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NMR determination of enantiopurity via chiral derivatisation. $de_{(measured)} = ee_{(substrate)}$?

Anthony Cawley, Julian P. Duxbury and Terence P. Kee *

School of Chemistry, University of Leeds, Leeds LS2 9JT, UK

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Abstract

It is not necessary to use 100% enantiopure '*probe*' compounds as chiral derivatising reagents.

1. Chiral derivatisation as a means of measuring enantiopurities (*ee*)

Various methods are available to determine the enantiopurities (*ee*s) of chiral molecules, including chiroptical, spectroscopic and chromatographic techniques each finding preference in certain circumstances. Many research chemists determine *ee*s using NMR spectroscopy since the experiments are generally simple, convenient, familiar to perform and interpret and, with care, can afford reasonable levels of precision.[1] Of the several types of NMR experiment available to assay enantiopurity, chiral derivatisation, relying upon conversion of *substrate* enantiomers to diastereomers upon interaction with a chiral *probe* compound, is one of the mostly commonly used.[1] Since precise *ee* measurement via NMR entails maximising diastereoisomer shift dispersion, signal resolution and signal-to-noise ratios whilst minimising line-broadening, all of which lead to more accurate integration of signals, choice of solvent, temperature, nucleus of observation (commonly ^{1}H, ^{19}F, ^{31}P), acquisition parameters such as pulse delay times, peak-shape manipulation and digital resolution but arguably the most important variable is the enantiopurity of the chiral probe.[†1]

Established practice requires a chiral probe compound to be (as close as possible to) 100% enantiopure so that the ratio of diastereoisomers (*de*) produced corresponds exactly to the ratio of enantiomers (*ee*) in the substrate compound; under these conditions, $de_{(measured)} = ee_{(substrate)}$.[‡] However, it is not necessary

* Corresponding author. Tel: 0113 2336421; fax: 0113 2336565; e-mail: T.P.Kee@chem.leeds.ac.uk

† Enantiopurity measurements do not always require a derivatising agent which is chiral. Achiral reagents have found use in cases where exploitation of Horeau's principle is possible.[2]

‡ Enantiopurity of the chiral agent is less crucial when it is used as a solvating agent.[3]

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for the chiral probe compound to be 100% enantiopure, only enantioenriched to a level that can be measured with precision using an independent technique such as HPLC or GC. Once the enantiopurity of the probe [$ee_{(probe)}$] is known, the enantiopurity of any derivatised substrate [$ee_{(substrate)}$], along with associated error limits, can be calculated from the NMR measurement. Whilst this, in itself, may be self-evident to chemists who work with enantiomerically enriched compounds, it is nevertheless important for all workers, especially students, to recognise both; (i) how to perform the required calculations and (ii) recognise and use such calculations in their enantiopurity assays. Both are illustrated below.

2. $\frac{ee_{(substrate)}=[de_{(measured)}]\times 100}{ee_{(probe)}}$. Derivation and error limits

Consider an NMR experiment to measure the enantiomeric excess of a given substrate $ee_{(substrate)}$ using a chiral derivatising probe of enantiomeric excess $ee_{(probe)}$. A combination of substrate and probe molecules leads to the formation of four possible diastereoisomers; $R_{(substrate)}, R_{(probe)}$, $R_{(substrate)}, S_{(probe)}$; $S_{(substrate)}, R_{(probe)}$ and $S_{(substrate)}, S_{(probe)}$,[§] which exist as two pairs of enantiomers.

If the probe is 100% enantiopure then only two of the above diastereoisomers exist, one from each mutual enantiomeric pair and consequently $de_{(measured)} = ee_{(substrate)}$. However, should the probe contain a fraction of the opposite enantiomer, then the remaining two diastereoisomers become populated and contribute to the peak areas of their enantiomers. Under these conditions, $de_{(measured)} < ee_{(substrate)}$.

Consider the probe molecule to exist as x parts of the dominant enantiomer and $(100 - x)$ parts of the lesser enantiomer; thus, $ee_{(probe)} = [2x - 100]$. Similarly, if the substrate consists of y parts of the dominant enantiomer and $(100 - y)$ parts of the other, $ee_{(substrate)} = [2y - 100]$. Consequently, $x = \frac{50+ee_{(probe)}}{2}$ and $y = \frac{50+ee_{(substrate)}}{2}$.

The relative concentrations of each of the four possible diastereoisomers are then given by combining the populations of each possible combination of probe and substrate; $[y\times x]$; $[y\times(100x)]$; $[(100-y)\times x]$ and $[(100 - y) \times (100 - x)]$. Since this set of four diastereoisomers represents two pairs of enantiomers, NMR is able only to differentiate each mutual pair such that the integration of appropriate NMR signals represents the concentrations of $[R_{(substrate)} - R_{(probe)} + S_{(substrate)} - S_{(probe)}]$[§] under one resonance versus $[R_{(substrate)} - S_{(probe)} + S_{(substrate)} - R_{(probe)}]$[§] under the other; consequently;

$$xy + (100 - y)(100 - x) \propto \mathbf{a} \tag{1}$$

$$y(100 - x) + (100 - y)x \propto \mathbf{b} \tag{2}$$

Recognising the equation for $de_{(measured)} = (\mathbf{a} - \mathbf{b})/(\mathbf{a} + \mathbf{b}) \times 100$, where **a** and **b** represent the respective NMR integrations under each of the two observable signals (where **a>b**) allows us to represent $de_{(measured)}$ in terms of x and y through the proportionalities of Eqs 1 and 2 respectively to afford ultimately Eq. 3.

$$de_{(measured)} = \frac{100 - 2x - 2y + (4xy)}{100} \tag{3}$$

Substituting $x = \frac{50+ee_{(probe)}}{2}$ and $y = \frac{50+ee_{(substrate)}}{2}$ into Eq. 3 followed by elimination and rearrangement affords Eq. 4.

§ *R* and *S* Descriptors are used here to represent substrate and probe enantiomers and do not necessarily imply that each has only a single stereogenic centre.

$$ee_{(substrate)} = \frac{de_{(measured)} \times 100}{ee_{(probe)}} \tag{4}$$

If it is possible, with due consideration of experimental variables, to achieve a level of precision in the NMR determination of $de_{(measured)}$ of $\pm m\%$ whilst the degree of precision in $ee_{(probe)}$ is $\pm n\%$, then the error limits for $ee_{(substrate)}$ can be expressed from Eq. 4 as Eq. 5.

$$\Delta ee_{(substrate)} = \frac{\Delta de_{(measured)} \times \Delta ee_{(probe)}}{100} \tag{5}$$

Substituting in above for the maximum and minimum values of both $de_{(measured)}$ and $ee_{(substrate)}$ affords the expression in Eq. 6 which subsequently evolves into Eq. 7.

$$\Delta ee_{(substrate)} = \frac{[(de_{(measured)} + m) \times (ee_{(probe)} + n)] - [(de_{(measured)} - m) \times (ee_{(probe)} - n)]}{100} \tag{6}$$

$$\Delta ee_{(substrate)} = \pm\frac{1}{100}[n \times de_{(measured)} + m \times ee_{(probe)}] \tag{7}$$

Finally, one can combine Eqs 4 and 7 to afford the final, well-recognised, expression which allows calculation of the actual enantiopurity, with limits of precision, from known enantiopurity values of probe and measured values from NMR spectroscopy (Eq. 8).

$$ee_{(substrate)} = \frac{[de_{(measured)} \times 100]}{ee_{(probe)}} \pm \frac{1}{100}[n \times de_{(measured)} + m \times ee_{(probe)}] \tag{8}$$

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References

1. See for example; Parker, D. *Chem. Rev.* **1991**, *91*, 1441–1457; Hulst, R; Kellogg, R. M.; Feringa, B. L. *Recl. Trav. Chim. Pays Bas.* **1995**, *114*, 115 and references therein.
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