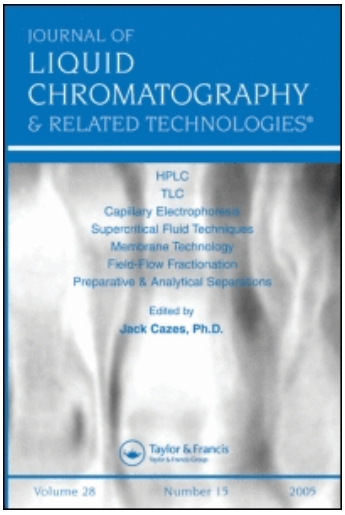

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Liquid Chromatographic Chiral Separations by Crown Ether-Based Chiral Stationary Phases

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Abstract: Liquid chromatographic chiral stationary phases (CSPs) based on chiral crown ethers have been successfully utilized in the separation of the enantiomers of various racemic compounds containing a primary amino group. Especially, CSPs based on chiral crown ethers incorporating chiral binaphthyl unit or tartaric acid unit have been most successful. In this review, the development of CSPs based on chiral crown ethers incorporating a chiral binaphthyl unit or a tartaric acid unit, their applications to the resolution of various primary and non-primary amino compounds with the variation of the type and the content of organic, acidic, and inorganic modifiers in an aqueous mobile phase or with a non-aqueous mobile phase and the efforts to improve the chiral recognition efficiency or the stability of the CSPs have been discussed.

Keywords: Chiral separation, Chiral stationary phase, Chiral crown ether, Liquid chromatography

INTRODUCTION

Liquid chromatographic chiral separations by chiral stationary phases (CSPs) have been known as the most accurate, convenient, and economic means of assaying the enantiomeric composition of chiral compounds and separating the two enantiomers of chiral compounds for both analytical and preparative purposes. Consequently, various efforts have been devoted to the development

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of effective CSPs and, as a result, various CSPs have been developed.[1,2] Among others, crown ether-based CSPs have been most successfully utilized for the separation of the two enantiomers of racemic compounds containing a primary amino group without derivatization.[3–5]

Since crown ethers were first introduced by Pederson in 1967,[6] various chiral crown ethers have been developed by incorporating appropriate chiral units, such as chiral binaphthyl units,[7,8] chiral biphenanthryl units,[9] chiral helicene derivatives,[10] tartaric acid,[11] or carbohydrates[12] as chiral barrier(s) into crown ethers. Phenolic pseudo chiral crown ethers have also been developed.[13] However, chiral crown ethers which have been utilized as successful chiral selectors of CSPs are limited to those incorporating a chiral binaphthyl unit or a tartaric acid unit and phenolic pseudo chiral crown ethers.

In this review paper, the discussion will be focused on the development and application of two different-types most successful crown ether-based CSPs. The first type is the CSPs based on chiral crown ethers incorporating a chiral 1,1′-binaphthyl unit. The second type is the CSPs based on (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid, a chiral crown ether incorporating two tartaric acid units.

CSPs BASED ON CHIRAL CROWN ETHERS INCORPORATING A CHIRAL 1,1′-BINAPHTHYL UNIT

Developments and Structural Characteristics

Chiral crown ethers incorporating optically active 1,1′-binaphthyl units were first prepared by Cram and coworkers.[7,8] Especially, optically active bis(1,1′-binaphthyl)-22-crown-6 compounds immobilized on silica gel[14] or polystyrene[15] have been utilized as CSPs for the resolution of racemic amines, α-amino acids, and amino acid esters in the late 1970s. However, the chiral recognition efficiency of these CSPs was not sufficient for general use. In contrast, optically active (3,3′-diphentl-1,1′-binaphthyl)-22-crown-6, which was also first synthesized by Cram,[16] coated dynamically onto octadecylsilica gel, has been very successful as a CSP (CSP **1**, Figure 1) for the resolution of various racemic primary amino compounds.[17]

CSP **1** was actually prepared by Shinbo and coworkers in 1987 by dissolving optically active (3,3′-diphenyl-1,1′-binaphthyl)-22-crown-6 in a mixed solvent of methanol-water and then eluting the solution through an octadecylsilica gel column (LiChrosorb RP-18, 125 × 4 mm I.D.).[17] Because of the high chiral recognition efficiency, CSP **1** has been commercialized as a brand name, Crownpak CR (Daicel Chemical Industries, Tokyo, Japan). However, the use of a mobile phase with CSP **1** is limited to a mobile phase containing less than 15% methanol in water because of the dynamically coated nature of the CSP. When a mobile phase

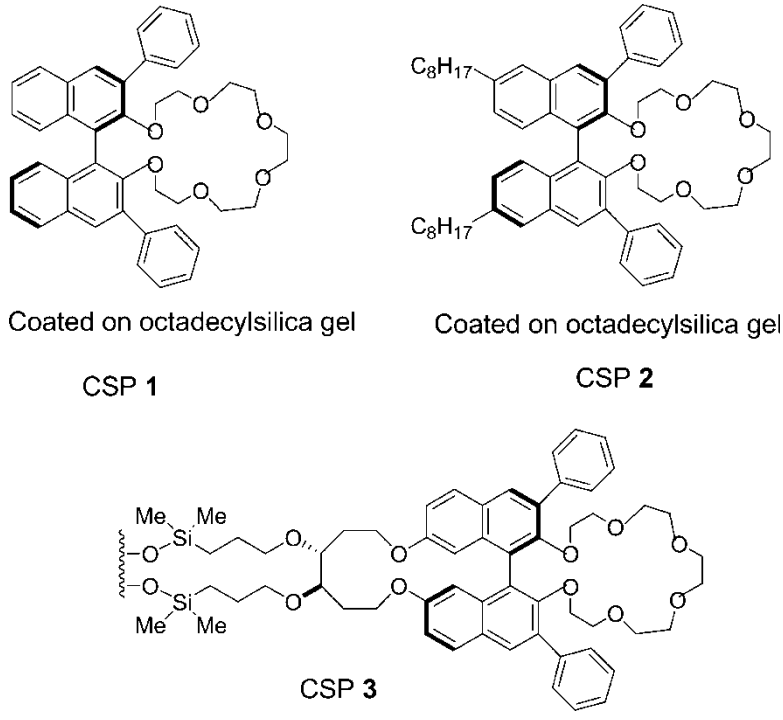


Figure 1. Structures of CSPs **1**, **2**, and **3**.

containing more than 15% methanol in water is used, CSP **1** is no longer useful because the chiral crown ether coated on octadecylsilica gel leaches from the column.

As a more durable CSP, Shinbo and coworkers developed CSP **2** (Figure 1) by coating (6,6'-dioctyl-3,3′-diphenyl-1,1′-binaphthyl)-20-crown-6 dynamically on octadecylsilica gel.[18] Two octyl groups attached to the binaphthyl ring of the chiral crown ether selector of the CSP are expected to improve the lipophilic interaction between the octadecyl chains of the stationary phase and the chiral crown ether. Actually the stability of CSP **2** was improved, but the use of a mobile phase containing more than 40% methanol in water was not recommended with CSP **2**.[18]

The intrinsic drawback of CSP **1** and CSP **2,** which stemmed from the dynamically coated nature of the chiral crown ether selector, can be overcome by developing a covalently bonded CSP. Indeed, a new crown ether-based CSP (CSP **3**, Figure 1) was prepared by covalently bonding (3,3′-diphenyl-1,1′-binaphthyl)-20-crown-6 to silica gel.[19] As expected, CSP **3** was found to be compatible with the use of a mobile phase containing any percentage of methanol in water and with the use of even 100% methanol as a mobile phase.[19]

Applications

Analyte Characteristics-Resolution of Primary Amino Compounds

Crown ether-based CSPs have been utilized mostly for the resolution of racemic primary amino compounds. CSP **1** was originally utilized for the resolution of various α-amino acids and 1-phenylethylamine.[17] After the commercialization, many users used the chiral column packed with CSP **1** for the resolution of biologically important primary amino compounds including, for example, chiral primary amines,[20,21] chiral cyclic amines,[22] amino alcohols,[21,23] and β-amino acids.[24] Some chiral drugs shown in Figure 2, such as aminoglutethimide, baclofen (muscle relaxant), primaquine (antimalarial),[23] and a new fluoroquinolone antibacterial agent named gemifloxacin mesylate[25] were also resolved with CSP **1**. Resolution of racemic dipeptides on CSP **1** demonstrated that the distance between the primary amino group and the chiral center is important for good enantioselectivity.[26]

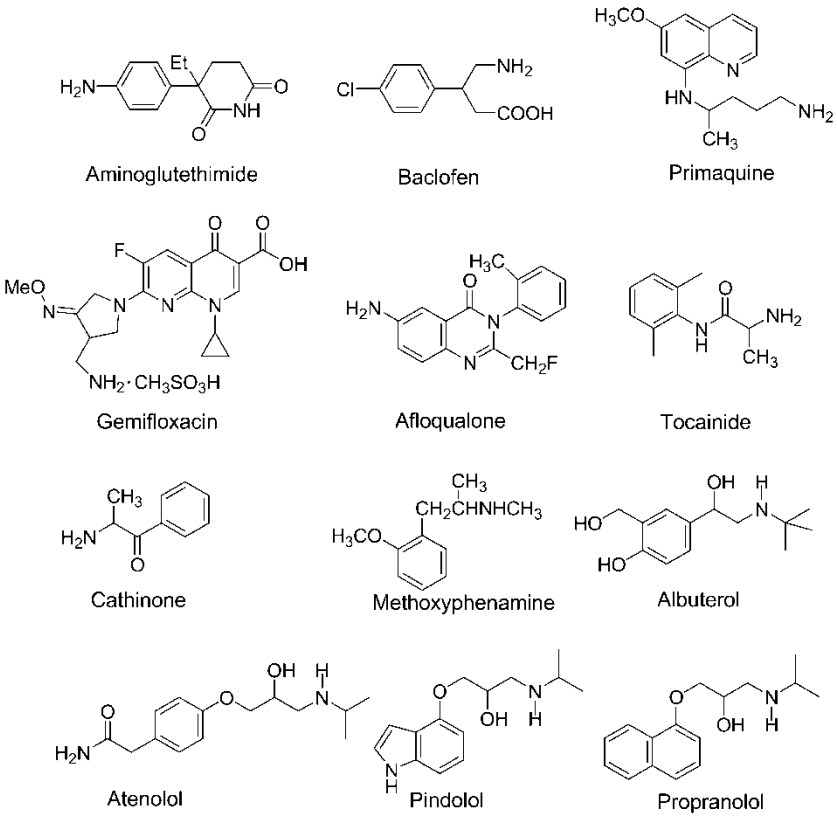


Figure 2. Chiral drugs resolved on crown ether-based CSPs.

Good enantioselectivity was closely related to the close proximity of the primary amino group to the chiral center. In this instance, the resolution of primaquine on CSP **1** is quite interesting in that its primary amino group is quite remote from the chiral center. In addition, the resolution of aminoglutethimide is quite interesting in that the primary amino group is located on the aromatic ring.

Even though CSP **2** is more compatible with the use of mobile phase than CSP **1**, CSP **2** was not commercialized. Consequently, the reports for the use of CSP **2** are rare and only the resolutions of α-amino acids, 1-phenylethylamine, and 3-aminocaprolactam were reported.[18]

CSP **3** was also quite successful in the resolution of various racemic primary amino compounds. α-Amino acids,[19] amino alcohols,[27] racemic non-cyclic and cyclic primary amines,[27] various fluoroquinolone antibacterials,[28] tocainide (antiarrhythmic agent) and its analogues,[29] and aryl α-amino ketones including cathinone[30] were resolved quite excellently on CSP **3**.

Organic Modifier in Mobile Phase

For the resolution of racemic primary amino compounds on CSP **1**, CSP **2**, and CSP **3**, water or water-organic solvent (organic modifier) mixture containing a small amount of acidic modifier has been used as a mobile phase. As an organic modifier, a water miscible solvent such as methanol, ethanol, or acetonitrile has been used.[20–30] However, in general, methanol or acetonitrile has been most widely utilized as an organic modifier. The organic modifier concentration in the aqueous mobile phase was a very important factor influencing the chromatographic behaviors for the resolution of primary amino compounds on CSP **1**, CSP **2**, and CSP **3**. When the organic modifier concentration in the aqueous mobile phase was increased, the retention factors (k') for the resolution of primary amino compounds on CSP **1**, CSP **2**, and CSP **3** usually decreased.[18,19,23,27–30] The separation factors (α) usually increased as the organic modifier concentration in aqueous mobile phase was increased.[18,19,27–29] However, for the resolution of aryl α-amino ketones on CSP **3**, the resolution factors (α) decreased as the organic modifier concentration in the aqueous mobile phase was increased.[30] As an example, the chromatographic parameters for the resolution of phenylglycine and methionine on CSP **3** are shown in Table 1.[19] As shown in Table 1, the retention factors (k'_1) decrease while the separation factors (α) increase as the organic modifier concentration in water is increased.

Even though the reason for the trends of the separation factors (α) for the resolution of racemic primary amino compounds on CSP **1**, CSP **2**, and CSP **3** with the variation of the organic modifier concentration in aqueous mobile phase was not clear, the trends of the retention factors (k') was proposed to stem from the balance between the lipophilic interaction of analytes with a CSP and the hydrophilic interaction of analytes with a mobile phase.[30]

Table 1. The chromatographic trends for the resolution of phenylglycine and methionine on CSP **3** with the variation of the organic modifiers and their content in water at the constant concentration of ammonium acetate (1.0×10^{-3} M) at 20°C

	Phenylglycine			Methionine		
Mobile phase	k_1	α	R_S	k_1	α	R_S
100% $H_2O + H_2SO_4$ (10 mM)	1.15	3.94	4.33	0.95	2.22	2.12
20% MeOH + H_2SO_4 (10 mM)	1.06	5.44	5.60	0.93	3.70	3.60
50% MeOH + H_2SO_4 (10 mM)	0.65	5.86	7.55	0.68	4.76	5.50
80% MeOH + H_2SO_4 (10 mM)	0.37	7.70	10.00	0.55	6.42	10.50
20% Ethanol + H_2SO_4 (10 mM)	0.87	5.01	6.10	0.72	3.63	3.80
50% Ethanol + H_2SO_4 (10 mM)	0.57	5.32	7.80	0.60	4.20	4.40
80% Ethanol + H_2SO_4 (10 mM)	0.35	6.86	8.60	0.46	5.96	7.60
20% $CH_3CN + H_2SO_4$ (10 mM)	1.06	7.00	12.80	0.89	4.24	6.00
50% $CH_3CN + H_2SO_4$ (10 mM)	0.69	9.32	14.60	0.59	6.34	7.20
80% $CH_3CN + H_2SO_4$ (10 mM)	0.51	10.49	15.00	0.52	7.54	11.60

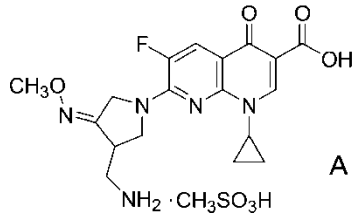
Flow rate: 0.5 ml/min. Detection: 225 nm UV. k_1: Retention factor of the first eluted enantiomer. α: Separation factor. R_S: Resolution factor. (Source: Ref. 19).

In reverse-phase chromatography, the lipophilic interaction of analytes with the stationary phase is an important factor for the retention of analytes. As the organic modifier concentration in water increases, the polarity of the mobile phase decreases and, consequently, the lipophilic interaction of analytes with the stationary phase should decrease. In this instance, the retention factors (k') should decrease as the organic modifier concentration in water increases.[30] The decreasing trends of the retention factors (k') should be more significant with more lipophilic analytes. For example, the decreasing trends of the retention factors (k') for the resolution of highly lipophilic fluoroquinolone antibacterials with the variation of the organic modifier concentration in aqueous mobile phase are very significant, as shown in Table 2.[28]

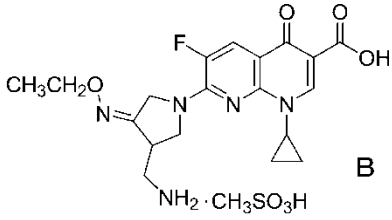
Acidic Modifier in Mobile Phase

Acidic modifier added to the aqueous mobile phase is an important factor influencing the enantioselectivities on crown ether-based CSPs. For the resolution of primary amino compounds on crown ether-based CSPs, the tripodal complexation of the protonated primary amino group ($R\text{-}NH_3^+$) inside the cavity of the crown ether ring of the CSP via three $^+N\text{-}H \cdots O$ hydrogen bonds has been known to be essential for chiral recognition.[3–5] Consequently, acidic modifier added to the mobile phase is used to protonate the primary amino groups of analytes. As an acidic modifier, perchloric, sulfuric, acetic, trifluoroacetic, nitric, hydrochloric, methanesulfonic, and

Table 2. The trends for the resolution of gemifloxacin (A) and its O-ethyloxime analogue (B) on CSP **3** with the variation of the type and the content of organic modifier in water at the constant concentration of ammonium acetate (1.0×10^{-3} M) at 20°C



A



B

	A			B		
Mobile phase	k_1	α	R_S	k_1	α	R_S
30% $CH_3OH + H_2SO_4$ (10 mM)	21.73	1.57	1.40	38.19	1.74	1.25
50% $CH_3OH + H_2SO_4$ (10 mM)	5.16	1.72	2.08	6.96	1.95	2.57
80% $CH_3OH + H_2SO_4$ (10 mM)	1.74	1.90	3.68	1.94	2.12	4.28
30% $CH_3CN + H_2SO_4$ (10 mM)	10.44	1.98	3.56	16.34	2.27	5.36
50% $CH_3CN + H_2SO_4$ (10 mM)	4.14	2.00	5.06	5.25	2.27	6.57
80% $CH_3CN + H_2SO_4$ (10 mM)	2.07	1.96	6.11	2.10	2.20	8.00

Flow rate: 0.8 ml/min. Detection: 254 nm UV. k_1: Retention factor of the first eluted enantiomer. α: Separation factor. R_S: Resolution factor. (Source: Ref. 28).

phosphoric acid can be used.[3] For the resolution of primary amino compounds on CSP **1** and CSP **2**, in general, perchloric acid has been most widely and successfully utilized.[17,18,20–24,26] However, sulfuric acid was most effective for the resolution of fluoroquinolone antibacterials on CSP **1**.[25] For the resolution of racemic primary amino compounds on CSP **3**, perchloric, acetic, trifluoroacetic, and sulfuric acid were equally effective, but sulfuric acid was most widely utilized as an acidic modifier.[19,27–30]

The chromatographic behaviors for the resolution of racemic primary amino compounds on CSP **1**, CSP **2**, and CSP **3** have been influenced by the acidic modifier concentration in the aqueous mobile phase. For example, on highly lipophilic CSPs such as CSP **1**, an increase in the acidic modifier concentration in the aqueous mobile phase improved both the retention (k') and the separation factors (α) for the resolution of α-amino acids.[17] The rationale for the retention behaviors for the resolution of α-amino acids on CSP **1** with the variation of the acidic modifier concentration was proposed by Shinbo and coworkers.[17,18] Under acidic conditions, protonated amino acid (A^+) is distributed between the mobile phase and the stationary phase as in the following equation:

$$A^+ + X^- \rightleftharpoons \overline{AX}$$

$$A^+ + \bar{C} + X^- \rightleftharpoons \overline{ACX}$$

where X^- is the acid anion in mobile phase, and C, AX, and ACX are the crown ether, the ion pair between A^+ and X^- and the ternary complex formed from A^+, C, and $X,^-$respectively. The bars above the letters denote the stationary phase. As the acidic modifier concentration in aqueous mobile phase increases, the concentration of the acid anion, X^-, in the mobile phase increases and, consequently, the equilibrium shown in the above equation should shift to the right. In this instance, the retention of the two enantiomers should increase.

The equilibrium shown in the above equation is expected to shift to the right more effectively with a more lipophilic acid anion, X^-, because the ion pair, AX, and the ternary complex, ACX, should approach the stationary phase more effectively. Indeed, the retention factors (k') for the resolution of phenylglycine and methionine on CSP **1** was found to increase as the lipophilicity of the acid anion, X^-, increases in the order: $Cl^- < NO_3^- < ClO_4^-$.[17] In the resolution of 1-aminoindan-2-ol on CSP **1** at an identical mobile phase pH of 2.0, the retention factors (k') were also found to increase as the lipophilicity of the acid anion, X^-, increases in the order: $H_2PO_4^- < NO_3^- < CF_3CO_2^- < ClO_4^-$.[31]

The equilibrium expressed by the above equation seems not to move to the right so effectively on a less lipophilic CSP, such as CSP **3**, as the acidic modifier concentration in the aqueous mobile phase increases. In the resolution of α-amino acids on CSP **3**, the retention factors (k') showed a maximum at a certain concentration of acidic modifier.[19] However, in the resolution of amines, amino alcohols, and other related primary amino compounds on CSP **3**, the retention factors (k') decreased significantly as the sulfuric acid concentration in aqueous mobile phase was increased.[27] In addition, the retention factors (k') for the resolution of fluoroquinolone antibacterials,[28] tocainide and its analogues,[29] and aryl α-amino ketones[30] on CSP **3** also decreased quite significantly as the sulfuric acid concentration in the aqueous mobile phase was increased. As the acidic modifier concentration in the aqueous mobile phase increases, the ionic strength of the mobile phase is expected to increase. On a less lipophilic CSP, the protonated analytes are expected to be distributed to the mobile phase more significantly than to the stationary phase because of the relatively more significant hydration of the ionic analytes by the aqueous mobile phase.[3,4]

Inorganic Cationic Modifier in Mobile Phase

Machida and coworkers investigated the effect of inorganic cationic modifier in the aqueous mobile phase for the retention of lipophilic analytes such as alanine-β-naphthylamide and 1-(1-naphthyl)ethylamine on CSP **1**.[32] Because of the highly lipophilic nature of CSP **1**, the retention times for the resolution of alanine-β-naphthylamide and 1-(1-naphthyl)ethylamine were quite long. In order to reduce the long retention times, Machida and coworkers added various types of inorganic cationic modifiers to the

aqueous mobile phase and found that the retention times decreased with the addition of cation in the order: $Li^+ < Na^+ < NH_4^+ < K^+$.[32] The inorganic cationic modifier in the aqueous mobile phase is expected to compete with the protonated primary ammonium ion ($R\text{-}NH_3^+$) of analytes for the complexation inside the cavity of the crown ether ring of the CSP. The stability of the complex between crown ether and cations depends significantly on the size of the cavity relative to that of the cation (Li^+, 1.36Å; Na^+, 1.94Å; K^+, 2.66Å; NH_4^+, 2.84Å). The diameter of 18-crown-6 ether ring is estimated as 2.6Å and, consequently, forms the most stable complex with K^+.[32]

The retention times of the two enantiomers for the resolution of α-amino acids,[19] amines,[27] amino alcohols,[27] fluoroquinolone antibacterials,[28] tocainide and its analogues,[29] and aryl α-amino ketones[30] on CSP **3** were successfully controlled by adding ammonium ion (NH_4^+) to the mobile phase. For the resolution of amines,[27] amino alcohols,[27] homocysteine thiolactone,[27] and fluoroquinolone antibacterials[28] on CSP **3**, K^+ was also utilized as an inorganic cationic modifier in an aqueous mobile phase to reduce the retention time of analytes and was found to reduce the retention times more significantly than NH_4^+.

Column Temperature

The chromatographic behaviors for the resolution of racemic primary amino compounds on CSP **1**, CSP **2**, and CSP **3** were also influenced by the column temperature. As the column temperature was lowered, the retention (k') and the separation factors (α) were found always to increase.[17–19,23,26,29,30] At lower temperatures, the diastereomeric complexes formed between the individual enantiomers of an analyte and the chiral crown ether moiety of the CSP are expected to become energetically more favorable; this is more significant with the more stable diastereomeric complex. In this instance, the retention (k') and the separation factors (α) should increase as the column temperature is lowered.

CSPs BASED ON CHIRAL CROWN ETHERS INCORPORATING TARTARIC ACID UNITS

Developments and Structural Characteristics

Lehn and coworkers developed various chiral crown ethers incorporating one, two, or three tartaric acid units.[11] Especially, (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid (**4**, Figure 3) incorporating two tartaric acid units has been widely utilized since the early 1990s as a chiral selector for the resolution of racemic primary amino compounds by capillary electrophoresis.[22,33–35] In contrast, the first utilization of (+)-(18-crown-6)-2,3,11,12-tetracarboxylic

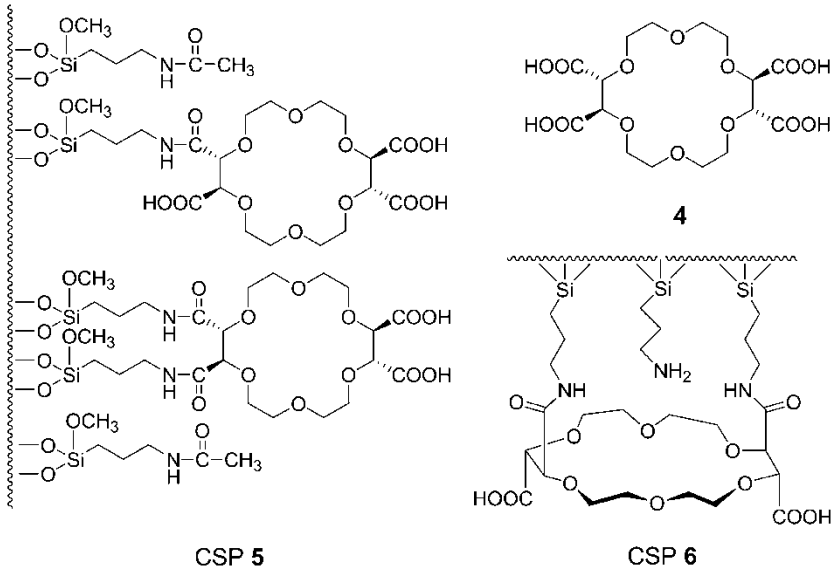


Figure 3. Structures of (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid **4** and CSPs **5** and **6**.

acid **4** as a chiral selector of liquid chromatographic CSPs was first reported in 1998 by Machida[36] and Hyun[37,38] independently.

Machida and coworkers attached (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid **4** to aminopropylsilica gel in the presence of 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ) and then treated the modified silica gel with acetic anhydride in the presence of pyridine.[36] The resulting CSP (CSP **5**, Figure 3) has some ambiguity in its structure because the mode of connecting the chiral selector to the aminopropylsilica gel in the presence of a coupling agent, EEDQ, allows a structural variety in the amide linkage. In contrast, Hyun and coworkers developed a structurally well defined CSP (CSP **6**, Figure 3).[38] (+)-(18-Crown-6)-2,3,11,12-tetracarboxylic acid **4** was converted to the dianhydride by treating with acetyl chloride and then (+)-(18-crown-6)-2,3,11,12-tetracarboxylic dianhydride was treated with aminopropylsilica gel in the presence of triethylamine to afford CSP **6** (Figure 3). CSP **6** is believed to have a *syn*-diamide structure based on a study concerning the stereoselective *syn*-opening of the dianhydride by primary amino compounds in the presence of triethylamine.[39]

Very recently, the antipode of CSP **6** derived from (−)-(18-crown-6)-2,3,11,12-tetracarboxylic acid was also prepared and demonstrated to be very useful for the determination of the enantiomeric purity of enantiomerically enriched samples.[40]

Applications

Resolution of Primary Amino Compounds

CSP **5** was successfully applied to the resolution of various α-amino acids, alanine-β-naphthylamide, amino alcohols, 1-(1-naphthyl)ethylamine, α-methyltryptamine, and primary amino drugs, including afloqualone (muscle relaxant) and primaquine (antimalarial).[36] Resolution of afloqualone on CSP **5** is quite interesting in that an atropisomer is resolved. Even though the resolution of α-amino acids on CSP **5** was quite successful, among various α-amino acids tested for their resolution, asparagine, aspartic acid, isoleucine, threonine, and valine were not resolved at all on CSP **5**.[36] In contrast, all natural and non-natural α-amino acids, including DOPA, thyroxine, and kynurenine, were resolved on CSP **6** with reasonable separation factors (α) except for proline, which does not contain a primary amino group.[38,40] In addition, the resolution factors (R_S) for the resolution of α-amino acids on CSP **6** were greater than 1.00, except for asparagine, cysteine, and isoleucine.[38]

CSP **6** was quite successful in the resolution of various amines,[41] amino alcohols,[41] fluoroquinolone antibacterial agents,[37,42,43] tocainide and its analogues,[44], β-amino acids,[45–47], aryl α-amino ketones,[48] and di- and tri-peptides.[49] In the resolution of the enantiomers of a dipeptide (Ala-Phe), all four stereoisomers were separated simultaneously.[49] CSP **6** was also successful in the resolution of baclofen,[40] which was not resolved on CSP **5**.[36] In addition, CSP **6** was demonstrated to be very useful for the exact determination of the enantiomeric composition or enantiomeric purity of enantiomerically enriched samples. Especially, the use of CSP **6** with its antipode was very useful. For example, in the resolution of enantiomerically enriched thyroxine (L:D = 90:1) and DOPA (L:D = 100:1) on CSP **6** derived from (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid, the second small peak, corresponding to (D)-thyroxine or (D)-DOPA, is embedded in the first big peak and, consequently, the exact determination of the enantiomeric purity is somewhat difficult. However, with the use of the antipode CSP, the small peak corresponding to (D)-thyroxine or (D)-DOPA is eluted first and is well separated because of the reversal of the elution order and, consequently, the enantiomeric composition is determined without any difficulty.[40]

Resolution of Non-primary Amino Compounds

It has been believed that only racemic primary amino compounds can be resolved on crown ether-based CSPs because the tripodal complexation of the primary ammonium ion ($R\text{-}NH_3^+$) of analytes inside the cavity of the chiral crown ether ring of the CSP is essential for the chiral recognition. In this instance, the resolution of racemic non-primary amino compounds on CSP **6** is not expected. However, surprisingly, CSP **6** was successfully applied to the resolution of secondary amino compounds, including

methoxyphenamine (bronchodilator) and four β-blockers including albuterol, atenolol, pindolol, and propranolol with the use of a non-aqueous mobile phase consisting of acetic acid-triethylamine-methanol-acetonitrile (0.1:0.1:50:50, v/v/v/v).[50] In the chiral recognition of primary amino compounds on CSP **6**, the two free carboxylic acid groups of the CSP have been proposed to act as chiral barriers, enantioselective hydrogen bonding sites, or ionic interaction sites based on the NMR studies[51,52] and an X-ray crystallographic study.[53] In this instance, the enantioselective hydrogen bonding interaction or ionic interaction between the carboxylate ion of CSP **6** and the analytes, in addition to the two $^{+}$N-H$\cdots$O hydrogen bonds of the protonated secondary ammonium ions of analytes with the crown ether ring oxygens of the CSP, was proposed to be responsible for the resolution of secondary amino compounds on CSP **6**.[50]

Resolution of β-blockers on CSP **6** was improved significantly when a slightly modified mobile phase consisting of trifluoroacetic acid-triethylamine-ethanol-acetonitrile (0.1:0.1:50:50, v/v/v/v) was used.[54] Under the modified mobile phase condition, eleven β-blockers were resolved quite well on CSP **6**, the separation (α) and the resolution factors (R_S) being in the range of 1.13–1.85 and 1.36–5.79, respectively.

More recently, *N*-(3,5-dinitrobenzoyl)-α-amino acids were reported to be resolved on CSP **6** with a mobile phase consisting of acetic acid-triethylamine-acetonitrile (0.05:0.25:100, v/v/v) as a non-aqueous mobile phase.[55] In addition, *N*-(3,5-dinitrobenzoyl)-α-amino acid amides were found to be resolved even better than *N*-(3,5-dinitrobenzoyl)-α-amino acids on CSP **6**.[56] As examples, resolution of *N*-(3,5-dinitrobenzoyl)valine and *N*-(3,5-dinitrobenzoyl)alanine *N*-propylamide on CSP **6** are presented in Figure 4. *N*-(3,5-Dinitrobenzoyl)-α-amino acids and their amides do not contain any primary or secondary amino groups. In this instance, the resolution of *N*-(3,5-dinitrobenzoyl)-α-amino acids and their amides on CSP **6** is quite unusual and very surprising. For the chiral recognition, the two N-H hydrogens of the amide tethers of the CSP, the carbonyl oxygen of the amide group of analytes, and the nitro group on the benzoyl group of analytes were proposed to play significant roles,[56] but the exact chiral recognition mechanism is not yet clear.

Organic Modifier in Mobile Phase

For the resolution of racemic primary amino compounds on CSP **5** and CSP **6**, water or a water-organic solvent (organic modifier) mixture containing a small amount of acidic modifier was also utilized as a mobile phase. As a water miscible organic modifier, methanol, ethanol, acetonitrile, 2-propanol, and tetrahydrofuran can be used.[36–38,40–49] However, methanol has been most widely utilized as an organic modifier in the aqueous mobile phase on CSP **6**.[37,38,40–47,49]

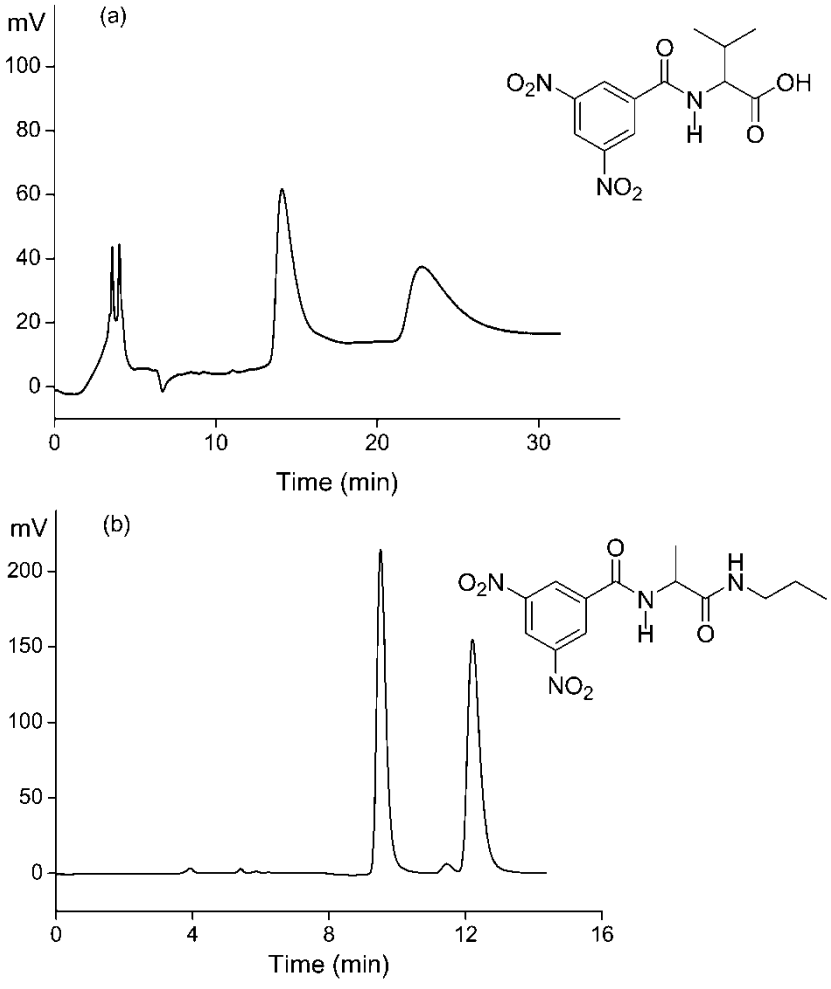
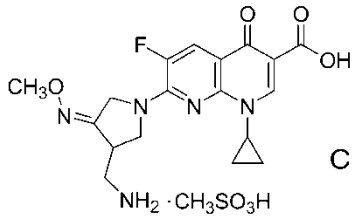


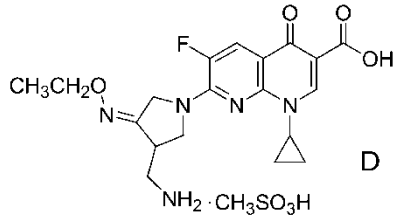
Figure 4. Chromatograms for the resolution of (a) *N*-(3,5-dinitrobenzoyl)valine and (b) *N*-(3,5-dinitrobenzoyl)alanine *N*-propylamide on CSP **6**. (a) Mobile phase: acetic acid-triethylamine-acetonitrile (0.05:0.25:100,v/v/v). Flow rate, 0.5 ml/min; detection, 254 nm UV; temperature, 20°C. Source: Ref. 55. (b) Mobile phase: acetic acid-triethylamine-acetonitrile (0.01:0.05:100,v/v/v). Flow rate, 0.5 ml/min; detection, 254 nm UV; temperature, 20°C. Source: Ref. 56.

The chromatographic behaviors for the resolution of α-amino acids,[38] amines,[41] amino alcohols,[41] fluoroquinolone antibacterial agents,[42,43] tocainide and its analogues,[44] and β-amino acids[45] on CSP **6**, the retention (k'), the separation (α) and the resolution factors (R_S) generally improved as the organic modifier concentration in the aqueous mobile phase was increased. As an example, Table 3 (entry a) shows the chromatographic behaviors for the

Table 3. The chromatographic trends for the resolution of fluoroquinolone compounds (C and D) on CSP **6** with the variation of methanol and sulfuric acid concentration in water at 20°C



C



D

	C			D		
Mobile phase	k_1	α	R_S	k_1	α	R_S
a						
20% $CH_3OH + H_2SO_4$ (10 mM)	0.70	2.56	2.75	2.16	1.29	0.60
50% $CH_3OH + H_2SO_4$ (10 mM)	0.76	2.83	3.72	1.93	1.42	1.72
80% $CH_3OH + H_2SO_4$ (10 mM)	1.60	3.41	4.86	4.41	1.53	2.50
100% $CH_3ON + H_2SO_4$ (10 mM)	4.16	4.05	6.37	14.09	1.54	2.64
20% $C_2H_5OH + H_2SO_4$ (10 mM)	0.62	2.61	2.50	1.79	1.32	0.86
50% $C_2H_5OH + H_2SO_4$ (10 mM)	0.97	2.97	3.40	1.74	1.43	1.40
b						
80% $CH_3OH + H_2SO_4$ (1 mM)	2.89	3.49	6.25	8.47	1.49	2.00
80% $CH_3OH + H_2SO_4$ (5 mM)	1.84	3.51	6.17	5.09	1.52	3.10
80% $CH_3OH + H_2SO_4$ (10 mM)	1.60	3.41	4.86	4.41	1.53	2.50
80% $CH_3OH + H_2SO_4$ (20 mM)	1.57	3.43	5.83	4.32	1.54	2.80

Flow rate: 0.8 ml/min. Detection: 254 nm UV. k_1: Retention factor of the first eluted enantiomer. α: Separation factor. R_S: Resolution factor. (Source: Ref. 42).

resolution of fluoroquinolone antibacterial agents on CSP **6** with the variation of methanol concentration. Especially, the trends of the retention factors (k') on CSP **6** with the variation of the organic modifier concentration in the aqueous mobile phase are exactly opposite to those on CSP **1**, CSP **2**, and CSP **3**. However, the retention factors (k') for the resolution of highly lipophilic analytes such as alanine-β-naphthylamide and 1-(1-napthyl)ethylamine on CSP **5**[36] and for the resolution of a highly lipophilic 1-(6,7-dimethyl-1-naphthyl)ethylamine on CSP **6**[41] decreased as the organic modifier concentration in aqueous mobile phase was increased.

The trends of the retention factors (k') on CSP **6** was also proposed to stem from the balance between the lipophilic interaction of analytes with a CSP and the hydrophilic interaction of analytes with the mobile phase, based on the study concerning the effect of the analyte lipophilicity on the resolution of various α-amino acids containing lipophilically different alkyl groups at the chiral center.[3,57] Compared to the chiral selectors of CSP **1**,

CSP **2**, and CSP **3**, the chiral selectors of CSP **5** and CSP **6** are less lipophilic. In addition, CSP **5** or CSP **6** does not contain octadecyl groups at the silica gel surface. In this instance, CSP **5** or CSP **6** is less lipophilic than CSP **1**, CSP **2**, or CSP **3**. On less lipophilic CSP **5** or CSP **6**, the hydrophilic interaction of analytes with the mobile phase should be more significant than the lipophilic interaction with the CSP. As the organic modifier in the aqueous mobile phase increases, the mobile phase becomes less polar and more hydrophobic. Under this condition, the hydrophilic interaction of analytes with mobile phase becomes less favorable and, consequently, analytes are eluted slower and slower as the organic concentration in aqueous mobile phase is increased. However, for the resolution of highly lipophilic analytes such as alanine-β-naphthylamide and 1-(1-napthyl)ethylamine on CSP **5** and for the resolution of highly lipophilic analyte 1-(6,7-dimethyl-1-naphthyl)ethylamine CSP **6**, the lipophilic interaction of analytes with the CSP is still more significant than the hydrophilic interaction of analytes with mobile phase and, in this event, the retention factors (k') should decrease as the organic modifier concentration in the aqueous mobile phase is increased.

Acidic Modifier in Mobile Phase

As an acidic modifier in the aqueous mobile phase, sulfuric acid was most widely and successfully utilized for the resolution of α-amino acids,[38,57] amines,[41] amino alcohols,[41] fluoroquinolone antibacterial agents,[37,42,43] tocainide and its analogues,[44] and aryl α-aminoketones[48] on CSP **6**, even though perchloric acid was still useful. However, in the resolution of β-amino acids on CSP **6**, acetic acid was much more effective as an acidic modifier than sulfuric acid, in terms of both enantioselectivity (α) and resolution (R_S).[46,47]

The effect of the acidic modifier concentration in the aqueous mobile phase on the retention factors (k') for the resolution of racemic primary amino compounds on CSP **6** was generally opposite to that on CSP **1**, CSP **2**, or CSP **3**. The retention factors (k') for the resolution of phenylglycine,[38] fluoroquinolone antibacterial agents,[42,43] β-amino acids,[45,47] and tocainide and its analogues[44] on CSP **6** decreased as the acidic modifier concentration in the aqueous mobile phase was increased. As an example, Table 3 (entry b) shows the retention behaviors for the resolution of fluoroquinolone antibacterial agents on CSP **6** with the variation of sulfuric acid concentration in aqueous mobile phase. In relatively less lipophilic CSP **6**, the hydrophilic interaction of analytes with mobile phase is predominant over the lipophilic interaction of analytes with the CSP. As the acidic modifier concentration in the aqueous mobile phase increases, the ionic strength of mobile phase increases and, consequently, the hydrophilic interaction of analytes with the mobile phase increases. In this instance, polar-protonated analytes are eluted faster and faster as the acidic modifier concentration in the aqueous mobile phase increases. However, the separation (α) and the resolution factors (R_S) did

not show any significant trends on CSP **6** with the variation of the acidic modifier concentration in aqueous mobile phase.

Column Temperature

For the resolution of racemic primary amino compounds on CSP **5** or CSP **6**, the retention (k') and the separation factors (α) always increased as the column temperature was decreased.[36,41–43,45] These trends are exactly identical to those on CSP **1**, CSP **2**, or CSP **3**. Based on the chromatographic parameters, including the retention (k') or the separation factors (α) for the resolution of amines and amino alcohols on CSP **6** with the variation of the column temperature, both the differential enthalpy ($\Delta\Delta H$) and differential entropy ($\Delta\Delta S$) of absorption for the two enantiomers were calculated to be negative from van't Hoff plots.[41] In this instance, the negative differential free energy ($\Delta\Delta G$) value corresponding to the separation factor (α) of greater than 1.00 should be negative and the negative $\Delta\Delta G$ is entirely dependent on $\Delta\Delta H$. Consequently, the enantioselectivities for the resolution of racemic primary amino compounds on crown ether-based CSPs were reported to be enthalpy controlled.

In contrast, in the resolution of β-blockers on CSP **6**, the separation factors (α) were found, very surprisingly and unusually, to increase as the column temperature was increased.[54] From the van't Hoff plots, both $\Delta\Delta H$ and $\Delta\Delta S$ values for the resolution of five β-blockers on CSP **6** were calculated to be positive. In this instance, the negative $\Delta\Delta G$ values are entirely dependent on $\Delta\Delta S$ values. Consequently, the resolution of β-blockers on CSP **6** is entropy controlled.[54]

Enantioselectivity-Improved CSPs

As efforts to improve the chiral recognition efficiency of CSP **6**, CSP **7** (Figure 5) was prepared.[58] In CSP **6**, the possible hydrogen bondings between the N-H hydrogens of the two connecting amide tethering groups of the CSP and the crown ether ring oxygens of the CSP were expected to hinder the tripodal complexation of the ammonium ions ($R\text{-}NH_3^+$) of analytes inside the cavity of the crown ether ring of the CSP. In this instance, removal of the two N-H amide hydrogens of CSP **6** was expected to improve the chiral recognition efficiency of the CSP. Based on this rationale, CSP **7** was prepared by simply treating (+)-(18-crown-6)-2,3,11,12-tetracarboxylic dianhydride with 3-(*N*-methylamino)propylsilica gel in the presence of 2,6-lutidine in methylene chloride.[58] CSP **7** was successfully applied to the resolution of α-amino acids,[58] amines,[58] amino alcohols,.[58] tocainide and its analogues,[44] β-amino acids,[46] and aryl α-amino ketones.[48] In the resolution of α-amino acids and amino alcohols, CSP **6** and CSP **7** were complementary, but in the resolution of amines, CSP **7** was always superior to CSP **6**.[58] In the resolution of tocainide and

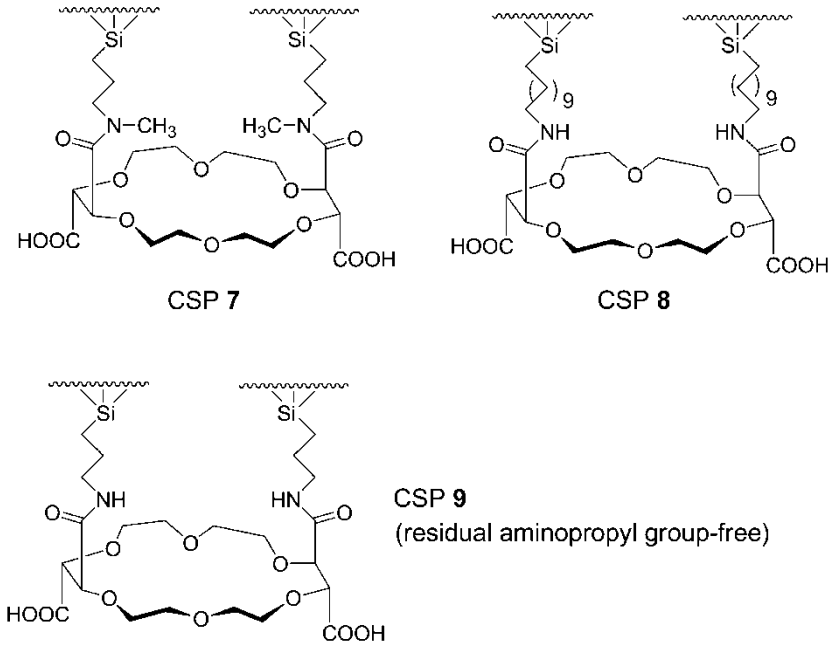


Figure 5. Structures of CSPs **7**, **8** and **9**.

its analogues, CSP **7** was generally better than CSP **6**.[44] In the β-amino acids, CSP **7** was also better than CSP **6**, especially when sulfuric acid was used as an acidic modifier in the aqueous mobile phase, but CSP **7** was worse than CSP **6** when acetic acid was used as an acidic modifier.[46] In the resolution of aryl α-amino ketones, CSP **6** and CSP **7** were equally effective.[48] Especially, CSP **7** was demonstrated to be very useful for the exact determination of the enantiomeric purity of optically active cathinone, one of the aryl α-amino ketones.[48]

The short spacer of three methylene units connecting the chiral selector to the silica gel in CSP **6** might cause steric hindrance by the silica gel during the enantioselective complexation of analytes inside the cavity of the crown ether ring of the CSP and, consequently, diminish the chiral recognition. In an effort to develop an improved CSP by increasing the spacer length, CSP **8** (Figure 5), containing a relatively long spacer group of eleven methylene units, was prepared by treating (+)-(18-crown-6)-2,3,11,12-tetracarboxylic dianhydride with 11-aminoundecylsilica gel.[59] CSP **8** was, indeed, superior to CSP **6** in the resolution of α-amino acids, β-amino acids, amines, and amino alcohols in terms of both the separation (α) and the resolution factors (R_S).

CSP **6** intrinsically contains unreacted residual aminopropyl groups on the surface of the stationary phase. The residual aminopropyl groups of CSP **6** are protonated under the acidic mobile phase conditions. The resulting primary

ammonium ions are expected to compete with the primary ammonium ions of the analytes for complexation inside the cavity of the crown ether ring of the CSP and, consequently, the chiral recognition ability of the CSP can be diminished.[60] In this instance, the removal of the residual primary aminopropyl groups of CSP **6** can improve the enantioselectivity of CSP **6**. Based on this rationale, CSP **9** (Figure 5) without extra free residual aminopropyl groups on silica gel surface was developed by bonding *N*,*N*′-triethoxysilylpropyl *syn*-diamide of (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid to silica gel.[60] CSP **9** was applied to the resolution of various racemic α-amino acids, amines, and amino alcohols and the chromatographic resolution results on CSP **9** were found to be generally superior to those on CSP **6** as expected.

Stability-Improved CSPs

Crown ether-based CSPs have been used under acidic mobile phase conditions to protonate the primary amino groups of analytes. CSPs based on (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid have also been utilized under acidic mobile phase conditions. Under highly acidic mobile phase conditions, these CSPs were not considered to be stable enough for long term use, because the silyloxy linkage connecting the chiral selector to silica gel can be cleaved under highly acidic mobile phase conditions.

In an effort to improve the stability of CSP **6** or CSP **7**, CSP **10** (Figure 6) was prepared by adding a second point of attachment through the nitrogen atom of the amide tethering group of the singly tethered CSP.[61] CSP **10** was thought to be intrinsically the type of CSP **7,** since both CSPs contain a tertiary amide linkage. CSP **10** was quite effective for the resolution of α- and β-amino acids, amino alcohols, and amines, but the chiral recognition efficiency of CSP **10** was slightly worse than that of CSP **7**. The structural difference between the tertiary N-CH_3 group of CSP **7** and the second tertiary N-alkyl tethering group of CSP **10** seems to be responsible for the inferior chiral recognition efficiency of CSP **10**. However, the stability of CSP **10** was greater than that of CSP **7** because of the doubly tethered nature of the CSP.

To maintain the structural integrity of the secondary N-H amide nature of CSP **6** or the tertiary N-CH_3 amide nature of CSP **7**, a new method of attaching the second tethering group of the CSP to silica gel through a carbon atom of the first tethering group of the corresponding singly tethered CSP was recently developed.[62] The method was applied to the preparation of CSP **11** (Figure 6). CSP **11** was applied successfully to the resolution of various racemic α-amino acids, primary amino alcohols, and primary amines. The chiral recognition efficiency of CSP **11** was mostly superior to CSP **6**. The stability of CSP **11** was also greater than that of CSP **6**.[62]

The method of attaching the second tethering group to silica gel utilized in the preparation of CSP **11** was also applied to the preparation of CSP **12** (Figure 6).[63] CSP **12** maintains the N-CH_3 amide linkage of CSP **7**. CSP

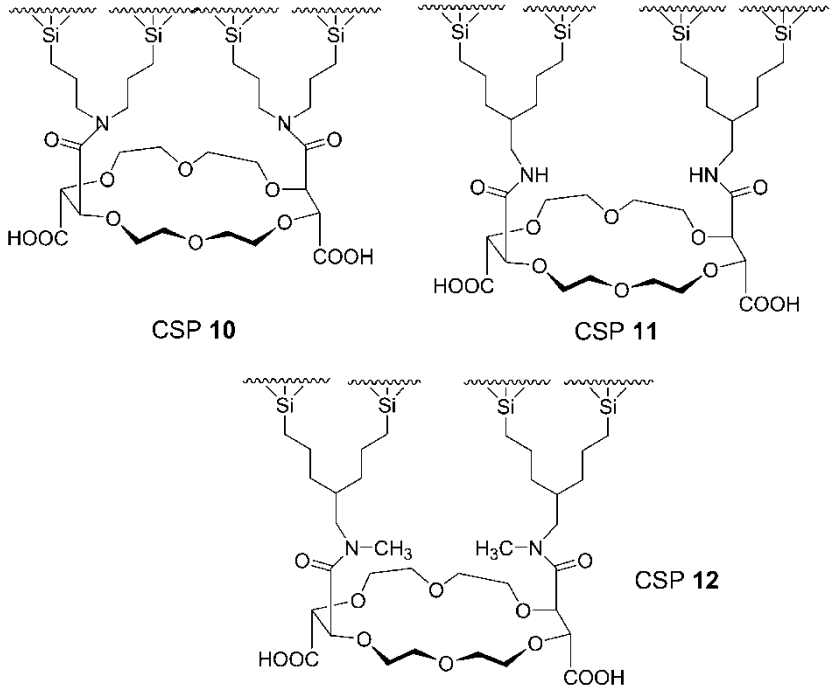


Figure 6. Structures of CSPs **10**, **11** and **12**.

12 was quite effective for the resolution of various racemic α-amino acids, amines, and amino alcohols. The chiral recognition efficiency of CSP **12** was greater than that of CSP **7**, especially in terms of the resolution factors (R_S). In addition, the stability of CSP **12** was greater than that of CSP **7**.

CONCLUSION

Chiral crown ether-based CSPs have been known to be useful for the resolution of racemic primary amino compounds. However, recently, application of chiral crown ether-based CSPs was extended to the resolution of secondary amino compounds and non-amino compounds with the use of non-aqueous mobile phases. The efforts to extend the use of chiral crown ether-based CSPs further are still underway and, consequently, these CSPs are expected to be applied more widely in the future. The development of the doubly tethered CSPs is important since the stability-improved CSPs are very valuable for production scale resolution of enantiomers. Consequently, chiral crown ether-based CSPs are expected to be applicable for preparative purposes in the near future.

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