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Enantiomeric Separations of Ruthenium(II) Polypyridyl Complexes Using High-Performance Liquid Chromatography (HPLC) with Cyclodextrin Chiral Stationary Phases (CSPs)

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Rapid, highly efficient, analytical resolution of the enantiomers of eight different monomeric ruthenium(II) polypyridyl complexes has been achieved using HPLC with cyclodextrin chiral stationary phases. This technique also proved capable of separating both of the diastereomers and the enantiomers of one dinuclear complex in a single run, whereas similar efforts with another dinuclear complex gave only one stereoisomer cleanly. Factors such as the stereochemistry of the chiral selectors, solvent polarity, and salt effects can be altered to provide precise control of the enantioselective interactions. The ability to quickly and quantitatively determine the enantiopurity of a given ruthenium complex allowed facile reexamination and optimization of the commonly used bulk resolution procedures based on diastereomeric coprecipitation with sodium arsenyl (+)-tartrate or sodium arsenyl (–)-tartrate salts.

Introduction

The helical chirality inherent in octahedral transition-metal complexes bound by three bidentate ligands has fascinated chemists for over a century.[1,2] The right- and left-handed configurations of these metal complexes are referred to as Δ and Λ enantiomers, respectively (part a of Figure 1).[3] Derivatives of $[Ru(bpy)_3]^{2+}$ and $[Ru(phen)_3]^{2+}$ (bpy = 2,2′-bipyridine and phen = 1,10-phenanthroline) have enjoyed a unique amount of attention, owing to the robust nature of the complexes and the favorable electrochemical and photophysical properties.[4,5] In addition, the skeletal rigidity and variable functionality of such ruthenium(II) complexes has led to their application as catalysts for asymmetric synthesis.[6–8]

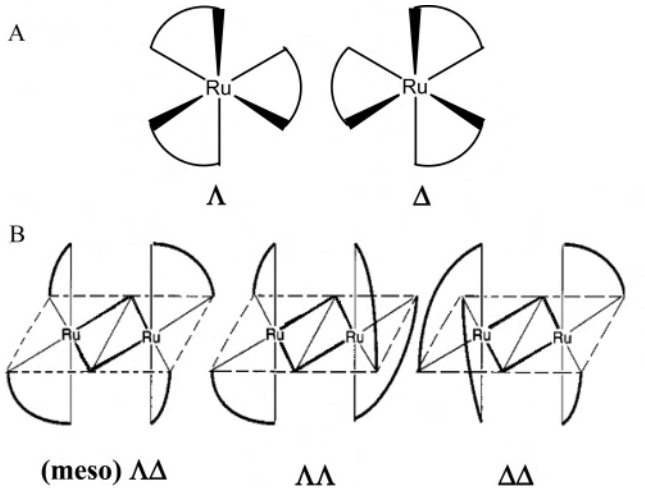

Figure 1. Stereoisomers of ruthenium(II) trisdiimine complexes, (a) enantiomeric mononuclear complexes and (b) distereomeric (meso) and enantiomeric dimeric complexes.

They have shown potential as DNA probes or cleavage agents, in part, because of their stereoselective interactions with DNA.[9–19] They can also be used as building blocks in the synthesis of a variety of higher nuclearity, supermolecular

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assemblies.[20−33] As seen in part b of Figure 1, having only two such metal centers quickly increases the stereochemical complexity. Many of these applications require stereochemically pure compounds or, at least, a knowledge of the stereochemical composition.

Keene and co-workers developed the first general chromatographic method for the separation of the geometric isomers, diastereomers, and enantiomers of metal-polypyridyl complexes containing up to three chiral centers.[31,32,34−42] Their method relies on cation exchange and ion pairing chromatography, in which the nature of the anionic additive to the mobile phase is typically the determining factor. Diastereomers can be separated on cation-exchange resins by the addition of nonchiral anionic additives (e.g., benzenesulfonate, tolulene-4-sulfonate), whereas the resolution of enantiomers requires anionic chiral selectors such as (+)- and (−)-O,O′-dibenzoyl-tartrate or (+)- and (−)-O,O′-di-*p*-toluoyl-tartrate. The biggest disadvantage of this method is the relatively long times that are sometimes required to complete a separation. More recently, they have shown that DNA-based columns can be useful for the resolution of chiral ruthenium complexes.[43]

Lacour and co-workers[44,45] and more recently Gruselle et al.[46] have shown that ruthenium complexes can be resolved on silica using Δ- or Λ-[tris(tetrachlorocatecholato)P(V)] as a chiral ion-pairing agent. Resolutions of ruthenium complexes were also reported using capillary electrophoresis (CE).[47−53] Kane-Maguire et al. used capillary zone electrophoresis (CZE) with enantiopure tartrate salt as the chiral selector in the running buffer to separate enantiomers of 16 transition-metal complexes,[48] which have different metal centers (Ru^{2+}, Ni^{2+}, Cr^{3+}, and Co^{3+}) and bidentate ligands (bipyridine, phenanthroline, and oxalate). Chiral discrimination of $[Ru(phen)_3]^{2+}$ and $[Ru(bpy)_3]^{2+}$ was also achieved using CZE with double-stranded DNA dissolved in the run buffer.[49] The DNA was shown to have different binding affinities to the enantiomers. Unfortunately, the CE technique often has reproducibility problems, and it is incapable of working as a preparative method.

Because of its flexibility, broad selectivity, and high efficiency, HPLC with chiral stationary phases (CSPs) is the dominant method for enantiomeric separations and analyses. It is widely used both as an analytical method and as a preparative tool. Vos and co-workers were among the first to explore HPLC as a method to resolve ruthenium(II) polypyridyl complexes.[54,55] They used a teicoplanin chiral stationary phase to resolve a series of chiral monomeric ruthenium(II) complexes, including $[Ru(L)_3]^{2+}$(L = 2,2′-bipyridine (bpy), 1,10-phenanthroline (phen), and 4,7-diphenyl-1,10-phenanthroline (dpphen)) and some mixed-ligand complexes. They also separated the diastereomers and enantiomers of one dinuclear complex at both the analytical and semi-preperative scale using this technique.

Since bonded cyclodextrin stationary phases developed in our laboratory were first commercialized in 1983, they have

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proven to be successful for separating enantiomers.[56−63] Among them, the aromatic-derivatized cyclodextrin CSPs are multimodal and capable of working in three operational modes, which extends the range of enantiomers resolved. Although they have been widely applied to resolve many different classes of compounds, this class of stationary phases has not been applied to the transition-metal polypyridyl complex enantiomers, to our knowledge.

In this work, we explore the use of enantioselective cyclodextrin-based HPLC as an analytical method to resolve eight chiral ruthenium(II) monomer complexes and to analyze the enantiomeric excess (ee) of these enantiomers obtained by other resolution procedures. Furthermore, these HPLC methods are shown to be applicable for the separation and resolution of the diastereomers and enantiomers of dinuclear complexes, such as those represented in part b of Figure 1. Finally, the development of HPLC methods for quantifying the ee of such complexes gives us a rapid and more-accurate assessment of the enantiomeric composition of the ruthenium complexes than the commonly applied circular dichroism (CD) and high-field NMR (with chiral-shift reagents) methods.[44,64−67]

In particular, we report that the *R*-naphthylethyl-carbamate-derivatized β-cyclodextrin stationary phase shows high enantioselectivity for this entire class of compounds. Other factors including the composition of the polar-organic mobile phase and the ligand structure were shown to have profound effects on the resolution efficiency. Using this method, we can also quickly and quantitatively evaluate the efficiency of resolution methods for such cationic complexes, including the commonly used method of diastereoselective coprecipitation with the chiral dianions, $[As_2((+)\text{-tartrate})_2]^{2-}$ and $[As_2((-)\text{-tartrate})_2]^{2-}$.

Experimental Section

Materials. The compounds arsenic(III)oxide, L(+)- and D(-)-tartaric acid, tetra-*n*-butylammonium chloride hydrate, hydrazine monohydrate, palladium on carbon (Pd/C, 10%), acetic acid (HOAc), triethylamine (TEA), sodium chloride, potassium nitrate, ammonium chloride, ammonium trifluoroacetate, and ammonium nitrate were purchased from Alfa Aesar (Ward Hill, MA) or Aldrich Chemical (St. Louis, MO) and used without further purification. Ammonium hexafluorophosphate was purchased from Oakwood Products (West Columbia, SC). Acetonitrile (ACN) and methanol (MeOH) of HPLC grade were purchased from EMD (Gillbstown, NJ). Water was obtained from Millpore (Billerica, MA).

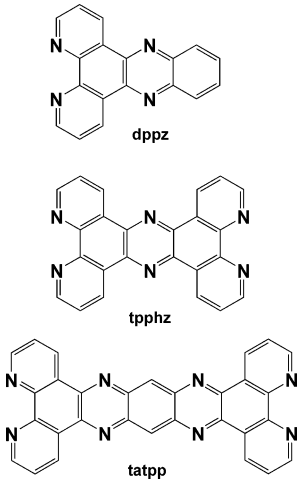

Figure 2. Structures of some of the polypyridyl ligands used in this study.

The compounds: 5-nitro-1,10-phenanthroline,[68] $Ru(phen)_2Cl_2$,[69,70] $[Ru(phen)_3]Cl_2$ (**1**),[71] $[Ru(phen)_2phendione]Cl_2$ (**4**)[10,72] (phendione = 1,10-phenanthroline-5,6-dione), $[Ru(phen)_2tatpp](PF_6)_2$ (**5**),[73] $[Ru(phen)_2(py)_2]Cl_2$ (**6**)[74] (py = pyridine), $[Ru(dppz)_3]Cl_2$ (**7**),[67] $[Ru(bpy)_3]Cl_2$ (**8**),[75] $[Ru_2(phen)_4(tpphz)]Cl_2$ (**9**),[22] and $[Ru_2(phen)_4(tatpp)]Cl_4$ (**10**),[76] were prepared according to literature procedures. The structures of the dppz, tpphz, and tatpp ligands are shown in Figure 2.

Cyclobond I (β-cyclodextrin), II (γ-cyclodextrin), III (α-cyclodextrin), AC (acetylated β-cyclodextrin), DM (dimethylated β-cyclodextrin), RSP (hydroxypropyl ether β-cyclodextrin), DMP (dimethylphenyl carbamate β-cyclodextrin), RN, and SN (i.e., *R*- or *S*-naphthylethyl carbamate derivatives of β-cyclodextrins) CSPs were obtained from Advanced Separation Technologies (Whippany, NF, USA). All of the columns are 250 × 4.6 mm (i.d.).

Equipment. The chromatographic separations were carried out on two HPLC systems. The first was a HP (Agilent Technologies, Palo Alto, CA) 1050 system with a UV VWD detector, an autosampler, a quaternary pump, and Chemstation software. The second system consisted of a circular dichroism (CD) detector (Jasco CD-2095, JASCO Corporation, Tokyo, Japan) and a Shimadzu LC-6A pump (Shimadzu, Tokyo, Japan). ^{1}H NMR spectra were obtained on a Bruker JEOL Eclipse Plus 500 MHz spectrometer, using CD_3CN as the solvent. Chemical shifts are given in ppm and referenced to TMS.

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Synthesis and Resolutions. Sodium Arsenyl (+) or (-) Tartrate. These salt compounds were prepared using slight modifications of the literature procedures.[77,78] L(+)-tartaric acid (20 g, 0.133 mol) and NaOH (5.33 g, 0.133mol) were dissolved in water (150 mL), and the solution was heated to reflux. As_2O_3 (13.1 g, 0.066 mol) was added, and the resulting slurry refluxed for 45 min, during which the solution became clear. The solution was filtered while hot, and 300 mL ethanol was added to the filtrate, which resulted in some precipitation. The resulting mixture was cooled to 4 °C for 12 h, upon which a large mass of white crystals formed. The crystals were isolated by filtration, washed with cold ethanol, and air-dried. Yield 29 g (90%). In an analogous manner, $Na_2[As_2((-)\text{-tartrate})_2]\cdot 3H_2O$ could be prepared in a similar yield from D(−)-tartaric acid. Both salts were identical to those reported by Marcovich and Tapscott in all respects.[77]

Resolution Procedure for $[Ru(phen)_3]Cl_2$. The following resolution procedure was widely applicable for most monomeric ruthenium complexes. Metatheses were conducted as follows: hexafluorophosphate salts of the complexes were converted to chloride salts by dissolving the complex in a minimal volume of dry acetone and dropwise addition of a saturated solution of tetra-*n*-butylammonium chloride hydrate in acetone. The chloride salt of the complex precipitates immediately and is collected by filtration, rinsed with acetone and diethyl ether, and dried in vacuo at 60 °C for 2 h. Arsenyltartrate salts were decomposed by dissolution in hot 2 M HNO_3, and the resulting solution was treated with a saturated solution of aqueous NH_4PF_6 to precipitate the hexafluorophosphate salts. These precipitates are isolated by filtration, washed with cold water, and dried in vacuo at 60 °C for 2 h.

Racemic $[Ru(phen)_3]Cl_2$ (1.0 g) was dissolved in 25 mL hot water (80 °C). A solution of $Na_2[As_2((+)\text{-tart})_2]\cdot 3H_2O$ (2.25 g in 30 mL hot water) was added into the racemic solution while stirring vigorously. The solution was chilled at 4 °C overnight. The solution was filtered, and the precipitate was treated by method A and the filtrate by method B.

Method A. The precipitate of enantioenriched Λ-$[Ru(phen)_3][As_2((+)\text{-tart})_2]$ was converted to the hexafluorophosphate salt and then chloride salt as described above. Yield 0.41 g (62% ee). The chloride salt was then dissolved in 15 mL water and warmed to 80 °C and treated with $Na_2[As_2((+)\text{-tart})_2]\cdot 3H_2O$ (1.25 g in 15 mL hot water) and chilled to 4 °C overnight. The precipitate was isolated by filtration and washing with cold water and ethanol. The precipitate was converted to the hexafluorophosphate salt as described above. Yield 0.42 g Λ-$[Ru(phen)_3][PF_6]_2$ (64%; 99.8% ee).

Method B. The filtrate was warmed to 80 °C, and a solution of $Na_2[As_2((-)\text{-tart})_2]\cdot 3H_2O$ (1.25 g in 15 mL hot water) was added while stirring. The solution was chilled overnight, and the precipitate isolated by filtration and washing with cold water and ethanol. The precipitate was converted to the hexafluorophosphate salt as described above. Yield 0.53 g Δ-$[Ru(phen)_3][PF_6]_2$ (82%; 99.5% ee).

$[Ru(phen)_2nitrophen](PF_6)_2$ (2). $Ru(phen)_2Cl_2$ (1.05 g, 1.97 mmol) was dissolved in 50 mL of a 1:1 mixture of water and ethanol and heated to reflux under N_2 atmosphere. Once refluxing, 5-nitro-1,10-phenanthroline (0.525 g, 2.3 mmol) was added in portions, and the mixture was refluxed for 12 h. After cooling, the solution was filtered, and the product was precipitated as a hexafluorophosphate salt upon the addition of aqueous NH_4PF_6. The product was isolated by filtration, washed with water, and oven dried at 60 °C. Yield 1.2 g *rac*-**2** (70%). Anal. Calcd for $RuC_{36}H_{23}N_7O_2P_2F_{12}\cdot H_2O$: C, 43.47; H, 2.53; N, 9.85. Found: C, 43.65; H, 2.35; N, 10.11. ^{1}H NMR (500 MHz, CD_3CN): 9.15 (1H, s), 9.06 (1H, d, ^3J = 7.8 Hz), 8.76 (1H, d, ^3J = 7.3 Hz), 8.59−8.63 (3H, m), 8.26 (2H, s), 8.25 (2H, s), 8.20 (1H, d, ^3J = 6.5 Hz), 8.15 (1H, d, ^3J = 5.05 Hz), 8.04−8.06 (2H, m), 7.98−8.0 (2H, m), 7.71−7.76 (2H, m), 7.60−7.66 (4H, m). UV−vis: MeCN [λ_{max}, nm (ϵ $M^{-1}cm^{-1}$)]: 445 (18 200).

The complex was resolved as described in method B for $[Ru(phen)_3]Cl_2$. The Δ enantiomer was obtained in 70% yield (94.5% ee). CD for Δ-$[Ru(phen)_2(nitrophen)](PF_6)_2$ (Δ-**2**) [CH_3CN, λ_{max}, nm ($\Delta\epsilon$, $M^{-1}cm^{-1}$)]: 415 (+15.6), 464 (−15.3). The Λ complex was also obtained by method B by reversing the order of arsenyl tartrate addition (first the (−) salt, then the (+) salt). Yield 72% (95.6% ee). CD for Λ-$[Ru(phen)_2(nitrophen)](PF_6)_2$ (Λ-**2**) [CH_3CN, λ_{max}, nm ($\Delta\epsilon$, $M^{-1}cm^{-1}$)]: 415 (−15.6), 464 (+15.4).

Δ- or Λ- $[Ru(phen)_2(aminophen)](PF_6)_2$ (Δ-3 or Λ-3). A solution containing Δ- or Λ-$[Ru(phen)_2(nitrophen)]Cl_2$ (0.4 g, 0.53 mmol) and 10% Pd/C catalyst (0.5 g) in 50 mL ethanol was purged with N_2 gas for 15 min. The reaction mixture was heated to 68−75 °C. To this mixture, 6 mL $N_2H_4\cdot H_2O$ in 20 mL ethanol was added dropwise over a period of 1 h while refluxing the solution. The reflux was continued for another 6 to 8 h. The solution was cooled overnight and filtered over Celite, washing with additional ethanol. The sample was concentrated by removing excess ethanol via rotary evaporation and was treated with an aqueous solution of NH_4PF_6. The reddish-orange precipitate was filtered and oven dried at 60 °C. Yield 80%. Anal. Calcd for $RuC_{36}H_{25}N_7P_2F_{12}$:C, 44.82; H, 2.82; N, 10.16. Found: C, 45.16; H, 2.63; N, 10.24. ^{1}H NMR (500 MHz, CD_3CN): 8.56−8.60 (5H, m), 8.25 (2H, s), 8.24 (2H, s), 8.21 (1H, d, ^3J = 8.5 Hz), 8.06 (1H, d, ^3J = 5.05 Hz), 8.02 (2H, apparent triplet, ^3J = 5.5), 7.98 (2H, apparent triplet, ^3J = 5.1, 4.1), 7.56−7.66 (6H, m), 7.39 (1H, dd, ^3J = 8.5 Hz, ^4J = 5.9 Hz), 7.19 (1H, s), 5.57 (2H, br. s).

LC Analysis. For the LC analysis, the flow rate and the detection wavelength were 1 mL/min and 254 nm, respectively. All of the analytical separations utilized an injection volume of 5 μL of solution containing 1 mg/mL of the ruthenium complex of interest in methanol or acetonitrile. The mobile phase was degassed by ultrasonication under vacuum for 5 min. All of the experiments were repeated three times at room temperature. The chloride (Cl^-) salts and hexafluorophosphate (PF_6^-) salts of ruthenium(II) complexes were dissolved in methanol and acetonitrile, respectively. Three parameters, retention factor (k'), selectivity (α), and resolution (Rs), were assayed to analyze and optimize the separations. The retention factor 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 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 $Rs = 2(t_{r2} - t_{r1})/(w_1 + w_2)$, where w is the base-peak width. For baseline separations, Rs values must be equal to or greater than 1.5. For the complexes, which could not be baseline separated (Rs < 1.5), the flow rate was decreased to 0.5 mL/min, to achieve better efficiency. Once the resolution was satisfactory, efforts were made to minimize the retention time (factor).

Results and Discussion

Performance of Cyclodextrin-Based CSPs. Most studies reporting chromatographic enantiomeric separations of tris-

(77) Marcovich, D.; Tapscott, R. E. *J. Am. Chem. Soc.* **1980**, *102*, 5712−5717.
(78) Henderson, G. G.; Ewing, A. R. *J. Chem. Soc.* **1895**, *67*, 102−108.

Table 1. Comparison of the Enantioseparation of Δ- and Λ-$[Ru(phen)_3]^{2+}$ with Three Aromatic-Derivatized Cyclobond CSPs

name of CSP	k_1'	α	Rs	mobile phase (v/v)
Cyclobond RN	0.467	1.97	2.4	80 MeOH/20 ACN/0.2 NH_4NO_3
Cyclobond SN	4.821	1.07	0.7	100 MeOH/0.4 HOAc/0.8 TEA
Cyclobond DMP	12.040	1.13	1.0	100 MeOH/0.4 HOAc/1.2 TEA

(diimine) ruthenium(II) complexes are limited to mononuclear species,[31,45,48−52] with the notable exception of Keene and co-workers, who have reported the column chromatographic resolution of numerous dimeric and even trimeric complexes.[37,39,43] It should be noted that these resolutions typically first require the separation of diastereomers. Our purpose was to develop a broadly effective HPLC technique for the facile separation of enantiomers not only of mononuclear complexes but also for diastereomers and enantiomers that are found in dinuclear species of ruthenium polypyridyl complexes. The ruthenium(II) complexes (eight mononuclear and two dinuclear ones) in this study include both homoleptic and heteroleptic diimine complexes that have diverse ligand structures (Figure 2). Among them, $[Ru(phen)_3]Cl_2$ (**1**) was used as a model compound to screen CSPs and operational modes.

Three native cyclodextrin and six derivatized β-cyclodextrin stationary phases were evaluated for their ability to separate $[Ru(phen)_3]Cl_2$. Three of them (Cyclobond RN, SN, and DMP) showed enantioselectivity. It is particularly noteworthy that only Cyclobond RN, SN, and DMP are aromatic-derivatized among these nine CSPs. The ruthenium-complex enantiomers are separated by aromatic-derivatized but not by nonaromatic-derivatized cyclodextrin, which suggests that the π-stacking interaction provided by the aromatic group of CSPs plays an important role and is necessary in chiral recognition. In addition to providing π-stacking interaction, the derivatized functional groups of CSPs have the effect of extending the mouth, which could allow the accommodation of larger analytes. Because of the different structure of these aromatic functional groups, Cyclobond RN, SN, and DMP showed different enantioselectivities. Table 1 shows the optimized enantioseparation results for $[Ru(phen)_3]Cl_2$ on these three CSPs. Among the three columns, R-naphthylethyl carbamate β-cyclodextrin (Cyclobond RN) CSP is the best stationary phase for $[Ru(phen)_3]Cl_2$. The capability of cyclodextrin in discriminating ruthenium(II) complex enantiomers was also reported by Kano's group, using NMR.[65] The conclusion that "cyclodextrin has an asymmetrically twisted cavity in which a guest having a helix configuration is well fit" is in accord with our results that cyclodextrin CSPs are capable of well discriminating enantiomers of helical ruthenium(II) polypyridyl complexes.

Polar-organic and reversed-phase modes were both examined, and enantioseparations were observed with both. Figure 3 shows the chromatograms for the separation of $[Ru(phen)_3]Cl_2$ in these two modes using optimized conditions. From this figure, it is apparent that in the polar-organic mode, the separation is both faster and more selective. The longer retention time in the reversed-phase mode may be due to the formation of a strong inclusion complex between the phenanthroline aromatic ring and the derivatized cyclodextrin.

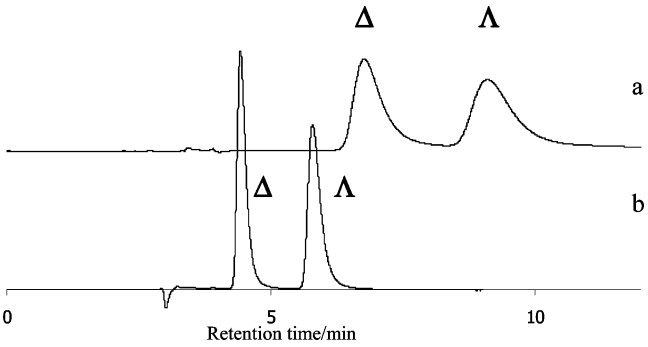


Figure 3. Comparison of the enantiomeric separation of Δ- and Λ-$[Ru(phen)_3]^{2+}$ in two different chromatographic modes on Cyclobond RN CSP. (a) The reversed-phase mode, the mobile phase is 20% acetonitrile/80% buffer (buffer, 0.1% triethylammonium acetate in water, pH 4.1); (b) polar-organic mode, the mobile phase is 80 methanol%/20% acetonitrile/0.2% NH_4NO_3.

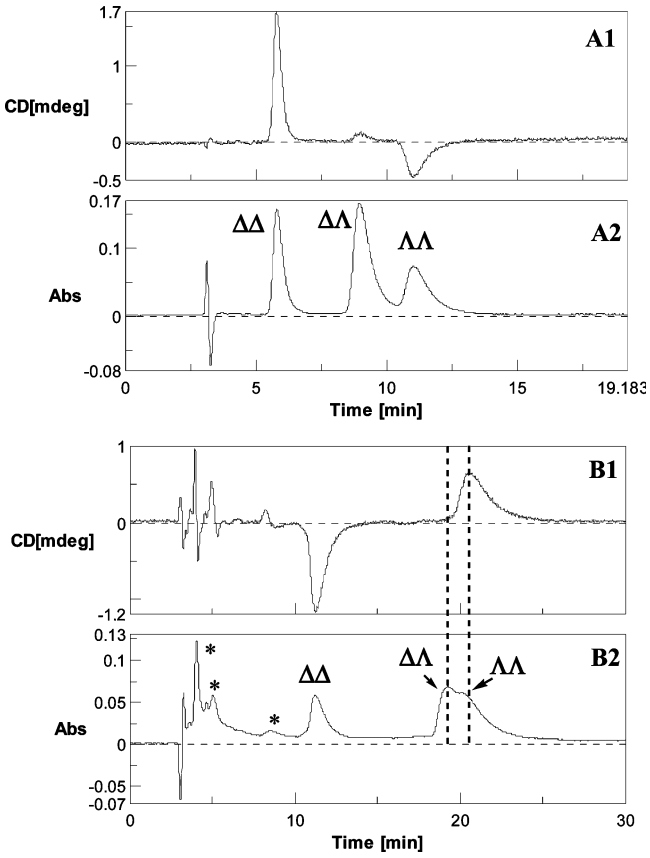


Figure 4. Chromatograms of $[Ru_2(phen)_4(tpphz)]Cl_4$, **9** (A1 and A2); and $[Ru_2(phen)_4(tatpp)]Cl_4$, **10** (B1 and B2) in the optimized mobile phase on Cyclobond RN CSP. The top (A1 and B1) and bottom (A2 and B2) chromatograms are circular dichroism and UV signal at 254 nm, respectively. For **9**, the mobile phase is 60% methanol/40% acetonitrile/0.3% NH_4NO_3. For **10**, the mobile phase is 70% methanol/30% acetonitrile/0.4% NH_4NO_3. * denotes impurities.

Chromatographic Resolution of Mononuclear Ruthenium(II) Complexes. Analogous HPLC studies on the Cyclobond RN column in the polar-organic mode were carried out for all 10 of the ruthenium(II) complexes. The optimized enantioseparation results (including k_1', α, Rs, and the mobile-phase composition) for the 8 monomeric com-

Table 2. Summary of the Optimized Results of Ruthenium(II) Polypyridyl Complexes on Cyclobond RN CSP

number	name	k_1'	α	Rs	mobile phase (v/v)	elution order
1	$[Ru(phen)_3](Cl_2)$	0.467	1.97	2.4	80 MeOH/20 ACN/0.2 NH_4NO_3	Δ, Λ
2	$[Ru(phen)_2$nitrophen$](Cl_2)$	0.412	2.14	2.2	80 MeOH/20 ACN/0.2 NH_4NO_3	Δ, Λ
3	$[Ru(phen)_2$aminophen$](Cl_2)$	0.422	1.94	2.2	80 MeOH/20 ACN/0.2 NH_4NO_3	Δ, Λ
4	$[Ru(phen)_2$phendione$](Cl_2)$	0.912	1.69	1.7	95 MeOH/5 ACN/0.4 NH_4NO_3	Δ, Λ
5[a]	$[Ru(phen)_2$tatpp$](PF_6)_2$	1.561	1.40	1.5	100 MeOH/0.4 NH_4NO_3	Δ, Λ
6	$[Ru(phen)_2py_2](Cl_2)$	1.913	1.23	1.5	100 MeOH/0.4 NH_4NO_4	Δ, Λ
7	$[Ru(dppz)_3](Cl_2)$	1.067	2.74	3.9	70 MeOH/30 ACN/0.2 NH_4NO_3	Δ, Λ
8[a]	$[Ru(bpy)_3](Cl_2)$	1.102	1.27	1.5	95 MeOH/5 ACN/0.4 NH_4NO_3	Δ, Λ
9[b]	$[Ru_2(phen)_4$(tpphz)$](Cl_4)$	0.865	2.95	3.5	60 MeOH/40 ACN/0.3 NH_4NO_3	ΔΔ, ΔΛ, ΛΛ
10[b]	$[Ru_2(phen)_4$(tatpp)$](Cl_4)$	2.510	2.16	2.8	70 MeOH/30 ACN/0.4 NH_4NO_3	ΔΔ, ΔΛ, ΛΛ

[a] The flow rate is 0.5 mL/min. In other conditions, the flow rate is 1.0 mL/min. [b] For the dimer, the results were calculated for one pair of enantiomers.

plexes are given in Table 2, and their chromatograms are available in the Supporting Information (Figure S1). Enantioselectivity values ranges between 1.23 and 2.95. Good enantioselectivity and chromatographic efficiencies provided complete baseline enantiomeric separations within minutes for all 8 complexes. These baseline resolution results demonstrate that accurate measurements of enantiomeric excess (ee) can be achieved with this method using HPLC and the Cyclobond RN CSP. For example, the ee of Λ-[Ru(phen)$_2$aminophen]Cl_2 was determined to be 98.9% from its chromatogram (Figure S2, Supporting Information). Comparable sensitivities and short analysis times are generally difficult or impossible with direct analysis via polarimetry, circular dichroism, or NMR with chiral shift reagents.

All of the above resolutions were conducted on relatively small samples (~5 μg) using a typical analytical column (25 cm × 0.46 cm, i.d.). To test the preparative capabilities of this chiral stationary phase (CSP), 2 mg of [Ru(phen)$_2$nitrophen]-Cl_2 was dissolved in 100 μL methanol and injected onto the analytical column. The two enantiomers were readily separated in a single run in approximately 12 min. Using typical assumptions on the scalability of HPLC separations, conservative estimates show that separations of 100 mg racemate on a standard semiprep column (25 × 5.08 cm, i.d.) of the same type could be made in a single run. Vos and co-workers have shown that HPLC separations on the order of 30 mg racemate are possible with semi-prep columns (25 × 1 cm, i.d.) using the Teicoplanin CSP.[55]

Chromatographic Separation of Diastereomers and Enantiomers in Dinuclear Ruthenium(II) Complexes. Dinuclear complexes, such as **9** and **10**, contain two chiral centers, and therefore they are often prepared as a mixture of both diastereomers (e.g., ΔΔ and ΛΛ vs ΔΛ and ΛΔ) and enantiomers; making the separation problem considerably more challenging. For **9** and **10**, the problem is simplified somewhat in that the ΔΛ complex is a meso structure, and thus only three stereoisomers (ΔΔ, ΛΛ and ΔΛ) need be separated. Figure 4 shows selected chromatograms of $[Ru_2(phen)_4$(tpphz)$]Cl_4$ (**9**, panels A1 and A2) and $[Ru_2(phen)_4$(tatpp)$]Cl_4$ (**10**, panels B1 and B2). Both CD and UV detectors were employed, and thus UV absorption and CD chromatograms were obtained simultaneously. For dimer **9**, three peaks are clearly seen in the UV chromatogram (panel A2), and the relative peak areas are consistent with statistical expectations. Figure 4 (panel A1) shows two of the three peaks to be chirooptic, and the assignment of these as ΔΔ-**9** and then ΛΛ-**9** are based on the sign of the CD and by injection of enantiopure samples. The meso structure, ΔΛ-**9**, can be assigned as the middle peak in the UV trace because its CD signal is expected to be nil, thus it was possible to separate all three stereoisomers in one chromatographic run in this case. However, the separations with **10** were less successful, as seen in Figure 4 (panel B2), in which two overlapping peaks are seen at the end of the chromatogram. From the CD data, we can assign the first large peak to the ΛΛ enantiomer and the two overlapping UV peaks as first the ΔΛ complex and then the ΔΔ stereoisomer because only the latter peak is chiroptic (as seen by the dotted lines relating the UV to CD data). As with **9**, the meso complex comes between the two enantiomers; however, here only one pure enantiomer is obtained (ΛΛ) because ΔΛ and ΔΔ are only partially separated in this one-run experiment. The elution order (ΔΔ, ΔΛ, ΛΛ) is the same for both dimers as determined by the injection of enantiopure samples. Note that the signs of the CD signals are reversed for **9** (panel A1) and **10** (panel B1), which seems odd but is simply due to different null points in the CD spectra of these two complexes at this wavelength (254 nm). The appearance of several impurity peaks early in chromatogram of **10** are ascribed to the instability of this complex toward normal-phase chromatography.[76] Prior attempts to purify crude isolates of **10** by column chromatography on silica or alumina (typically with MeCN eluent) are known to cause some decomposition to an uncharacterized side-product(s),[76] and are likely to be occurring to a lesser extent here.

Effects of the Ligand Structure, Complex Charge, and Counterion. The enantiomeric elution order indicated in Table 2 was established by injecting a single enantiomer standard under the same experiment conditions as well as the CD measurements. All of the mononuclear ruthenium(II) complexes show the same elution order, that is, the Δ isomer eluted first. This means that the Λ enantiomer binds to derivatized β-cyclodextrin with greater affinity. The fact that **1**−**8** have the same elution order indicates that this method may be useful for determining the absolute configuration of related metal complexes with analogous ligands. **2**−**6** are mixed ligand complexes and contain two phenanthroline ligands. The dppz in $[Ru(dppz)_3]Cl_2$ (**7**) and the bpy ligand in $[Ru(bpy)_3]Cl_2$ (**8**) are also diimine ligands, similar to the phenanthroline ligand. The fact that such complexes

often show similar LC enantioselectivity also was reported previously. On the silica-bonded teicoplanin LC stationary phase, $[Ru(bpy)_3]^{2+}$, $[Ru(phen)_3]^{2+}$, and $[Ru(dpphen)_3]^{2+}$ showed the same elution order, with the Δ isomer being retained more strongly.[54] In a CE, $[Ru(bpy)_3]^{2+}$ and $[Ru(phen)_3]^{2+}$ also showed similar enantioselectivity.[48,51] For the dinuclear complexes (**9** and **10**), the same elution order (i.e., ΔΔ eluted before ΛΛ) was obtained. The meso (ΔΛ) stereoisomer was eluted between ΔΔ and ΛΛ forms, which means that the meso form has intermediate binding strength for the cyclodextrin-based stationary phase (Figure 4 (B2)).

The elution order also was determined on the *S*-naphthylethyl carbamate *β*-cyclodextrin (Cyclobond RN) CSP. The configuration of the naphthylethyl carbamate moieties is opposite on the RN and SN *β*-cyclodextrin stationary phases. The elution order for all except $[Ru(dppz)_3]^{2+}$ (**7**) reversed, which means the Δ enantiomer shows a greater affinity for this chiral stationary phase. This indicates that the stereogenic configuration of the naphthylethyl carbamate group is a major factor for chiral recognition, and the cyclodextrin plays a secondary role. In the polar-organic mode, chiral recognition is mainly through external interaction (outer sphere) between the analyte and derivatized cyclodextrin. In fact, it has been shown that both the attached chiral naphthylethyl carbamate moiety and the chiral base-cyclodextrin molecule contribute to chiral recognition.[79,80] Furthermore, they can do so in a synergistic or antagonistic fashion.[79,81] Clearly, in the case of all of the ruthenium(II) complexes, the *R*-naphthylethyl carbamate groups and the underlying *β*-cyclodextrin act synergistically to produce enhanced enantiomeric separations. If the cyclodextrins played no role in the enantiomeric selectivity for these complexes, then the *S*-naphthylethyl carbamate-*β*-cyclodextrin CSP would produce equivalent enantiomeric separations but of the opposite retention order. This, however, is not the case.

The cyclodextrin-based stationary phase binds ruthenium(II) complexes with a marked dependence upon the ligand structure, as seen from the data in Table 2. As these data were obtained with varying mobile-phase compositions, it is difficult to make specific structure-binding correlations; however, with an 80% methanol/20% acetonitrile/0.2% NH_4NO_3 mobile phase, the k_1' of **1**, **2**, **3**, **4**, and **5** are 0.467, 0.412, 0.422, 0.225, 0.185, respectively. **2**, **3**, and **4** are retained less than **1**, which is likely due to their stronger hydrogen-bonding interactions with the methanol mobile phase. **5** was much less retained than **1**, possibly due to steric effect from the bulky (tatpp) ligand.

As seen from the results in Table 2, the retention is greatly affected by the overall charge of the complex cations. The retention for the quadruple-charged dinuclear complexes, **9** ($k_1' = 0.865$) and **10** ($k_1' = 2.32$), is considerably stronger than of the doubly charged mononuclear complexes, **1** ($k_1' = 0.267$) and **2** ($k_1' = 0.191$), when chromatographed using the same mobile phase (60% methanol/40% acetonitrile/0.3% NH_4NO_3). We presume that this is primarily due to the stronger electrostatic interaction with the commonly formed nitrate-cyclodextrin inclusion complex in the CSP. The role of the initial counteranion in the complex salt is apparently unimportant in the separation process. When the anion with $[Ru(phen)_3]^{2+}$ was changed intentionally to Br^-, F^-, BF_4^-, PF_6^-, and $CF_3SO_3^-$, the same retention times and enantioselectivity (within experimental error) were obtained under the same experimental conditions. It seems likely that the mobile-phase nitrate anion rapidly exchanges with these counterions, and it is this ion-pair that dictates the chiral recognition process. Keene has showed that the anion present in the mobile phase is critical for effective separations in ion-pairing chromatography on cation exchange resins.

Table 3. Effect of the Salt Type in the Mobile Phase on Enantioseparation[a]

salt type	k_1'	α	Rs
potassium nitrate	5.844	1.48	1.5
ammonium trifluoroacetate	1.475	1.62	1.5
ammonium nitrate	2.449	1.67	2.0
sodium chloride	3.114	1.54	1.5
triethylammonium acetate	9.701	1.34	1.0
ammonium chloride	2.471	1.56	1.6

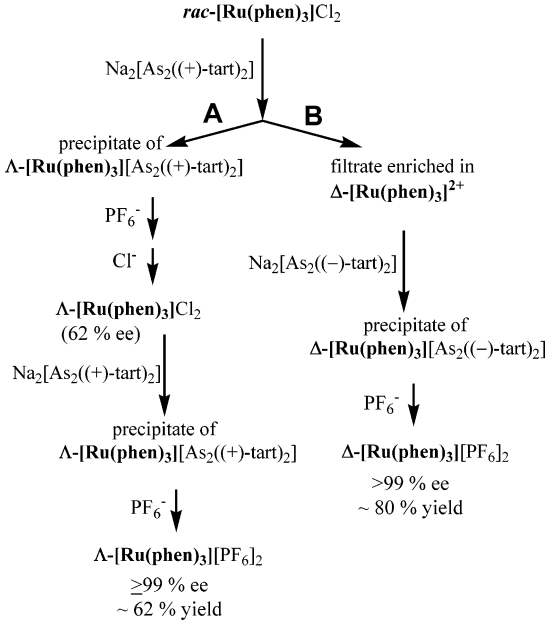
[a] Note: The mobile phase is 0.0125 M salt in methanol. All of the results were obtained on Cyclobond RN CSP, and the analyte is $[Ru(phen)_3]Cl_2$.

Effects of the mobile phase were investigated by changing salt type, salt concentration, and modifier solvent concentration. Different salts (potassium nitrate, ammonium trifluoroacetate, ammonium nitrate, sodium chloride, triethylammonium acetate, and ammonium chloride) were used as the additive in 100% methanol, at identical concentration (0.0125 M). Enantioseparation of $[Ru(phen)_3]Cl_2$ was obtained with all of these additives. However, the mobile phases, that utilized different salt additives show different retention and selectivity (Table 3). Among them, ammonium nitrate (NH_4NO_3) produced the highest selectivity and excellent resolution values. Furthermore, increasing the salt concentration was found to increase the enantioselectivity and retention (Figure S3, Supporting Information).

In addition, adding an organic modifier solvent, such as acetonitrile, affects separation greatly. To study the effect of acetonitrile concentration, the percentage of acetonitrile was increased from 0 to 100% (Figure S4, Supporting Information). Because ammonium nitrate will precipitate at high percentages of acetonitrile, an acetic acid/triethylamine salt, which is commonly used in the polar-organic mode, was used at the concentration of 1.0% acetic acid/1.0% triethylamine. The retention factor curve shows a U shape. When the percentage of acetonitrile is below 30%, increasing the concentration of acetonitrile decreases the retention. When the percentage of acetonitrile is above 30%, the reverse trend is obtained; and also, the selectivity decreases slightly from 1.341 to 1.146 with increasing acetonitrile concentration. Generally, in the polar-organic mode on cyclodextrin CSPs, the retention time can be decreased by increasing the methanol concentration because methanol competes with

(79) Stalcup, A. M.; Chang, S.-C.; Armstrong, D. W. *J. Chromatogr.* **1991**, *540*, 113−128.
(80) Berthod, A.; Chang, S.-C.; Armstrong, D. W. *Anal. Chem.* **1992**, *64*, 395−404.
(81) Berthod, A.; Chang, S.-C.; Armstrong, D. A. *Anal. Chem.* **1991**, *64*, 395−404.

Scheme 1. Resolution Procedures for Λ- and Δ-$[Ru(phen)_3]^{2+}$ Cations.



analytes for hydrogen-bonding sites in the stationary phase.[82] This can explain the phenomenon in the range of 30 to 100% acetonitrile. However, the mechanism by which a small amount of acetonitrile reduces retention is still not clear. Nonetheless, adding an appropriate amount of acetonitrile can decrease the retention, while maintaining good selectivity. On the basis of the salt and acetonitrile concentration studies, enantioseparation could be optimized by controlling acetonitrile and ammonium nitrate concentrations to achieve the shortest retention time, good selectivities, and baseline resolutions.

Large Scale Resolution of Ruthenium(II) Diimine Complexes. Whereas it is possible to use preparative-scale HPLC to isolate enantiopure complexes, large-scale resolution (1 − 0.5 g) for many trisdiimine metal complexes can often be achieved via diastereoselective precipitation with chiral anions, such $[As_2((+)\text{-tart})_2]^{2-}$, $[Sb_2((+)\text{-tart})_2]^{2-}$, [(−)-O,O′-dibenzoyl-L-tartrate]$^{2-}$,[24,83] and Δ and Λ-[P^V-(tetrachlorocatecholate)$_3$]$^-$. In particular, sodium arsenyl-(+)-tartrate and potassium antimonyl-(+)-tartrate have been frequently employed with good success.[1,2,10,74,84−88] The resolution procedures vary in detail but follow the same general procedures outlined in Scheme 1. In this section, we reexamine this general procedure, using chiral HPLC to evaluate the enantiopurity of the complex at each step. First, however, some comments on the tartrate salts are appropriate.

Both the arsenyl and antimonyl tartrate salts contain a dimeric dianion with arsenic or antimony atoms at the axial positions of a twisted (C_2 symmetric), flattened spheroid.[89] A crystal structure of Λ-$[Fe(phen)_3][Sb_2((+)\text{-tart})_2]$ is known and reveals how the left-handed twist of the dianion allows closer ion pairing with the Λ dication than with the Δ dication.[90] The closer ion pairing leads to selective precipitation of the Λ complex when L-(+)-tartrate salts are used and the Δ complex with D-(−)-tartrate salts. The absolute structure of the Λ configuration of **1** (PF_6 salt) has also been determined by crystallography and correlated with its CD spectrum.[91]

The tartrate salts are readily prepared from the metal oxide (As_2O_3 or Sb_2O_3) and the appropriate tartaric acid (+ or −). For example, sodium arsenyl (+)-tartrate was first reported in 1895 by Henderson[78] and later in 1980, and the (+)- and (−)-tartrate salts were synthesized by Marcovich and Tapscott.[77] Neither report gave yields, and the reaction conditions varied from heating from 15 min to several days. We find that the Henderson procedure,[78] with minor modifications, reliably gives the arsenyl salts, $Na_2[As_2((+)\text{-tart})_2]\cdot 3H_2O$ and $Na_2[As_2((-)\text{-tart})_2]\cdot 3H_2O$ in ∼90% yield. The details of this procedure are reported in the experimental section.

Most resolution procedures for $[Ru(phen)_3]Cl_2$ and related complexes are based on the procedures first developed by Dwyer and Gyarfas in 1949.[85−87,92] This procedure is depicted in Scheme 1 (Method A) and essentially involves two diastereoselective precipitations of the cationic complex with the same chiral anion. For example, treatment of $[Ru(phen)_3]Cl_2$ with $Na_2[As_2((+)\text{-tart})_2]\cdot 3H_2O$ gives an initial precipitate of Λ-$[Ru(phen)_3][As_2((+)\text{-tart})_2]$, which we find to be approximately 62% ee. This salt can be metathesized to the water-soluble chloride salt and retreated with $Na_2[As_2((+)\text{-tart})_2]\cdot 3H_2O$ to give Λ-$[Ru(phen)_3][PF_6]_2$ (after metathesis), which is ≥99% ee by chiral HPLC. The overall yield of the Λ complex is 64% (0.42 g based on 1.3 g racemate). Similar results are obtained for the Δ complex if $Na_2[As_2((-)\text{-tart})_2]\cdot 3H_2O$ is used instead.

Scheme 1 also shows a modification to this procedure (method B) that gives the chiral product in greater yield with considerably less manipulation of the intermediates (mainly the metathesis reactions). Method B is based on the observation by Hiort and co-workers,[10] who noted that the Δ enantiomer of the cationic complex can be isolated from the initial filtrate (assuming the initial solution was treated with $Na_2[As_2((+)\text{-tart})_2]\cdot 3H_2O$) by precipitation with hexafluorophosphate anion, metathesis to the chloride salt, and treatment of this solution with $Na_2[As_2((-)\text{-tart})_2]\cdot 3H_2O$. We have found that this procedure can be further streamlined, in that, the initial filtrate can be treated directly with $Na_2[As_2((-)\text{-tart})_2]\cdot 3H_2O$, without bothering to isolate and

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metathesize the complex. The precipitate of Δ-$[Ru(phen)_3]$-$[As_2((-)\text{-tart})_2]$ can then be converted to the PF_6^- salt, giving Δ-$[Ru(phen)_3][PF_6]_2$ in >99% ee and 80% yield (0.53 g based on 1.3 g racemate). Of course, the two methods can be used together to obtain reasonable amounts of enantiopure Δ- and Λ-$[Ru(phen)_3]Cl_2$ and the waste products can be recycled to improve the yield even further.

We examined the enantiopurity of a number of ruthenium complexes after resolution by both methods A and B (Table S1, Supporting Information) and find that typically method B works better, faster, and gives higher recovery (yield) than method A. Interestingly, whereas both method A and B resolve most of the trischelate complexes tested, we were unable to significantly resolve $[Ru(bpy)_3]^{2+}$ using either method A (9.4% ee) or method B (4.5% ee).

As has been shown by ourselves[67,93] and others,[84,87,94,95] the stereochemistry at these ruthenium centers is very robust and difficult to racemize. Dwyer showed that oxidation to the Ru(III) analogue and reduction back to the ruthenium-(II) center leaves the stereochemistry unaltered.[84] Photolysis appears to be the only efficient manner to racemize a particular enantiomer in this class of ruthenium(II) complexes.[96−98] Such stability suggests these complexes may have utility as chiral selectors in their own right, and we are beginning to explore this possibility.

Conclusion

The stability and interesting photonic properties of ruthenium trisdiimine complexes have led to an extensive body of work on the preparation of monomeric, oligomeric, and polymeric molecules containing this structural unit. The chirality of the trisdiimine complex inevitably leads to situations in which the absolute stereochemistry needs to be controlled and the optical purity assessed. The chiral HPLC method developed in this work shows that this class of complexes can be reliably separated and examined for optical purity. The factors that affect the separation efficiency have been parametrized such that the stereochemical makeup of most monomeric and many dimeric complexes can be quantitatively examined. We used this tool to reexamine and improve on the most-common resolution procedures and can now report on their efficiency with high accuracy.

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Supporting Information Available: Chromatograms for Figures S1−S3 and Table S1. This material is available free of charge via the Internet at http://pubs.acs.org.

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