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NMR Spectroscopy

Determination of Labile Chiral Supramolecular Ion Pairs by Chromatographic NMR Spectroscopy**

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The existence of supramolecular complexes in solution and particularly of ion pairs has been neatly highlighted by NMR diffusometry.[1] Nonetheless, intriguing cases that defy this simple and effective approach are isomeric mixtures. NMR diffusometry relies on and requires measurable differences in the hydrodynamic radius. Isomers can be identified by this method if their shape differs significantly,[2] but otherwise remains a challenge, particularly because of their tendency to produce highly overlapping ^{1}H NMR spectra. Improvements to the resolution of diffusion-ordered spectroscopy (DOSY) have been proposed by addition of a soluble matrix capable of either amplifying the diffusion differences[3] or of reducing the spectral degeneracy.[4] For instance, epimers could be characterized by DOSY following the addition of cyclodextrin.[3f] Chromatographic NMR[5] is a version of matrix-assisted DOSY, the separation capabilities of which are modulated and boosted by addition of a solid chromatographic phase to the mixture under study, assisted by high-resolution magic-angle spinning (HRMAS) to recover resolution.[6] In favorable cases, it has been demonstrated that this technique may be even capable to outperform liquid chromatography (LC),[6b] because of a larger flexibility on the choice of the liquid/solid phase ratio, which has direct effects on the resolution of the method.[7] In the following, we demonstrate that silica-enhanced diffusion-ordered NMR spectroscopy (DOSY) is capable of resolving the spectra of isomeric mixtures of ion pair supramolecular species, chiral metal(II) tris(diimine) complexes ML_3,[8] and TRISPHAT counterions, even for cases that elude classic liquid chromatography.

Previously, it was shown that chiral TRISPHAT anion **1** (tris(tetrachlorobenzenediolato)phosphate(V), Figure 1) is configurationally stable[9] and that its Δ or Λ enantiomers can be readily synthesized and separated on large scale.[10]

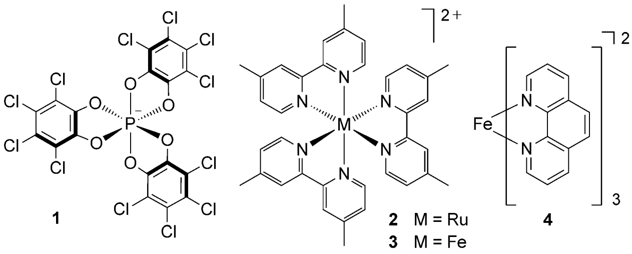

Figure 1. TRISPHAT **1** (Δ enantiomer shown), [Ru(Me_2bpy)$_3$] **2**, [Fe(Me_2bpy)$_3$] **3** (Λ enantiomers shown), and [Fe(phen)$_3$] **4**. (Me_2bpy = 4,4′-dimethyl-2,2′-bipyridine; phen = 1,10-phenanthroline).

When associated with ruthenium and iron tris(diimine) complexes, this anion is a valuable resolving and asymmetry-inducing reagent, respectively.[11,12] The asymmetric interactions occurring between the ions can be characterized and quantified by circular dichroism (CD) and by NMR spectroscopy as anions **1** are in addition good chiral solvating agents.[13]

The first step in the present study was to reproduce by chromatographic NMR spectroscopy the diastereomeric separation of the mixture of [Ru(Me_2bpy)$_3$] **2** enantiomers (Me_2bpy = 4,4′-dimethyl-2,2′-bipyridine) paired to anions Δ-**1**, which have been previously shown to be separable by TLC on bare silica gel.[12a,14,15] The ^{1}H NMR spectrum of the two diastereomeric ion pairs gives rise to two well distinct sets of peaks in the aromatic region (Figure 2 A). Integration reveals that in this case the mixture has a diastereomeric ratio (d.r.) of 3.5:1 in favor of the heterochiral pair [Λ-**2**][Δ-**1**]$_2$ over homochiral species [Δ-**2**][Δ-**1**]$_2$. The spectra of the diastereomerically pure species, which were separated by chromatographic purification on silica gel and characterized by CD, are also reported for comparison.

The assignment of these spectra has been discussed previously in the framework of the analysis of the TRISPHAT role as a chiral solvating and resolving reagent.[12a,16] A first qualitative comparison of solution-state DOSY and chromatographic NMR spectroscopy on bare silica (Figure 2 B and 2 C) shows a clear separation of the two forms for the latter only. The peaks at about 8.15–8.25 ppm merge in this experiment, because of its somewhat reduced resolution, leading to one unique spot located somewhere in between the two values of the diffusion coefficients of the diastereomers because of monoexponential modeling of the diffusion decay. Note that it is possible that more advanced processing may result in an even better resolved spectra. Chromatographic NMR is performed on samples of heterogeneous composition, so that the mobility is described by an apparent diffusion coefficient, the nature of which may be rather complex to

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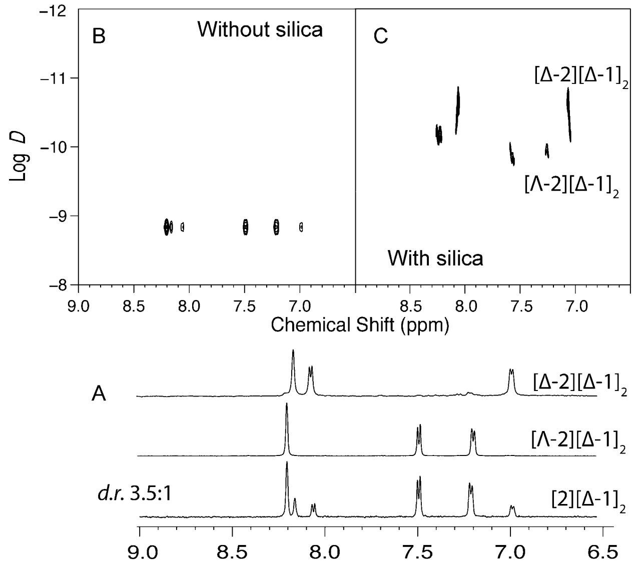

Figure 2. NMR spectra (9.0–6.5 ppm, CD$_2$Cl$_2$) of ruthenium pairs [**2**][Δ-**1**]$_2$. A) 1D ^{1}H NMR spectra in solution; pure homochiral ΔΔ$_2$ (top) and heterochiral ΛΔ$_2$ (middle) diastereomers and mixture of the two ion pairs (bottom, d.r. 3.5:1). B) Solution-state DOSY (left, recorded with a diffusion time of 100 ms and pulsed field gradient (PFG) durations of 1.2 ms). C) Silica-based chromatographic NMR spectroscopy (right, diffusion time of 340 ms and 2.4 ms PFG duration) of the diastereomeric mixture.

describe, unless in the presence of fast equilibration. This latter is also the only condition for which the chromatographic NMR spectra are exploitable. Remarkable differences between LC and chromatographic NMR analysis on porous silica may depend on a critical parameter: the phase ratio between the solution volume and the phase mass.[7b,c] In short, dramatic changes in the differential mass transport properties of a mixture (i.e. the differences in apparent diffusion coefficients of the components) were observed for solution volumes smaller or larger than the silica porous volume.[7a] This phenomenological evidence was justified on the basis of distinguishable average diffusional behavior when the solution was confined inside the silica pores or accessing the interparticle void as well. This can be described by the filling factor *f*, the ratio V$_\text{solution}$/V$_\text{porous}$. Agreement between LC and chromatographic NMR spectroscopy could be recovered for large values of *f*, while smaller values of *f* may correspond to better or worse separation for the NMR experiment.

For Ru(Me$_2$bpy)$_3$ pairs [**2**][Δ-**1**]$_2$, the best separation by chromatographic NMR spectroscopy of the diastereomeric signals was achieved for $f = 0.5$, that is, with confinement of the solution inside the pores, consistently with the mild differences in retention observed in TLC for these two compounds. Here (Figure 2C), the homochiral pair [Δ-**2**][Δ-**1**]$_2$ is the most delayed ($D_\text{app} = 1.4\ 10^{-10}$ and 2.0×10^{-11} m^{2}s^{-1} for the two cases). This result differs from previous chromatographic separations achieved on this system, where the homochiral system was eluted first, and is possibly another consequence of the different phase ratio.[12a]

Chiral divalent tris(diimine) iron complexes are, contrary to the ruthenium analogs, configurationally labile. To our knowledge, only one separation of diastereomers of this kind by TLC on silica gel has been reported; this example involved a rigid tetradentate bis-diimine ligand wrapped around the metal atom and assuring thus an unusual configurational stability.[17,18] In all other instances, a single species is observed on silica gel even in the presence of two anions **1**. The ^{1}H NMR spectrum in CD$_2$Cl$_2$ of the Fe(Me$_2$bpy)$_3$ (**3**) TRISPHAT salts [**3**][Δ-**1**]$_2$ is reported in Figure 3.[16] It shows the presence of both diastereomers, identified by characteristic spectral patterns as discussed before. Because of the metal lability, one predominant configuration is however obtained (Pfeiffer effect) as intermolecular (interionic) diastereoselective interactions occur and favor the formation of the thermodynamically preferred ion pair.[19] In CD$_2$Cl$_2$, a 5.3:1 diastereomeric ratio is measured in favor of the homochiral ion pair.[20] The DOSY of this CD$_2$Cl$_2$ solution is again not capable of clearly distinguishing the two diastereomers, while chromatographic NMR spectroscopy on bare silica separates them readily. Even so, the average self-diffusion properties of the two diastereomers are clearly differentiated, corresponding to an appreciable separation in the DOSY chart of two logarithmic units. The “NMR elution order” is consistent with the previous case, and again contrary to the LC one. Moreover, in this case a viable separation along the diffusion dimension is achieved for large ranges of choices for the filling factor, contrary to what was observed with Ru(Me$_2$bpy)$_3$ **2**, indicative of a more intrinsic tendency to be separated on bare silica. This difference is justified by a very weak interaction of the homochiral iron complex with the silica surface, ascertained by noticing that similar apparent diffusion coefficients are observed for this configuration in both solution DOSY and chromatographic NMR spectroscopy,

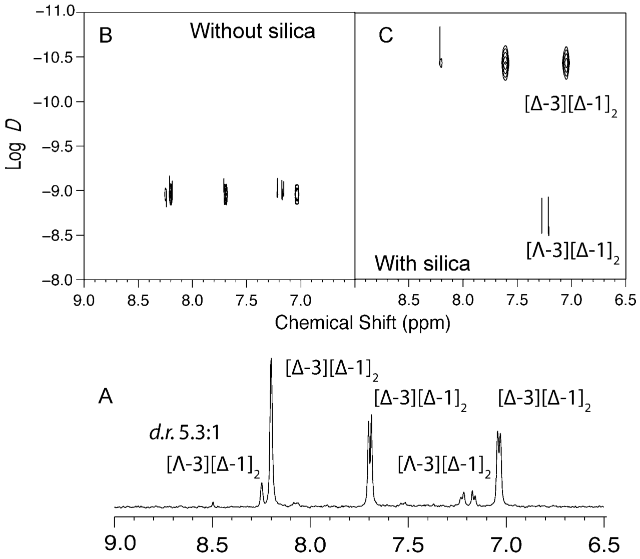

Figure 3. Spectra (9.0–6.5 ppm, CD$_2$Cl$_2$) of equilibrating iron diastereomeric pairs [Δ-**3**][Δ-**1**]$_2$ and [Λ-**3**][Δ-**1**]$_2$. A) ^{1}H NMR spectrum (d.r. 5.3:1). B) Solution state DOSY spectra (80 ms diffusion time and 1 ms PFG duration). C) Chromatographic NMR spectroscopy (right, bare silica, $f = 1.0$, 350 ms diffusion time and 2.4 ms PFG duration).

probably because of a very low polarity. Conversely, the heterochiral diastereomer does interact with the chromatographic phase, resulting in an immediate and efficient source of discrimination.

Figure 4 illustrates the NMR analysis for the TRISPHAT salt of Fe(phen)$_3$ **4**, demonstrating a displacement of the equilibrium even more strongly towards the homochiral isomer (d.r. 32.3:1). Interestingly, the mobility of the two diastereomers in solution is appreciably different in this case (Figure 4B), and they can be vaguely distinguished in the DOSY spectrum of the solution. In this case, because of the high selectivity and the associated difficulty in detecting all NMR peaks, only preparations with relatively large solution volumes produced viable Chromatographic NMR spectra. In fact, using a filling factor of 2.0, it was possible to obtain an exploitable Chromatographic NMR spectrum (Figure 4). Note that the fact that the isomer in lower concentration is also the one diffusing more slowly contributes to enhance its relative contribution in DOSY. A remarkable difference of the Chromatographic NMR behavior of [**4**][Δ-**1**]$_2$ with the two previous cases is an inversion of the NMR "elution order", since the homochiral isomer is the one showing a behavior similar to the usual TLC elution order. In the reported chromatographic separations mentioned above of configurationally stable ML$_3$ ion pairs with TRISPHAT anions, the homochiral salts were less retained.[12a,13,17] The precise reasons for the observed inversion of the elution order for the homochiral and heterochiral FeL$_3$ ion pairs, salts [**3**][Δ-**1**]$_2$ and [**4**][Δ-**1**]$_2$, and the discrepancy with LC observed in the case of the ruthenium complex [**2**][Δ-**1**]$_2$ are not immediately apparent and will be the object of further studies.

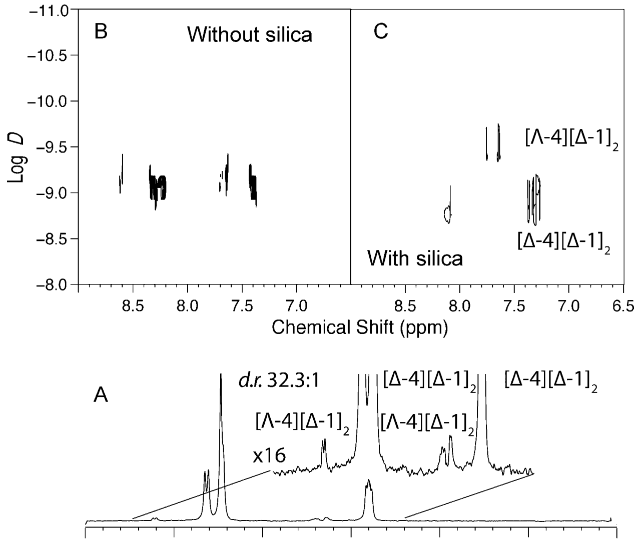

Figure 4. Spectra (9.0–6.5 ppm, CD$_2$Cl$_2$) of equilibrating iron diastereomeric pairs [Δ-**4**][Δ-**1**]$_2$ and [Λ-**4**][Δ-**1**]$_2$. A) ^{1}H spectrum (d.r. 32.3:1). B) Solution-state DOSY spectra with a diffusion time of 100 ms and a PFG duration of about 1.2 ms. C) Chromatographic NMR spectroscopy (bare silica, $f = 2.0$) with a diffusion time of 180 ms and a PFG duration of 2 ms.

In conclusion, chromatographic NMR spectroscopy using bare silica as the matrix support could provide a quick alternative and complement to LC for the characterization of ion pairs and probably other supramolecular architectures. This success could be due to either the larger range of solution/solid rations available or to more specific concentration effects on the diasteroimeric equilibration. Moreover, it appears that the technique could reveal specificities of the interactions of ion pairs with the surface of solids, and it could work as a complement to other HRMAS-based techniques that have been designed to follow chiral solute–solid chromatographic interactions.[21]

Experimental Section

The ammonium TRISPHAT salts, precursor diimine metal complexes, and the corresponding ion pairs were prepared according to literature conditions.[10,12a,16] In general, solutions of the corresponding [ML$_3$][PF$_6$]$_2$ salts (0.07 mmol) in CH$_2$Cl$_2$ (10 mL) were added to solutions of [cinchonidinium][Δ-**1**] (0.14 mmol) in acetone (5 mL). After 10 minutes of stirring and the addition of silica gel (1.0 g), the crude product was concentrated in vacuo to afford a powder that was placed on top of a chromatography column (SiO$_2$, 3 × 18). Elution with CH$_2$Cl$_2$ afforded the corresponding ion pairs either as two fractions (Ru complex **2**) or a single adduct (Fe complexes **3** and **4**).

All ^{1}H NMR spectra were recorded on a Bruker 400 Advance spectrometer, operating at a frequency of 400.13 MHz, with a HRMAS probehead equipped with gradients along the MAS directions producing a maximum of 60 G cm^{-1}. Deuterated solvents were purchased from Eurisotop and filtered on basic alumina prior to dissolution of the ion pairs. Silica gel Lichrospher Si100 (Merck) was used, with a porous volume of 1.25 mL mg^{-1}. The sample concentration was of 20 mg mL^{-1}. HRMAS spectra were recorded using standard zirconia rotors (4 mm outside diameter), with a sample volume limited to 50 µL for chromatographic NMR measurements and 12 µL for pure solution measurements. DOSY experiments were recorded using the BPP-LED pulse sequence[22] (see Figure captions for the parameter values).

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