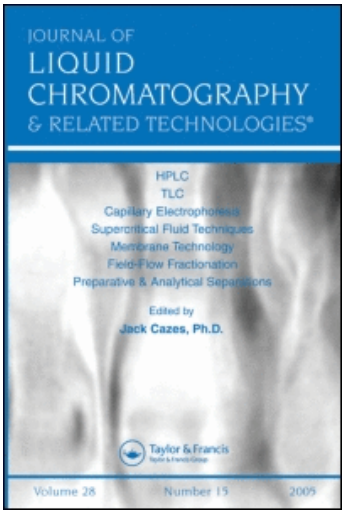

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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Online publication date: 25 May 2004

To cite this Article Hyun, Myung Ho , Tan, Guanghui and Cho, Yoon Jae(2004) 'Liquid Chromatographic Resolution of Aryl α-Amino Ketones on Chiral Stationary Phases Based on (+)-(18-Crown-6)-2,3,11,12-Tetracarboxylic Acid', Journal of Liquid Chromatography & Related Technologies, 27: 11, 1671 — 1680
To link to this Article: DOI: 10.1081/JLC-120037363
URL: http://dx.doi.org/10.1081/JLC-120037363

JOURNAL OF LIQUID CHROMATOGRAPHY & RELATED TECHNOLOGIES®
Vol. 27, No. 11, pp. 1671–1680, 2004

Liquid Chromatographic Resolution of Aryl α-Amino Ketones on Chiral Stationary Phases Based on (+)-(18-Crown-6)-2,3,11,12-Tetracarboxylic Acid

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ABSTRACT

Liquid chromatographic two chiral stationary phases (CSPs) based on (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid were successfully applied in the resolution of aryl α-amino ketones, including cathinone, the (*S*)-enantiomer of which is a psychoactive alkaloid found in the leaves of the khat plant. The chromatographic resolution behaviors were found to be dependent on the type, and the content, of organic and acidic modifiers in aqueous mobile phase. In addition, one of the two CSPs was demonstrated to be quite useful in the determination of the enantiomeric purity of cathinone.

Key Words: Chiral phase; Chiral separation; Crown ether; Aryl α-amino ketones; Cathinone.

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DOI: 10.1081/JLC-120037363 1082-6076 (Print); 1520-572X (Online)

INTRODUCTION

Chiral stationary phases (CSPs) based on chiral crown ethers have been known to be very useful in the resolution of racemic compounds containing a primary amino group.[1,2] For example, CSPs prepared by covalently bonding (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid **1** (Fig. 1) to aminopropyl silica gel were quite successful in the resolution of racemic compounds containing a primary amino group.[3,4] Especially, CSP **2** (Fig. 1) developed in our laboratory, was very effective in the resolution of racemic α- and β-amino acids,[5–7] α-amino acid derivatives,[5,8] racemic amines, racemic amino alcohols,[9] and fluoroquinolone antibacterial agents.[10,11]

Recently, as an effort to improve the chiral recognition efficiency of CSP **2**, we prepared a new CSP (CSP **3**, Fig. 1) by simply replacing the two N–H hydrogens of the connecting amide tethers of CSP **2** with a methyl group.[12] CSP **2** and CSP **3** were reported complementary with each other in the resolution of α- and β-amino acids[12,13] and amino alcohols,[12] while CSP **3** was found to be

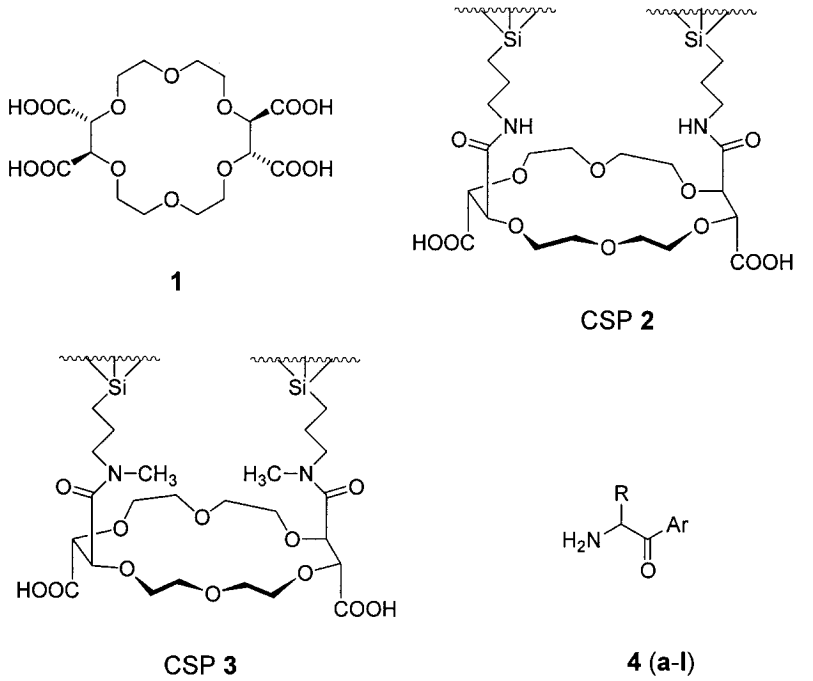

Figure 1. The structures of (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid **1**, CSP **2** and CSP **3** and aryl α-amino ketones **4**.

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always greater than CSP **2** in the resolution of racemic amines.[12] Now, we are in the process of extending the utility of CSP **2** and CSP **3** further, for the resolution of other important chiral compounds containing a primary amino group.

Aryl α-amino ketones, which have been used as precursors of biologically active medicines, such as ephedrine,[14] belong to an interesting family of drugs, such as bupropion, employed in the clinical treatment of nicotine dependence.[15] Especially, cathinone (**4a**, R = CH_3, Ar = phenyl), the (*S*)-enantiomer of α-aminopropiophenone, is a psychoactive alkaloid found in the leaves of the khat plant and, consequently, cathinone is forensically significant for both legal and intelligence purposes.[16,17] Previously, various efforts have been devoted to the synthesis of optically active cathinone.[18–20] Various efforts have also been devoted to the chromatographic resolution of racemic cathinone. For example, capillary electrophoresis with added cyclodextrins was applied in the resolution of racemic cathinone.[16] More recently, racemic cathinone has been resolved with HPLC on a CSP based on (3,3′-diphenyl-1,1′-binaphthyl)-20-crown-6, dynamically coated on octadecyl silica gel (Crownpak CR)[21] and on a CSP based on cellobiohydrolase (CBH-I).[22] However, CSP **2** and CSP **3** have not been applied in the resolution of aryl α-amino ketones. In this study, we wish to extend the use of CSP **2** and CSP **3** for the resolution of aryl α-amino ketones, including racemic cathinone.

EXPERIMENTAL

Chromatography was performed with an HPLC system consisting of a Waters Model 515 pump, a Rheodyne Model 7725i injector with a 20 μL sample loop, a Youngin M 720 Absorbance detector (variable wavelength), and a YoungLin Autochro Data Module (Software: YoungLin Autochro-WIN 2.0 plus). The chiral columns (150 × 4.6 mm^2 I.D. stainless steel column), packed with the CSP **2** and with CSP **3**, were available from previous studies.[5,12] Column temperature was controlled by using a Julabo F30 Ultratemp 2000 cooling circulator. Racemic and optically active aryl α-amino ketones, including cathinone (**4**, Fig. 1) used in this study, were prepared from corresponding racemic and optically active α-amino acids via the known procedure.[19,20]

RESULTS AND DISCUSSION

As a preliminary study, we resolved several selected aryl α-amino ketones on CSP **2** and CSP **3** with the variation of the type and the content of organic and acidic modifiers in aqueous mobile phase and, finally, we concluded that 80% ethanol in water containing 10 mM sulfuric acid, is

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most useful as a mobile phase. The liquid chromatographic results on CSP **2** and CSP **3**, for the resolution of 12 aryl α-amino ketones **4**, prepared from five different α-amino acids, such as alanine, valine, leucine, methionine, and phenylalanine with the use of 80% ethanol in water containing 10 mM sulfuric acid as a mobile phase, are summarized in Table 1. Complexation of primary ammonium ion (R-NH_3^+) inside the cavity of the chiral crown ether ring has been reported to be essential for the chiral recognition of racemic compounds containing a primary amino group by chiral crown ethers.[23] In this instance, sulfuric acid added to the mobile phase is believed to protonate the amino group of aryl α-amino ketones to produce ammonium ions (R-NH_3^+), and to improve their complexation inside the cavity of the crown ether ring of the CSP. The elution orders, shown in Table 1, were determined by injecting optically active aryl α-amino ketones, which were prepared from optically active α-amino acids, and then by comparing the chromatograms with those for racemic aryl α-amino ketones.

As shown in Table 1, the liquid chromatographic resolution of aryl α-amino ketones is quite excellent on both CSP **2** and CSP **3**. The retention factors (k_1) are always greater on CSP **3** than on CSP **2**. These retention

Table 1. Resolution of aryl α-amino ketones **4** on CSP **2** and CSP **3** with a mobile phase of 80% ethanol in water containing sulfuric acid (10 mM) as an acidic modifier.[a]

	Analyte 4		CSP 2			CSP 3		
	Ar	R	k_1'[b]	α[c]	R_S[d]	k_1'[b]	α[c]	R_S[d]
4a	C_6H_5	CH_3 (cathinone)	1.23 (*S*)	1.48	1.47	2.44 (*S*)	1.47	1.50
4b	C_6H_5	$CH(CH_3)_2$	0.11 (*S*)	2.12	2.13	0.41 (*S*)	2.82	4.92
4c	C_6H_5	$CH_2CH(CH_3)_2$	0.34 (*S*)	1.95	3.11	1.37 (*S*)	1.47	2.54
4d	C_6H_5	$CH_2CH_2SCH_3$	0.84 (*S*)	1.57	2.29	2.42 (*S*)	1.46	2.34
4e	C_6H_5	$CH_2C_6H_5$	1.03 (*S*)	1.55	3.55	2.85 (*S*)	1.54	4.11
4f	4-$CH_3C_6H_4$	CH_3	1.22 (*S*)	1.55	2.80	2.46 (*S*)	1.55	3.03
4g	4-$CH_3C_6H_4$	$CH(CH_3)_2$	0.16 (*S*)	2.08	1.89	0.59 (*S*)	2.00	2.28
4h	4-$CH_3C_6H_4$	$CH_2CH(CH_3)_2$	0.31 (*S*)	1.99	2.88	1.24 (*S*)	1.63	3.00
4i	4-$CH_3C_6H_4$	$CH_2CH_2SCH_3$	0.78 (*S*)	1.65	2.98	2.25 (*S*)	1.56	3.08
4j	4-$CH_3C_6H_4$	$CH_2C_6H_5$	0.86 (*S*)	1.58	3.09	2.26 (*S*)	1.69	3.17
4k	1-Naphthyl	$CH(CH_3)_2$	0.25 (*S*)	2.20	3.87	0.80 (*S*)	2.22	5.84
4l	2-Naphthyl	$CH(CH_3)_2$	0.26 (*S*)	2.19	3.77	0.90 (*S*)	2.55	7.17

[a]Flow rate, 0.5 ml/min; detection, 210 nm UV; temperature, 20°C.
[b]Retention factor of the first eluted enantiomer. The absolute configuration of the first eluted enantiomer was presented in the parenthesis.
[c]Separation factor.
[d]Resolution factor.

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behaviors are consistent with those for the resolution of α-amino acids, amines, and amino alcohols on CSP **2** and CSP **3**.[12] The resolution factors (R_S) are also generally greater on CSP **3** than on CSP **2**. In contrast, the enantioselectivities denoted by the separation factors (α) are not much different on the two CSPs.

When the aryl group of analytes **4** was changed from phenyl to 4-methylphenyl and to naphthyl, no significant change was observed in the retention (k_1) and the separation factors (α), as shown in Table 1. However, the resolution factors (R_S) are quite improved by increasing the size of the aryl group of analytes **4** from phenyl to 4-methylphenyl and to naphthyl (see the resolution of **4b**, **4g**, **4k**, and/or **4l**). As an alkyl group of analytes **4**, the isopropyl group shows the best resolution results in terms of separation factor (α).

As an effort to elucidate the chromatographic resolution behaviors with the variation of the type and the content of organic and acidic modifiers in aqueous mobile phase and the column temperature, we selected two analytes (**4a** and **4f**) and resolved them on CSP **3**. The chromatographic resolution results are summarized in Table 2.

As shown in Table 2, ethanol is most effective among three different organic modifiers, such as acetonitrile, methanol, and ethanol, in terms of both the separation (α) and the resolution factors (R_S) (see entry a in Table 2). When the content of ethanol in aqueous mobile phase is low, the resolution was not observed at all (see entry b in Table 2). However, when the content of ethanol increased, the separation (α) and the resolution factors (R_S) also increased. In addition, the retention factors (k_1) also increased as the content of ethanol in aqueous mobile phase increased. As the content of ethanol in aqueous mobile phased increases, the polarity of the mobile phase is expected to decrease and, consequently, the polar interaction between the mobile phase and analyte might decrease. In this instance, the retention factors (k_1) should increase as the content of ethanol in aqueous mobile phase increases.

As an acidic modifier, sulfuric acid is most effective in terms of the resolution factor (R_S), even though perchloric acid, acetic acid, and sulfuric acid are equally effective in terms of the separation factor (α) (see entry c in Table 2). As the content of sulfuric acid in aqueous mobile phase increases, the retention factors (k_1) decrease (see entry d in Table 2). As the content of acidic modifier in aqueous mobile phase increases, the ionic strength of the mobile phase is expected to increase. In this instance, the interaction between the mobile phase and the analyte molecule increases and, consequently, the analyte is expected to elute faster. The resolution factors (R_S) also increase as the content of acidic modifier in aqueous mobile phase increases, but the separation factors (α) do not show significant changes.

The effect of the column temperature on the chromatographic behaviors for the resolution of the two selected analytes on CSP **3** is shown in Table 2 (see

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Table 2. Resolution of aryl α-amino ketone **4a** and **4f** on CSP **3** with the variation of the type and the content of organic and acidic modifiers in aqueous mobile phase and the column temperature.[a]

		4a			**4f**		
Entry	Chromatographic condition	k_1'[b]	α[c]	R_S[d]	k_1'[b]	α[c]	R_S[d]
a	80% CH_3CN in H_2O + 10 mM H_2SO_4, 20°C	0.40	1.16	0.32	0.34	1.27	1.38
	80% CH_3OH in H_2O + 10 mM H_2SO_4, 20°C	2.28	1.39	1.47	2.42	1.43	2.82
	80% C_2H_5OH in H_2O + 10 mM H_2SO_4, 20°C	2.44	1.47	1.50	2.46	1.55	3.03
b	30% C_2H_5OH in H_2O + 10 mM H_2SO_4, 20°C	0.50	1.00		0.60	1.00	
	50% C_2H_5OH in H_2O + 10 mM H_2SO_4, 20°C	0.82	1.24	0.61	0.84	1.32	1.50
	80% C_2H_5OH in H_2O + 10 mM H_2SO_4, 20°C	2.44	1.47	1.50	2.46	1.55	3.03
c	80% C_2H_5OH in H_2O + 10 mM $HClO_4$, 20°C	1.46	1.48	1.20	1.58	1.54	2.57
	80% C_2H_5OH in H_2O + 10 mM CH_3CO_2H, 20°C	2.94	1.42	1.33	3.00	1.58	2.55
	80% C_2H_5OH in H_2O + 10 mM H_2SO_4, 20°C	2.44	1.47	1.50	2.46	1.55	3.03
d	80% C_2H_5OH in H_2O + 1 mM H_2SO_4, 20°C	2.73	1.51	1.35	2.82	1.58	2.57
	80% C_2H_5OH in H_2O + 5 mM H_2SO_4, 20°C	2.51	1.46	1.41	2.54	1.56	2.93
	80% C_2H_5OH in H_2O + 10 mM H_2SO_4, 20°C	2.44	1.47	1.50	2.46	1.55	3.03
e	80% C_2H_5OH in H_2O + 10 mM H_2SO_4, 5°C	5.07	1.51	1.53	5.17	1.58	3.02
	80% C_2H_5OH in H_2O + 10 mM H_2SO_4, 10°C	2.75	1.50	1.52	4.10	1.57	2.91
	80% C_2H_5OH in H_2O + 10 mM H_2SO_4, 20°C	2.44	1.47	1.50	2.46	1.55	3.03

[a]In every case, (*S*)-enantiomer was eluted first. Flow rate, 0.5 ml/min; detection, 210 nm UV.
[b]Retention factor of the first eluted enantiomer.
[c] Separation factor.
[d]Resolution factor.

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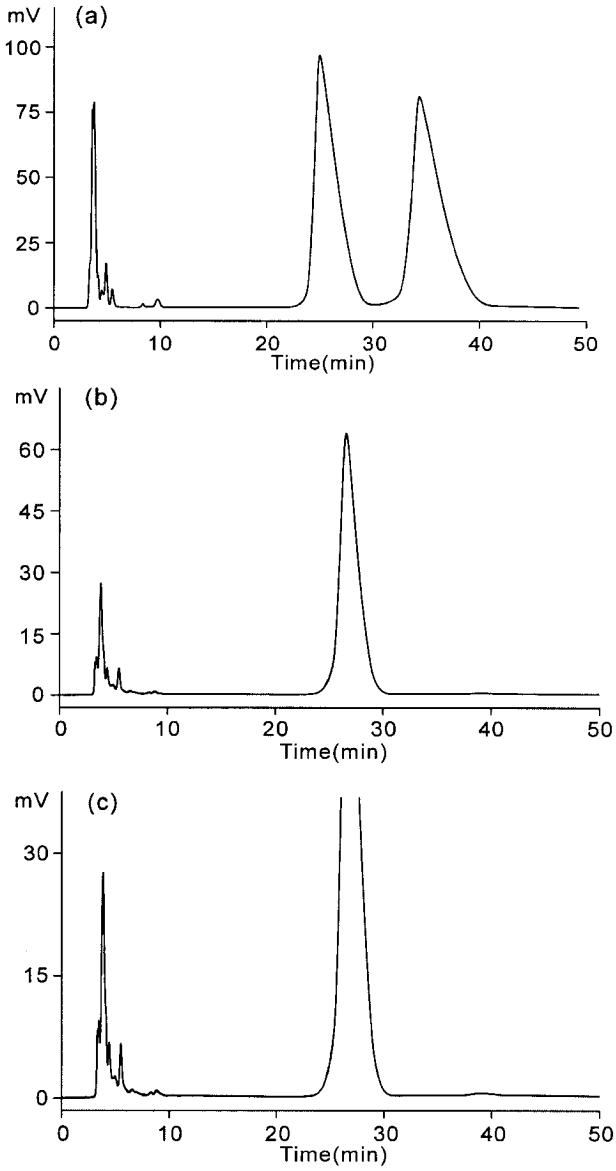

Figure 2. (a) Chromatogram for the resolution of racemic cathinone (**4a**) on CSP **3**. (b) Chromatogram for the resolution of (*S*)-cathinone prepared from (*S*)-alanine on CSP **3**. (c) The expanded chromatogram of (b). Chromatograms were obtained with the mobile phase of 80% ethanol in water containing sulfuric acid (10 mM). Flow rate, 0.5 ml/min; detection, 210 nm UV; temperature, 20°C.

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entry e in Table 2). As shown in Table 2, the retention factors (k_1) increase as the column temperature decreases. At lower temperature, the formation of the diastereomeric complexes of analytes inside the cavity of the crown ether ring of the CSP should be more effective and, consequently, the retention factors (k_1) increases. However, the separation (α) and the resolution factors (R_S) did not show any significant change with the variation of the column temperature.

Finally, the usefulness of CSP **2** or CSP **3** in the determination of enantiomeric purity of aryl α-amino ketones **4**, was demonstrated by comparing the chromatograms for the resolution of the racemic and optically active analytes on the CSPs. For example, the chromatogram for the resolution of racemic cathinone (**4a**) on CSP **3** is presented in Fig. 2(a). The chromatogram for the resolution of optically active (*S*)-cathinone prepared from (*S*)-alanine in this study is also presented in Fig. 2(b), and its expanded chromatogram is presented in Fig. 2(c). Based on the computer-generated peak areas corresponding to the two enantiomers shown in Fig. 2(b) or 2(c), the enantiomeric purity of the (*S*)-cathinone was calculated to be 99.0% ee ($R:S = 99.8:0.8$). The enantiomeric purity of the starting (*S*)-alanine was also 99.9% ee. Consequently, the enantiomeric purity of (*S*)-cathinone prepared from (*S*)-alanine is concluded to be maintained intact, during the synthetic procedure and under the chromatographic condition.

In summary, CSP **2** or CSP **3** based on (+)-18-(crown-6)-2,3,11,12-tetracarboxylic acid was excellent in the resolution of aryl α-amino ketones **4** including cathinone. The chromatographic behaviors for the resolution of aryl α-amino ketones **4** were dependent on the type and the content of organic and acidic modifiers in aqueous mobile phase. Especially, 80% ethanol in water containing 10 mM sulfuric acid was most useful as a mobile phase. CSP **2** or CSP **3** was also demonstrated to be useful in the determination of the enantiomeric purity of aryl α-amino ketones **4** including cathinone.

ACKNOWLEDGMENT

This work has been supported by KISTEP (NRL Program: M1-0318-00-0005).

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Received January 20, 2004
Accepted February 10, 2004
Manuscript 6316