Amine *(R)* and *(S)* Chirality Analyzers: Chemosensors & ^{19}F Benchtop NMR Spectroscopy

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Introduction

Both naturally occurring and synthetic molecules often exhibit a natural handedness known as chirality. This has been found to have important implications in biological activity and metabolism of these molecules. For example, in a chiral drug's molecule structure there are two enantiomeric forms (figure 1), while one hand can prove to be very beneficial, the other hand can have either a diminished, absent, or in some cases even harmful effect.[1] This was exemplified by the cautionary 1950s case study - thalidomide. This pharmaceutical was introduced due to its strong efficacy in alleviating morning sickness for pregnant women, however, it was later found that it also exhibited tragic teratogenic side effects, resulting in babies born with missing or deformed limbs, respiratory problems and decreased life expectancy.[2]

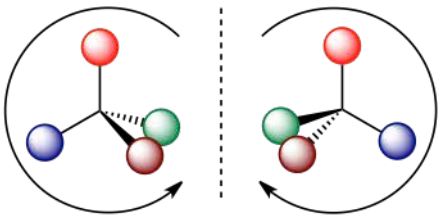

Figure 1. Representation of enantiomers

Thalidomide was sold as a racemate, it contained both enantiomers in a 50:50 ratio. Subsequent investigation revealed that only the *(R)*-(+)-enantiomer of thalidomide was safe, while the *(S)*-(–)-enantiomer had serious toxic and teratogenic effects. Nowadays, nearly 75% of new drugs entering the market are composed of a single enantiomer rather than a racemic mixture, and the proportion of single-enantiomer drugs is rapidly growing.[3] Thus, rapid identification and differentiation of chiral compounds is increasingly important to synthetic, medicinal, biological chemistry.

Although not resolvable without chemical derivativization, Nuclear Magnetic Resonance (NMR) spectroscopy has been used to discriminate between enantiomers. Even with additional sample preparation, NMR has been shown to offer a low-cost, simple, and rapid alternative to X-ray crystallography or chiral chromatography. However, given the capital cost, significant operating expenditure, the space, and power consumed to maintain a traditional super-conducting, high-field NMR technology, this powerful technique is significantly underutilized in this regard.

A recently published chemosensory approach,[4] uses fluorinated metal-ligand complexes to determine the chirality of various amines by ^{19}F NMR.

The ligand creates a chiral environment, in which the binding of enantiomeric amines creates 'static' diastereomeric complexes on the NMR timescale with distinct and characteristic ^{19}F NMR shifts.

The molecular design of a chemosensor (see figure 2) capable of producing a detectable signal in the NMR time scale is not straightforward. The system must be strategically designed to include:

(a) a sterically constricted environment to allow size discrimination; while

(b) the binding must be strong enough to generate a static complex on the NMR time scale; and

(c) the resultant signal must be sufficiently resolved to allow easy quantification.

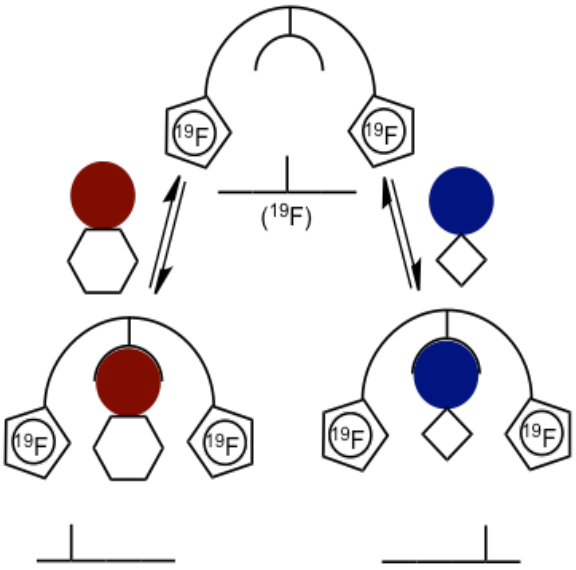

Figure 2. Schematic representation of chemosensor monitoring the change in ^{19}F chemical shift

Fluorine-19 is an excellent nuclide to produce detectable signals in NMR spectroscopy because:

(a) the nuclei is very receptive and has high sensitivity for NMR spectroscopy,

(b) fluorine-19 is 100% natural abundant,

(c) the resonances are typically well resolved owing to an extremely wide chemical shifts range (*i.e.*, +250 and –300 ppm) and naturally sharp resonances.[5]

Herein we describe the molecular design of such palladium pincer complexes (scheme 1), and the extension of this powerful, cost-saving method to benchtop NMR detection, to afford an accessible and affordable amine chirality quantification tool. The benchtop NMR shows efficacy in the differentiation of enantiomers of chiral amines, the simultaneous sensing of multiple chiral amines, and the estimation of enantiomeric ratio (*er*) and enantiomeric excess (*ee*).

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Scheme 1. Palladium pincer complex with fluorine probes

Identification and Differentiation of Chiral Amines

Figure 3 shows the identification of different chiral amines using the palladium complex 1 and ^{19}F NMR Spectroscopy. Upon complexation with each of the chiral amines, a different characteristic ^{19}F NMR resonance is produced (the peaks are doublets due to coupling with the adjacent H on the ligand pendant arm). The enantiomers of α-methylbenzylamine (figure 3a – c) and α-ethylbenzylamine (figure 3d – f) can be clearly differentiated in racemic mixtures.

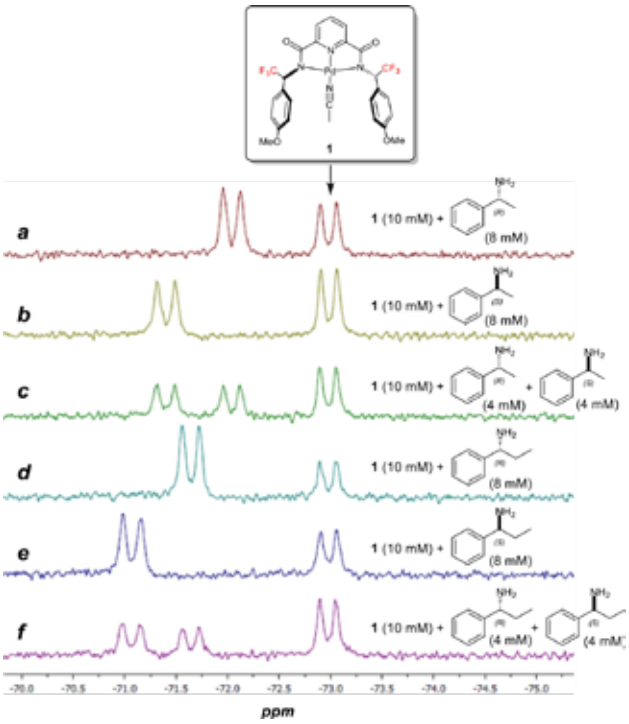


Figure 3. Palladium pincer complex with fluorine probes

Simultaneous Sensing of Multiple Chiral Amines

As shown in Figure 4a and 4b, in addition to the simple enantiomeric pairs, multiple enantiomers can also be sensed simultaneously using complex 1. Each enantiomer gives rise to a resonance at the same ppm value as in the single-enantiomer spectra, allowing straightforward identification of each peak. Combining the four analytes (both enantiomers of each of two chiral amines) results in overlapping peaks, as shown in Figure 4c. Even though individual peaks are not easily identified, the spectrum clearly indicates the presence of multiple species. Using a peak-fitting tool (available in most NMR processing software[6]) the overlapping peaks can be deconvoluted, allowing the identification of each individual peak as shown in Figure 4d.

For comparison, figure 4e shows a high-field NMR spectrum of the same sample as in Figure 4c. Predictably, while both spectra contain the same quantitative information, the 400 MHz NMR spectrum shows better peak dispersion than the 60 MHz spectrum.

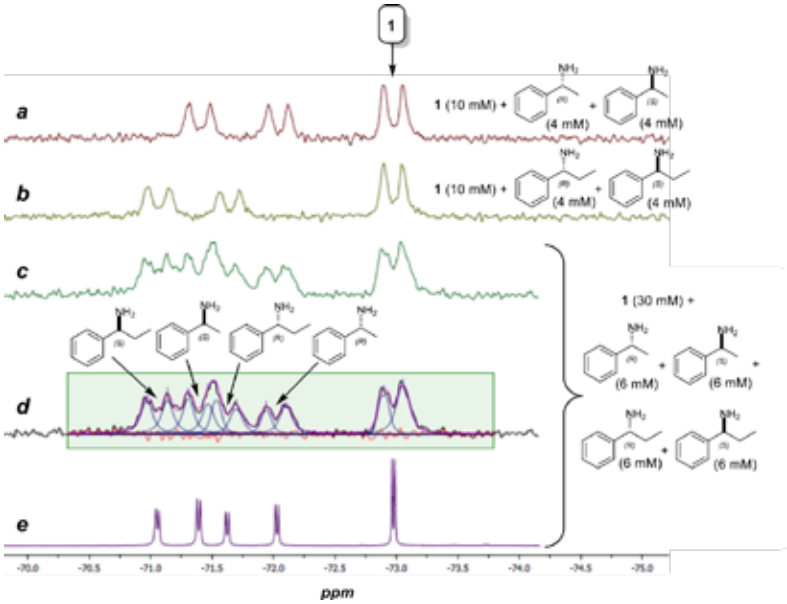
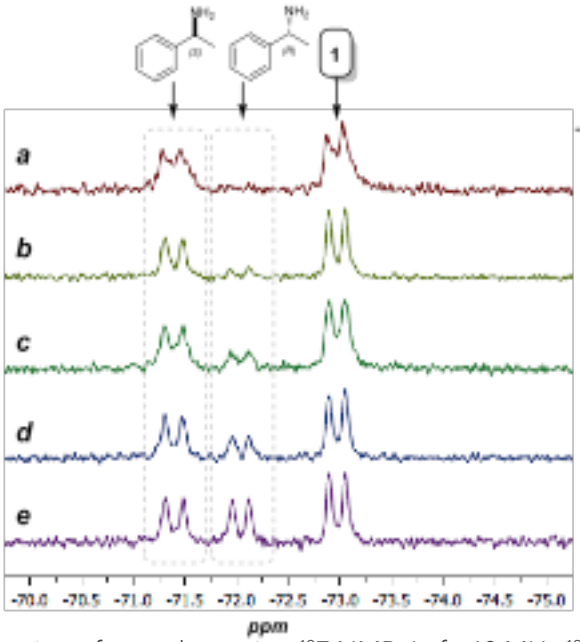


Figure 4. a – c) 60 MHz ^{19}F NMR spectra (64 scans) of complex 1 with mixtures of chiral amines. **d)** Peak fitting results of spectrum 4c (black = actual spectrum; blue = simulated peaks; magenta = sum of simulated peaks; red = residual error). **e)** 400 MHz ^{19}F NMR spectrum (64 scans) of same sample as spectrum 4c.

Estimation of Enantiomeric Ratio (*er*) and Enantiomeric Excess (*ee*)

Owing to the inherently quantitative nature of NMR Spectroscopy, the concentration of each species present in the sample can be estimated by integrating the NMR peaks directly.[7] Figure 5 shows the spectra of complex 1 with a series of mixtures of *(R)*-α-methylbenzylamine and *(S)*-α-methylbenzylamine with different enantiomeric ratios. Integrating the two enantiomeric amine peaks and comparing their areas gives an estimate of the enantiomeric ratio (*er*) and enantiomeric excess (*ee*), as shown in figure 5.



Entry	Actual er (*S*:*R*)	Est'd er (*S*:*R*)	Actual ee (%)	Est'd ee (%)
a	90:10	93:7	80%	86%
b	80:20	84:16	60%	68%
c	70:30	75:25	40%	50%
d	60:40	69:31	20%	38%
e	50:50	53:47	0%	6%

Figure 5. Estimation of *er* and *ee* using ^{19}F NMR. Left: 60 MHz ^{19}F NMR spectra (64 scans) of complex 1 (ca. 10 mM in $CDCl_3$) with *(R)*-α-methylbenzylamine and *(S)*-α-methylbenzylamine (ca. 8 mM total). Right: Actual and estimated *er* and *ee* values.

Using relative integral values as a rough estimate, the *er* values obtained by this method are correct within 10% *(S)*, and the *ee* values obtained by this method are correct within 20%. This method requires the acquisition of only a single NMR spectrum and can very quickly provide a good estimate of the *er* and *ee* of a sample in a couple of minutes.

If more accuracy is desired, a calibration curve can be constructed as shown in figure 6. This method can more accurately account for any error in the measurement. The estimated *er* and *ee* values obtained by the above method display a linear relationship with the actual *er* and *ee* values. This allows the initial estimate to be further adjusted towards a more accurate value.

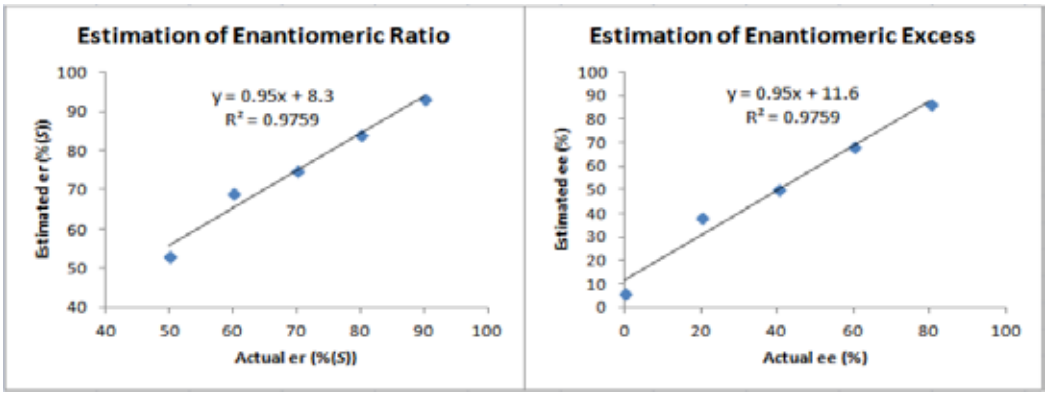


Figure 6. Calibration curves obtained from the data displayed in figure 5, showing linear relationship between actual and estimated *er* and *ee* values.

Conclusions

The compact footprint, low-cost, and ease-of-use of the NMReady benchtop spectrometer render it well-suited as an analyzer for ^{19}F NMR chirality sensing of amines when paired with these cleverly designed palladium pincer chemosensors. The ^{19}F NMR sensing method shown in this application note enables the identification, differentiation, and quantification of multiple chiral amines without elaborate derivatization. In combination with benchtop NMR spectroscopy, this chemosensory method has the potential to accelerate advances in synthetic and medicinal chemistry by facilitating the rapid determination of enantiomeric purity.

Experimental Details

For Figure 3, complex 1 (ca. 10 mM) and several chiral amines (ca. 8 mM) were dissolved in $CDCl_3$, for a total sample volume of ca. 0.5 mL. ^{19}F NMR spectra (64 scans) were acquired on an 60 MHz NMReady spectrometer.

For Figure 4c – e, complex 1 (ca. 30 mM) and four amines (ca. 6 mM) were dissolved in $CDCl_3$, for a total sample volume of ca. 0.5 mL. ^{19}F NMR spectra (64 scans) were acquired on an NMReady spectrometer and a 400 MHz Bruker spectrometer. Peak fitting was performed in MestReNova using standard parameters.

For Figure 5, complex 1 (ca. 10 mM), *(R)*-α-methylbenzylamine, and *(S)*-α–methylbenzylamine (ca. 8 mM total) were dissolved in $CDCl_3$, for a total sample volume of ca. 0.5 mL. ^{19}F NMR spectra (64 scans) were acquired on an NMReady spectrometer. Peaks were integrated in MestReNova. Enantiomeric ratio (*er*) was calculated by normalizing the total area of the two α-methylbenzylamine peaks to 100. Enantiomeric excess (*ee*) was calculated from the er.

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