ORIGINAL ARTICLE

Chiral recognition ability of cellulose derivatives bearing pyridyl and bipyridyl residues as chiral stationary phases for high-performance liquid chromatography

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Cellulose derivatives bearing pyridyl and bipyridyl residues were synthesized, and their recognition abilities as chiral stationary phases for high-performance liquid chromatography were evaluated. Compared with cellulose derivatives bearing these residues at the 2-, 3- and 6-positions of a glucose ring, the regioselectively substituted derivatives exhibited relatively high chiral recognition. The recognition ability of the derivatives was significantly influenced by the coordination of a Cu(II) ion to the bipyridyl residues. In addition, the derivatives were also used for ligand-exchange chromatography with an eluent containing a copper salt to directly separate amino acids without derivatization.

INTRODUCTION

Chirality is an attractive property in many scientific fields, including pharmaceuticals, agrochemicals, fragrances, foods, ferroelectric liquid crystals and organic nonlinear optical molecules. Particularly, obtaining both enantiomers with high optical purity is indispensable during the development of chiral drugs, and the use of a single enantiomer is frequently required in the pharmaceutical industry.[1–8] The chiral separation of racemic compounds is a potential way to obtain pure optically active compounds.[9]

During the past three decades, direct enantioseparation using chiral stationary phases (CSPs) by high-performance liquid chromatography (HPLC) has advanced significantly as a practical method suitable for both analytical and preparative purposes.[10–18] Chiral separation by HPLC is based on the different interaction behavior between two enantiomers on the CSPs.

Among the large number of CSPs for HPLC derived from chiral small molecules and polymers with chiral recognition abilities, the benzoates and phenylcarbamates of polysaccharides, including cellulose and amylose, have been recognized as the most powerful CSPs for the resolution of a wide range of racemates.[19–25] However, not all chiral compounds can be resolved using the present polysaccharide-based CSPs. Therefore, a critical challenge to the development of novel CSPs based on polysaccharides must still be met.

In this study, cellulose derivatives bearing pyridyl **1** and bipyridyl **2–6** residues (Figures 1 and 2) were synthesized, and their recognition abilities as CSPs for HPLC were evaluated and compared with the commercially available cellulose-based CSPs. The ability to change through a coordination of Cu(II) ion to the bipyridyl groups of the derivatives **2–6** was also investigated. In addition, the derivatives bearing bipyridyl residues were applied to the resolution of amino acids in ligand-exchange chromatography.

EXPERIMENTAL PROCEDURE

Materials

Cellulose (Avicel, DP ~200) and ammonium acetate were purchased from Merck (Darmstadt, Germany). Methacrolein, formamide, pyridinium tribromide and copper(II) triflate were obtained from Aldrich (Milwaukee, WI, USA). Lithium chloride, 2-acetylpyridine, hydrobromic acid, 2-pyridinecarboxylic acid, 4-pyridinecarboxylic acid and thionyl chloride were purchased from Wako (Osaka, Japan). Benzoyl chloride, triphenylmethyl chloride, potassium permanganate, dichloromethane, 3-pyridinecarboxylic acid, acetic acid and copper(II) sulfate were from Kishida (Osaka, Japan). Triethylamine, 6-chloro-3-pyridinecarboxylic acid and oxalyl chloride were from Tokyo Kasei (Tokyo, Japan). Chiralcel OD (25×0.46 cm ID), Chiralcel OB (25×0.46 cm ID) and 3,5-dimethylphenyl isocyanate were kindly supplied by Daicel Chemical Industries (Tokyo, Japan). Macroporous spherical silica gel (Daiso gel SP-1000) with a mean particle size of 7 μm and a mean pore diameter of 100 nm was kindly

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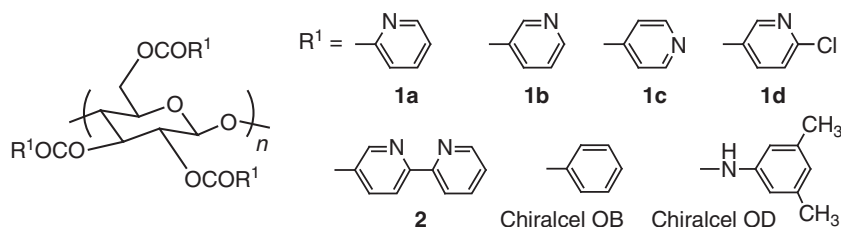E-mail: okamoto@apchem.nagoya-u.ac.jp

Figure 1 Structures of cellulose derivatives bearing pyridyl groups **1**, bipyridyl group **2**, Chiralcel OB and Chiralcel OD.
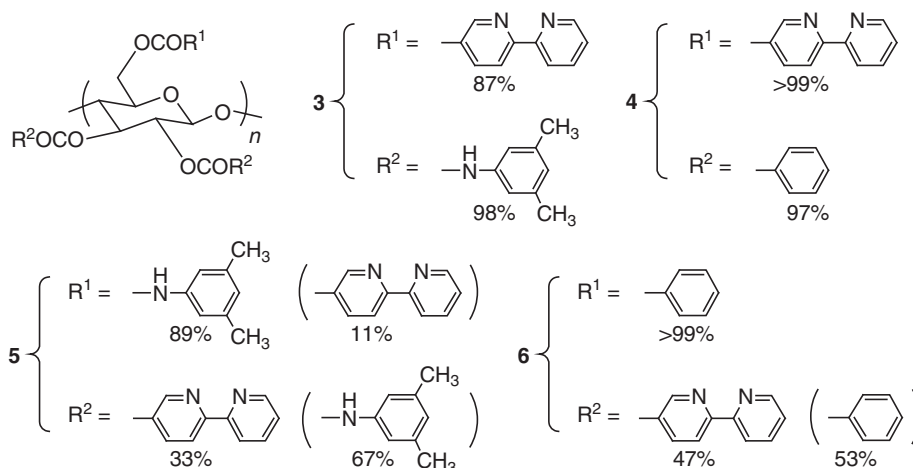

Figure 2 Structures of regioselectively substituted cellulose derivatives bearing bipyridyl group **3–6**.
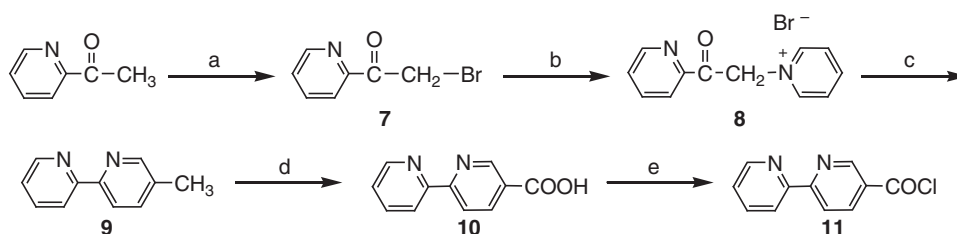

Figure 3 Synthesis of 2,2′-bipyridine-5-carboxylic acid chloride **11**. (a) Pyridinium tribromide, HBr, AcOH, 60 °C for 6 h; (b) pyridine, tetrahydrofuran, 25 °C for 6 h; (c) methacrolein, NH_4OAc, formamide, 70 °C for 6 h; (d) $KMnO_4$, H_2O, 70 °C for 3 h, 90 °C for 12 h; and (e) $SOCl_2$, 70 °C for 3 h.

supplied by Daiso Chemical (Tokyo, Japan). Pyridine, N,N-dimethylacetamide, toluene and tetrahydrofuran were purchased from Kanto (Tokyo, Japan) as anhydrous solvents. The racemates were commercially available or were prepared by the usual method.[26]

Synthesis of 2-(bromoacetyl)pyridine (7)
Following established procedures,[27,28] a solution of 2-acetylpyridine (10 g) in acetic acid (21 ml) containing 32% hydrobromic acid was cooled to 0 °C, and a solution of pyridinium tribromide (25 g) in acetic acid (300 ml) was added and allowed to react at 60 °C for 6 h (Figure 3). The reaction system was cooled to room temperature, and diethyl ether (600 ml) was added to the system. After 12 h at 0 °C, the precipitates were filtered, washed with acetone and vacuum-dried to yield **7** as a yellow solid (21 g, >99%). The product **7** was used in the next step without further purification.

Synthesis of N-(2-pyridylcarbonylmethyl)pyridinium bromide (8)
Pyridine (10 ml) was added to a dispersion of compound **7** (20 g) in tetrahydrofuran (350 ml) under a nitrogen atmosphere, and the reaction mixture was stirred at 25 °C for 6 h.[29] The precipitate was filtered, washed

with tetrahydrofuran and vacuum-dried to give **8** as a white solid (23 g, 82%). The obtained **8** was used for the preparation of compound **9** without further purification.

Synthesis of 5-methyl-2,2′-bipyridine (9)
Under a nitrogen atmosphere, methacrolein (8 g) was added to formamide (80 ml) containing compound **8** (23 g) and ammonium acetate (14 g), and the reaction mixture was stirred at 70 °C for 6 h.[30] The precipitate was extracted with diethyl ether and washed with NaCl aqueous solution. The organic layer was dried over anhydrous $MgSO_4$ and evaporated *in vacuo* to give product **9** as a yellow oil (5 g, 36%).

Synthesis of 2,2′-bipyridine-5-carboxylic acid (10)
The compound **9** (8 g) was dispersed in water (18 ml), and $KMnO_4$ (35 g) was added in seven equal portions.[31] After heating at 70 °C for 3 h and at 90 °C for 12 h, the mixture was filtered and washed with hot water. The pale pink filtrate was then slowly acidified with 1 M HCl to obtain a white precipitate. The precipitate **10** was collected by filtration and dried *in vacuo* (7 g, 36%).

Synthesis of 2,2′-bipyridine-5-carboxylic acid chloride (11)

Compound **10** (0.7 g) was stirred in $SOCl_2$ (40 ml) and the mixture was heated at 70 °C.[31] After 3 h, the remaining $SOCl_2$ was removed under reduced pressure. The obtained acid chloride **11** was used for the synthesis of cellulose derivatives without further purification.

Synthesis of cellulose 2,3,6-tris(pyridinecarboxylate)s (1a–d)

The acid chlorides 2-pyridinecarboxylic acid chloride, 3-pyridinecarboxylic acid chloride, 4-pyridinecarboxylic acid chloride and 6-chloro-3-pyridinecarboxylic acid chloride were prepared from 2-pyridinecarboxylic acid, 3-pyridinecarboxylic acid, 4-pyridinecarboxylic acid and 6-chloro-3-pyridinecarboxylic acid, respectively, through the reaction with oxalyl chloride in toluene at 60 °C. The cellulose derivatives (**1a–d**) (Figure 1) were synthesized by the reaction of cellulose with an excess amount of the corresponding acid chlorides in an N,N-dimethylacetamide-LiCl-pyridine-triethylamine mixture at 80 °C for 24 h. The derivatives were isolated as methanol-insoluble solids and vacuum-dried. Although **1a** was insoluble in any solvent, **1b–d** could be dissolved in organic solvents, such as N,N-dimethylacetamide, pyridine and dimethyl sulfoxide. The ^{1}H nuclear magnetic resonance (NMR) spectra for **1b–d** showed that the hydroxy groups of the cellulose were almost quantitatively converted into ester moieties. **1b**: ^{1}H NMR (dimethylsulfoxide (DMSO)-d_6): $\delta = 3.5–5.8$ (br, glucose protons, 7H), 6.8–8.9 (aromatic, 12H). **1c**: ^{1}H NMR (DMSO-d_6): $\delta = 3.4–5.8$ (br, glucose protons, 7H), 7.2–8.8 (aromatic, 12H). **1d**: ^{1}H NMR (DMSO-d_6): $\delta = 3.3–5.8$ (br, glucose protons, 7H), 6.9–8.8 (aromatic, 9H).

Synthesis of cellulose 2,3,6-tris(2,2′-bipyridine-5-carboxylate) (2)

Cellulose (0.4 g) was dissolved in a N,N-dimethylacetamide-LiCl-pyridine mixture at 80 °C, and then an excess amount of **11** (2.5 g) was added to the solution. The reaction was continued at 80 °C for 24 h. The cellulose derivative **2** was isolated as a methanol-insoluble fraction and vacuum-dried (1.5 g, 79%). The ^{1}H NMR spectrum of **2** indicated that the degree of substitution of bipyridyl group was 94%. ^{1}H NMR (pyridine-d_5): $\delta = 3.9–6.4$ (br, glucose protons, 7H), 6.9–9.6 (aromatic, 21H).

Synthesis of regioselectively substituted cellulose derivatives (3–6)

The regioselectively substituted cellulose derivatives (**3–6**) having a bipyridyl group (Figure 2) were prepared as described below.[32,33] First, the cellulose was dissolved in N,N-dimethylacetamide-LiCl-pyridine mixture at 80 °C, and then 2.0 equivalent of triphenylmethyl chloride, on the basis of a glucose unit, was added and allowed to react with the primary hydroxy group at the 6-position for 24 h. The cellulose derivative protected at the 6-position was isolated as the methanol-insoluble fraction and vacuum-dried. The 6-O-trityl cellulose was reacted with an excess amount of the corresponding acid chlorides or 3,5-dimethylphenyl isocyanate in pyridine at 80 °C. The obtained derivatives were suspended in methanol containing 0.1 M hydrochloric acid to deprotect the trityl group at ~50 °C. The deprotected cellulose derivatives were dissolved in pyridine and allowed to react with an excess amount of corresponding acid chlorides or 3,5-dimethylphenyl isocyanate at 80 °C. After 24 h, the regioselectively substituted cellulose derivatives (**3–6**) were isolated as a methanol-insoluble fraction and vacuum-dried. The obtained derivatives were analyzed by ^{1}H NMR spectroscopy. The cellulose derivatives **3** and **4** had 87 and >99% bipyridyl groups at the 6-position of a glucose unit, respectively. In the derivatives **5** and **6**, however, the degree of substitution values of bipyridyl groups at the 2- and 3-positions were 33 and 47%, respectively. The degree of substitution values of **5** and **6** were low, probably because the acid chloride **11** could not efficiently react with the cellulose derivatives bearing the bulky trityl group. **3**: ^{1}H NMR (DMSO-d_6): =1.6–2.3 (CH_3 (12H)), 3.4–5.4 (br, glucose protons, 7H), 6.0–9.2 (aromatic, NH, 15H). **4**: ^{1}H NMR (DMSO-d_6): $\delta = 3.4–5.7$ (br, glucose protons, 7H), 6.8–9.0 (aromatic, 17H). **5**: ^{1}H NMR (DMSO-d_6): $\delta = 1.7–2.5$ (CH_3 (6H)), 3.3–5.9 (br, glucose protons, 7H), 6.1–10.3 (aromatic, NH, 18H). **6**: ^{1}H NMR (DMSO-d_6): $\delta = 3.3–5.9$ (br, glucose protons, 7H), 6.6–9.0 (aromatic, 19H).

Preparation of stationary phases

The packing materials were prepared according to the previously described method.[34] The macroporous silica gel obtained from Daiso Chemical was treated with a large excess of (3-aminopropyl)triethoxysilane in toluene in the presence of a catalytic amount of pyridine at 80 °C for 20 h. The cellulose derivatives bearing pyridyl and bipyridyl residues were dissolved in chloroform, tetrahydrofuran, pyridine or N,N-dimethylacetamide, and the solutions were coated on the aminopropyl silica gel as uniformly as possible. The solvents were then evaporated under reduced pressure. The remaining polymer solutions were adsorbed onto the silica gel by the same procedure. The total coating amount of the cellulose derivatives to the silica gel was 25 wt%. Because **1a** was insoluble in any solvent, the packing material based on **1a** could not be prepared. The obtained packing materials were packed into stainless steel tubes (25×0.46 cm ID or 25×0.20 cm ID) by a conventional high-pressure slurry packing technique using a model CCP-085 Econo packer pump (Chemco, Osaka, Japan). The plate number was ~3000 for the columns with 25×0.46 cm ID and ~1500 for the columns with 25×0.20 cm ID for benzene using a hexane/2-propanol (90/10, v/v) mixture as the eluent, at flow rates of 0.5 and 0.1 ml min^{-1}, respectively. The non-retained compound 1,3,5-tri-*tert*-butylbenzene was used to estimate the dead time (t_0).[35]

Instruments

Chromatographic experiments were performed using a JASCO PU-980 Intelligent HPLC pump equipped with ultraviolet (JASCO 970-UV) and polarimetric (JASCO OR-990) detectors (JASCO, Tokyo, Japan) at room temperature or 60 °C. A solution of a racemate (3 mg ml^{-1}, 1–10 μl) was injected into the chromatographic system by a Rheodyne Medel 7125 injector (Rheodyne, Rohnert Park, CA, USA). The ^{1}H NMR (400 MHz) spectra were taken in pyridine-d_5 and DMSO-d_6 at 80 °C using a Varian Gemini-2000 spectrometer (Varian, Palo Alto, CA, USA).

RESULTS AND DISCUSSION

Enantioseparation on cellulose derivatives bearing pyridyl and bipyridyl groups

Pyridinecarboxylic acid chlorides and 2,2′-bipyridine-5-carboxylic acid chloride were synthesized according to reported procedures[27–31] and reacted with cellulose to obtain the derivatives **1a–d** and **2–6** bearing pyridyl and bipyridyl residues, respectively. The derivatives **1–6** except for **1a** were adsorbed onto aminopropyl silica gel, and the obtained packing materials were packed in stainless steel tubes by the slurry method.[34]

The chiral recognition ability on the cellulose derivatives bearing pyridyl and bipyridyl groups was examined for the following 10 racemates: 2-phenylcyclohexanone (**12**), Tröger base (**13**), *trans*-stilbene oxide (**14**), 1,2,2,2-tetraphenylethanol (**15**), 2,2′-dihydroxy-6,6′-dimethylbiphenyl (**16**), benzoin (**17**), flavanone (**18**), cobalt(III) tris(acetylacetonate) (**19**), 1-(9-anthryl)-2,2,2-trifluoroethanol (**20**) and *trans*-cyclopropanedicarboxylic acid dianilide (**21**) (Figure 4). Figure 5 shows a chromatogram of the resolution of **16** on the CSP prepared from the cellulose derivative **5**. The enantiomers eluted at the retention times of 23.1 and 33.0 min showed complete separation. The retention factors, $k_1 (=(t_1–t_0)/t_0)$ and $k_2 (=(t_2–t_0)/t_0)$, were 2.77 and 4.65, respectively, and the separation factor $\alpha (=k_2/k_1)$ was 1.68.

The results of the resolutions of **12–21** on derivatives **1b–d** are summarized in Table 1. The recognition ability of **1b** and **1c** was much lower than that of the commercially available Chiralcel OB, consisting of the cellulose tribenzoate (Figure 1), although the elution orders of the separated enantiomers were the same in these CSPs. Because the pyridyl groups of **1b** and **1c** were located far from a chiral glucose unit, these polar groups themselves may cause a non-enantioselective interaction with the racemates.[34] This explanation was supported by the notably higher capacity factors of **1b** and **1c** for **16**, **20** and **21** compared with that of Chiralcel OB, indicating that the nitrogen of the pyridyl residues in cellulose derivatives **1b** and **1c** had a hydrogen bonding interaction with the racemates bearing hydroxy and amide groups. On the other hand, the cellulose derivative **1d**, which has a
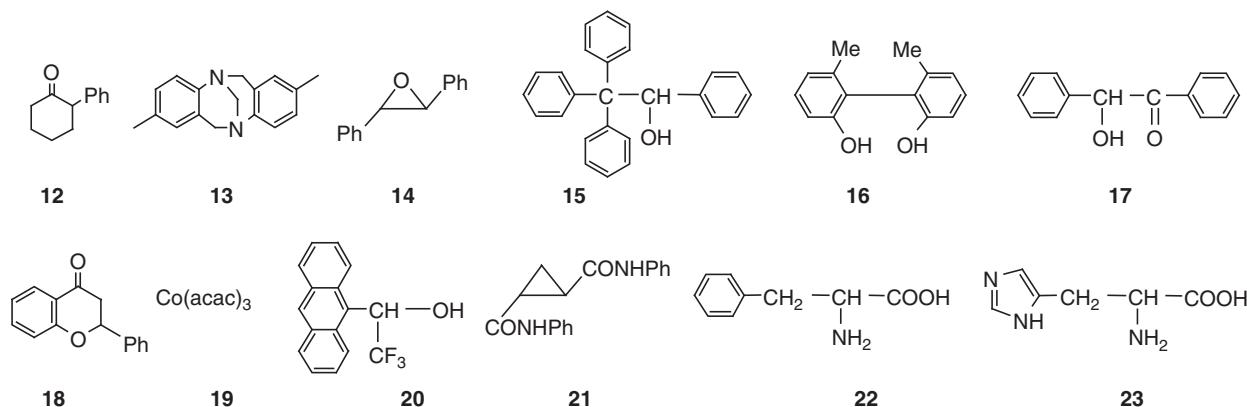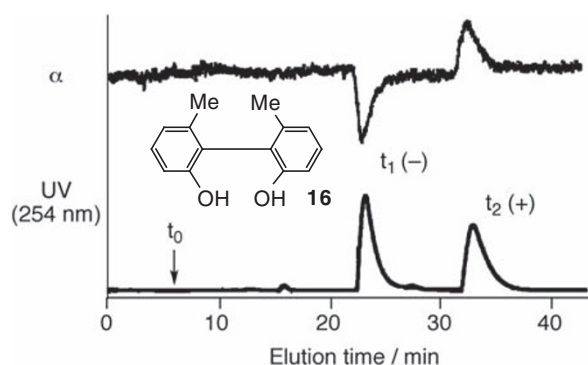
Figure 4 Structures of racemates 12–23.

Figure 5 Chiral separation of rac-2,2′-dihydroxy-6,6′-dimethylbiphenyl 16 on cellulose derivative 5. Column dimensions: 25×0.20 cm (inner diameter); flow rate: 0.1 ml min^{-1}; eluent: hexane/2-propanol (90:10).

Table 1 Resolutions of 12–21 on cellulose derivatives bearing pyridyl residues 1b–d and Chiralcel OB[a]

	1b		1c		1d		Chiralcel OB[b]	
Racemates	k_1'	α	k_1'	α	k_1'	α	k_1'	α
12	1.54 (−)	~1	1.25 (−)	1.11	1.82 (−)	1.08	3.95 (−)	1.64
13	0.49 (+)	~1	0.27 (+)	~1	0.48	1.00	1.29 (−)	~1
14	0.55 (+)	~1	0.50 (+)	1.14	0.50 (+)	1.17	0.91 (+)	1.44
15	2.36	1.00	0.57 (+)	~1	0.73	1.00	2.21 (+)	1.20
16	11.6 (−)	1.09	6.82 (−)	1.12	1.61 (−)	1.11	1.03 (−)	1.30
17	6.03 (−)	~1	5.16 (+)	~1	4.43 (−)	1.05	4.16 (+)	1.23
18	1.99	1.00	1.64	1.00	2.61	1.00	2.53 (−)	~1
19	0.56	1.00	0.13	1.00	0.61	1.00	1.27 (+)	~1
20	6.63	1.00	3.25	1.00	2.03	1.00	2.14	1.00
21	4.07	1.00	2.80 (−)	~1	1.62	1.00	2.21 (+)	1.26

The signs in parentheses represent the optical rotation of the first-eluted enantiomers.
[a]Column dimensions: 25×0.20 cm (inner diameter); flow rate: 0.1 ml min^{-1}; eluent: hexane/2-propanol (90:10).
[b]Column dimensions: 25×0.46 cm (inner diameter); flow rate: 0.5 ml min^{-1}.

chloro group at the para position, exhibited smaller capacity factors and a higher recognition ability than 1b. This suggests that the chloro substituent may inactivate a hydrogen bonding interaction at the nitrogen of the pyridyl residues due to its steric hindrance and electron-withdrawing property.

Table 2 shows the resolution results of 12–21 on the derivatives 2–6. The cellulose derivative 2 bearing bipyridyl residues at all 2-, 3- and 6-positions of a glucose ring showed high capacity factors for the racemates, probably due to hydrogen bonding with the bipyridyl groups. However, its recognition ability was very low, and only 12 could be separated with a small α-value. The bipyridyl groups may be too far from the main chain to function as effective chiral recognition sites.[34] On the other hand, the regioselectively substituted derivatives 3–6 exhibited a relatively higher chiral recognition ability than the totally substituted cellulose derivative 2. Compared with a commercially available cellulose-based chiral column, Chiralcel OD, which consists of cellulose tris(3,5-dimethylphenylcarbamate) and is known to be one of the most powerful CSPs (Figure 1), the racemates 12, 13, 16 and 19 and the racemates 12 and 16 were more efficiently separated on 3 and 5, respectively. Meanwhile, 4 and 6 could separate the racemates 13 and 20 and the racemates 13 and 21, respectively, with larger α-values than Chiralcel OB.

Enantioseparation on cellulose derivatives 2–6 having coordinated Cu(II) ion

The chiral recognition abilities of the derivatives (2–6) were also evaluated after the coordination of Cu(II) ion (Table 3). The chromatographic behavior was significantly altered before and after the coordination of Cu(II) ion to the bipyridyl residues. Although the recognition ability on these derivatives was decreased for many racemates after coordination, the racemates 14 and 18 were newly resolved on 2 and 4, respectively, and some racemates were more efficiently resolved compared with before coordination (Figure 6). In addition, the elution order of the enantiomers of 15 was reversed on the CSPs prepared from 3. To understand the background of these changes in recognition ability, the structures of 2 before (Figure 7a) and after (Figure 7b) the coordination of Cu(II) ion have been estimated on the basis of the left-handed 3/2-helical structure of cellulose tribenzoate determined by X-ray structural analysis.[36] Because the higher-ordered structure of 2 is not stabilized by an effective intramolecular interaction, such as hydrogen bonds, the orientation of the side chains of 2 may be easily changed through the coordination of Cu(II) ions to the bipyridyl residues (Figure 7b). Moreover, because of the property change due to the coordination itself, this structural difference of the cellulose derivative 2 is expected to provide a change in the chiral recognition ability, resulting from environmental change around the interaction sites, such as ester and carbamate groups.

Table 2 Resolutions of 12–21 on cellulose derivatives bearing bipyridyl residue 2–6 and Chiralcel OD[a]

Racemates	2[b]		3		4		5		6		Chiralcel OD[b]
	k_1'	α	k_1'	α	k_1'	α	k_1'	α	k_1'	α	α
12	2.76 (−)	1.14	1.58 (−)	1.27	3.53 (−)	1.23	1.80 (−)	1.26	3.30 (−)	1.14	1.14 (−)
13	1.11 (+)	~1	0.98 (+)	1.70	1.21 (+)	1.30	1.93 (+)	~1	1.25 (+)	1.29	1.26 (+)
14	0.91 (+)	~1	0.74 (+)	1.24	0.94 (+)	1.22	0.82 (+)	1.32	1.04 (+)	1.15	2.11 (−)
15	2.02 (−)	~1	1.92 (−)	1.20	4.17 (+)	~1	2.11 (+)	~1	3.37	1.00	1.27 (+)
16	7.64	1.00	3.20 (−)	1.97	4.49 (−)	1.14	2.77 (−)	1.68	4.12 (−)	1.07	1.47 (−)
17	6.65	1.00	4.08 (+)	1.09	3.42	1.00	5.71 (+)	1.20	5.72 (−)	1.06	1.57 (+)
18	4.23	1.00	2.24 (−)	~1	4.07 (−)	~1	2.76	1.00	4.25	1.00	1.40 (−)
19	0.71	1.00	0.93 (+)	1.22	1.54 (+)	~1	1.12 (+)	~1	1.00	1.00	1.13 (+)
20	7.47	1.00	2.45 (−)	~1	2.63 (−)	1.08	3.44 (−)	1.32	4.97	1.00	2.87 (−)
21	2.58	1.00	1.71 (−)	1.28	3.63	1.00	2.81	1.00	2.56 (−)	1.38	2.63 (+)

The signs in parentheses represent the optical rotation of the first-eluted enantiomers.
[a]Column dimensions: 25×0.20 cm (inner diameter); flow rate: 0.1 ml min^{-1}; eluent: hexane/2-propanol (90:10).
[b]Column dimensions: 25×0.46 cm (inner diameter); flow rate: 0.5 ml min^{-1}.

Table 3 Resolutions of 12–21 on cellulose derivatives 2–6 having coordinated Cu(II) ion[a]

Racemates	2-Cu[b]		3-Cu		4-Cu		5-Cu		6-Cu	
	k_1'	α	k_1'	α	k_1'	α	k_1'	α	k_1'	α
12	1.59 (−)	~1	0.80 (−)	1.28	2.18 (−)	~1	2.24 (−)	1.17	2.31 (−)	~1
13	0.53	1.00	0.56 (+)	1.57	0.81 (+)	~1	1.26 (+)	~1	0.84 (+)	~1
14	1.17 (+)	1.25	0.93 (+)	1.15	1.29 (+)	1.38	0.98 (+)	1.27	1.11 (+)	~1
15	1.56	1.00	1.00 (+)	1.17	5.90 (+)	~1	2.50 (+)	1.00	4.33	1.00
16	0.66	1.00	5.72 (−)	1.55	2.11	1.00	25.6 (−)	~1	2.26 (−)	~1
17	5.68	1.00	5.22 (+)	1.13	5.37	1.00	10.5 (+)	1.13	5.57	1.00
18	2.42	1.00	2.42 (−)	~1	2.61 (+)	1.06	3.55	1.00	2.88	1.00
19	1.08	1.00	0.90 (+)	1.17	2.28	1.00	1.18	1.00	1.70	1.00
20	2.19	1.00	7.13 (−)	1.15	2.28	1.00	5.46	1.00	2.45	1.00
21	0.22	1.00	4.82	1.00	1.88	1.00	5.21	1.00	2.28	1.00

The signs in parentheses represent the optical rotation of the first-eluted enantiomers.
[a]Column dimensions: 25×0.20 cm (inner diameter); flow rate: 0.1 ml min^{-1}; eluent: hexane/2-propanol (90:10).
[b]Column dimensions: 25×0.46 cm (inner diameter); flow rate: 0.5 ml min^{-1}.
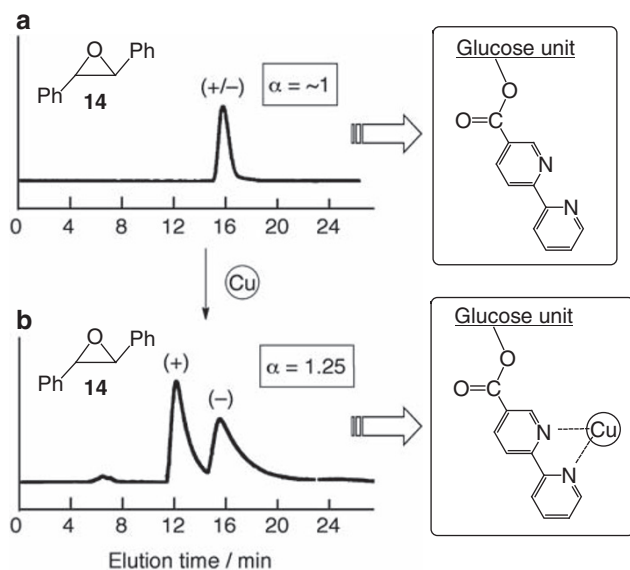

Figure 6 Chromatograms for the resolution of trans-stilbene oxide **14** on **2** with a coordinated Cu(II) ion.

Chiral ligand-exchange chromatography using cellulose derivatives bearing bipyridyl group

In 1971, Davankov and Rogozhin[37] developed the first ligand-exchange-type CSP, which was prepared by immobilizing L-proline onto poly(styrene-co-divinylbenzene) beads. The ligand-exchange-type CSPs were effective for the resolution of chiral compounds bearing polydentate ligands, such as amino acids.[38] However, the resolution of amino acids was rarely carried out on polysaccharide-based CSPs. Here, the cellulose derivatives bearing bipyridyl residues have been applied for the separation of amino acids using ligand-exchange chromatography, because Cu(II) coordinated in the bipyridyl group is expected to interact with amino acids through the multiple coordination bonds.

Aromatic amino acids, such as phenylalanine (**22**) and histidine (**23**), could be separated on the cellulose derivative **6** with α-values of 1.12 and 1.37, respectively. Figure 8 shows the resolution of histidine on **6** using an H_2O/CH_3OH (90:10) mixture, including NH_4OAc (50 mM) and $Cu(OAc)_2$ (0.5 mM), as an eluent.[39,40] Although the individual enantiomers showed rather broad overlapping peaks, **6** could partially separate the amino acid. On the other hand, the aliphatic amino acids, such as alanine, leucine, isoleucine, methionine, threonine, valine, proline and serine, could not be separated on these
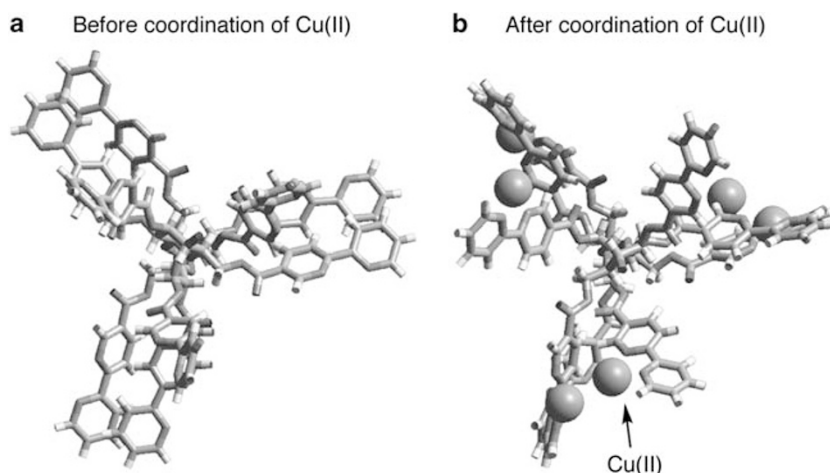
a Before coordination of Cu(II)   **b** After coordination of Cu(II)

Cu(II)

Figure 7 Schematic representation of the structural change of **2** before (**a**) and after (**b**) coordination of Cu(II) to the bipyridyl residue. The view is perpendicular to the helix axis, and **2** and Cu(II) are represented by cylinders and van der Waals spheres, respectively.
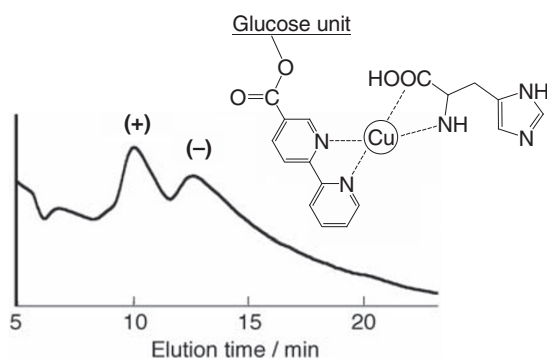

Figure 8 Chiral separation of *rac*-histidine **23** on **6**. Column dimensions: 25×0.20 cm (inner diameter); flow rate: 0.1 ml min^{-1}; eluent: H_2O-CH_3OH (90:10), NH_4OAc (50 mM), $Cu(OAc)_2$ (0.5 mM); pH: 5.5; temperature: 60 °C.

derivatives. Therefore, both the coordination bond and the π–π interaction are expected to have an important role for the separation of amino acids. This result expands the possibility of chiral separation using polysaccharide derivatives. Because the derivatives **2–5** could not separate any amino acids, the environment around the bipyridyl groups is expected to be very important for ligand-exchange chromatography using these derivatives.

CONCLUSIONS

Cellulose derivatives bearing pyridyl and bipyridyl residues were synthesized, and their chiral recognition abilities as CSPs for HPLC were evaluated. Although the cellulose derivatives bearing pyridyl **1** and bipyridyl **2** residues at all 2-, 3- and 6-positions of a glucose ring showed low chiral recognition, the regioselectively substituted derivatives **3–6** exhibited relatively high chiral recognition. After the coordination of Cu(II) ion to the bipyridyl residues, their recognition abilities were changed depending on the difference of the higher-order structures of the cellulose derivatives. In addition, the derivatives can be used for ligand-exchange chromatography, and aromatic amino acids, such as phenylalanine and histidine, could be separated on cellulose derivative **6**.

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