

Rapid Determination of Enantiomeric Excess *via* NMR Spectroscopy

Demonstrator Notes

Demonstrator Skills and Knowledge Required

As the demonstrator you should be comfortable with the following:

1. Use of automated pipettes
2. Preparation of accurate concentration solutions
3. Preparation of NMR samples
4. Analysis of NMR spectra on NMR processing software
5. Assignments of the NMR spectra given in the lab manual
6. Construction of calibration curve
7. Use of graphing software

Laboratory Introduction for Students

The following points should be covered with the students during the laboratory introduction prior to them commencing laboratory work:

- 1. Purpose of experiment**
 - a. To infer *ee* of a chiral primary amine through the formation of diastereoisomers using a three component boronic acid based assembly.
- 2. Health and safety**
 - a. α -Methylbenzylamine is toxic and corrosive. All other reagents are either an irritant or harmful.
 - b. Gloves and PPE should be worn for experiment.
- 3. Experimental key points**
 - a. Need to accurately measure out the volumes of the solutions and reagents.
 - b. Ensure stock solutions have been sufficiently dried with 4 \AA molecular sieves to remove water.
 - c. Correctly label the NMR tubes with a system they will be able to understand when the data is returned.

Health and Safety

Reagent	Hazard
(R)-BINOL 18531-94-7	Toxic if Swallowed Causes Serious Eye Irritation
2-Formylphenylboronic acid (FPBA) 40138-16-7	Causes Skin, Eye and Respiratory Irritation
(R)- α -Methylbenzylamine 3886-69-9	Harmful if Swallowed or by Skin Contact Causes Severe Skin Burns and Eye Damage
(S)- α -Methylbenzylamine 2627-86-3	Harmful if Swallowed Toxic by Skin Contact Causes Severe Skin Burns and Eye Damage
<i>d</i> -Chloroform (CDCl ₃) 865-49-6	Harmful if Swallowed Causes Skin Irritation Suspected of Causing Cancer (Cat. 2) Causes Damage to Organs Through Prolonged or Repeated Exposure (Cat. 2)
4 \AA molecular sieves (beads) 70955-01-0	Causes Skin, Respiratory and Serious Eye Irritation

Students will be doing the following

First session (in the chemistry laboratory):

1. Each group makes host solution: **50 mM** FPBA and BINOL. The solution requires:

Chemical	Amount
BINOL	143 mg
FPBA	75 mg
CDCl ₃	10 ml

- a. **MAKING THE HOST IS THE TOP PRIORITY AS THE REAGENTS TAKE CA. 30 MINS TO DISSOLVE**
- b. This solution will need sonication or gentle warming (40 °C) to fully dissolve the components.
- c. Solutions should be dried over molecular sieves for ten minutes.

2. Each group makes five **60 mM** amine solutions of known ee for their calibration curves:

Known ee Solutions of α -Methylbenzylamine

Known ee solution number	ee (%)	Concentration (mM)	Desired Volume (ml)	Volume of R-MBA (μ l)	Volume of S-MBA (μ l)
1	75	60	10	68	10
2	50			58	19
3	25			48	29
4	0			39	39
5	-25			29	48
6	-50			19	58
7	-75			10	68

- a. Ensure students do not use contaminated equipment especially when measuring out enantiopure MBA.
- b. Solutions should be dried over molecular sieves for ten minutes.

3. Each group prepares five NMR samples of known *ee* using solutions from steps (1) and (2) above.
 - a. 0.3 mL of each solution will be combined into a vial and then transferred into a clean NMR tube.
4. Each group prepares five NMR samples of unknown *ee* using host solution (made in step 1) and the solutions amine of unknown *ee* prepared by the technician.
 - a. 0.3 mL of each solution will be combined into a vial and then transferred into a clean NMR tube.
5. Students sometimes get a small amount of their NMR sample on the outside of the tube
 - a. The tubes should be wiped with a small amount of acetone on a tissue before submitting for analysis
6. NMR samples are submitted.

Second session (In the IT Suite):

This session will be conducted in a computer cluster. Students will analyse the electronic copies of the NMR spectra to construct a calibration curve from the 'known' samples and then calculate the *ee*'s of the 'unknown' samples. A video on how to use MNova and TopSpin is available and there are detailed written notes on the use of MNova and TopSpin for the analysis of spectra obtained from this experiment. However, the students may find it useful to see a demonstration of the software using a projector. The students need to know how to use the following functions in the NMR analysis software:

- Loading the data file
- Zooming in and out of a spectrum and increase/decrease of intensity
- Integration of a signal
- Normalise the integration value
- Pick peaks in the spectrum

Students need to:

1. Interpret spectra of individual components, these are provided within the student's lab manual.
 - a. Identify the following peaks:
 - i. BINOL: alcohol protons – 5.0 ppm (singlet)
 - ii. Host Solution: aldehyde proton of 2-formylphenylboronic acid – 9.9 ppm (singlet)
 - iii. Amine: benzylic proton – 4.1 ppm (quartet)
2. Interpret spectra of their ten samples from the first session.
 - a. Identify the following peaks: imine protons and benzylic protons
 - i. imine at 8.1 ppm for *R* and 8.3 ppm for *S*
 - ii. benzylic at 5.0 ppm for *S* and 5.1 ppm for *R*

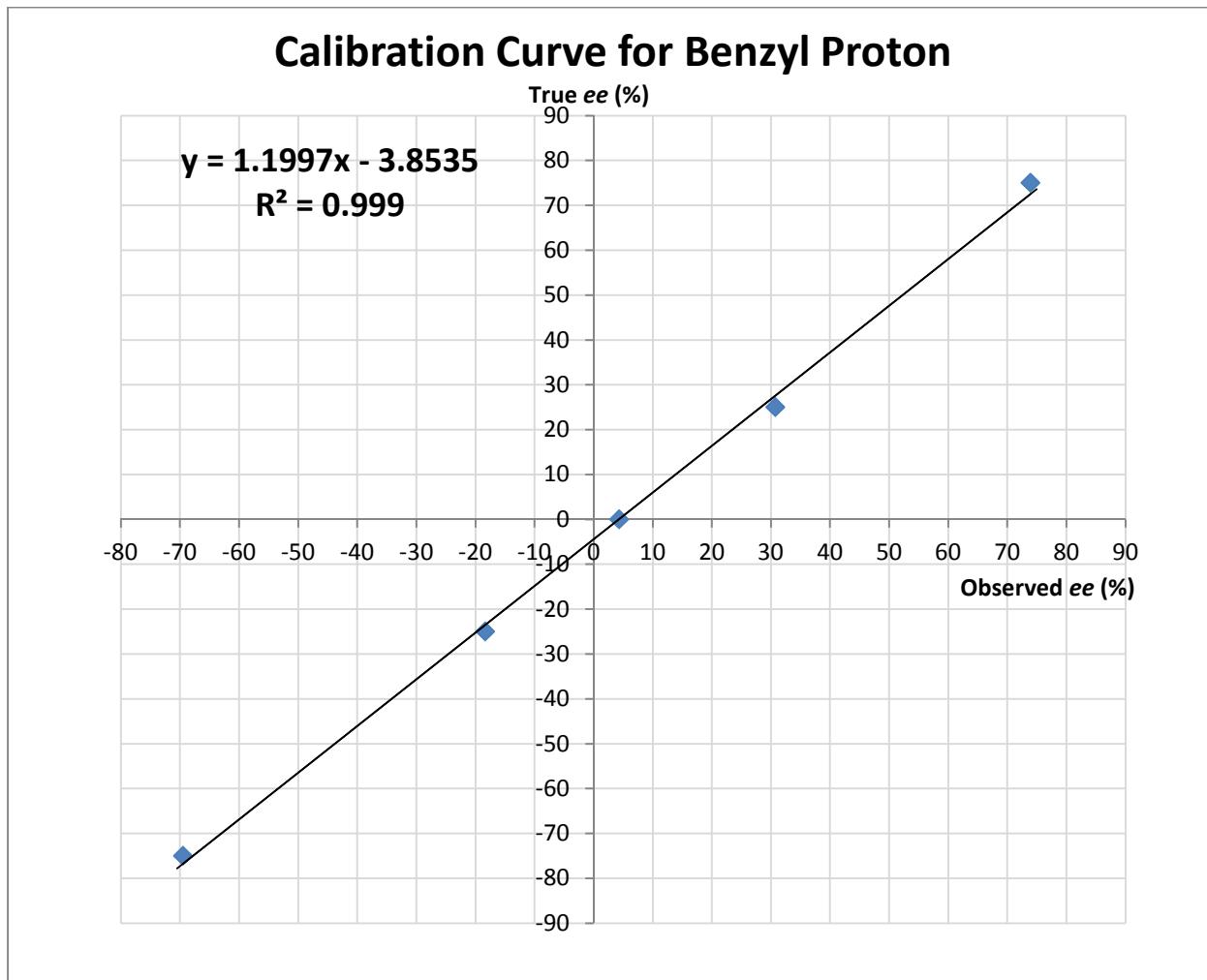
3. Integrate the imine and benzylic peaks
4. Use the values obtained for integration to infer the *ee* of the starting amine, they are provided with the following formula:

$$ee = \frac{I_R - I_S}{I_R + I_S}$$

Where I_x is the integration of the signal.

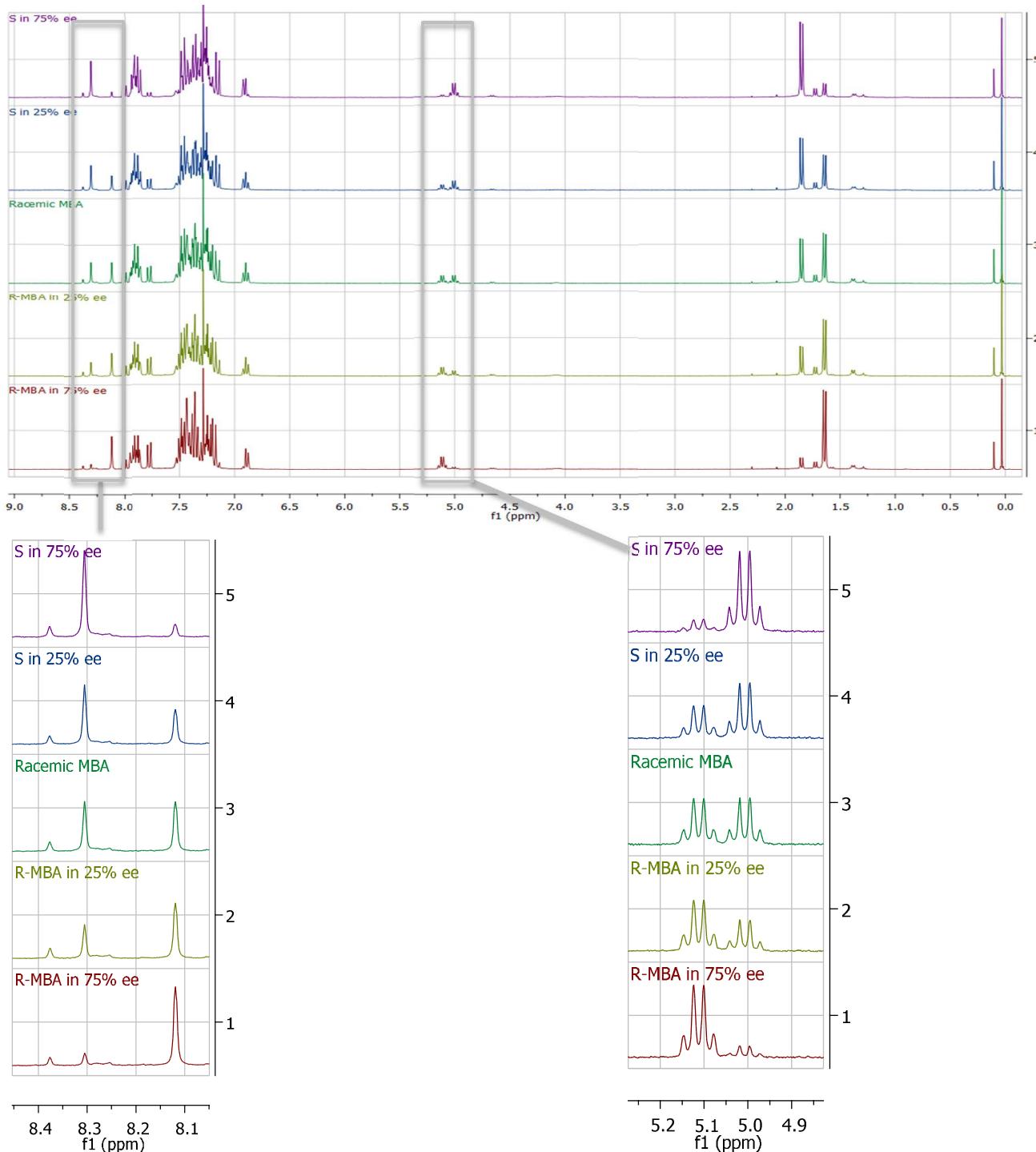
5. Each group constructs a calibration curve(s), see below for an example of a plotted curve.
 - a. Plot the best-fit line in the graphing software/calculator, ideally R^2 values should be >0.97
 - b. Critical evaluation of data is encouraged for the assessment. Students should indicate anomalous points.
 - c. If data points are anomalous and leading to skewing of data, then they could be omitted from the calibration curve. It should be pointed out this is not typically done in research.
6. Each group uses the best-fit equation(s) from their calibration curve(s) to calculate the *ee* of the unknown samples.

Example Calibration Curve



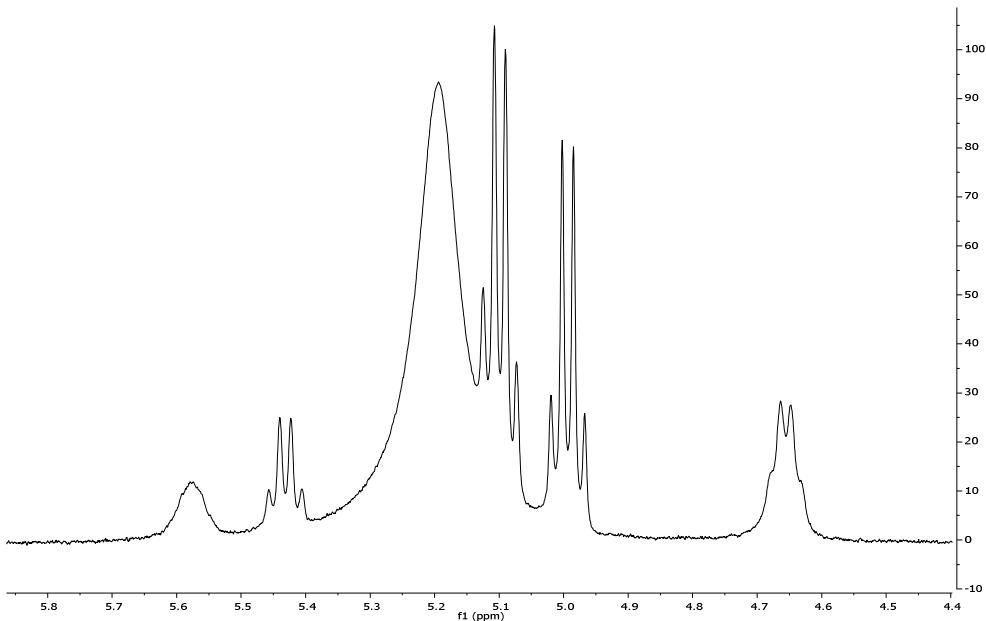
Reference Spectra

The following page shows the spectra for varying *ee* solutions. It is possible to use the imine signals (at 8.1 ppm for *R* and 8.3 ppm for *S*, both singlets), the benzyl signals (at 5.0 ppm for *S* and 5.1 ppm for *R*, both quartets) or the methyl signals (at 1.6 ppm for *R* and 1.9 ppm for *S*, both doublets) to analyse the *diastereomeric ratio of the two complexes*. Instruct the students to use the methyl signals if there is a problem with either the benzylic or imine resonances.

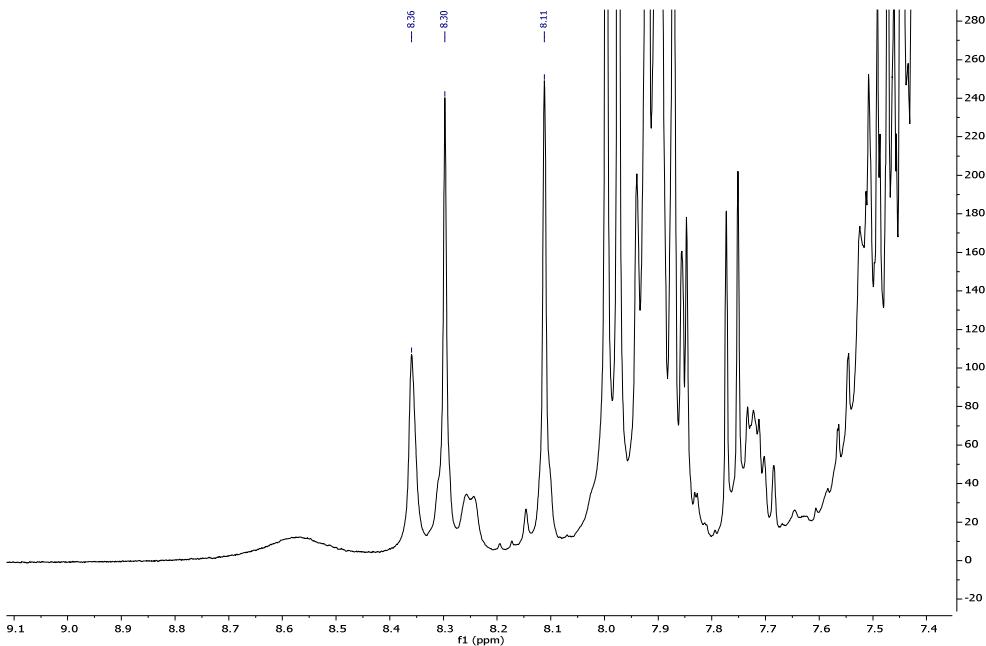


Common Errors in Student Spectra

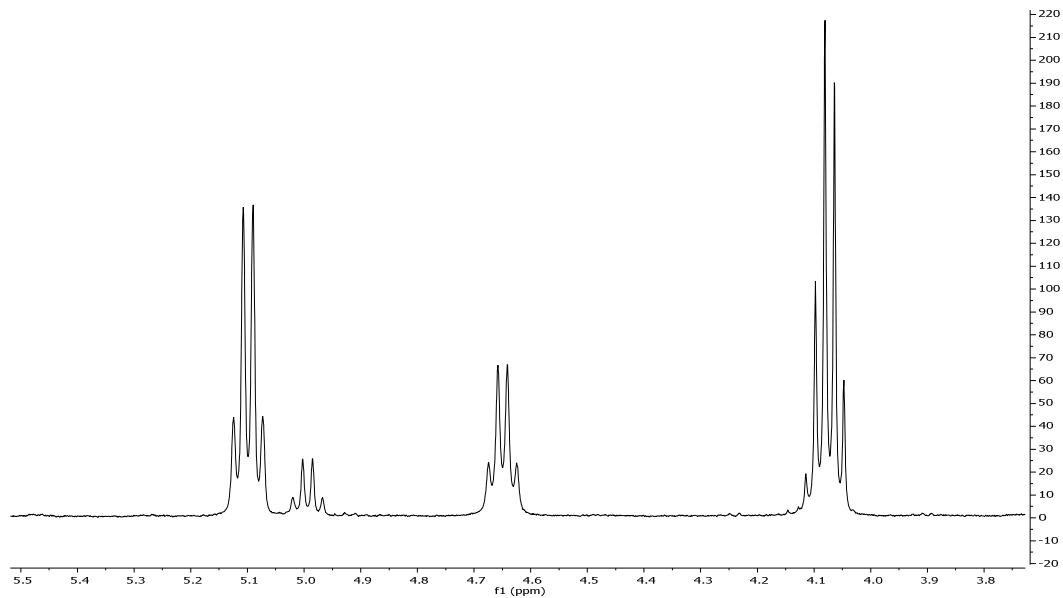
Unbound BINOL will overlap the benzylic signals, rendering analysis of these signals unreliable. Unbound MBA gives a benzylic signal at 4.1 ppm. The presence of water in the NMR mixture can lead to several extra resonances at 1.7 ppm (d), 4.7 ppm (q) and 8.4 ppm (s). Make sure that students do not accidentally integrate these signals.



Broad peak at 5.2 ppm is due to excess BINOL in the sample, peak at 4.6 ppm is due to water adduct



Extra imine peak at 8.35 ppm is due to the presence of water adduct



Peak at 4.1 ppm is due to free amine, peak at 4.6 ppm is due to the water adduct