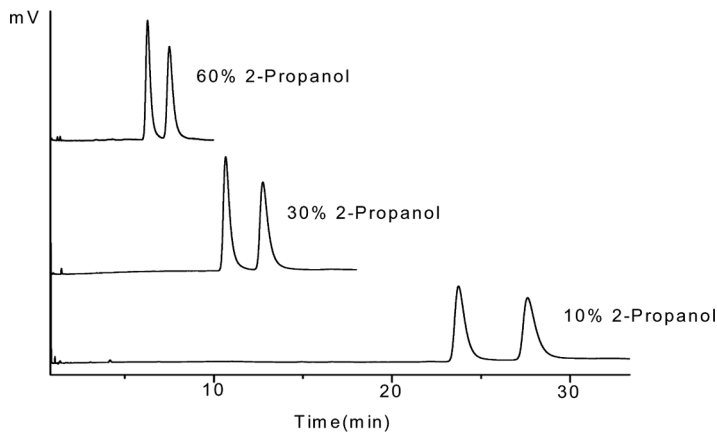

Figure 3. Comparison of the chromatograms for the resolution of *N*-(3,5-dinitrobenzoyl)leucine with the use of 10%, 30%, and 60% 2-propanol in hexane containing 0.5% acetic acid as a mobile phase. Flow rate: 0.5 mL/min. Detection: 254 nm UV. Temperature: 20°C.

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