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Chiral Selector with Multiple Hydrogen-Bonding Sites in a Macrocyclic Cavity

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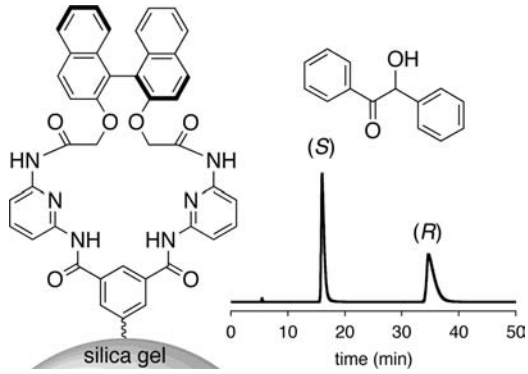
ABSTRACT



Chiral macrocycles with the hydrogen bond donor/acceptor sites in the cavity were synthesized and covalently bonded to silica gel to give chiral stationary phases (CSPs), which showed excellent abilities to resolve various chiral compounds, such as benzoin and $Co(acac)_3$, in HPLC. Various organic solvents could be used as the mobile phase to optimize the resolution efficiency of CSPs, and in some cases, even MeCN, MeOH, and CO_2 could be used for the complete resolution of enantiomers.

There is an increasing demand for the accurate determination of the enantiomeric purity with an increase in the number of chiral drugs. Among various methods for the determination of the enantiomeric purity, the HPLC method with a chiral stationary phase (CSP) column has gained a dominant position because of high sensitivity and reliability.[1] In addition to the analytical utility, chiral HPLC can also give optically active compounds by the preparative resolution of enantiomers. Recently, various types of CSPs have been developed, where a chiral selector is covalently or noncovalently bound to silica gel.[1−7] The advantage of the former, covalently immobilized CSPs, is that the combination of mobile phases (solvents) can be tuned to maximize the

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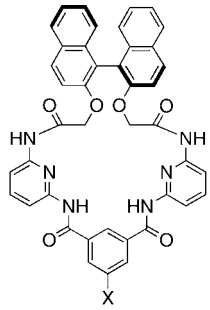
resolution efficiency. Polysaccharides,[2] antibiotics,[3] binaphthyls,[4,5a] macrocycles such as cyclodextrins,[1d] crown ethers,[5] and cyclophanes,[6] and others[1,7] have been used as chiral selectors. Among them, the binaphthyl structure is one of the most attractive chiral units because of its moderate rigidity and flexibility as well as its synthetic accessibility.

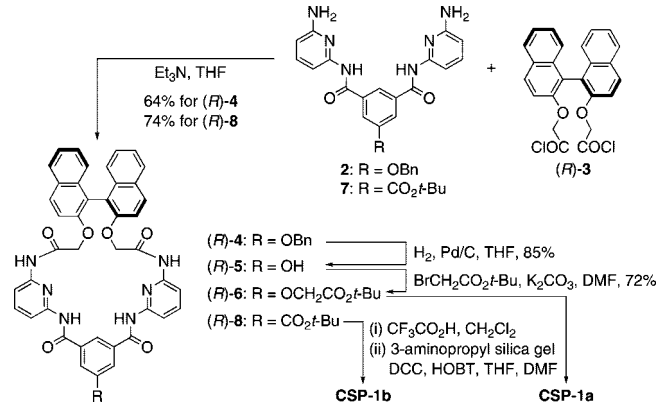
In addition to the chiral unit, a binding unit is also a key factor in the design of a chiral selector. Selector molecules covalently attached to the silica surface in a highly accumulated state may aggregate via the intermolecular interactions, which would result in the compensation and masking of the binding sites. A highly organized structure such as a helix, which has been well-demonstrated by Okamoto and Yashima,[1b,2a] would be needed to avoid the aggregation of a linear binding motif. We envisioned that such a binding unit where the binding site is located inside a macrocyclic cavity could retain a chiral binding environment independently even in a highly accumulated state, which would lead to the highly efficient resolution. Although chiral selectors bearing crown ether motifs have been developed, the scope is rather limited because they have only the hydrogen bond acceptor (ether) functionality. On the other hand, an NMR chiral solvating agent called Chirabite-AR that we have recently developed can bind a wide range of compounds by using both hydrogen bond donor and acceptor sites inside the macrocyclic cavity.[8] In view of the unique characteristics of the receptor, we decided to investigate whether it could be used as a chiral selector. Here we report the synthesis of **CSP-1** and the chromatographic resolution of chiral compounds.



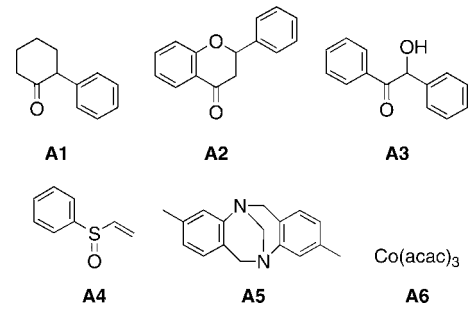
Chirabite-AR: X = NO_2
CSP-1a: X = $OCH_2CONH(CH_2)_3$-silica
CSP-1b: X = $CONH(CH_2)_3$-silica

Initially, we attempted to convert the NO_2 group in Chirabite-AR to an amino group by the Pd-catalyzed hydrogenation; however, the corresponding amine obtained was found to be unstable for an unknown reason. We therefore searched for a stable precursor bearing a functional group connectable to silica or a silane coupling agent, and found that the hydroxy and carboxyl groups can be good substitutes for the amino group. The synthetic routes to **CSP-1a** and **CSP-1b** are shown in Scheme 1, where mild synthetic methods are employed to avoid the decomposition of the four amide groups forming the macrocyclic structure. Diamines **2** and **7** were prepared from 5-benzyloxyisophthalic acid and 5-*tert*-butyloxycarbonylisophthalic acid, respectively, according to the reported procedure.[9,10]

Scheme 1. Preparation of **CSP-1**



The coupling of **2** and **7** with acid chloride **3** afforded macrocycles **4** and **8**, respectively. The deprotection of the benzyl group in **4** followed by the attachment of the *tert*-butyl ester moiety to **5** gave **6**. The *tert*-butyl ester group in **6** or **8** was cleaved by treatment with trifluoroacetic acid. The condensation of the resulting acids with 3-aminopropyl silica gel afforded **CSP-1a** or **CSP-1b**, which were packed in a stainless steel column (ϕ 0.46 × 25 cm). In both cases, the coverage of the macrocycle on silica was adjusted to 0.18 mmol/g as determined by elemental analysis.



The ability of **CSP-1** to resolve chiral analytes **A1**−**A6** was evaluated by HPLC. The results are summarized in Table

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1, and representative chromatograms are shown in Figure 1. We confirmed that racemic compounds **A1**−**A6** could be resolved successfully without derivatization. Among them, $Co(acac)_3$ (**A6**), which happened to be used as one of our standard samples for the CSP assay, gave intriguing results.[11] As shown in Figure 1b and Table 1, **A6** was resolved

Table 1. Chromatographic Data for the Resolution of Racemic Compounds on **CSP-1**[a]

		separation factor α[b] (peak separation)[c]			
CSP	analyte	eluent I	eluent II	eluent III	eluent IV
CSP-1a	**A1**	1.14 (B)	1.31 (B)	1.00 (C)	1.00 (C)
CSP-1a	**A2**	1.07 (B)	1.07 (C)	1.00 (C)	1.00 (C)
CSP-1a	**A3**	1.87 (A)	2.26 (A)	2.19 (A)	1.54 (B)
CSP-1a	**A4**	1.00 (C)	1.24 (B)	1.17 (B)	1.00 (C)
CSP-1a	**A5**	1.00 (C)	1.00 (C)	1.37 (B)	1.00 (C)
CSP-1a	**A6**	1.57 (B)	1.50 (B)	1.61 (B)	1.00 (C)
CSP-1b	**A1**	1.15 (A)	1.44 (A)	1.26 (C)	1.00 (C)
CSP-1b	**A2**	1.09 (B)	1.07 (B)	1.00 (C)	1.06 (C)
CSP-1b	**A3**	1.83 (A)	2.43 (A)	2.38 (A)	1.70 (A)
CSP-1b	**A4**	1.06 (B)	1.51 (A)	1.04 (C)	1.08 (C)
CSP-1b	**A5**	1.08 (B)	1.08 (B)	1.25 (B)	1.00 (C)
CSP-1b	**A6**	1.75 (A)	1.67 (A)	1.61 (A)	1.20 (B)

[a] **CSP-1a** or **CSP-1b** packed in a stainless steel column (ϕ 0.46 × 25 cm) was used for HPLC: flow rate 1.0 mL/min, detection 254 nm except for **A1** (225 nm), 25 °C, eluent I = hexane/*i*-PrOH (9:1), eluent II = hexane/$CHCl_3$ (7:3 except for **A1** and **A4** (4:6)), eluent III = MeCN, eluent IV = MeOH. [b] Calculated from k_2'/k_1', where $k_1' = (t_1 - t_0)/t_0$ and $k_2' = (t_2 - t_0)/t_0$. The retention times for the faster and slower moving enantiomers are designated as t_1 and t_2, respectively, and that for 1,3,5-tri-*tert*-butylbenzene used as a void volume marker is designated as t_0. [c] A = complete or almost complete separation, B = partial separation, C = little or no separation.

successfully. Although the detailed mode of chiral recognition is unknown despite the computational calculations, the outside lone-pair orbitals of the carbonyl O atoms of **A6** are likely to be accessible to the cavity of **CSP-1** via hydrogen bonding even if the carbonyl O atoms remain coordinated to the Co atom.

Table 1 also indicates that various solvents can be tested to optimize the resolution efficiency of the covalently immobilized CSPs. In the case of **CSP-1b**, for example, the complete resolution of **A1**, **A3**, and **A6** was achieved by using hexane/*i*-PrOH, while the excellent resolution of **A1**, **A3**, **A4**, and **A6** was realized by using hexane/$CHCl_3$. When a highly polar solvent, MeCN, was used, surprisingly, **A3** and **A6** could be resolved completely by **CSP-1b**. This success was totally unexpected because it is known that the ability of Chirabite-AR to discriminate between enantiomers in NMR is deteriorated in $CDCl_3$ containing d_4-methanol or in d_6-acetone.[8b] The use of a more polar solvent, MeOH, drastically diminished the peak separation in most cases. These results suggest that the hydrogen bonds are formed in the macrocyclic cavity of **CSP-1** even in polar solvents

(11) Several CSPs have been reported to be effective for the resolution of **A6**. For example, see ref 1b.

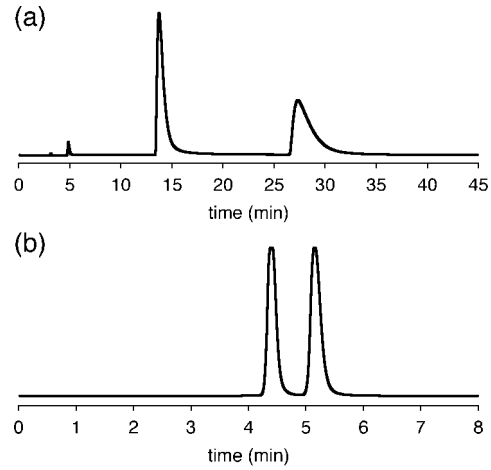

Figure 1. Selected chromatograms for the resolution of (a) **A3** on **CSP-1a** with hexane/$CHCl_3$ (7:3) and (b) **A6** on **CSP-1b** with MeCN. For details of the analytical conditions, see the footnote for Table 1.

and that they are stronger on the surface of silica than in a bulk solution.

Comparisons between **CSP-1a** and **CSP-1b** clearly indicate the importance of the linker, the ether or amide group (Table 1 and Supporting Information Tables S1−S6). In many cases, the separation factors (α) are larger for **CSP-1b** than for **CSP-1a**. One of the exceptions is the resolution of sulfoxide **A4** in MeCN, which was resolved by **CSP-1a**, but not by **CSP-1b**. The hydrogen bond donor ability of **CSP-1b** is expected to be higher than that of **CSP-1a** because it is known that the presence of the NO_2 group in Chirabite-AR improves the hydrogen bond donor ability of the lower amide groups of the macrocycle.[8b] The most impressive results were obtained for benzoin (**A3**), which was resolved completely by **CSP-1b** by using any of the solvent systems that were examined, even MeOH (Table 1). We confirmed that, in all the eluents I−IV, the enantiomers of **A3** eluted in the same order: (*S*)-**A3** followed by (*R*)-**A3**. The binding constants (K_a) of Chirabite-AR for (*R*)- and (*S*)-**A3** in $CDCl_3$ were determined by NMR titrations to be 240 and 40 M^{-1}, respectively. These results mean that (*R*)-**A3**, which has a higher affinity for the (*R*)-host, eluted more slowly in all cases, which in turn suggests that the mechanism of chiral recognition is basically unchanged. We consider that, because of the preorganized binding site in the macrocyclic structure, hydrogen bonds that are essential for chiral recognition are retained in all the eluents (I−IV).[12]

Chiral HPLC with CO_2 as the mobile phase is very important from the viewpoint of green chemistry.[13] The remarkable solvent tolerance of **CSP-1** as demonstrated above prompted us to examine the CO_2-based mobile phase.

(12) The NMR titrations for determining the binding constant of Chirabite-AR for **A3** in MeCN or MeOH could not be performed because of the insolubility of the reagent.

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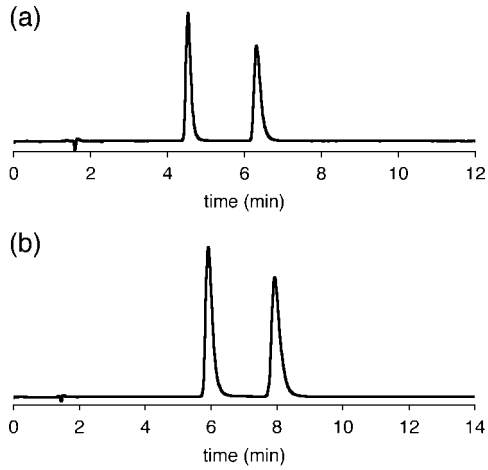

Figure 2. Chromatograms for the resolution of (a) **A3** and (b) **A6** on **CSP-1a**, using the CO_2-based mobile phase: flow rate 2.5 mL/min, detection 220 nm, 25 °C, CO_2/*i*-PrOH (9:1 for **A3**, 8:2 for **A6**), 10 MPa.

The results are shown in Figure 2. Complete resolution of **A3** and **A6** was achieved by **CSP-1a**, and the peak separation was much better than we had expected. These results convinced us of the excellence of **CSP-1**.

In summary, we have demonstrated that the synthetic macrocycles with multiple hydrogen bonding sites in the cavity can function as excellent chiral selectors. **CSP-1**, having a distinct site preorganized for chiral recognition, can resolve a variety of chiral compounds including an unexpected one, $Co(acac)_3$, without derivatization of the analytes. Various organic solvents can be used as the mobile phase to optimize the resolution efficiency, including even the highly polar solvents such as MeCN and MeOH as well as the CO_2-based mobile phase. **CSP-1**, showing excellent performance and durability, is very promising, and the alteration and tuning of the macrocyclic structure will lead to a further enhancement in the chiral HPLC performance.

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Supporting Information Available: Synthetic procedures for **CSP-1a** and **CSP-1b**, determination of the coverage of the macrocycle on silica, copies of ^1H and ^{13}C NMR, and copies of chromatograms. This material is available free of charge via the Internet at http://pubs.acs.org.

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