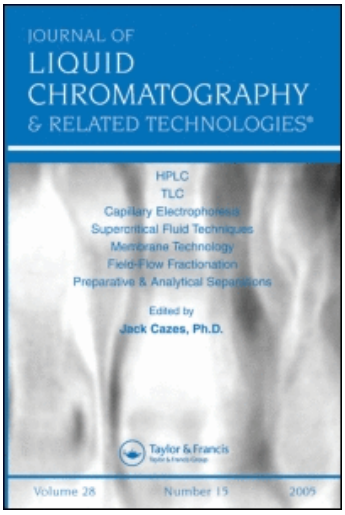

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Abstract: Chiral stationary phases, CSP 1 and CSP 2, with a reverse stereochemical configuration, were prepared by covalently bonding (+)- and (−)-(18-crown-6)-2,3,11,12-tetracarboxylic acid (18-C-6-TA) to silica gel, respectively. These CSPs were used to resolve the enantiomers of α-amino acids and primary amino compounds, affording reasonable and quite similar resolution behaviors except for the elution orders. The elution orders of the two enantiomers for the resolution of α-amino acids and other primary amino compounds were always opposite on the two CSPs. The reverse elution orders on the two CSPs with the antipode of the chiral selector were demonstrated to be very useful in the determination of the enantiomeric purity of optically enriched analytes.

Keywords: Chiral stationary phases, Enantiomer separation, 18-Crown-6-tetracarboxylic acid, Reversal of elution order

INTRODUCTION

Chiral stationary phases (CSPs) derived from chiral crown ethers have attracted considerable interest since Cram and his coworkers first introduced

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chiral crown ether type CSPs immobilized on a silica gel or on polystyrene in the 1970's.[1,2] Many studies using crown ethers as chiral selectors have been used to resolve racemic α-amino acids and primary amines by liquid-liquid extraction and high performance liquid chromatography.[3,4] Among the several chiral crown ethers developed by Cram, (3,3′-diphenyl-1,1′-binaphthyl)-20-crown-6 as a chiral selector was dynamically coated on an octadecylsilica gel to afford the Crownpak CR CSP.[5] The Crownpak CR column of the chiral crown ether type has proven to be useful for the chromatographic resolution of not only amino acids but also primary amino compounds.[5–8] However, since the Crownpak CR is prepared by a dynamic coating of a chiral crown ether on a reversed-phase packing, it has an intrinsic drawback in the use of mobile phase.[5,6] The use of a mobile phase containing more than 15% methanol deteriorates the CSP performance due to leaching of the chiral crown ether selector. In particular, hydrophobic analytes have significantly long retention times.[7,8] Moreover, the use of other organic solvents except methanol is not permitted because they can compromise the safety of the column, which cause the column to over-pressurize and lose capacity. Recently, CSPs derived from (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid (18-C-6-TA), which was first prepared by Behr and co-workers,[9] have been developed.[10–15] The covalently bonded type column, CSP 1, shown in Figure 1, has been successfully used to resolve the enantiomers of primary amines, as well as α-amino acids.[11–14] In particular, since CSP 1 is prepared by covalently bonding (+)-18-C-6-TA to an aminopropyl silica gel, this crown ether type chiral column has the advantage of the use of a variety of mobile phases along with the wide applicability of the hydrophobic analytes.[13,14] More recently, detailed NMR studies on the chiral recognition mechanism between (+)-18-C-6-TA as a chiral selector and α-amino acid in the solution state were reported.[16] However, in the determination of enantiomeric composition of optically active primary amino compounds containing very small amounts of second eluting enantiomers on CSP 1, some difficulties have sometimes been encountered because the small second HPLC peaks are sometimes embebded in the first big peaks. To solve these difficulties, the reversal of the elution orders of the two enantiomers is absolutely required.[17,18] In an effort to solve these difficuties, in this study, we report the preparation of a new CSP 2 by covalently

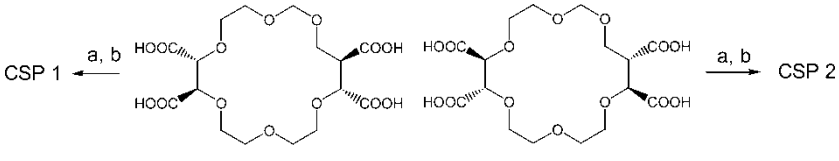

Figure 1. Covalently-bonded CSP 1 and CSP 2 derived from (+)- and (−)-18-C-6-TA, respectively; (a) acetyl chloride (b) aminopropyl silica gel, triethylamine.

bonding (−)-18-C-6-TA and the comparison of the enantiomer resolutions on CSP 1 and CSP 2.

EXPERIMENTAL

CSP 1 and CSP 2 were prepared by bonding (+)- and (−)-18-C-6-TA to aminopropyl silica gel, [Kromasil, EKA Chemicals, 100 Å, 5 μm; Micro analysis, found: C, 6.30%, N, 1.72%, calculated: 1.22 mmol/g (based on N)], respectively, as described previously.[11,12] The modified silica gels [Microanalysis, found: C, 8.70%; N 1.03% calculated: 0.28 mmol/g (based on C)] for CSP 1 and [Microanalysis, found: C, 9.02%; N 1.20% calculated: 0.29 mmol/g (based on C)] for CSP 2 were slurry packed into 250 × 4.6 mm stainless steel column, respectively. Chromatography was performed at room temperature using an HPLC Breeze system consisting of a Waters model 1525 binary pump, a Rheodyne model 7125 injector with a 20 μL loop, and a dual absorbance detector (Waters 2487 detector). The HPLC grade methanol was obtained from J.T. Baker. Water was purified using a milli-Q water purification system (Bedford, MA, USA). Sulfuric acid was obtained from the Fluka Company (Switzerland). All the analytes were obtained from either Aldrich or Sigma.

RESULTS AND DISCUSSION

For the purpose of comparison, CSP 1 and CSP 2 were prepared under identical conditions by covalently bonding (+)- and (−)-18-C-6-TA to an aminopropyl silica gel, respectively, as reported previously (Figure 1).[11,12] Each 18-C-6-TA was converted into its dianhydride by treating it with acetyl chloride. In order to prepare the modified silica gel, the dianhydride compound was treated in dry methylene chloride at 0°C under nitrogen with triethylamine and 3-aminopropyl silica gel. The CSP 1 and CSP 2 columns packed with the covalently bonded silica gels prepared from (+)- and (−)-18-C-6-TA, respectively, were used to resolve several α-amino acids and primary amino compounds.

Table 1 shows the chromatographic results of the enantiomeric resolution of α-amino acids on CSP 1 and CSP 2. All α-amino acids used in this study were base line resolved, with good separation factors ($\alpha = 1.27-2.74$) on CSP 1 and CSP 2. The separation factors for the α-amino acids on CSP 1 were similar to those on CSP 2. Probably, the longer retention times on CSP 2 might be due to the slightly higher loading of the chiral selector to the aminopropyl silica gel in CSP 2. The elution orders were determined by injecting configurationally known enantiomers. The elution orders of the α-amino acids shown in Table 1 were consistent, the (D)-enantiomers of all the analytes investigated in this study being selectively retained on CSP 1 derived from (+)-18-C-6-TA, while

Table 1. Enantiomer resolution of the α-amino acids on CSP 1 and CSP 2

		CSP 1				CSP 2			
Entry	Analyte	α	k'_1	Rs	Conf.[a]	α	k'_1	Rs	Conf.[a]
1	Alanine	1.34	0.77	1.58	D	1.32	1.15	1.51	L
2	Aspartic acid	1.31	1.02	1.11	D	1.31	1.49	1.10	L
3	Isoleucine	1.44	0.29	1.23	D	1.44	0.44	1.25	L
4	Leucine	1.62	0.42	1.78	D	1.65	0.63	1.80	L
5	Methionine	1.66	0.70	2.30	D	1.64	1.02	2.25	L
6	Norleucine	1.53	0.36	1.75	D	1.47	0.50	1.60	L
7	Norvaline	1.53	0.33	1.74	D	1.50	0.52	1.71	L
8	Phenylglycine	2.74	0.82	7.18	D	2.57	1.31	7.05	L
9	Phenylalanine	1.60	0.44	2.01	D	1.54	0.67	1.92	L
10	Serine	2.10	0.71	4.02	L	2.14	1.01	4.30	D
11	Threonine	1.27	0.19	1.01	L	1.30	0.25	1.08	D
12	Tryptophan	1.49	0.51	1.62	D	1.45	0.71	1.59	L
13	Tyrosine	1.55	0.45	2.80	D	1.52	0.65	2.65	L
14	Valine	1.56	0.16	1.49	D	1.51	0.26	1.41	L
15	Diphenylalanine	2.00	0.11	2.56	D	2.04	0.16	2.67	L
16	DOPA	1.62	0.55	2.74	D	1.55	0.75	2.40	L
17	5-Hydroxy tryptophan	1.49	0.61	1.46	D	1.55	0.63	1.56	L
18	Thyroxine	1.88	1.50	2.65	D	1.90	1.72	2.90	L
19	m-Tyrosine	1.61	0.55	2.07	D	1.56	0.80	2.01	L

Mobile phase = 80% methanol in water (V/V) containing 10 mM H_2SO_4; Flow rate = 1 mL/min; UV detection at 210 nm.

[a]The configuration of the second eluted isomer.

the (L)-enantiomers being selectively retained on CSP 2 derived from (−)-18-C-6-TA, with the exception of serine, threonine (entry 10 and 11 in Table 1), and serine and threonine methyl esters.

Since both threonine and serine analytes have a hydroxy group on the β-carbon, it is considered that the β-hydroxy moiety might directly influence the chiral recognition interaction with the crown ether as a chiral selector. Based on our previous NMR results and molecular dynamic calculations, it is considered that much stronger hydrogen bonding between the β-hydroxy moiety of threonine (or serine) and the COOH of 18-C-6-TA, instead of the hydrogen bonding between the carbonyl oxygen of threonine (or serine) and the COOH of 18-C-6-TA, might be formed.[16] This hydrogen bonding interaction between the β-hydroxy moiety of threonine (or serine) and the COOH of 18-C-6-TA, which is meant to be a favorable interaction in the spatial disposition upon forming the diastereomeric complex, could reverse the elution orders of these analytes. The separation factors (α = 1.30, 1.30) of the enantiomers of threonine and its methyl ester

were observed to be lower than those ($\alpha = 2.14, 3.10$) of serine and its methyl ester on CSP 2. It is possible that the interference of the methyl group on the β-carbon in the threonine structure with the chiral interaction between threonine and 18-C-6-TA might be responsible for these reduced enantioselectivities.

Table 2 shows the enantiomer resolution of some primary amino compounds on CSP 1 and CSP 2. All the primary amino compounds investigated in this study were well resolved, except for the kynurenine and α-methyltryptamine analytes (entry 3 and 5). Generally, CSP 2 for all the analytes shows a slightly higher enantioselectivity with increased retention times than CSP 1. Again, the elution orders of the examined analytes are exactly opposite on the two CSPs, and the typical examples of the reversal of elution order on CSP 1 and CSP 2 are demonstrated in Figure 2 and Figure 3. The chromatograms for the determination of the enantiomeric purity of enantiomerically enriched thyroxine (L:D = 90:1) and DOPA samples (L:D = 100:1) on CSP 1 and CSP 2 are shown in Figure 2 and Figure 3, respectively. The (D)-enantiomers are strongly retained on the CSP 1 derived from (+)-18-C-6-TA, while the (L)-enantiomers are strongly retained on the CSP 2 derived from (−)-18-C-6-TA. Especially, as shown in Figure 3, the second small peak corresponding to (D)-DOPA is slightly embedded in the first big peak on CSP 1 and, consequently, the exact determination of the enantiomeric purity is somewhat difficult.[17,18] In contrast, the two peaks on CSP 2 are well separated because of the reversal of the

Table 2. Enantiomer resolution of the primary amino compounds on CSP 1 and CSP 2

		CSP 1				CSP 2			
Entry	Analyte	α	k_1'	Rs	Conf.[a]	α	k_1'	Rs	Conf.[a]
1	Alanine-β-naphthylamide	1.48	1.72	3.49	R	1.55	3.09	3.70	S
2	Baclofen	1.25	3.08	1.98	S	1.26	5.92	2.00	R
3	Kynurenine	1.10	1.43[b]	0.47	–	1.12	2.33[b]	0.59	–
4	α-Methylbenzylamine	1.37	0.95	2.47	S	1.30	2.16	2.36	R
5	α-Methyltryptamine	1.10	3.76[b]	0.70	–	1.13	5.09[b]	0.66	–
6	Mexiletine	1.17	0.28	1.39	–	1.20	0.69	1.42	–
7	1-(1-Naphthyl) ethylamine	1.22	0.85	1.98	R	1.27	2.05	2.09	S
8	Norepinephrine	1.20	1.07	1.60	R	1.21	1.52	1.63	S
9	Octopamine	1.29	0.61	1.30	–	1.35	0.81	1.60	–

Mobile phase = 80% methanol in water (v/v) containing 10 mM H_2SO_4; Flow rate = 1 mL/min; UV detection at 210 nm;

[a]the configuration of the second eluted isomer;

[b]100% Methanol containing 10 mM H_2SO_4 was used as a mobile phase.

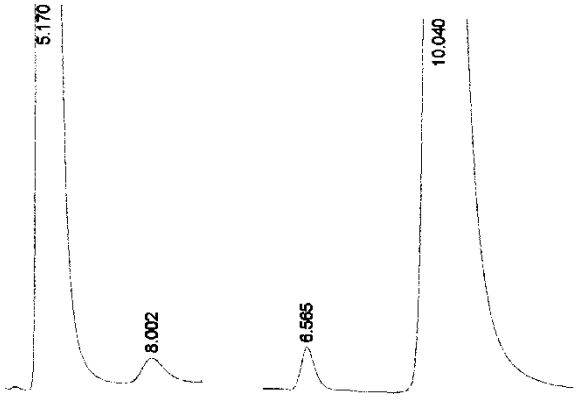

Figure 2. Chromatograms showing the resolution of an enantiomerically enriched Thyroxine (L:D = 90:1) on CSP 1 (the left) and CSP 2 (the right); Injection amount 1 μg. The chromatographic conditions are given in Table 1.

elution order and, consequently, it is much easier to determine the enantiomeric purity on CSP 2. These results demonstrate that the change in the elution order is often desirable when attempting to detect the minor enantiomers in the front of the major isomer of the analyte.[17]

In conclusion, CSP 1 and CSP 2 derived from (+)- and (−)-18-C-6-TA with a reverse configuration, respectively, were prepared and successfully used to resolve several primary amino compounds as well as α-amino acids. Since the chiral selectors of CSP 1 and CSP 2 are covalently bonded to the

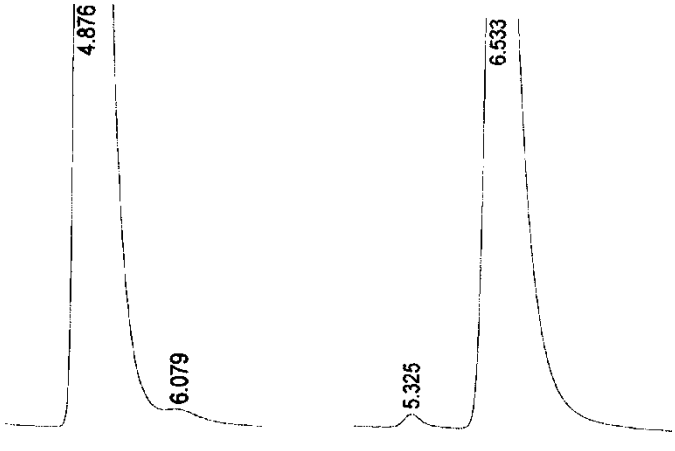

Figure 3. Chromatograms showing the resolution of an enantiomerically enriched DOPA (L:D = 100:1) on CSP 1 (the left) and CSP 2 (the right); Injection amount 1 μg. The chromatographic conditions are given in Table 1.

silica gel, various mobile phases can be used without any deterioration. Therefore, it is expected, that they will be quite useful for resolving the enantiomers of hydrophobic compounds and preparative separations.[14] In addition, it is expected that CSP 1 and CSP 2, with the antipode of the chiral selector, will be effective for determining the enantiomeric purity, due to the advantage of a reversal of the elution order.

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