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Improved chiral stationary phase derived from (*S*)-naproxen for the liquid chromatographic resolution of enantiomers

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Abstract

An (*S*)-naproxen-derived chiral stationary phase (CSP) containing a tertiary N-phenyl amide linkage was prepared. The CSP was applied to the resolution of various π-acidic racemates, including N-(3,5-dinitrobenzoyl) derivatives of α-amino esters and 3,5-dinitroanilide derivatives of anti-inflammatory drugs related to α-arylpropionic acids. From the comparison of the resolution results this CSP was found to show greater enantioselectivities than any other (*S*)-naproxen-derived CSPs reported so far in resolving various π-acidic racemates.

Keywords: Enantiomer separation; Chiral stationary phases, LC; Naproxen; Anti-inflammatory drugs; Amino esters

1. Introduction

(*S*)-Naproxen, an optically active anti-inflammatory drug, has been used as a chiral selector in chiral liquid chromatography. Since (*S*)-naproxen immobilized on silica gel via a secondary amide linkage was first reported as a π-basic chiral stationary phase (CSP **1**, Fig. 1) in resolving various π-acidic racemates [1,2], several CSPs based on (*S*)-naproxen immobilized on silica gel via an ester linkage or an ionic linkage [3] have been successfully utilized. Recently, Pirkle et al. [4] reported that (*S*)-naproxen immobilized on silica gel via a tertiary dialkylamide linkage (doubly tethered CSP **2**, Fig. 1) shows the best resolving ability among others reported previously.

In this area, our effort has also been focused on the use of (*S*)-naproxen as a chiral selector in chiral liquid chromatography. Consequently, two new CSPs based on 3,5-dinitroanilide derivative of (*S*)-naproxen and 3,5-dimethylanilide derivative of (*S*)-naproxen were prepared and applied in resolving various racemates [5–7]. During this process, we found that N-alkyl-N-arylamide derivatives of racemic naproxen are resolved better than other amide derivatives such as N-alkylamide, N-arylamide and N,N-dialkylamide derivatives on a π-acidic CSP derived from N-(3,5-dinitrobenzoyl)-(*S*)-leucine. In addition, N-alkyl-N-phenylamide derivatives of racemic naproxen were found to be resolved better than other N-alkyl-N-arylamide derivatives [8]. These results, in connection with the reciprocity concept of chiral recognition [9], prompted us to develop an improved CSP (CSP **3**, Fig. 1) de-

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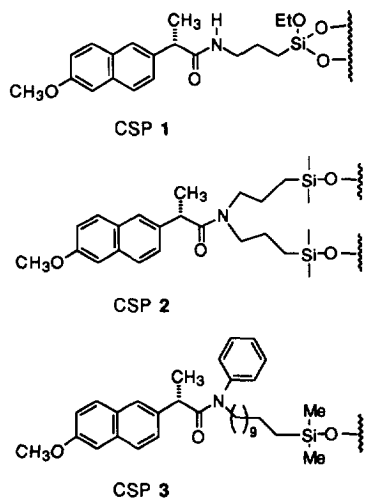

Fig. 1. Structures of CSP **1**, **2**, and **3**.

rived from the N-alkyl-N-phenylamide derivative of (*S*)-naproxen.

2. Experimental

2.1. General

^{1}H NMR spectra were obtained with a Varian Gemini 300 spectrometer (300 MHz). Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane as the internal standard. IR spectra were recorded on a Mattson Polaris Fourier transform (FT) IR spectrometer. Elemental analyses were performed at the OCRC Center, Sogang University, Seoul, South Korea.

The analytes used in this study were available from previous studies [5–7]. (*S*)-Naproxen [(+)-6-methoxy-α-methyl-2-naphthaleneacetic acid] was purchased from Aldrich and used without further purification. Solvents for HPLC analysis were of HPLC grade. All reactions were performed under an argon atmosphere.

Chromatography was performed with a Model 510 pump, a Model U6K universal liquid chromatograph injector, a Model 441 absorbance detector with 254-nm UV filter and a Model 740 data module recorder (all from Waters). All chromatographic data were collected using 2-propanol–hexane (10:90) as the mobile phase at a flow-rate of 2 ml/min at 21°C. The column void volume was measured by injecting 1,3,5-tri-*tert.*-butylbenzene [10].

2.2. Preparation of CSP 3

CSP **3** was prepared by the procedure shown in Fig. 2. The detailed synthetic procedure is as follows.

2.2.1. N-Phenyl-N-(10-undecenyl)-(S)-α-(6-methoxy-2-naphthyl)propionamide (4)

A solution of (*S*)-naproxen (1.84 g, 0.008 mol) and 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ) (2.47 g, 0.01 mol) were dissolved in 50 ml of dichloromethane in a two-necked, round bottomed flask. To the stirred solution was slowly added N-(10-undecenyl)aniline (1.96 g, 0.008 mol), which was prepared by reaction between aniline and undecenoyl chloride followed by reduction with $LiAlH_4$ (EEDQ) in 15 ml of dichloromethane. The whole mixture was stirred for 16 h at room temperature. The reaction mixture was washed successively with 50 ml of 2 *M* HCl, saturated K_2CO_3 solution and brine. The organic solution was dried over anhydrous Na_2SO_4 and evapo-

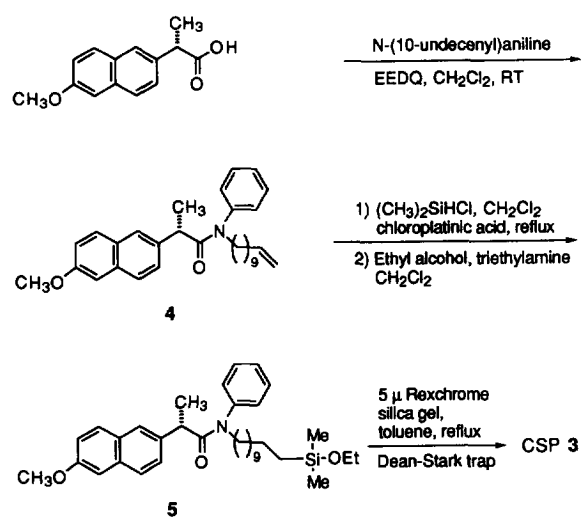

Fig. 2. Scheme for the preparation of CSP **3**.

rated. The residue was purified by column chromatography on silica gel [ethyl acetate–hexane–dichloromethane (1:40:1–1:25:1, v/v)] to afford **4** as a colourless oil (2.26 g, 61%) (for structures see Fig. 2). The enantiomeric purity of amide **4** was greater than 98% *ee* by HPLC analysis on a chiral column (Regis Chemical, Morton Grove, IL, USA) derived from N-(3,5-dinitrobenzoyl)-(*S*)-leucine. ^{1}H NMR (C^2HCl_3): δ 1.20–1.75 (m, 14H), 1.46 (d, 3H), 1.98–2.08 (m, 2H), 3.62–3.76 (m, 3H), 3.91 (s, 3H), 4.91–5.05 (m, 2H), 5.70–5.90 (m, 1H), 6.93–7.63 (m, 11H). IR (CH_2Cl_2): 3076, 2928, 1658, 1608, 1495 cm^{-1}.

2.2.2. *N-Phenyl-N-(11-ethoxydimethylsilylundecyl)-(S)-α-(6-methoxy-2-naphthyl)-propionamide (5)*

The (*S*)-naproxen amide derivative **4** (2.26 g, 0.0049 mol) was dissolved in 50 ml of dichloromethane. To the stirred solution were added chlorodimethylsilane (22 ml) and $H_2PtCl_6 \cdot 6H_2O$ (40 mg in 1 ml of try tetrahydrofuran). The reaction mixture was heated at reflux for 2 h. The excess chlorodimethylsilane and dichloromethane were removed by simple distillation and then under reduced pressure. The residue was dissolved in 50 ml of dichloromethane. To the stirred solution was slowly added 10 ml of absolute ethanol–triethylamine (1:1, v/v). The mixture was stirred at room temperature for 30 min and then concentrated under vacuum. The residue was purified by column chromatography [ethyl acetate–hexane (1:30–1:20, v/v)] on silica gel to afford silyl compound **5** (2.07 g, 75%) as a colourless, viscous oil. The enantiomeric purity of **5** was greater than 98% *ee* on a chiral column derived from N-(3,5-dinitrobenzoyl)-(*S*)-leucine. ^{1}H NMR (C^2HCl_3): δ 0.10 (s, 6H), 0.55–0.65 (m, 2H), 1.16–1.35 (m, 21H), 1.44 (d, 3H), 3.61–3.72 (m, 5H), 3.91 (s, 3H), 6.92–7.63 (m, 11H). IR (CH_2Cl_2): 3430, 3076, 2925, 1658, 1608, 1495 cm^{-1}.

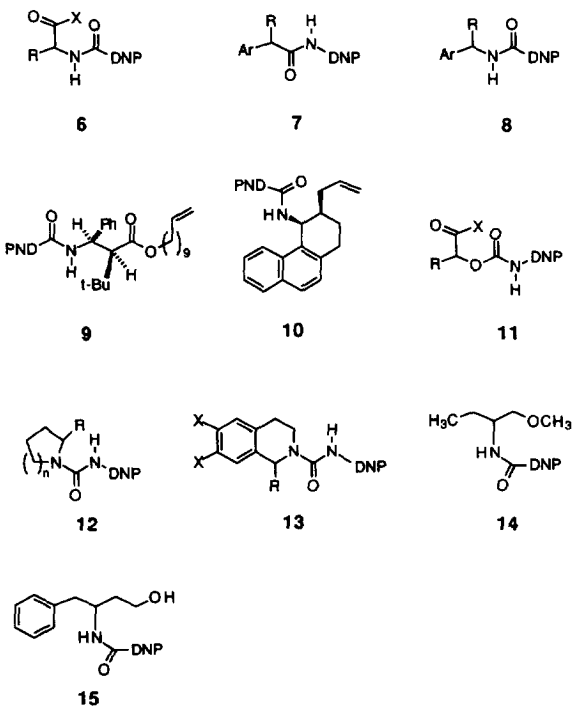

Fig. 3. Structures of the racemic compounds resolved on CSP **3** in this study. DNP = 3,5-dinitrophenyl.

2.2.3. *Preparation of CSP 3 and HPLC column packing*

Rexchrom silica gel (5 μm, 4.5 g) (Regis Chemical) was suspended in 100 ml of toluene in a 250-ml flask equipped with a Dean–Stark trap, a condenser and a magnetic stirring bar. The heterogeneous mixture was heated to reflux to remove water azeotropically. After the complete azeotropic removal of water, a solution of silyl compound **5** (2.0 g, 0.0036 mol) in 20 ml of dry toluene was added and the whole heterogeneous mixture was refluxed for 72 h. The bonded phase was then filtered and washed successively with toluene, methanol, acetone, ethyl acetate, hexane and diethyl ether. Elemental analysis of the thoroughly dried bonded phase (C 3.28, H 0.48, N 0.12%) showed a loading of 0.08 mmol of chiral selector per gram of stationary phase based on either C or N. The bonded phase was slurried in methanol and packed into a 250 × 4.6 mm I.D. stainless-steel HPLC column using a conventional slurry packing method with an Alltech slurry packer. After washing the HPLC chiral column with 100 ml of dichloromethane, a

solution of 2 ml of hexamethyldisilazane in 50 ml of dichloromethane was eluted through the column to protect the unreacted residual silanol groups of the bonded phase and then 100 ml of dichloromethane were eluted to wash out unreacted hexamethyldisilazane.

3. Results and discussion

In designing CSP **2**, which has been reported to show so far the best resolving ability for various π-acidic racemates among various (*S*)-naproxen-derived CSPs, the primary concern pointed out by Pirkle et al. [4] was to avoid the supposedly superfluous secondary amide NH hydrogen of CSP **1**. Removing the secondary amide NH hydrogen of CSP **1** was expected to enhance the enantioselectivity of the CSP because the NH hydrogen of CSP **1** might lead to interactions with analytes which increase their retention without distinguishing between enantiomers. In this context, CSP **3** prepared as shown in Fig. 2 is expected to show greater enantioselectivity than **1** because CSP **3** does not contain the superfluous amide NH hydrogen. In addition, we found that the N-undecenyl-N-phenylamide of racemic naproxen was resolved much better [separation factor $\alpha = 2.65$ using 2-propanol–hexane [20:80)] than the N,N-dipropylamide of racemic naproxen [separation factor $\alpha = 1.48$ using 2-propanol–hexane (20:80)] on a π-acidic CSP derived from N-(3,5-dinitrobenzoyl)-(*S*)-leucine. Considering that the N,N-dipropylamide of racemic naproxen is the actual chiral selector of CSP **2**, it is expected that CSP **3** derived from N-undecenyl-N-phenylamide of (*S*)-naproxen will show better enantioselectivity than CSP **2** at least for the two enantiomers of racemic N-(3,5-dinitrobenzoyl)-α-amino acid derivatives.

Based on the rationale, CSP **3** was prepared starting from commercially available (*S*)-naproxen via the procedure shown in Fig. 2. It should be noted that an unsymmetrical tertiary amide can have two geometrical isomers because of the

Table 1
Comparison of the resolution of various π-acidic racemates on CSP **2** and **3**

Analyte	R (or Ar)	X (or R)	CSP **2**[a]			CSP **3**[b]		
			k_1'[c]	α[d]	Config.[e]	k_1'[c]	α[d]	Config.[e]
6a	CH_3	OCH_3	1.60	2.47	*R*	6.40	3.10	*R*
6b	$CH(CH_3)_2$	OCH_3	0.56	3.11	*R*	5.89	3.85	*R*
6c	$CH_2CH(CH_3)_2$	OCH_3	4.06	1.79	*R*	5.83	3.88	*R*
6d	Benzyl	OCH_3	0.74	2.30	*R*	10.70	3.25	*R*
7a	6-Methoxy-2-naphthyl	CH_3	2.88	1.19	*S*	7.81	1.31	*S*
7b	4-Isobutylphenyl	CH_3				3.94	1.46	
7c	3-Benzoylphenyl	CH_3				4.51	1.27	
7d	3-Phenoxyphenyl	CH_3				5.76	1.41	
7e	5-Benzoyl-2-thienyl	CH_3				7.38	1.11	
8a	Phenyl	CH_3	8.99	1.23	*R*	14.26	1.21	*R*
8b	1-Naphthyl	CH_3	10.67	1.86	*R*	16.21	1.85	*R*
9[f]			4.12	6.67		4.68	8.23	
10			7.95	5.86		27.03	8.00	

See Fig. 3 for structures of the compounds mentioned.
[a] The chromatographic data are quoted from Ref. [4].
[b] See Experimental for the chromatographic conditions.
[c] Capacity factor of the first-eluted enantiomer.
[d] Separation factor.
[e] Absolute configuration of the second-eluted enantiomer.
[f] The actual compound resolved on CSP **2** is the methyl ester [4] instead of the 10-undecenyl ester **9**.

barrier to OC–NS rotation [11]. Consequently, CSP **3** is also envisaged to have two geometrical isomeric structures. However, we assumed that **3** exists in only one geometrical isomeric structure based on the proton NMR spectra of tertiary amide **4** showing only one set of signals even at −60°C. Between two possible geometrical structures of CSP **3**, the preferred one is not clearly confirmed yet, even though the one shown here seems likely due to the steric hindrance, as one of referees suggested. The elucidation of the exact geometrical structure of CSP **3** needs further studies.

CSP **3** thus prepared was excellent in resolving various π-acidic racemates. The performance of CSP **3** in resolving various racemic π-acidic racemates including racemic N-(3,5-dinitrobenzoyl)-α-amino acid derivatives is compared with that of CSP **2** reported by Pirkle et al. [4] in Table 1. As shown in Table 1, CSP **3** is much better than CSP **2** in resolving racemic N-(3,5-dinitrobenzoyl)-α-amino esters (**6**), as expected from the reciprocity conception of chiral recognition. CSP **3** is also better than CSP **2** in resolving the 3,5-dinitroanilide derivative of racemic naproxen (**7a**) and shows good enantioselectivities for the 3,5-dinitroanilide derivatives of other anti-inflammatory drugs (**7b–e**) related to α-arylpropionic acids. In addition, CSP **3** shows great enantioselectivities for π-acidic compounds **9** and **10**. The enantioselectivities for racemic 3,5-dinitrobenzamide derivatives of α-arylalkylamines (**8**) on CSP **3** are comparable to that on CSP **2**. From these results, we conclude that CSP **3** is generally better than CSP **2** in resolving various π-acidic racemates.

In addition to the π-acidic racemates shown in Table 1, 3,5-dinitrobenzoyl carbamates of α-hydroxycarboxylic esters (**11**), cyclic amines (**12** and **13**) and N-(3,5-dinitrobenzoyl) derivatives of

Table 2
Resolution of 3,5-dinitrobenzoyl carbamates of α-hydroxycarboxylic esters and cyclic amines and N-(3,5-dinitrobenzoyl)amides of amino alcohol derivatives on CSP **3**[a]

Analyte	R	X	*n*	k'_1[b]	k'_2[c]	α[d]
11a	CH_3	OCH_2CH_3		2.29	2.99	1.31
11b	$(CH_2)_3CH_3$	OCH_2CH_3		1.82	2.37	1.30
11c	$(CH_2)_5CH_3$	OCH_2CH_3		1.70	2.19	1.29
11d	$(CH_2)_{11}CH_3$	OCH_2CH_3		1.39	1.74	1.25
11e	CH_3	OCH_3		2.79	3.68	1.32
11f	CH_3	$O(CH_2)_3CH_3$		1.93	2.56	1.33
11g	CH_3	$O(CH_2)_{11}CH_3$		1.40	1.89	1.35
11h	Phenyl	OCH_3		3.61	4.72	1.31
11i	Phenyl	$O(CH_2)_3CH_3$		2.53	3.37	1.33
11j	Phenyl	$OCH(CH_3)_2$		2.34	3.00	1.28
12a	CH_3		1	3.70	4.29	1.16
12b	$(CH_2)_7CH_3$		1	2.47	3.04	1.23
12c	$(CH_2)_7CH_3$		2	2.59	3.05	1.18
13a	CH_3	H		5.17	5.87	1.14
13b	Benzyl	H		4.55	7.00	1.54
13c	CH_3	OCH_3		9.63	11.71	1.22
13d	Benzyl	OCH_3		8.50	12.67	1.49
13e	Phenyl	OCH_3		11.41	16.00	1.40
14				5.37	15.52	2.89
15				5.75	7.84	1.36

[a] See Experimental for the chromatographic conditions.
[b] Capacity factor of the first-eluted enantiomer.
[c] Capacity factor of the second-eluted enantiomer.
[d] Separation factor.

some amino alcohol derivatives (**14** and **15**) are also resolved with reasonable separation factors on CSP **3**. The results are summarized in Table 2. The resolution of α-hydroxycarboxylic acid and cyclic amine derivatives on CSP **3** is especially interesting because they are important as biologically active substances, chiral building blocks or intermediates for the asymmetric synthesis of biologically active materials [12,13].

In conclusion, CSP **3** has been found to be better than any other CSPs derived from (*S*)-naproxen in resolving various π-acidic racemates. From this study, we were able to show that tethering the chiral selector to silica gel through the tertiary amide bond can enhance the enantioselectivity of naproxen-based CSPs, as Pirkle et al. [4] proposed, but it is not necessary to use the doubly tethered tertiary amide bond. To rationalize the chiral recognition mechanism exerted by CSP **3**, we may need to study the resolution trends of a homologous series of π-acidic racemates and/or need to prepare a short tethered version of CSP **3**. A study concerning the chiral recognition mechanism exerted by CSP **3** is in progress.

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