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Resolution of Aryl α-Aminoalkyl Ketones on a Doubly Tethered Liquid Chromatographic Chiral Stationary Phase Based on (+)-(18-Crown-6)-2,3,11,12-tetracarboxylic Acid

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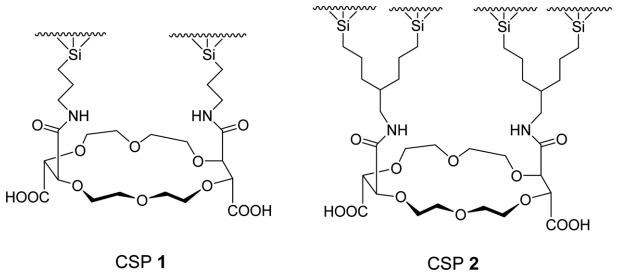
ABSTRACT. A doubly tethered chiral stationary phase (CSP) based on (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid was applied to the resolution of various aryl α-aminoalkyl ketones with the use of 80% ethanol in water containing 10 mM sulfuric acid as a mobile phase. The chiral resolution was quite successful, the separation factors (α) and the resolutions (R_S) being in the range of 1.39-2.05 and 3.18-5.22, respectively. The separation factors (α) on the doubly tethered CSP were slightly worse than those on the corresponding singly tethered CSP. However, the resolutions (R_S) on the doubly tethered CSP were generally greater than those on the corresponding singly tethered CSP. The chromatographic behaviors for the resolution of aryl α-aminoalkyl ketones on the doubly tethered CSP were demonstrated to be dependent on the type and the content of the organic and acidic modifiers in aqueous mobile phase and the column temperature.

Key words: Aryl α-aminoalkyl ketone, Chiral stationary phase, (+)-(18-Crown-6)-2,3,11,12-tetracarboxylic acid, Liquid chromatography, Resolution

INTRODUCTION

Liquid chromatographic chiral stationary phase (CSP **1**, *Fig.* 1) prepared by covalently bonding (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid to aminopropylsilica gel was very successful in the resolution of various racemic primary amino compounds including α- and β- and γ-amino acids,[1-3] racemic amines and amino alcohols,[4] fluoroquinolone antibacterial agents,[5] and tocainide (antiarrhythmic agent) and its analogues.[6] For the chiral recognition of primary amino compounds on CSP **1**, the enantioselective complexation of the primary ammonium ions ($R\text{-}NH_3^+$) of analytes inside the cavity of the crown ether ring of the stationary phase has been known to be essential and consequently, acidic modifier was usually added to aqueous mobile phase to protonate the primary amino group of analytes.[1-6] Under the acidic mobile phase condition, the stability of CSP **1** was not ensured. In order to improve the stability of CSP **1**, CSP **2** (*Fig.* 1) was prepared by adding a second tethering group to silica gel through a carbon atom of the first tethering group of CSP **1**.[7] CSP **2** was found to show higher stability than CSP **1**[7] and, additionally, CSP **2** was found to be generally greater than CSP **1** in the resolution of α-amino acids,[7] amines,[7] amino alcohols.[7] β-amino acids,[8] and tocainide (antiarrhythmic agent) and its analogues.[9]

Aryl α-aminoalkyl ketones (**3**, *Fig.* 2) belong to an interesting family of biologically active chiral compounds. For example, the (*S*)-enantiomer of α-aminopropiophenone (**3a**) named as cathinone is a psychoactive alkaloid found in the leaves of the khat plant and, consequently, it has attracted forensic attention.[10] Aryl α-aminoalkyl ketones (**3**) including racemic cathinone have been successfully resolved on CSPs based on (3,3'-diphenyl-1,1'-binaphthyl)-20-crown-6.[11-13] CSP **1** was also successful in the resolution of aryl α-aminoalkyl ketones (**3**) including racemic cathinone.[14] However, CSP **2** has not been applied to the resolution of aryl α-aminoalkyl ketones (**3**). In this report, we wish to demonstrate that CSP **2** is also effective for the resolution of aryl α-aminoalkyl ketones (**3**) especially in terms of the resolutions (R_S).



CSP **1** CSP **2**

***Fig.* 1.** Structures of CSP **1** and CSP **2**.

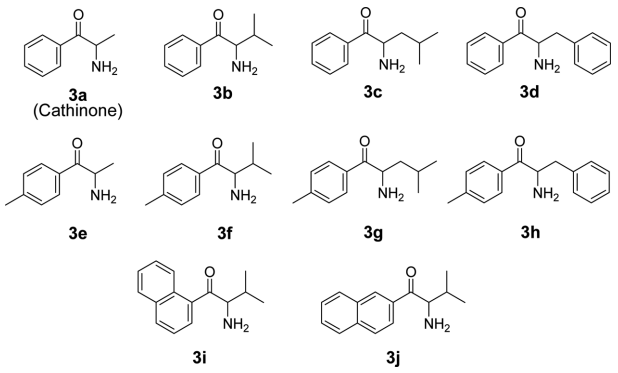


***Fig.* 2.** Structures of aryl α-aminoalkyl ketones **3** used in this study.

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). Column temperature was controlled by using a Julabo F30 Ultratemp 2000 cooling circulator. The chiral column (150 mm × 4.6 mm I.D. stainless steel column) packed with CSP **2** were available from the previous study.[7] Racemic and optically active cathinone and aryl α-aminoalkyl ketones (**3**) prepared from corresponding racemic and optically active α-amino acids were available from the previous study.[14]

RESULTS AND DISCUSSION

Aryl α-aminoalkyl ketones (**3**) were resolved on CSP **1** with the use of 80% ethanol in water containing 10 mM sulfuric acid as a mobile phase.[11] In order to compare the chiral recognition efficiency of CSP **2** with that of CSP **1**, aryl α-aminoalkyl ketones (**3**) were resolved on CSP **2** under the identical mobile phase condition. The chromatographic resolution results on CSP **2** are summarized and compared to those on CSP **1** in *Table* 1.

The retention times of the first eluted enantiomers denoted by the retention factors (k_1) on CSP **2** were always greater than those on CSP **1** as shown in *Table* 1. The improved lipophilicity of CSP **2** originated from the second tethering group is expected to increase the lipophilic interaction between the CSP and the analytes under reversed mobile phase condition. In this instance, the retention of the analytes on CSP **2** should be greater than that on CSP **1**. Another interesting result to note is the fact

***Table* 1.** Comparison of the resolution of aryl α-aminoalkyl ketones **3** on CSP **1** and CSP **2** with the use of 80% ethanol in water containing sulfuric acid (10 mM) as a mobile phase.[a]

Analyte	CSP **1**			CSP **2**		
	k_1'[b]	α[c]	R_S[d]	k_1'[b]	α[c]	R_S[d]
3a	1.23 (S)	1.48	1.47	8.11 (S)	1.38	3.08
3b	0.11 (S)	2.12	2.13	1.85 (S)	1.73	3.73
3c	0.34 (S)	1.95	3.11	3.70 (S)	1.55	4.00
3d	1.03 (S)	1.55	3.55	8.30 (S)	1.36	3.15
3e	1.22 (S)	1.55	2.80	8.08 (S)	1.40	3.64
3f	0.16 (S)	2.08	1.89	2.30 (S)	1.59	3.86
3g	0.31 (S)	1.99	2.88	3.75 (S)	1.55	4.57
3h	0.86 (S)	1.58	3.09	8.00 (S)	1.36	2.90
3i	0.25 (S)	2.20	3.87	2.48 (S)	2.13	4.42
3j	0.26 (S)	2.19	3.77	2.92 (S)	1.95	5.83

[a]The chromatographic data on CSP **1** were quoted from Reference [11]. 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.

that the retention factors on CSP **1** and CSP **2** are dependent quite much on the size of the alkyl group at the chiral center of analytes. When the alkyl group at the chiral center of analytes is methyl (**3a** and **3e**), the retention factors are largest on both CSP **1** and CSP **2**. However, when the alkyl group at the chiral center of analytes is changed from methyl to isopropyl (**3b** and **3f**), the retention factors are reduced significantly on both CSP **1** and CSP **2**. The enantioselective complexation of the primary ammonium ions ($R\text{-}NH_3^+$) of analytes inside the cavity of the crown ether ring of the stationary phase might be hindered by the sterically large isopropyl group at the chiral center of analytes and consequently, the retention factors are expected to be reduced. When the alkyl group at the chiral center of analytes is changed from isopropyl to isobutyl (**3c** and **3g**) and then to benzyl (**3d** and **3h**), the retention factors are increased on both CSP **1** and CSP **2**. Especially, the retention factors for analytes **3d** and **3h** are increased quite much. The isobutyl group is expected to be sterically less demanding than the isopropyl group because the sterically large isopropyl moiety of the isobutyl group is remote from the chiral center by one methylene unit. In addition, the isobutyl group is more lipophilic than the isopropyl group. Consequently, the retention factors for analytes **3c** and **3g** should be greater than those for analytes **3b** and **3f**. The benzyl group at the chiral center of analytes **3d** and **3h** is expected to be sterically much less demanding than the isobutyl group because of the flat nature of the phenyl moiety. In this instance, the retention

factors for analytes **3d** and **3h** should be quite long. In contrast, the effect of the size or lipophilicity of the aryl group of analytes **3** on the retention times is not so significant. As the lipophilicity of the aryl group of analytes is increased, the retention times were found to increase only slightly (see **3b**, **3f**, **3i** and **3j** in *Table* 1). The separation factors (α) on CSP **2** are slightly worse than those on CSP **1**. However, the resolutions (R_S) on CSP **2** are much greater than those on CSP **1** except for the analytes (**3d** and **3h**) containing benzyl substituent at the chiral center. Even though the reason is not clear, the improved lipophilicity of CSP **2** might be responsible for the slightly diminished separation factors and the significantly improved resolutions on CSP **2** compared to those on CSP **1**.

As an effort to elucidate the chiral recognition behaviors for the resolution of aryl α-aminoalkyl ketones (**3**) on CSP **2**, three selected analytes (**3a**, **3e** and **3f**) were resolved on CSP **2** with the variation of the type and the content of organic and acidic modifiers in aqueous mobile phase and with the variation of column temperature.

The chromatographic resolution results with the variation of the type and the content of organic modifiers in aqueous mobile phase containing 10 mM sulfuric acid are summarized in *Table* 2. Among three different organic modifiers including acetonitrile, methanol and ethanol, ethanol is most effective in terms of the separation factors and the resolutions for the resolution of **3a** and **3e**. However, the three organic modifiers are almost equally effective for the resolution of **3f**. When the content of ethanol in aqueous mobile phase was decreased, all of the three chromatographic parameters including the retention factors, separation factors and resolutions decreased significantly. *Fig.* 3 demonstrates the significant decrease in the retention factor, separation factor and resolution for the resolution of **3a** on CSP **2**. As the content of ethanol in aqueous mobile phase is decreased, the polarity of the mobile phase is expected to increase and, consequently, the polar

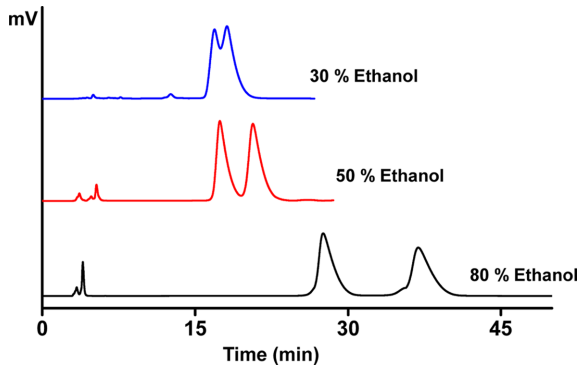


***Fig.* 3.** Chromatograms for the resolution of **3a** (cathinone) on CSP **2** with the variation of methanol content in water containing 10 mM sulfuric acid as a mobile phase. Flow rate: 0.5 ml/min. Detection: 254 nm UV. Column temperature: 20 °C.

interaction between the analyte and the mobile phase might increase. In this instance, the retention factors should decrease as the content of ethanol in aqueous mobile phase is decreased. However, the trends of the separation factors and the resolutions with the variation of the content of ethanol in aqueous mobile phase are not clear.

The chromatographic results for the resolution of selected analytes (**3a**, **3e** and **3f**) on CSP **2** with the variation of the type and the content of acidic modifier in 80% ethanol in water are summarized in *Table* 3. Even though each of perchloric acid, trifluoroacetic acid and sulfuric acid was useful as an acidic modifier, sulfuric acid was found to be generally better than the other two acids in terms of the separation factors and the resolutions. However, the reason is not clear yet. When the content of sulfuric acid was decreased from 10 mM to 5 mM and then to 1 mM, the retention factors increased significantly while the separation factors and resolutions decreased only slightly. The representative chromatograms for the resolution of **3f** on CSP **2** with the variation of the content of sulfuric acid in 80% ethanol are presented in *Fig.* 4. As the content of sulfuric acid in aqueous mobile phase is decreased, the ionic

***Table* 2.** Resolution of selected aryl α-aminoalkyl ketones (**3a**, **3e** and **3f**) on CSP **2** with the variation of the type and the content of organic modifier in aqueous mobile phase containing 10 mM sulfuric acid.[a]

Organic modifier in water	**3a**			**3e**			**3f**		
	k_1	α	R_S	k_1	α	R_S	k_1	α	R_S
80% CH_3CN	2.76	1.16	1.73	2.69	1.22	3.13	0.69	1.55	3.88
80% CH_3OH	5.28	1.26	2.36	5.25	1.29	2.90	1.06	1.62	3.71
80% CH_3CH_2OH	8.11	1.38	3.08	8.08	1.40	3.64	2.30	1.59	3.86
50% CH_3CH_2OH	4.76	1.23	1.54	5.27	1.23	1.85	1.99	1.29	1.78
30% CH_3CH_2OH	4.59	1.09	0.44	4.23	1.09	0.52	1.59	1.13	0.81

[a]Flow rate: 0.5 ml/min. Detection: 254 nm UV. Column temperature: 20 °C. k_1: Retention factor of the first eluted enantiomer. α: Separation factor. R_S: Resolution.

***Table* 3.** Resolution of selected aryl α-aminoalkyl ketones (**3a**, **3e** and **3f**) on CSP **2** with the variation of the type and the content of acidic modifier in 80% ethanol in water as a mobile phase.[a]

Mobile phase	3a			3e			3f		
	k_1	α	R_S	k_1	α	R_S	k_1	α	R_S
10 mM $HClO_4$	9.01	1.32	2.30	8.42	1.35	2.79	2.26	1.56	3.64
10 mM CF_3COOH	7.43	1.37	2.50	7.09	1.39	2.71	2.08	1.53	3.03
10 mM H_2SO_4	8.11	1.38	3.08	8.08	1.40	3.64	2.30	1.59	3.86
5 mM H_2SO_4	11.45	1.37	2.63	11.02	1.39	3.11	3.20	1.55	3.38
1 mM H_2SO_4	26.99	1.35	2.12	25.88	1.38	2.57	8.25	1.41	2.64

[a]Flow rate: 0.5 ml/min. Detection: 254 nm UV. Column temperature: 20 °C. k_1: Retention factor of the first eluted enantiomer. α: Separation factor. R_S: Resolution.

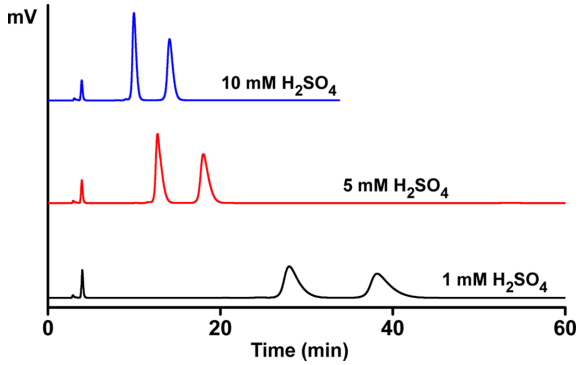


***Fig.* 4.** Chromatograms for the resolution of **3f** on CSP **2** with the variation of sulfuric acid content in 80% ethanol in water as a mobile phase. Flow rate: 0.5 ml/min. Detection: 254 nm UV. Column temperature: 20 °C.

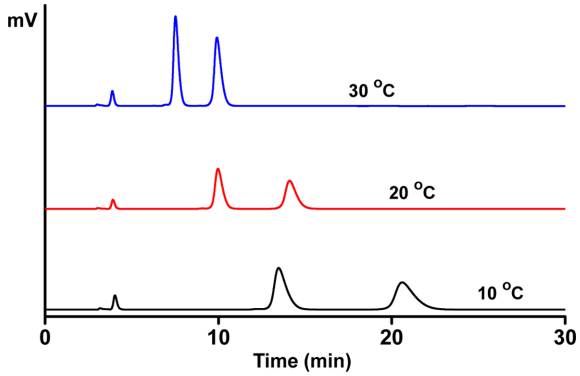


***Fig.* 5.** Chromatograms for the resolution of **3f** on CSP **2** with the variation of the column temperature. Mobile phase: 80% ethanol in water containing 10 mM sulfuric acid. Flow rate: 0.5 ml/min. Detection: 254 nm UV.

strength of the mobile phase is expected to decrease. In this instance, the polar interaction between the mobile phase and the analyte decreases and, consequently, the retention of the analyte should increase.

The effect of the column temperature on the resolution of three selected analytes (**3a**, **3e** and **3f**) on CSP **2** is summarized in *Table* 4. As the column temperature is increased, the retention factors decrease quite much. The decreasing trends of the retention factor for the resolution of **3f** on CSP **2** are well demonstrated in *Fig.* 5. As the column temperature is increased, the formation of the diastereomeric complex of the analyte inside the cavity of the crown ether ring of the stationary phase will become less effective and, consequently, the retention factors should decrease. In contrast, the separation factors and the resolutions did not change significantly with the variation of the column temperature.

In summary, CSP **2** prepared by introducing the second tethering group into the corresponding singly tethered CSP (CSP **1**) was applied to the resolution of aryl α-aminoalkyl ketones. CSP **2** was found to be generally greater than CSP **1** in terms of the resolutions (R_S), but the former was slightly worse than the latter in terms of the separation factors (α). In addition, CSP **2** was found to show greater retention factors (k_1) than CSP **1**. The improved lipophilicity of CSP **2** was proposed to be responsible for

***Table* 4.** Resolution of selected aryl α-aminoalkyl ketones (**3a**, **3e** and **3f**) on CSP **2** with the variation of the column temperature in 80% ethanol in water containing 10 mM sulfuric acid as a mobile phase.[a]

Column temperature	3a			3e			3f		
	k_1	α	R_S	k_1	α	R_S	k_1	α	R_S
10 °C	13.81	1.40	2.72	12.71	1.44	3.11	3.45	1.68	3.88
20 °C	8.11	1.38	3.08	8.08	1.40	3.64	2.30	1.59	3.86
30 °C	1.64	1.35	3.13	4.85	1.38	3.30	1.49	1.53	3.20

[a]Flow rate: 0.5 ml/min. Detection: 254 nm UV. k_1: Retention factor of the first eluted enantiomer. α: Separation factor. R_S: Resolution.

the greater retention factors. The chromatographic behaviors for the resolution of aryl α-aminoalkyl ketones on CSP **2** were demonstrated to be dependent on the type and the content of organic and acidic modifiers in aqueous mobile phase and the column temperature.

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