Enantioseparation of Racemic 1,1'-Binaphthyl-2,2'-diamine by Preparative Liquid Chromatography

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The same kind of chiral stationary phase with a commercialized chiral column was used to make preparative chiral columns and was applied to resolve racemic *N*-acetyl-1-naphthylethylamide (**3**) by preparative liquid chromatography. An improved chromatographic condition to resolve racemic **3** on the CSP was examined by changing flow rate and kind of the mobile phase and the sample injection volume. The optimized separation conditions were applied to resolve racemic 1,1'-binaphthyl-2,2'-diamine (**4**).

Key Words : Preparative liquid chromatography, Chiral separation, *N*-Acetyl-1-naphthylethylamide, 1,1'-Binaphthyl-2,2'-diamine, Separation condition

Introduction

The main purpose of preparative chromatography is to offer separation and purification of the samples. Verzele and Bidlingmeyer developed many preparative chromatographic techniques related to column size and mobile phase speed for good resolution.[1-3] Unger also tried similar research with Verzele and Bidlingmeyer and found that column size and flow rate of the mobile phase deeply affected the resolution on preparative chromatography.[4,5] Guiochon studied the effect of the amount of a sample injection on the preparative chromatography, and found the amount was very important to the large scale resolution.[6] Research related to preparative chromatography parameters such as, size of column, choice of stationary and mobile phase, column packing density and particle size of stationary phase has been carried out.[7] The applications, however, are fairly limited due to the kinds and properties of analytes and stationary phase until now.

As the importance of the optically pure compound increased,[8] the importance of the large scale separation of chiral materials also increased. Many researches related to resolution of racemic compounds by prep. LC has been reported.[9-13] Polysaccharides cellulose and amylose based prep. columns developed by Okamoto are most widely used in the world and have been applied to resolve various chiral compounds.[14,15] These stationary phases were also applied to separate and purify chiral foods, drugs, agricultural chemicals and many important chemicals by a SMB (simulated moving bed) method.[16] Pirkle-type chiral stationary phases are also used as a large scale separation.[17]

In this study, the same kind of chiral stationary phase[18] with a commercialized chiral column[19] (CSP 1) was used to make preparative chiral columns. The preparative chiral stationary phase was largely prepared by a very similar synthetic procedure with CSP 1 and was packed into four 7.8 mm ID (inner diameter) and 250 mm length empty columns under different packing pressures, 2000, 4000, 6000 and 8000 psi in order to find the optimum packing condition. Since the 8000 psi was the most suitable packing pressure, a 10 mm ID and 250 mm length preparative chiral column was additionally prepared under 8000 psi. A preparative liquid chromatography (prep. LC) experiment was performed to get the optimum analysis conditions by using the prep. columns. To check the influence of the flow rate of the mobile phase and the sample injection volume of racemic *N*-acetyl-1-naphthylethylamide (3) on the prep LC separation, the flow rate was varied from 4, 6 and 8 mL/min and the injection volume of a 10% (w/w) solution of **3** by 0.2, 0.4, 0.6 and 0.8 mL. The racemic **3** was used as a testing analyte of CSP 1 in previous analytical HPLC.[18] From comparing the results of each condition, the optimum condition was found. This condition was applied to the resolution of racemic 1,1'-binaphthyl-2,2'-diamine (**4**) which is used as an important starting material for asymmetric synthesis and chiral separation.

Experimental Section

General Methods. The ^{1}H-NMR spectra were recorded on to a Varian Unity Inova 300WB Spectrometer (300Mz). The IR spectra were measured with a Mattson Galaxy 7020 Polaris FT-IR Spectrometer. The melting points were taken on a capillary melting point apparatus. Elemental analysis data were obtained with a Carlo Erba EA 1108 Elemental Analyzer.

The reagents used in this study were purchased from the Aldrich Chemical Co. Test racemic materials were prepared according to the procedure described previously.[20] The solvents for the HPLC analysis were of Merck HPLC grade. All reactions were performed under a nitrogen atmosphere.

Preparation of Preparative CSP 1. The synthetic method of the preparative CSP 1 was very similar to that of the analytical CSP 1[18] except for size of silica gel and the amount of chemical used. The commercialized CSP is produced by K-MAC with the name of CHIRALRYOO-PGO-1.[19] The synthesis of N-3,5-dinitrobenzoyl-(R)-phenylglycinol (**1**)

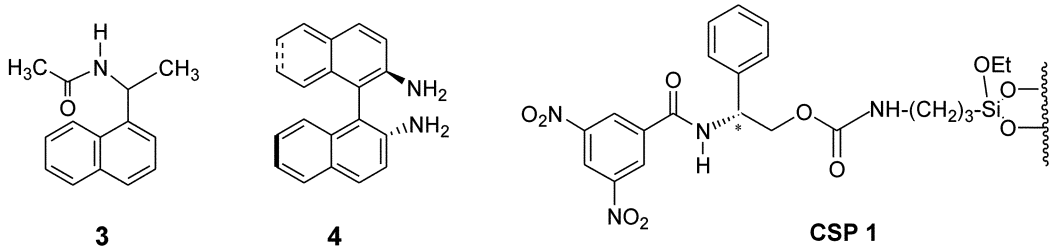

Figure 1. Structures of racemic samples (*N*-acetyl-1-naphthylethylamide (**3**), 1,1-binaphthyl-2,2-diamine (**4**)) and the chiral stationary phase (CSP 1) used in the study.

and (R)-N-(3,5-dinitrobenzoyl)phenylglycinol (triethoxysilyl) propylcarbamate (**2**) could be found in the previous work.[18]

CSP 1. The 80.0 g of 10 μm Kromasil silica gel (100 Å) and 500 mL toluene were added to a 1000 mL round bottom flask. From the resulting slurry, water was removed azeotropically by using a Dean-Stark trap with reflux for 24 hrs. After the water was completely removed, the silyl compound (**2**) (20.0 g, 34.6 mmole) was added to the slurry solution and the whole mixture was heated to reflux for 80 hrs and stirred mechanically instead of by a spin bar. The reaction flask was cooled to room temperature under nitrogen atmosphere, then the modified silica gel was washed with benzene, methanol, acetone, ethyl acetate, methylene chloride and hexane and dried. The dried silica gel was packed into a 250 mm length, 10 mm I.D and four 250 mm length, 7.8 mm I.D stainless steel columns under different pressure. The methyl alcohol was used as a packing solvent and the packing time of each column was about 20 minutes.

Preparative Chromatography. The prep. HPLC system consisting of a Waters Prep LC controller, Waters 2487 Dual λ Absorbance Detector (254 nm UV filter), and Waters Delta Prep 4000 preparative chromatography system was controlled by Win 98 Millennium 32 program. All chromatographic data were obtained using 2-propanol/hexane (20 : 80) as a mobile phase and the column void volume was checked by injecting 1,3,5-tri-tert-butylbenzene, a presumed unretained solute[21] which was obtained from the Aldrich Chemical Co. Three grams of *N*-acetyl-1-naphthylethylamide (**3**) were synthesized by a known method,[20] and 1,1-binaphthyl-2,2'-diamine (**4**) was obtained from the Aldrich Chemical Co. The structures of the two samples are shown in Figure 1. The sample solutions were prepared by dilution of 1.00 g of **3** or 0.80 g of **4** with 10 mL methanol. The flow rate of the mobile phase was varied from 4, 6, and 8 mL/min and the injection volume of a 10%(w/v) solution of racemic *N*-acetyl-1-naphthylethylamide (**3**) by 0.2, 0.4, 0.6, and that of a 8%(w/v) solution of racemic 1,1'-binaphthyl-2,2'-diamine (**4**) by 0.1, 0.2, 0.5, and 0.8 mL.

Results and Discussion

Preparation of a Preparative Chiral Stationary Phase (CSP 1). A commercialized CSP developed by our laboratory was prepared in bulk (about 80 g) with some modifications in order to obtain a preparative separation of racemic **3** and **4**. Each synthetic step is quite simple and easy, but, in the last step, connecting the silyl compound (**2**) to 80.0 g of 10 μm Kromasil silica gel had to be done using a mechanical stirrer instead of a magnetic stirrer because the silica gel was broken by a spin bar in the course of magnetic stirring. From the elemental analysis result of the bonded silica gel (Anal. found ($C_{21}H_{25}N_4$): C, 3.81; N, 0.92. Calcd.; 0.15 mmole/g (based on C), 0.14 mmole/g (based on N)), it was calculated that the average amount of chiral selector bonded to the silica gel was about 0.15 mmole per 1g of silica gel. The bonded chiral stationary phase was packed into four 250 mm length, 7.8 mm I.D stainless steel columns under 2000, 4000, 6000 and 8000 psi and the preparative chiral columns were tested by sample **3**.

As shown in Table 1, as the packing pressure of the column increased, the resolution and number of theoretical plate increased continuously but the operating pressure of the system also largely increased. In such a situation, the packing pressure did not exceed 8000 psi. From these results, a 250 mm length 10 mm I.D stainless steel column was packed under 8000 psi and the preparative chiral column was also tested by sample **3** and the results are shown in the right end column of Table 1. A comparison between the different sized columns packed under the same

Table 1. Resolution of racemic *N*-acetyl-1-naphthylethylamide (**3**) on five different preparative columns[a]

Column size	7.8 mm ID × 250 mm length				10 mm × 250 mm
Packing pressure	2000 psi	4000 psi	6000 psi	8000 psi	8000 psi
N^2/m	14600	16200	24300	26800	36800
operating pressure (psi)	906	912	927	1008	786
α	1.52	1.47	1.53	1.50	1.51
Rs	1.26	1.41	1.64	1.73	1.79

[a]Flow rate; 6 mL/min, injection volume; 0.2 mL, mobile phase; 20% IPA in hexane. [b]Number of theoretical plate (N/m)

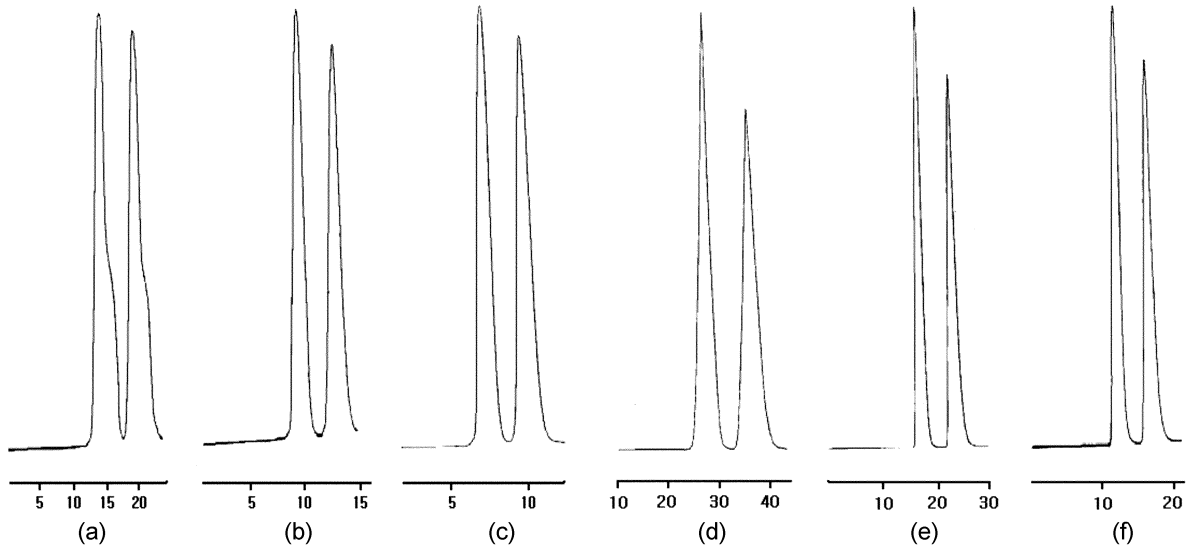

Figure 2. Chromatograms of the separation of racemic *N*-acetyl-1-naphthylethylamide (**3**) on preparative CSP 1 at different flow rates (F/R). Mobile phase; 20% 2-propanol in hexane. Sample injection volume (I/V); 0.2 mL (20 mg) (a) column size; 7.8 mm ID × 250 mm length, F/R 4 mL/min, (b) F/R 6 mL/min, (c) F/R 8 mL/min. (d) column size; 10 mm ID × 250 mm length, F/R 4 mL/min, (e) F/R 6 mL/min, (f) F/R 8 mL/min.

8000 psi showed that a small operating pressure, an improved resolution (*Rs*), and a high number of theoretical plates (*N*) were found in the larger column. Therefore, the 250 mm length, 10 mm I.D chiral preparative column packed under 8000 psi was mainly used in this study as CSP 1.

Determination of an Optimum Flow Rate of Mobile Phase in Preparative Chromatography. To determine the optimum flow rate for the preparative separation of racemic *N*-acetyl-1-naphthylethylamide (**3**) on the two different sized CSP 1, preparative chiral chromatographic experiments were performed by changing the flow rate of the mobile phase with the following rates, 4, 6 and 8 mL/min.

In Figure 2, the elution rate was about twice as fast in the small sized column (elution time; 2(a)-24 min, 2(d)-40 min and 2(c)-12 min, 2(f)-20 min), but the resolution was better in the larger column. A comparison of the inner volume between the two columns showed that the larger one was twice that of the smaller one. The larger columns empty volume was about 20 cm^3 ($\pi \times (0.5\ cm)^2 \times 25\ cm = 19.63\ cm^3$), on the other hand, the smaller column was about 12 cm^3 ($\pi \times (0.39\ cm)^2 \times 25\ cm = 11.94\ cm^3$). In addition, the packing capacity of the general packed column is 60%(ε_T: 0.4), [22] the empty volume of the larger packed column is about 7.85 cm^3 ($0.4 \times 19.63 = 7.85\ cm^3$), whereas that of the smaller one is about 4.78 cm^3 ($0.41 \times 1.94 = 4.78\ cm^3$). Therefore, the elution rate was small in larger one and it was in accordance with the chromatographic results.

Table 2. Resolution of racemic *N*-acetyl-1-naphthylethylamide (**3**) on preparative CSP 1 at different flow rate[a]

Flow-rate (mL/min)	7.8 mm ID				10 mm ID			
	k_1	α	Rs	operating pressure	k_1	α	Rs	operating pressure
4	10.03	1.48	1.18	858	17.0	1.49	1.47	696
6	7.19	1.49	1.73	1008	10.0	1.51	1.79	786
8	5.41	1.48	1.57	1122	7.85	1.50	1.71	882

[a]Sample injection volume; 0.2 mL (20 mg), mobile phase; 20% IPA in hexane.

The actual operating pressure and some chromatographic parameters calculated from the chromatograms in Figure 2 were arranged in Table 2. When the same amount (20 mg) of samples were introduced to the two columns, it was observed that as the flow rate of the mobile phase increased, the selectivity factor (a) was not influenced but the resolution (Rs) values changed. Also, the operating pressure of the LC system increased and the retention factor

Table 3. Relation between sample injection volume and the resolution (Rs) in the enantioseparation of racemic *N*-acetyl-1-naphthylethylamide (**3**) on preparative CSP 1[a]

Flow rate (mL/min)	Injection volume (Amount of sample **3**)											
	0.2 mL (20 mg)			0.4 mL (40 mg)			0.6 mL (60 mg)			0.8 mL (80 mg)		
	k_1	α	Rs	k_1	α	Rs	k_1	α	Rs	k_1	α	Rs
4	17.0	1.49	1.47	16.0	1.51	0.81	15.1	1.50	0.62	14.7	1.49	0.49
6	10.0	1.52	1.79	9.4	1.51	1.05	9.4	1.52	0.83	10.2	1.51	0.55
8	7.85	1.50	1.71	7.45	1.49	0.97	7.45	1.49	0.83	7.65	1.51	0.55

[a]Mobile phase; 20% IPA in hexane.

Table 4. Relation between sample injection volume and the resolution (Rs) in the enantioseparation of racemic 1,1-binaphthyl-2,2'-diamine (**4**) on preparative CSP 1[a]

Injection volume (Amount of sample **4**)	0.2 mL (16 mg)	0.1 mL (8.0 mg)	0.05 mL (4.0 mg)	0.02 mL (1.6 mg)	0.01 mL (0.8 mg)	5 μL (0.4 mg)	2.5 μL (0.2 mg)
Rs	0	0.41	0.46	0.53	0.62	0.63	0.61

[a]Mobile phase; 20% IPA in hexane, flow rate; 6 mL/min.

decreased continuously. The best resolution appeared when the flow rate of the mobile phase was 6 mL/min. When the mobile phase speed was 4 mL/min in the smaller sized column (Figure 2(a)), the resolution was at its worst and the shoulder peak appeared every time. The bad resolution at 4 mL/min may be a result from the band broadening effect caused by the relatively low speed, but reasons for the distorted peak are not known.

A comparison of the two different sized columns in the separation of racemic **3** showed the larger (10 mm ID) column with good resolution in a low operating pressure. It was thought that there were many chiral selectors which could interact with analytes in the larger column.

Determination of an Optimum Sample Injection Volume in Preparative Chromatography. To determine the optimum sample injection volume for the preparative separation of racemic *N*-acetyl-1-naphthylethylamide (**3**) on CSP 1, preparative chiral chromatographic experiments were performed by changing the sample injection volume using the following: 0.2 mL (20 mg), 0.4 mL (40 mg), 0.6 mL (60 mg) and 0.8 mL (80 mg) while retaining the same mobile phase speed, 6 mL/min. The results were summarized in Table 3.

The resolution (Rs) of the 40 mg injection sample at 6 mL/min was 1.05 and that of the 20 mg injection sample at the same flow rate was 1.79. In addition, the resolution (Rs) improved from 0.80 to 1.47 when the injection volume of the sample decreased from 0.4 mL(40 mg) to 0.2 mL(20 mg) at a mobile phase speed of 4 mL/min. The same phenomena was also found at 8 mL/min. Therefore, the sample injection volumes are limited in the preparative separation. In this case, the selectivity factor for the enantioseparation of racemic *N*-acetyl-1-naphthylethylamide (**3**) on CSP 1 was about 1.5. The sample **3** could be effectively separated if 20-40 mg samples were injected at once on a 10 mm ID, 250 mm length preparative column. On the other hand, the resolution values (Rs) were similar to each other between mobile phase speeds of 6 mL/min and 8 mL/min. It is thought that 8 mL/min is more useful than 6 mL/min from a practical point of view.

Resolution of Racemic 1,1'-Binaphthyl-2,2'-diamine (4) on Preparative CSP 1. The application of the optimum conditions to a large scale separation of racemic 1,1'-binaphthyl-2,2'-diamine (**4**) which is used as a starting material for an asymmetric catalyst[23] was performed. The selectivity factor (α) for sample **4** on 4.2 mm ID, 250 mm length analytical CSP 1 was about 1.15, therefore the preparative resolution would be more difficult than sample **3** (α = 1.50). The results for the resolution of racemic **4** on 10 mm ID, 250 mm length preparative CSP 1 were shown in Table 4.

As shown in Table 4, there was no resolution in the case of 16 mg sample injection. However, the best resolution was found when 0.8 mg of **4** was introduced and there was no improvement in the resolution even if it was smaller than the 0.8 mg of sample that was injected.

In conclusion, the preparative chiral chromatographic experiments were performed with two well known chiral samples, *N*-acetyl-1-naphthylethylamide (**3**) and 1,1-binaphthyl-2,2'-diamine (**4**) which have low selectivity factors (α = 1.50 and 1.15) by using CSP 1. When the size of the column was 10 mm ID, with a 250 mm length and the selectivity factor of a sample was about 1.5, the best flow rate of mobile phase was about 8 mL/min and optimum injection amount of sample was 20-40 mg. While the selectivity factor of a sample was about 1.15 on the same column, the optimum flow rate was about 6 mL/min and the optimum injection amount of the sample was 0.8 mg.

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