

## Preparation and Application of a New Liquid Chromatographic Chiral Stationary Phase Based on Cefaclor

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A new HPLC chiral stationary phase (CSP) was prepared by bonding *N*-(3,5-dinitrobenzoyl)cefaclor to silica gel. The effective  $\pi$ - $\pi$  donor-acceptor interaction between the CSP and analytes was demonstrated to be important for the chiral recognition by applying the new CSP to the resolution of *N*-(substituted benzoyl)leucine ethyl esters and *N*-(substituted benzoyl)leucine propyl amides. The new CSP was also successfully applied to the resolution of anilide derivatives of *N*-acetyl- $\alpha$ -amino acids, anilide derivatives of *O*-ethoxycarbonyl-2-hydroxycarboxylic acids and 3-substituted 1,4-benzodiazepin-2-ones.

**Key Words :** Cefaclor, Chiral stationary phase, *N*-(3,5-Dinitrobenzoyl)- $\alpha$ -amino acids, *O*-Ethoxycarbonyl-2-hydroxycarboxylic acids, 3-Substituted 1,4-benzodiazepine-2-ones

### Introduction

Liquid chromatographic chiral stationary phases (CSPs) have been known to be very useful for the separation of enantiomers in both analytical and preparative scale.<sup>1</sup> During the last three decades, various effective CSPs have been developed. For example, CSPs based on natural polymeric chiral molecules,<sup>2</sup> macrocyclic glycopeptide antibiotics,<sup>3</sup> chiral crown ethers<sup>4</sup> and other optically active small chiral molecules<sup>5</sup> have been developed and successfully applied to liquid chromatographic resolution of racemic compounds.

In our laboratory, we have been continuously interested in the development of effective CSPs for the liquid chromatographic resolution of racemic compounds and as results, very successful CSPs based on  $\alpha$ -amino acids,<sup>6</sup> chiral crown ethers,<sup>7</sup> and other small chiral molecules<sup>8</sup> have been developed. As continuous efforts to develop other effective CSPs, we directed our attention to the use of cefaclor (**1**, Figure 1). Cefaclor, which belongs to the family of antibiotics known as the cephalosporins, is readily available in an optically active form. In addition, cefaclor contains a free amino group and a free carboxylic acid group and consequently, it can be readily modified through these two functional groups. In this instance, cefaclor might be utilized as a successful chiral selector of a CSP. However, cefaclor has not been used as a chiral selector of a liquid chromatographic CSP. In this study, we wish to explore the usefulness of cefaclor as a chiral selector material of a Pirkle-type CSP.

### Experimental

**General.** <sup>1</sup>H NMR spectrum was recorded with a Varian Mercury 300 spectrometer. Chemical shifts were reported in parts per million (ppm) relative to tetramethylsilane as an internal standard. IR spectrum was measured with a Jasco

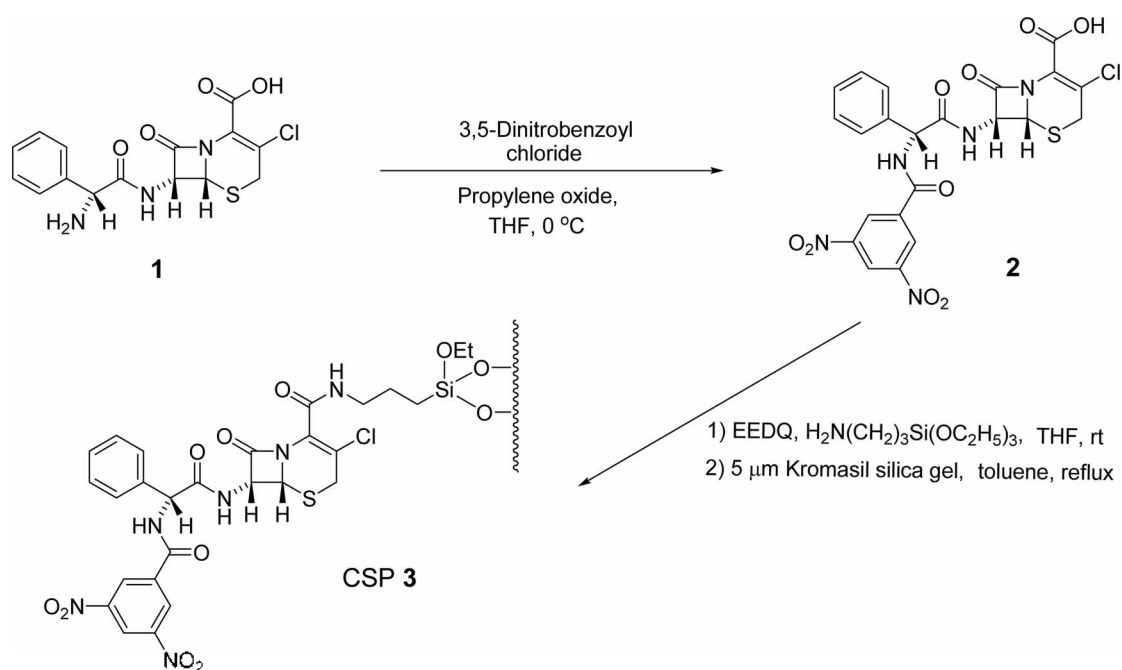
FT/IR-300E. Optical rotation was taken on a Rudolph Research Analytical AUTOPOL IV Polarimeter (Flanders, NJ, USA).

Chromatography was performed with an HPLC system consisting of a Waters model 510 HPLC Pump, a Rheodyne model 7725i injector with a 20  $\mu$ L sample loop, a Waters 2487 Absorbance Detector and a YoungLin Autochro Data Module (Software: YoungLin Autochro-WIN 2.0 plus). The temperature of the chiral column was maintained at 20 °C by using a Julabo F30 Ultratemp 2000 cooling circulator.

Racemic and optically active analytes described in Tables 1, 2, 3 and 4 were available from previous studies or prepared according to the procedure reported in the previous studies.<sup>8a,9-11</sup> Each of racemic and optically active samples was dissolved in tetrahydrofuran (usually 1.0 mg/mL) and then used for the resolution on the CSP derived from cefaclor. The usual injection volume was 1.0  $\mu$ L.

**Preparation of cefaclor-based CSP.** Cefaclor-based CSP was prepared starting from cefaclor (donated by the Korean Food and Drug Administration: KFDA) as shown in Figure 1. All reactions were performed under an argon atmosphere.

***N*-(3,5-Dinitrobenzoyl)cefaclor, 2.** Cefaclor (**1**, 2.0 g, 5.44 mmol) was dissolved in 25 mL of dried tetrahydrofuran in 100 mL round bottom flask. To the solution cooled at 0 °C was slowly added propylene oxide (1.14 mL, 16.31 mmol). The mixture was stirred for 30 min. and then 3,5-dinitrobenzoyl chloride (1.38 g, 5.98 mmol) dissolved in 20 mL of tetrahydrofuran was slowly added through syringe. The whole mixture was stirred at room temperature for 20 h. The reaction mixture was evaporated and then the residue was crystallized in a mixed solvent of tetrahydrofuran and ethyl acetate to afford a white solid material (1.61 g, 53% yield). mp 212-214 °C;  $[\alpha]_D^{14.6} +42.46$  (c 0.04, CHCl<sub>3</sub>); <sup>1</sup>H NMR (Acetone-*d*<sub>6</sub>)  $\delta$ 3.55 (d, 1H), 3.94 (d, 1H), 5.23 (d, 1H), 5.92-5.96 (m, 1H), 5.98 (d, 1H), 7.36-7.38 (m, 2H), 7.39-7.43 (m,



**Figure 1.** Preparation of CSP 3 starting from cefaclor 1.

1H), 7.59-7.62 (m, 2H), 8.54 (s, 3H), 9.08 (dd, 2H), 9.16 (q, 1H); IR (KBr pellet,  $\text{cm}^{-1}$ ) 3270, 3150, 1790, 1540.

**Preparation of CSP 3.** Compound 2 (0.50 g, 1.00 mmol) and EEDQ (2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline, 2.27 g, 1.10 mmol) were dissolved in 40 mL of dried

**Table 1.** Resolution of racemic *N*-(substituted benzoyl)leucine ethyl esters (**4**) and *N*-(substituted benzoyl)leucine propyl amides (**5**) on CSP 3<sup>a</sup>

**4**

**5**

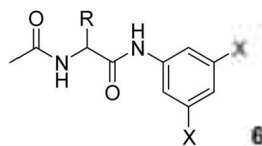
| Analytes  | Ar                  | $k_1$    | $k_2$    | $\alpha$ |
|-----------|---------------------|----------|----------|----------|
| <b>4a</b> | 3,5-Dinitrophenyl   | 7.85     | 7.85     | 1.00     |
| <b>4b</b> | 4-Nitrophenyl       | 4.79     | 5.45     | 1.14     |
| <b>4c</b> | Phenyl              | 2.39 (S) | 2.93 (R) | 1.23     |
| <b>4d</b> | 4-Methylphenyl      | 2.26 (S) | 2.85 (R) | 1.26     |
| <b>4e</b> | 4-Methoxyphenyl     | 2.25 (S) | 2.84 (R) | 1.26     |
| <b>4f</b> | 3,5-Dimethoxyphenyl | 5.17 (S) | 6.83 (R) | 1.32     |
| <b>5a</b> | 3,5-Dinitrophenyl   | 5.15     | 5.15     | 1.00     |
| <b>5b</b> | 4-Nitrophenyl       | 2.21     | 2.45     | 1.11     |
| <b>5c</b> | Phenyl              | 0.91 (S) | 1.16 (R) | 1.27     |
| <b>5d</b> | 4-Methylphenyl      | 0.85 (S) | 1.16 (R) | 1.37     |
| <b>5e</b> | 4-Methoxyphenyl     | 1.72 (S) | 2.38 (R) | 1.39     |
| <b>5f</b> | 3,5-Dimethoxyphenyl | 2.31 (S) | 3.53 (R) | 1.53     |

<sup>a</sup>Mobile phase: 20% isopropyl alcohol in hexane. Flow rate: 1.0 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.  $\alpha$ : Separation factor.

tetrahydrofuran in 100 mL round bottom flask. The mixture was stirred for 20 min. and then 3-aminopropyltriethoxysilane (0.26 mL, 1.10 mmol) was slowly added. The whole reaction mixture was stirred at room temperature for 6 h and then evaporated. Without further purification, the residue was dissolved in 30 mL of toluene. Meanwhile a 250 mL flask equipped with a Dean-Stark trap, a condenser and a magnetic stirring bar was charged with Kromasil silica gel (2.8 g, 5  $\mu\text{m}$ , 100 Å available from Eka Chemicals.) and toluene (100 mL). The mixture was heated to reflux until the complete azeotropic removal of water. To the heterogeneous solution was added the residue dissolved previously in 30 mL of toluene. The whole mixture was heated to reflux for 72 h and then cooled to room temperature. The modified silica gel (CSP 3) was collected by filtering and then washed successively with toluene, methanol, 1N HCl, acetone, ethyl acetate, methylene chloride, hexane and diethyl ether. Finally, CSP 3 was dried under high vacuum. Elemental analysis of CSP 3 (Found: C, 4.01%; H, 0.40%; N, 0.48%) showed a loading of 0.12 mmol of selector (based on C). CSP 3 was slurried in methanol and packed into a 150 mm  $\times$  4.6 mm I.D. stainless-steel HPLC column using a conventional slurry packing method with an Alltech slurry packer. The chiral column packed with CSP 3 was found to show equal chiral recognition efficiency during the period of more than one year.

## Results and Discussion

In order to utilize cefaclor (**1**) as a chiral selector of a liquid chromatographic CSP, we directed our attention to the free amino group and the carboxylic acid group. In Pirkle-type CSPs, enantioselective  $\pi$ - $\pi$  donor-acceptor interaction

**Table 2.** Resolution of anilide derivatives of *N*-acetyl- $\alpha$ -amino acids, **6**, on CSP **3'**

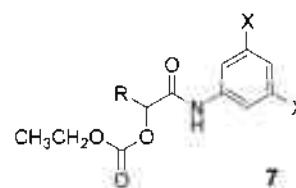
| Analytes  | R   | X                | $k_1^b$ | $k_2^c$ | $\alpha^d$ |
|-----------|---|------------------|---------|---------|------------|
| <b>6a</b> | CH <sub>3</sub>                                 | H                | 4.37    | 5.52    | 1.26       |
|           |   | CH <sub>3</sub>  | 2.00    | 2.63    | 1.31       |
|           |   | OCH <sub>3</sub> | 5.96    | 8.57    | 1.44       |
| <b>6b</b> | CH <sub>2</sub> CH <sub>3</sub>                 | H                | 2.59    | 3.50    | 1.35       |
|           |   | CH <sub>3</sub>  | 1.40    | 2.03    | 1.45       |
|           |   | OCH <sub>3</sub> | 4.20    | 6.67    | 1.59       |
| <b>6c</b> | CH <sub>2</sub> CH <sub>2</sub> CH <sub>3</sub> | H                | 2.05    | 2.86    | 1.40       |
|           |   | CH <sub>3</sub>  | 1.12    | 1.66    | 1.48       |
|           |   | OCH <sub>3</sub> | 3.21    | 5.14    | 1.60       |
| <b>6d</b> | (CH <sub>2</sub> ) <sub>3</sub> CH <sub>3</sub> | H                | 1.79    | 2.54    | 1.41       |
|           |   | CH <sub>3</sub>  | 0.98    | 1.47    | 1.50       |
|           |   | OCH <sub>3</sub> | 2.75    | 4.42    | 1.61       |
| <b>6e</b> | (CH <sub>2</sub> ) <sub>5</sub> CH <sub>3</sub> | H                | 1.36    | 1.87    | 1.38       |
|           |   | CH <sub>3</sub>  | 0.78    | 1.14    | 1.46       |
|           |   | OCH <sub>3</sub> | 2.23    | 3.49    | 1.57       |
| <b>6f</b> | CH(CH <sub>3</sub> ) <sub>2</sub>               | H                | 1.76    | 2.49    | 1.41       |
|           |   | CH <sub>3</sub>  | 1.00    | 1.59    | 1.59       |
|           |   | OCH <sub>3</sub> | 2.88    | 4.74    | 1.64       |
| <b>6g</b> | CH <sub>2</sub> C <sub>6</sub> H <sub>5</sub>   | H                | 1.49    | 2.74    | 1.41       |
|           |   | CH <sub>3</sub>  | 1.95    | 3.14    | 1.61       |
|           |   | OCH <sub>3</sub> | 5.61    | 10.23   | 1.82       |
| <b>6h</b> | C <sub>6</sub> H <sub>5</sub>                   | H                | 4.46    | 6.81    | 1.53       |
|           |   | CH <sub>3</sub>  | 2.45    | 3.72    | 1.52       |
|           |   | OCH <sub>3</sub> | 6.63    | 10.74   | 1.62       |

<sup>a</sup>Mobile phase: 20% isopropyl alcohol in hexane. Flow rate: 1.0 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.  $\alpha$ : Separation factor.

between a CSP and analytes has been known to be essential for the chiral recognition.<sup>12</sup> For the effective  $\pi$ - $\pi$  donor-acceptor interaction, Pirkle-type CSPs have been usually designed to contain  $\pi$ -acidic and/or  $\pi$ -basic aromatic groups. Even though cefaclor already contains one aromatic group, we intended to introduce more  $\pi$ -acidic aromatic group through the amino group. Anchoring cefaclor derivative to silica gel was thought to be done through the carboxylic acid group.

Cefaclor-based CSP (CSP **3**) was prepared as shown in Figure 1. To summarize the preparation of CSP **3** briefly, cefaclor was treated with 3,5-dinitrobenzoyl chloride to introduce effective  $\pi$ -acidic aromatic group and then the resulting *N*-(3,5-dinitrobenzoyl)cefaclor was connected to silica gel through the propylamide tethering group to afford CSP **3**. CSP **3** contains a strong  $\pi$ -acidic aromatic group and consequently, CSP **3** is expected to be useful for the enantio-separation of  $\pi$ -basic racemic compounds.

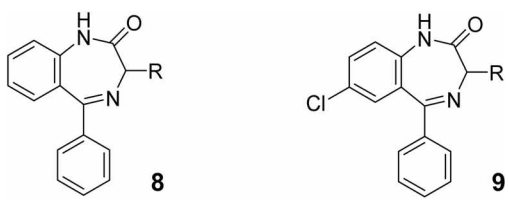
In order to demonstrate the importance of the effective  $\pi$ - $\pi$  donor-acceptor interaction between CSP **3** and analytes, the

**Table 3.** Resolution of anilide derivatives of *O*-ethoxycarbonyl-2-hydroxycarboxylic acids, **7**, on CSP **4'**

| Analytes  | R   | X                | $k_1^b$ | $k_2^c$ | $\alpha^d$ |
|-----------|---|------------------|---------|---------|------------|
| <b>7a</b> | CH <sub>3</sub>                                 | H                | 2.12    | 2.42    | 1.14       |
|           |   | CH <sub>3</sub>  | 1.89    | 2.28    | 1.20       |
|           |   | OCH <sub>3</sub> | 4.82    | 6.22    | 1.29       |
| <b>7b</b> | (CH <sub>2</sub> ) <sub>3</sub> CH <sub>3</sub> | H                | 1.40    | 1.61    | 1.15       |
|           |   | CH <sub>3</sub>  | 1.24    | 1.50    | 1.21       |
|           |   | OCH <sub>3</sub> | 3.09    | 4.11    | 1.33       |
| <b>7c</b> | (CH <sub>2</sub> ) <sub>5</sub> CH <sub>3</sub> | H                | 1.16    | 1.31    | 1.12       |
|           |   | CH <sub>3</sub>  | 1.03    | 1.22    | 1.18       |
|           |   | OCH <sub>3</sub> | 2.47    | 3.14    | 1.27       |
| <b>7d</b> | (CH <sub>2</sub> ) <sub>7</sub> CH <sub>3</sub> | H                | 1.00    | 1.11    | 1.11       |
|           |   | CH <sub>3</sub>  | 0.87    | 1.01    | 1.15       |
|           |   | OCH <sub>3</sub> | 2.14    | 2.66    | 1.24       |
| <b>7e</b> | (CH <sub>2</sub> ) <sub>9</sub> CH <sub>3</sub> | H                | 0.88    | 0.96    | 1.09       |
|           |   | CH <sub>3</sub>  | 0.77    | 0.87    | 1.14       |
|           |   | OCH <sub>3</sub> | 1.89    | 2.32    | 1.23       |
| <b>7f</b> | CH(CH <sub>3</sub> ) <sub>2</sub>               | H                | 1.43    | 1.72    | 1.20       |
|           |   | CH <sub>3</sub>  | 1.27    | 1.67    | 1.31       |
|           |   | OCH <sub>3</sub> | 3.09    | 4.49    | 1.45       |
| <b>7g</b> | CH <sub>2</sub> C <sub>6</sub> H <sub>5</sub>   | H                | 2.40    | 3.05    | 1.27       |
|           |   | CH <sub>3</sub>  | 2.12    | 2.94    | 1.39       |
|           |   | OCH <sub>3</sub> | 5.36    | 8.43    | 1.57       |
| <b>7h</b> | C <sub>6</sub> H <sub>5</sub>                   | H                | 2.67    | 3.25    | 1.22       |
|           |   | CH <sub>3</sub>  | 2.27    | 3.09    | 1.36       |
|           |   | OCH <sub>3</sub> | 5.27    | 7.76    | 1.47       |

<sup>a</sup>Mobile phase: 20% isopropyl alcohol in hexane. Flow rate: 1.0 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.  $\alpha$ : Separation factor.

CSP was applied to the resolution of *N*-(substituted benzoyl)lucine ethyl esters, **4**, and *N*-(substituted benzoyl)lucine propyl amides, **5**. The chromatographic resolution results are summarized in Table 1. The elution orders shown in Table 1 were determined by injecting configurationally known samples. When the *N*-(substituted benzoyl) group was relatively  $\pi$ -acidic, resolution was not observed at all (analytes **4a** and **5a**) or resolved quite poorly (analytes **4b** and **5b**). However, the *N*-(substituted benzoyl) group of analytes changes from the benzoyl (analytes **4c** and **5c**) group to the 4-methyl (analytes **4d** and **5d**) or 4-methoxybenzoyl group (analytes **4e** and **5e**) and then to the 3,5-dimethoxybenzoyl group (analytes **4f** and **5f**), the separation factor ( $\alpha$ ) improves quite much. From these results, the effective  $\pi$ - $\pi$  donor-acceptor interaction between the  $\pi$ -basic *N*-(substituted benzoyl) group of analytes and the  $\pi$ -acidic 3,5-dinitrobenzoyl group of CSP **3** seems to be very important for the chiral recognition. However, the chiral recognition mechanism is not clear yet. One thing to note is the retention factors. As

**Table 4.** Resolution of racemic 3-substituted 1,4-benzodiazepin-2-ones, **8** and **9**, on CSP 3<sup>a</sup>


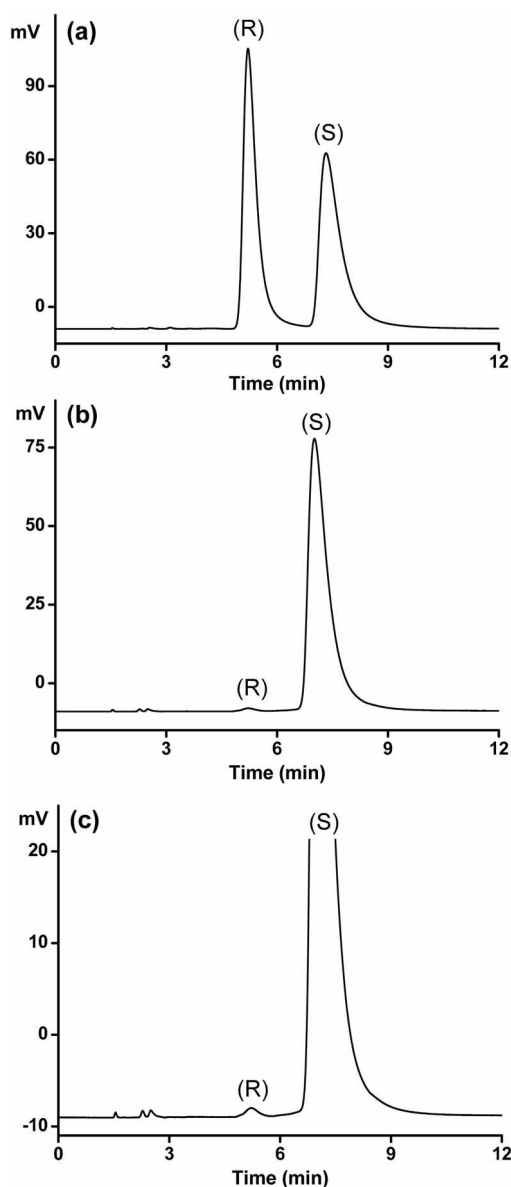
| Analytes  | R   | $k_1$    | $k_2$    | $\alpha$ |
|-----------|---|----------|----------|----------|
| <b>8a</b> | CH <sub>3</sub>                                   | 3.82 (R) | 5.21 (S) | 1.39     |
| <b>8b</b> | (CH <sub>2</sub> ) <sub>2</sub> CH <sub>3</sub>   | 2.66 (R) | 3.86 (S) | 1.45     |
| <b>8c</b> | (CH <sub>2</sub> ) <sub>3</sub> CH <sub>3</sub>   | 2.63 (R) | 3.83 (S) | 1.45     |
| <b>8d</b> | CH(CH <sub>3</sub> ) <sub>2</sub>                 | 2.15 (R) | 3.28 (S) | 1.53     |
| <b>8e</b> | CH <sub>2</sub> CH(CH <sub>3</sub> ) <sub>2</sub> | 2.51 (R) | 3.94 (S) | 1.57     |
| <b>8f</b> | CH <sub>2</sub> C <sub>6</sub> H <sub>5</sub>     | 4.76 (R) | 7.65 (S) | 1.61     |
| <b>9a</b> | CH <sub>3</sub>                                   | 3.13 (R) | 4.27 (S) | 1.37     |
| <b>9b</b> | (CH <sub>2</sub> ) <sub>3</sub> CH <sub>3</sub>   | 2.02 (R) | 3.05 (S) | 1.51     |
| <b>9c</b> | CH(CH <sub>3</sub> ) <sub>2</sub>                 | 1.82 (R) | 2.78 (S) | 1.52     |
| <b>9d</b> | CH <sub>2</sub> CH(CH <sub>3</sub> ) <sub>2</sub> | 2.08 (R) | 3.21 (S) | 1.55     |
| <b>9e</b> | CH <sub>2</sub> C <sub>6</sub> H <sub>5</sub>     | 3.83 (R) | 5.93 (S) | 1.55     |

<sup>a</sup>Mobile phase: 20% isopropyl alcohol in hexane. Flow rate: 1.0 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.  $\alpha$ : Separation factor.

shown in Table 1, the retention factor,  $k_1$ , for the resolution of the *N*-(3,5-dinitrobenzoyl) derivative of leucine ethyl ester (**4a**) or leucine propyl amide (**5a**) is greater than that for the resolution of other analytes. The non-enantioselective  $\pi$ - $\pi$  interaction between the phenyl group of CSP 3 and the *N*-(3,5-dinitrobenzoyl) group of analytes and the non-enantioselective hydrogen bonding interaction between the nitro group on the benzoyl ring of analytes and the various N-H groups of CSP 3 might be responsible for the unexpected large retention factors for the resolution of *N*-(3,5-dinitrobenzoyl)leucine ethyl ester (**4a**) or *N*-(3,5-dinitrobenzoyl)leucine propyl amide (**5a**).

CSP 3 was quite successful for the resolution of anilide derivatives of *N*-acetyl- $\alpha$ -amino acids, **6**, and for the resolution of anilide derivatives of *O*-ethoxycarbonyl-2-hydroxycarboxylic acids, **7**. The chromatographic results are summarized in Table 2 and Table 3. All analytes are resolved quite well. As the anilide group is changed from the simple anilide group to the 3,5-dimethylanilide group and then to the 3,5-dimethoxyanilide group, the separation of the two enantiomers denoted by the separation factor,  $\alpha$ , improves. From these results, it is concluded that the effective  $\pi$ - $\pi$  donor-acceptor interaction between the 3,5-dinitrobenzoyl group of CSP 3 and the anilide group of analytes is quite important for the chiral recognition.

CSP 3 was also applied to the resolution of 3-substituted 1,4-benzodiazepin-2-ones. Two enantiomers of 3-substituted 1,4-benzodiazepin-2-ones such as camazepam, lorazepam, lormetazepam and oxazepam, which belong to a class of widely used anxiolytics and/or tranquilizer,<sup>13</sup> have been



**Figure 2.** (a) Chromatogram for the resolution of racemic 3-isobutyl-1,3-dihydro(2H)-5-phenyl-1,4-benzodiazepine-2-one (**8e**) on CSP 3. (b) Chromatogram for the resolution of (*S*)-**8e** prepared from (*S*)-leucine on CSP 3. (c) The expanded chromatogram of (b). Flow rate: 1.0 mL/min. Detection: 254 nm UV. Column temperature: 20 °C.

known to show different pharmacological activity.<sup>14</sup> Consequently, enantiomeric composition or enantiomeric purity of 3-substituted 1,4-benzodiazepin-2-ones is very important. As shown in Table 4, CSP 3 was quite successful in the resolution of two types of 3-substituted 1,4-benzodiazepin-2-ones, **8** and **9**, the separation factor,  $\alpha$ , for 11 analytes being in the range of 1.37-1.61. The retention factors are not so large and consequently, CSP 3 are expected to be useful in the determination of the enantiomeric composition of 3-substituted 1,4-benzodiazepin-2-ones in terms of saving analytical time and reducing solvent. One example for the practical usefulness of CSP 3 in the determination of enantiomeric purity of 3-substituted 1,4-benzodiazepin-2-ones is demonstrated by

the chromatograms shown in Figure 2. The comparison of the chromatogram for the resolution of racemic 3-isobutyl-1,3-dihydro(2H)-5-phenyl-1,4-benzodiazepine-2-one (**8e**) (Figure 2a) with that of (*S*)-**8e** prepared from (*S*)-leucine (Figure 3b or Figure 3c) shows that (*S*)-enantiomer is contaminated with a small amount of (*R*)-enantiomer. Based on the peak areas corresponding to the two enantiomers shown in Figure 2b or 2c, the enantiomeric purity of (*S*)-3-isobutyl-1,3-dihydro(2H)-5-phenyl-1,4-benzodiazepine-2-one was calculated to be 99.0% ee (R:S = 0.5:99.5). The enantiomeric purity of (*S*)-3-isobutyl-1,3-dihydro(2H)-5-phenyl-1,4-benzodiazepine-2-one was exactly consistent with that of the starting (*S*)-leucine.

Between the two types of 3-substituted 1,4-benzodiazepin-2-ones, **8** and **9**, analytes **8** are generally resolved better than analytes **9** in terms of separation factors,  $\alpha$ , as shown in Table 4. In addition, the retention factors for the resolution of analytes **8** are greater than those of corresponding analytes **9**. From these results, we expect that  $\pi$ - $\pi$  donor-acceptor interaction between the 3,5-dinitrobenzoyl group of the CSP and the benzo ring of the analytes is important for the chiral recognition even though the exact chiral recognition mechanism is not clear yet. The non-substituted benzo ring of analytes **8** might be more  $\pi$ -basic than the chlorine substituted benzo ring of analytes **9** because of the electron withdrawing inductive nature of the chlorine group. In this instance, the  $\pi$ - $\pi$  donor-acceptor interaction of the 3,5-dinitrobenzoyl group of CSP **3** with the non-substituted benzo ring of analytes **8** should be more effective than that with the chlorine-substituted benzo ring of analytes **9** and consequently, analytes **8** are resolved better and retained longer than analytes **9**.

In summary, we prepared a new Pirkle-type CSP (CSP **3**) by bonding *N*-3,5-dinitrobenzoyl derivative of readily available optically active cefaclor to silica gel. The new CSP was demonstrated to be quite useful in the resolution of *N*-(substituted benzoyl)leucine ethyl esters, *N*-(substituted benzoyl)leucine propyl amides, anilide derivatives of *N*-acetyl- $\alpha$ -amino acids, anilide derivatives of *O*-ethoxycarbonyl-2-hydroxycarboxylic acids and 3-substitute 1,4-benzodiazepine-2-ones. Even though the chiral recognition mechanism is not clear yet, the effective  $\pi$ - $\pi$  donor-acceptor interaction between the CSP and analytes was demonstrated to be very important for the chiral recognition.

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