Experiment Code

Rapid Determination of Enantiomeric Excess by NMR Spectroscopy

Objectives

By the end of this experiment you should be able to:

- Use volumetric pipettes to prepare solutions of accurate concentrations
- Prepare a series of solutions which can be used to produce a calibration curve
- Determine an unknown enantiomeric excess (ee) using a calibration curve
- Analyse an NMR spectrum using NMR processing software
- Calculate an enantiomeric excess (ee) from your NMR data

Time Permitted

2 practical sessions (1 session in the laboratory, 1 session in an IT Suite)

Background

Chiral molecules are important due to their prevalence in biological systems such as proteins (including structural proteins, receptors and enzymes), carbohydrates, hormones and DNA. Additionally, many pharmaceutical compounds are chiral molecules which can be synthesised as a mixture of stereoisomers, sometimes different stereoisomers can interact differently within biological systems such as the body.



Figure 1 - Two enantiomers of DOPA

One such example of this is 2-amino-3-(3,4-dihydroxyphenyl)propanoic acid (DOPA), which is chiral; . The enantiomers (which are non-superimposable mirror images) L-DOPA and D-DOPA are shown above (figure 1). Whilst L-DOPA has been shown to be a useful drug in combating Parkinson's disease, its opposite enantiomer (D-DOPA) has no observable effect.

Therefore, in order to make a more cost-effective and active formulation, L-DOPA alone is required. A stereoselective synthesis (a synthetic route that only produced one enantiomer) would be the most effective way to achieve this. The asymmetric synthesis of L-DOPA reported by Knowles in 1983,¹ for which he was

jointly awarded a Nobel Prize in 2001,² is shown below (scheme 1). The step in which chirality is introduced is achieved *via* asymmetric hydrogenation using a chiral ruthenium catalyst and molecular hydrogen.³ However, asymmetric transformations do not always give one enantiomer exclusively, i.e. a non-racemic mixture of enantiomers can be produced. This means that a method of determining the amount of each enantiomer present is required.



Scheme 1 Knowles' stereoselective synthesis of L-DOPA¹

Historically, optical rotation was used for the determination of the enantiomeric excess (*ee*). Optical rotation measures the angle of rotation of plane-polarized light by a solution of the compound. If a reference sample is available, then this can be used to determine the *ee* of an unknown sample. This has been superseded by high performance liquid chromatography (HPLC) using a chiral stationary phase. In HPLC, a sample is passed through a chiral stationary phase using a solvent as the mobile phase. For analytical HPLC this is done at high pressures, typically with small quantities of sample (<0.1 mg). Each enantiomer will interact differently with the chiral stationary phase, leading to different retention times which are usually represented as a chromatogram. The enantiomers form transient diastereoisomeric interactions with the stationary phase, leading to difference in residence or 'retention time'.⁴

However, HPLC can be a time-consuming process and therefore efforts have been made to develop methods which can determine the *ee* of a compound more rapidly. One technique which could be used is ¹H NMR spectroscopy, because data can be acquired quickly (less than 5 minutes per sample). However enantiomers have identical NMR spectra and therefore NMR spectroscopy cannot be used to determine the relative proportions of enantiomers in a mixture directly. Diastereoisomers have different physical and chemical properties, which means that the protons are in different magnetic environments and will produce different ¹H NMR spectra. Therefore, NMR spectroscopy can be used to measure the relative proportions of diastereoisomers in a mixture.⁴

Thus if a mixture of enantiomers is converted into a mixture of diasteroisomers then NMR spectroscopy can be used to determine the ratio of diastereoisomers in the mixture, which can be assumed to be the same as the ratio of enantiomers (figure 2). This can only be achieved by forming derivatives with **chiral reagents of known stereochemistry**. The derivatising reagent needs to be enantiopure (a single enantiomer) because if it was not, then two pairs of diastereoisomers would be formed, each with their corresponding enantiomer, which would lead to inaccurate results.



Figure 2 - Demonstration of the relationship between enantiomers and diastereoisomers

There are several ways to convert an enantiomer into a pair of diastereoisomers, such as derivatization with Mosher's acid chloride (scheme 2), the formation of ammonium salts with chiral acids such as camphor sulfonic acid, and the formation of complexes with chiral shift reagents such as europium tris[3-(heptafluoropropylhydroxymethylene)-(+)-camphorate] (Eu(hfc)₃).



Scheme 2 Derivatisation of a chiral secondary alcohol with an enantiopure Mosher's acid chloride to form diastereoisomers

Due to the historic use of optical rotation in the field of measuring chiral integrity we determine a value called enantiomeric excess (*ee*), which is a measure of how much more of one enantiomer there is, relative to the total amount of sample.⁴ Enantiomeric excess is defined as:

$$ee=rac{n_A-n_B}{n_A+n_B} imes 100\%$$

Where:

 n_A = moles of major enantiomer A

 n_B = moles of minor enantiomer B

Enantiomeric excess ranges in values from 0% ee for racemic mixtures to 100% ee for an enantiopure sample.

Introduction

In this experiment 2-formylphenylboronic acid (FPBA) and (*R*)-BINOL are used to form a chiral boronate ester, which then produce a pair of diastereomeric imines upon exposure to α -methylbenzylamine (MBA, a chiral amine) (scheme 3). In aprotic solvents, this assembly is stabilised by donation of a lone pair of electrons on the nitrogen into the empty p-orbital of the boron.⁵



Scheme 3 Assembly formation of diastereoisomers to infer the ee of a chiral amine

There are three key chemical concepts in this assembly; axial chirality of BINOL, the binding of boronic acids with diols and the reversible formation of stable imines from aldehydes and primary amines. Firstly, BINOL does not contain a chiral tetrahedral carbon atom,; it instead exhibits axial chirality, which is introduced through restricting the rotation of a carbon-carbon single bond between the two naphthyl units to form stable atropisomers. BINOL is important for this assembly because it is a chiral diol and boronic acids are known to bind strongly to certain diols because they form stable cyclic boronic esters. The third key concept is imine formation; – it is well studied that when exposed to an amine, an aldehyde will reversibly form an imine in equilibrium with the parent aldehyde. Imine formation is exploited in this assembly as the binding mode for the amine being studied.

For this experiment some specific terminology is used which is derived from supramolecular chemistry, these are the terms 'host' and 'guest'. A host is a molecule or structure that non-covalently binds a molecule, and the guest is the compound that is bound, as illustrated in (Figure 3). According to this definition, the assembly formed in this experiment is not formally a host-guest complex, but the terminology best describes the interaction. Therefore, the BINOL-FPBA compound will be described as the host and the MBA is the guest.



Figure 3 - Theoretical example of host-guest interactions

During this experiment reactions will be performed on small scale by adding a solution of amine in $CDCl_3$ to a solution of FPBA and (*R*)-BINOL in $CDCl_3$ and the resulting complex observed by NMR spectroscopy. Therefore, in this experiment you will first make the FPBA+BINOL solution (known as the 'host' solution) and then solutions of (*R*)- and (*S*)-methylbenzylamine of a known enantiomeric excess (*ee*). When these two solutions are mixed together in the correct ratio, the assembly shown in scheme 3 is formed. The ratio of diastereoisomers can be determined and this will be used to construct a calibration curve.

After production of a calibration curve, you will be provided with a solution of unknown *ee* from which you will prepare NMR samples with the "host" solution. You will measure the diastereoisomeric ratio (*dr*) to determine *ee* using your calibration curve to convert spectroscopically determined values of *dr* into *ee* of your unknown amine solution.

During this experiment you will use a modified version of the calculation of *ee*. Enantiomeric excess will still use the formula defined earlier, but n_A and n_B will remain the same signal in the ¹H NMR spectroscopy rather than n_A being the major enantiomer and n_B being the minor.

$$\frac{n_A - n_B}{n_A + n_B} \times 100\%$$

 n_A = moles of enantiomer A

 n_B = moles of enantiomer B

This means that enantiomeric excess ranges in values from 0% *ee* for racemic mixtures to 100% *ee* for an enantiopure sample of A through to -100% *ee* for an enantiopure sample of B.

Safety & Physical Data

The hazard statements and physical data for the reagents used in this experiment are listed below:

Substance	Hazards	Physical Data
(R)-BINOL	Toxic if Swallowed	$RMM = 286.32 \text{ g mol}^{-1}$
	Causes Serious Eye Irritation	
2-Formylphenylboronic acid	Causes Skin, Eye and Respiratory Irritation	RMM = 149.94 g mol ⁻¹
(FPBA)		
(<i>R</i>)- α -Methylbenzylamine	Harmful if Swallowed or by Skin Contact	RMM = 121.18 g mol ⁻¹
	Causes Severe Skin Burns and Eye Damage	d = 0.94 g mL ⁻¹
(S)-α-Methylbenzylamine	Harmful if Swallowed	RMM = 121.18 g mol ⁻¹
	Toxic by Skin Contact	d = 0.94 g mL ⁻¹
	Causes Severe Skin Burns and Eye Damage	
<i>d</i> -Chloroform (CDCl ₃)	Harmful if Swallowed	
	Causes Skin Irritation	
	Suspected of Causing Cancer	
	Causes Damage to Organs Through Prolonged or	
	Repeated Exposure	
4Å molecular sieves (beads)	Causes Skin, Respiratory and Serious Eye Irritation	

Acetone	Highly flammable liquid and vapour	
	Causes serious eye irritation	
	May cause drowsiness or dizziness	

The hazards of the diastereomeric assembly formed in this experiment needs to be considered. The assembly should be considered toxic, irritant and corrosive. Ensure that gloves are worn when handling reagents and their solutions. Gloves are to be changed immediately should any reagent or solution be spilt on them.

Procedure

First session (in the chemistry laboratory)

In groups of five students, make 10 mL of the 'host' solution in deuterated chloroform (CDCl₃) that is 50 mM for both 2-formylphenylboronic acid (2-FPBA) and (R)-[1,1'-binaphthalene]-2,2'-diol (R-BINOL) i.e. you will make one solution which contains **both** FPBA and BINOL. (Use a 10 mL volumetric flask.) You need to calculate the amount of FPBA and BINOL you will need. Check the results of your calculation with a demonstrator before making the solution.

This solution will need <u>gentle</u> warming (use a beaker of hot water) in order to fully dissolve the components. After the correct volume of $CDCl_3$ has been added, add enough activated 4Å molecular sieves to cover the bottom of the volumetric flask (10 should be sufficient), stopper the flask and allow the solution to dry for ten minutes with occasional swirling. This host solution will be shared among the members of the group.

Each member of the group should make a 60 mM solution of α-methylbenzylamine (MBA) in CDCl₃ to a known *ee*. Decide as a group who is going to make which solution so that 5 different solutions are made. 7 different solutions are listed in the following table – you only need to make 5 of them.

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

Known ee Solutions of α-Methylbenzylamine

Use a 10 mL volumetric flask. This will require using a volumetric pipette to add (*R*)- α -MBA and (*S*)- α -MBA – speak to the demonstrator if you are unsure how to use a volumetric pipette. **Use a new pipette tip every time MBA is dispensed.** After the correct volume of CDCl₃ has been added, add enough activated 4Å molecular sieves to cover the bottom of the volumetric flask (10 should be sufficient), stopper the flask and allow the solution to dry for ten minutes with occasional swirling.

- 3. Prepare an NMR sample of known *ee* by combining 0.3 mL of the host solution with 0.3 mL of the amine solution in a vial. (Use a graduated glass pipette for this. Transfer this solution carefully over to an NMR tube using a Pasteur pipette. Cap and label the NMR tube and submit for analysis. <u>You may wish to devise a labelling system as a group so that it is easier to analyse the spectra and avoid mix-ups</u>. Your group should end up with a total of 5 known *ee* NMR samples.
- 4. Prepare an NMR sample of unknown *ee* by combining 0.3 mL of the host solution with 0.3 mL of the unknown *ee* amine solution provided by the technician as described above. Decide as a group who is going to make which unknown solution so that a total of 5 different unknown solutions are analysed. Transfer the solution to an NMR tube, cap and label the tube and submit for analysis. Your group should end up with a total of 5 unknown NMR samples.
- 5. As a final step, once you have prepared, all of your NMR samples for submission please wipe the outsides of each tube using a tissue with a small amount of acetone on it.

The NMR spectra for the samples your group have prepared will be recorded for you, and the data files will be made available for you to analyse at the start of the second session in the IT suite.

In order to prepare for the next session please take the time to read the lab manual, especially the introduction to NMR processing software given below (appendix 2) and watch the video recording on how to use the software.

Points to consider before the computer session

- What sources of error were present in carrying out the laboratory session?
- How widely applicable do you think this assembly could be. What limitations do you see in the types of substrates which could be analysed using it?
- Why did you add molecular sieves to your solutions?
- What protons are responsible for the signals observed in the ¹H NMR spectra of the starting materials?
- What signals would you expect to see in the NMR spectrum of the assembly and where would these approximately come in a ¹H NMR spectrum?

Second session (in the IT Suite)

- 1. Below are the NMR spectra of components used in the assembly.
- 2. Identify the following signals:
 - a. BINOL: alcohol protons
 - b. HOST SOLUTION: aldehyde proton of 2-formylphenylboronic acid
 - c. MBA: benzylic proton

Think carefully about these signals and what they can tell you about assembly formation.

3. Now compare the individual components to the ten spectra produced by your group.

- a. Identify the imine protons and benzylic protons of the two diastereoisomers in each spectrum.
 Take care when identifying the correct imine peaks. Compare all of the spectra to determine which peaks are of interest.
- 4. Integrate the imine and benzylic peaks in all five samples of known ee. Use these integrations to calculate *ee.* Use the following formula:

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

Where I_x is the integration of the signal. If you are unsure as to which peaks belong to *R* and *S*, use the table of known *ee* amine solutions to see which should be in excess in your spectra.

- 5. Construct 2 calibration curves using all five solutions of **known** *ee* one curve using data from the imine signals and another curve using data from the benzylic signals. For each curve:
 - a. Plot the observed *ee* of the sample on the x axis (this observed *ee* was calculated from the integrations you found in step 3.)
 - b. Plot the exact (known) *ee* of the sample on the y axis.
 - c. **Calculate** the lines of best fit for your data. You can use MS Excel or Sigmaplot trend lines to do this.
- 6. Integrate the imine and benzylic peaks in all five samples of **unknown** *ee*. Use these integrations to calculate *ee*.
- 7. Now take the observed *ee*'s for the unknown samples (calculated in step 5) and use your calibration curves to determine the "true" *ee* of the samples.

The Report

For this experiment you are not expected to hand in your lab book, the mark will be 100% based on the proforma which you are required to complete. The blank proforma for this experiment is available for download through the Chemistry Canvas server.

Once completed, this should be submitted electronically via the online submission portal, which can also be found on Canvas.

For ease of processing, files uploaded must be either in Microsoft Word format or converted to PDF.

The file should be named with the experiment code followed by an underscore ("_") followed by your name, e.g. "CODE_JBloggs.doc" or "CODE_JSmith.pdf"

All graphs should be produced in either Excel or Sigmaplot and pasted into the Word document containing your proforma, either at the end of the file or at an appropriate point. You must ensure that the graphs are appropriately sized and labelled prior to submission.

Appendix

Reference Spectra

Below are the reference spectra for (*R*)-BINOL, MBA and the host solution (BINOL + 2-formylphenylboronic acid). The important signals to note are the alcohol protons of BINOL, the aldehyde proton of 2 formylphenylboronic acid and the methine proton of methylbenzylamine.

<u>(R)-BINOL</u>



Host Solution

Т



α -Methylbenzylamine



Introduction to NMR processing

Extracting your data

When you receive your data back you should get it as a .zip folder. In order to be able to access all of the spectra contained within this folder you will first need to extract the files. This can be done by right clicking and selecting "Extract All"

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Figure 4 Screenshot showing the inside of a typical NMR data set; tell the NMR processing software you wish to open the **.fid** file

Once you have extracted the folder, you may open it to find separate folders inside, each of which will have a separate title. They may be titled as "02-10.1.Teaching" or similar, you need to open each of these folders and find the .fid file inside (this is shown in Figure 4).

Opening a spectrum in MNova 10

Now you have located your .fid file for your data set, opening the .fid file is done using the open function within the MNova software or alternatively dragging the .fid file into the MNova software window. Upon doing this a spectrum should be displayed. In the top left hand corner of the spectrum it should display the folder name e.g. 02-10.1.Teaching and also the title you gave the sample e.g. 25% known amine. A representative spectrum is shown in Figure 5.



Figure 5 Screen upon opening a spectrum

Basic functions in MNova 10

There are several buttons which you will need to be able to carry out the analysis of your spectra. They are shown in Figure 6.



Figure 6 Basic controls required for the analysis of your NMR spectra in MNova 10

It is important that you know how to use these basic functions of the program before you begin the analysis of your spectra. You may wish to spend a few minutes getting to grips with manipulating your spectra using the tools shown above before you commence data analysis. Use the video on Canvas to assist you.

Integration of a spectrum in MNova 10

In order to construct your calibration curves and determine the *ee* of the unknown amine solution, you will need to be able to calculate the ratio of diastereoisomers in each of your spectra. You will therefore need to integrate specific protons within your spectra and put these numbers into the equation given above.

Using the integration drop down menu (shown in Figure 6) select manual integration. You can then drag over the signal you wish to integrate, for example the benzylic protons have been integrated in Figure 7. Make sure you drag over each separate signal one at a time. Upon doing this the program will give you the relative integrations below each peak you have selected. By default, the peak you integrated first will be given a value of 1 and the others a value in comparison to this. These values can be used directly in the equation; to determine which peak is related to *R* and to *S* compare to the table of known *ee* solutions above. Alternatively, you can change which peak is assigned as one proton by right clicking on your chosen integral, selecting to edit the integral and changing the normalised integral value.



Figure 7 Integration of the benzylic region

Opening a spectrum in TopSpin 3.5

Now you have located your .fid file for your data set, opening the .fid file is done by opening the folder containing your .fid file in TopSpin or alternatively dragging the .fid file into the TopSpin software window. Upon doing this a spectrum should be displayed. In the top left hand corner of the spectrum it should display the title you gave the sample e.g. 25% known amine. A representative spectrum is shown in Figure 8. The peaks may have been automatically picked and integrated, however these will need to be altered and reanalysed as outlined below.



Figure 8 Screen upon opening a spectrum

Basic functions in TopSpin 3.5

There are several buttons which you will need to be able to carry out the analysis of your spectra. The tools to manipulate the zoom level and the intensity level of your peaks are shown in Figure 9.



Figure 9 Basic controls required for the analysis of your NMR spectra in TopSpin 3.5

Selecting the Pick Peaks sub menu on the right brings up the following menu of tools on the top of the spectrum,



Figure 10 Peak picking tools in TopSpin 3.5

whereas selecting the Integrate button brings up the following integration tools.



Figure 11 Integration tools in TopSpin 3.5

It is important that you know how to use these basic functions of the program before you begin the analysis of your spectra. You may wish to spend a few minutes getting to grips with manipulating your spectra using the tools shown above before you commence data analysis. Use the video on Canvas to assist you.

Integration of a spectrum in TopSpin 3.5

In order to construct your calibration curves and determine the *ee* of the unknown amine solution, you will need to be able to calculate the ratio of diastereoisomers in each of your spectra. You will therefore need to integrate specific protons within your spectra and put these numbers into the equation given above.

Using the integration sub menu (shown in Figure 11) select the button to allow you to integrate a specific region on the spectrum. You can then drag over the signal you wish to integrate, for example the benzylic protons have been integrated in Figure 12. Make sure you drag over each separate signal one at a time. Upon doing this the program will give you the relative integrations below each peak you have selected. By default, the peak you integrated first will be given a value of 1 and the others a value in comparison to this. These values can be used directly in the equation; to determine which peak is related to *R* and to *S* compare to the table of known *ee* solutions above. Alternatively, you can change which peak is assigned as one proton by (while in the integration mode) right clicking on your chosen integral, selecting to calibrate the current integral and changing the calibrated value.



Figure 12 Integration of the benzylic region

References

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5. Perez-Fuertes, Y.; Kelly, A. M.; Fossey, J. S.; Powell, M. E.; Bull, S. D.; James, T. D., Simple protocols for NMR analysis of the enantiomeric purity of chiral primary amines. *Nat. Protocols* **2008**, *3* (2), 210-214.