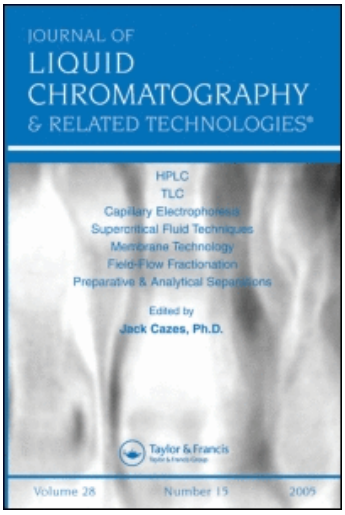

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Development of New LC Chiral Stationary Phases Based on Ruthenium *Tris*(diimine) Complexes

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Development of New LC Chiral Stationary Phases Based on Ruthenium *Tris*(diimine) Complexes

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Abstract: Enantiopure ruthenium(II) *tris*(diimine) complexes have been covalently bonded to silica and evaluated as chiral stationary phases for LC. Three binding chemistries were tested and the CSP synthesized by reductive amination of the aldehyde-functionalized silica provided the largest enantioselectivity. The Ru complex bonded CSP showed selectivity toward a variety of racemic compounds. Circular dichroism (CD) detection was used to confirm the enantiomeric separations. This CSP worked especially well for the enantiomeric separation of binaphthyl type compounds in the normal phase mode and appeared to be selective for acidic compounds in the polar organic mode. Effects of mobile phase composition on the enantioseparations were also studied.

Keywords: Binding chemistry, Chiral stationary phase, Enantioresolution, Ru *tris*(diimine) complex

INTRODUCTION

Enantiomeric separation has been an important topic for several decades. Among various chiral separation methods, LC with chiral stationary phases (CSPs) has been dominant due to its good reproducibility, wide selectivity, easy operation, and ability to do preparative and semipreparative separations.[1–8] CSPs are produced by immobilizing pure

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enantiomers (chiral selectors) to solid support (silica) via chemical bonding or physical absorption. Although a large number of papers related to the development of CSPs have been published, only a few types of CSPs dominate the field of LC enantiomeric separations. However, researchers continue to investigate new types of chiral stationary phases, in hopes of finding a more universal stationary phase or a more specific stationary phase, which is especially powerful for selected groups of compounds.

One type of chiral selector that has not received much attention in LC is that of transition metal complexes.[9–17] The helical chirality of the enantiomers of ruthenium *tris*(diimine) complexes is shown schematically in Figure 1. The earliest study involving a metal ligand complex as a stationary phase showed that the Δ- and Λ-enantiomers of a metal complex were absorbed on a clay support in stereoregular manners and similar behavior was observed between "enantiomers" of different types, such as Δ-$[Ni(phen)_3]^{2+}$ and Λ-$[Ru(phen)_3]^{2+}$.[9] Yamagishi[12] reported that a column of Λ-$[Ru(phen)_3]^{2+}$ absorbed into montmorillonite gave "optical resolution" of tris(chelated) and bis(chelated) metal complexes. Later, the same group extended the applicability of this column to chiral aromatic compounds, by showing separation of 2,3-diphenylpyrazine and binaphthyl enantiomers.[15] Also, they used spherically shaped synthetic hectorite instead of montmorillonite in order to improve the column efficiency.[16] However, all the reported columns were prepared by simply absorbing the metal complexes onto the clay and some mobile phases had to be avoided so as to prevent desorption of the chiral selectors from the clay.

To our knowledge, the CSPs based on Ru complexes, which are covalently linked to the support have not been reported. In the present

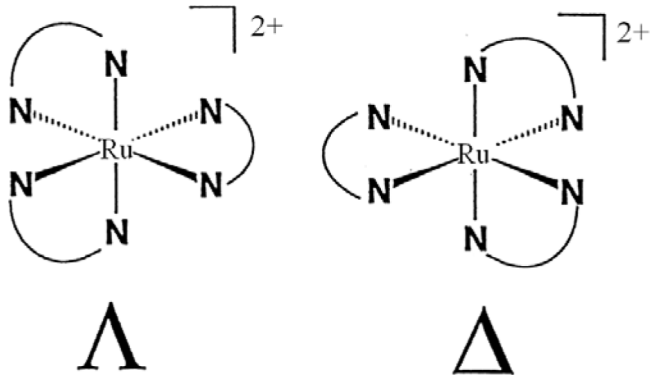


Figure 1. Mirror image relationship of Ru(II) *tris*(diimine) enantiomers.

work, we have attached chiral, non-racemic Ru(II) *tris*(diimine) complexes to high surface area silica gel in an attempt to improve the performance of this class of CSPs. Three different chemistries linking the Ru complex to 5 μm spherical silica gel were studied, in order to improve the chiral selector loading. The CSP prepared via an amination reaction between aldehyde functionalized silica and the peripheral amino group of Ru *tris*(phenanthroline) complex gave the best performance. The column was then evaluated as a chiral stationary phase for HPLC by injecting 155 racemic analytes. Three operation modes (normal phase mode, polar organic mode, and reversed phase mode) were tested. Effects of mobile phases composition on the separations were studied.

EXPERIMENTAL

Chemicals

Anhydrous N,N-dimethylformamide (DMF), anhydrous toluene, 3-(triethoxysilyl)propyl isocyanate, 1,6-diisocyanatohexane, sodium periodate, sodium cyanoborohydride, sodium monophosphate, phosphoric acid, ammonium nitrate, ammonium chloride, and most racemic analytes used in this study were purchased from Sigma-Aldrich (Milwaukee, WI, USA). Acetonitrile, 2-propanol, n-heptane, ethanol, and methanol of HPLC grade were obtained from EMD (Gibbstown, NJ). Tetramethylammonium nitrate (TMAN), ammonium trifluoroacetate, ammonium nitrate, ammonium chloride, and ammonium acetate were purchased from Sigma-Aldrich (St. Louis, MO). Water was obtained from Millipore (Billerica, MA). Kromasil silica (5 μm spherical diameter, 100 Å and 200 Å pore size) was obtained from Supelco (Bellefonte, PA).

Synthesis of $[Ru(phen)_2aminophen](PF_6)_2$ and $[Ru(phen)_2phendiamine](PF_6)_2$

The compounds: 5-nitro-1,10-phenanthroline(nitrophen),[18] $Ru(phen)_2Cl_2$,[19,20] $[Ru(phen)_2phendione]Cl_2$ (phendione = 1,10-phenanthroline-5,6-dione),[21] and $[Ru(phen)_2nitrophen](PF_6)_2$ were prepared as previously reported.[22]

Racemates of $[Ru(phen)_2nitrophen](PF_6)_2$ and $[Ru(phen)_2phendione](PF_6)_2$ were converted to chloride salts by metatheses, and resolved as reported.[22] $[Ru(phen)_2nitrophen]Cl_2$ was reduced to $[Ru(phen)_2(aminophen)]Cl_2$ (aminophen = 5-amino-1,10-phenanthroline), described in Ref. [22]. $[Ru(phen)_2(phendiamine)]Cl_2$ (phendiamine = 5,6-diamino-1, 10-phenanthroline) was prepared according to Ref. [23].

Preparation of Ru Complex Bonded Chiral Stationary Phases

Three different linkage strategies for chemically attaching Ru tris(diimine) complex to 5 μm diameter silica gel were conducted and the synthetic schemes of three binding methods are shown in Figure 2.

The first binding method[3,4] involved reacting Ru complex with excess 3-(triethoxysilyl)propyl isocyanate in anhydrous DMF at 90°C for five hours. Then, the product was added to dry silica gel and heated at 105°C overnight. The mixture was cooled, filtered, and washed, as indicated previously. The second approach involved three steps and

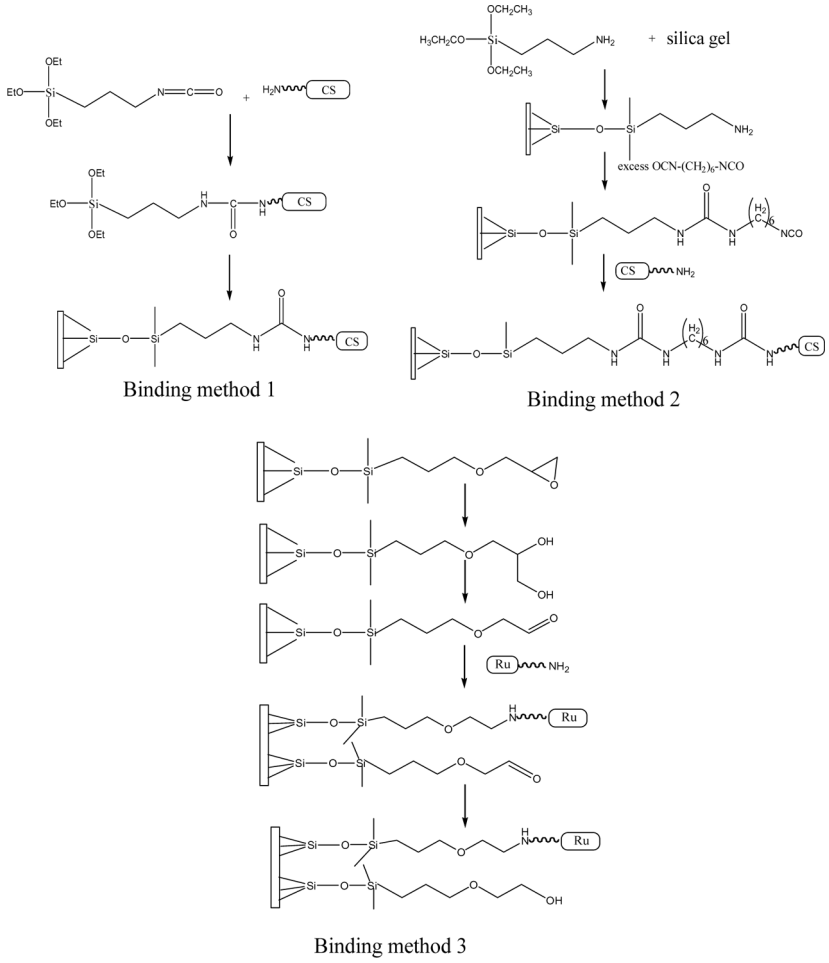


Figure 2. Schemes of three synthetic methods of Ru compelx-based CSPs.

was analogous to those reported previously for antibiotic stationary phases.[3] 3-Aminopropyl-triethoxysilane was added to silica-toluene slurry dropwise after the silica was dewatered using Dean-Stack trap. The mixture was refluxed for 4 h and then cooled, filtered, and washed with toluene, methanol, and acetone. 1,6-Diisocyanatohexane was added to dry amino-silica toluene slurry while kept in an ice bath. Then, the slurry mixture was heated to 70°C for 4 h. The excess reactant was removed by vacuum filtration and the solid product was washed with anhydrous toluene. The Ru-complex in pyridine was then added and the mixture was heated to 70°C and allowed to react overnight. Finally, the product was filtered, washed, and dried. In the third linkage chemistry, the diol-silica was prepared according to the literature.[24] The diol groups were oxidized in 60 mM sodium periodate in water/methanol (4:1) at room temperature. Then, the aldehyde-silica was filtered and dried. Ru complex was added to the resulting aldehyde-silica in anhydrous methanol and refluxed overnight. Finally, the remaining aldehyde moieties were reduced by sodium cyanoborohydride ($NaCNBH_3$) in phosphate buffer (pH = 3).[25] The elemental analysis results are shown in Table 1 (See Results and Discussion section). The CSP was slurry packed into a 25 cm × 0.46 cm (i.d.) stainless steel column.

Column Evaluation

The first chromatographic system was an HP (Agilent Technologies, Palo Alto, CA, USA) 1050 system, which consists of a UV VWD detector, an autosampler, a quaternary pump, and Chemstation software. The second HPLC system was composed of a pump (Shimadzu, LC-6A) and a circular dichroism detector (Jasco, CD-2095 plus). For the LC analysis, the injection volume, the flow rate, and the detection wavelength are 5 μL, 1 mL/min, and 254 nm, respectively. Separations were carried out at room temperature (~22°C) if not specified. The mobile phase was degassed by ultrasonication under vacuum for 5 min. The analytes were dissolved in ethanol, or the appropriate mobile phases. Each sample was analyzed in duplicate. In the normal phase mode, heptane/ethanol was used as the mobile phase. The mobile phase of the polar organic mode was composed of acetonitrile/methanol and a small amount of salt. Water/acetonitrile was used as the mobile phase in the reversed phase mode.

Calculations

The retention factor (k') was calculated using the equation $k' = (t_r - t_0)/t_r$, where t_r is the retention time, and t_0 is the dead time, which is determined

by the peak of the refractive index change due to the sample solvent. Selectivity (α) was calculated by $\alpha = k'_2/k'_1$, where k'_1 and k'_2 are the retention factors of the first and second eluted enantiomers, respectively. The resolution (Rs) was determined using $Rs = 2 \times (t_{r2} - t_{r1})/(w_1 + w_2)$, where w is the base peak width.

RESULTS AND DISCUSSION

Evaluation of Binding Chemistries

Normally, bonded chiral stationary phases are more stable and robust than coated ones, because the adsorbed chiral selectors may be soluble in some solvents and can be removed by the mobile phases employed to affect the separation. The objective of this work is to synthesize the first LC CSP in which a chiral Ru complex was covalently bonded to a modern silica gel support.

The initial step was to compare the performances of different Ru complexes as chiral selectors. Two Ru *tris*(diimine) complexes with amino groups were selected: $[Ru(phen)_2aminophen](PF_6)_2$ and $[Ru(phen)_2phendiamine](PF_6)_2$ (structures shown in Figure 3). It was found that amino groups of these compounds were poorer nucleophiles than typical aliphatic amines due to the electron withdrawing nature of Ru^{2+}, and the fact that they are attached to an aromatic ring system. Therefore, nucleophilic coupling reactions that are commonly used to immobilize other chiral selectors to the surface of silica gel,[3,4] tend to be more difficult for these metal complexes. Λ-$[Ru(phen)_2aminophen](PF_6)_2$ and Λ-$[Ru(phen)_2phendiamine](PF_6)_2$ were chemically bonded to silica gel using the same binding method (binding method **1** in Figure 2). The carbon percentage of the final products is 4% for $[Ru(phen)_2aminophen](PF_6)_2$-bonded silica, and 7% for $[Ru(phen)_2phendiamine](PF_6)_2$-bonded silica, respectively. This indicates that replacing $[Ru(phen)_2aminophen](PF_6)_2$ with

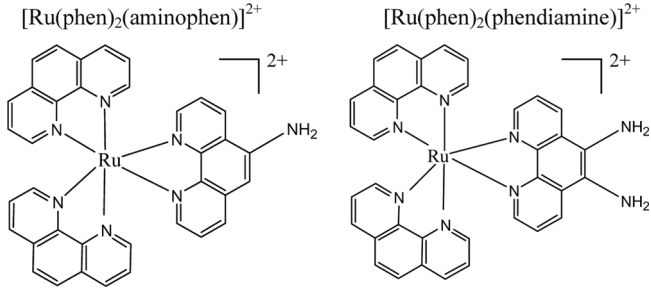


Figure 3. Structures of two Ru *tris*(diimine) complexes as chiral selectors.

$[Ru(phen)_2phendiamine](PF_6)_2$ effectively improved the chiral selector loading. The enantiomeric performances of these two CSPs were evaluated by HPLC using the same experimental conditions. The chromatograms of cis-4,5-diphenyl-2-oxazolidinone separated on the $[Ru(phen)_2aminophen](PF_6)_2$-bonded CSP and the $[Ru(phen)_2phendiamine](PF_6)_2$-bonded CSP are shown in Figure 4. It is evident that the CSP using Λ-$[Ru(phen)_2(phendiamine)]^{2+}$ provided higher selectivity and resolution, although similar retention was observed on these two columns. Both elemental analysis and chromatographic results show that $[Ru(phen)_2(phendiamine)]^{2+}$ is more suitable for preparing stationary phases, providing higher chiral selector loading and better enantioselectivity. Therefore, $[Ru(phen)_2(phendiamine)]^{2+}$ was selected to study further binding chemistries. In addition, the surface area of silica gel plays an important role in synthesis. Replacing 200 Å silica (pore size, surface area is $\sim 180\,m^2/g$) with 100 Å silica (pore size, surface area is $\sim 450\,m^2/g$) significantly improved the carbon loading from 3% to 7% using the same Ru complex. Therefore, 100 Å silica was used for subsequent studies.

Special attention was paid to the chemical binding procedures in order to obtain stationary phases with good chiral selector loading. To determine the best immobilization process, reactions were carried out using three different methods (Figure 2), named isocyanate binding (Method **1**), diisocyanate binding (Method **2**), and aldehyde binding (Method **3**), which are commonly used to prepare macrocyclic glycopeptide and cyclodextrin CSPs.[3,4] Small scale reactions were conducted due

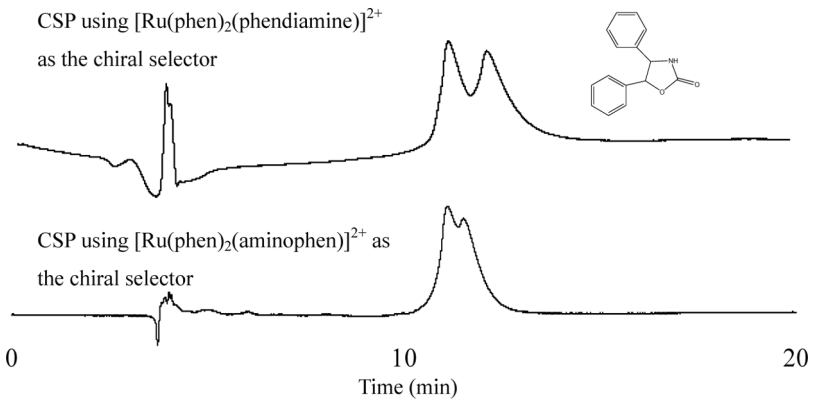


Figure 4. Comparison between two CSPs using Λ-$[Ru(phen)_2(aminophen)]^{2+}$ and Λ-$[Ru(phen)_2(phendiamine)]^{2+}$ as the chiral selectors. The analyte and mobile phase is cis-4,5-Diphenyl-2-oxazolidinone, and 95%water/5%acetonitrile, respectively.

to limited availability of enantiopure Ru complex. The elemental analyses of three products are shown in Table 1. Also, comparison of the color of the final products allows a qualitative comparison of the binding methods, since these Ru complexes have a pronounced orange color. Diisocyanate binding chemistry (method **2**) gave a product with the highest carbon loading (13.5%), but the color of the final product was the lightest. This is explained by the fact that the high percentage of carbon originates mainly from the large quantity of the diisocyanate linkage present. Comparing isocyanate binding (method **1**) and aldehyde binding (method **3**), visual observation shows the darkness of products is similar and carbon percent of method **3** is higher than method **1** (9.2% versus 7.2%, shown in Table 1). These two methods were then utilized to provide sufficient media to pack into 25 cm columns (i.e., 3.5 grams of each, respectively), which were evaluated using the same analytes and mobile phases. The chromatograms of 1,1′-bi-2-naphthol separated on the CSPs prepared via isocyanate and aldehyde binding methods are shown in Figure 5. The enantioselectivity of the analyte separated on the CSP that utilized the aldehyde binding is greater than that achieved on isocyanate bound CSP.

Evaluation of the CSP Prepared Via Aldehyde Binding Method

In this study, the best CSP was obtained by binding $[Ru(phen)_2(phendiamine)]^{2+}$ to 100 Å silica via aldehyde binding chemistry.

A set of 155 chiral compounds with a wide variety of functionalities was used to test this Ru complex-bonded CSP. Three operation modes (normal phase, polar organic mode, and reversed phase) were studied. It should be noted that acidic compounds with a –COOH group are not eluted with heptane/ethanol, or acetonitrile/water, due to strong charge-charge interaction with the Ru complex-bonded stationary phase. It was also observed that salts (such as ammonium nitrate) are necessary to elute these acidic compounds in the polar organic mode. Other compounds without acidic groups did not give retention in the polar organic mode. Therefore, 35 acidic compounds were studied only in the

Table 1. Elemental analysis results of Ru complex-silica products synthesized by three different methods

	Description	C%	H%	N%
Method **1**	isocyanate	7.2	1.2	1.5
Method **2**	diisocyanate	13.5	2.2	4.5
Method **3**	aldehyde	9.2	1.6	0.4

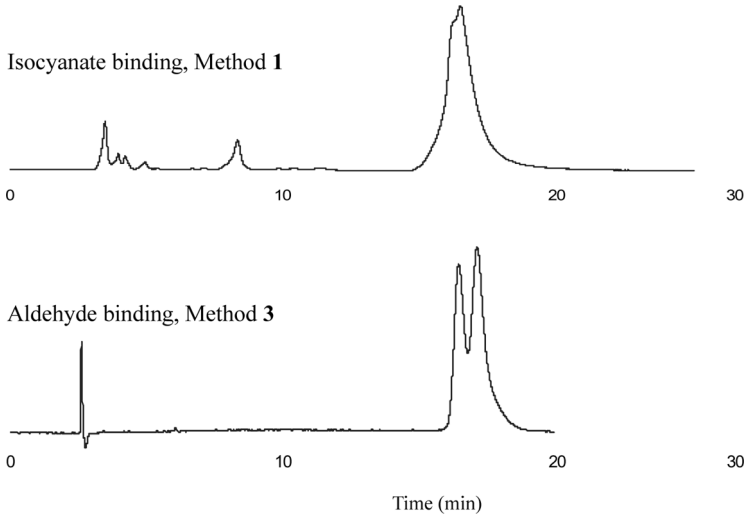
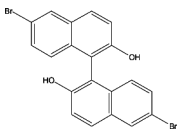
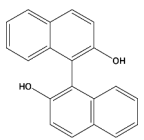
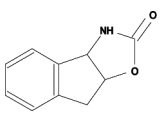
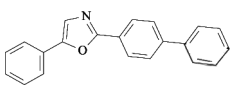
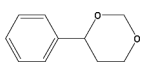
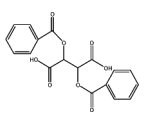


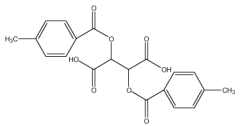
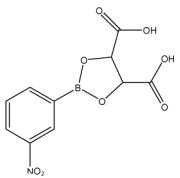
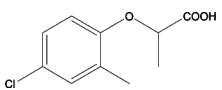
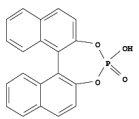
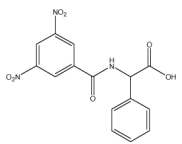
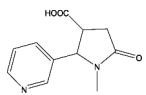
Figure 5. Comparison between two binding methods. The chromatograms were obtained on the CSPs prepared via isocyanate binding (method **1** in Figure 2) and aldehyde binding (method **3** in Figure 2), respectively. The chiral selectors ($[Ru(phen)_2(phendiamine)]^{2+}$) and silica (pore size: 100 Å) used are the same for two CSPs. The analyte and mobile phase is 1,1′-bi-2-naphthol and 80%heptane/20%ethanol, respectively.

polar organic mode, and the other 120 compounds were injected in the normal phase and reversed phase modes. Table 2 lists the chromatographic data for all compounds separated in three chromatographic modes. The chromatographic data include retention factor (k'_1), selectivity (α), and resolution (Rs). Examples of optimized separations in all three modes are shown in Figure 6.

Overall, sixteen racemic compounds were separated on the best Ru complex-based CSP using three mobile phase modes mentioned above. The polar organic mode appeared to produce more successful separations than the other two modes. In the polar organic mode, eight compounds are separated, compared to five compounds separated in the normal phase, and four in the reversed phase, respectively. Four compounds are baseline separated ($R_s \geq 1.5$) and twelve were partially separated ($0.4 < R_s < 1.5$). One compound (No. 3 or No. 17 in Table 2) was partially enantioseparated in both the normal phase mode and the reversed phase mode. Although the enantioseparation capability of Ru complex-based CSP is relatively limited, Ru-CSP is selectively effective for acidic compounds, considering 8 racemic analytes were separated out of 35 tested.

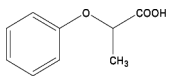
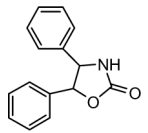
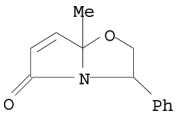
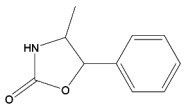
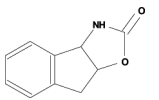
Table 2. Chromatographic data of enantiomers resolved by the Ru complex-based CSP

Mode	No.	Analyte name	Analyte structure	k1′	α	Rs	Mobile phase
Normal phase	1	6,6′-Dibromo-1,1′-bi-2-naphthol		11.69	1.17	1.5	A
	2	1,1′-Bi-2-naphthol		23.70	1.08	1.5	B
	3[c]	(3a(R,S)-cis)-(±)-3,3a,8,8a-Tetrahydro-2H-indeno[1,2-d]oxazol-2-one		2.72	1.08	0.6	C
	4	2-(4-Biphenylyl)-5-phenyloxazole		1.22	1.02	0.5	B
	5	4-Phenyl-1,3-dioxane		0.92	1.03	0.6	D
Polar organic	6	2,3-Dibenzoyl-DL-tartaric acid		17.34	1.07	1.5	E[a]

7	O,O′-di-p-toluoyl-DL-tartaric acid		15.49	1.07	1.5	E[a]
8	3-nitrophenylboronic acid tartaric acid ester		5.41	1.28	1.4	F
9	2-(4-chloro-2-methyl-phenoxy)propionic acid		3.14	1.03	0.6	F
10	(R/S)-(-/+)-1.1′-Binaphthyl-2,2′-diyl hydrogenphosphate		4.57	1.02	0.6	F
11	N-(3,5-Dinitrobenzoyl)-DL-Phenylglycine		2.14	1.02	0.5	F
12	trans-4-Cotininecarboxylic acid		1.28	1.05	0.6	F

(*Continued*)

Table 2. Continued

Mode	No.	Analyte name	Analyte structure	k1′	α	Rs	Mobile phase
	13	2-Phenoxypropionic acid		1.56	1.02	0.5	F
Reversed phase	14	cis-4,5-Diphenyl-2-oxazolidinone		3.70	1.05	0.9	G[b]
	15	2,3-Dihydro-7a-methyl-3-phenylpyrrolo[2,1-b]oxazol-5(7aH)-one		3.74	1.04	0.6	H[b]
	16	4-Methyl-5-phenyl-2-oxazolidinone		2.48	1.03	0.6	H[b]
	17[c]	(3a(R,S)-cis)-(±)-3,3a,8,8a-Tetrahydro-2H-indeno[1,2-d]oxazol-2-one		2.36	1.02	0.5	H[b]

Note: [a]The flow rate and the column temperature are 0.5 mL/min and 0°C in order to improve the resolution.
[b]The flow rate is 0.8 mL/min to avoid high pressure (>300 bar), which could damage silica support. The mobile phase compositions are: (A) 90%heptane/10%isopropanol; (B) 95%heptane/5%isopropanol; (C) 80%heptane/20%ethanol; (D) 60%heptane/40%ethanol; (E) 100%methanol/15 mM NH_4NO_3; (F) 100%methanol/12.5 mM NH_4NO_3; (G) 90%water/10%acetonitrile; (H) 98%water/2%acetonitrile.
[c]Enantiomers of this analyte were separated in the normal phase mode (No. 3) and reversed phase mode (No. 17).

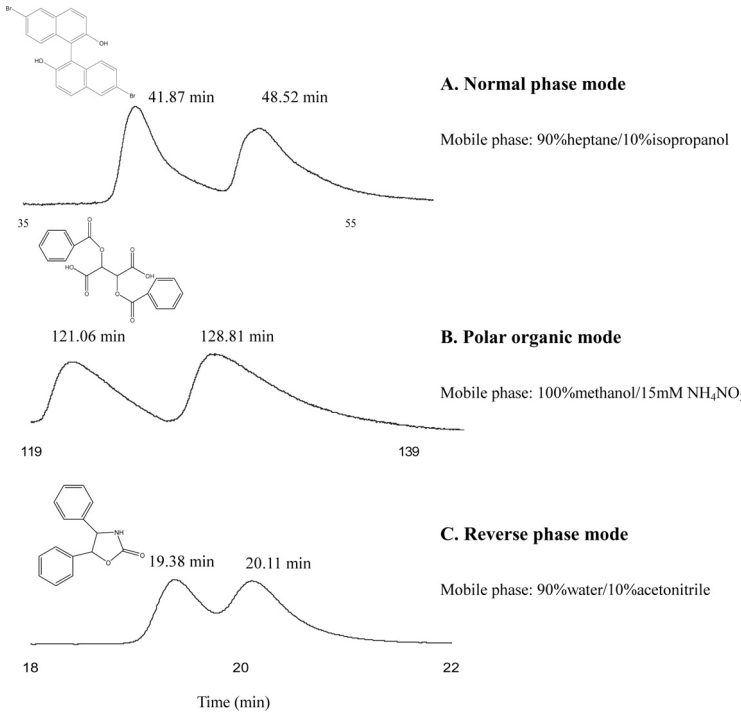


Figure 6. Optimized separations in three operation modes. Chromatographic data: (a) $k'_1 = 11.69$ $\alpha = 1.17$, $R_s = 1.5$; (b) $k'_1 = 17.34$, $\alpha = 1.07$, $R_s = 1.5$ (flow rate = 0.5 mL/min); (c) $k'_1 = 3.70$, $\alpha = 1.05$, $R_s = 0.9$ (flow rate = 0.8 mL/min).

A closer examination of the data reveals some interesting facts. Ru complex bonded stationary phase appears to be multimodal in that they can be used in normal phase, polar organic, and reversed phase modes. The CSP was not irreversibly altered when changing from one mobile phase mode to another. Previous papers[22,26–28] also indicate that the stereochemistry at these ruthenium centers is very robust and these compounds are not easily racemized or decomposed in ordinary protic or aprotic solvents.

The Ru complex-bonded CSP provides the best resolution for compounds of binaphthyl types in the normal phase mode. This indicates that different retention mechanisms may be involved in different mobile phase modes. In the normal phase mode where the non-polar solvents are used as the mobile phase, π-π interactions can play an important role in the chiral recognition process. The phenanthroline ring linked to Ru^{2+} is very electron deficient and π-π interactions are strong when it associates with π-basic compounds, such as 1,1′-bi-2-naphthol. Also, the carbamate linker provides additional sites for dipolar interactions. The helical chirality of Ru complex possibly provides a good fit with those analytes of helical

chirality (such as binaphthyl compounds). Steric interaction may be important as well. These results are in agreement with previous reports on the $[Ru(phen)_3]^{2+}$-clay column, which was shown to separate 1,1′-bi-2-naphthol.[15]

In the polar organic mode, the dominant interactions between the analyte and CSP usually involve some combination of hydrogen bonding, electrostatic, and dipolar interactions. The fact that Ru complex column shows enantioselectivity toward acidic compounds demonstrates that electrostatic interaction between positively-charged Ru complex and negatively-charged acidic analyte plays an important role in chiral recognition. In addition, electrostatic interactions between a solute and the stationary phase may be associated with a slow adsorption-desorption process, giving rise to broad and more poorly defined peaks.

In addition, the circular dichroism detector was used to check for partial separation of compounds with appropriate retentions ($1 < k' < 6$) in the normal phase and reversed phase modes. A positive-negative signal split in the circular dichroism chromatogram was observed in many cases (shown in Figure 7), where only one single peak was obtained in the UV chromatograms. This occurred when there was chiral recognition between the CSP and racemic analyte, but only moderate enantioselectivity and low efficiency. It is found that the Ru-complex CSP shows

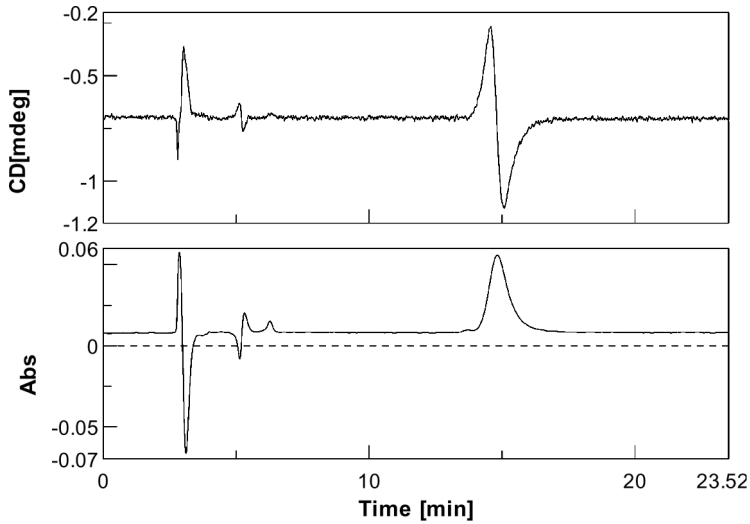
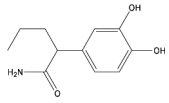
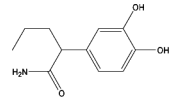
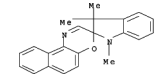
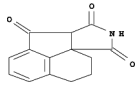
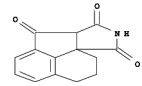
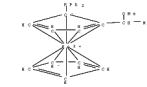
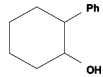
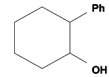
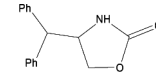
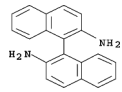
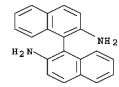
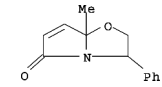
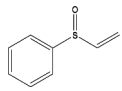
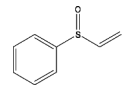
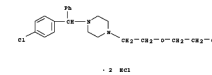
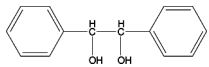
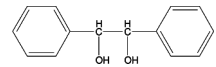
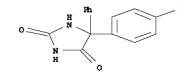
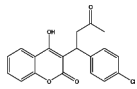
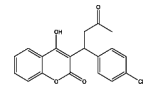
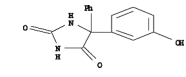
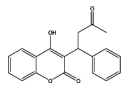
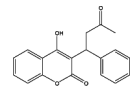
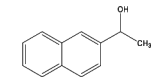
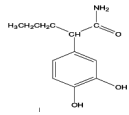
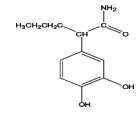
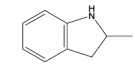
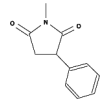
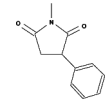
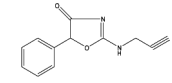
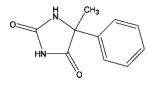
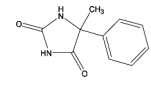
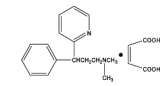


Figure 7. UV and CD chromatograms of the analyte retained on the Ru-complex column. The analyte is 4-(4-methoxy-phenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid allyl ester. The mobile phase and wavelength is 80%heptane/20%ethanol, and 254 nm, respectively.

Table 3. Enantioselectivity of compounds observed by the circular dichroism (CD) detector

Normal phase		Reversed phase			
No.	Analyte structure	No.	Analyte structure	No.	Analyte structure
1		1		25	
2		2		26	
3		3		27	
4		4		28	
5		5		29	
6		6		30	
7		7		31	
8		8		32	
9		9		33	
10		10		34	
11		11		35	

(*Continued*)

Table 3. Continued

Normal phase		Reversed phase			
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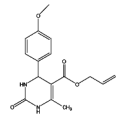
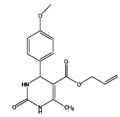
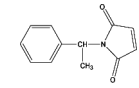
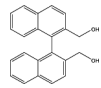
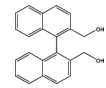
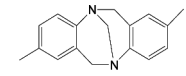
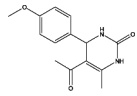
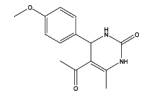
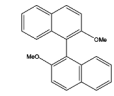
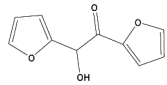
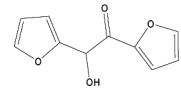
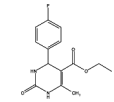
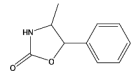
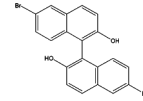
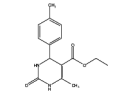
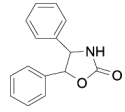
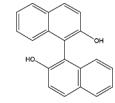
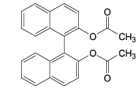
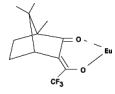
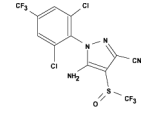
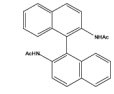
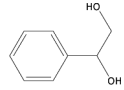
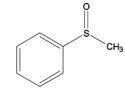
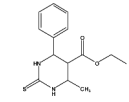
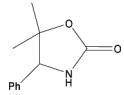
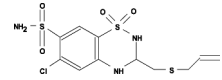
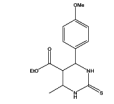
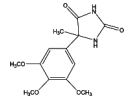
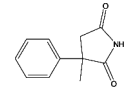
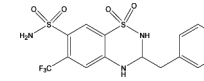
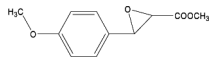
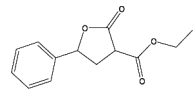
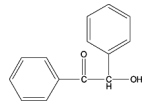
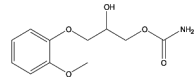
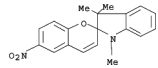
12		12		36	
13		13		37	
14		14		38	
15		15		39	
16		16		40	
17		17		41	
18		18		42	
19		19		43	
20		20		44	
21		21		45	

(*Continued*)

Table 3. Continued

Normal phase		Reversed phase			
No.	Analyte structure	No.	Analyte structure	No.	Analyte structure
22		22		46	
		23		47	
		24			

enantioselectivity to a wide variety of compounds when using CD detection. All the compounds showing selectivity in normal phase and reversed phase modes are listed in Table 3. In summary, 47 compounds were slightly separated in reversed phase conditions, compared to 22 in the normal phase mode. Considering its broader selectivity, the reversed phase mode is more successful than the normal phase mode.

Effect of Mobile Phase Composition and Temperature on Enantioseparation

In order to optimize enantioseparations on the Ru complex bonded stationary phase, effects of mobile phase composition have been studied. In the normal phase mode, the concentration and nature of the alcohol modifier in the mobile phase affects the retention and selectivity of analytes.[29,30] Figure 8 shows effects of varying alcohol types. Five different alcohols were studied with a constant mobile phase composition. Increasing alkyl chain length of primary and secondary alcohols enhances the retention, illustrated by comparison of ethanol, 1-propanol, and 1-butanol, or comparison of isopropanol and 2-butanol. Using five alcohols, the enantioselectivities are different, ranging between 1.10–1.21. The concentration of the alcohol modifier also affects enantioseparation greatly (results shown in Figure 9). The retention factor of the analyte increased from 1.60 to 16.59, when reducing the ethanol percentage from 40% to 5%. The improvement in enantioresolution resulted from much longer retention, because selectivity remained unchanged.

In the polar organic mode, additives, such as acetic acid, triethylamine, and ammonium nitrate are often added. These additives in the

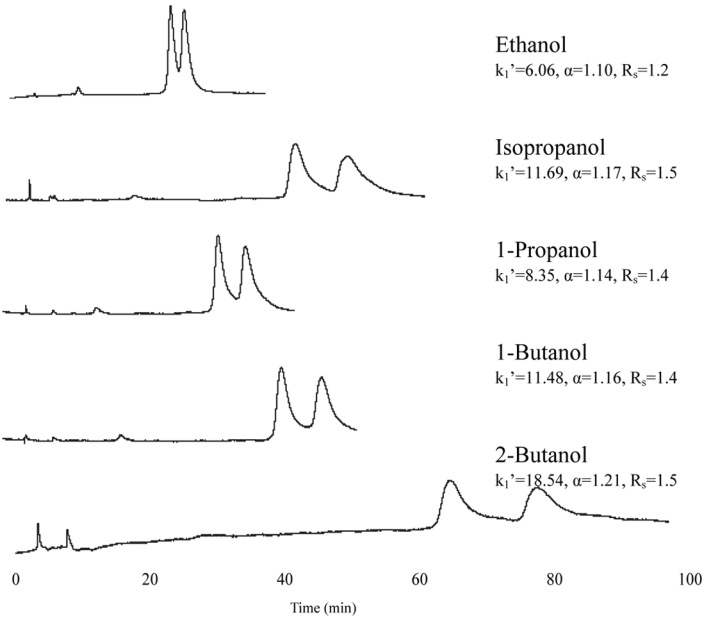


Figure 8. Effect of nature of alcohol on enantioseparations of 6,6′-dibromo-1,1′-bi-2-naphthol in the normal phase mode. The mobile phase is composed of 90% heptane/10%alcohol and all the other chromatographic conditions are kept the same.

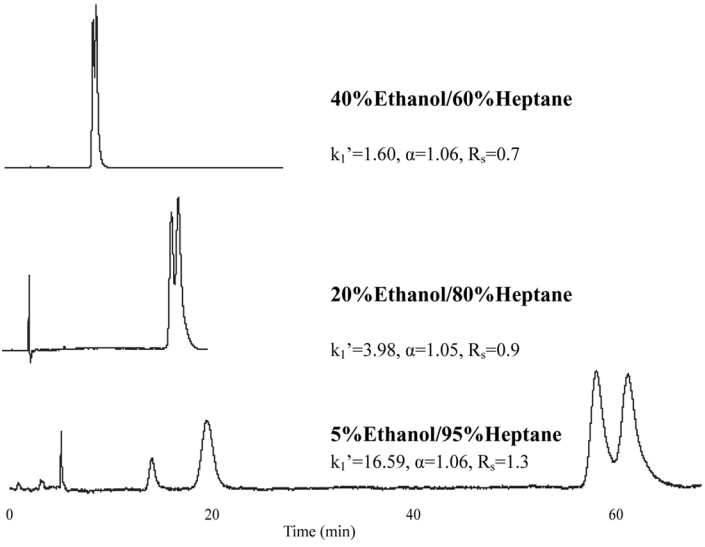


Figure 9. Effect of concentration of alcohol. The analyte is 6,6′-dibromo-1,1′-bi-2-naphthol.

Table 4. Effect of salt type in the mobile phase on enantioseparation

Salt type	k_1'	α	Rs
NH_4NO_3	11.95	1.15	1.3
$N(CH_3)_4NO_3$	8.22	1.07	0.9
NH_4Cl	33.42	1.09	1.1
NH_4COOCF_3	12.76	1.09	1.2
NH_4COOCH_3	No elution		

Note: O,O′-Di-p-toluoyl-DL-tartaric acid is separated on CSP 3. The mobile phase is 100%methanol/12.5 mM salt.

mobile phase can usually shorten the retention time and improve chromatographic efficiency. In these studies, additives were necessary to elute acidic compounds by competing with analytes for strong binding sites. Different salts were tested as additives, while the salt concentration remained the same (12.5 mM). The chromatographic results are listed in Table 4, and it is evident that the type of salt additives affects retention and resolution significantly. The highest selectivity and resolution were obtained with the mobile phase containing ammonium nitrate. Furthermore, increasing the salt concentration was found to decrease retention (data not shown), which is a trend usually observed in the polar organic mode on other chiral stationary phases.

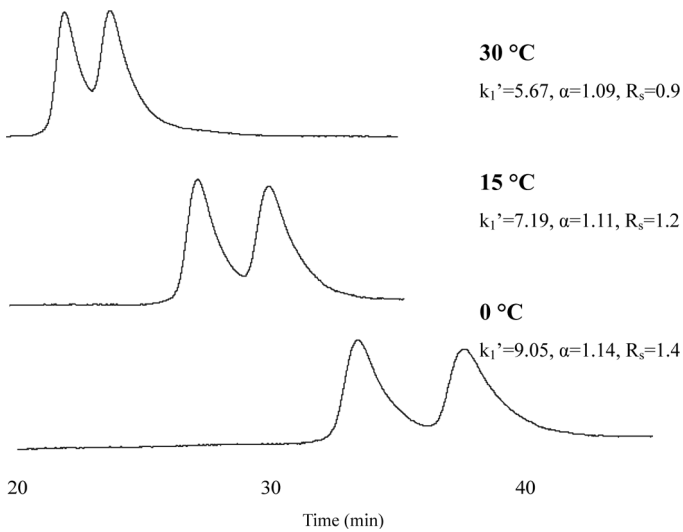


Figure 10. Effect of temperature on enantioresoluion of 6,6′-dibromo-1,1′-bi-2-naphthol. The mobile phase is 90%heptane/10%ethanol.

In order to study the effect of temperature on enantioseparation of Ru complex bonded CSP, a study of varying temperature between 0°C–30°C was carried out. Chromatograms at three specific temperatures are shown in Figure 10. When increasing temperature, enantioresolution decreases due to lower retention and selectivity. Resolution of chiral separation is often improved by lowering the column temperature.

CONCLUSIONS

Silica gel based Ru complex bonded chiral stationary phases have been developed for the first time. The best Ru complex column was obtained using a reductive amination binding reaction and high surface area silica. Extensive liquid chromatographic studies with UV and CD detectors show Ru complex bonded CSP provide enantioselectivity toward a wide variety of compounds, in particular, compounds with acidic groups. While the Ru complex bonded CSP did not have high efficiency, the bonded phase is likely more robust than its coated predecessors. The development of the first transition metal complex bonded chiral stationary phases and chromatographic studies provide insight concerning chiral stationary phase bonding strategies.

ACKNOWLEDGMENT

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