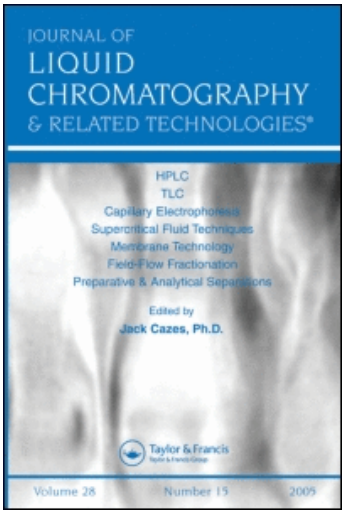

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Abstract: A new liquid chromatographic chiral stationary phase (CSP) was prepared by bonding *N*-(3,5-dimethoxybenzoyl)cefaclor to spherical silica gel. The new CSP was successfully utilized for the resolution of racemic *N*-(3,5-dinitrobenzoyl)-α-amino acid derivatives and racemic 3-substituted 1,4-benzodiazepin-2-ones. For the resolution of racemic *N*-(3,5-dinitrobenzoyl)-α-amino acid derivatives on the CSP, a chiral recognition mechanism utilizing the enantioselective π−π donor–acceptor interaction between the π-basic 3,5-dimethoxybenzoyl group of the CSP and the π-acidic 3,5-dinitrobenzoyl group of analytes was proposed.

Keywords: Cefaclor, Chiral stationary phase, Enantiomer separation, Liquid chromatography

INTRODUCTION

Two enantiomers consisting of chiral drugs have been often known to show different pharmacological activities in living systems.[1] In this

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instance, the exact determination of the enantiomeric composition or enantiomeric purity of chiral compounds is very important. Among various methods, liquid chromatographic separation of enantiomers on chiral stationary phases (CSPs) has been known to be the most accurate, convenient, and economic means for the exact determination of the enantiomeric composition of enantiomeric purity of chiral compounds.[2] For this purpose, various CSPs have been successfully utilized for the separation of enantiomers.[2] For example, CSPs based on natural polymeric chiral molecules,[3,4] α-amino acids,[5] macrocyclic antibiotics,[6] chiral crown ethers,[7–9] and other optically active small chiral molecules[10–14] have been prepared and successfully applied for the liquid chromatographic resolution of racemic compounds.

During our efforts to develop other effective CSPs, our attention was directed to cefaclor (**1**, Figure 1). Cefaclor, known as a cephalosporins antibiotic is commercially available in an optically active form. In addition, cefaclor contains a free amino group and a carboxylic acid group. In this instance, cefaclor is expected to be readily modified and easily bonded to spherical silica gel through these two functional groups to afford a silica based CSP. Actually, *N*-(3,5-dinitrobenzoyl) cefaclor bonded to spherical silica gel have been prepared, and the resulting CSP has been found to be quite successful for the liquid chromatographic resolution of various π-basic chiral compounds.[15] The stereoselective π−π interaction between the π-acidic *N*-(3,5-dinitrobenzoyl) group of the CSP and the π-basic group of analytes was proposed to be responsible

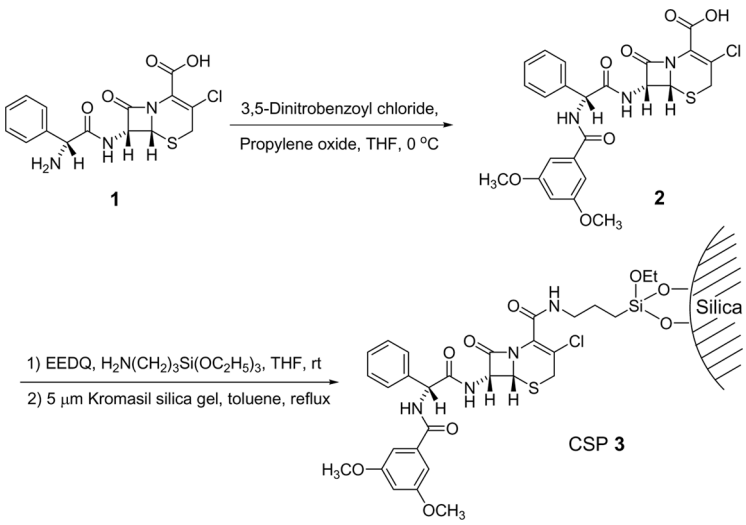


Figure 1. Scheme for the preparation of CSP **3** starting from cefaclor (**1**).

for the enantioseparation. However, the CSP was not useful for the resolution of π-acidic chiral compounds.

In this study, we wish to prepare another CSP by bonding *N*-(3,5-dimethoxybenzoyl) cefaclor to spherical silica gel. The new CSP is expected to be utilized as an HPLC CSP for the resolution of racemic π-acidic analytes.

EXPERIMENTAL

General

^{1}H NMR spectra were recorded with a Varian Mercury 300 spectrometer. Chemical shifts were reported in parts per million (ppm) relative to tetramethylsilane as an internal standard. IR spectra were measured with a Jasco FT/IR-300E. 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 510 HPLC Pump, a Rheodyne model 7725i injector with a 20 μL sample loop, a Waters 2487 Absorbance Detector, and a Young-Lin Autochro Data Module (Software: YoungLin Autochro-WIN 2.0 plus). The chiral column temperature was maintained at 20°C by using a Julabo F30 Ultratemp 2000 cooling circulator.

Racemic and optically active analytes used in this study were available from previous studies or prepared according to the procedure reported in the previous studies.[16] Each of the racemic and optically active samples were dissolved in tetrahydrofuran (usually 1.0 mg/mL) and then used for the resolution on the cefaclor derived CSP. The usual injection volume was 1.0 μL.

Preparation of the CSP Based on Cefaclor

The CSP based on cefaclor 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.

Preparation of *N*-(3,5-dimethoxybenzoyl) Cefaclor, **2**

Cefaclor (**1**, 2.0 g, 5.44 mmol) was dissolved in 25 mL of dried tetrahydrofuran in a 100 mL round bottom flask. The solution was cooled to 0°C and then, propylene oxide (1.14 mL, 16.31 mmol) was slowly added to the cooled solution. The mixture was stirred for 30 min and then, 3,5-dimethoxybenzoyl chloride (1.20 g, 5.98 mmol) dissolved in 20 mL

of tetrahydrofuran was slowly added through a syringe. The whole mixture was stirred at room temperature for 20 hr. The reaction mixture was evaporated and the residue was crystallized in a mixed solvent of tetrahydrofuran and ethyl acetate to afford a white solid material (2.66 g, 89% yield). mp 160–164°C; $[\alpha]_D^{14.5} + 51.29$ (c 0.11, $CHCl_3$); ^1H NMR (Acetone-d_6) 3.57 (d, 1H), 3.81 (s, 6H), 3.93 (d, 1H), 5.22 (d, 1H), 5.89–5.95 (m, 2H), 6.63 (t, 1H), 7.10 (d, 2H), 7.32–7.41 (m, 4H), 7.56–7.60 (m, 1H), 8.14 (d, 1H), 8.46 (d, 1H); IR (KBr pellet, cm^{-1}) 3280, 1790, 1590, 1360.

Preparation of CSP **3** and Column Packing

Compound **2** (0.53 g, 1.00 mmol) and 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ, 2.27 g, 1.10 mmol) were dissolved in 40 mL of dried tetrahydrofuran in a 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 hr and then evaporated. Without further purification, the residue was dissolved in 30 mL of toluene. At the same time, 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 μm, 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 hr and then cooled to room temperature. The modified silica gel (CSP **3**) was collected by filtering and washed successively with toluene, methanol, 1N HCl solution, acetone, ethyl acetate, methylene chloride, hexane, and diethyl ether. Finally, the modified silica gel was dried under high vacuum. Elemental analysis of the modified silica gel (Found: C, 13.66%; H, 1.82%; N, 3.68%) showed a loading of 0.45 mmol of selector (based on C). The modified silica gel was slurried in methanol and packed into a 150 mm × 4.6 mm I.D. stainless steel HPLC column, using a conventional slurry packing method with an Alltech slurry packer.

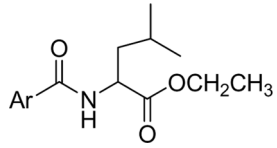
RESULTS AND DISCUSSION

CSP **3** contains a strong π-basic *N*-(3,5-dimethoxybenzoyl) group and consequently, CSP **3** is expected to be useful in the separation of the two enantiomers of π-acidic racemic analytes through the effective π-π donor–acceptor interaction. In order to see the importance of the π-π donor–acceptor interaction between the π-basic 3,5-dimethoxybenzoyl

group of the CSP and the π-acidic group of analytes, CSP **3** was applied to the resolution of *N*-(substituted benzoyl)leucine ethyl esters, **4**, and the chromatographic resolution results are summarized in Table 1. As shown in Table 1, *N*-(3,5-dinitrobnziyl)leucine ethyl ester (**4a**) was resolved best and *N*-(3- or 4-nitobenzoyl) leucine ethyl ester (**4b** or **4c**) was slightly resolved. However, *N*-benzoylleucine ethyl ester (**4d**) or *N*-(4-methylbenzoyl) leucine ethyl ester (**4e**) was not resolved at all. These results clearly demonstrate the importance of the effective π−π donor–acceptor interaction between the π-basic 3,5-dimethoxybenzoyl group of CSP **3** and the π-acidic *N*-(substituted benzoyl) group of analytes for the chiral recognition.

CSP **3** was also applied to the resolution of various *N*-(3,5-dinitrobenzoyl) leucine amides **5** and the chromatographic resolution results were summarized in Table 2. As shown in Table 2, the resolution of *N*-(3,5-dinitrobenzoyl) leucine *N*′-ethyl (**5a**) or *N*′-propylamide (**5b**) on CSP **3** is worse than that of *N*-(3,5-dinitrobenzoyl)leucine ethyl ester (**4a**). However, the resolution of *N*-(3,5-dinitrobenzoyl)leucine *N*′,*N*′-diethyl (**5c**), *N*′,*N*′-dipropyl (**5d**), *N*′-ethyl-*N*′-phenyl (**5e**) or *N*′-propyl-*N*′-phenylamide (**5f**) on CSP **3** is greater than that of *N*-(3,5-dinitrobenzoyl)-leucine ethyl ester (**4a**). From these results, we can conclude that the N-H hydrogen ($R_2 = H$) of analyte **5a** or **5b** is not involved in the chiral recognition. Instead, the N-H hydrogen ($R_2 = H$) of analyte **5a** or **5b** might be used as a non-enantioselective hydrogen bonding donor site and

Table 1. Resolution of racemic *N*-(substituted benzoyl)leucine ethyl esters (**4**) on CSP **3**[a]

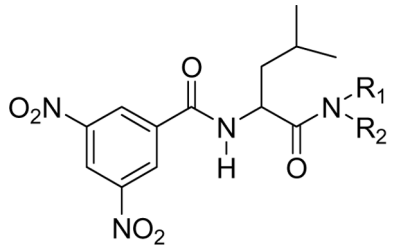


4

	Ar	k_1	k_2	α
4a	3,5-Dinitrophenyl	2.23 (S)	2.78 (R)	1.25
4b	3-Nitrophenyl	1.07 (S)	1.67 (R)	1.07
4c	4-Nitrophenyl	1.30 (S)	1.36 (R)	1.05
4d	Phenyl	0.57		1.00
4e	4-Methylphenyl	0.71		1.00

[a]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. α: Separation factor.

Table 2. Resolution of racemic *N*-(3,5-dinitrobenzoyl)leucine amides (**5**) on CSP **3**[a]



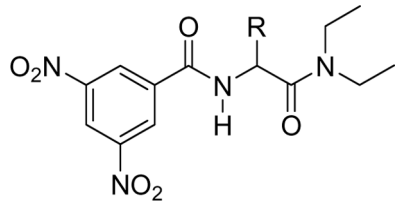
5

	R_1	R_2	k_1	k_2	α
5a	CH_2CH_3	H	3.50 (S)	3.80 (R)	1.08
5b	$CH_2CH_2CH_3$	H	2.17 (S)	2.55 (R)	1.17
5c	CH_2CH_3	CH_2CH_3	2.26 (S)	3.00 (R)	1.32
5d	$CH_2CH_2CH_3$	$CH_2CH_2CH_3$	1.39 (S)	2.30 (R)	1.65
5e	CH_2CH_3	Phenyl	3.16 (S)	4.04 (R)	1.28
5f	$CH_2CH_2CH_3$	Phenyl	2.55 (S)	3.42 (R)	1.34

[a]Mobile phase: 20% ethyl 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. α: Separation factor.

consequently, it might diminish the chiral recognition. When the N-H hydrogen ($R_2 = H$) was replaced with an alkyl group or a phenyl group, the chiral recognition was quite much improved. Between the alkyl and the phenyl group, which were utilized to replace the N-H hydrogen, the former was more effective for the chiral recognition. The electron donating inductive effect of the alkyl group might enhance the electron density at the amide carbonyl oxygen. In this instance, the enantioselective hydrogen bonding interaction between the analytes and the CSP is expected to be facilitated, and consequently, the chiral recognition is improved. Overall, it is concluded that *N*-(3,5-dinitrobenzoyl)leucine *N*′,*N*′-dialkylamides are resolved best on CSP **3** among other *N*-(substituted benzoyl) leucine esters and amides. Table 3 shows that various *N*-(3,5-dinitrobenzoyl)-α-amino acid *N*′,*N*′-diethylamides are resolved quite well on CSP **3**. The relatively long retention factors for the resolution of tyrosine derivatives might be stemmed from the non-enantioselective hydrogen bonding interaction of the 4-hydroxy group of the analyte with certain hydrogen bonding acceptor sites of the CSP or with the residual hydroxyl group on the surface of silica gel.

Table 3. Resolution of racemic *N*-(3,5-dinitrobenzoyl)-α-amino acid *N*′,*N*′-diethylamides on CSP **3**[a]



R	k_1	k_2	α
CH_3 (Alanine)	4.22 (S)	5.70 (R)	1.35
$(CH_3)_2CH$ (Valine)	2.20 (S)	2.45 (R)	1.11
$(CH_3)_2CHCH_2$ (Leucine)	2.26 (S)	3.00 (R)	1.32
C_6H_5 (Phenylglycine)	3.44 (S)	5.18 (R)	1.51
$C_6H_5CH_2$ (Phenylalanine)	3.58 (S)	4.67 (R)	1.30
4-OH-$C_6H_4CH_2$ (Tyrosine)	8.08 (S)	11.02 (R)	1.36

[a]Mobile phase: 20% ethyl 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. α: Separation factor.

The elution orders of the two enantiomers shown in Tables 1, 2, and 3 were determined by injecting configurationally known samples. In every case, the (*R*)-enantiomers were eluted second. Considering the elution orders of the two enantiomers and based on a CPK space filling molecular model study focusing on the importance of the π−π donor–acceptor interaction between the CSP and analytes and the contribution of the high electron density on the amide carbonyl oxygen to the chiral recognition described above, a chiral recognition mechanism for the resolution of *N*-(3,5-dinitrobenzoyl)-α-amino esters or amides on CSP **3** is proposed, as shown in Figure 2. The model compound of the chiral selector of CSP **3**, *N*-(3,5-dimethoxybenzoyl) cefaclor derivative, and the analyte, *N*-(3,5-dinitrobenzoyl) leucine ethyl ester, shown in Figure 2 are presumed in their lowest energy conformation based on previous study.[15] It should be noted that the notation for the absolute stereochemistry at the three chiral centers of cefaclor is known to be *R*, *R*, and *R*, respectively. As shown in Figure 2, the model compound of the chiral selector of CSP **3**, *N*-(3,5-dimethoxybenzoyl) cefaclor derivative, interacts with (*R*)-*N*-(3,5-dinitrobenzoyl) leucine ethyl ester through the face-to-face π−π interaction between the 3,5-dimethoxybenzoyl group of the chiral selector and the 3,5-dinitrobenzoyl group of the

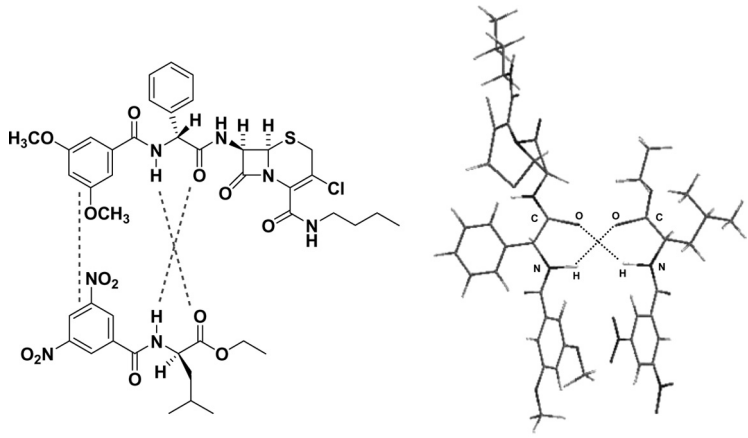


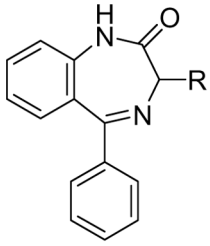
Figure 2. Chiral recognition model proposed for the more stable complex formed between the chiral selector of CSP **3** and the analyte, (*R*)-*N*-(3,5-dinitrobenzoyl) leucine ethyl ester. (a) The simple presentation showing the $\pi-\pi$ donor–acceptor interaction and the simultaneous two hydrogen bonding interactions between the CSP and the analyte. (b) The tree dimensional stick molecular model showing the face-to-face $\pi-\pi$ donor–acceptor interaction and the simultaneous two hydrogen bonding interactions between the CSP and the analyte.

analyte and through the simultaneous two hydrogen bonding interactions. In this instance, the phenyl group at the chiral center of the chiral selector and the isobutyl group at the chiral center of the (*R*)-analyte are directed outward from the interaction sphere as shown in Figure 2(b), the steric hindrance between the phenyl and the isobutyl group at the chiral centers being minimized. However, when the model compound of the chiral selector of CSP **3**, *N*-(3,5-dimethoxybenzoyl) cefaclor derivative, interacts with (*S*)-*N*-(3,5-dinitrobenzoyl) leucine ethyl ester, the isobutyl group at the chiral center of the analyte is expected to be directed inward into the interaction sphere and consequently, some steric hindrance should be experienced. Overall, the chiral selector of the CSP is expected to interact with the (R)-enantiomer more effectively than with the (S)-enantiomer and consequently, the (R)-enantiomer should be retained longer in the chiral column while the (S)-enantiomer is eluted faster.

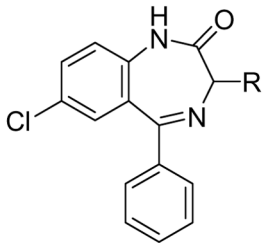
CSP **3** was also successfully applied to the resolution of 3-substituted 1,4-benzodiaepin-2-ones. 3-Substituted 1,4-benzodiaepin-2-ones such as camazepam, lorazepam, lormetazepam, and oxazepam belong to a class of anxiolytics and/or tranquilizer.[17] Two enantiomers consisting of them have been known to show different pharmacological activity,[18] and consequently, the exact determination of enantiomeric composition

or purity of 3-substituted 1,4-benzodiaepin-2-ones is very important. Previously, CSPs based on π-acidic chiral selectors containing 3,5-dinitrobenzoyl group(s) has been successfully utilized in the resolution of racemic 3-substituted 1,4-benzodiaepin-2-ones.[19–21] The CSP based on the *N*-(3,5-dinitrobenzoyl) cefaclor derivative was also successful in the resolution of 3-substituted 1,4-benzodiaepin-2-ones.[15] However, CSPs based on π-basic chiral selectors have been rarely utilized in the resolution of 3-substituted 1,4-benzodiaepin-2-ones. In this study, CSP **3** was applied to the resolution of the two types of 3-substituted 1,4-benzodiazepin-2-ones, **6** and **7** and the chromatographic resolution results are summarized in Table 4. Previously, between the two types of 3-substituted 1,4-benzodiazepin-2-ones, **6** and **7**, analytes **6** were reported to be generally resolved better than analytes **7** in terms of separation factors, α, on a π-acidic CSP based on *N*-(3,5-dinitrobenzoyl) cefaclor

Table 4. Resolution of racemic 3-substituted 1,4-benzodiazepin-2-ones **6** and **7** on CSP **3**[a]



6



7

Entry	R	k_1	k_2	α
6a	CH_3	2.62 (R)	2.74 (S)	1.05
6b	$(CH_2)_2CH_3$	1.80 (R)	1.97 (S)	1.09
6c	$(CH_2)_3CH_3$	1.62 (R)	1.82 (S)	1.12
6d	$CH(CH_3)_2$	1.42 (R)	1.69 (S)	1.19
6e	$CH_2CH(CH_3)_2$	1.64 (R)	1.84 (S)	1.12
6f	$CH_2C_6H_5$	2.74 (R)	3.00 (S)	1.09
7a	CH_3	2.23 (R)	2.40 (S)	1.07
7b	$(CH_2)_3CH_3$	1.41 (R)	1.61 (S)	1.14
7c	$CH(CH_3)_2$	1.23 (R)	1.46 (S)	1.19
7d	$CH_2CH(CH_3)_2$	1.40 (R)	1.61 (S)	1.15
7e	$CH_2C_6H_5$	2.31 (R)	2.60 (S)	1.12

[a]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. α: Separation factor.

derivative.[15] In contrast, analytes **7** are generally resolved better than analytes **6** on CSP **3** as shown in Table 4. The chloride substituted benzo ring of analytes **7** is relatively more acidic than the non-substituted benzo ring of analytes **6**, because of the electron withdrawing inductive nature of the chloride group. In this instance, the $\pi-\pi$ donor–acceptor interaction of the 3,5-dimethoxybenzoyl group of CSP **3** with the chloride substituted benzo ring of analytes **7** should be more effective than that with the non-substituted benzo ring of analytes **6** and consequently, analytes **7** are resolved better than analytes **6**.

CONCLUSION

A new CSP based on cefaclor was utilized for the liquid chromatographic separation of the two enantiomers of racemic compounds. The CSP contains a π-basic 3,5-dimethoxybenzoyl group. Consequently, the CSP was useful in the resolution of racemic compounds containing a π-acidic 3,5-dinitrobenzoyl group such as *N*-(3,5-dinitrobenzoyl)-α-amino acid derivatives, the (R)-enantiomers being eluted always second. From these results, a chiral recognition mechanism utilizing effective $\pi-\pi$ donor–acceptor interaction between the CSP and the analytes was proposed. The CSP was also successful in the resolution of 3-substituted 1,4-benzodiazepin-2-ones.

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