Department of Chemistry and Chemistry Institute for Functional Materials[2], Busan National University, Busan, Korea, Department of Analytical Chemistry[1], Shenyang Pharmaceutical University, Shenyang, China

Preparation and application of a novel Pirkle-type chiral stationary phase in liquid chromatography

DANDAN ZHANG[1], FAMEI LI[1], MYUNG HO HYUN[2]

Received June 19, 2006, accepted August 7, 2006

Dandan Zhang, Department of Analytical Chemistry, Shenyang Pharmaceutical University, Wenhua road 103, Shenyang, China
Dandanzhang625@hotmail.com

Pharmazie 62: 258–261 (2007) *doi: 10.1691/ph.2007.4.6113*

A new high-performance liquid chromatographic Pirkle-type chiral stationary phase (CSP) was prepared starting from *R*-(+)-1,1′-binaphthyl-2,2′-diamine. The CSP prepared was successfully applied in resolving various *N*-(3,5-dinitrobenzoyl)-α-amino amides and *N*-acyl-1-aryl-1-aminoalkanes and found to be effective. The chiral recognition mechanism was proposed to be π-π interaction and simultaneously hydrogen bonding interactions between the CSP and the analytes.

1. Introduction

During the last two decades various chiral stationary phases (CSPs) for the liquid chromatographic resolution of enantiomers have been developed and commercialized. For example, CSPs based on helical polymers (Nakano 2001), proteins (Sunanda and Narayanan 1992), cellulose derivatives (Oguni et al. 1995), cyclodextrins (Riering and Sieber 1996), chiral crown ethers (Shinbo et al. 1987; Hyun et al. 1998, 1999), macrocyclic antibiotics (Timothy et al. 2001) and other low molecular weight chiral molecules (Gasparrini et al. 2001; Hyun and Min 1997) have been developed and commercialized for the direct liquid chromatographic separation of enantiomers. Consequently, liquid chromatographic resolution of enantiomers on CSPs is now quite popular as a means of determining the enantiomeric purity of optically active compounds (Haginaka 2002; Roussel et al. 2004). Among others, Pirkle-type CSPs were one type of CSPs that are widely used. Pirkle-type CSPs have been successfully used for the enantioresolution of various π-acidic and π-basic compounds and are known to separate enantiomers through the enantioselective π-π donor-acceptor interaction and hydrogen bonding between CSPs and racemic analytes (Pirkle et al. 1980, 1985; Welch 1994).

In this paper, we describe the preparation of a new Pikle-type CSP, which was successfully applied for the separation of the enantiomers of various *N*-(3,5-dinitrobenzoyl)-α-amino amides and *N*-acyl-1-aryl-1-aminoalkanes.

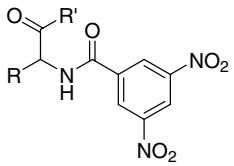
2. Investigations, results and discussion

In order to investigate the effect of the mobile phase on the chiral recognition, we examined two types of mobile phases for the resolution of analytes on CSP. One type of mobile phase was the mixture of isopropyl alcohol in hexane, which has been generally used for the enantioseparation on Pirkle-type CSPs. The use of 10% isopropyl alcohol in hexane as a mobile phase was not practical because the analysis time was too long. The use of 30% isopropyl alcohol in hexane significantly reduced analysis time and consequently was useful especially for the resolution of racemic analytes which show long retention on the column even though the enantioselectivities were slightly reduced. However, in general the use of 20% isopropyl alcohol in hexane was most practical in terms of analysis time and enantioselectivity. The second type of mobile phase tested for the resolution of the racemic analytes on the CSP was a mixture of methylene chloride in hexane. However, this mobile phase (e.g. 50% methylene chloride in hexane) was very poor in terms of peak shapes, analysis time and enantioselectivities. The chromatographic results for the enantioresolution are summarized in Table 1 and Table 2, the data were both obtained with 20% isopropanol hexane as a mobile phase.

The chromatographic results for the resolution of the enantiomers of *N*-(3,5-dinitrobenzoyl)-α-amino amides on the new CSP are listed in Table 1. As shown in Table 1, *N*-(3,5-dinitrobenzoyl) phenylglycine derivatives (such as **1c**, **d**, **e**) were better resolved on the CSP as denoted by the separation factors (α) than other *N*-(3,5-dinitrobenzoyl)-α-amino amides. As reported previously (Hyun 1995) we assume, at this stage, that the phenyl group at the chiral center of the analytes might act as a π-donor site for the donor-acceptor π-π interaction with the π-acidic *N*-(3,5-dinitrobenzoyl) group of the CSP in the chiral recognition. The representative chromatograms for the enantioresolution of some *N*-(3,5-dinitrobenzoyl) phenylglycine derivatives is illustrated in Fig. 1. The π-acidic *N*-(3,5-dinitrobenzoyl) group of analytes might also be considered to act as a π-acceptor site for the donor-acceptor π-π interaction with the binaphthyl group attached nitrogen atom in the CSP in the chiral recognition.

The chromatographic results for the resolution of the enantiomers of *N*-acyl-1-aryl-1-aminoalkanes on the new CSP are listed in Table 2. It is well known that the π-basi-

Table 1: Resolution of *N*-(3,5-dinitrobenzoyl)-α-amino amides on the new-synthesized CSP with a mobile phase of 20% isopropanol in hexane. Flow rate: 1.0 ml/min



Analytes	R′	R	k_1[a]	α[b]	Rs[c]
1a	$N(CH_3)_2$	CH_3	4.80	1.02	0.26
1b		$CH_2CH(CH_3)_2$	1.72	1.05	1.03
1c		C_6H_5	3.37	1.08	1.27
1d		$CH_2C_6H_5$	2.49	1.07	1.18
1e		$CH_2C_6H_4OH$	4.90	1.06	1.04
1f	$N(CH_2CH_3)_2$	CH_3	1.93	1.00	–
1g		$CH_2CH(CH_3)_2$	0.91	1.02	0.40
1h		C_6H_5	1.80	1.05	0.74
1i		$CH_2C_6H_5$	1.34	1.05	0.57
1j		$CH_2C_6H_4OH$	2.70	1.04	0.52
1k	$NHCH_2CH_2CH_3$	CH_3	1.19	1.00	–
1l		$CH_2CH(CH_3)_2$	0.58	1.00	–
1m		C_6H_5	1.20	1.18	2.10
1n		$CH_2C_6H_5$	0.97	1.03	–
1o		$CH_2C_6H_4OH$	2.14	1.04	0.38

[a] Retention factor of the first eluted enantiomer. [b] Separation factor. [c] Resolution factor

city of the aryl substituent, its conformational disposition, the size of the alkyl substituent and the length of the *N*-acyl "tail" all influenced the magnitude of α, the separation factor (Pirkle et al. 1984; Welch 1994). The analytes with phenyl as the aryl substituent were resolved worse as denoted by the separation factors (α) than the corresponding analytes with naphthyl and biphenyl as the aryl substituent. This indicates that the increase in the π-basicity of the aryl group of the analytes will increase the π-π interaction with the π-acidic *N*-(3,5-dinitrobenzoyl) group in the CSP which induced the enhancement of the chiral recognition.

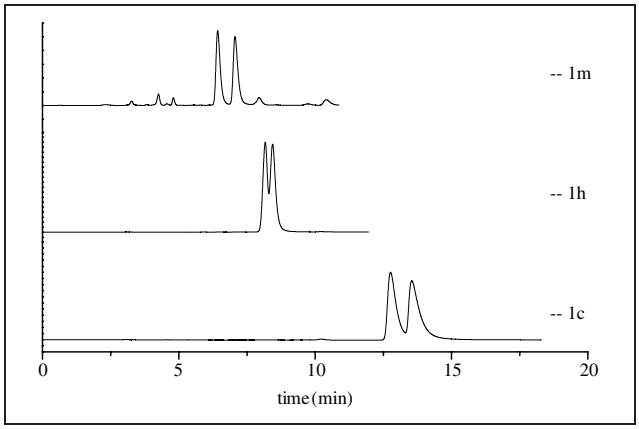
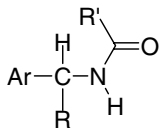


Fig. 1: Chromatograms for the resolution of *N*-(3,5-dinitrobenzoyl)-a-amino amides on the new-synthesized Pirkle-type CSP with a mobile phase of 20% isopropanol in hexane. Flow-rate: 1.0 ml/min. Detection: 254 nm

As shown in Table 2, with the increase of the length of the alkyl "tail" of the acyl group, the retention time of the analytes decreased significantly, while the enantioseparations were improved. The representative chromatograms for the enantio-resolution of *N*-acyl-1-aryl-1-aminoalkanes are shown in Fig. 2. The lenghth of the alkyl "tail" of the acyl group can influence the steric interaction between the analytes and the CSP, this indicates that the steric interaction might be one of the interactions responsible for chiral discrimination.

Table 2: Resolution of *N*-acyl-1-aryl-1-aminoalkanes on the new-synthesized CSP with a mobile phase of 20% isopropanol in hexane. Flow rate: 1.0 ml/min



Analytes	Ar	R	R′	k_1[a]	α[b]	Rs[c]
2a	naphthyl	CH_2CH_3	CH_3	5.15	1.00	–
2b			CH_2CH_3	3.76	1.08	0.82
2c			$(CH_2)_2CH_3$	2.90	1.10	1.18
2d			$(CH_2)_5CH_3$	1.96	1.12	1.22
2e			$(CH_2)_{10}CH_3$	1.51	1.14	1.82
2f	naphthyl	$CH(CH_3)_2$	CH_3	4.79	1.00	–
2g			CH_2CH_3	3.28	1.09	1.18
2h			$(CH_2)_2CH_3$	2.52	1.13	1.54
2i			$(CH_2)_5CH_3$	1.81	1.21	2.67
2j			$(CH_2)_{10}CH_3$	1.40	1.27	3.24
2k	biphenyl	CH_3	CH_3	4.99	1.02	–
2l			CH_2CH_3	3.26	1.06	0.91
2m			$(CH_2)_2CH_3$	2.43	1.07	1.10
2n			$(CH_2)_5CH_3$	1.70	1.10	1.43
2o			$(CH_2)_{10}CH_3$	1.32	1.11	1.56
2p	phenyl	CH_3	CH_3	2.74	1.00	–
2q			CH_2CH_3	2.04	1.00	–
2r			$(CH_2)_2CH_3$	1.92	1.00	–
2s			$(CH_2)_5CH_3$	1.09	1.04	0.18
2t			$(CH_2)_{10}CH_3$	0.84	1.05	0.29

[a] Retention factor of the first eluted enantiomer. [b] Separation factor. [c] Resolution factor

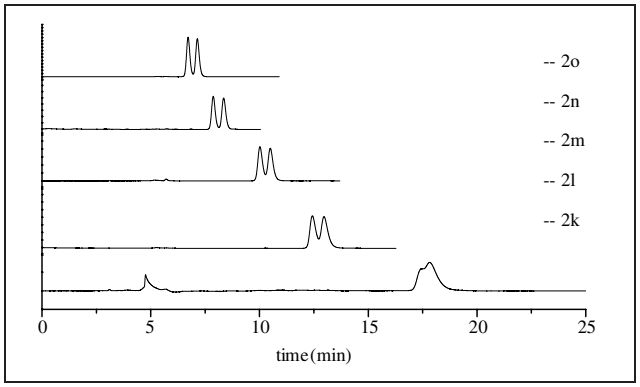


Fig. 2: Chromatograms for the resolution of *N*-acyl-1-aryl-1-aminoalkanes on the new-synthesized Pirkle-type CSP with a mobile phase of 20% isopropanol in hexane. Flow-rate: 1.0 ml/min. Detection: 254 nm

The carbonyl and amino groups in the structures of the two-type analytes may interact with the complimentary chiral sites in the CSP structure by hydrogen bonding, which is also one of the interactions responsible for chiral discrimination.

The chiral recognition mechanism between the chiral selector of the synthesized chiral stationary phase (CSP) and two type analytes was proposed. The major attractive interactions between the CSP and two type analytes are π-π interaction, hydrogen bonding and steric interaction.

3. Experimental

3.1. Materials

R-(+)-1,1′-Binaphthyl-2,2′-diamine, 3,5-dinitrobenzoyl chloride, triethylamine, 3-(triethoxysilyl) propyl isocyanate, dichloromethane were purchased from Sigma-Aldrich Co. (USA). The isopropanol, methylenechloride and hexane were of HPLC grade from Fisher Scientific Korea Ltd. (Korea). All solvents were filtered through a 0.45 μm filter and degassed before use. All the analytes for enantioresolution used in this study were synthesized in the Department of Chemistry and Chemistry Institute for Functional Materials (Busan National University, Busan, Korea).

3.2. Apparatus

Chromatography was performed on a liquid chromatograph equipped with a Younglin M720 detector and a Waters 515 solvent delivery system. The detection was set at 254 nm. All chromatographic experiments were carried out at a flow-rate of 1.0 ml/min at room temperature. Column void volume was measured by injecting 1,3,5-tri-*tert*-butylbenzene.

3.3. Sample preparation

The solutions of all analytes 1.0 mg/mL were prepared by dissolving the compound in ethanol. Each solution of 2 μL was injected onto the HPLC system.

3.4. Preparation of the new Pirkle-type CSP

The new CSP (**4**), was prepared starting from *R*-(+)-1,1′-binaphthyl-2,2′-diamine as shown in the Scheme. The detailed procedures are as follows.

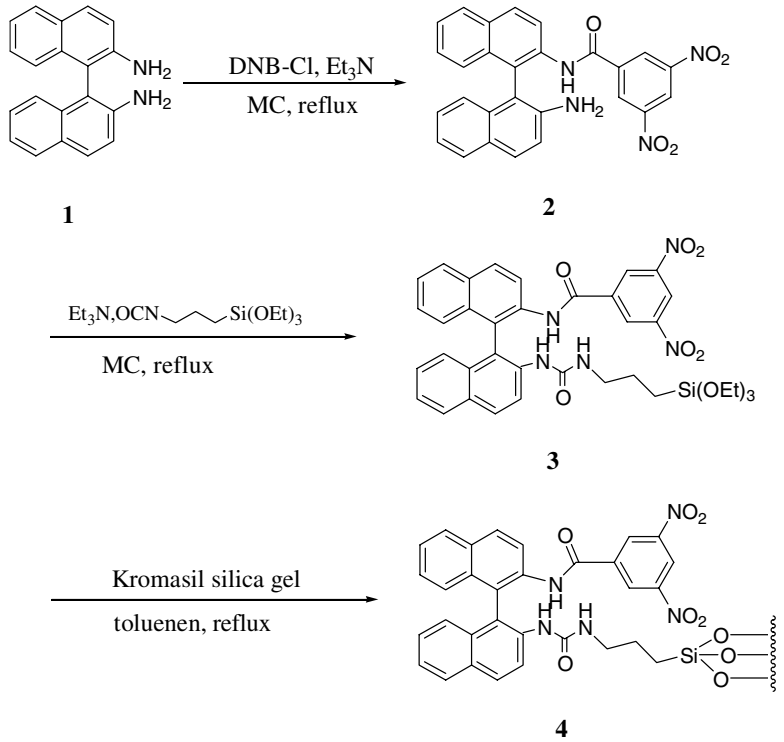
3.4.1. Preparation of N-[2′-amino-[1,1′]-binaphthyl-2-]-3,5-dinitrobenzoylamine (**2**)

R-(+)-1,1′-Binaphthyl-2,2′-diamine (1.21 g, 4.26 mmol) and triethylamine (0.59 ml, 4.26 mmol) were dissolved in 100 ml of methylene chloride in a 250 ml two-necked round bottom flask. To the stirred solution, a solution of 3,5-dinitrobenzoyl chloride (DNB-Cl) (0.98 g, 4.26 mmol) in 80 ml methylene chloride was dropped as slowly as possible under nitrogen at room temperature. The reaction mixture was stirred under reflux for 10 h and then washed with 1 mol/L HCl. The organic solution was dried over anhydrous $MgSO_4$, filtered and evaporated. The residue was purified by column chromatography on silica gel to afford **2** (1.79 g, yield 88%) as a red powder.

3.4.2. Preparation of N-[2′-(3-butyl-ureid)-[1,1′]-binaphthyl-2-]-3,5-dinitrobenzoylamine (**3**)

To a stirred solution of **2** (1.79 g, 3.74 mmol) in 100 ml methylene chloride 3-(triethoxysilyl)-propyl isocyanate (0.92 ml, 3.74 mmol) and triethylamine (0.52 ml, 3.74 mmol) were added. The whole reaction mixture was stirred for 10 h under reflux. Then the mixture was washed with 2 mol/L HCl, dried over anhydrous $MgSO_4$, filtered and evaporated. The residue was purified by column chromatography on silica gel to afford **3** (1.3 g, yield 48%) as a yellow powder.

Scheme



3.4.3. Preparation of CSP and column packing

A flask equipped with a Dean-Stark trap and a condenser was charged with Regis Rexchrom silica gel (4.5 g, particle size: 5 μm, surface area: 212 m^2/g) and toluene (100 ml). After heating the heterogeneous mixture under reflux until azeotropic removal of water was complete, compound **3** (1.3 g, 1.79 mmol) was added and then the whole mixture was heated to reflux for 72 h. The silica gel was filtered and washed extensively with toluene, ethyl acetate, methanol, acetone, diethylether and hexane successively. Then the modified silica gel was slurried in methanol and packed into a 4.6 mm × 250 mm stainless steel HPLC column using a conventional method with an Alltech HPLC Slurry Packer.

References

Gasparrini F, Misiti D, Villani C (2001) High-performance liquid chromatography chiral stationary phases based on low-molecular-mass selectors. J Chromatogr A 906: 35–50.

Haginaka J (2002) Pharmaceutical and biomedical applications of enantioseparations using liquid chromatographic techniques. J Pharm Biomed Anal 27: 357–372.

Hyun MH, Min CS, Cho YJ (1995) Examples of liquid chromatographic resolution of π-acidic racemates on a π-acidic chiral stationary phase. J High Resol Chromatogr 18: 63–65.

Hyun MH, Min CS (1997) Unusual high enantioselectivity by a new HPLC chiral stationary phase. Tetrahedron Lett 38: 1943–1946.

Hyun MH, Jin JS, Lee W (1998) Liquid chromatographic resolution of racemic amino acids and their derivatives on a new chiral stationary phase based on crown ether. J Chromatogr A 822: 155–161.

Hyun MH, Jin JS, Koo HJ, Lee W (1999) Liquid chromatographic resolution of racemic amines and amino alcohols on a chiral stationary phase derived from crown ether. J Chromatogr A 837: 75–82.

Nakano T (2001) Optically active synthetic polymers as chiral stationary phases in HPLC. J Chromatogr A 906: 205–225.

Narayanan SR (1992) Immobilized proteins as chromatographic supports for chiral resolution. Analysis 10: 251–262.

Oguni K, Oda H, Ichida A (1995) Development of chiral stationary phases consisting of polysaccharides derivatives. J Chromatogr A 694: 91–100.

Pirkle WH, House DW, Finn JM (1980) Broad spectrum resolution of optical isomers using chiral high-performance liquid chromatographic bonded phases. J Chromatogr A 192: 143–158.

Pirkle WH, Hyun MH, Bank B (1984) A rational approach to the design of highly-effective chiral stationary phases. J Chromatogr A 316: 585–604.

Pirkle WH, Pochapsky TC, Mahler GS, Field RE (1985) Chromatographic separation of the enantiomers of a-carboalkoxyindolines and *N*-aryl-α-amino esters on chiral stationary phases derived from *N*-(3,5-dinotrobenzoyl)-α-amino acids. J Chromatogr 348: 89–96.

Riering H, Sieber M (1996) Covalently bonded permethylated cyclodextrins, new selectors for enantiomeric separations by liquid chromatography. J Chromatogr A 728: 171–177.

Roussel C, Del Rio A, Pierot-Sanders I, Piras P, Vanthuyne N (2004) Chiral liquid chromatography contribution to the determination of the absolute configuration of enantiomers. J Chromatogr A 1037: 311–328.

Shinbo T, Yamaguchi T, Nishimura K, Sugiura M (1987) Chromatographic separation of racemic amino acids by use of crown ether-coated reversed-phase packings. J Chromatogr 405: 145–153.

Ward TJ, Farris III AB (2001) Chiral separation using the macrocyclic antibiotics: a review. J Chromatogr A 906: 73–89.

Welch CJ (1994) Evolution of chiral stationary phase design in the pirkle laboratories. J Chromatogr A 666: 3–26.