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# Enantioseparation on Riboflavin Derivatives Chemically-Bonded to Silica Gel as Chiral Stationary Phases for HPLC

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## Abstract

Acetylated and/or 3,5-dimethylphenylcarbamated riboflavins were prepared and the resulting riboflavin derivatives as well as natural riboflavin were regioselectively immobilized on silica gel through chemical bonding at the 5'-O- or 3-N-position of the riboflavin to develop novel chiral stationary phases (CSPs) for enantioseparation by high-performance liquid chromatography (HPLC). The chiral recognition abilities of the obtained CSPs were significantly dependent on the structures of the riboflavin derivatives, the position of the chemical bonding on the silica gel, and the structures of the racemic compounds. The CSPs bonded at the 5'-O-position on the silica gel tended to well separate helicene derivatives, while the CSPs bonded at the 3-N-position composed of acetylated and 3,5-dimethylphenylcarbamated riboflavins showed a better resolving ability toward helicene derivatives and bulky aromatic racemic alcohols, respectively, and some of them were completely separated into the enantiomers. The observed difference in the chiral recognition abilities of these riboflavin-based CSPs is discussed based on the difference in their structures, including the substituents of riboflavin and the positions immobilized on the silica gel.

**Keywords:** riboflavin, chiral recognition, chiral stationary phase, helicene

## Introduction

The separation of enantiomers by high-performance liquid chromatography (HPLC) has been recognized as one of the most useful techniques not only for obtaining both enantiomers on a large scale, but also for the precise determination of the enantiomeric excess of the chiral analytes including chiral drugs.<sup>1-4</sup> The development of a chiral stationary phase (CSP) showing an efficient chiral recognition for a variety of enantiomers is of key importance for this purpose. Therefore, a number of chiral stationary phases (CSPs) for HPLC has been prepared in the past few decades, and more than 100 CSPs have been commercialized,<sup>5</sup> which mostly consist of synthetic or polysaccharide-derived helical polymers<sup>6-11</sup> or are derived from optically-active synthetic or naturally-occurring small molecules including amino acids, crown ethers, and cinchona alkaloids.<sup>12-15</sup>

Riboflavin (vitamin B<sub>2</sub>) is an important unit of cofactors of biologically-active flavoenzymes<sup>16</sup> and provides a variety of functions, such as catalytic and redox activities and a photoluminescent ability. Hence the readily-available natural riboflavin consisting of a diverse functional heterocyclic isoalloxazine ring together with an optically-active ribityl group and its derivatives have been considered as a promising class of novel asymmetric organocatalysts<sup>17,18</sup> and chiral materials for sensing<sup>19</sup> and separating enantiomers, but successful examples for the use as a CSP are still rare except for one precedent by Gil-Av et al.;<sup>20</sup> they found that natural riboflavin had a high chiral recognition ability toward carbohelicenes ([7] to [14]helicene) when coated on silica gel as a CSP for HPLC using a mixture of *n*-hexane and

CH<sub>2</sub>Cl<sub>2</sub> as the eluent. Recently, Papadimitrakopoulos and coworkers have also demonstrated that the flavin mononucleotide (FMN), a phosphorylated analog of riboflavin, self-assembled and wrapped around single-walled carbon nanotubes (SWCNTs) in a chirality and handedness selective way and enriched the left-handed helical SWCNTs.<sup>21</sup> These results suggest that riboflavin and its derivatives may have the potential as promising CSPs for separation of polyaromatic racemic compounds through  $\pi$ - $\pi$  interactions including charge-transfer complexation between the analytes and isoalloxazine ring of the riboflavin<sup>20</sup> when they are chemically-bonded to silica gel.<sup>22-25</sup> Riboflavin has reactive hydroxy and amino groups, and modification with various substituents and further chemical bonding to silica gel are possible.

In this study, we prepared five new riboflavin-based CSPs composed of natural riboflavin and its acetylated and/or 3,5-dimethylphenylcarbamated derivatives by regioselective

immobilization at the 5'-O- or 3-N-position of the riboflavin on silica gel (Fig. 1), and their chiral recognition abilities were evaluated. Among the possible derivatization methods of the hydroxy groups of the ribityl unit, we employed 3,5-dimethylphenylcarbamoylation because the CSPs composed of the tris(3,5-dimethylphenylcarbamate)s of cellulose and amylose developed by Okamoto et al. exhibit an excellent chiral resolving ability for a wide range of racemic compounds and are recognized as the most frequently used CSPs.<sup>4-7,9-11</sup>

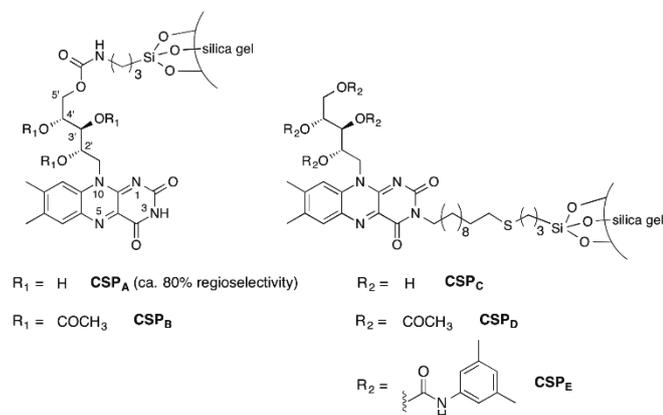


Fig. 1. Structures of riboflavin-based chiral stationary phases.

## Materials and Methods

### Instruments

The NMR spectra were measured using a Varian VXR-500S spectrometer (Varian, Palo Alto, CA) operating at 500 MHz for <sup>1</sup>H and 125 MHz for <sup>13</sup>C using tetramethylsilane (TMS) for CDCl<sub>3</sub> as the internal standard. The IR spectra were recorded on a JASCO FT/IR-680 spectrometer (JASCO, Tokyo, Japan). The chromatographic separations of enantiomers were performed using a JASCO PU-2080 Plus liquid chromatograph equipped with Multi UV-Vis (JASCO MD-2010 Plus or MD-2018 Plus) and CD detectors (JASCO CD-1595 or CD-2095 Plus) at ca. 25 °C. A solution of racemate was injected into the chromatographic system using a Rheodyne Model 7725i injector (20 μL loop). The thermogravimetric (TG) analyses were conducted on a SEIKO EXSTAR6000 TG/DTA 6200 (Seiko Instruments Inc., Chiba, Japan) under a heating rate of 10 °C/min in a nitrogen flow of 200 mL/min.

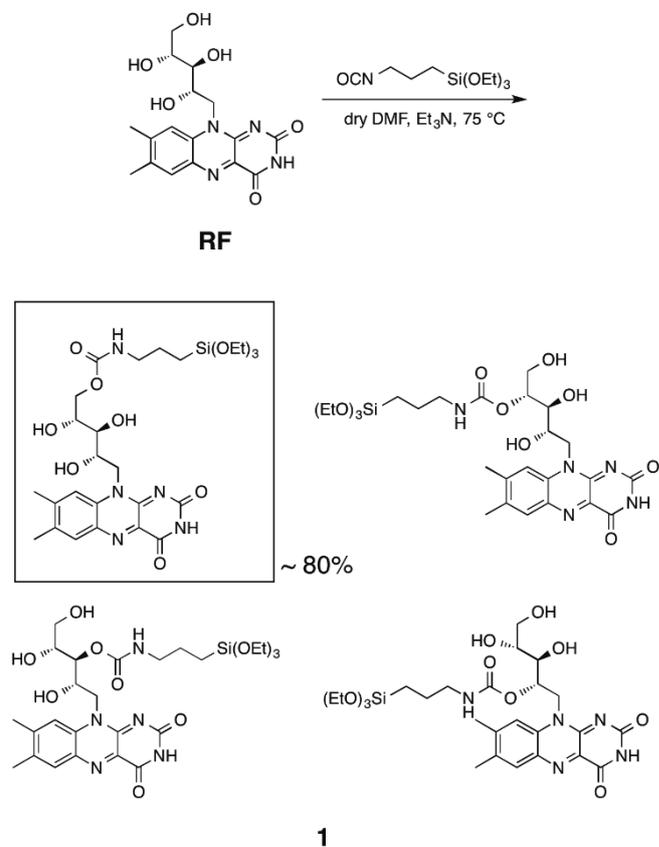
### Chemicals and Reagents

Anhydrous dimethylformamide (DMF), dimethyl sulfoxide (DMSO), chloroform (CHCl<sub>3</sub>), pyridine, and tetrahydrofuran (THF) (water content <0.005%), methanol (MeOH), ethanol (EtOH), acetone, *n*-hexane, *N,N*-dimethyl-4-aminopyridine (DMAP), and 1,8-diazabicyclo [5.4.0]-7-undecene (DBU) were purchased from Wako (Osaka, Japan). Riboflavin (RF), 3-(triethoxysilyl)propyl isocyanate, 11-bromo-1-undecene (5), *p*-toluenesulfonic acid monohydrate, 2,2'-azobis(isobutyronitrile) (AIBN), and 3,5-dimethylphenyl isocyanate were purchased from Tokyo Kasei (TCI, Tokyo, Japan). Hydrogen chloride in diethyl ether (1.0 M) was obtained from Aldrich (Milwaukee, WI). Triethylamine and acetic anhydride were obtained from Kishida (Osaka, Japan). Tetraacetylriboflavin (TARF),<sup>26</sup> (3-mercaptopropyl)triethoxy

silanized silica gel (M-silica, 8.3 wt%) with a mean particle size of 7 μm and a mean pore diameter of 12 nm,<sup>27</sup> and 5'-O-trityl riboflavin (TrRF)<sup>28</sup> were prepared according to the previously reported methods. The solvents used in the chromatographic experiments were of HPLC grade. The racemates were commercially available or were prepared by the usual or reported methods.<sup>29-31</sup> The synthesis of new [6]helicene derivatives (26–28) will be reported elsewhere. Porous spherical silica gel (Daiso gel SP-120-7P, N-silica) with a mean particle size of 7 μm and a mean pore diameter of 12 nm was kindly supplied from Daicel (Tokyo, Japan).

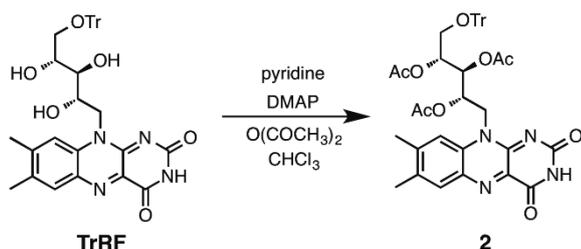
## Synthesis

**Synthesis of Compound 1.** The synthesis of 1 was carried out according to Scheme 1. To a solution of RF (1.08 g, 2.87 mmol) in anhydrous DMF (400 mL) were added 3-(triethoxysilyl)propyl isocyanate (710 μL, 2.88 mmol) and triethylamine (400 μL, 2.87 mmol) under nitrogen. The reaction mixture was stirred at 80 °C for 24 h, and then the solvent was evaporated to give 1 (1.71 g). The residue was used for the next reaction without further purification (see below for immobilization of the compound 1 on silica gel, Scheme 8). Among four possible carbamate derivatives, the main-product was found to be the 5'-carbamated riboflavin (Scheme 1) produced by the reaction at the primary hydroxy group at the 5'-O-position and its regioselectivity was about 80% estimated from its <sup>1</sup>H NMR spectrum (see Spectrum S1 in the Supporting Information (SI)).



Scheme 1. Synthesis of 1.

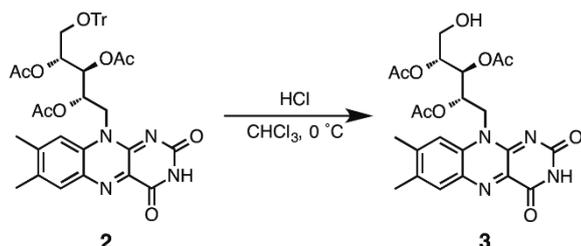
**Synthesis of Compound 2.** The synthesis of **2** was carried out according to Scheme 2. To a solution of **TrRF** (1.10 g, 2.78 mmol), which had been prepared according to the reported method,<sup>28</sup> and DMAP (261 mg, 2.09 mmol) in  $\text{CHCl}_3$  (93 mL) were added anhydrous pyridine (13 mL) and acetic anhydride (71 mL) under nitrogen. After the reaction mixture was stirred at room temperature for 24 h, to this was further added acetic anhydride (20 mL) and anhydrous pyridine (3.8 mL). After stirring at room temperature for 24 h, the solvents were evaporated. The residue was dissolved in EtOAc (80 mL) and the solution was washed with brine (50 mL x 3) and then dried over anhydrous  $\text{MgSO}_4$ . After filtration, the solvent was evaporated to dryness. The residue was purified by column chromatography ( $\text{SiO}_2$ , EtOAc–*n*-hexane = 0:10 to 5:5, 9:1 (v/v)) and then subjected to SEC fractionation to give **2** as an orange solid (3.2 g, 88% yield).



**Scheme 2.** Synthesis of **2**.

Spectroscopic data of **2**:  $^1\text{H NMR}$  (500 MHz,  $\text{CDCl}_3$ , 25 °C):  $\delta$  8.34 (s, CONH, 1H), 8.04 (s, ArH, 1H), 7.56 (s, ArH, 1H), 7.42-7.38 (m, OCArH, 9H), 7.24-7.13 (m, OCArH, 6H), 5.69-5.59 (m,  $\text{NCH}_2\text{CH}$ ,  $\text{NCH}_2\text{CHCH}$ , 2H), 5.38-5.34 (m,  $\text{NCH}_2\text{CHCHCH}$ , 1H), 3.46-3.16 (m,  $\text{OCOCH}_2$ , 2H), 2.53 (s,  $\text{ArCH}_3$ , 3H), 2.44 (s,  $\text{ArCH}_3$ , 3H), 2.37 (s,  $\text{CH}_3\text{COO}$ , 3H), 1.90 (s,  $\text{CH}_3\text{COO}$ , 3H), 1.68 (s,  $\text{CH}_3\text{COO}$ , 3H).

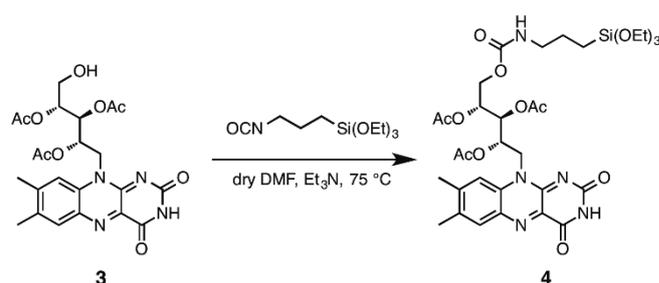
**Synthesis of Compound 3.** The compound **3** was prepared according to Scheme 3. The compound **2** (2.19 g, 2.94 mmol) was dissolved in anhydrous  $\text{CHCl}_3$  (34 mL) under nitrogen and the solution was stirred at 0 °C for 30 min. To this was added diethyl ether containing HCl (1.0 M) (11 mL) and the reaction mixture was stirred at 0 °C for 45 min so as to remove the trityl group. The solution was then neutralized with aqueous  $\text{NaHCO}_3$  (50 mL) and extracted with  $\text{CHCl}_3$  (20 mL). The organic layer was washed with water (50 mL) and brine (50 mL), and then dried over anhydrous  $\text{MgSO}_4$ . After filtration, the solvent was evaporated to dryness. The residue was purified by column chromatography ( $\text{SiO}_2$ , MeOH– $\text{CHCl}_3$  = 0:10 to 1:9 (v/v)) to give **3** as a yellow solid (1.11 g, 75% yield).



**Scheme 3.** Synthesis of **3**.

Spectroscopic data of **3**:  $^1\text{H NMR}$  (500 MHz,  $\text{CDCl}_3$ , rt.):  $\delta$  8.08 (s, ArH, 1H), 7.76 (s, ArH, 1H), 5.73-5.65 (m,  $\text{NCH}_2\text{CH}$ , 1H), 5.30-5.24 (m,  $\text{NCH}_2\text{CHCH}$ , 1H), 4.30-4.25 (m,  $\text{OHCH}_2$ , 2H), 3.64 (s,  $\text{NCH}_2\text{CHCHCH}$ , 1H), 2.58 (s,  $\text{ArCH}_3$ , 3H), 2.47 (s,  $\text{ArCH}_3$ , 3H), 2.12 (s,  $\text{CH}_3\text{COO}$ , 3H), 2.09 (s,  $\text{CH}_3\text{COO}$ , 3H), 1.97 (s,  $\text{CH}_3\text{COO}$ , 3H).

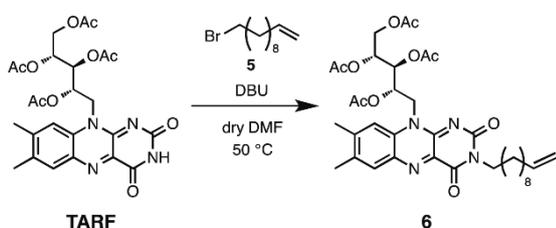
**Synthesis of Compound 4.** The synthesis of **4** was carried out according to Scheme 4. To a solution of **3** (502 mg, 1.00 mmol) in anhydrous DMF (10 mL) were added 3-(triethoxysilyl)propyl isocyanate (737  $\mu\text{L}$ , 2.98 mmol) and triethylamine (413  $\mu\text{L}$ , 2.98 mmol) under nitrogen. After the reaction mixture was stirred at 75 °C for 24 h, the solvents were evaporated. The residue was washed with *n*-hexane (200 mL) and purified by column chromatography ( $\text{SiO}_2$ , EtOAc–*n*-hexane = 0:10 to 5:5, 9:1 (v/v)) and then subjected to SEC fractionation to give **4** as an orange solid (280 mg, 38% yield).



**Scheme 4.** Synthesis of **4**.

Spectroscopic data of **4**: Mp: 206.7 °C (dec); IR (KBr,  $\text{cm}^{-1}$ ): 3173, 3019, 2810, 1742, 1663, 1579, 1542, 1226, 104;  $^1\text{H NMR}$  (500 MHz,  $\text{CDCl}_3$ , 25 °C):  $\delta$  8.50 (s, CONH, 1H), 8.03 (s, ArH, 1H), 7.57 (s, ArH, 1H), 5.72-5.62 (m,  $\text{NCH}_2\text{CH}$ , 1H), 5.48-5.38 (m,  $\text{NCH}_2\text{CHCH}$ ,  $\text{NCH}_2\text{CHCHCH}$ , 2H), 4.46-4.21 (m,  $\text{OCOCH}_2$ , 2H), 3.86-3.78 (q,  $J = 7.1$  Hz,  $\text{SiOCH}_2$ , 6H), 3.35-3.25 (t,  $J = 6.8$  Hz,  $\text{OCONHCH}_2$ , 2H), 2.57 (s,  $\text{ArCH}_3$ , 3H), 2.45 (s,  $\text{ArCH}_3$ , 3H), 2.29 (s,  $\text{CH}_3\text{COO}$ , 3H), 2.22 (s,  $\text{CH}_3\text{COO}$ , 3H), 2.08 (s,  $\text{CH}_3\text{COO}$ , 3H), 1.74-1.71 (m,  $\text{OCONHCH}_2\text{CH}_2$ , 2H), 1.31-1.20 (t,  $\text{SiOCH}_2\text{CH}_3$ , 9H), 0.70-0.65 (m,  $\text{SiCH}_2$ , 2H);  $^{13}\text{C NMR}$  (125 MHz,  $\text{CDCl}_3$ , 25 °C): 170.77, 170.44, 170.03, 169.88, 159.42, 154.49, 150.87, 148.28, 137.16, 136.20, 134.79, 133.14, 131.38, 115.67, 70.63, 69.59, 69.16, 62.03, 58.62, 45.54, 45.18, 25.26, 21.61, 21.19, 20.94, 20.47, 19.60, 18.42, 7.70; HRMS (ESI+):  $m/z$  calcd for  $\text{C}_{33}\text{H}_{47}\text{N}_5\text{O}_{13}\text{Si}$  ( $M + \text{Na}^+$ ) 772.2837; found 772.2803.

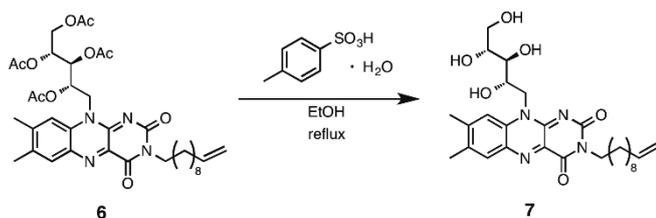
**Synthesis of Compound 6.** The compound **6** was prepared according to Scheme 5. To a solution of **5** (4.4 mL, 20 mmol) and **TARF** (2.20 g, 4.04 mmol)<sup>26</sup> in anhydrous DMF (190 mL) was added DBU (1.2 mL, 8.0 mmol) under nitrogen. After the reaction mixture was stirred at 50 °C for 20 h, the solvent was evaporated, and the residue was washed with *n*-hexane (100 mL x 2). The residue was then dissolved in  $\text{CHCl}_3$  (250 mL) and the solution was washed with water (300 mL x 4), and then dried over anhydrous  $\text{MgSO}_4$ . After filtration, the solvent was evaporated to dryness, and the residue was purified by column chromatography ( $\text{SiO}_2$ ,  $\text{CHCl}_3$ –*n*-hexane = 1:1 to 1:0 (v/v)) then MeOH/ $\text{CHCl}_3$  = 0/100 to 5/95 (v/v)) and MeOH– $\text{CHCl}_3$  = 0:10 to 1:9 (v/v)) to give **6** as an orange solid (2.1 g, 73% yield).



**Scheme 5.** Synthesis of **6**.

Spectroscopic data of **6**: Mp: 67.5-69.3 °C; IR (KBr,  $\text{cm}^{-1}$ ): 2927, 2855, 1751, 1663, 1588, 1551, 1221, 1050;  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ , 25 °C):  $\delta$  8.03 (s, ArH, 1H), 7.53 (s, ArH, 1H), 5.86-5.76 (ddt,  $J = 17.1, 10.3, 6.7$  Hz,  $\text{CH}_2\text{CH}=\text{CH}_2$ , 1H), 5.72-5.62 (m,  $\text{NCH}_2\text{CH}$ , 1H), 5.50-5.44 (m,  $\text{NCH}_2\text{CHCH}$ , 1H), 5.44-5.37 (m,  $\text{NCH}_2\text{CHCHCH}$ , 1H), 5.02-4.95 (m,  $\text{CH}=\text{CH}_2\text{H}_E$ , 1H), 4.94-4.89 (m,  $\text{CH}=\text{CH}_2\text{H}_Z$ , 1H), 4.47-4.21 (m,  $\text{OCOCH}_2$ , 2H), 4.09-4.12 (t,  $J = 7.6$  Hz,  $\text{NCH}_2\text{C}_8\text{H}_{16}\text{CH}=\text{CH}_2$ , 2H), 2.55 (s,  $\text{ArCH}_3$ , 3H), 2.44 (s,  $\text{ArCH}_3$ , 3H), 2.30 (s,  $\text{CH}_3\text{COO}$ , 3H), 2.22 (s,  $\text{CH}_3\text{COO}$ , 3H), 2.08 (s,  $\text{CH}_3\text{COO}$ , 3H), 2.05-2.00 (m,  $\text{CH}_2\text{CH}=\text{CH}_2$ , 2H), 1.73 (s,  $\text{CH}_3\text{COO}$ , 3H), 1.74-1.66 (m,  $\text{NCH}_2\text{C}_7\text{H}_{14}$ , 2H), 1.40-1.26 (m,  $\text{NCH}_2\text{C}_7\text{H}_{14}$ , 12H);  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ , 25 °C):  $\delta$  170.76, 170.45, 170.02, 169.79, 159.75, 155.14, 149.26, 147.45, 139.43, 136.54, 135.97, 134.78, 133.08, 131.29, 115.45, 114.20, 70.54, 69.57, 69.13, 62.03, 44.55, 42.26, 33.96, 29.61, 29.57, 29.49, 29.26, 29.08, 27.90, 27.14, 21.53, 21.21, 20.95, 20.84, 20.47, 19.56; HRMS (ESI+):  $m/z$  calcd for  $\text{C}_{36}\text{H}_{48}\text{N}_4\text{O}_{10}$  ( $\text{M} + \text{Na}^+$ ) 719.3268; found 719.3286.

**Synthesis of Compound 7.** The synthesis of **7** was carried out according to Scheme 6. The compound **6** (7.97 g, 11.4 mmol) and *p*-toluenesulfonic acid monohydrate (6.61 g, 34.7 mmol) were dissolved in EtOH (820 mL) and the solution was refluxed for 16 h under nitrogen. The reaction mixture was then cooled to -20 °C. The precipitated solid was collected by filtration and washed with EtOH (60 mL) to give **7** as an orange solid (3.94 g, 65% yield).

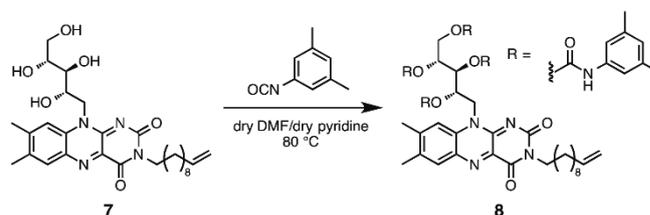


**Scheme 6.** Synthesis of **7**.

Spectroscopic data of **7**: Mp: 164.0-167.9 °C; IR (KBr,  $\text{cm}^{-1}$ ): 3400, 2925, 1546;  $^1\text{H}$  NMR (500 MHz,  $\text{DMSO}-d_6$ , 25 °C):  $\delta$  7.94 (s, ArH, 1H), 7.93 (s, ArH, 1H), 5.84-5.72 (ddt,  $J = 17.3, 10.1, 6.9$  Hz,  $\text{CH}_2\text{CH}=\text{CH}_2$ , 1H), 5.18-5.05 (s, OH, 1H), 5.02-4.95 (m,  $\text{CH}=\text{CH}_2\text{H}_E$ , 1H), 4.94-4.89 (m,  $\text{CH}=\text{CH}_2\text{H}_Z$ , 1H), 4.89-4.80 (m,  $\text{NCH}_2\text{CH}$ , OH, 2H), 4.79-4.77 (m, OH, 1H), 4.64-4.60 (m,  $\text{NCH}_2\text{CHCH}$ , 1H), 4.51-4.49 (m, OH, 1H), 4.30-4.21 (m,  $\text{NCH}_2\text{CHCHCH}$ , 1H), 3.90-3.83 (t,  $J = 7.6$  Hz,  $\text{NCH}_2\text{C}_8\text{H}_{16}\text{CH}=\text{CH}_2$ , 2H), 3.70-3.42 (m,  $\text{NCH}_2$ ,  $\text{OCOCH}_2$ , 4H), 2.48 (s,  $\text{ArCH}_3$ , 3H), 2.40 (s,  $\text{ArCH}_3$ , 3H), 2.05-1.95 (m,  $\text{CH}_2\text{CH}=\text{CH}_2$ , 2H), 1.62-1.18 (m,  $\text{NCH}_2\text{C}_7\text{H}_{14}$ , 14H);  $^{13}\text{C}$  NMR (125 MHz,  $\text{DMSO}-d_6$ , 25 °C): 159.41, 154.65, 149.42, 146.12, 138.82, 135.86, 135.79, 134.28, 132.05, 130.68, 117.41, 114.62, 114.60, 73.55, 72.78, 68.79,

63.41, 47.07, 33.16, 28.90, 28.81, 28.77, 28.50, 28.26, 27.34, 26.45, 20.77, 18.76; HRMS (ESI+):  $m/z$  calcd for  $\text{C}_{28}\text{H}_{40}\text{N}_4\text{O}_6$  ( $\text{M} + \text{Na}^+$ ) 551.2846; found 551.2863.

**Synthesis of Compound 8.** The compound **8** was prepared according to Scheme 7. To a solution of **7** (1.17 g, 2.22 mmol) in anhydrous DMF (100 mL) were added anhydrous pyridine (60 mL) and 3,5-dimethylphenyl isocyanate (2.0 mL, 14 mmol) under nitrogen. The reaction mixture was stirred at 80 °C for 20 h, and then the solvents were evaporated. The residue was dissolved in  $\text{CHCl}_3$  (100 mL) and the solution was washed with water (150 mL x 3), and then dried over  $\text{Na}_2\text{S}_2\text{O}_4$ . After filtration, the solvent was evaporated to dryness. The residue was purified by column chromatography ( $\text{SiO}_2$ ,  $\text{EtOAc}-n$ -hexane = 1:9 to 4:6 (v/v)) and then subjected to SEC fractionation to give **8** as a yellow solid (1.3 g, 54% yield).

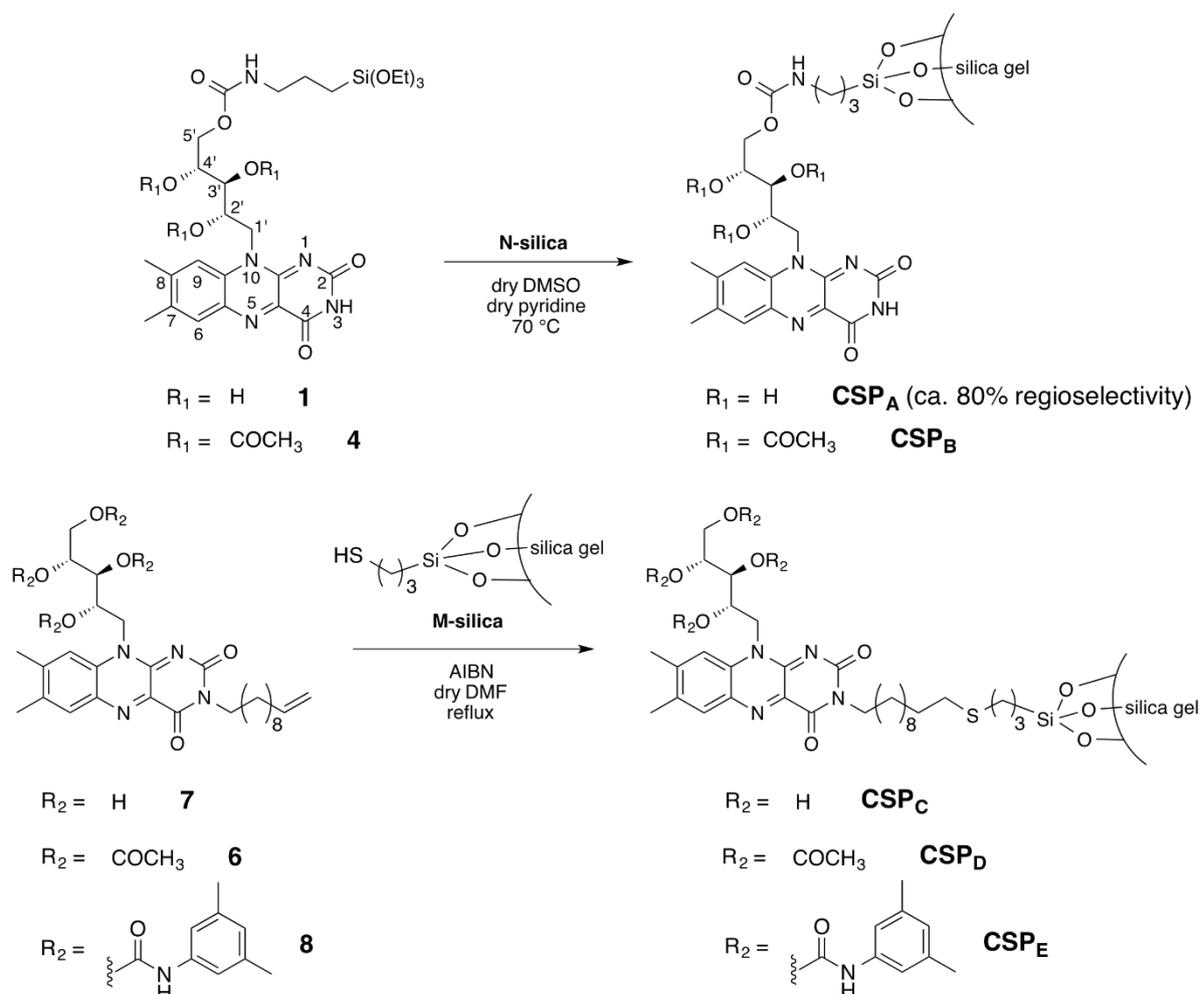


**Scheme 7.** Synthesis of **8**.

Spectroscopic data of **8**: Mp: 217.8-219.0 °C; IR (KBr,  $\text{cm}^{-1}$ ): 3313, 2924, 1735, 1650, 1585, 1549, 1216;  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ , 25 °C):  $\delta$  7.99 (s, ArH, 1H), 7.63 (s, ArH, 1H), 7.21 (s,  $\text{OCONHArH}$ , 2H), 7.05 (s,  $\text{OCONHArH}$ , 2H), 6.85 (s,  $\text{OCONHArH}$ , 2H), 6.70 (s,  $\text{OCONHArH}$ , 1H), 6.67 (s,  $\text{OCONHArH}$ , 1H), 6.65 (s,  $\text{OCONHArH}$ , 1H), 6.60 (s,  $\text{OCONHArH}$ , 2H), 6.48 (s,  $\text{OCONHArH}$ , 2H), 6.00-5.91 (br,  $\text{NCH}_2\text{CH}$ , 1H), 5.86-5.77 (ddt,  $J = 17.1, 10.5, 6.7$  Hz,  $\text{CH}_2\text{CH}=\text{CH}_2$ , 1H), 5.61-5.44 (m,  $\text{NCH}_2\text{CHCH}$ ,  $\text{NCH}_2\text{CHCHCH}$ , 2H), 5.02-4.95 (m,  $\text{CH}=\text{CH}_2\text{H}_E$ , 1H), 4.94-4.89 (m,  $\text{CH}=\text{CH}_2\text{H}_Z$ , 1H), 4.76-4.38 (m,  $\text{OCOCH}_2$ , 2H), 4.52-4.48 (br,  $\text{NCH}_2$ , 2H), 4.11-3.88 (m,  $\text{NCH}_2\text{C}_8\text{H}_{16}\text{CH}=\text{CH}_2$ , 2H), 2.40 (s,  $\text{ArCH}_3$ , 3H), 2.29-2.24 (m,  $\text{OCONHArCH}_3$ , 12H), 2.21-2.12 (m,  $\text{OCONHArCH}_3$ , 12H), 2.04-1.98 (m,  $\text{CH}_2\text{CH}=\text{CH}_2$ , 2H), 1.69-1.24 (m,  $\text{NCH}_2\text{C}_7\text{H}_{14}$ , 14H);  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ , 25 °C): 159.91, 156.48, 153.07, 152.76, 152.72, 152.60, 148.88, 147.68, 139.42, 139.40, 138.98, 138.82, 138.63, 138.61, 137.87, 137.28, 136.66, 136.38, 135.87, 135.21, 132.76, 131.66, 126.14, 125.81, 125.28, 125.06, 122.10, 117.10, 117.01, 116.65, 116.18, 115.52, 114.22, 114.20, 107.07, 71.71, 71.24, 63.56, 46.07, 42.28, 33.96, 29.65, 29.60, 29.49, 29.26, 29.08, 27.91, 27.24, 21.56, 21.50, 21.44, 21.35, 19.41; HRMS (ESI+):  $m/z$  calcd for  $\text{C}_{64}\text{H}_{76}\text{N}_6\text{O}_{10}$  ( $\text{M} + \text{Na}^+$ ) 1139.5582; found 1139.5577.

Immobilizations of compounds **1**, **4**, **6**, **7**, and **8** on silica gel were carried out according to Scheme 8.

**Immobilization of Compounds 1 and 4 on Silica Gel.** A typical experimental procedure is described below. N-Silica (901 mg) was dispersed in a solution of **1** just after the preparation (ca. 2.8 mmol) in anhydrous DMSO (4.0 mL) and pyridine (1.5 mL) under nitrogen and the mixture was heated at 70 °C. After 25 h, anhydrous DMF (60 mL) was added to this, and the resulting



**Scheme 8.** Immobilization of **1**, **4**, **6**, **7**, and **8** on silica gel.

silica gel was collected by filtration, washed with DMF (20 mL), MeOH (60 mL), acetone (80 mL), and *n*-hexane (60 mL), and dried in vacuo at 120 °C overnight, yielding the **CSP<sub>A</sub>** (1.14 g). The content of **1** chemically bonded to silica gel was estimated by TG analysis and was 13.4 wt%. In the same way, the compound **4** was chemically bonded to N-silica (**CSP<sub>B</sub>**), and its content was estimated to be 13.2 wt%.

**Immobilization of Compounds 6, 7, and 8 on Silica Gel.** A typical experimental procedure is described below. M-silica (1.00 g, 8.3 wt%)<sup>27</sup> was dispersed in a solution of **7** (444 mg, 0.638 mmol) in THF (30 mL) under nitrogen, and to this was added AIBN (35.7 mg, 0.217 mmol). The reaction mixture was refluxed for 24 h, and AIBN (35.5 mg, 0.216 mmol) was further added. After refluxing for 15 h, the resulting silica gel was collected by filtration, washed with THF (50 mL x 2) and *n*-hexane (50 mL), and dried in vacuo at 120 °C overnight. The content of **7** chemically bonded to silica gel was estimated by TG analysis and was 11.8 wt%. In order to improve the amount of **7** bonded to silica gel, the immobilization procedure was repeated two times, giving **CSP<sub>C</sub>** (0.92 g); the content of **7** was 18.4 wt% thus estimated by TG analysis. In the same way, the compounds **6** and **8** were chemically bonded to M-silica, and their contents were estimated to be 15.9 (**CSP<sub>D</sub>**) and 24.7 wt% (**CSP<sub>E</sub>**), respectively.

**Preparation of Chiral Columns.** Each column packing material was packed into a stainless-steel tube (25 cm x 0.20 cm (i.d.)) by conventional high-pressure slurry packing technique using a Chemco Slurry-Packing Apparatus Model 124A (Chemco, Osaka, Japan).<sup>29</sup> The plate numbers of the columns were 1500–2200 for benzene with *n*-hexane–2-propanol (90:10, v/v) as the eluent at a flow rate of 0.1 mL/min. The dead time ( $t_0$ ) was estimated using 1,3,5-tri-*tert*-butylbenzene as the nonretained compound.<sup>32</sup> The CSPs are stable and maintained their chiral recognition abilities at least for more than one month.

## Results and Discussion

### Synthesis of Riboflavin Derivatives and Immobilization on Silica Gel.

In order to regioselectively immobilize natural riboflavin and its derivatives (**1**, **4**, **6–8**) on the silica gel (7 μm particle size, 12 nm pore size) surface via chemical linkages, a 3-(triethoxysilyl)propylcarbamate or a 1-undecenyl residue was introduced at the 5'-O- (**1** and **4**) or 3-N-position (**6–8**) of the riboflavins, respectively (Scheme 8). The precursors were then allowed to react with unmodified silica gel in DMSO in the presence of pyridine or (3-mercaptopropyl)triethoxy silanized silica gel in DMF in the presence of AIBN,<sup>27</sup> producing the riboflavin-based CSPs regioselectively immobilized on the silica



**TABLE 1.** Chromatographic resolution results of racemates on riboflavin-based CSPs with *n*-hexane–2-propanol as the eluent<sup>a</sup>

Racemates	CSP <sub>A</sub>			CSP <sub>B</sub>			CSP <sub>C</sub>		
	<i>k</i> <sub>1</sub>	$\alpha$	<i>R</i> <sub>s</sub>	<i>k</i> <sub>1</sub>	$\alpha$	<i>R</i> <sub>s</sub>	<i>k</i> <sub>1</sub>	$\alpha$	<i>R</i> <sub>s</sub>
<b>13</b>	2.77	1	—	3.05	1	—	2.65	1	—
<b>16</b>	11.8	1	—	16.8	1	—	9.38	1	—
<b>18</b>	4.29	1.11 (–)	0.60	4.49	1.05 (–)	0.48	2.54	ca. 1 (–)	—
<b>19</b>	3.18	1	—	3.35	1	—	5.89	1	—
<b>20</b>	2.51	1.04 (–)	0.27	1.78	1.02 (–)	0.22	20.4	1	—
<b>21<sup>b</sup></b>	6.73	ca. 1 (+)	—	7.83	ca.1 (+)	—	4.89	ca. 1 (–)	—
<b>22<sup>b</sup></b>	4.93	1.06 (+)	0.14	4.93	1.07 (+)	0.17	5.23	ca. 1 (–)	—
<b>23<sup>b</sup></b>	21.3	ca. 1 (–)	—	20.5	ca. 1 (–)	—	16.0	1.54 (–)	1.54
<b>24</b>	3.00	ca. 1 (–)	—	2.98	ca. 1 (–)	—	1.67	ca. 1 (–)	—
<b>30</b>	3.49	1.07 (–)	0.28	2.89	ca. 1 (–)	—	2.02	ca. 1 (–)	—

Racemates	CSP <sub>D</sub>			CSP <sub>E</sub>		
	<i>k</i> <sub>1</sub>	$\alpha$	<i>R</i> <sub>s</sub>	<i>k</i> <sub>1</sub>	$\alpha$	<i>R</i> <sub>s</sub>
<b>13</b>	3.00	ca. 1 (–)	—	1.25	1	—
<b>16</b>	8.17	1	—	5.26	1.11 (–)	1.02
<b>18</b>	2.04	1.05 (–)	0.23	0.78	1.38 (–)	1.28
<b>19</b>	2.31	1	—	2.11	1.12 (–)	0.46
<b>20</b>	1.66	1.11 (–)	0.30	1.54	1	—
<b>21<sup>b</sup></b>	5.30	ca. 1 (–)	—	2.69	ca. 1 (+)	—
<b>22<sup>b</sup></b>	6.71	ca. 1 (–)	—	2.31	1.25 (–)	0.66
<b>23<sup>b</sup></b>	32.2	1.27 (+)	0.79	5.82	1.49 (–)	1.26
<b>24</b>	1.93	ca. 1 (+)	—	2.12	ca. 1 (–)	—
<b>30</b>	2.35	ca. 1 (+)	—	2.44	ca. 1 (–)	—

<sup>a</sup>Conditions: column, 25 x 0.20 (i.d.) cm; eluent, *n*-hexane–2-propanol (90:10); flow rate, 0.1 mL/min; temperature, 25 °C. The signs in parentheses represent the CD detection (254 nm) of the first-eluted enantiomer. <sup>b</sup>Eluent, *n*-hexane–2-propanol (80:20); flow rate, 0.5 mL/min.

cyclic dianilide **18** (Fig. 2) with high  $\alpha$  values ( $\alpha = 1.11$  and  $1.38$ , respectively) probably through an intermolecular hydrogen bond formation between the polar carbamate residues introduced at the ribityl group of the CSP<sub>E</sub> and the hydroxy or amide residues of the analytes, while the CSP<sub>A</sub>, CSP<sub>B</sub>, and CSP<sub>D</sub> only partially resolved **18** (Table S1). A similar trend was observed in the *n*-hexane–CH<sub>2</sub>Cl<sub>2</sub> system for CSP<sub>E</sub> that partially separated the bulky aromatic alcohols **12** and **16**. In contrast to the resolutions in *n*-hexane–2-propanol (90:10, v/v), the CSP<sub>A</sub> and CSP<sub>B</sub> immobilized at the 5'-O-position partially resolved the Tröger base (**10**) in addition to **16** in *n*-hexane–CH<sub>2</sub>Cl<sub>2</sub> (90:10, v/v) (Table S2), indicating that not only hydrogen bonding, but also hydrophobic or  $\pi$ -stacking interactions may contribute during the chiral recognition process in this eluent system. The CSP<sub>C</sub> bearing the free ribityl group showed a poor chiral recognition in both eluent systems and could not separate the ten tested racemates. It should be noted that the bulky aromatic racemic alcohols with an acidic hydroxy group **13** and **16** strongly interacted with the five CSPs in *n*-hexane–CH<sub>2</sub>Cl<sub>2</sub>, in particular on the CSP<sub>C</sub> and CSP<sub>D</sub>, **16** could not elute ( $k_1 > 100$ ) under the present conditions.

Based on the above results together with the previously reported intriguing chiral recognition abilities of riboflavin<sup>20</sup> and FMN<sup>21</sup> for the racemic helicenes and SWCNTs, respectively, we employed 13 racemates (**19–31**) (Chart 1) including the cyclic dibenzamide (**19**) and dianilide (**20**), bulky aromatic racemic alcohols (**21–23**), and helicene derivatives including the [6] and [7]helicenes (**24–30**) and a metal-containing [8]helicene analog (**31**) to further evaluate the specific chiral recognition abilities of the riboflavin-based CSPs in *n*-hexane–2-propanol and *n*-hexane–CH<sub>2</sub>Cl<sub>2</sub> eluent systems; the chromatographic resolution results showing a more or less enantioselectivity ( $\alpha > 1$ ) on either CSP for the racemates are summarized in Table 1 and Table 2, respectively. For comparison, some resolution results in Tables S1 and S2 are also shown.

**TABLE 2.** Chromatographic resolution results of racemates on riboflavin-based CSPs with *n*-hexane–CH<sub>2</sub>Cl<sub>2</sub> as the eluent<sup>a</sup>

As expected, an analogous cyclic dibenzamide (**19**) and more bulky aromatic alcohols (**22** and **23**) were partially or almost completely resolved on the phenylcarbamated CSP<sub>E</sub> in *n*-hexane–2-propanol (Table 1). The four-membered cyclic dianilide (**20**) was not resolved on CSP<sub>E</sub>, but **20** as well as the bulky alcohols (**22** or **23**) were partially resolved on CSP<sub>A</sub>, CSP<sub>B</sub>, and CSP<sub>D</sub> and the reversed elution order was observed for **23** on CSP<sub>D</sub>. The fact that the relatively less bulky 2,2'-dihydroxy-6,6'-dimethylbiphenyl (**13**) and 1,1'-bi-2-naphthol (**21**) were not separated at all on all of the CSPs independent of the eluents (Tables 1 and 2) clearly indicated the important role of the steric or bulky effect, in other words, the molecular size for the efficient separation of aromatic racemic alcohols on the riboflavin-based CSPs. A similar molecular size effect was suggested by Gil-Av during the separation of a series of achiral polyaromatic compounds on silica gel coated with natural riboflavin.<sup>20</sup> Interestingly, CSP<sub>C</sub> exhibited an exceptionally high chiral recognition ability only for the bulky racemic alcohol **23** ( $\alpha = 1.54$ ) and completely resolved it (Fig. 3B), although the CSP<sub>C</sub> showed a poor chiral recognition for the other racemates in both eluent systems. In addition, most of the CSPs could not resolve the polycyclic fully aromatic [6] and [7]helicenes (**24** and **30**) except for CSP<sub>A</sub> bearing mostly free hydroxy groups at the ribityl chain, which partially separated [7]helicene (**30**) into enantiomers.

Interestingly, all of the eight helicene derivatives (**24–31**) were efficiently recognized by the 5'-O-bonded CSP<sub>A</sub> and CSP<sub>B</sub> except for **29** on CSP<sub>B</sub> in *n*-hexane–CH<sub>2</sub>Cl<sub>2</sub>, showing not complete, but partial separations in the same elution order, while interacting strongly with the CSPs as supported by high  $k_1$  values (Table 2). However, CSP<sub>C</sub> and CSP<sub>E</sub> immobilized at the 3-N-position could not resolve the helicenes at all, indicating that the interaction of the original isoalloxazine ring of CSP<sub>A</sub> and CSP<sub>B</sub> with the polyaromatic helicenes may be the major driving force for their efficient chiral recognition of the helicenes rather

than the ribityl moiety, since **CSP<sub>A</sub>** and **CSP<sub>C</sub>** possess the same chirality of the ribityl chain is essential for the chiral recognition of

Racemates	<b>CSP<sub>A</sub></b>			<b>CSP<sub>B</sub></b>			<b>CSP<sub>C</sub></b>		
	$k_1$	$\alpha$	$R_s$	$k_1$	$\alpha$	$R_s$	$k_1$	$\alpha$	$R_s$
<b>10</b>	10.5	1.03 (+)	0.23	11.2	1.03 (+)	0.09	3.93	1	—
<b>12</b>	2.80	1	—	0.76	1	—	3.88	1	—
<b>13</b>	36.9	1	—	18.6	ca. 1 (+)	—	40.9	1	—
<b>13<sup>b</sup></b>	16.8	ca. 1 (+)	—	—	—	—	13.8	ca. 1 (—)	—
<b>16</b>	88.3	1.17 (—)	0.28	90.0	1.12 (—)	0.13	>100	—	—
<b>16<sup>b</sup></b>	65.7	1	—	—	—	—	—	—	—
<b>16<sup>d</sup></b>	17.1	1	—	18.4	1	—	9.61	1	—
<b>21</b>	15.3	1	—	1.68	1	—	1.34	1	—
<b>22<sup>c</sup></b>	29.8	1.07 (+)	0.28	19.9	1.10 (+)	0.26	5.82	ca. 1 (+)	—
<b>22<sup>d</sup></b>	4.07	1.08 (+)	0.32	—	—	—	—	—	—
<b>23<sup>c</sup></b>	>100	—	—	>100	—	—	31.4	1.15 (—)	0.54
<b>23<sup>d</sup></b>	20.1	1	—	16.5	ca. 1 (+)	—	11.6	1.28 (—)	1.23
<b>24<sup>c</sup></b>	3.37	1.05 (+)	0.26	2.90	1.06 (+)	0.28	0.87	ca. 1 (+)	—
<b>25</b>	3.66	1.04 (—)	0.26	3.46	1.05 (—)	0.23	2.92	ca. 1 (—)	—
<b>26</b>	25.0	1.08 (—)	0.50	21.5	1.04 (—)	0.42	33.0	ca. 1 (—)	—
<b>26<sup>b</sup></b>	4.43	1.07 (—)	0.43	3.05	1.09 (—)	0.44	0.89	ca. 1 (—)	—
<b>27</b>	21.0	1.07 (+)	0.24	18.9	1.06 (+)	0.22	52.8	ca. 1 (—)	—
<b>27<sup>b</sup></b>	7.71	1.03 (+)	0.18	—	—	—	1.56	ca. 1 (—)	—
<b>28</b>	30.4	1.09 (—)	0.52	24.1	1.05 (—)	0.47	36.7	ca. 1 (—)	—
<b>28<sup>b</sup></b>	4.47	1.07 (—)	0.35	2.91	1.08 (—)	0.35	0.81	ca. 1 (—)	—
<b>29</b>	4.45	1.08 (—)	0.32	3.54	ca. 1 (—)	—	3.39	ca. 1 (—)	—
<b>30</b>	>100	—	—	>100	—	—	5.09	1	—
<b>30<sup>c</sup></b>	5.29	1.07 (+)	0.34	5.30	1.07 (+)	0.35	1.21	1	—
<b>31<sup>e</sup></b>	—	—	—	—	—	—	—	—	—
<b>31<sup>c</sup></b>	6.66	1.16 (+)	0.38	6.40	1.10 (+)	0.62	7.00	ca. 1 (+)	—

Racemates	<b>CSP<sub>D</sub></b>			<b>CSP<sub>E</sub></b>		
	$k_1$	$\alpha$	$R_s$	$k_1$	$\alpha$	$R_s$
<b>10</b>	1.66	1	—	5.45	1	—
<b>12</b>	4.55	1	—	3.05	1.07 (—)	0.51
<b>13</b>	18.9	1	—	19.3	1	—
<b>16</b>	>100	—	—	71.0	1.14 (—)	0.38
<b>16<sup>d</sup></b>	9.22	1	—	16.0 <sup>c</sup>	1.09 (—) <sup>c</sup>	0.44 <sup>c</sup>
<b>21</b>	56.8	ca. 1 (—)	—	0.96	1	—
<b>21<sup>d</sup></b>	8.81	ca. 1 (—)	—	—	—	—
<b>22<sup>c</sup></b>	6.58	ca. 1 (—)	—	2.37	1.26 (—)	0.65
<b>23<sup>c</sup></b>	26.1	1.27 (+)	0.94	5.87	1.49 (—)	1.36
<b>23<sup>d</sup></b>	14.1	1.22 (+)	0.62	—	—	—
<b>24<sup>c</sup></b>	0.80	ca. 1 (—)	—	0.70	ca. 1 (—)	—
<b>25</b>	2.28	1.13 (+)	1.13	2.04	ca. 1 (—)	—
<b>26</b>	1.30	ca. 1 (+)	—	16.7	ca. 1 (+)	—
<b>27</b>	1.86	1.17 (—)	0.60	20.3	ca. 1 (—)	—
<b>27<sup>b</sup></b>	—	—	—	4.14	ca. 1 (—)	—
<b>28</b>	1.43	ca. 1 (+)	—	14.2	ca. 1 (—)	—
<b>29</b>	1.92	1.05 (+)	0.21	2.32	ca. 1 (—)	—
<b>30</b>	4.29	1.09 (—)	0.86	3.41	1	—
<b>30<sup>c</sup></b>	1.00	ca. 1 (—)	—	0.87	1	—
<b>31<sup>e</sup></b>	10.4	1.36 (—)	1.92	—	—	—
<b>31<sup>c</sup></b>	4.86	1.27 (—)	1.56	2.26	ca. 1 (+)	—

<sup>a</sup>Conditions: column, 25 x 0.20 (i.d.) cm; eluent, *n*-hexane–CH<sub>2</sub>Cl<sub>2</sub> (90:10); flow rate, 0.1 mL/min; temperature, 25 °C. The signs in parentheses represent the CD detection (254 nm) of the first-eluted enantiomer except for **24**, **30**, and **31**, which were detected at 350 nm. <sup>b</sup>Eluent, *n*-hexane–CH<sub>2</sub>Cl<sub>2</sub> (90:10) containing 1% 2-propanol; flow rate, 0.1 mL/min. <sup>c</sup>Eluent, *n*-hexane–CH<sub>2</sub>Cl<sub>2</sub> (80:20); flow rate, 0.5 mL/min. <sup>d</sup>Eluent, *n*-hexane–CH<sub>2</sub>Cl<sub>2</sub> (80:20) containing 1% 2-propanol; flow rate, 0.5 mL/min. <sup>e</sup>Flow rate, 0.5 mL/min.

ribityl group except for the 5'-O-position of **CSP<sub>A</sub>**. Obviously, the the present riboflavin-based CSPs. The elution order of

[7]helicene (**30**) on **CSP<sub>A</sub>** and **CSP<sub>B</sub>** was identical to that on the reported riboflavin-coated silica gel CSP,<sup>20</sup> judging from the Cotton effect sign of the first-eluted enantiomer of **30** at 350 nm.<sup>20,33</sup>

More interestingly, most of the helicene derivatives, in particular, the [6]helicene derivatives (**25** and **27**), [7]helicene (**30**), and an [8]helicene analog (**31**) were better resolved on the 3-N-bonded **CSP<sub>D</sub>** composed of the fully-acetylated ribityl residue, and **25** and **31** (Fig. 3C) were completely separated. It is noteworthy that the elution order of the helicene derivatives (**24–31**) on **CSP<sub>D</sub>** was totally reversed without exception when compared to that on **CSP<sub>A</sub>** and **CSP<sub>B</sub>** in *n*-hexane–CH<sub>2</sub>Cl<sub>2</sub> (Table 2). The difference between the acetylated **CSP<sub>B</sub>** and **CSP<sub>D</sub>** is the position immobilized on the silica gel (5'-O- and 3-N-positions, respectively). Therefore, the results reveal the critical role of the immobilization positions of the riboflavin-based CSPs during the enantioselectivity of the helicenes as well as their elution order.

Again, **CSP<sub>E</sub>** exhibited a good chiral recognition ability for bulky aromatic racemic alcohols (**22** and **23**) ( $\alpha = 1.26$  and  $1.49$ , respectively) and completely resolved **23** (Fig. 3D), although **CSP<sub>E</sub>** showed poor enantioselectivities ( $\alpha = \text{ca. } 1$ ) toward the racemic helicene derivatives. The other CSPs also separated either **22** or **23**; the latter bulky alcohol was almost base-line resolved on **CSP<sub>D</sub>** with the reversed elution order to that on **CSP<sub>E</sub>**. The retention factors of bulky racemic alcohols (**13**, **16**, **22**, and **23**) as well as helicene derivatives (**26–28**) significantly decreased when *n*-hexane–CH<sub>2</sub>Cl<sub>2</sub> (90:10 or 80/20) containing 1% 2-propanol was used as the eluent (Table 2), while the separation factors slightly decreased or were improved depending on the CSPs.

A comparison of the enantioseparation results on the five riboflavin-based CSPs summarized in Tables 1 and 2 in *n*-hexane–2-propanol and *n*-hexane–CH<sub>2</sub>Cl<sub>2</sub> eluent systems revealed their specific and relative chiral recognition ability; **CSP<sub>A</sub>** and **CSP<sub>B</sub>** chemically bonded at the 5'-O-position on the silica gel showed a relatively high chiral recognition toward the helicene derivatives via specific  $\pi$ - $\pi$  interactions including charge-transfer complexation between the unmodified isoalloxazine ring and the polyaromatic helicenes and could also partially separate some bulky aromatic alcohols and cyclic amides. On the other hand, **CSP<sub>E</sub>** bearing the 3,5-dimethylphenylcarbamated ribityl unit bonded at the 3-N-position on silica gel specifically and better resolved the bulky aromatic alcohols and cyclic amides, some of which were completely separated into enantiomers through an intermolecular hydrogen bond formation, although **CSP<sub>E</sub>** showed a poor chiral recognition ability for the helicene derivatives. The chiral recognition power of these CSPs seems to be complementary. **CSP<sub>C</sub>** immobilized at the 3-N-position on the silica gel is structurally similar to **CSP<sub>A</sub>** with respect to the ribityl pendant, but showed a poor chiral recognition ability and specifically resolved only one bulky aromatic alcohol **23**. Among the riboflavin-based CSPs, **CSP<sub>D</sub>** bearing the fully acetylated ribityl residue immobilized at the 3-N-position showed quite unique and high chiral recognition abilities for a variety of helicenes and bulky aromatic racemic alcohols with the reversed elution order compared to that on the other CSPs. The enantioselectivity and elution order of the racemates are significantly influenced not only by the position immobilized on the silica gel, but also by the substituents of the ribityl unit.

These findings will contribute to the development of more efficient CSPs composed of novel riboflavins with more suitable substituents and also riboflavin-based polymers. In fact, we recently prepared the first optically-active riboflavin-containing polymer with the fully acetylated riboflavin as the main-chain, which enantioselectively catalyzed the asymmetric oxidation of sulfides whose enantioselectivity was much higher than that catalyzed by the corresponding riboflavin monomer<sup>18</sup> and also detected the chirality of chiral primary and secondary amine vapors in the solid state.<sup>19</sup> We believe that such riboflavin-based optically-active polymers will show a better chiral recognition ability than the present riboflavin-based CSPs because such

polymers may form a preferred-handed helical structure<sup>18,34,35</sup> and the research along this line is now in progress in our laboratory.

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## Supporting information

Additional supporting information may be found in the online version of this article at the publisher's website.

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## Graphical Abstract

### Riboflavin-based Chiral Stationary Phases

