Enantiomeric Separations of Ruthenium (II) Polypyridyl Complexes Using HPLC With Cyclofructan Chiral Stationary Phases

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ABSTRACT The enantiomeric separation of 21 ruthenium (II) polypyridyl complexes was achieved with a novel class of cyclofructan-based chiral stationary phases (CSPs) in the polar organic mode. Aromatic derivatives on the chiral selectors proved to be essential for enantioselectivity. The R-napthylethyl carbamate functionalized cyclofructan 6 (LARIHC CF6-RN) column proved to be the most effective overall, while the dimethylphenyl carbamate cyclofructan 7 (LARIHC CF7-DMP) showed complementary selectivity. A combination of acid and base additives was necessary for optimal separations. The retention factor vs. acetonitrile/methanol ratio plot showed a U-shaped retention curve, indicating that different interactions take place at different polar organic solvent compositions. The separation results indicated that π–π interactions, steric effects, and hydrogen bonding contribute to the enantiomeric separation of ruthenium (II) polypyridyl complexes with cyclofructan chiral stationary phases in the polar organic mode. *Chirality 27:64–70, 2015.* © 2014 Wiley Periodicals, Inc.

KEY WORDS: cyclofructan; ruthenium (II) polypyridyl complexes; enantiomeric separation; chiral stationary phase; LARIHC

INTRODUCTION

A plethora of ruthenium(II) polypyridyl complexes have been synthesized due to their robust nature and distinctive electrochemical and photophysical characteristics. Recently, these complexes have been widely used for cellular imaging and therapeutics, as they are known to have specific interactions with DNA.[1–4] In addition, ruthenium(II) complexes are effective catalysts for organic synthesis and dye sensitizers for solar cells.[5–8] They are also used to construct various supramolecular assemblies.[9–11] Ruthenium(II) polypyridyl complexes exhibit axial chirality and the right- and left-handed configurations of the octahedral complexes are referred to as Δ- and Λ-enantiomers. Enantiomers of ruthenium(II) complexes exhibit very different biological activities when used as DNA intercalating agents, stabilizers of G-Quadruplex DNA, and inhibitors of enzyme activity.[12–14] As a catalyst, enantiomers of ruthenium(II) complexes dramatically influence the stereochemistry of chiral products.[15] Therefore, there is a great need for analytical methods by which ruthenium(II) polypyridyl complex enantiomers can be separated and evaluated.

The separation of geometric isomers, diastereomers, and enantiomers of ruthenium complexes have been achieved by chromatographic methods and capillary electrophoresis using chiral selectors.[16–22] Capillary electrophoresis is not suitable for preparative-scale separations and, as such, high-performance liquid chromatography (HPLC) with chiral stationary phases (CSPs) has proven to be the best way to separate enantiomers of organometallic compounds due to the technique's broad selectivity, high efficiency, and ability to transition to preparative-scales. For example, the enantiomeric separation of ruthenium polypyridyl complexes has been obtained with macrocyclic glycopeptide CSPs and cyclodextrin (CD) CSPs.[20–22]

Cyclofructans (CFs) are structural isomers of CDs. They are naturally occurring chiral crown ethers which consist of β-(2-1) linked D-fructofuranose units. Recently, isopropyl carbamate CF6 (LARIHC CF6-P), R-naphthylethyl-carbamate CF6 (LARIHC CF6-RN), and dimethylphenyl carbamate CF7 (LARIHC CF7-DMP) have been developed as bonded chiral stationary phases for HPLC. These CF CSPs provide excellent selectivities for many racemic compounds (spiroindoline phytoalexins, binaphthyl catalysts, tetrahydrobenzimidazoles, chiral acids, amines, amino compounds, metal complexes, neutral compounds, etc).[23–29] The LARIHC CF6-P shows unique selectivity and broad applicability for amine-containing racemates. LARIHC CF6-RN, and LARIHC CF7-DMP exhibit complementary selectivities. Previous studies showed that CFs behave very differently compared to CDs when used as chiral selectors. Sulfated cyclofructan 6 was superior to sulfated cyclodextrins in the enantiomeric separation of four basic pharmaceuticals by capillary electrophoresis.[30] The R-naphthylethyl functionalized CF6 CSP proved more suitable for the enantiomeric separation of binaphthyl catalysts when compared to the R-naphthylethyl functionalized CD CSP.[25] It is necessary to develop new CSPs and apply them comprehensively to strive for better or complementary enantiomeric separations.

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In the present work, LARIHC CF6-P, LARIHC CF6-RN, and LARIHC CF7-DMP columns were utilized for the enantiomeric separation of 21 ruthenium(II) polypyridyl complexes, 19 of which have not been separated previously by any means. The dependence of separation performance on the chiral selector structure, mobile phase composition, type, and concentration of additives and the structure of analytes were investigated to explore the chiral separation mechanism.

EXPERIMENTAL

Materials and Chemicals

Triethylamine (TEA), trimethylamine, ethanolamine, butylamine, acetic acid (AA), trifluoroacetic acid, formic acid, ammonium nitrate, tetramethylammonium nitrate, ammonium trifluroacetate, and tetramethylammonium acetate were purchased from Aldrich Chemical (St. Louis, MO) and used without further purification. Water was obtained from Millipore (Billerica, MA). Acetonitrile (ACN) and methanol (MeOH) of HPLC grade were purchased from VWR (Boston, MA).

The 21 racemic ruthenium (II) polypyridyl complexes used in this study were produced according to the literature.[31–37] The structures of the ligands and ruthenium(II) polypyridyl complexes are depicted in Figure 1A,B, respectively. Figure 1C gives the structures and the names of each individual ruthenium(II) polypyridyl complex.

Isopropyl carbamate CF6 (LARIHC CF6-P), R-naphthylethyl-carbamate CF6 (LARIHC CF6-RN), and dimethylphenyl carbamate CF7 (LARIHC CF7-DMP) columns, 25 × 0.46 cm (i.d.), were obtained from AZYP (Arlington, TX).

Chromatographic Conditions

An Agilent 1200 HPLC (Agilent Technologies, Palo Alto, CA) was used in this study. It consisted of a 1200 diode array detector, autosampler, and quaternary pump. All separations were carried out at room temperature unless stated otherwise. For all HPLC experiments, the injection volume was 5 μL and the flow rate was 1.0 mL/min in isocratic mode. The UV wavelength of 254 nm was employed for detection. The chloride (Cl^-) salts and hexafluorophosphate (PF_6^-) salts of ruthenium(II) polypyridyl complexes were dissolved in methanol and acetonitrile, respectively. Samples were dissolved at 1.0 mg/mL concentration and subsequently diluted with methanol or acetonitrile for LC injection. Each sample was analyzed in duplicate. The "dead time" t_0 was determined by the peak of the refractive index change due to the unretained sample solvent.

RESULTS AND DISCUSSION

Enantiomeric Separations of Ruthenium(II) Polypyridyl Complexes With LARIHC CSPs

The enantiomeric separation of 21 ruthenium(II) polypyridyl complexes was evaluated in the polar organic mode using LARIHC CF6-P, LARIHC CF6-RN, and LARIHC CF7-DMP CSPs. The results showed that the racemates of ruthenium (II) polypyridyl complexes are only separated by aromatic derivatized cyclofructans (i.e., LARIHC CF6-RN and LARIHC CF7-DMP) but not by nonaromatic derivatized cyclofrutans (i.e., LARIHC CF6-P). This indicates that π–π interaction between the aromatic groups of the CSPs and polypyridyl groups of the analytes is one major factor for chiral recognition. This is consistent with previous studies on the enantiomeric separation of ruthenium(II) polypyridyl complexes with three native and six derivatized β-cyclodextrin CSPs, where only the three aromatic derivatized CSPs showed enantiomeric selectivity.[22] In the case of the R- and S-naphthylethyl derivatized CD CSPs, it has been shown that the stereogenic configuration of the derivative group on the oligosaccharide is more important than the cyclodextrin molecule, even though the attached chiral

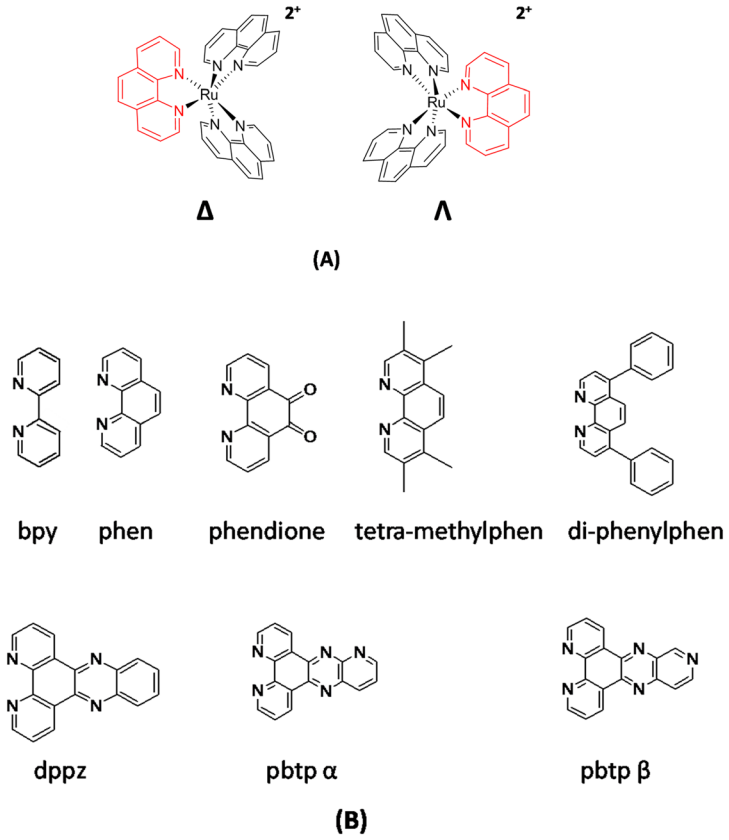

Fig. 1. Structures of (**A**) stereochemistry of $[Ru(phen)_3]^{2+}$, (**B**) the polypyridyl ligands, and (**C**) the cation of ruthenium (II) polypyridyl complexes.

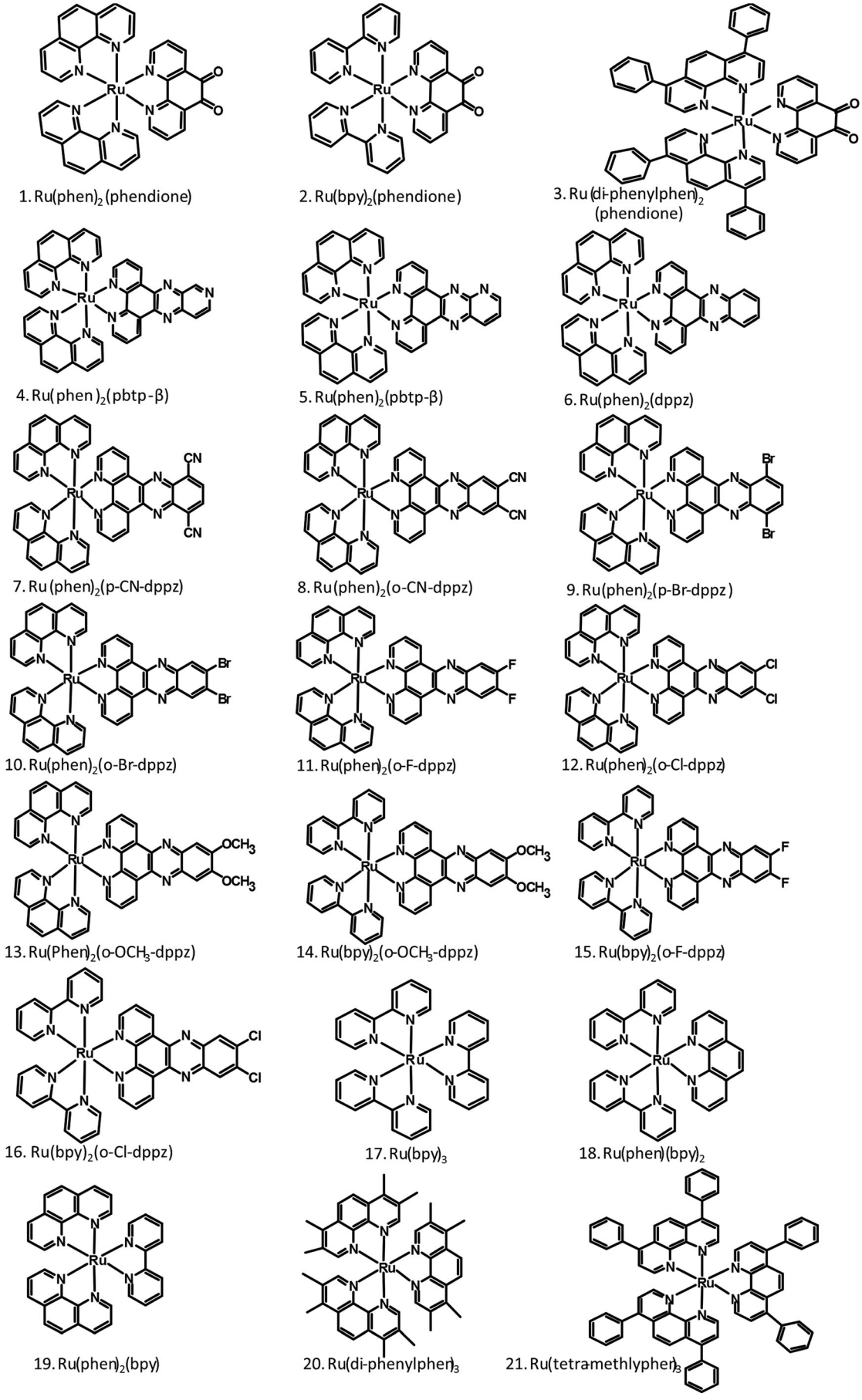

Fig. 1. (Continued)

moiety and cyclodextrin molecule contribute to the chiral recognition in a synergistic or antagonistic fashion.[38] Similarly, the need for aromatic derivatized cyclofructan (CF) chiral selectors indicated that the external interaction (i.e., outer portion of the macrocycle) between the analytes and derivative group play a significant role in chiral recognition as well.

Table 1 shows the optimized enantiomeric separation conditions for the 21 ruthenium(II) polypyridyl complexes on the LARIHC CF6-RN and CF7-DMP columns. The aromatic derivatized columns provided excellent enantiomeric separations. Both LARIHC columns yielded a 100% success rate in separating these analytes, meaning every compound was at least partially separated on each column. Further, R_S values as high as 6.2 (LARIHC CF6-RN, compound **9**) were obtained. All but one of these racemates (compound **17**) was greater than baseline separated on one or both CSPs.

The LARIHC CF6-RN generally gave higher selectivity values (1.08–2.28) than the CF7-DMP CSP (1.04–1.26). However, enantiomers of compound **2** [Ru(bpy)$_2$(phendione)](Cl$_2$) were baseline separated on the CF7-DMP CSP with good selectivity ($\alpha = 1.30$), while only partially separated by the CF6-RN CSP ($\alpha = 1.08$). This indicates that LARIHC CF6-RN and CF7-DMP show complementary enantioselectivities in the chiral separation of ruthenium(II) polypyridyl complexes. This complementary nature has been noted before, as the CF6-RN CSP provided a higher enantioselectivity for chiral amines and the CF7-DMP CSP had greater success separating chiral acids.[24] The complementary characteristic between the CF6-RN and CF7-DMP columns facilitates separating a large number of different compounds.

Effect of Additives on the Enantiomeric Separation

Enantiomeric separations of ruthenium(II) polypyridyl complexes were observed mainly in polar organic mode and were not successful in the normal phase due to the lack of solubility of the analytes in the mobile phase. The mobile phase for the polar organic mode usually is composed of acetonitrile, methanol, and small amounts of triethylamine (TEA) and acetic acid (AA). In order to evaluate the effects of additives, the separation of compound **14** [Ru(bpy)$_2$(o-OCH$_3$-dppz)](PF$_6$)$_2$ was investigated using both the LARIHC CF6-RN and LARIHC CF7-DMP columns and a mobile phase of ACN/MeOH = 30/70, in which baseline separation can be achieved. Figure 2 shows the chromatograms of compound **14** on the LARIHC CF6-RN column with different amounts of TEA and AA in the mobile phase. When no additive was used (Fig. 2G), the analyte did not elute within 1 h. The same was true when just an acid or a base was used (Fig. 2E,F). Therefore, it was found to be necessary to use a combination of acid (AA) and base (TEA) in the mobile phase. As shown in Figure 2A–C, having a molar excess of base (Fig. 2A) or acid (Fig. 2B,C) did not eliminate the peak tailing and selectivity did not change. However, when equimolar amounts of acid and base were used (Fig. 2D), efficiency increased greatly, resulting in the great resolution. Next, keeping the acid/base

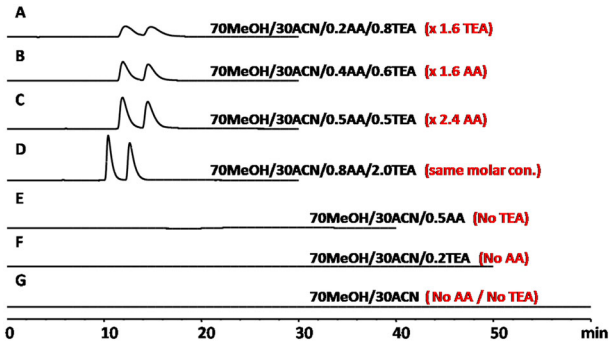

Fig. 2. The separation of [Ru(bpy)$_2$(o-OCH$_3$-dppz)](PF$_6$)$_2$ enantiomers on LARIHC CF6-RN at various AA/TEA (v/v) ratio. Molar excess of acid or base listed in parenthesis. Flow rate: 1 mL/min, UV detection: 254 nm.

TABLE 1. Summary of the optimized enantiomeric separations of 21 ruthenium (II) polypyridyl complexes on LARIHC CF6-RN and LARIHC CF7-DMP

			LARIHC CF6-RN			LARIHC CF7-DMP		
Code	Name	Mobile phase	k_1	α	R_S	k_1	α	R_S
1	[Ru(phen)$_2$(phendione)](Cl$_2$)	100MeOH/1.6AA/2.4TEA	2.50	1.30	2.3	1.44	1.14	1.6
2	[Ru(bpy)$_2$(phendione)](Cl$_2$)	100MeOH/1.6AA/2.4TEA	1.31	1.08	0.6	0.82	1.30	2.2
3	[Ru(di-phenylphen)$_2$(phendione)](Cl$_2$)	100MeOH/1.6AA/2.4TEA	1.00	1.21	2.5	0.51	1.24	3.0
4	[Ru(phen)$_2$(pbtp β)](PF$_6$)$_2$	95MeOH / 5ACN/0.05 M N(CH$_3$)$_4$NO$_3$	1.27	1.54	2.2	3.35	1.25	3.9
5	[Ru(phen)$_2$(pbtp α)](PF$_6$)$_2$	95MeOH / 5ACN/0.05 M N(CH$_3$)$_4$NO$_3$	1.31	1.50	3.0	3.10	1.26	4.2
6	[Ru(phen)$_2$(dppz)](PF$_6$)$_2$	30ACN / 70MeOH/ 1.6AA / 4TEA	4.52	1.53	3.7	2.37	1.25	4.4
7	[Ru(phen)$_2$(p-CN-dppz)](PF$_6$)$_2$	30ACN / 70MeOH/ 1.6AA / 4TEA	4.28	1.87	5.5	3.38	1.19	3.0
8	[Ru(phen)$_2$(o-CN-dppz)](PF$_6$)$_2$	30ACN / 70MeOH/ 1.6AA / 4TEA	5.38	1.44	3.1	3.08	1.21	3.3
9	[Ru(phen)$_2$(p-Br-dppz)](PF$_6$)$_2$	30ACN / 70MeOH/ 1.6AA / 4TEA	5.17	2.00	6.2	4.23	1.24	3.5
10	[Ru(phen)$_2$(o-Br-dppz)](PF$_6$)$_2$	30ACN / 70MeOH/ 1.6AA / 4TEA	5.55	1.59	4.0	3.60	1.27	3.6
11	[Ru(phen)$_2$(o-F-dppz)](PF$_6$)$_2$	30ACN / 70MeOH/ 1.6AA / 4TEA	4.24	1.53	3.3	2.42	1.25	3.3
12	[Ru(phen)$_2$(o-Cl-dppz)](PF$_6$)$_2$	30ACN / 70MeOH/ 1.6AA / 4TEA	4.38	1.54	3.9	3.43	1.26	4.0
13	[Ru(Phen)$_2$(o-OCH$_3$-dppz)](PF$_6$)$_2$	30ACN / 70MeOH/ 1.6AA / 4TEA	4.59	1.51	3.7	2.30	1.20	3.2
14	[Ru(bpy)$_2$(o-OCH$_3$-dppz)](PF$_6$)$_2$	30ACN / 70MeOH/ 1.6AA / 4TEA	4.58	1.23	1.8	1.59	1.11	1.9
15	[Ru(bpy)$_2$(o-F-dppz)](PF$_6$)$_2$	30ACN / 70MeOH/ 1.6AA / 4TEA	3.97	1.23	1.5	2.11	1.17	2.5
16	[Ru(bpy)$_2$(o-Cl-dppz)](PF$_6$)$_2$	30ACN / 70MeOH/ 1.6AA / 4TEA	4.10	1.24	1.7	3.72	1.18	3.1
17	[Ru(bpy)$_3$](PF$_6$)$_2$	100MeOH/1.6AA/4TEA	6.79	1.06	0.6	1.44	1.04	0.5
18	[Ru(phen) (bpy)$_2$](PF$_6$)$_2$	100MeOH/1.6AA/4TEA	7.86	1.12	1.0	2.52	1.07	0.8
19	[Ru(phen)$_2$(bpy)](PF$_6$)$_2$	100MeOH/1.6AA/4TEA	8.44	1.22	1.9	2.88	1.13	1.5
20	[Ru(di-phenylphen)$_3$](PF$_6$)$_2$	100MeOH/1.6AA/4TEA	0.86	1.56	1.9	1.57	1.06	1.0
21	[Ru(tetra-methlyphen)$_3$](PF$_6$)$_2$	100MeOH/1.6AA/4TEA	1.24	2.28	4.3	2.27	1.20	0.9

TABLE 2. Effect of types of bases and acids in the mobile phase (ACN/MeOH/acid/base) on enantiomeric separation of $[Ru(bpy)_2(o\text{-}OCH_3\text{-}dppz](PF_6)_2$ with LARIHC CF6-RN and LARIHC CF7-DMP chiral stationary phases*

	LARIHC CF6-RN			LARIHC CF7-DMP		
	k_1	α	R_S	k_1	α	R_S
BASE ADDITIVES						
Trimethylamine	1.59	1.33	1.6	3.45	1.13	1.8
Ethanolamine	1.44	1.30	1.5	4.96	1.15	1.9
Butylamine	1.76	1.29	1.5	4.48	1.14	1.7
Triethylamine	1.31	1.33	1.6	2.34	1.12	1.9
ACID ADDITIVES						
Acetic acid	1.31	1.33	1.6	2.34	1.12	1.9
Trifluoroacetic acid	1.63	1.26	1.5	1.31	1.11	1.6
Formic acid	4.56	1.24	1.8	2.20	1.13	2.2

*MeOH/ACN: 70/30, concentration of acid: 1.0%, molar ratio of acid/base: 1, acetic acid used when testing bases and TEA used when testing acids, flow rate: 1 mL/min, UV detection: 254 nm.

molar ratio at one, an evaluation of several different bases and acids (Table 2) was performed to understand the influence on the separation of compound **14**. Overall, comparing the data in Table 2, it was determined that a mix of acetic acid and triethylamine resulted in the greatest selectivities and efficiencies in the shortest amount of time. Also, it appears that the acidic additives used in the polar organic mode have a greater influence on the selectivity, efficiency, and retention of the analytes than basic additives.

To further improve peak efficiency, mobile phases with increasing ionic strength (equal molar concentrations of AA and TEA) were prepared. Figure 3A–D shows that an increase in ionic strength decreased the retention time and led to improved peak efficiencies. The combination of 280 mM AA and 280 mM TEA was found to be the optimized additive ratio for 80% of the analytes in this study.

Considering that equimolar amount of acids and bases gave the optimum separation, the effect of simple ammonium salts added to the mobile phase on the enantiomeric separation was also investigated on both the LARIHC CF6-RN and LARIHC CF7-DMP columns. Note that these ammonium and tetraalkylammonium additives can only be tested in their equimolar salt form due to the nonaqueous mobile phases being used. Further, such ammonium salts have proven to be the best additives for separating similar chiral ruthenium(II)

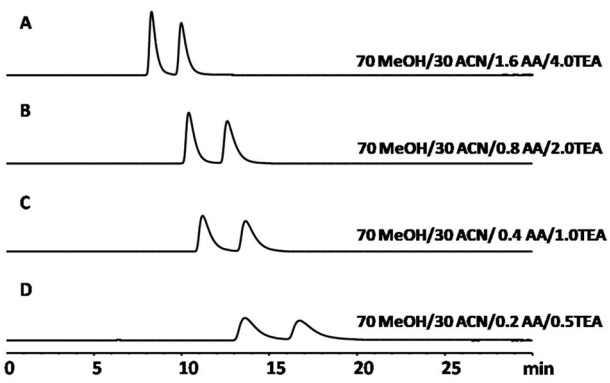

Fig. 3. The separation of $[Ru(bpy)_2(o\text{-}OCH_3\text{-}dppz](PF_6)_2$ enantiomers on LARIHC CF6-RN column at various AA:TEA (v:v) amount. Molar ratio of AA/TEA: 1, flow rate: 1 mL/min, UV detection: 254 nm.

TABLE 3. Effect of salt type in the mobile phase on enantiomeric separation of $[Ru(bpy)_2(o\text{-}OCH_3\text{-}dppz](PF_6)_2$ with LARIHC CF6-RN and LARIHC CF7-DMP chiral stationary phases

	LARIHC CF6-RN			LARIHC CF7-DMP		
Salts	k_1	α	R_S	k_1	α	R_S
NH_4NO_3	1.17	1.40	1.5	3.34	1.11	1.1
$N(CH_3)_4NO_3$	0.52	1.35	1.3	3.09	1.12	1.4
NH_4COOCF_3	0.62	1.22	0.8	2.75	1.10	1.0
$N(CH_3)_4COOCH_3$	7.52	1.15	0.9	6.50	1.14	1.6

MeOH/ACN: 70/30, concentration of salt: 0.025 mol/L, flow rate: 1 mL/min, UV detection: 254 nm.

complexes on cyclodextrin and macrocyclic glycopeptides CSPs.[21,22] Ammonium nitrate, tetramethylammonium nitrate, ammonium trifluroacetate, and tetramethylammonium acetate were tested as additives in a 70/30 methanol/acetonitrile mobile phase. Compound **14** was enantiomerically separated with all the above-mentioned additives and the separation results are listed in Table 3. Tetramethylammonium nitrate resulted in the best compromise of short analysis time and good resolution and was determined to be the most useful salt. This is in agreement with past reports using macrocyclic glycopeptide CSPs and cyclodextrin CSPs.[21,22] However, for the CF-based CSPs used here, AA/TEA additives are most useful, so the tetramethylammonium nitrate was only used as a secondary option.

Effects of Mobile Phase Composition on the Enantiomeric Separation

It has been reported that the use of acetonitrile as a modifier produced the best resolution in the polar organic mode.[23] Hence, the effect of the percentage of acetonitrile in the mobile phase containing AA/TEA additives was examined. Figure 4A shows that the retention factor of compound **14** slightly decreased with increased the concentrations of ACN until it reached 50/50 ACN/MeOH. Then retention gradually increased in the range of 50–80% ACN. Subsequently, the retention time steeply increased when the concentration of ACN exceeded 80%. The increase of the retention factor with the concentration of ACN is probably attributed to the competition of the methanol with the analyte for hydrogen bonding sites on the stationary phase. Similar competition between methanol and analytes occurred on cyclodextrin CSPs in the polar organic mode.[39]

The dependence of retention factor on the concentration of acetonitrile was also carried out by using another additive, ammonium nitrate (see Fig. 4B). Here, a U shape retention curve was observed and the retention factor decreased with increasing acetonitrile concentration from 0–40%. It reached a minimum in the range of 40–60% and then increased again with acetonitrile concentration increasing from 60% to 85% (ammonium nitrate does not dissolve at higher acetonitrile concentrations). The retention factor increased with acetonitrile percentage due to the competition of the methanol with the analyte for hydrogen bonding sites at high concentrations of acetonitrile. However, the opposite trend is observed when the ACN is below 40%. This suggests that other interactions dominated in the chromatographic separation. The reason for increased retention in high methanol concentrations when using an ammonium salt is unclear, but this trend was

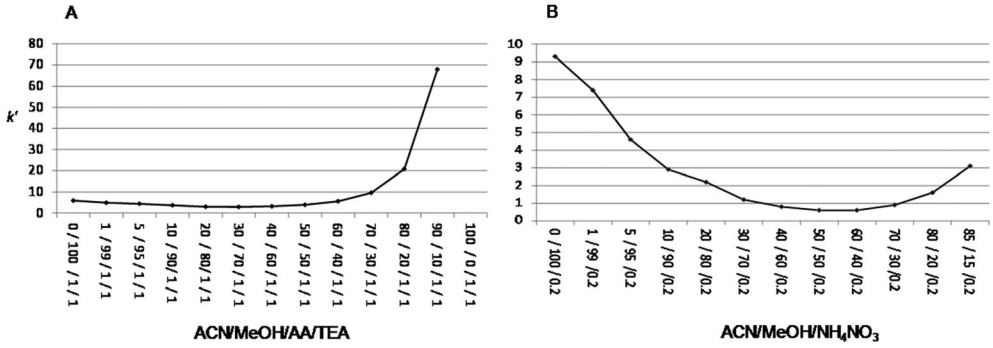

Fig. 4. The retention factor of compound $[Ru(bpy)_2(o\text{-}OCH_3\text{-}dppz](PF_6)_2$ vs. acetonitrile concentration on LARIHC CF6-RN. The mobile phase composition: (**A**) ACN/MeOH/AA/TEA (v/v), (**B**) ACN/MeOH/NH_4NO_3 (w/w).

observed on CD CSPs as well.[22] It should be noted that this "dual" retention behavior provides a second means of method development, separation, and optimization.

Influence of the Enantiomer Structure on the Enantiomeric Separation

The dependence of retention and separation on the structure of the racemic ruthenium(II) polypyridyl complexes was investigated to explore the enantiomeric separation mechanism. The PF_6^- anion of the ruthenium(II) polypyridyl complexes was exchanged to Cl^- and the analysis was carried out on an LARIHC CF6-RN column under the same mobile phase conditions. When the separation of $[Ru(phen)_2(o\text{-}Cl\text{-}dppz)](PF_6)_2$ is compared with that of $([Ru(phen)_2(o\text{-}Cl\text{-}dppz)](Cl)_2$, the chromatographic data, such as retention time, selectivity, and efficiency did not change. This is likely attributed to the exchange of anions from the mobile phase additives with initial counter anions of the complex.

Table 1 summarizes the chromatographic data for all ruthenium (II) polypyridyl complexes. Compounds **1** and **3** are structurally related compounds, which contain one phendione ligand but different aromatic moieties for the remaining ligands. Compound **1** was retained much longer than compound **3**, while compound **3** had much greater resolution values. Apparently, the steric bulk of the "diphenylphen" ligands on compound **3** decreased nonenantioselective retention interactions, while enhancing enantioselectivity.

The effect of the positions of substituent groups (*ortho* and *para*) on a given ligand (dppz) was also investigated. Analytes **7** and **8** have cyano (CN) substituents at the *ortho* (o) and *para* (p) positions in the dppz ligand, respectively. Analytes **9** and **10** have bromo (Br) substituents in those positions. Interestingly, higher resolutions were obtained for the *para* substituted analytes on the LARIHC CF6-RN CSP, whereas the analytes with *ortho* substitutions were better separated on the LARIHC CF7-DMP CSP. This highlights the complementary nature of these two CSPs.

Analytes which have *ortho* substituted electron-withdrawing groups (compounds **10**, **11**, **12**, **15**, **16**) generally showed higher selectivity and resolution than the analytes which have electron-donating groups (compounds **13**, **14**) on the CF7-DMP CSP. It appears that the electron-withdrawing groups (-CN, -Br, -Cl, -F) on the dppz ligands makes these analytes more π-acidic and the π–π interaction between π-acidic analytes and the π-basic dimethylphenyl groups is enhanced. This leads to improved separation of those analytes.

A series of ruthenium(II) polypyridyl complexes (**17–19**) which possess different numbers of "phen" and "bpy" ligands were separated on both columns. The retention factors and selectivities of these analytes increased in the order of **17** < **18** < **19**. Thus, as the size of the conjugated system increases, so does enantioselectivity.

CONCLUSION

We found greater than baseline separations for 20 of 21 chiral ruthenium (II) polypyridyl complexes (of which 19 have never been separated) using cyclofructan-based CSPs. Additives played an essential role in the enantioselectivity and retention. It appears that π–π interactions are of great importance for these separations, as aliphatic derivatives of cyclofructan were unable to discriminate between the enantiomers. It is anticipated that these separations will be very important with the growing interest in chiral metallo-organic complexes and future studies will focus on determining elution patterns of Δ- and Λ-enantiomers which will be helpful in preparative separations.

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