Chirality and NMR

Enantiomeric molecules in principle do not differ in their physical properties unless they are placed in a chiral environment. In optical spectroscopy, linearly-polarized light is used to determine the rotation angle in order to determine purity of optically active compounds. Protons attached to chiral centers will give rise to separate signals only if the molecule is placed in a non-chiral environment.

Protons at prochiral centers (e.g. methylen protons) may be enantiotopic or diastereotopic. **Enantiotopic** protons can be converted into each other by a “drehspiegel” operation and will give rise to a single peak; they are isochronous. To reveal whether protons are enantiotopic or diasterotopic one proton may be substituted by a (so far non-existing group) X. If the resulting molecules are diastereoisomers, the protons are called diastereotopic. They may than (but not necessarily) resonate at different frequencies (e.g. methylen protons at β-positions of amino acids). Whether or not two separate signals are observed often depends on the spatial separation to the nearest chiral center. In the case a pair of enantiomers is formed the protons are called enantiotopic. Diastereotopic protons can only be found in molecules that contain at least one chiral center.

Enantiotopic protons (belonging to the R- and S-form) can only be distinguished in the presence of a chiral environment. Such a chiral environment may for example be a chiral solvent but could also be a chiral molecule that complexes to the molecule of interest:

- **chiral solvents**

  ![2,2,2-Trifluoro-1-phenylethanol](image)
  ![1-Phenylethylamine](image)

  2,2,2-Trifluoro-1-phenylethanol  
  1-Phenylethylamine

Since these solvents are non-deuterated another (external) substance must be added for enabling locking on deuterium.
Lanthanide shift reagents contain unpaired electrons, which by interaction of the electrons of the metal with the protons of the substance of interest lead to (large) changes in the resonance frequency (paramagnetic contribution to the chemical shift).

Pr\(^{3+}\) : High-field shift of the signals (\(\rightarrow\))

Eu\(^{3+}\) : Low-field shift of the signals (\(\leftarrow\))

By adding lanthanide shift reagents highly overlapped (crowded) regions of the spectrum can be better dispersed.

**Commonly used shift reagents and their properties:**

Chiral shift reagents form diastereotopic complexes with the compounds, which differ in their physical properties. Some lanthanide shift reagents contain chiral ligands and the resulting complexes with chiral molecules are diastereotopic. Provided sufficient chiral lanthanide shift reagent has been added the enantiotopic protons are shifted into opposite directions until they are (completely) resolved.

But because shift reagents contain paramagnetic material proton-electron dipolar relaxation will lead to (significant) signal broadening. Therefore it is highly
recommended to add only little quantities until the signal separation of R- and S-signals is sufficient.

A chiral shift reagent that does not induce line-broadening is the Pirkle reagent. It is commercially available in both forms (R(-) and S(+)).

1-(9-Antryl)-2,2,2-trifluorethanol

**Determination of optical purity in compound 2a using Pirkle’s reagent**

1. Initially, a $^1$H NMR-spectrum of racemic (2a) is recorded. (Lower trace spectrum in Fig. 2)
   - The methylene protons attached to Si display a singlet signal ($\delta$=0.25ppm).
   - The methoxy group shows up as a singlet ($\delta$=3.3ppm).
2. Thereafter another $^1$H NMR Spectrum is recorded after 20mg of Pirkl’s reagent was added. (middle-trace spectrum in Fig. 2)
   - The methylgroups at Si are still not completely resolved ($\delta$=0.25ppm).
   - The methoxy protons are clearly separated and can be integrated. ($\delta$=3.3ppm).
3. After adding another 20mg of Pirkle’s reagent another $^1$H NMR-spectrum is measured. (bottom-trace spectrum in Fig. 2)
   - Even now the methyl protons at Si are not fully separated.
   - The methoxy protons are even better separated ($\delta$=3.3ppm).

In order to determine the optical purity it is sufficient that one set of signals is sufficiently well separated (in our case the methoxy protons).
Fig. 2: $^1$H NMR-Spectrum of (2a)

Determination of optical purity of alcohols and amines
Mosher’s reagent

Mosher’s reagent enables the determination of absolute stereochemistry of secondary alcohols or amines.

- **Preparation:** All proton chemical shifts should be assigned in the molecule.
- **Reaction:** Take two samples of the molecule of interest and treat them with either R(-)-Mosher’s reagent or S(+)-Mosher’s reagent.

**Mosher’s reagent:** 2-Methoxy-2-(trifluoromethyl)-2-phenylacetic acid chloride

- **Preparation:** All proton chemical shifts should be assigned in the molecule.
- **Reaction:** Take two samples of the molecule of interest and treat them with either R(-)-Mosher’s reagent or S(+)-Mosher’s reagent.

**Mosher’s reagent:** 2-Methoxy-2-(trifluoromethyl)-2-phenylacetic acid chloride

![Mosher's reagent diagram](image)

- **Measurement:** Record 1-D proton spectra of both reaction products. The method relies on the large contribution of the ring current from the phenyl moiety of the reagent to the chemical shifts of the methylene protons. The magnitude of the effect is proportional to the distance of the methylene protons to the MTPA-moiety.

*Never use benzene-d6 or pyridine-d5 as the solvent (for obvious reasons!)*.

- **Interpretation:** Compute the differences in chemical shift for the methylene protons ($\Delta \delta = \delta_S - \delta_R$) (in Hz) between the R- or S-MTPA-esters. For all protons on one side of the stereocenter $\Delta \delta > 0$ and on the other side $\Delta \delta < 0$. The absolute stereochemistry at the chiral center can then be extracted by using the following picture:
Referencing of NMR-Spectra

In order to compare NMR spectra recorded at different places spectra need to be referenced correctly. Moreover, the exact conditions under which samples were prepared (pH, salt content etc.) and recorded (temperature) should be described and general standards have to be used. Standards may be directly added to the sample or given as an external reference. In the latter, the standard is filled into a small capillary, which is placed inside the tube. Unfortunately, the external reference does not experience identical conditions of susceptibility, pH, temperature or pH, and therefore internal standards are usually preferable. An ideal standard should not interfere (react!) with the sample. The signal ideally is a singlet, which resonates outside the region (e.g. tetramethylsilane, TMS), in which the signals commonly occur. In addition, temperature and pH sensitivity must be small and known.

Frequently used chemicals for referencing:

**TMS** Tetramethylsilane

\[
\begin{align*}
{^1}\text{H: } & \delta = 0 \text{ ppm} \\
{^{13}}\text{C: } & \delta = 0 \text{ ppm}
\end{align*}
\]

**Cyclosilane-d_{18}**

\[{^1}\text{H: } \delta = 0.327 \text{ ppm}\]

**DSS** 2,2-Dimethyl-2-silapentane-sulfonic acid sodium salt

3-Trimethylsilyl-1-propanesulfonic acid sodium salt

\[
\begin{align*}
{^1}\text{H: } & \delta = 0 \text{ ppm} \\
{^{13}}\text{C: } & \delta = 1.7 \text{ ppm}
\end{align*}
\]

**TSP** 3-(Trimethylsilyl)-propionic acid Sodium salt

\[
\begin{align*}
{^1}\text{H: } & \delta = 0 \text{ ppm} \\
{^{13}}\text{C: } & \delta = 1.7 \text{ ppm}
\end{align*}
\]

**Dioxane**

\[
\begin{align*}
{^1}\text{H: } & \delta = 3.75 \text{ ppm} \\
{^{13}}\text{C: } & \delta = 67.4 \text{ ppm}
\end{align*}
\]
Calibration of proton spectra

<table>
<thead>
<tr>
<th></th>
<th>(\delta) = 0 ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMS</td>
<td></td>
</tr>
<tr>
<td>TSP</td>
<td></td>
</tr>
<tr>
<td>DSS</td>
<td></td>
</tr>
<tr>
<td>Cyclosiland(_{18})</td>
<td>(\delta) = 0.327 ppm</td>
</tr>
<tr>
<td>Dioxan</td>
<td>(\delta) = 3.75 ppm</td>
</tr>
</tbody>
</table>

Be careful when referencing measurements in water or methanol: shifts are \(pH\) and temperature dependent!

Measurements in water:

TMS is not water insoluble and therefore TSP is mostly used. The resonance frequency of TSP is \(pH\) dependent. TSP may also interact with hydrophobic parts of the molecule, and the chemical shift will then be the population-weighted average, which of course depends on the concentration \((\delta \neq 0 \ \text{ppm})\). Another often-used possibility is to use the water signal for referencing. The water frequency is highly temperature and weakly \(pH\) dependent \((0.02 \ \text{ppm} / \ \text{pH-unit})\). Provided the exact temperature in the sample is known (which may not be trivial! Some experiments do deliver a considerable amount of heating, e.g. the TOCSY) the chemical shift of the water is calculated from the following formula:

\[
\delta_{(H_2O)} = 7.83 - \frac{T}{96.9} \quad \text{[ppm]}
\]

\(T\) = measuring temp. in Kelvin
at \(pH = 5.5\)

Measurements in organic solvents:

In most organic solvents TMS is used as an internal standard. It is added in small amounts (!) \((5 \ \text{drops of TMS to 30 ml solvent, one may also use a pipet and suck some TMS from the gas phase and add it to the NMR sample, never directly add the TMS liquid!})\). Because TMS is highly volatile it is better substituted by Cyclosiland\(_{18}\), whose boiling point is 208 °C, for high-temperature measurements. A less precise method is to use the solvent signal for referencing.
Calibration of $^{13}$C-spectra

<table>
<thead>
<tr>
<th>Solvent</th>
<th>$\delta$ [ppm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMS</td>
<td>0</td>
</tr>
<tr>
<td>TSP</td>
<td>1.7</td>
</tr>
<tr>
<td>Dioxan</td>
<td>67.4</td>
</tr>
</tbody>
</table>

$^{13}$C-spectra are usually referenced to the solvent line resulting in uncertainties as large as 1 ppm! In the case of CDCl$_3$ the solvent $^{13}$C signal occurs between 77.4 and 76.5 ppm, depending on the concentration and type of the solute.

Calibration of $^{15}$N-spectra

It is confusing that two major standards are nowadays used for referencing of $^{15}$N spectra. Whereas inorganic or organic molecules are usually referenced with respect to CH$_3$NO$_2$ or NH$_4$Cl in bio-NMR applications shifts are presented relative to NH$_3$. The two scales differ by a considerable amount:

<table>
<thead>
<tr>
<th>Scale :</th>
<th>NH$_4$Cl</th>
<th>CH$_3$NO$_2$</th>
<th>NH$_3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH$_4$Cl $\delta$[ppm] :</td>
<td>0</td>
<td>-352.9</td>
<td>+27.33</td>
</tr>
<tr>
<td>CH$_3$NO$_2$ $\delta$[ppm] :</td>
<td>+352.9</td>
<td>0</td>
<td>+380.23</td>
</tr>
<tr>
<td>NH$_4$NO$_3$ $\delta$[ppm] :</td>
<td>+348.9</td>
<td>-4</td>
<td>+376.23</td>
</tr>
</tbody>
</table>

$\delta_n$(CH$_3$NO$_2$) = $\delta_n$(NH$_4$Cl) - 352.9[ppm]  
= $\delta_n$(NH$_3$) - 380.23 [ppm]

Calibration of $^{31}$P-spectra

$^{31}$P-NMR-spectra are referenced against 85% phosphoric acid (added as an external standard in a capillary).

| H$_3$PO$_4$ (85% in H$_2$O) | $\delta$ = 0 ppm |

Calibration of $^{17}$O-spectra

H$_2$O is added as an external standard for $^{17}$O-NMR-spectroscopy:

| H$_2$O | $\delta$ = 0 ppm |
Be careful: Sometimes CH₃NO₂⁻ or (CH₃)₂CO is used for referencing! The resulting scales are very different:

\[ \delta^{17}_O(H_2O) = \delta^{17}_O(CH_3NO_2) + 605 \quad [\text{ppm}] \]
\[ = \delta^{17}_O((CH_3)_2CO) + 569 \quad [\text{ppm}] \]

**Calibration of \(^{19}\text{F}-\text{spectra}\)**

Mostly CFCl₃ is used as an external standard in \(^{19}\text{F}\) NMR spectroscopy:

| CFCl₃          | \(\delta = 0 \text{ ppm} \) |
---|---|

Unfortunately \(^{19}\text{F}\) chemical shifts are highly solvent dependent and hence the exact conditions of measurement must be presented!

Fig. \(^{19}\text{F}\) NMR spectrum of CFCl₃ in CDCl₃.

The fine structure of the \(^{19}\text{F}\)-signal of CFCl₃ is due to the different isotopes of chlorine: \(^{35}\text{Cl}\) and \(^{37}\text{Cl}\).
For referencing \(\delta_f\) of CF\(^{35}\text{Cl}_2^{37}\text{Cl}\) is set to 0 ppm.

Another, less frequently used standard is C₆F₆.

**Calibration of \(^{29}\text{Si}-\text{spectra}\)**

For \(^{29}\text{Si}\) NMR TMS is used as an internal standard:

| TMS          | \(\delta = 0 \) |
---|---|

In case the \(^{29}\text{Si}\) resonance of TMS overlaps with signals from the compound of interest a spectrum is measured without standard, after which one drop of TMS is added and another spectrum is taken.
Referencing without standard (indirect calibration)

Some nuclei are so insensitive that internal standards yield insufficient signal-to-noise. In these cases the chemical shift scale of the heteronucleus may be computed from the proton scale using the following formula:

\[ \nu_X^0 = \nu_H^0 \cdot \frac{\Xi_X}{\Xi_H} \]

\(\Xi_X\) denotes the tabulated standard values of resonance frequencies of X-nuclei. Therein \(\Xi_H\) is 100 MHz, \(\nu_X^0\) the frequency of 0 ppm for the X nucleus and \(\nu_H^0\) the frequency of 0 ppm \(^1\text{H}\).

The ratio of frequencies depends on the nature of the used proton standard:

<table>
<thead>
<tr>
<th></th>
<th>(^{13}\text{C})</th>
<th>(^{15}\text{N (rel. to NH\textsubscript{3})})</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMS</td>
<td>0.25145002</td>
<td>0.10132914</td>
</tr>
<tr>
<td>DSS</td>
<td>0.25144952</td>
<td>0.10132905</td>
</tr>
<tr>
<td>TSP</td>
<td>0.25144954</td>
<td>0.10132900</td>
</tr>
</tbody>
</table>

Fig: Taken from J.Cavanagh et al., Protein NMR Spectroscopy

Indirect referencing is more precise than the use of external standards!

Closing remarks:

The following rules should be obeyed when publishing chemical shifts:

- Don’t define your own standards or own rules, because comparing your data to those taken by others will be difficult (or impossible).
- Always note which signal has been used for referencing.
- When using indirect referencing exactly report how this was achieved.
- Always add: Temperature, pH- (for measurements in water), concentration, referencing mode....