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Preparation and application of an (*S*)-naproxen chiral stationary phase

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Abstract

A chiral stationary phase (CSP) for the liquid chromatographic separation of enantiomers was prepared by immobilizing the 3,5-dimethylanilide derivative of (*S*)-naproxen on silica gel through the 6-methoxy-2-naphthyl functionality of (*S*)-naproxen. The enantioselectivities exerted by this π-basic CSP for resolving π-acidic racemates were generally greater than those on the previously reported CSPs prepared by immobilizing an alkylamide of (*S*)-naproxen on silica gel through the alkylamide functionality. Based on the chromatographic resolution trends, two chiral recognition mechanisms are proposed. One mechanism applied for the resolution of N-(3,5-dinitrobenzoyl)-α-amino esters is proposed to utilize the 6-alkoxy-2-naphthyl group of the CSP as a π-basic interaction site for enantioselective $\pi-\pi$ complexation with the 3,5-dinitrobenzoyl group of the analyte and the other mechanism is proposed to utilize the 3,5-dimethylanilide group of the CSP in resolving N-(3,5-dinitrobenzoyl)-α-arylalkylamines.

1. Introduction

The two enantiomers of racemic drugs often show different pharmacological effects in living systems [1]. In consequence, the enantiomeric composition of pharmaceuticals has been an important issue in the drug development and in the clinical use of drugs and there has been a widespread need for techniques that afford a means of determining enantiomeric composition and absolute configuration and obtaining each of the two enantiomers in an enantiomerically pure form [2,3]. Among various techniques, chiral liquid chromatography based on chiral stationary phases (CSPs) has been widely accepted as a convenient and accurate means to meet such a need [4].

The resolution of racemates by chiral liquid chromatography is now fairly common because various CSPs are available [5]. Among others, Pirkle-type CSPs have been known to resolve two enantiomers through the $\pi-\pi$ interaction between the CSP and the analytes [6]. To utilize the effective $\pi-\pi$ interaction, Pirkle-type CSPs are usually designed to contain a π-basic or a π-acidic aryl functional group [7,8]. In relation to this point, (*S*)-naproxen which is a well known anti-inflammatory drug sold as an optically active

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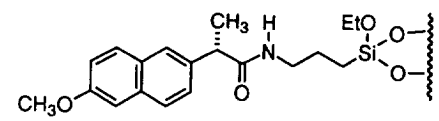
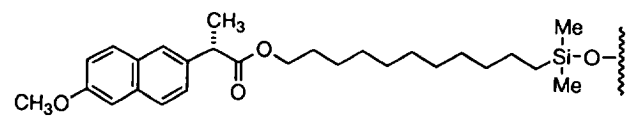
form, is an attractive candidate for new Pirkle-type CSPs because the 6-methoxy-2-naphthyl group of the compound can be utilized as a strong π-basic aryl functionality. Previously, (*S*)-naproxen has been used as a π-basic chiral selector in chiral liquid chromatography. For example, CSPs such as **1**, **2** and **3** consisting of (*S*)-naproxen immobilized on silica gel via an amide linkage [9,10], an ester linkage or an ionic linkage [11] have been reported. Recently, Pirkle *et al.* [12] reported doubly tethered CSP **4** prepared by immobilizing (*S*)-naproxen diallylamide to silica gel through the diallylamide functionality and compared its efficiencies with CSP **1**. All of these (*S*)-naproxen-derived CSPs utilize the carboxylic acid functionality of (*S*)-naproxen in immobilization.

In contrast, in this paper we report a CSP (**5**) prepared by immobilizing the 3,5-dimethylanilide of (*S*)-naproxen on silica gel through the 6-methoxy-2-naphthyl functionality of (*S*)-naproxen, the connection mode of which has been verified as useful in preparing other (*S*)-naproxen-derived CSPs in our laboratory [13,14]. Previously, it has been reported that arylamide derivatives of ibuprofen, which has a structure similar to that of naproxen, are resolved better than simple alkylamide derivatives on the π-



CSP 1



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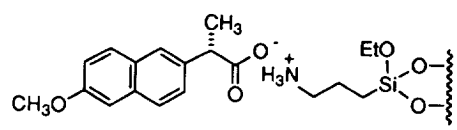

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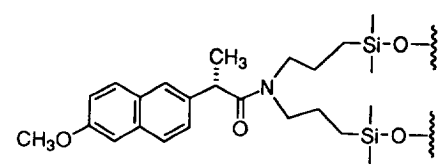
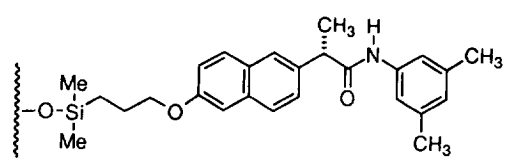


CSP 4



CSP 5

FORMULA A.

acidic CSP derived from (*R*)-N-(3,5-dinitrobenzoyl)phenylglycine and the resolutions are improved as the electron-donating power of the substituent on the aromatic derivatizing group increases [15]. We also found that arylamide derivatives of racemic naproxen are generally resolved better than simple alkylamide derivatives on the same CSP. In consequence, CSP **5** derived from (*S*)-naproxen arylamide containing two electron-donating substituents such as a methyl functionality on the aromatic ring of the aryl amide group is expected to be better than the CSPs derived from alkylamide derivatives of naproxen in resolving π-acidic racemic analytes based on the reciprocity concept of chiral recognition [16].

In addition, CSP **5** actually contains two π-basic functional groups that can act as a π-donor site, i.e., 6-alkoxy-2-naphthyl and 3,5-dimethylphenyl groups. Either of the two π-basic functional groups of CSP **5** may be used for face-to-face $\pi-\pi$ complexation with π-acidic racemates, which is known to be essential for chiral recognition by Pirkle-type CSPs [6]. Therefore, the role of the two π-basic aromatic functional groups contained in CSP **5** in resolving π-acidic racemates is of interest and is discussed herein.

2. Experimental

^{1}H NMR spectra were recorded on 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 measured with a Mattson Polaris Fourier transform (FT) IR spectrometer. Melting points were taken on a Rigaku TAS 100 thermal analyzer. Optical rotations were measured on a Rudolph Autopol III automatic polarimeter. Elemental analyses were performed at the OCRC Centre, Sogang University, Seoul, Korea.

The analytes used in this study were either available from prior studies or prepared in the manner described previously [14]. (*S*)-Naproxen, (+)-6-methoxy-α-methyl-2-naphthaleneacetic acid, was purchased from Aldrich. Solvents for HPLC analysis were of HPLC grade. All reagents purchased from commercial suppliers were of analytical-reagent grade and used without further purification, unless indicated otherwise. All reactions were performed under an argon atmosphere.

HPLC analyses were performed using a HPLC system consisting of a Waters Model 510 pump, a Rheodyne Model 7125 injector with a 20-μl sample loop, a Youngin (Seoul, Korea) Model 710 absorbance detector with a 254-nm UV filter and a Youngin D520B computing integrator. The volume of sample injected was usually 5 μl.

2.1. Preparation of CSP 5

(S) - α - (6-Methoxy-2-naphthyl)propion - 3,5-dimethylanilide (6)

(*S*)-Naproxen (7.38 g, 0.032 mol) and thionyl chloride (9.35 ml, 0.128 mol) were dissolved in 100 ml of benzene and then the stirred mixture was refluxed for 2 h. After cooling to room temperature, the mixture was evaporated to dryness using a rotary evaporator. The residue was dissolved in 80 ml of dry dichloromethane and then a mixture of 3,5-dimethylaniline (3.99 ml, 0.032 mol) and triethylamine (4.46 ml, 0.032 mol) diluted in 20 ml of dry dichloromethane was added slowly with stirring. The reaction mixture was stirred at room temperature for 2 h and then washed successively with 100 ml of saturated $NaHCO_3$ solution, 100 ml of 2 *M* HCl and brine. The organic solution was dried over anhydrous Na_2SO_4 and evaporated. The residue was purified by flash column chromatography on silica gel [ethyl acetate–hexane–dichloromethane (1:5:1–1:3:1, v/v/v)] to afford a white solid. Crystallization of the white solid in hexane afforded amide **6** (10.41 g) in 98% yield. The enantiomeric purity of amide **6** was greater than 98% by the HPLC analysis on a previously described CSP [17]; m.p. 153–155°C. ^{1}H NMR (C^2HCl_3), δ 1.67 (d, 3H), 2.23 (s, 6H), 3.84 (q, 1H), 3.94 (s. 3H), 6.70 (s, 1H), 6.99–7.78 (m, 9H). IR (KBr) cm^{-1}, 3436, 3305, 1661, 1607, 1532. $[\alpha]_D^{25}$ +103.7 (*c* 1.0, CH_2Cl_2).

(S) - α - (6-Hydroxy-2-naphthyl)propion - 3,5-dimethylanilide (7)

Amide **6** (5.0 g, 0.015 mol) was placed in a 250-ml round-bottomed flask with 70 ml of dry dichloromethane. The solution was cooled to −78°C and then a solution of BBr_3 (3.58 ml, 0.038 mol) in 15 ml of dry dichloromethane was added over 1 h with stirring. The reaction mixture was allowed to warm to room temperature over 1 h and then stirred for an additional 1 h. At this stage, the formation of a white solid was observed. The mixture was cooled to 0°C and then water was added slowly with stirring until no more white fumes (probably HBr gas) evolved. After stirring for an additional 3 h, the white solid was filtered off and dissolved in a mixture of acetone (130 ml) and ethyl acetate (600 ml). The organic solution was washed with brine, dried over anhydrous Na_2SO_4 and then evaporated. The residue was purified by flash column chromatography on silica gel [acetone–dichloromethane (1:20, v/v)] to afford hydroxy compound **7** (4.16 g, 87% yield) as a white solid. The enantiomeric purity of hydroxy compound **7** was greater than 98% by the HPLC analysis on a previously described CSP [17]; m.p. 161–163°C. ^{1}H NMR (acetone-$^2H_6$), δ 1.57 (d, 3H), 2.24 (s, 6H), 3.97 (q, 1H), 6.70 (broad s, 1H), 7.15–7.80 (m, 9H), 9.06 (s, 1H). IR (KBr) cm^{-1}, 3298, 2974, 1618, 1547. $[\alpha]_D^{25}$ + 105.4 (*c* 1.0, CH_3OH).

(S) - α - (6-Allyloxy-2-naphthyl)proprion - 3,5-dimethylanilide (8)

Hydroxy compound **7** (2.0 g, 0.0063 mol) was dissolved in 100 ml of dry CH_3CN and then stirred with K_2CO_3 (1.12 g, 0.0081 mol) at room temperature for 2 h in a 150-ml round-bottomed flask. Allyl bromide (1.35 ml, 0.0156 mol) was added to the reaction mixture and then the whole mixture was heated to reflux. After refluxing for 2 h, the solvent was evaporated and the residue was dissolved in diethyl ether. The diethyl ether solution was washed successively with 100 ml of 1 *M* NaOH solution and 100 ml of brine, dried over anhydrous $MgSO_4$ and concentrated. The residue was purified by silica gel column chromatography [ethyl acetate–hexane (1:6–1:3, v/v)] to afford allyloxy compound **8** (2.14 g, 95% yield) as a white solid. The enantiomeric purity of allyloxy compound **8** was greater than 98% by the HPLC analysis on a previously described CSP [17]; m.p. 136–137°C. ^{1}H NMR (C^2HCl_3), δ 1.67 (d, 3H), 2.24 (s, 6H), 3.84 (q, 1H), 4.66–4.69 (m, 2H), 5.32–5.38 (m, 1H), 5.45–5.53 (m, 1H), 6.08–6.09 (m, 1H), 6.71 (s, 1H), 7.00–7.76 (m, 9H). IR (KBr) cm^{-1}, 3281, 1653, 1604, 1531. $[\alpha]_D^{25}$ + 88.6 (*c* 1.0, CH_2Cl_2).

(S) - α - [6-(3-Ethoxydimethylsilylpropoxy) - 2-naphthyl)]proprion - 3,5 - dimethylanilide (9)

Compound **8** (2.0 g, 0.0056 mol) was placed in a 100-ml round-bottomed flask with 50 ml of dry dichloromethane. Dimethylchlorosilane (25 ml) was added to the reaction mixture, followed by addition of $H_2PtCl_6 \cdot 6H_2O$ (*ca.* 10 mg) dissolved in 1 ml of dry tetrahydrofuran. The reaction mixture was heated to reflux and checked periodically by TLC. After checking that there was no starting material in the reaction mixture, dichloromethane and excess dimethylchlorosilane were removed by simple distillation and then under reduced pressure. The residue was dissolved in 60 ml of dry dichloromethane and then 10 ml of triethylamine—absolute ethanol (1:1, v/v) were added slowly with stirring. The mixture was stirred at room temperature for 1 h, concentrated and chromatographed on silica gel [ethyl acetate–hexane (1:6, v/v)] to afford silyl compound **9** (2.26 g, 88% yield) as a highly viscous, colourless liquid. The enantiomeric purity of **9** was greater than 98% by the HPLC analysis on a previously described CSP [17]. ^{1}H NMR (C^2HCl_3), δ 0.15 (s, 6H), 0.74–0.79 (m, 2H), 1.20 (t, 3H), 1.64 (d, 3H), 1.88–1.96 (m, 2H), 2.21 (s, 6H), 3.69 (q, 2H), 3.81 (q, 1H), 4.05 (t, 2H), 6.68 (s, 1H), 7.04–7.72 (m, 9H). IR (CH_2Cl_2) cm^{-1}, 3299, 2934, 1659, 1605, 1559. $[\alpha]_D^{25}$ + 65.9 (*c* 0.8, CH_2Cl_2).

*Preparation of CSP **5** and HPLC column packing*

A 100-ml flask equipped with a Dean–Stark trap, a condenser and a magnetic stirring bar was

charged with Regis Rexchrom silica gel (5 μm, 4.5 g) and toluene (90 ml). After heating the heterogeneous silica gel–toluene mixture to reflux until the complete azeotropic removal of water, silyl compound **9** (1.28 g, 0.0028 mol) dissolved in 20 ml of toluene was added and the whole mixture was heated at reflux for 83 h. The modified silica gel was filtered, washed successively with toluene, methanol, acetone, ethyl acetate, hexane and diethyl ether and then dried under vacuum. Elemental analysis of the modified silica gel (C 10.48, H 1.07, N 0.42%) showed a loading of 0.34 mmol of chiral selector per gram of stationary phase based on C or 0.30 mmol of chiral selector per gram of stationary phase based on N. 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. After washing the HPLC chiral columns thus packed with 100 ml of dichloromethane, a solution of 2 ml of hexamethyldisilazane in 50 ml of dichloromethane was eluted through the column to end-cap the residual silanol groups and then dichloromethane was eluted to wash out unreacted hexamethyldisilazane.

3. Results and discussion

CSP **5** was prepared as shown in Fig. 1. To summarize, (*S*)-naproxen was converted into the acid chloride and then treated with 3,5-dimethylaniline in the presence of triethylamine to afford the 3,5-dimethylanilide derivative **6** of (*S*)-naproxen. Treatment of **6** with boron tribromide at −78°C afforded the 6-hydroxy-2-naphthyl com-

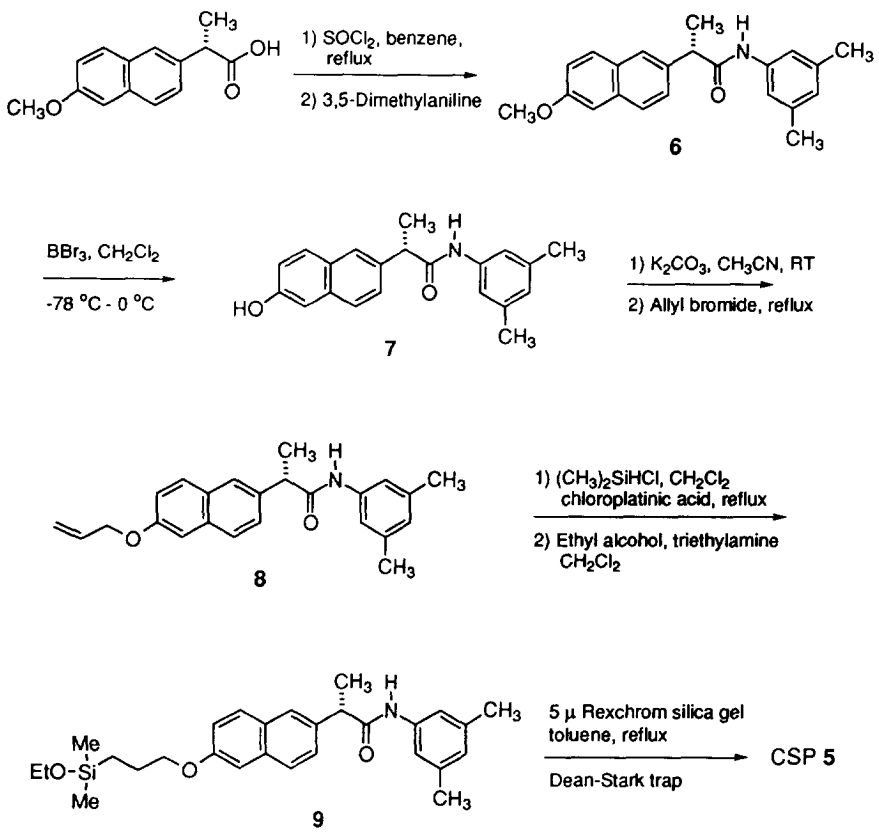


Fig. 1. Scheme for the preparation of CSP **5**.

pound **7**. Reaction of **7** with K_2CO_3 in acetonitrile at room temperature and then with allyl bromide under reflux gave the 6-allyloxy-2-naphthyl compound **8**. Finally, hydrosilylation of **8** to give silyl compound **9** and treatment of **9** with 5-μm silica gel produced CSP **5**. In each step, no racemization was observed by HPLC analysis on a previously described CSP [17].

The enantioselectivities exerted by CSP **5** for resolving various π-acidic racemates shown in Fig. 2 are summarized in Table 1. As shown in Table 1, N-(3,5-dinitrobenzoyl) derivatives of α-amino esters (analytes **10a–o**), α-amino amides (analytes **10p–r**) and α-arylalkylamines (analytes **11**) and 3,5-dinitroanilide derivatives of anti-inflammatory drugs related to α-arylpropionic acids (analytes **12**) are resolved with reasonable to good separation factors on CSP **5**. In addition, N-(3,5-dinitrobenzoyl) derivatives of other racemic compounds (analytes **13–15**) are resolved with reasonable separation factors on CSP **5**. By comparing the separation factors shown in Table 1 with those reported previously by Pirkle *et al.* [12], we found that CSP **5** is generally better than CSP **1** in terms of chiral recognition denoted by separation factors (α values) but worse than CSP **4** except for resolving N-(3,5-dinitrobenzoyl)leucine alkyl esters. In resolving N-(3,5-dinitrobenzoyl)leucine alkyl esters, CSP **5** showed the best resolution results. The elution orders shown in Table 1 for resolving N-(3,5-dinitrobenzoyl) derivatives of racemates on CSP **5** were identical with those on CSPs **1** and **4**.

In an effort to elucidate the chiral recognition mechanism manifested by CSP **5**, N-(3,5-dinitrobenzoyl) derivatives of a series of α-alkylglycine ethyl esters (analytes **10a–e**) and leucine alkyl esters (analytes **10g–m**) were prepared and resolved on CSP **5**. The use of homologous series of analytes has been often utilized as mechanistic probes to investigate the origins of enantioselectivity exerted by a certain Pirkle-type CSP. For example, increasing or decreasing trends in the separation factors of a homologous series of analytes with variation of the length of the substituent have been used as evidence for the intercalation of alkyl substituents of analytes between the adjacent strands of the bonded phase during the chiral recognition [18,19]. However, the separation factors for resolving N-(3,5-dinitrobenzoyl) derivatives of a series of α-alkylglycine ethyl esters (analytes **10a–e**) and leucine alkyl esters (analytes **10g–m**) on CSP **5** remain almost constant as

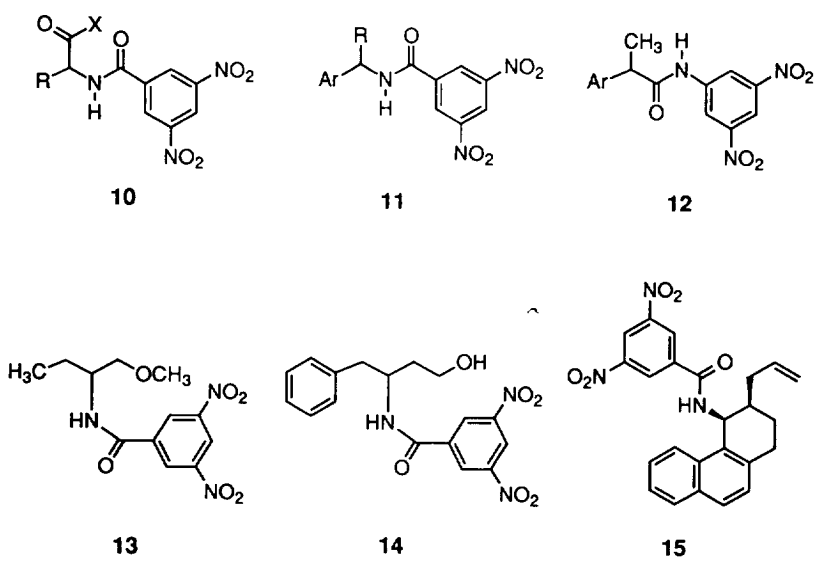


Fig. 2. Structures of the racemic compounds resolved on CSP **5** in this study.

Table 1
Resolution of various π-acidic racemates on CSP **5**

Analyte	R (or Ar)	X (or R)	k_1'[a]	k_1'[b]	α[c]	Configuration[d]
10a	CH_3	OCH_2CH_3	14.77	23.01	1.56	*R*
b	$(CH_2)_2CH_3$	OCH_2CH_3	13.89	30.57	2.20	
c	$(CH_2)_4CH_3$	OCH_2CH_3	12.67	26.29	2.07	
d	$(CH_2)_6CH_3$	OCH_2CH_3	11.74	25.82	2.20	
e	$(CH_2)_7CH_3$	OCH_2CH_3	10.99	24.07	2.19	
f	$CH(CH_3)_2$	OCH_2CH_3	12.44	25.39	2.04	*R*
g	$CH_2CH(CH_3)_2$	OCH_3	15.07	37.98	2.52	*R*
h	$CH_2CH(CH_3)_2$	OCH_2CH_3	12.56	32.15	2.56	*R*
i	$CH_2CH(CH_3)_2$	$O(CH_2)_2CH_3$	11.79	29.45	2.50	
j	$CH_2CH(CH_3)_2$	$O(CH_2)_3CH_3$	11.39	28.73	2.52	
k	$CH_2CH(CH_3)_2$	$O(CH_2)_5CH_3$	10.83	26.72	2.47	
l	$CH_2CH(CH_3)_2$	$O(CH_2)_9CH_3$	8.90	21.98	2.47	
m	$CH_2CH(CH_3)_2$	$O(CH_2)_{15}CH_3$	7.36	18.16	2.47	
n	Phenyl	OCH_3	28.45	32.99	1.16	*R*
o	Benzyl	OCH_3	37.25	57.70	1.55	*R*
p	CH_3	$NH(CH_2)_2CH_3$	4.21	4.94	1.17	*R*
q	$CH(CH_3)_2$	$NH(CH_2)_2CH_3$	2.05	3.35	1.63	*R*
r	$CH_2CH(CH_3)_2$	$NH(CH_2)_2CH_3$	2.31	3.95	1.71	*R*
11a	Phenyl	CH_3	35.52	38.67	1.19	*R*
b	Phenyl	CH_2CH_3	36.04	49.23	1.37	
c	Phenyl	$(CH_2)_4CH_3$	34.88	46.83	1.34	
d	Phenyl	$(CH_2)_7CH_3$	27.03	38.64	1.43	
e	Phenyl	$(CH_2)_{11}CH_3$	21.81	33.31	1.53	
f	4-CH_3-Phenyl	CH_3	30.19	35.00	1.16	
g	4-$CH_3(CH_2)_2$-phenyl	CH_3	27.09	31.06	1.15	
h	4-$CH_3(CH_2)_7$-phenyl	CH_3	22.41	23.51	1.05	
i	4-$CH_3(CH_2)_{11}$-phenyl	CH_3	19.42	19.42	1.00	
j	4-Methoxyphenyl	CH_3	42.96	53.49	1.25	*R*
k	4-Methoxyphenyl	$(CH_2)_7CH_3$	35.92	51.13	1.42	
l	4-Methoxyphenyl	$(CH_2)_{16}CH_3$	25.38	39.63	1.56	
m	α-Naphthyl	CH_3	40.63	60.61	1.49	*R*
12a	3-Phenoxyphenyl		11.45	13.65	1.19	
b	3-Benzoylphenyl		13.19	16.38	1.24	
c	6-Methoxy-2-naphthyl		17.54	20.12	1.15	*S*
13			15.98	24.88	1.56	
14			16.66	19.03	1.14	
15			35.68	116.67	3.27	

All data were collected using 20% 2-propanol in hexane at a flow-rate of 2.0 ml/min. Void volumes were measured using 1,3,5-tri-*tert.*-butylbenzene.

[a] Capacity factors of the first-eluted enantiomers.

[b] Capacity factors of the second-eluted enantiomers.

[c] Separation factors.

[d] Absolute configuration of the second-eluted enantiomers. For blank entries, the elution orders have not been established.

the length of the alkyl chain of analytes increases as shown in Table 1. Note that relatively small separation factor for resolving N-(3,5-dinitrobenzoyl)alanine ethyl ester (**10a**, α-alkyl = methyl) on CSP **5** compared with the separation factors of other N-(3,5-dinitrobenzoyl)-α-alkylglycine

ethyl esters may stem from conformational factors, as described previously [19]. From these results, we excluded the possibility of a chiral recognition mechanism involving the process of intercalating the ester alkyl tail or the α-alkyl tail of N-(3,5-dinitrobenzoyl)-α-alkylamino alkyl esters between the adjacent strands of the bonded phase. Instead, we propose, from a study of the CPK molecular models, a possible chiral recognition mechanism that does not involve the intercalation process for resolving N-(3,5-dinitrobenzoyl)-α-alkylamino alkyl esters.

Fig. 3 shows the proposed chiral recognition model for resolving N-(3,5-dinitrobenzoyl)-α-alkylamino alkyl esters on CSP **5**. In this model, CSP **5** and the analyte are represented in conformations which are presumed to be of relatively low energy and hence preferentially populated [11,18]. CSP **5** and the analyte interact through the face-to-face $\pi-\pi$ complexation between their respective 6-alkoxy-2-naphthyl (NAPH) and 3,5-dinitrophenyl (DNP) groups and the simultaneous face-to-edge $\pi-\pi$ interaction, which has received increased attention as an associative force between aromatic rings [18,19], between the 3,5-dimethylphenyl (DMP) group of the CSP and the DNP group of the analyte and through the hydrogen bonding between the amide NH hydrogen of the CSP and the carbonyl oxygen of the 3,5-dinitrobenzoyl group of the analyte. In this event, the alkoxy oxygen of the ester functionality of the *R*-enantiomer can also interact with the amide NH hydrogen of the CSP and in consequence the diastereomeric *R,S*-complex shown in Fig. 3 is energetically more favorable than the *S,S*-complex. Note that in this model both of the ester alkyl group (Y) and the α-aklyl substituent (denoted by R in the model) of the analyte are away from the connecting tether of the CSP and in consequence the intercalation of either the Y or the R group of the analyte between the connecting tethers of the CSP is not possible. When the ester alkoxy group of the analyte is changed to an amide NH group, the second hydrogen bonding shown in Fig. 3 between the alkoxy oxygen of the ester functionality of the *R*-enantiomer and the amide NH hydrogen of the CSP is not possible. This may be the reason for the shorter retention times and lower separation factors observed for resolving N-(3,5-dinitrobenzoyl)-α-aminoamides (analytes **10p–r**) on CSP **5** than those for resolving N-(3,5-dinitrobenzoyl)-α-amino esters shown in Table 1.

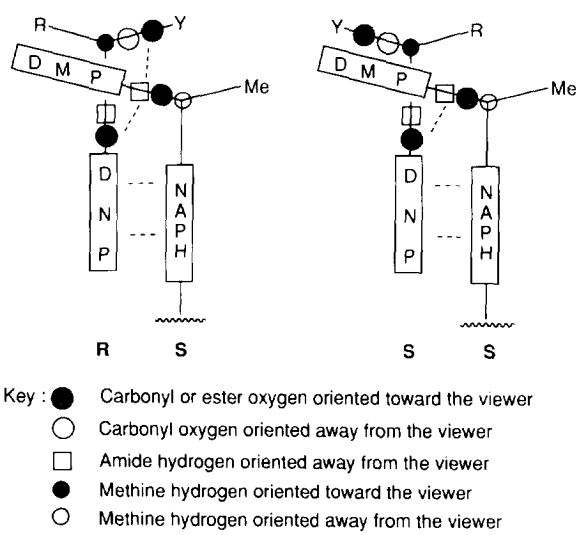


Fig. 3. Proposed chiral recognition model for resolving N-(3,5-dinitrobenzoyl)-α-alkylamino alkyl esters on CSP **5**. The *R,S*-complex is more stable than the *S,S*-complex.

The separation factors for resolving a series of N-(3,5-dinitrobenzoyl)-α-phenylalkylamines (analytes **11a–e**) on CSP **5** increase whereas those for resolving a series of N-(3,5-dinitrobenzoyl)-α-(*p*-alkylphenyl)ethylamines (analytes **11f–i**) decrease as the alkyl substituent of analytes increases in length, as shown in Table 1, and these trends are illustrated with more relevant data included in Figs. 4 and 5. As shown in Fig. 4, the capacity factors of the *S*-enantiomers of N-(3,5-dinitrobenzoyl)-α-phenylalkylamines decrease more rapidly than those of the more retained *R*-enantiomers as the length of the α-alkyl substituent of the analyte increases and in consequence separation factors increase continuously. The discrepancy in the continuous increase of separation factors at $n = 2$ noted in Fig. 4 may also originate from conformational reasons, as described previously [19]. However, Fig. 5 shows that the capacity factors of the more

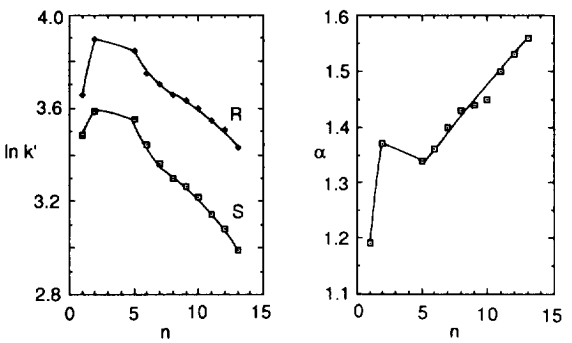


Fig. 4. Trends in the retention (ln k') of the two enantiomers and the enantioselectivity (α) for resolving N-(3,5-dinitrobenzoyl)-α-phenylalkylamines on CSP **5**. The length of the alkyl substituent [$-(CH_2)_nH$] at the chiral centre of the analyte is denoted by n on the abscissa. For chromatographic conditions, see Experimental and the footnote to Table 1.

retained R-enantiomers of N-(3.5-dinitrobenzoyl)-α-(p-alkylphenyl)ethylamines decrease more rapidly than the S-enantiomers as the length of the p-alkyl substituent of the analyte increases, and in consequence the separation factors decrease and finally no resolution is observed with the p-alkyl substituent reaches n-decyl.

From the trends shown in Figs. 4 and 5, we can imagine that the alkyl substituent at the chiral centre of the S-enantiomers of N-(3,5-dinitrobenzoyl)-α-phenylalkylamines intercalates between the strands of bonded phase during the chiral recognition and the intercalation process experiences more difficulty as the alkyl substituent increases in length. Similarly, the p-alkyl substituent of the R-enantiomers of N-(3,5-dinitrobenzoyl)-α-(p-alkylphenyl)ethylamine intercalates between the strands of bonded phase during the chiral recognition and the separation factors decrease as the p-alkyl substituent increases in length.

A possible chiral recognition mode which involves such intercalation processes and explains the trends shown in Figs. 4 and 5 is proposed as shown in Fig. 6. In this model, the CSP has the same conformation as drawn in the model shown in Fig. 3 and the analyte is presumed to be in its lowest energy conformation as described previously [20]. As shown in Fig. 6, CSP **5** utilizes the DMP instead of the NAPH group as a π-basic interaction site for the face-to-face $\pi-\pi$ complexation with the DNP group of the analyte and the carbonyl oxygen for the simultaneous hydrogen bonding with the NH hydrogen of the analyte. The alternative hydrogen bonding interaction between the NH hydrogen of the CSP and the carbonyl oxygen of the analyte seems also to be possible. However, from study of the CPK molecular models we found that the hydrogen bonding interaction between the carbonyl oxygen of the CSP and the

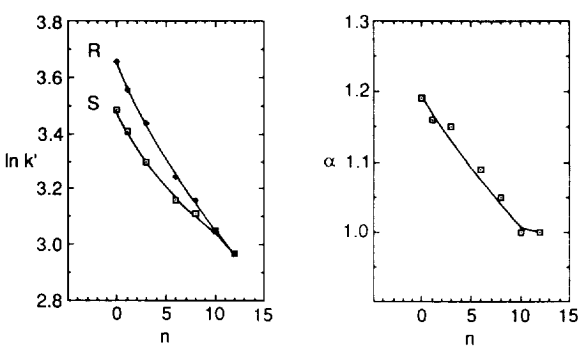


Fig. 5. Trends in the retention (ln k') of the two enantiomers and the enantioselectivity (α) for resolving N-(3,5-dinitrobenzoyl)-α-(p-alkylphenyl)ethylamines on CSP **5**. The length of the p-alkyl substituent [$-(CH_2)_nH$] of the analyte is denoted by n on the abscissa. For chromatographic conditions, see Experimental and the footnote to Table 1.

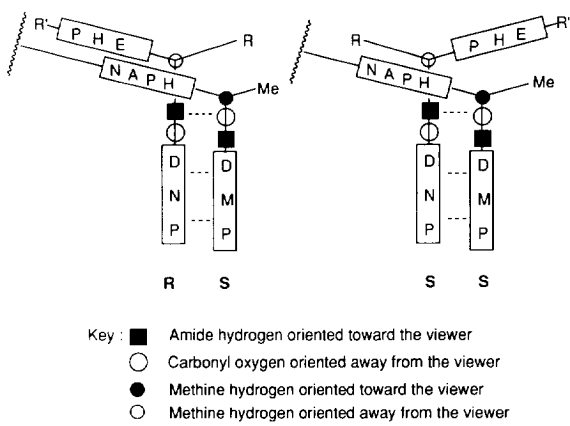


Fig. 6. Proposed chiral recognition model for resolving N-(3,5-dinitrobenzoyl)-α-arylalkylamines on CSP **5**. The R,S-complex is more stable than the S,S-complex.

NH hydrogen of the analyte shown in Fig. 6 might make possible the secondary hydrogen bonding interaction between the NH hydrogen of the CSP and the oxygen of the nitro group of the analyte, a hydrogen bonding interaction similar to which has been proposed previously [20], and consequently we presumed the model shown in Fig. 6 to be more plausible.

In this instance, the alkyl substituent (denoted by R in the model) at the chiral centre of the *R*-analyte is oriented to the direction of the methyl group of the CSP whereas that of the *S*-analyte is more parallel to the connecting tether of the CSP. As a consequence, the retention time of the initially less retained *S*-enantiomer decreases more rapidly than that of the initially more retained *R*-enantiomer and the separation factor (α) increases as the length of the alkyl substituent at the chiral centre of the analyte increases as shown in Fig. 4. However, the origin of the more retention of the *R*-enantiomer than that of the *S*-enantiomer on CSP **5** is not clear at present. Based on the chiral recognition model shown in Fig. 6, we propose that the two aromatic functional groups such as the phenyl group (PHE) of the *R*-enantiomer of the analyte and the NAPH group of the CSP may induce an attractive interaction between them. In this event, the diastereomeric *R,S*-complex should be more favorable than the *S,S*-complex.

In resolving N-(3,5-dinitrobenzoyl)-α-(*p*-alkylphenyl)ethylamines on CSP **5**, the *p*-alkyl group of the *R*-enantiomer is directed alongside the connecting tether of the CSP whereas that of the *S*-enantiomer is oriented towards the direction of the methyl group of the CSP as shown in Fig. 6. In this instance, the retention time of the *R*-enantiomer of the analyte decreases more rapidly than that of the *S*-enantiomer because of the suppressed intercalation process as the *p*-alkyl group of the analyte increases in length and consequently the separation factor decreases. All of these are consistent with the trends shown in Fig. 5.

The use of different π-basic aromatic groups of CSP **5** in resolving N-(3,5-dinitrobenzoyl)-α-alkylamino alkyl esters and N-(3,5-dinitrobenzoyl)-α-arylalkylamines illustrated in Figs. 3 and 6 may be evidenced by comparison of the enantioselectivities with those obtained with a CSP derived from the 3,5-dinitroanilide derivative of (*S*)-naproxen. Previously, we prepared a CSP that has the same structure as that of CSP **5** except for the 3,5-dimethylanilide group by immobilizing the 3,5-dinitroanilide derivative of (*S*)-naproxen on silica gel and reported the resolution of various N-(3,5-dinitrobenzoyl)-α-alkylamino alkyl esters and N-(3,5-dinitrobenzoyl)-α-arylalkylamines on the CSP [14]. From the comparison of the reported resolution results on the CSP derived from the 3,5-dinitroanilide derivative of (*S*)-naproxen with those on CSP **5**, we found that the enantioselectivities for resolving N-(3,5-dinitrobenzoyl)-α-arylalkylamines on the CSP derived from the 3,5-dinitroanilide derivative of (*S*)-naproxen are much worse than those on CSP **5** whereas those for resolving N-(3,5-dinitrobenzoyl)-α-alkylamino alkyl esters on the CSP derived from the 3,5-dinitroanilide derivative of (*S*)-naproxen are comparable to those on CSP **5**. These results might be rationalized based on the chiral recognition mechanisms shown in Figs. 3 and 6 as follows. N-(3,5-Dinitrobenzoyl)-α-arylalkylamines which are proposed to be resolved through the effective $\pi-\pi$ complexation between the π-acidic DNP group of the analyte and the π-basic DMP group of CSP **5** as shown in the chiral recognition model in Fig. 6 cannot make an effective $\pi-\pi$ donor–acceptor complex with the CSP derived from the 3,5-dinitroanilide of (*S*)-naproxen because the CSP lacks the DMP group and are resolved very poorly. However, N-(3,5-dinitrobenzoyl)-α-alkylamino alkyl esters, which are expected to be resolved through the effective $\pi-\pi$ complexation between the π-acidic DNP group of the analyte and the π-basic NAPH group of CSP **5** as shown in the chiral recognition model in Fig. 3, can induce an effective $\pi-\pi$ donor–acceptor interaction with the CSP derived from the 3,5 dinitroanilide of (*S*)-naproxen utilizing the 6-alkoxy-2-naphthyl group of the CSP and are resolved well on this CSP. Consequently, these results support the proposed chiral recognition models utilizing different π-basic aromatic

groups of CSP **5** in resolving N-(3,5-dinitrobenzoyl)-α-alkylamino alkyl esters and N-(3,5-dinitrobenzoyl)-α-arylalkylamines shown in Figs. 3 and 6.

In conclusion, we have demonstrated that CSP **5** prepared by immobilizing the 3,5-dimethylanilide of (*S*)-naproxen on silica gel through the 6-methoxy-2-naphthyl functionality of (*S*)-naproxen shows greater enantioselectivities in resolving some π-acidic racemates than the previously reported CSPs prepared by immobilizing an alkylamide of (*S*)-naproxen on silica gel through the alkylamide functionality. From the chromatographic resolution trends and the study based on the CPK molecular models, we proposed two chiral recognition mechanisms, shown in Figs. 3 and 6, which utilize either the NAPH group or the DMP group of the CSP for the enantioselective π–π complexation with the DNP group of the analytes in resolving N-(3,5-dinitrobenzoyl)-α-alkylamino alkyl esters or N-(3,5-dinitrobenzoyl)-α-arylalkylamines, respectively. However, we had some difficulty in clearly portraying three-dimensional structures of the chiral recognition models in two-dimensional representations. Therefore, some parts of the chiral recognition models shown in Figs. 3 and 6 are exaggerated or distorted and readers who wish to see clear three-dimensional structures of the molecular models shown in Figs. 3 and 6 are advised to use CPK molecular models. Finally, we should mention that the chiral recognition models shown in Figs. 3 and 6 might be modified or improved as more spectroscopic and/or crystallographic data for the diastereomeric complex formed between the chiral selector molecule of the CSP and the analyte molecule are collected.

Acknowledgements

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