APPLICATIONS OF TRITIUM AND TRITIUM NMR SPECTROSCOPY

A Thesis presented to the University of Surrey for the degree of Doctor of Philosophy in the Faculty of Science.

by

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ABSTRACT

Tritium labelled compounds are widely used in the physical and life sciences. The preparation of such compounds is a very important area of chemistry. Although the methods are, on the whole, fairly simple and straightforward, there is still a need for improvement. The same applies as far as the analysis of the tritium distribution is concerned. High resolution $^3$H NMR spectroscopy of solutions is now a well established technique. However many compounds exist as solids which are either insoluble or sparingly soluble in organic solvents, consequently there is a need to develop high resolution $^3$H NMR spectroscopy of solids.

The thesis therefore consists of four chapters. The first is a review dealing with the properties of tritium, the various labelling methods and tritium nuclear magnetic resonance spectroscopy.

In the second chapter an account is given of the way in which complex metal tritides and tritiated methyl iodide, an important reagent which enables one to introduce three tritium atoms in a single step and therefore obtain products of very high specific activity, are prepared.

In the third chapter an account is given of the synthesis and attempted tritiation of two potential 5-HT re-uptake inhibitors, prior to their use in biological studies. Whilst the synthesis of the two compounds $N$-[[1-[(1-bromo-2-naphthalenyl)methyl]-4-piperidinyl]amino]carbonyl]-3-pyridinecarboxamide, and $N$-[[1-[(1-bromo-6-fluoro-2-naphthalenyl)methyl]-4-piperidinyl]amino]carbonyl]-3-pyridinecarboxamide, was successful the tritiation procedure led to unforeseen difficulties.

Finally in the fourth chapter solid state tritium NMR spectroscopy, using a magic angle accessory, was developed and used in the analysis of several tritiated compounds.
ACKNOWLEDGEMENTS

During the course of the research important contributions have been made by a number of individuals. I would like to take this opportunity to acknowledge their efforts.

It is with considerable pleasure that I acknowledge the help, advice and constant support given to me by Professor John Jones, Professor Victor Pike, Dr Maurice Coombs and Dr Roger Bolton throughout this project. I would also like to extend my appreciation and thanks to Mr Jim Bloxsidge for his many helpful discussions and support on all aspects of NMR spectroscopy, and to Mrs Leonor Carroll for her practical assistance, support and friendship in the Radiochemistry laboratory.

In addition, thanks go to Mike, Steve, Ronnie, Gareth, Richard, Jean-Michel, Lotte, Mark and Steve for their sense of humour and constant support during the difficult times. On a more personal level, I am deeply grateful to my parents and Dr Ghislaine Dell for their love and encouragement throughout.
## ABBREVIATIONS

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<tr>
<td>$^2$H (D)</td>
<td>Deuterium</td>
</tr>
<tr>
<td>$^3$H (T)</td>
<td>Tritium</td>
</tr>
<tr>
<td>5-HT</td>
<td>5-hydroxytryptamine (serotonin)</td>
</tr>
<tr>
<td>acac</td>
<td>acetylacetonate</td>
</tr>
<tr>
<td>AMS</td>
<td>Accelerator mass Spectrometry</td>
</tr>
<tr>
<td>Bq</td>
<td>Becquerel</td>
</tr>
<tr>
<td>Ci</td>
<td>Curie</td>
</tr>
<tr>
<td>CNS</td>
<td>Central nervous system</td>
</tr>
<tr>
<td>CSA</td>
<td>Chemical shift anisotropy</td>
</tr>
<tr>
<td>d</td>
<td>Day</td>
</tr>
<tr>
<td>DMF</td>
<td>$N,N$-dimethylformamide</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethylsulphoxide</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>$E_{\text{ave}}$</td>
<td>Average energy</td>
</tr>
<tr>
<td>EC</td>
<td>Electron capture</td>
</tr>
<tr>
<td>$E_{\text{max}}$</td>
<td>Maximum energy</td>
</tr>
<tr>
<td>eV</td>
<td>Electron volt</td>
</tr>
<tr>
<td>FID</td>
<td>Free induction decay</td>
</tr>
<tr>
<td>G-Protein</td>
<td>Guanine nucleotide binding protein</td>
</tr>
<tr>
<td>GTP</td>
<td>Guanosine triphosphate</td>
</tr>
<tr>
<td>$h$</td>
<td>Planck’s constant</td>
</tr>
<tr>
<td>HPLC</td>
<td>High pressure liquid chromatography</td>
</tr>
<tr>
<td>HTO</td>
<td>Tritiated Water</td>
</tr>
<tr>
<td>Hz</td>
<td>Hertz</td>
</tr>
<tr>
<td>LD$_{50}$</td>
<td>Median lethal dose</td>
</tr>
<tr>
<td>MAS</td>
<td>Magic angle spinning</td>
</tr>
<tr>
<td>Min</td>
<td>Minute</td>
</tr>
<tr>
<td>NADP</td>
<td>Nicotinamide-adenine dinucleotide phosphate</td>
</tr>
<tr>
<td>NBS</td>
<td>$N$-bromosuccinimide</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
</tr>
<tr>
<td>NUP</td>
<td>4-nicotinoylureidopiperidine</td>
</tr>
<tr>
<td>p.s.i.</td>
<td>Pounds per square inch</td>
</tr>
<tr>
<td>pCa</td>
<td>p-chloroamphetamine</td>
</tr>
<tr>
<td>PET</td>
<td>Positron emission tomography</td>
</tr>
<tr>
<td>Ph</td>
<td>Phenyl</td>
</tr>
<tr>
<td>ppm</td>
<td>Parts per million</td>
</tr>
<tr>
<td>rpm</td>
<td>Revolutions per minute</td>
</tr>
<tr>
<td>STP</td>
<td>Standard temperature and pressure</td>
</tr>
<tr>
<td>TMEDA</td>
<td>$N,N,N',N'$-tetramethylethylenediamine</td>
</tr>
<tr>
<td>wt</td>
<td>Weight</td>
</tr>
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<td>y</td>
<td>Year</td>
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1.1 INTRODUCTION

Since their discovery in the period 1927 - 1932, the stable isotopes of hydrogen ($^2$H), carbon ($^{13}$C), nitrogen ($^{15}$N), oxygen ($^{17}$O and $^{18}$O), and sulphur ($^{32}$S and $^{34}$S) have rapidly assumed an important role in chemical and biochemical studies, especially for nitrogen and oxygen where there are no complementary radioisotopes with convenient long half-lives. The incorporation of stable and radioactive isotopes into biologically active molecules has now become a standard strategy for the investigation of biochemical mechanisms in vitro and in vivo. This complementary use of stable and radio-isotopes has been shown to be an increasingly potent research tool throughout the life sciences, particularly with the current advances in detection and analytical techniques.

Radioisotopes have found more widespread uses than their stable counterparts, partly due to their increased availability, and partly due to the development of liquid scintillation counting which now allows the use of tritium ($^3$H) and $^{14}$C at tracer levels. Consequently, this allows radioisotope incorporation to be much lower than of those of their stable counterparts. Therefore, radiolabelling has now become the preferred method for studying many research problems, in particular the metabolic fate of compounds that are of interest to the pharmaceutical industry.

Methods for the detection and quantitation of radioisotopes have changed little over the past 30 years; the common method in use today is still decay counting using liquid scintillation counting or autoradiography. Recent developments in the area of accelerator mass spectrometry (AMS) are expected to change this. Unlike liquid scintillation and autoradiography, AMS measures radioisotopes not through their radioactive decay, but by counting the number of radioactive atoms in a sample. Preliminary studies are such that the instrument is able to measure small numbers of
radioisotope atoms in a background of non-radioactive atoms. For example $^{14}$C can be detected in attomole ($10^{-18}$) to zeptomole ($10^{-21}$) concentrations in a background of naturally occurring $^{12}$C. In addition to radiocarbon, other isotopes that can be measured by AMS include $^3$H, and $^{129}$I. In effect AMS can be likened to a highly sensitive particle counter and can be used for the same purposes as liquid scintillation counting but with a million-fold higher sensitivity. However, this new technique should not be allowed to detract from the valuable contribution made by other analytical techniques including radio-HPLC, mass spectrometry and NMR spectroscopy, which are invaluable in determining the purity and identification of radiolabelled organic compounds.

1.2 RADIOISOTOPES

Virtually all radioisotopes used nowadays are produced artificially. This means nuclear reactions have to be used to turn stable isotopes into radioactive ones. In the biological sciences the most commonly used radioactive isotopes emit $\beta^-$, or $\beta^-$ plus $\gamma$, radiation. This is because these radioisotopes are the easiest to detect. All $\beta^-$-decay processes result in a change in the element accompanied by a negligible change in mass. This transformation is due to the ‘weak’ nuclear forces in the nucleus, which permit neutrons to change into protons and vice versa.
1.2.1 Negatron (β') Emitters

In these neutron-excess radioisotopes, the decay transformation takes the form of β⁻ or negatron emission and can be represented by:

\[ {}^{14}_{6}\text{C} \rightarrow {}^{14}_{7}\text{N} + \beta^- + \bar{\nu} + \text{Energy} \ (E_{\beta\text{max}}) \]

i.e.

\[ \text{n} \rightarrow \text{p}^+ + \beta^- + \bar{\nu} + \text{Energy} \ (E_{\beta\text{max}}) \]

The β⁻-particle and the anti-neutrino (\( \bar{\nu} \)) are formed at the moment of radioactive decay and are thrown out of the nucleus with associated kinetic energy. The β⁻-particle has the same properties as an electron travelling at speed and is easily detected. On the other hand, the anti-neutrino carries no charge and virtually no rest mass; its detection is therefore very difficult. More importantly, the anti-neutrino carries a fraction of the radioactive decay energy. β⁻-Particles are therefore emitted with a whole range of energies up to a maximum value (\( E_{\beta\text{max}} \)), corresponding to the accompanying emission of an anti-neutrino with no kinetic energy. A typical β⁻-energy spectrum is shown in Figure 1.1, in general the average \( E_\beta \) is approximately one-third of \( E_{\beta\text{max}} \).

![Figure 1.1](image)  

**Figure 1.1** The β⁻ radiation spectrum of tritium.
1.2.2 Positron (\(\beta^+\)) Emitters

In neutron-deficient radioisotopes the reverse of the \(\beta^-\) process occurs, but only if the nuclear instability has sufficient energy available. The process itself is known as positron emission (\(\beta^+\)) and can be represented by:

\[
\begin{align*}
{}^7_6\text{C} & \rightarrow {}^7_7\text{B} + \beta^+ + \bar{\nu} + \text{Energy}(E_{\beta_{\text{max}}}) \\
\text{i.e.} & \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ p^+ & \rightarrow n + \beta^+ + \bar{\nu} + \text{Energy}(E_{\beta_{\text{max}}})
\end{align*}
\]

The emission of a positron from a neutron-deficient nucleus effectively creates a neutron from a proton. The ejected positron has kinetic energy that like its negatron counterpart is on average one third its maximum value and this time the remaining energy is taken by a neutrino. This kinetic energy propels the positron some distance, the exact distance depending on the density of the local matter. When the positron is almost at rest it annihilates with an electron and sends out two penetrating \(\gamma\)-rays of equal and specific energy (511 keV - equivalent to the rest mass of an electron), in almost exactly opposite directions (so as to conserve near zero momentum), as shown in Figure 1.2.

![Figure 1.2: Positron annihilation.](image)

It is this special feature of positron-electron annihilation, the simultaneous emission of two 511 keV \(\gamma\)-rays in opposite directions that endows the technique of Positron Emission Tomography (PET) with quantitative accuracy and spatial resolution.\(^2\)\(^{,}\)\(^11\)
However, when there is not enough energy from the nuclear instability, relief from neutron deficiency is sought by drawing an electron into the nucleus. This process is known as electron capture (EC). The relative proportions of decay by positron emission or electron capture are fixed for any neutron deficient radioisotope. EC is of no value to PET because no pair of $\gamma$-rays are generated for detection. Indeed, electron capture is a disadvantage since it contributes to radiation dose, but not to acquired data.

### 1.2.3 Commonly Used Radioisotopes

Radioisotopes are used in all disciplines of the biological sciences because of the high sensitivity they give to experiments. The most important radioisotopes used are the low energy $\beta^-$ emitters as shown in Table 1.1. Carbon-14 and tritium ($^3$H) are the most commonly used radioisotopes due to the high carbon and hydrogen composition of most organic molecules, whilst, phosphorus-32 and 33 see uses with nucleotides and phospholipids, and iodine-131 in protein studies. Furthermore, with the improvements in medical imaging made over the last decade, in particular the gamma detectors used in sophisticated cameras for positron emission tomography (PET), the discipline of nuclear medicine has seen rapid growth, especially in areas involving the study of physiological, biochemical, and pharmacological functions at the molecular level in living organisms (including man), whether in health or in disease. Although the number of positron-emitting isotopes is large, it is the ‘organic’ radioisotopes, carbon-11, nitrogen-13, oxygen-15 and fluorine-18 that generally see the most use in PET studies. Table 1.2 shows some of the more medically relevant positron-emitters.
Table 1.1  Common negatron emitters used in the life sciences.\textsuperscript{14}

<table>
<thead>
<tr>
<th>Isotope</th>
<th>Half-Life</th>
<th>Maximum energy of $\beta^-$ particles (MeV)</th>
<th>Maximum specific radioactivity (Ci/milliatom)</th>
<th>Decay product</th>
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<tbody>
<tr>
<td>$^{14}$C</td>
<td>5730 y</td>
<td>0.155</td>
<td>0.062</td>
<td>$^{14}$N</td>
</tr>
<tr>
<td>$^3$H</td>
<td>12.3 y</td>
<td>0.018</td>
<td>29</td>
<td>$^3$He</td>
</tr>
<tr>
<td>$^{35}$S</td>
<td>87.4 d</td>
<td>0.167</td>
<td>14488</td>
<td>$^{35}$Cl</td>
</tr>
<tr>
<td>$^{32}$P</td>
<td>14.3 d</td>
<td>1.71</td>
<td>9120</td>
<td>$^{32}$S</td>
</tr>
<tr>
<td>$^{33}$P</td>
<td>25.2 d</td>
<td>0.25</td>
<td>5200</td>
<td>$^{33}$S</td>
</tr>
<tr>
<td>$^{131}$I</td>
<td>8.04 d</td>
<td>0.61</td>
<td>2600</td>
<td>$^{131}$Xe</td>
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</table>

Table 1.2  Common positron emitters used in the life sciences.\textsuperscript{13}

<table>
<thead>
<tr>
<th>Isotope</th>
<th>Half-Life (Min)</th>
<th>Maximum energy of $\beta^+$ particles (MeV)</th>
<th>$\beta^+$ Emission (%)\textsuperscript{a}</th>
<th>Decay product</th>
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<tr>
<td>$^{11}$C</td>
<td>20.4</td>
<td>0.96</td>
<td>99.8</td>
<td>$^{11}$B</td>
</tr>
<tr>
<td>$^{13}$N</td>
<td>9.96</td>
<td>1.19</td>
<td>100</td>
<td>$^{13}$C</td>
</tr>
<tr>
<td>$^{15}$O</td>
<td>2.03</td>
<td>1.723</td>
<td>99.9</td>
<td>$^{15}$N</td>
</tr>
<tr>
<td>$^{18}$F</td>
<td>109.8</td>
<td>0.635</td>
<td>96.9</td>
<td>$^{18}$O</td>
</tr>
<tr>
<td>$^{76}$Br</td>
<td>966</td>
<td>3.98</td>
<td>57</td>
<td>$^{76}$Se</td>
</tr>
</tbody>
</table>

\textsuperscript{a} = Remainder by electron capture.
1.3 TRITIUM

The element hydrogen has three known isotopes: protium ($^1$H), deuterium ($^2$H, D), and tritium ($^3$H, T); two heavier isotopes have been reported but not substantiated.$^{15, 16}$ Both deuterium and tritium effectively duplicate the highly diversified chemistry of the parent element hydrogen, and not only act as tracers for the element but also as labels for a carbon skeleton. Unlike hydrogen and deuterium, tritium is a radioactive isotope and, as such, decays by the emission of low energy $\beta^-$ radiation as follows:

$$^3\text{H} \rightarrow ^3\text{He}^+ + ^0\beta^-$$

The $\beta^-$ radiation decay spectrum of tritium has already been illustrated in Figure 1.1.

Tritium arises in nature by the action of primary cosmic rays (high-energy protons) or cosmic ray neutrons on a number of elements. Natural tritium was first detected in the atmosphere$^{17}$ and was later shown to be present in rainwater.$^{18}$ Because of its relatively short radioactive half-life, naturally produced tritium does not accumulate indefinitely and as such the amount of tritium found in nature is very small. The principal reactions induced by cosmic radiation in the upper atmosphere, which produce tritium at the rate of approximately $1.3 \times 10^{11}$ atoms per year per square metre of the earth's surface are:$^{14}$

$$^{14}\text{N} + ^1\text{n} \rightarrow ^3\text{T} + ^{12}\text{C}$$

$$^{14}\text{N} + ^1\text{H} \rightarrow ^3\text{T} + \text{fragments}$$

$$^2\text{D} + ^2\text{D} \rightarrow ^3\text{T} + ^1\text{H}$$

Tritium is now commercially produced on a large scale in reactors, where any nuclide can easily be exposed to a high flux of neutrons under controlled conditions. Amongst the various possibilities, it is found that lithium is favoured because of its high
cross section for thermal neutrons. This means that the lithium nucleus splits easily when exposed to neutrons of an energy lower than molecular bond energies yielding tritium and an α-particle (\(^{4}\text{He}\)).

\[
^{6}\text{Li} + ^{1}\text{n} \rightarrow ^{1}\text{H} + ^{4}\text{He}
\]

1.3.1 Properties of Tritium

Tritium’s physical and nuclear properties have been the subject of various reviews\(^{19,20,21}\) and a standard book\(^{22}\) provides a comprehensive survey of the preparation and uses of tritium compounds. Selected properties are given in table 1.3.

**Table 1.3** Some important physical properties of tritium.\(^{22}\)

<table>
<thead>
<tr>
<th>Production</th>
<th>(^{6}\text{Li}(\text{n,}\alpha)^{3}\text{H})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radiation</td>
<td>(\beta^- (100%))</td>
</tr>
<tr>
<td>Half-life</td>
<td>12.3 y</td>
</tr>
<tr>
<td>Decay constant</td>
<td>(1.8 \times 10^{-9}\text{s}^{-1})</td>
</tr>
<tr>
<td>Max (\beta^-) energy ((E_{\beta\text{Max}}))</td>
<td>18.6 keV</td>
</tr>
<tr>
<td>Mean (\beta^-) energy ((E_{\beta\text{Mean}}))</td>
<td>5.7 keV</td>
</tr>
<tr>
<td>Max specific activity (per site)</td>
<td>29.12 Ci/mmol (1.07 TBq/mmol)</td>
</tr>
<tr>
<td>Dissociation energy ((T_2 \rightarrow 2T))</td>
<td>4.59 eV</td>
</tr>
<tr>
<td>Ionisation energy ((T \rightarrow T^+ + e^-))</td>
<td>13.35 eV</td>
</tr>
<tr>
<td>Energy required to break C-T bond</td>
<td>3.858 eV/Molecule</td>
</tr>
<tr>
<td>Energy required to break C-H bond</td>
<td>3.793 eV/Molecule</td>
</tr>
<tr>
<td>Vol. of 1 Ci of (T_2) gas at STP.</td>
<td>0.385 ml</td>
</tr>
</tbody>
</table>
1.3.2 Isotope Effects

Isotope effects can be of two kinds, kinetic where issues of rate come into play and equilibrium, where one is dealing with thermodynamic aspects. Within the first category one can be dealing with (i) primary, (ii) secondary or (iii) solvent effects. When a reaction takes place it proceeds in a number of steps, one of which is the slowest – the rate-determining step. If that involves the cleavage of for example, a C-H bond, the reaction with the corresponding compound involving a C-D bond will be slower. This is what is known as the primary hydrogen isotope effect which frequently is of the order of 3-7 at room temperature. When the isotopic substitution is at a site adjacent to that undergoing reaction a secondary effect, much lower than the primary, is observed. If we change the solvent for example, from H₂O to D₂O a solvent isotope effect is observed.²³ A review on isotope effects in chemical reactions concentrating on hydrogen isotope effects has been published as part of the American Chemical Society’s series of chemical monographs.²⁴

For isotopes other than hydrogen, the isotope effects are much reduced because the mass ratios are closer to 1. For example,¹⁴C is only 17% heavier than¹²C and rate differences of only approximately 10% occur for carbon bond rupture. Mass differences between ³²P and ³¹P and between ³⁵S and ³²S are even smaller.⁹

1.4 BIOLOGICAL HAZARDS OF TRITIUM

Because tritium decays with emission of low-energy radiation (E_{\beta\text{Mean}} = 5.7 keV), it does not constitute an external radiation hazard. However, tritium does present a serious hazard through ingestion and subsequent exposure of vital body tissue to internal
radiation. When exposed to tritiated water vapour via inhalation people absorb 98-99% of the activity taken in through the respiratory system. Distribution throughout the body fluids occurs within 90 minutes. Molecular tritium, T₂ or HT is much less readily assimilated. Approximately 0.004% of such inhaled activity is absorbed, probably after preliminary oxidation in the lungs.

It is generally assumed that ingested tritiated water is rapidly absorbed and uniformly distributed, with the result that the entire organism is uniformly irradiated. Experiments using mice have shown that this may not be the case, as ingestion of low-level tritiated water causes liver damage. Furthermore, the ingestion of tritium used as a tracer in organic compounds that are metabolised in specific pathways can concentrate tritium, resulting in the possibility of localised radiation damage. For example, tritiated thymidine concentrates selectively in the DNA of cells resulting in selective damage to cell nuclei.

The body excretes tritium, usually as HTO, with a biological half-life of 8-14 days (10.5 on average), which can be reduced significantly with forced fluid intake, particularly using diuretics. For humans, the estimated maximum permissible total body burden is 37 MBq (1 mCi), while the median lethal dose (LD₅₀) of tritium assimilated by the body is estimated to be 370 GBq (10 Ci).

The monitoring and control of the uptake of tritium by personnel is most efficiently monitored by urinalysis, normally by liquid scintillation counting, on a routine basis. Surface contamination is normally detected by means of smears, which are simple disks of filter paper wiped over the suspected surfaces and counted again by liquid scintillation.
1.5 TRITIUM LABELLING METHODS

Any compound containing hydrogen should be able to be labelled by one or more of the methods listed below. In practice tritium attached to atoms other than carbon is often labile because of the acidity/basicity of the bond, and consequently most of the preparative methods described are primarily for the labelling of organic molecules, and in particular involve the formation of carbon-tritium bonds. The three general categories for tritium labelling of organic compounds are:-

1. Isotope Exchange Reactions
2. Direct Chemical Synthesis
3. Biochemical Methods

1.5.1 Isotope Exchange Reactions

The preparation of tritium labelled compounds by hydrogen isotope exchange reactions involves the catalytic exchange of a hydrogen atom, from the molecule of interest, for a tritium atom from the tritium source. The source of tritium for these exchange reactions can either be tritium gas or a suitable tritiated solvent, usually tritiated water. Exchange reactions are frequently regarded as the most important general technique for the introduction of tritium into compounds. The method allows the labelling of very complex molecules which otherwise could not be labelled. However, it should be noted that most exchange labelling methods involving tritium normally result in a “generally” labelled compound in which the labelling is seldom specific. The preparation of tritium-labelled compounds by exchange can be classified into the following reaction types:
1.5.1.1 Isotope Exchange with Tritium Gas (Wilzbach Labelling)

Hydrogen isotope exchange of organic molecules using tritium gas was discovered by Wilzbach in 1956, and is consequently called “Wilzbach labelling”.\(^{29,30}\) A book discussing the technique and the preparation of compounds in some detail is available.\(^{31}\) Wilzbach labelling consists simply of exposing the compound to tritium gas at virtually 100% isotopic abundance, for a number of days or weeks. During this time the radiation induces exchange reactions to occur between hydrogen atoms in the compound and the tritium gas. Unfortunately this simple technique, while furthering the use of tritium compounds at the time, soon became less used due to its limitations, especially the problems associated in purifying the compounds in order to obtain a radiochemically pure product, combined with the low molar specific radioactivities usually attainable due to the slow rate of tritium incorporation (1% per day of the total activity present under STP).\(^{32}\)

1.5.1.2 Catalytic Isotope Exchange with Tritium Gas

The use of metal catalysts such as Pd or Pt in an intimate mixture with the desired compound exposed to tritium gas has been shown to increase the specific activities obtained in Wilzbach labelling by up to 2,000 times.\(^{33,34}\) However, this increase varies considerably with the type of compound, and the relative degree of labelling is higher for aromatics than it is for aliphatics. In recent years, with the development of tritium NMR spectroscopy, Williams and co-workers\(^{35,36}\) have analysed the regiospecificity of catalytic isotope exchange reactions with tritium gas in the presence of Pd or Pt on a number of simple aromatics.
The analysis of these exchange labelled organic substrates has led to the following observations. For the exchange of organic compounds with tritium gas over unsupported sodium borohydride-reduced platinum (IV) oxide, the authors state that:\(^{35}\)

- In substituted benzenoid compounds predominantly meta/para exchange takes place.
- In alkyl protons situated in an aromatic framework strong $\alpha$-CH exchange occurs, the extent of exchange decreasing with the remoteness from the aromatic centre.
- In pyridine exchange is predominantly in the 2,6 positions.
- In 5-membered heterocycles $\alpha$-exchange takes place.
- In naphthalene $\beta$-exchange occurs.
- In alkanes exchange is random.

For palladium catalysts, a number of the labelling patterns are unique, and depend on both the particular metal and its physical form. For palladium black-catalysed exchange with tritium gas the following observations have been made:\(^{36}\)

- Alkyl exchange is the major process in the labelling of alkylbenzenes.
- Alkyl groups on aromatic rings and in alkanes are readily and uniformly labelled.
- Aromatic rings are preferentially labelled in the least hindered positions.
- Molecules containing heteroatoms are usually labelled mostly at positions adjacent to the heteroatom.
- Under conditions of high substrate to tritium gas molar ratio (ca 250:1) hydrogenation of aromatic substrates is a minor factor.

Any change in the physical form of the catalyst for Pd or Pt has been shown to have a substantial effect on the exchange pattern observed for various organic substrates.\(^{37, 38}\) The use of platinum-loaded microporous aluminophosphate molecular sieves generally yields higher radiochemical purities and greater aromatic exchange.\(^{39}\)
1.5.1.3 Catalytic Isotope Exchange in Solution with Tritium Gas

Catalysed hydrogen isotope exchange in solution with tritium gas is a method developed by Evans for the isotopic labelling of a wide variety of compounds. The method is based upon the fact that hydrogen atoms in certain positions within a molecule become exchangeable when the compound is stirred in the presence of a metal hydrogen transfer catalyst, usually Pd or Pt although Ir has also been used successfully. The exchange is normally achieved using neutral or basic conditions, although acidic conditions are used for the exchange labelling of benzylic hydrogen atoms. However, under acidic conditions rapid exchange between water and tritium gas also occurs - this exchange is at least 15 times slower under the corresponding alkaline conditions.

All the positions readily labelled are near centres of high electron density, e.g. on a carbon atom, which has a ketonic function, on a carbon atom adjacent to a benzene ring, at the 8-position of purines. It appears then that the compound is bound to the catalyst at this point of high electron density. Since tritium gas is also adsorbed onto the catalyst, exchange can take place, followed by desorption of the tritiated compound and tritium-hydrogen gas. This type of exchange is thought to occur through a free radical mechanism.

The results obtained by this catalysed gas-exchange technique show the products to be frequently of high radiochemical and chemical purity, of high specific activity, and unlike most exchange reactions, specific labelling may also be achieved in many compounds. The literature shows this method to have found applications with a wide variety of organic molecules particularly benzylic compounds, steroids, drugs, purine nucleotides and nucleosides. Recent developments have seen the use of high-temperature
solid-state catalytic isotope exchange [HSCIE] with tritium gas, used to prepare α-amino acids at high specific activity.\textsuperscript{46}

Other recent communications\textsuperscript{47, 48} have described the exchange labelling of a variety of compounds using deuterium or tritium gas, catalysed by several organoiridium complexes. The exchange is often rapid, efficient and highly regioselective, and proceeds under very mild conditions. Regioselectivity of exchange is associated with several functional groups, and the proposed mechanism of exchange, involves initial coordination of the metal to the functional heteroatom followed by oxidative addition to the activated C-H bond. The method is usually selective for aryl C-H bonds, but certain structural features may promote labelling of specific alkyl sites. Modifications of the phosphine ligands on the iridium catalyst do influence the rate and selectivity of exchange, and may even allow control of the regioselectivity of labelling coupled with the correct choice of reaction conditions. For example labelling of type A compounds has been shown to be best achieved using catalysts like [IrH\textsubscript{2}(acetone)\textsubscript{2}(PPh\textsubscript{3})\textsubscript{2}]BF\textsubscript{4}, which contain monodentate phosphine ligands, and type B compounds are labelled using catalysts related to [(cod)Ir(dppe)]BF\textsubscript{4}, whose phosphine ligand is bidentate\textsuperscript{47, 48} as shown in Figure 1.3.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.3.png}
\caption{Organoiridium regioselective exchange of type A and B compounds.}
\label{fig:1.3}
\end{figure}

\textsuperscript{1}Y = N or O; X = C, N, or S, and R, R' = H, C, N, O, and either may be part of a cyclic subsystem, including a fused ring.
1.5.1.4 Catalytic Isotope Exchange with Tritiated Solvents

Isotope exchange reactions using tritiated solvents such as acetic acid and water, are widely used for the introduction of a tritium label into organic compounds, especially into physiologically active compounds. This is due to the complexity of their structures, which often makes it difficult to modify their structure prior to incorporating the label. Furthermore, the main advantage of using a labelled solvent as the isotope source is the elimination of unwanted hydrogenation reactions often observed when using a gaseous tritium source, in the presence of Pd or Pt catalysts, which are also active hydrogenation catalysts. The following isotope exchange reactions using tritiated solvents are the most widely used:

- Heterogeneous isotope exchange, catalysed by VIII group metals (Pt, Pd, Co, Ni, Fe, Ru, Rh, Ir, and Os);
- Homogeneous isotope exchange, catalysed by acids, base, and soluble transition metal salts.

The use of organic solvents, particularly anhydrous aprotic solvents (such as dioxan), afford a considerable reduction in the autoradiolysis of tritiated water, as a result of a decrease in radioactivity per unit volume. Therefore, this makes it possible to use tritiated water with very high molar radioactivity levels.

Heterogeneous metal-catalysed exchange reactions have been employed under a variety of conditions to label a wide range of compounds, including amino acids, steroids, and heterocyclic compounds. In particular, the catalysts have ranged from evaporated metal films to unreduced metal oxides, whilst reaction temperatures have varied from -60°C to 150°C, and reaction times have covered the range from 0.5-200 hours. Of the group VIII transition metals usually used to catalyse these exchange
reactions in supported or unsupported forms, Pt is the most active and as such the most extensively used.\textsuperscript{54}

The following characteristics have been observed for the properties of sodium borohydride-reduced platinum (IV) oxide catalyst using HTO as the isotope source in exchange labelling of some simple organic substrates.\textsuperscript{35}

- Very high chemical and radiochemical purity.
- Predominantly meta/para aromatic exchange in alkylbenzenes and halobenzenes.
- \(\alpha\)-CH exchange in alkylbenzenes, diminishing along the alkyl chain.
- Rapid exchange next to a group capable of binding to the catalyst surface (e.g. ortho-attack in N-methylaniline).

The following reaction intermediates have been proposed in heterogeneous metal catalysed exchange labelling. Where aromatic exchange occurs, the initial step is believed to involve \(\pi\)-complex adsorption, and subsequent exchange may take place through either a dissociative (Figure 1.4A) or an associative (Figure 1.4B) intermediate.\textsuperscript{55}

The distinctive \(\alpha\)-CH exchange observed for alkylbenzenes is thought to occur via a \(\pi\)-allylic mechanism involving the intermediate in Figure 1.4C.\textsuperscript{56} Alkyl exchange occurs by direct dissociation and exchange through a hydrocarbon radical adsorbed on the catalyst surface.\textsuperscript{57} This is proposed to involve rapid interconversion of \(\alpha,\beta\)-intermediates between mono-, di-, and possible tri-adsorbed alkyl species (Figures 1.4D and 1.4E).\textsuperscript{58} A theoretical model also supports the outlined \(\alpha,\beta\)-process rather than \(\alpha,\alpha\)- or \(\alpha,\gamma\)-intermediates.\textsuperscript{59} When ortho-exchange is observed using heterogeneous catalysts, for example in benzaldehyde and aniline, it is thought that initial adsorption onto the catalyst is similar to that in Figure 1.4F, and the ortho-positions are held in close proximity to the catalyst surface where they are readily exchanged.\textsuperscript{33}
Figure 1.4  Possible reaction intermediates in heterogeneous metal catalysed exchange. (A) Dissociative $\pi$-complex intermediate, (B) Associative $\pi$-complex intermediate, (C) $\pi$-Allylic intermediate, (D) $\sigma$-Bonded intermediate for exchange in the alkyl chain, (E) $\alpha,\beta$-Diadsorbed alkyl exchange intermediate, (F) Initial adsorption in exchange of strongly adsorbing molecules.

Recent work has made it clear that the adsorption of a substrate onto a catalyst surface can change the structure of the surface, and that minor differences in regioselectivity of exchange processes with HTO or $T_2$ as the isotope source may be due to these surface modifications.

Homogeneous catalysts, like heterogeneous catalysts, have also been employed to label a wide variety of organic compounds. In fact, under such conditions arene and
benzylic hydrogens are usually more reactive to isotope exchange than alkanes. Benzene, the least reactive arene, is about twice as reactive as the most reactive alkane, cyclohexane. Typical homogeneous metal exchange catalysts include; Na₂PtCl₄, Na₃IrCl₆, RhCl₃, RuCl₃, Rh(acac)₃, and Ru(acac)₃. These catalysts frequently require a slightly higher temperature for exchange than their heterogeneous counterparts, to compensate for their slower exchange rates. Although these catalysts all have a common feature, in that more than one hydrogen atom can be exchanged during a single residence of the substrate on the catalyst, they also show different selectivities towards certain substrates. For example recent studies using rhodium and ruthenium homogeneous catalysts (usually as the trichloride), have shown that they can catalyse the ortho-exchange labelling of aromatic acids, amides, aralkylamines, and arilides, with high regioselectivity. Previously, ortho-labelled compounds had typically been prepared using multistep syntheses such as the reduction of ortho-thalliated acids, or the dehalogenation of ortho-halobenzoic acids. Where reduced regiospecificity is observed in more complex organic substrates using homogeneous rhodium or ruthenium catalysts this is usually due to the following:

- The presence of electron-donating groups such as hydroxyl and amino, which can promote exchange via a competing electrophilic aromatic substitution process.
- The presence of another group which, like the carboxyl group, can involve itself in a complexation-decomplexation sequence and as such directs isotope incorporation to an additional site.
- The presence of activated methylene groups.

It has been hypothesised, by analogy with the proposed mechanism of the ortho-lithiation reaction, that the regioselectivity observed in rhodium and ruthenium homogeneous metal-catalysed exchange labelling for certain aromatic species, such as
carboxylates, arises via an initial coordination of the catalytic metal with the non-bonding electrons of the substrate carboxyl group. Subsequent reaction, leading to exchange labelling at the nearby ortho-position of the substrate, would then be greatly facilitated over exchange at the non-adjacent meta- or para-positions. Therefore it has been suggested that the reaction mechanism proceeds via a cyclometallation reaction involving the formation of a five-membered ring as shown in Figure 1.5.

![Figure 1.5](image.png)

Figure 1.5 Showing the proposed cyclometallation ring for RhCl₃-catalysed ortho labelling.

This would appear likely, since carboxylate complexes of rhodium and ruthenium in various oxidation states are well known and since aryl-metal bonds are implicated in both heterogeneous and homogeneous catalytic exchange labelling of aromatic hydrocarbons. Therefore, if the regioselectivity of these Rh and Ru catalysed reactions do arise via the process shown in Figure 1.5, other metals possessing coordination chemistry resembling that of rhodium and ruthenium might also yield similar specific or selective ortho-exchange labelling. Furthermore, there have also been instances where heterogeneous catalysed exchange has been difficult, but homogeneous exchange occurs readily.

A large number of organic compounds can also be specifically labelled under homogeneous conditions by taking advantage of the weakly acidic character exhibited by some carbon-hydrogen bonds. Therefore, treatment with a sufficiently strong base leads to ionisation, forming an intermediate carbanion, which itself is able to abstract a triton
from an appropriately tritiated solvent (HTO), yielding a radiolabelled product. The extent to which this exchange takes place depends on two factors; (i) the acidity of the carbon acid, and (ii) the basicity of the medium.

Conversely, the treatment of many organic compounds with a strong acid results in protonation. Therefore, on subsequent deprotonation (assuming that the hydrogen isotope lost is not the same as the one introduced), this can provide a means of exchange. A variety of acids have been employed to label a range of essentially electron rich aromatic and heterocyclic compounds.\textsuperscript{22,54}

An alternative to using highly acidic media is to use the reported high temperature-dilute acid (HTDA) exchange method. However, exchange labelling is also possible in a neutral medium for some compounds.\textsuperscript{78,79} Lewis acids of the type SbCl\textsubscript{5}, AlCl\textsubscript{3}, BBr\textsubscript{3}, and EtAlCl\textsubscript{2} have also been used as exchange labelling catalysts\textsuperscript{80,81} with HTO, ethylaluminium dichloride being the most effective. Recent developments have illustrated the advantages of using a polymer-supported acid or base catalyst. These catalysts have been used to tritiate a range of compounds, including compounds containing nitro groups, which poison metal catalysed exchange reactions.\textsuperscript{82,83}

1.5.2 Direct Chemical Synthesis

Where practicable, chemical synthesis is frequently the most reliable way of labelling compounds with tritium, particularly when molecules with site specific labelling and/or high specific molar activities are required. These requirements are often unattainable by using the isotope exchange procedures previously discussed. The syntheses are usually carried out on a micro or semi-micro chemical scale, often with quite large amounts of radioactivity, usually in the form of tritium gas or tritiated water.
In addition to the more specific chemical syntheses discussed in the next chapter, these general reactions can advantageously be used for the preparation of tritium compounds at very high specific activities:

- Catalytic reduction of unsaturated precursors.
- Catalytic halogen-tritium replacement.
- Decarboxylation of acid precursors.

**1.5.2.1 Catalytic Reduction of Unsaturated Precursors**

Hydrogenation of unsaturated compounds by the catalytic addition of tritium gas or tritium-hydrogen mixtures is one of the most convenient and simplest methods for the introduction of tritium into a compound. This is because most carbon-carbon double bonds, whether substituted by electron donating or withdrawing substituents, can be catalytically hydrogenated usually in quantitative or near quantitative yields. Almost all known alkenes add hydrogen, although conditions may vary according to steric hindrance. Among the double bonds most difficult to hydrogenate or which cannot be hydrogenated at all, are those common to two rings such as in some steroids. Many functional groups may be present in the molecule, e.g., OH, COOH, NH\(_2\), CHO, COR, COOR, or CN. Some of these groups are also susceptible to catalytic reduction, but it is usually possible to find conditions under which the carbon-carbon double bonds can be reduced selectively.\(^{84}\) To achieve maximum specific activities it is necessary to block any labile hydrogen positions such as OH, COOH, NH\(_2\), by the preparation of suitable derivatives, e.g., esters, ethers, or acetates, thus preventing the competing exchange reaction.
Catalytic reductions are best performed in dry aprotic solvents, e.g., tetrahydrofuran, dioxan, chloroform, ethyl acetate, benzene, although protic solvents, such as ethanol have been used successfully. The catalysts used can be divided into two broad classes:

- Heterogeneous catalysts - these have been the catalysts traditionally used. Among the most effective are Pd/C (probably the most common), Pd or Pt supported on CaCO₃, BaSO₄, Al₂O₃, along with Rh/Al₂O₃, Adams catalyst (reduced PtO₂), Lindlar catalyst (Pd/CaCO₃ poisoned with Pb(OAc)₃), and Raney nickel.

- Homogeneous catalysts - the most important is chlorotris(triphenylphosphine)rhodium RhCl(Ph₃P)₃ (Wilkinson’s catalyst), which catalyses the hydrogenation of many olefinic compounds without disturbing groups such as COOR, NO₂, CN, or COR present in the same molecule. Among other homogeneous catalysts are chlorotris(triphenylphosphine)hydridoruthenium(II) (Ph₃P)₃RuClH, which is specific for terminal double bonds (other double bonds are hydrogenated slowly or not at all), and pentacyanocobaltate(II) Co(CN)₅³⁻, which is effective for double and triple bonds only when they are part of conjugated systems (the conjugation may be with C=C, C=O, or an aromatic ring).

The mechanism of the heterogeneous catalytic hydrogenation of double bonds is not thoroughly understood, although it is believed to involve associative adsorption of hydrogen isotope gas and the π-bond electron density of the unsaturated substrate onto the catalyst surface at the interface.

Although heterogeneous catalysts are usually easier to separate from the reaction mixture, they may also give unwanted labelling due to exchange or double bond migration, in which the two carbon atoms bound to the catalyst may not be those of the original double bond - the initial site of substrate attachment. Therefore, subsequent
elimination of the reduced product can reveal isotope additions at all carbons along the alkyl chain. Non-specific exchange on alkyl chains can occur, and this seems to be dependent on the type of metal and experimental conditions employed. In the hydrogenation of alkenes platinum catalysts lead to very little double bond migration. In fact this isomerisation is favoured by low hydrogen isotope gas pressure, poor reaction mixture agitation, high catalyst-to-substrate ratios, and high catalyst loadings especially of those metals deemed to have a greater catalytic activity.

With most catalysts such as Pd/C, triple bonds are reduced more easily than a similar double bond. However, it is possible to just reduce a triple bond to a double bond (usually with stereoselective syn addition), or to reduce a triple bond without affecting a double bond present in the same molecule. A suitable catalyst for this purpose is the Lindlar catalyst (Pd-CaCO₃-PbO), as illustrated below in Figure 1.6 for the partial reduction of stearolic acid to give [9, 10-T]-oleic acid.

\[
\text{CH}_3\text{(CH}_2\text{)}_7\text{C}═\text{C}═\text{(CH}_2\text{)}_7\text{COOH} \xrightarrow{\text{Lindlar Catalyst}} \text{CH}_3\text{(CH}_2\text{)}_7\text{C}═\text{C}═\text{(CH}_2\text{)}_7\text{COOH}^\text{T}\text{T}
\]

Figure 1.6 The partial reduction of stearolic acid to [9, 10-T]-oleic acid.

Homogeneous catalytic hydrogenation and its heterogeneous counterpart have quite different mechanisms. For example, the mechanism of RhCl(Ph₃P)₃ catalytic reduction involves the reaction of the catalyst with the hydrogen isotope gas to form a metal hydride ((PPh₃)₂RhH₂Cl), which rapidly transfers two hydrogen isotope atoms to the alkene. The starting point of this catalytic cycle is with the complex I or its chlorine bridged dimer (which has identical catalytic properties as the monomer). Displacement of a triphenylphosphine ligand from complex I by a poorly coordinating solvent molecule to give complex II occurs via an associative pathway. Oxidative addition of the
hydrogen isotope gas yields complex III, in which the hydride ligands are mutually cis. Insertion of the alkene substrate displaces the labilised solvent ligand producing the π-alkene complex IV. This is followed by the syn addition of the two hydrogen isotopes to the double bond via complex V, to yield the alkane and complex II. There is evidence that this addition is not concerted but takes place in a stepwise fashion. This catalytic cycle is outlined in Figure 1.7.

![Catalytic Cycle Diagram](image)

**Figure 1.7** Proposed catalytic cycle for Wilkinson’s catalyst.

P = PPh₃ ligand, S = solvent molecule, and R, R', R'', R''' = any alkyl substituent.

Wilkinson’s catalyst has been shown by the use of a mixture of H₂ and D₂ gas to produce only di-deuterated and non-deuterated compounds. No mono-deuterated products are found, indicating that (unlike the case of heterogeneous catalysis) H₂ or D₂ has been added to one olefin molecule and that no exchange takes place. Homogeneous catalysts often have the advantage of better reproducibility and better selectivity. They are also less susceptible to catalyst poisoning. Optically active homogeneous (as well as heterogeneous) catalysts have been used to achieve partially asymmetric (enantioselective) hydrogenations. In recent years these have been
developed to such a point that optical purities greater than 90% have been achieved.\textsuperscript{110,111} An example is the reduction of 3-phenyl-2-benzoylaminoacrylic acid (1) \([R^1, R^2 = \text{Ph}, R^3 = \text{H}]\) in the presence of Rh\textsuperscript{III}/CHIRAPOS, while the reduction of 3-aryl-2-acetylaminoacrylic acids (1) \([R^1 = \text{Ph or Nap}, R^2 = \text{Ac}, R^3 = \text{H}]\) has been achieved using either Rh\textsuperscript{III}/(R)-PROPHOS or (S,S)-BPPM as chiral catalysts as shown in Figure 1.8.\textsuperscript{112}

\[
\begin{align*}
\text{H} & \quad \text{COOR}^3 \\
\text{NHCOR}^2 \\
R^1: \text{Ph, Nap} \\
R^2: \text{Me, Ph} \\
R^3: \text{H, Me} \\
\text{L} = \\
\end{align*}
\]

![Diagram](image)

**Figure 1.8** Enantioselective reduction of aminoacrylic acids by chiral rhodium complexes.

Most catalytic reductions of double or triple bonds whether heterogeneous or homogeneous have been shown to be syn, with the hydrogen isotope entering from the less hindered side of the molecule.\textsuperscript{113} The results from stereospecificity investigations show that this addition is usually 80 to 100% syn, though some of the anti addition product is normally also found, and in rare cases predominates. Catalytic hydrogenation of alkynes nearly always is stereoselective, giving the cis olefin (usually at least 80%), even when it is thermodynamically less stable. Thus this is a useful method for the preparation of cis olefins,\textsuperscript{114} although if steric hindrance is too great, the trans olefin may be formed.
1.5.2.2 Catalytic Halogen-Tritium Replacement

Catalytic dehalogenations with tritium are another widely used route for the preparation of specifically labelled molecules, especially for aromatic compounds. In this reaction both polar and non-polar solvents may be used, but it is preferable again to use solvents with no labile hydrogens in order to maintain high specific activities. It can be seen from the equation below that only half the tritium is incorporated into the desired compound, with the remaining half lost as the tritiated halide. It is necessary to neutralise this tritium halide by the use of a base - triethylamine is usually employed, although other amines or hydroxides may be used.

\[
RX + T_2 \rightarrow TX + RT
\]

\[
TX + OH^- \rightarrow THO + X^-
\]

Failure to neutralise this halide results in poisoning of the catalyst coupled with a dramatic drop in the reaction rate. A high degree of specific labelling is normally obtained if the rate of halogen - tritium replacement is fast; vigorous agitation of the reaction solution, correct choice of catalyst, and halogen to be replaced normally achieve this.

As a general rule for halogenated aromatics iodine is replaced much faster than bromine which again is replaced faster than chlorine (I > Br >> Cl), with fluorine seldom undergoing catalytic replacement with tritium gas. Although supported palladium catalysts are the preferred choice (particularly Pd/C) for catalytic halogen-tritium replacement, platinum and Raney nickel have also been employed. In the case of slow reactions, tritiated water may build up in the reaction solution. This tritiated water is normally at very high specific activity, and may undergo catalysed isotope exchange with the substrate in the presence of the metal catalyst. Although this exchange is normally
slow at room temperature, nevertheless some non-specific labelling has been observed with some catalytic halogen-tritium replacements.\textsuperscript{116,117}

1.5.2.3 Decarboxylation of Acid Precursors

The final general direct synthesis procedure involves the intramolecular conversion of labile tritium into non-labile tritium. This is typically achieved by the decarboxylation of compounds labelled with tritium on the labile carboxyl hydrogen. The reaction itself depends on the principle that the tritium atom attached to the carboxyl group is the atom that replaces the carboxyl group when carbon dioxide is eliminated during the decarboxylation process.

\[
\text{RCOOH} + \text{THO} \rightarrow \text{RCOOT} \rightarrow \text{RT} + \text{CO}_2 + \text{H}_2\text{O}
\]

The carboxyl hydrogen atom is usually labelled by warming the compound in tritiated water, followed by removal of the solvent prior to decarboxylation. Tritium gas can also be used, although the compound is usually dissolved or suspended in a non-hydroxylic solvent such as dioxan in the presence of a mild catalyst such as rhodium or alumina to increase the rate of exchange. An example of this decarboxylation labelling procedure is in the preparation of labelled pyrimidines as shown in Figure 1.9.

\[
\text{Y = Halogen, Methyl}
\]

**Figure 1.9** Labelling of pyrimidines via decarboxylation.\textsuperscript{22}
The usual method of decarboxylation of $\alpha,\beta$-unsaturated carboxylic acids (particularly aromatic acids) has been through the use of copper salts under basic conditions at high temperature.\textsuperscript{118, 119, 120} The mechanism of the decarboxylation is believed to involve an arylcopper intermediate\textsuperscript{118, 121, 122} (as shown in Figure 1.10) followed by protonation.

\begin{figure}[h]
\centering
\includegraphics[width=0.8\textwidth]{aromatic_decarb.png}
\caption{Proposed arylcopper intermediate.\textsuperscript{118}}
\end{figure}

Recent work involving microwave assisted deuterium labelled decarboxylation experiments by Frederikson,\textsuperscript{123} has shown that good yields of labelled decarboxylated product are obtained in short reaction times (approximately 15 minutes). However, because none of the reactions led to 100% incorporation of label, it has been proposed that there is a competing protonation source, probably from water in the catalyst.

1.5.3 Microwave Labelling

The use of microwave heating of small scale organic reaction mixtures is a technique that has only been available over the last 10 years, mainly due to the increased availability of good commercial laboratory microwave systems. It has already been shown that microwave heating can produce large reaction rate increases while decreasing reaction times on reactions such as esterifications, oxidations, nucleophilic displacements, Diels-Alder reactions, Claisen rearrangements and ene reactions.\textsuperscript{124} Some of these "microwave effect" improvements have been suggested to be due to the superheating of
organic solvents, for example acetonitrile (boiling point 82°C) can reach 120°C in a microwave reactor without boiling, or the microwaves may heat any metal catalyst exclusively to facilitate the reaction.

In the area of radiopharmaceuticals microwave techniques are attracting a great deal of interest, particularly in the area of PET where a number of radiolabelling procedures using microwaves have been reported.\textsuperscript{125, 126, 127} The use of microwaves has also been shown to improve yields while reducing reaction times for many alkylations using \textsuperscript{[11]}Calkyl halides.\textsuperscript{128} For catalysed H/D exchange, the use of microwaves has been shown to accelerate the rate of exchange,\textsuperscript{63, 123, 129} and in some cases it actually changes the degree of incorporation observed at particular sites, compared to the conventional thermally induced catalytic hydrogen isotope exchange procedure.

1.5.4 Biosynthetic Methods

Microorganisms have long been shown to be particularly suitable in work with deuterium and can serve similarly for labelling with tritium, as long as the radiation damage is kept low. Biosynthesis of tritiated compounds often has the advantage of stereospecific labelling although the yield is very small and the specific activity is low, in contrast to the chemical techniques previously discussed. Nevertheless, a variety of tritiated biological molecules including carbohydrates, amino acids, and hormones,\textsuperscript{22} have been prepared biosynthetically using tritium labelled precursors of simpler molecules (including tritiated water), and also from more complex intermediates prepared by chemical synthesis. Biosynthesis may utilise plants, animals, unicellular organisms, and bacteria, or isolated parts of them, as well as purified enzyme preparations.
1.6 TRITIUM NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

Since the first report of tritium nuclear magnetic resonance (NMR) spectroscopy by Tiers et al.\textsuperscript{130} in 1964, the technique has been developed by the pioneering work of Elvidge and Jones,\textsuperscript{131} into a powerful and essential tool in the analysis of tritiated compounds. As such, tritium NMR spectroscopy is now routinely used as a non-destructive method of confirming the labelling pattern ratios in tritiated compounds, and has also been of use in interpreting \textsuperscript{1}H NMR spectra.\textsuperscript{132} The \textsuperscript{3}H NMR signal integrals are also in general proportional to the number of contributing nuclei, so the technique is suited for the quantification of the tritium label distribution in any one compound.

The tritium nucleus (the triton), has a spin quantum number \( I = \frac{1}{2} \), identical to that of the proton. Therefore, like the proton it is a suitable nucleus for high resolution magnetic resonance spectroscopy. Some selected nuclear properties of commonly used isotopes, including tritium are given in Table 1.4. Tritium NMR spectroscopy is also facilitated by other properties of the triton, including its high nuclear magnetic moment (\( \mu_T \)) which causes the magnetogyric constant (\( \gamma_T = \mu_T/1h = 4.5414 \times 10^7 \text{ Hz T}^{-1} \)) to be higher than for any other nucleus in the periodic table. Thus at 2.114 T, at which field the \textsuperscript{1}H-NMR frequency is 90 MHz, the \textsuperscript{3}H-NMR frequency is 96 MHz.\textsuperscript{133} This higher NMR frequency results in slightly better spectral dispersion in \textsuperscript{3}H spectra compared with \textsuperscript{1}H spectra at the same field. A further consequence of the uniquely high magnetogyric constant for the triton is that it gives the tritium nucleus the highest sensitivity to NMR detection of all nuclei. This high receptivity coupled with effectively zero natural background abundance of tritium, results in detection of the isotope at very low levels. For example, a sample containing 0.5 mCi (18.5 MBq) at a single site in 100 µl of solvent will give a satisfactory signal to noise ratio after 60 minutes accumulation at 320 MHz,
Table 1.4  Nuclear properties of some commonly used isotopes.$^{131}$

<table>
<thead>
<tr>
<th>Isotope</th>
<th>Natural abundance (%)</th>
<th>Nuclear spin (I)</th>
<th>Magnetic moment (μN)</th>
<th>Resonance frequency (MHz at 2.114 T)</th>
<th>Magnetogyric ratio (γ/10^3 radians T⁻¹ s⁻¹)</th>
<th>Relative sensitivity of nuclei at constant field</th>
<th>Stability</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^3$H</td>
<td>99.58</td>
<td>$\frac{1}{2}$</td>
<td>0.0156</td>
<td>4.8371</td>
<td>26.7519</td>
<td>90.0</td>
<td>Stable</td>
</tr>
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<td>$^3$H</td>
<td>0.42</td>
<td>$\frac{1}{2}$</td>
<td>0.0156</td>
<td>4.8371</td>
<td>26.7519</td>
<td>90.0</td>
<td>Stable</td>
</tr>
<tr>
<td>$^3$C</td>
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<td>0</td>
<td>1.2125</td>
<td>2.2536</td>
<td>28.5336</td>
<td>13.8</td>
<td>Stable</td>
</tr>
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<td>$^3$C</td>
<td>0.11</td>
<td>0</td>
<td>1.2125</td>
<td>2.2536</td>
<td>28.5336</td>
<td>13.8</td>
<td>Stable</td>
</tr>
<tr>
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<td>$\frac{1}{2}$</td>
<td>0.0706</td>
<td>1.9324</td>
<td>2.0110</td>
<td>1.01 x 10^3</td>
<td>Stable</td>
</tr>
<tr>
<td>$^4$C</td>
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<td>$\frac{1}{2}$</td>
<td>0.0706</td>
<td>1.9324</td>
<td>2.0110</td>
<td>1.01 x 10^3</td>
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</tr>
<tr>
<td>$^1$N</td>
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<td>-0.4902</td>
<td>-2.7107</td>
<td>-2.2398</td>
<td>1.04 x 10^3</td>
<td>Stable</td>
</tr>
<tr>
<td>$^1$N</td>
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<td>0</td>
<td>-0.4902</td>
<td>-2.7107</td>
<td>-2.2398</td>
<td>1.04 x 10^3</td>
<td>Stable</td>
</tr>
<tr>
<td>$^15$O</td>
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<td>$\frac{1}{2}$</td>
<td>-0.22388</td>
<td>-3.6266</td>
<td>-3.6266</td>
<td>-1.04 x 10^3</td>
<td>Stable</td>
</tr>
<tr>
<td>$^15$O</td>
<td>0.2</td>
<td>0</td>
<td>-0.22388</td>
<td>-3.6266</td>
<td>-3.6266</td>
<td>-1.04 x 10^3</td>
<td>Stable</td>
</tr>
<tr>
<td>$^16$O</td>
<td>0.2</td>
<td>$\frac{1}{2}$</td>
<td>0.2</td>
<td>1.9581</td>
<td>10.8290</td>
<td>0.55 x 10^3</td>
<td>Stable</td>
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<tr>
<td>$^16$O</td>
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<td>$\frac{1}{2}$</td>
<td>0.2</td>
<td>1.9581</td>
<td>10.8290</td>
<td>0.55 x 10^3</td>
<td>Stable</td>
</tr>
<tr>
<td>$^31$P</td>
<td>1.0</td>
<td>$\frac{1}{2}$</td>
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<td>-1.2984</td>
<td>-1.2984</td>
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<tr>
<td>$^31$P</td>
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<td>-1.2984</td>
<td>-1.2984</td>
<td>2.46 x 10^4</td>
<td>Stable</td>
</tr>
</tbody>
</table>
while 27 µCi (1 MBq) per site is detectable after overnight (16 hours) accumulation at 320 MHz. With the continuing advancements in NMR spectroscopy design it means that the required activities for good signal to noise ratios are decreasing further with 1 µCi (37 kBq) per site in 100 µl of solvent now the possible detection limit for 16 hours accumulation using a modern Bruker AMX750 MHz NMR machine.\textsuperscript{134,135}

The chemical shifts of tritium are virtually the same as the \textsuperscript{1}H chemical shifts (and \textsuperscript{2}H chemical shifts).\textsuperscript{136} This follows from the facts that shielding of a hydrogen nucleus in a compound in solution is very largely a function of the local molecular environment, and that replacement of the hydrogen by an isotope will not materially affect that local environment. Experimental verification has been achieved from measuring the Larmor ratio, first using a variety of partially monotritiated compounds at constant field,\textsuperscript{136} and secondly using monotritiated tetramethylsilane (TMS),\textsuperscript{137} to give a Larmor ratio of $1.066639738 \pm 2 \times 10^{-9}$. These precise measurements show that there is a very small dependence of hydrogen nuclear shielding upon bond order but confirm that triton and proton chemical shifts are the same to within ±0.02 ppm.\textsuperscript{137} This is then the magnitude of the primary tritium isotope effect, not much different from routine NMR errors. The upfield shift in a tritium resulting from the $+I$ effect of another tritium, as in CHT\textsubscript{2} or CT\textsubscript{3} methyl groups, is approximately -0.02 ppm; the secondary isotope effect is -0.01 ppm for an additional vicinal triton.\textsuperscript{137} These shifts are large enough to be resolved at 320 MHz and enable multiple labelling to be identified.

The signals in a \textsuperscript{3}H-NMR spectrum will, as in a \textsuperscript{1}H-NMR spectrum, show the expected coupling to adjacent protons. This can be very useful in revealing the stereochemistry of the labelled site. However, the distribution of signal intensity amongst the lines of multiplets will necessitate inconveniently long acquisition times to build up an adequate signal to noise ratio. Therefore, tritium NMR spectra are acquired as proton
decoupled, so that only single lines are recorded, one per chemically distinct site. Furthermore, using samples of tritiated water, it has been shown that the integrated $^3$H-NMR signal intensity corresponds directly with the radioactivity determined by counting. Hence, tritium analyses derived from undecoupled $^3$H-NMR spectra are accurate to within normal NMR limits, while $^3$H-NMR signal intensities measured with simultaneous $^1$H decoupling, may be subject to errors arising from differential nuclear Overhauser effects, but these are small and can usually be ignored. 

Tritium NMR spectroscopy is undoubtedly the best method available for the determination and confirmation of the stereochemical position of any $^3$H label in a tritiated molecule. Hence, $^3$H-NMR spectroscopy has found one of its most important uses in tracer applications of tritium compounds, where a knowledge of the precise position and configuration of the label is essential. Increases in the sensitivity of the technique has allowed its use in addressing the kinetics, mechanisms, and stereochemistry of small molecules and reactions, while it is also finding increasing use in biochemical and biological studies.

With the exception of confirming the labelling patterns of tritiated molecules, the applications of tritium NMR spectroscopy have covered a wide experimental area, e.g. the study of biochemical transformation of steroids, the conformational analysis and proton-exchange kinetics. The technique has also been used in the probing and determination of protein structure and dynamics using both one and two dimensional proton-tritium nuclear Overhauser experiments. In recent years $^3$H-NMR spectroscopy has been used to study the binding of protein-substrate interactions. Examples include the study of the binding of tritium labelled maltose to its transport protein from E. coli, maltose binding protein. Further work in this area later confirmed the existence of two bound $\beta$-maltotriose resonances in rapid exchange, which were
assigned as two distinct sugar-protein complexes. Work with tritium labelled folic acid, methotrexate, and their corresponding complexes with *Lactobacillus casei* dihydrofolate reductase (DHFR), confirmed the presence of 3 different pH dependant conformational forms of the complex DHFR•NADP•folate. In contrast the methotrexate complex only exists in a single conformational state.
Chapter 2

Complex Metal Tritides
and Tritiated Methyl Iodide

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2.1 METAL TRITIDES - AN INTRODUCTION

One of the main methods for the introduction of tritium into organic molecules besides reduction of unsaturated precursors, decarboxylation of acids, catalytic halogen-tritium replacement and exchange reactions, is the reduction of polar double bonds with complex tritides. Of these, the most widely used are sodium or lithium borotritide and lithium aluminium tritide although, more specialised complex tritides are generally employed when chemoselectivity is required. These hydrides and complex hydrides of aluminium and boron reduce almost all functionalities such as aldehydes, ketones, esters, carboxylic acids, and nitriles. However, isolated carbon-carbon double bonds are reduced only exceptionally with lithium aluminium hydride activated by chlorides of iron, cobalt and nickel.\textsuperscript{158}

Although the synthesis and applications of lithium aluminium hydride and sodium borohydride have been known for fifty years,\textsuperscript{159, 160, 161} they have seen little use in the preparation of high specific activity tritiated compounds. In the past, reagents such as lithium or sodium borotritide were only accessible via exchange reactions, with the tritium content up to about 60%. Lithium aluminium tritide was made from lithium tritide (also prepared by an exchange reaction) and aluminium bromide, but this was again only available at low specific activities. Consequently, other complex lithium tritides were hardly used.\textsuperscript{162}

2.2 METAL BOROTRITIDES

Since the discovery of sodium borohydride, many additional hydride reagents and their applications have been developed.\textsuperscript{163} However, alkali metal borohydrides remain
the most widely used reducing agents in organic chemistry. In general, they are mild reagents and highly selective towards the reduction of aldehydes, ketones, acid chlorides and lactones. Labelled borohydrides are used as regioselective hydrogen isotope labelling reagents, because the labelling is strictly specific, with the label located only on the carbon atom originally forming the unsaturated group.

Sodium borohydride can reduce aldehydes, ketones, primary and secondary alkyl halides, tertiary amines and disulfonamides without affecting carboxylic acid functionalities.\textsuperscript{164, 165} It has a greater resistance to hydrolysis than the more powerful lithium aluminium hydride, and hence this allows reductions to be carried out in aqueous or alcoholic media.\textsuperscript{166} Lithium borohydride is an analogue with slightly greater reducing power and has been employed for the reduction of epoxides, esters and lactones.\textsuperscript{167} Potassium borohydride has milder reducing power than sodium borohydride and although it is less soluble in most organic solvents, it is not as hygroscopic as the other borohydrides and therefore is easier to handle.\textsuperscript{168}

There have been numerous reports on the synthesis of these metal borohydrides using their corresponding hydrides,\textsuperscript{161, 169} or by using methoxyborohydrides.\textsuperscript{170, 171} LiBH\textsubscript{4} and KBH\textsubscript{4} may also be prepared from NaBH\textsubscript{4} by its treatment with LiBr, LiCl, and KOH respectively.\textsuperscript{172, 173, 168} Davis \textit{et al.} prepared near pure NaBD\textsubscript{4} from the reaction of NaB(OCH\textsubscript{3})\textsubscript{4} with B\textsubscript{2}D\textsubscript{6}.\textsuperscript{174} High isotopic purity KBD\textsubscript{4} and NaBD\textsubscript{4} have also been prepared at high yields (up to 80\%) by Atkinson \textit{et al.},\textsuperscript{175} while the most successful LiBD\textsubscript{4} synthesis was based on the reaction between LiD and BF\textsubscript{3}-etherate. Unfortunately, in practice, with the exception of the use of LiT, none of the above procedures are particularly suitable for the preparation of highly tritiated borohydrides.

Consequently, the preparation of metal borotritides has been best left to the exchange method first developed by Brown \textit{et al.} in 1952.\textsuperscript{176} However, recent work by
Than and co-workers,\textsuperscript{177} has demonstrated the production of LiBT\textsubscript{4}, NaBT\textsubscript{4} and KBT\textsubscript{4} with a high tritium content (>70\%T) in a simple and facile synthesis.

The utility of alkali metal borohydrides has been greatly extended beyond simple reduction reactions in recent years. Techniques have been developed to effect the reduction of a wide range of functional groups in a variety of solvents. For example, solutions or suspensions of LiBH\textsubscript{4} or NaBH\textsubscript{4} in methanol or dimethylformamide in the presence of transition metal salts (Ni, Co, Sn, Pd, or La) have been shown to be useful reducing agents which do not perturb aromatic derivatives.\textsuperscript{178,179} NaBH\textsubscript{4} on alumina has been reported to be a very gentle reducing agent\textsuperscript{180} and can be employed in aprotic solvents, while a number of new reducing agents have been synthesised from alkali metal borohydrides for the reduction of specific molecules, each reagent having its own merits and characteristics, e.g. NaBH\textsubscript{3}CN,\textsuperscript{181} NaBH(OAc)\textsubscript{3},\textsuperscript{182} and KBH(\textit{t}-PrO)\textsubscript{3}.\textsuperscript{183} Furthermore tritiated NaBT\textsubscript{4} has also been shown to be of use as a source of tritium gas for small scale labelling without the need for sophisticated apparatus.\textsuperscript{184}

### 2.3 LITHIUM TRITIDES

The need for high specific activity tritium labelled compounds exists in many areas of the life sciences, and metal tritide reducing agents provide an attractive approach for the incorporation of tritium into molecules of biological importance. However, until recently the use of complex tritides has suffered because of the fact that sodium borohydride was the only available reagent available in an almost carrier-free state. However, work by Klusener \textit{et al.}\textsuperscript{185} has described the formation of alkali metal hydrides from their n-butyl compounds and hydrogen under very mild conditions and near ambient
temperature as detailed in Figure 2.1. These hydrides have subsequently been shown to be extremely reactive compared to their commercially available counterparts.\[186\]

\[
\text{n-BuLi } + \text{H}_2 \xrightarrow{\text{TMEDA/Hexane}} \text{LiH} + \text{BuH}
\]

\[
\text{t-BuONa(K) } + \text{n-BuLi } \xrightarrow{\text{TMEDA/Hexane (-30°C)}} \text{n-BuNa(K) } + \text{t-BuOLi}
\]

\[
\text{n-BuNa(K) } + \text{H}_2 \xrightarrow{\text{TMEDA/Hexane (-30°C)}} \text{Na(K)H} + \text{BuH}
\]

**Figure 2.1** The preparation of alkali metal hydrides under mild conditions.

In recent years, the emergence of new tritium labelling tools, in particular complex metal tritide reducing agents has evolved from the development of high specific activity lithium tritide. The complexity of target biological molecules has stimulated the development of these more sophisticated and selective tritiation reagents, and in the process opened up a whole new area for tritium labelling.\[152, \text{187, 188, 189, 190}\] Figure 2.2 shows some of the reducing agents currently synthesisable from lithium tritide.

The use of these reagents include the preparation of tritiated primary amines with tritiated methylborane \((\text{CH}_3\text{BT}_2)\) generated from the reaction of \(\text{AlT}_3\).\[187\] Andres et al.\[187\] have reported the synthesis of \(\text{LiAlT}_4\) and \(\text{LiEt}_3\text{BT}\) at maximum specific activity. Both of these are powerful reducing agents, but \(\text{LiAlT}_4\) in particular lacks selectivity. Because of this lack of selectivity recent work has concentrated on generating milder and more selective reducing agents containing boron, tin, silicon and even zirconium.\[184, 190\]
Figure 2.2 A selection of complex metal tritide reducing agents available from lithium tritide.

Some of these tritides can be employed in the reduction of specific functional groups, for example the use of Li(O-tBu)₃AlH allows the reduction of only keto groups in molecules containing both keto and ester functionalities.¹⁹¹ Site specific labelling is also possible in the reduction of double bonds to generate highly selective labelled products; e.g. the reduction of ethyl p-nitrocinnamate with [³H] lithium selectride¹⁶² yields a product that is tritiated selectively in the β-position as shown in Figure 2.3. This selective labelling is important with regard to the labelling of complex molecules in metabolically stable positions, since it now allows the introduction of tritium into positions remote from

Cp = Cyclopentadienyl
Schwartz' Reagent = Dicyclopentadienyl zirconium chloride hydride
Li₉-BBNT = [³H] lithium 9-boratabicyclo [3.3.1] nonane
the functional groups, especially if the functional group is to be transformed or removed later in the synthesis.

![Chemical structure](image)

**Figure 2.3** Selective labelling of ethyl p-nitrocinnamate.

The Schwartz reagent, because of its tolerance to a wide range of functional groups, opens up access to labelled trans-olefins. To give an example, the reaction of phenylacetylene with [³H]Schwartz reagent, followed by quenching of the intermediate trans-alkenyl zirconium compound with NBS, yields trans-β-bromo-α-tritiostyrene as shown in Figure 2.4.

![Chemical structure](image)

**Figure 2.4** Reduction of phenylacetylene with Schwartz reagent.

### 2.4 METHYLATION - AN INTRODUCTION

Labelled methyl groups have seen use in a variety of studies, to probe physical, biochemical, and physiological processes. In order to obtain this information, the methyl groups are frequently labelled with all of the available isotopes of hydrogen and carbon at one time or another. For example the action of methionine has been followed using $^{14}$C, $^{1}C$, $^{13}$C, $^{19}$H and $^{3}$H. Furthermore, since the discovery of methods to synthesise chiral methyl groups (i.e. containing one atom each of $^1$H, $^2$H and $^3$H), a host of
biochemical transformations have been studied by the incorporation of chirally labelled methyl groups, and the subsequent exploration of the stereochemistry of the reactions. Examples include the study of the stereochemistry of proton elimination in the biosynthesis of tritiated cycloartenol, and the configuration assignment of stereogenic (chiral) methyl groups in compounds containing a CHDT group.

Receptor binding assays of neurotransmitters and their agonists are now made possible by the use of tritiated ligands, some of which contain very highly labelled methyl groups. Therefore, the need for \(^3\)H-labelled compounds specifically labelled in the methyl group as a probe for biological studies is becoming more and more pressing.

According to the literature, a wide variety of organic compounds has been labelled in their methyl groups with tritium using several different procedures. These include catalysed exchange with tritium gas or tritiated water, direct labelling by the Wilzbach method, halogenation followed by tritiodidehalogenation, and catalytic reduction of the formyl group.

The most notable methods that show wider applicability are the numerous processes of methylation employing tritiated methylating reagents, particularly methyl iodide, methyl triflate and methyl nonoflate. Currently these reagents are generated from tritiated methanol, which itself is prepared by the catalytic reduction of carbon monoxide and/or carbon dioxide with tritium gas or tritium-hydrogen mixtures using a Fischer Tropsch type synthesis. A small-scale laboratory production has also been achieved using a “high pressure synthesis apparatus”, reacting tritium gas with carbon dioxide over a copper/zinc/chromium catalyst heated at 225°C at 500 p.s.i. However, since the preparation of tritiated methanol at close to 100% isotopic abundance involves stringent conditions, it is generally only amenable to production in commercial labelling
laboratories. Therefore, in recent years a few research groups have taken up the challenge to prepare high specific methylating agents “in house”.

2.5 TRITIATED METHYLIODIDE - LITERATURE REVIEW

Biologically active molecules frequently contain a methyl group. Therefore, it is highly desirable to have these methyl groups enriched with tritium especially at high specific activity. Methylation using [\(^3\)H]methyl iodide is particularly suited to providing these compounds, as it can often be performed as the last step of a synthetic sequence. A review article has been published for the preparation of tritium labelled methyl iodide.\(^{205}\)

Recent publications by Saljoughian and co-workers have outlined new syntheses of mono-tritiated methyl iodide\(^{206}\) and of high specific activity tritiated methyl iodide.\(^{207}\)

The preparative route for mono-tritiated methyl iodide involves the synthesis of a chloromethyl ester (in particular, chloromethyl benzoate), followed by catalytic tritium-halogen replacement, before this mono-tritiated methyl ester is cleaved with lithium iodide at 180°C to generate mono-tritiated methyl iodide as shown below in Figure 2.5.

\[
\begin{align*}
\text{O} & \quad \text{Cl} & \quad \text{O} & \quad \text{CH}_2\text{Cl} & \quad \text{O} & \quad \text{CH}_2\text{T} \\
\text{ZnCl}_2 & / \text{C}_6\text{H}_6 & \quad (\text{CH}_2\text{O})_n & \quad \text{T}_2 & / \text{Pd-C} & \quad \text{LiI} \quad \text{or LiI} / \text{DMF}
\end{align*}
\]

\[
\text{DMF} \quad \rightarrow \quad \text{CH}_2\text{TI}
\]

**Figure 2.5** Preparation of mono-tritiated methyl iodide.

Lithium iodide has previously been used for the cleavage of methyl esters to the corresponding carboxylic acids, with these earlier studies ignoring this methyl iodide generation.\(^{208, 209}\) This generation of mono-tritiated methyl iodide has been shown to be
an efficient and high yielding route resulting in good specific activities. Apart from being a more amenable route for laboratory production, this method also allows the production of chiral methyl iodide by the substitution of \[^{2}H\]paraformaldehyde (CHDO\)_n for (CH\_2O\)_n to form chloromonodeuteriomethyl p-phenylbenzoate prior to catalytic tritium-halogen replacement and cleavage in the manner illustrated in Figure 2.5.

Although this singly labelled route is important, particularly for the generation of chiral methyl iodide, it is usually desirable to incorporate more radioactivity both regio- and stereo-specifically in compounds of biological interest, and thereby monitor reaction mechanisms and metabolism under extremely dilute conditions. The preparative route chosen by Saljoughian and co-workers\(^{207}\) involves the following synthesis of biphenyl-4-yl chlorothioformate by the reaction of thionyl chloride with biphenyl-4-ol, followed by chlorination to yield the trichloromethyl biphenyl-4-yl ether. This then undergoes catalytic tritium-halogen replacement, before this tri-tritiated methyl biphenyl-4-yl ether is cleaved with hydriodic acid in glacial acetic acid at 127°C to yield the high specific activity tritiated methyl iodide as outlined below in Figure 2.6.

![Figure 2.6 Preparation of high specific activity tritiated methyl iodide.](image)

Although both these routes are attractive in many ways, in particular the former for its preparative route to chiral methyl iodide, they are also beset with practical problems. Therefore, it is apparent that these syntheses like many others described in the literature\(^{205}\) are unattractive for the routine on-line preparation of tritiated methyl iodide...
for a number of reasons including low yields, large number of synthetic steps, low specific activity, stringent reaction conditions, or large reaction scale. Subsequent investigations of carbon-14 and carbon-11 chemistry has highlighted the fact that it has been known for many years that $^{14}$C-methyl iodide can be prepared by the reduction of carbon dioxide to methanol with lithium aluminium hydride, followed by conversion to methyl iodide.$^{210}$

2.6 RESULTS AND DISCUSSION

2.6.1 Initial Experimental Methods

The development of Positron Emission Tomography (PET) has led to a huge growth in the development of rapid and efficient carbon-11 production systems especially for alkylating agents, and in particular methyl iodide. The current production procedure follows that of the analogous synthesis for $^{14}$C-methyl iodide, in that $^{11}$C-carbon dioxide is reduced with lithium aluminium hydride in tetrahydrofuran, followed by the removal of the solvent before hydrolysis of the complex with water to yield $^{11}$C-methanol [CH$_3$OH]. $^{11}$C-Methyl iodide is then prepared by treatment of $^{11}$C-methanol with hydriodic acid (57% w/v) under reflux. This procedure is now well documented in the literature.$^{211,212,213,214,215}$

However, this apparently elementary synthesis had never been applied to tritium labelling because LiAlPH$_4$ had not been available, until recently. In 1990 Andres and co-workers published details of a simple synthesis of lithium aluminium tritide.$^{187}$ Therefore, this method provided a starting point for the proposed initial synthetic route for the preparation of tritiated methyl iodide at various activities. The stages of this
synthetic route are outlined below in Figure 2.7 in which lithium tritide is formed from the reaction of n-butyl lithium with tritium gas in the presence of equimolar quantities of the base N,N',N'-tetramethylethylenediamine (TMEDA). The generated lithium tritide now undergoes reaction with aluminium bromide to generate lithium aluminium tritide, which is then used to reduce carbon dioxide, before the subsequent lithium aluminium tritide carbon dioxide complex intermediate is hydrolysed with water to generate the desired $[^3\text{H}]$methanol.

$$\begin{align*}
n-\text{BuLi} + \text{T}_2 & \xrightarrow{\text{TMEDA/Hexane}} \text{LiT} + n-\text{BuT} \\
4\text{LiT} + \text{AlBr}_3 & \xrightarrow{\text{Dry Diethyl Ether}} \text{LiAlT}_4 + 3\text{LiBr} \\
3\text{LiAlT}_4 + 4\text{CO}_2 & \xrightarrow{\text{i) Dry Diethyl Ether ii) }8\text{H}_2\text{O}} 4\text{CT}_3\text{OH} + 3\text{LiOH} + 3\text{Al(OH)}_3
\end{align*}$$

**Figure 2.7** Proposed initial route to $[^3\text{H}]$methanol.

Using deuterium as a model, LiAl$[^2\text{H}]$ was prepared from deuterium, followed by the subsequent reduction of carbon dioxide with the prepared $[^2\text{H}]$reagent to yield $[^2\text{H}]$methanol. However this early procedure was not without its problems:

- Moisture in the system.
- Incomplete reduction of CO$_2$.
- Incomplete removal of TMEDA.

The first difficulty was straightforward to overcome and was solved by ensuring that all reagents were fresh and that the solvents to be used were freshly distilled. Sodium-dried diethyl ether was used as a transfer solvent and this was kept over sodium wire until a couple of hours before use, when a small portion was transferred and dried over lithium aluminium hydride. Before use this solvent was filtered through dried neutral alumina to
remove the unwanted hydride. Coupled to this it was ensured that all glassware to be used was clean, and dried in an oven for at least 48 hours before use.

The incomplete reduction of carbon dioxide was more of a problem, because the reaction of \( \text{CO}_2 \) with lithium aluminium hydride can follow three courses, each of which represents a definite degree of reduction as outlined below in Figure 2.8.

\[
\begin{align*}
4\text{CO}_2 + 3\text{LiAlH}_4 & \rightarrow \text{LiAl(OCH}_3)_2 + 2\text{LiAlO}_2 \\
2\text{CO}_2 + \text{LiAlH}_4 & \rightarrow \text{LiAl(OCH}_2\text{O)}_2 \\
4\text{CO}_2 + \text{LiAlH}_4 & \rightarrow \text{LiAl(O}_2\text{CH)}_2
\end{align*}
\]

Figure 2.8 Reaction routes for the interaction of carbon dioxide with lithium aluminium hydride.\(^{216}\)

Hydrolysis or alcoholysis of these salts would give methanol, formaldehyde, or formic acid respectively. Attempts to control the reduction were made by ensuring the \( \text{CO}_2 \) used was dry - this was achieved by passing it through a drying column of phosphorous pentoxide and calcium carbonate. Varying the quantities of carbon dioxide used in the reduction process gave mixed results. Although the percentage of methanol generated increased when using dry \( \text{CO}_2 \), as might be expected, varying the amounts employed in the reduction stage however did not appear to have a particularly great effect. Although methanol was being generated as the major product, usually in excess of 70%, a significant amount of unwanted reduction products, particularly formic acid (\( \text{HCOOH} \)) was also being produced. Therefore in an attempt to solve this difficulty other reduction routes to the production of methanol were investigated and it was the use of diphenylcarbonate that finally solved the problem.

\[
\begin{align*}
\text{Ph}_2\text{O} + 3\text{LiAlH}_4 & \xrightarrow{12\text{H}_2\text{O}} 4\text{CH}_3\text{OH} + 8\text{OH}^{-} + 3\text{LiOH} + 3\text{Al(OH)}_3
\end{align*}
\]

Figure 2.9 Reaction of diphenylcarbonate with lithium aluminium hydride.
However the use of $N,N,N',N'$-tetramethylethylene diamine (TMEDA) which acts as a metal-chelating cosolvent, and also converts the n-butyllithium hexamer into a tetrasolvated tetramer, which is necessary for the formation of the lithium hydride species posed more of a problem. Careful examination of the lithium deuteride synthesis demonstrated that if the TMEDA was not completely removed from the reaction mixture, it formed a very stable complex with the lithium deuteride. Furthermore, this TMEDA complex transfers itself throughout the entire synthesis via a probable (LiAlD$_4$:TMEDA complex and on to a $[^2H]$-methanol:TMEDA complex, which on subsequent treatment with hydriodic acid to generate the desired methyl iodide, immediately forms a quaternary salt with the TMEDA present.

Consequently, although the proposed reaction scheme worked, it was shown that the TMEDA required for the initial stage of the synthesis must be completely removed from the system before methyl iodide generation in order for satisfactory synthesis of the latter. A number of options were available to try and remove this troublesome reagent including evaporation under high vacuum, lyophilisation, and centrifugation. Evaporation and lyophilisation both created extra difficulties in that extra glassware was required and a good vacuum was often difficult to obtain, coupled to the risk of water vapour depositing on the interior of the flask during the process of lyophilisation. Therefore, after numerous trials these procedures were disregarded in favour of centrifugation. It was found that lithium hydride, free from TMEDA, was readily prepared from the complex by repetitive centrifugation in pentane.

With the route to labelled methanol finalised, it was now possible to look into other methods of converting this labelled methanol into methyl iodide. The classical methods for the preparation of methyl iodide involve the iodination of methanol with either hydrogen iodide, or diphosphorous tetraiodide. However, in order to try and
avoid the use of the highly reactive hydrogen iodide species, and in view of its corrosive properties towards metal and plastics, attention turned to a new iodinating agent - alumina supported triphenylphosphine diiodide.

Triphenylphosphine diiodide was prepared and purified as described in the literature, before being adsorbed onto alumina (Brockmann activity I, 70-230 mesh). Figure 2.10 shows the proposed general reaction sequence for the reaction of triphenylphosphine diiodide with alcohols at elevated temperatures (160°C for methanol). Together with the desired alkyl halide (A) only triphenylphosphine oxide and hydrogen iodide (B) are formed as by-products. The hydrogen iodide is trapped out by the use of sodium hydroxide, while the non-volatile triphenylphosphine oxide remains on the alumina.

\[ R = \text{Alkyl group (e.g. Me, Et)} \]

Figure 2.10 Reaction sequence of triphenylphosphine diiodide with alcohols.

The iodination is achieved by distilling off the labelled methanol under a slow stream of nitrogen which is then passed through a pre-heated (160°C) column charged with the alumina supported triphenylphosphine diiodide reagent and closed with a plug of glass wool. The generated labelled methyl iodide is then passed through a second column containing sodium hydroxide and phosphorous pentoxide, and the product is then collected in a trap cooled to -78°C. This reaction procedure although offering an effective and easy to handle iodinating agent proved to be unreliable. The main problem was in maintaining a stable temperature of 160°C from the heating coil, (fabricated in-house)
around the column charged with the alumina-supported reagent, because triphenylphosphine diiodide is known to decompose at temperatures of 172°C, while one reference reports decomposition at 148°C. This then is the probable explanation for the occasional result observed using this iodination process, in which labelled methanol would frequently be the main product instead of the expected iodomethane. Therefore, after careful thought it was decided that although this route was promising, it had been shown not to be wholly successful, and as such was rejected in favour of the classical hydriodic acid approach for methyl iodide production. The basis of this decision was that rogue results were to be minimised wherever possible when tritium was to be employed as a label. It can also be seen from the literature that hydriodic acid has become the favoured reagent for the generation of [\(^{11}\)C]iodomethane from [\(^{11}\)C]methanol throughout the field of carbon-11 chemistry.

### 2.6.2 Discussion

The versatility of methyl iodide in labelling experiments has been demonstrated by its extensive use as a methylating agent, predominantly to form \(RR'N-CH_3\) and \(RO-CH_3\) bonds, examples of which can be found throughout the literature. The commercial methods for producing tritiated methyl iodide, highlighted in section 2.4, require stringent reaction conditions, for example high pressure, and are therefore unsuitable to a university laboratory environment. Although recent publications by Saljoughian and co-workers have outlined new syntheses of both mono-tritiated and tri-tritiated methyl iodide for small scale laboratory production, they are still beset with many practical difficulties, in particular the need for stringent reaction conditions and a large number of synthetic steps. Therefore it was evident that the numerous syntheses outlined in the
literature for the production of tritiated methyl iodide were unsuitable for our requirements.

However, by combining features of the early work by Klusener et al.\(^{185}\) outlining the production of alkali metal hydrides from their n-butyl compounds and hydrogen under mild conditions with those of the production of \(^{11}\text{C}\)-methyl iodide, has resulted in the development of a facile route to the production of tritiated methyl iodide outside a commercial environment. The generation and subsequent use of this tritiated methyl iodide are shown in sections 2.7.3 and 2.7.4 respectively.

The results show that although tritiated methanol has been generated as shown in Figure 2.13 (see page 66), the procedure is not without its shortcomings, in particular the failure to remove a significant percentage of the TMEDA after the first phase of the procedure, and the difficulty in handling such small quantities of highly hygroscopic reagents. Suspension impurities formed on hydrolysis of the diphenylcarbonate-lithium aluminium tritide/deuteride complex are probably responsible for the observed broadening at the base of the methyl peak in Figure 2.13 (see page 66), while the small peak at 8.79 ppm is due to incomplete reduction of diphenylcarbonate resulting in the generation of a formic acid impurity. Nonetheless, the procedure did result in a limited quantity of tritiated methyl iodide being generated, the \(^3\text{H}\)-NMR of which is shown in Figure 2.15 (see page 68). This resultant methyl iodide was subsequently used in the methylation reactions outlined in section 2.7.4.1, the resulting \(^1\text{H}\) and \(^3\text{H}\)-NMR spectra are shown in Figures 2.17 to 2.30 (see pages 73 to 79), and offer further evidence that \([^{3}\text{H}]\)methyl iodide was generated.

There are several possibilities for improving the overall radiochemical yield of the synthesis. The simplest of these would be to investigate the use of an alternative solvent in place of diethyl ether, for example dibutyl ether, in which lithium aluminium hydride
has a lower solubility. Other possibilities would require significant changes to the equipment, for example a suitable high vacuum line would allow the complete lyophilisation of the residual TMEDA after the initial stage of the synthesis. Automation of the procedure (similar to that already employed in PET chemistry) would allow easier manipulation of small quantities of reagents and radioactivity. However, this latter improvement is not within the scope of this laboratory.

Therefore it can be concluded, that despite the difficulties encountered in the preparation of \[^{1}H\]methanol and \[^{1}H\]methyl iodide, the procedure outlined in section 2.7.3.1 details their preparation using a manageable quantity of tritium gas in a university radiochemistry laboratory environment. A consequence of this successful development route for tritiated methanol and methyl iodide is that it opens up an in-house synthetic route to many other desirable tritiated precursors, for example, the route to the small scale preparation of numerous metal tritide reagents synthesisable from lithium tritide as illustrated in Figure 2.2. The production of \[^{3}H\]methyl iodide allows not only the development of other more reactive methylating agents such as \[^{3}H\]methyl triflate (CH$_3$SO$_2$CF$_3$) and \[^{3}H\]methyl nonoflate (CH$_3$SO$_2$C$_4$F$_9$), but also the use of \[^{3}H\]methyl Grignard reagents and palladium catalysed cross coupling reactions of methyl iodide with an organometallic reagent. This would increase the choice of synthetic labelling routes available.
2.7 EXPERIMENTAL

2.7.1 The Tritium Gas Line

The synthesis of tritiated compounds by catalytic exchange in solution with tritium gas was carried out on the tritium gas line as detailed in Figure 2.11. Hydrogen or deuterium may, of course, be substituted for tritium gas as starting material for the preliminary studies. The apparatus is designed to transfer the contents of an ampoule of tritium gas to the reaction vessel. It is constructed from stainless steel high performance chromatography equipment, which is connected to Pyrex glassware via glass-metal seals. The ‘business end’ of the apparatus is the tritiation tree which consists of a burette (5.0cm³, 28cm length) with a 330cm³ bulb at the base. The top of the burette is connected, via a four way junction piece (behind the plate on the diagram), to the manifold, tritium gas ampoule and the reaction flask, each of which may be isolated via Whitey swagelock™ SS-41S1 taps A, B, C, and D. The tree is connected to the main manifold at tap A. Connected to the base of the tree is a mercury reservoir (350cm³) which is in turn connected to the manifold at tap 7.

A vacuum pump is connected to the apparatus at tap 1 and the vacuum attained may be read from the Edwards vacuum gauge attached at tap 3. Volatile tritiated material is collected in the liquid nitrogen trap which is connected to the manifold by silicon rubber high vacuum tubing; an additional pair of ethylene glycol traps are present after the vacuum pump to purge the exhaust of any remaining volatile material. A helium balloon, connected via a Teflon 3-way Interflow tap, may be attached to the apparatus by the ground glass joint, 2. All glass joints are lubricated with grease. All stopcocks are made
of Teflon. The connection between the manifold and the tritium tree is silicon rubber high vacuum tubing.

Figure 2.11 Diagram of the tritium gas line system.
2.7.2 Operating the Tritium Gas Line

Before use all of the taps, joints, and silicon rubber high vacuum tubing on the tritium gas line should be thoroughly checked to ensure that they are clean and that the line is airtight. Following this routine check, all the necessary contamination checks are carried out around the area of work using swabs for liquid scintillation counting. The vacuum pump should be balanced and then switched back off, and all of the unconnected high vacuum tubing attached to the vacuum pump and traps. Before, the ethylene glycol traps are half-filled and liquid nitrogen placed in the Dewar trap.

A balloon of helium is attached to the apparatus at 2 and the reaction vessel containing catalyst, substrate, solvent, and stirrer bar is attached to B, (the stirrer bar is a short piece of paper clip encapsulated in a glass tube manufactured from the tip of a disposable Pasteur pipette). Before the attachment of a tritium gas ampoule at position X the presence of a magnet in the side-arm Y should be confirmed. Following attachment of the ampoule a 10-20cm³ round-bottomed glass flask with a greased high quality ground glass tap (5) and filled with helium is attached to the side arm at position Z. The vacuum pump may be started after ensuring that ALL taps are closed and that the traps are connected. The liquid nitrogen in the Dewar trap should also be re-filled to contain any solvent vapour from the reaction vessel.

Air can now be evacuated from the system, after ensuring that the Edwards vacuum gauge is on, and that tap 3 is open. The evacuation process starts by opening tap 1 followed by tap 4, and aligning the three-way tap 6 with taps 4 and A only. In order to evacuate the system as far as the manifold tap 7 is opened to the atmosphere and tap A opened, before tap C is CAREFULLY opened to the burette, so that the level of the mercury rises GENTLY until it is very close to the top, at which point tap C is closed.
The air is most effectively removed from the reaction flask by freezing the contents with liquid nitrogen prior to opening tap B. Tap D is now also opened to evacuate the entire manifold, reaction vessel and glassware connected to the tritium ampoule (tap 5 remains shut). Once a stable reading is obtained on the vacuum gauge then taps D, B, A, 4 and 1 respectively are closed.

Helium gas is now allowed into the system by opening the three way tap 2 until the Edwards vacuum gauge reads atmospheric pressure. Taps 4 and A, are opened followed by tap D in order to flush the side-arm and ampoule with helium after which this tap is again closed. Tap C is opened carefully to allow about 2cm$^3$ of helium into the burette before being shut. The reaction flask contents are allowed to thaw so that any gas trapped in the solvent is removed, before tap B is opened to allow the reaction vessel to be flushed with helium. Taps B, A, and 4 are closed, before the helium is shut off via the three way tap 2.

The system is re-evacuated by opening tap 1 and following the procedure previously outlined, before repeating the flush with helium gas. This ‘sweeping’ process is repeated a further two times, then all of the taps in the tritium tree A, B, C and D are closed and the air evacuated from the mercury reservoir by re-aligning the 3-way tap 6 so that it is fully open, and by turning tap 7 slowly to form a closed system (CARE). The line behind the tritium tree, leading to the reservoir is then swept with helium as before. This process is repeated a further two times (ensuring that the helium pressure is equal to or slightly less than that of the helium in the tritium tree). Finally all the taps except 6 and 3 are closed.

The glass breakseal of the tritium ampoule is broken by the magnet contained in the side-arm (Y), by the use of an external magnet. At this point taps D and B may be opened, the stirrer switched on and the reaction started. Or taps D and C are opened and
a drop in the mercury level in the burette provides visual confirmation that the breakseal has broken and tritium gas is being transferred into the burette. Taps 1 and 4 are opened, followed by the careful opening of tap 7, and as the mercury flows to fill the vacuum in the reservoir, it draws the tritium gas out of the ampoule and into the bulb below the burette. Tap 7 is closed to halt the flow of mercury followed by the closure of taps D, C, 4 and 1. Tap 7 is now very CAREFULLY opened to the atmosphere, and the pressures between the reservoir and the burette are allowed to slowly equilibrate in order to prevent breakage of the graduated burette. Once the pressures have equilibrated the volume of tritium gas that has been transferred may be measured from the graduations on the burette. If insufficient gas has been transferred, the process should be repeated. The gas can now be opened to the reaction mixture by opening taps B and C, the stirrer switched on, the vacuum pump switched off (air bleed), the nitrogen trap dewar removed and the traps disconnected.

N.B. It should be remembered that, for this procedure the side-arm glassware should be left evacuated during the final helium gas flush, otherwise an accurate tritium gas transfer reading is difficult. However, this reading is still subject to deviation when small volumes of tritium gas (37 GBq (1 Ci)) are used, due not just to the gas volume but also to the weight of the mercury and atmospheric pressure changes.

After the desired reaction time has elapsed, helium may be introduced into the reaction vessel from the gas reservoir at position Z, to ensure maximum incorporation of the tritium into the substrate. Where maximum incorporation is not an issue hydrogen or deuterium may be employed instead. The helium is initially transferred from the round-bottomed flask into the burette first by opening tap 5, and then by following the same procedure employed for the tritium gas transfer, and then with taps B, C, and 7 open to the atmosphere the helium is transferred into the reaction vessel. Stirring is recommenced
with taps B, C, and 7 open; the level in the burette can then be monitored, to check if any further tritium gas is taken up in the reaction.

Finally once the reaction is deemed to be complete all the taps are closed (except 6) and the reaction vessel is re-frozen (usually in liquid nitrogen). The traps and high vacuum tubing are re-attached before the vacuum pump is switched on again (as detailed at the start). Taps 1, 4, B and C are opened before 7 is CAREFULLY opened to the vacuum to evacuate the mercury reservoir, and transfer any residual tritium-helium mixture (along with any other unwanted gaseous component) back into the burette. When all of the gas mixture has been transferred, taps 7, B and C are closed followed by taps 4 and 1. Tap 7 is then opened carefully to the atmosphere to allow equilibration of the mercury reservoir as previously described. The vacuum pump is once again switched off (air bleed) and the dewar and traps removed. The reaction vessel is allowed to warm to room temperature and removed from the tritium gas line for immediate work up and purification. Finally a ‘clean-up’ reaction is attached to the line to mop up any residual tritium gas transferred back into the burette. A typical clean up reaction would be the hydrogenation of a cinnamic acid derivative in a suitable solvent with Pd/C catalyst.

2.7.3 Preparation of Tritiated Methyl Iodide

The outcome of the numerous early experimental attempts to produce labelled methyl iodide as outlined in section 2.6.1, finally led to the synthetic experimental route detailed in Figure 2.12. This synthesis essentially encompasses 4 distinct steps as illustrated. The first step, based on the early work by Klusener and co workers, involves the generation of an extremely reactive alkali metal tritide, under mild conditions and ambient temperature. The second step of the synthesis after purification of
the lithium tritide involves a classical complexation reaction with an ethereal solution of aluminium bromide to generate the desired tritiated lithium aluminium hydride. This vigorous reaction does not require an excess of the lithium tritide, since the reaction quantitatively consumes the hydride, this therefore allows the use of stoichiometric quantities. Although the generated ether solution of tritiated lithium aluminium hydride is saturated with lithium bromide, it has been previously reported in the case of LiAl\(^{1}H_{4}\) to behave normally in subsequent reactions.\(^{216}\) The third phase of the synthesis entails the reduction of diphenylcarbonate with the freshly prepared ethereal suspension of tritiated lithium aluminium hydride from the previous phase, followed by careful hydrolysis to yield an aqueous solution containing the desired tritiated methanol.

The reduction of carbonyl compounds with lithium aluminium hydride is thought to involve a hydride transfer from the nucleophilic attacking species, (the tetrahydroaluminate ion (AlH\(_{4}^{-}\))), onto the carbonyl carbon (as a place of the lowest electron density). However there is some controversy about how this proposed mechanism actually proceeds, especially with regard to which, if any, of the proposed intermediates are actual attacking species.\(^{223}\) The final stage entails the iodination of methanol with hydriodic acid under a stream of nitrogen and gentle reflux conditions, so that the methyl iodide formed is carried away from the reaction flask, through a drying column, to be collected in the desired solvent of choice for the following methylation reaction.
2.7.3.1 Experimental Outline

Butyl lithium (1.6M in hexane (Fluka, 300 µl, 0.48 mmol)) was carefully transferred into a suitable 2 ml gas line flask containing a clean glass encapsulated micro stirrer and 500 µl of dry hexane. To this solution is added 100 µl (0.625 mmol) of freshly distilled TMEDA (N,N,N',N'-Tetramethylethylenediamine). After this, the contents of the flask were frozen using liquid nitrogen in a dry atmosphere, before the flask was attached to the tritium gas line.

Normal gas line procedure was then employed, before a 1 Ci ampoule of tritium gas and a 5 ml round bottom flask fitted with a ground glass tap, and filled with deuterium gas were then attached to the tritium gas line after which the gas line was then evacuated before being saturated with He gas as outlined in section 2.7.2. The tritium gas ampoule was then opened in the normal manner and the gas allowed to fill the reservoir, before being opened to the reaction flask. Finally the reaction solution was stirred to increase the surface area in contact with the tritium gas and hence increase the reaction rate. After the reaction had been allowed to run for 12 hours, the reaction flask was
frozen. The flask containing the deuterium gas was opened, and the gas transferred into the burette using the procedure detailed in section 2.7.2, before once again being opened to the reaction flask, which is allowed to thaw before stirring is recommenced. The reaction was then allowed to continue overnight to ensure that it proceeds to completion.

On completion, the flask was frozen in an acetone slush bath (-95.4°C), made by cooling liquid acetone with liquid nitrogen, in order to freeze the hexane and leave any tritiated butane in liquid form. A reverse vacuum was then applied to the gas line to pull the butane and any residual tritium gas over into the reservoir as the flask was allowed to warm slightly. The vacuum was then sealed off and the tap to the reaction flask closed before normal atmospheric pressure was restored to the reservoir. This reverse vacuum freeze/thaw method was repeated a further two times to ensure that all the tritiated butane along with any residual tritium gas were trapped in the gas line reservoir for later clean up, before the reaction flask containing the lithium tritide/deuteride suspension was removed.

The reaction flask was removed from the gas line, again following the usual procedure highlighted in section 2.7.2. The lithium tritide/deuteride suspension was then very carefully transferred from the reaction flask into a 10 ml centrifuge tube, after which the reaction flask was washed repeatedly with dry pentane (Aldrich), and these washings were also transferred to the centrifuge tube. When the tube was 60-75% full with the lithium tritide/deuteride suspension/anhydrous pentane mixture, the tube was placed into a centrifuge, balanced, and spun for 20 minutes at 3000rpm. After this the majority of the upper pentane layer was carefully removed to leave the creamy white precipitate of lithium tritide/deuteride and a protective covering of anhydrous pentane at the base of the centrifuge tube. This procedure of “washing” with pentane was repeated another two
times, in order to remove the majority of the residual TMEDA left from the lithium tritide/deuteride formation.

After centrifugation the lithium tritide/deuteride suspension in pentane was slowly added to an anhydrous ethereal solution of aluminium bromide (70 mg) in a clean, dry 15ml pear shaped flask. The mixture was gently agitated and kept cool in order to generate the desired lithium aluminium tritide/deuteride (LiAlD$_4$ (LiAlD$_4$)). After formation of the freshly formed LiAlD$_4$ (LiAlD$_4$) suspension, the flask was kept cool by using a CO$_2$/acetone bath, while powdered dry diphenylcarbonate was carefully added. Upon cessation of effervescence the flask was allowed to warm up to room temperature, before a further small portion of diphenylcarbonate was added to ensure that the reaction had gone to completion. At this point, the reaction flask contents were frozen using liquid nitrogen in a dry atmosphere, and the residual solvent removed by lyophilisation. The removed solvent was collected in a liquid nitrogen trap to minimise the loss of any tritiated by-products. Upon removal of the solvent, distilled water (5 ml) was slowly added to the cooled flask containing the solid diphenylcarbonate-lithium aluminium tritide/deuteride complex, resulting in the instant hydrolysis of the complex, and generating the desired labelled methanol (6.73 GBq [182 mCi]). This solution of labelled methanol may, at this stage be stored frozen without any noticeable decomposition for up to six months. Figure 2.13 below shows the $^3$H-NMR spectrum obtained after the hydrolysis of the diphenylcarbonate-lithium aluminium tritide/deuteride complex.
Figure 2.13 $^3$H-NMR ($^1$H-decoupled) spectrum of [methyl-$^3$H] methanol in H$_2$O.

Labelled methyl iodide was then prepared by transferring an aliquot of the methanol solution into a two necked flat bottomed 4 ml flask, to which hydriodic acid 1ml (57% wt, Aldrich) was also added. The flask was then fitted with the specially designed glassware (fabricated in-house) for the sealed production, and extraction of anhydrous methyl iodide as illustrated in Figure 2.14. The system consists of capillary tubing, leading from the reaction flask, to a Pyrex glass column charged with drying agent (potassium hydroxide and phosphorous pentoxide). Following the drying column was a capillary loop, which was immersed in iced water to aid the cooling of the methyl iodide generated, before it was collected in the subsequent solvent traps. After the fitting of this glassware, the dry nitrogen flow was switched on and the reaction flask was immersed into a pre-heated oil bath for approximately 5 minutes. At this point the oil bath was removed and both the flask and the methyl iodide collection traps were cooled using liquid nitrogen. As the solution cooled to freezing, the dry nitrogen gas flow was reduced and the methyl iodide collection traps removed. The flow of dry nitrogen was then
stopped. The solution was then allowed to thaw, before being transferred to a small stoppered flask for analysis. The rest of the system is thoroughly cleaned and dried for later re-use. Figure 2.15 over the page shows the resultant $^3$H-NMR spectrum obtained for this reaction.

The following conditions were employed to generate the desired labelled methyl iodide.

- Gas flow rate of dry $N_2$: 25 ml min$^{-1}$
- Oil bath temperature: 110°C
- Drying column: phosphorus pentoxide/potassium hydroxide

Figure 2.14  Methyl iodide production system.
Analysis of the methyl iodide production system indicates that only approximately 28% of the methanol was actually converted to methyl iodide in this process. The low yield of methyl iodide may arise from a number of factors, including (a) the dilution of the 57% hydriodic acid by water in the dilute tritiated methanol solution, (b) the possibility of exchange between the water and the tritiated alcohol, (c) the incomplete removal of the TMEDA from the resultant lithium tritide suspension by centrifugation. From deuterium experiments it was felt that the first two possibilities would not account for the entirety of the observed low yield. However, the incomplete removal of the TMEDA, and its subsequent transfer throughout the entire synthetic route (via probable (LiAlD₄)₂(LiAlT₄)₂:TMEDA and methanol:TMEDA complexes), would have led to the immediate formation of a quaternary salt on subsequent treatment with hydriodic acid, instead of generating the desired methyl iodide. It was felt that this was the major cause of the low percentage conversion of methanol to methyl iodide.

Note – Further features of the work can be found in Appendix A.
2.7.4 Methylation Experiments.

The versatility of methyl iodide in labelling experiments has been demonstrated by its extensive use in methylation reactions, in particular its ability to readily methylate a variety of structural groups; e.g. methylation at phenolic oxygen has been used to label a number of important dopamine receptor ligands and serotonin uptake inhibitors for PET studies.

Reactions using methyl iodide are frequently mild and rapid, and the following methylation conditions are no exception. The reaction, employing dry DMSO, dry powdered potassium hydroxide and methyl iodide outlines a simple single reaction pot N- and/or O-methylation procedure suitable for a range of organic compounds. The process involves the addition of the substrate and methyl iodide to a mixture of dry powdered KOH in dry DMSO at room temperature. The use of DMSO as a dipolar aprotic solvent is well known, and excellent results have been obtained for example in the methylation of phenols, while little of the potassium hydroxide actually dissolves, its solubility in DMSO being low. Methylation is believed to occur via the formation of a potassium salt of the substrate, from the interaction of the substrate with the KOH in DMSO to remove a proton, and allowing normal nucleophilic attack of methyl iodide.

2.7.4.1 Experimental Outline

Tritiated methyl iodide was prepared as described previously in section 2.7.3.1, and stored in dry DMSO ready for use. 50 µmol of substrate plus 200 µmol of dry powdered potassium hydroxide (11.2 mg) were placed in a 5 ml round bottom flask containing a micro stirrer, under a dry nitrogen atmosphere. To which was added 5 ml of
the previously prepared methyl iodide/DMSO solution; the flask was then sealed, and left to stir at room temperature overnight. Previously, the corresponding reaction of CH₃I had been studied and it was found that the overnight time was more than sufficient for equilibrium to be reached.

The contents of the flask were then poured into 50 ml of distilled water in a conical flask and the product extracted with organic solvent (3x20 ml), (usually chloroform). The combined organic extracts were washed again with water (3x20 ml), before the extract was concentrated by gentle rotary evaporation (down to approximately 5 ml) at which stage the radioactivity of the solution was determined. Finally a steady gentle stream of nitrogen was blown over the surface of the solution (now in a pear shaped flask) to reduce the volume further (approximately 500 µl) thus effectively resulting in a DMSO solution of the product. A few drops of deuterated NMR solvent (internal standard) were added to the solution and a fraction of it (300 µl) was placed in the NMR tube for analysis (double containment in place).

The resultant §H-NMR spectra along with the solvent suppressed §H-NMR spectra for the methylated versions of the compounds given in Table 2.1 with their corresponding structures shown in Figure 2.16, are shown in Figures 2.17 to 2.30. The * in the §H-NMR spectra indicates a signal corresponding directly to that observed in the appropriate §H-NMR spectra.
Table 2.1  Specific activities of various $^{3}$H-methylated compounds.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Extraction solvent</th>
<th>Internal NMR standard</th>
<th>Position of methyl label (ppm)</th>
<th>Specific activity (Ci/mmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetanilide</td>
<td>Chloroform</td>
<td>d$_6$-Acetone</td>
<td>N-Me, $\delta$=2.73</td>
<td>5.4</td>
</tr>
<tr>
<td>p-acetophenetidide</td>
<td>Chloroform</td>
<td>d$_6$-Acetone</td>
<td>N-Me, $\delta$=3.09, Unknown, $\delta$=2.62</td>
<td>6.8</td>
</tr>
<tr>
<td>2-bromobenzyl alcohol</td>
<td>Dichloromethane</td>
<td>CDCl$_3$</td>
<td>O-Me, $\delta$=3.35</td>
<td>5.2</td>
</tr>
<tr>
<td>9-hydroxyxanthene</td>
<td>Diethyl Ether</td>
<td>CDCl$_3$</td>
<td>O-Me, $\delta$=2.89</td>
<td>10.6</td>
</tr>
<tr>
<td>2-phenylbenzimidazole</td>
<td>Petroleum Ether ($60^\circ$-$80^\circ$)</td>
<td>CDCl$_3$</td>
<td>N-Me, $\delta$=3.66</td>
<td>9.2</td>
</tr>
<tr>
<td>4-phenylphenol</td>
<td>Chloroform</td>
<td>CDCl$_3$</td>
<td>O-Me, $\delta$=3.79</td>
<td>3.8</td>
</tr>
<tr>
<td>2-(N-benzamido)-3-phenylprop-2-enoic acid</td>
<td>Chloroform</td>
<td>CDCl$_3$</td>
<td>N-Me, $\delta$=3.1, O-Me, $\delta$=3.61</td>
<td>8.4</td>
</tr>
</tbody>
</table>
Figure 2.16  Reaction summary of the methylation reactions performed using freshly prepared labelled methyl iodide.
Figure 2.17 $^3$H-NMR ($^1$H-decoupled) spectrum of [methyl-$^3$H] N-methylated acetanilide in DMSO and $d_6$-acetone.

Figure 2.18 $^1$H-NMR (DMSO solvent suppressed) spectrum of [methyl-$^3$H] N-methylated acetanilide in DMSO and $d_6$-acetone.
Figure 2.19 $^3$H-NMR ($^1$H-decoupled) spectrum of [methyl-$^3$H] N-methylated p-acetophenetidide in DMSO and d$_6$-acetone.

Figure 2.20 $^1$H-NMR (DMSO solvent suppressed) spectrum of [methyl-$^3$H] N-methylated p-acetophenetidide in DMSO and d$_6$-acetone.
Figure 2.21 \(^{3}\text{H-}\text{NMR (}^{1}\text{H-decoupled) spectrum of [methyl-}^{3}\text{H]}\) O-methylated 2-bromobenzyl alcohol in DMSO and \(\text{CDCl}_3\).

Figure 2.22 \(^{1}\text{H-}\text{NMR (DMSO solvent suppressed) spectrum of [methyl-}^{3}\text{H]}\) O-methylated 2-bromobenzyl alcohol in DMSO and \(\text{CDCl}_3\).
Figure 2.23 $^3$H-NMR ($^1$H-decoupled) spectrum of [methyl-$^3$H] O-methylated 9-hydroxyxanthene in DMSO and CDCl$_3$.

Figure 2.24 $^1$H-NMR (DMSO solvent suppressed) spectrum of [methyl-$^3$H] O-methylated 9-hydroxyxanthene in DMSO and CDCl$_3$. 
Figure 2.25 $^3$H-NMR ($^1$H-decoupled) spectrum of [methyl-$^3$H] N-methylated 2-phenylbenzimidazole in DMSO and CDCl$_3$.

Figure 2.26 $^1$H-NMR (DMSO solvent suppressed) spectrum of [methyl-$^3$H] N-methylated 2-phenylbenzimidazole in DMSO and CDCl$_3$. 
Figure 2.27 $^3$H-NMR ($^1$H-decoupled) spectrum of [methyl-$^3$H] O-methylated 4-phenylphenol in DMSO and CDC$_3$.

Figure 2.28 $^1$H-NMR (DMSO solvent suppressed) spectrum of [methyl-$^3$H] O-methylated 4-phenylphenol in DMSO and CDC$_3$. 

Figure 2.29 $^3$H-NMR ($^1$H-decoupled) spectrum of [methyl-$^3$H] N-methylated 2-(N-benzamido)-3-phenylprop-2-enoic acid in DMSO and CDCl$_3$.

Figure 2.30 $^1$H-NMR (DMSO solvent suppressed) spectrum of [methyl-$^3$H] N-methylated 2-(N-benzamido)-3-phenylprop-2-enoic acid in DMSO and CDCl$_3$. 
Chapter 3

Synthesis and Attempted Tritiation of Two Potential 5-HT Re-Uptake Inhibitors

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3.1 INTRODUCTION

3.1.1 Serotonin

Serotonin or 5-hydroxytryptamine (5-HT), discovered over 40 years ago, is a brain neurotransmitter that plays a role in various cognitive and behavioural functions including feeding, sleeping, and pain. It has also been implicated in various neuropsychiatric disorders including anxiety, depression, alcoholism, schizophrenia, sexual dysfunction, Alzheimer’s disease and migraine. 5-HT is synthesised in vivo from the amino acid, L-tryptophan, by sequential hydroxylation-decarboxylation, and stored in presynaptic vesicles.

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Firing of the neuron releases 5-HT into the synaptic cleft whereupon the 5-HT may have one of several fates which include: i) binding to a postsynaptic receptor, so activating a transduction pathway that leads to a physiological function; ii) binding to a presynaptic autoreceptor; and iii) re-uptake into the presynaptic neuron, via a transporter protein (re-uptake site), either for metabolism or re-storage in vesicles. 5-HT receptors can be found, in abundance, throughout the mammalian CNS. These may be grouped into 7 families (5-HT1-7) on the basis of their structural homologies and pharmacology. Over 14 receptors are now recognised; the receptors are all
transmembrane proteins, and coupled to G-proteins, except the 5-HT_3 receptor which is a ligand gated ion channel. 5-HT_1 receptors are defined as having nanomolar affinity for [³H]5-HT (in contrast to the others, which have lower affinity). The 5-HT_1 receptors can be further sub-divided, based on functional and ligand binding studies, into 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, 5-HT_{1D}, and 5-HT_{1F}. At present the 5-HT_{1A} receptor has been the most characterised in terms of molecular biology, pharmacology and biology; it plays a role in anxiety, hypertension and eating disorders. 5-HT_2 receptors have been implicated in a number of disorders including anxiety, depression and migraine. 5-HT_3 receptor antagonists have anti-emetic effects providing relief from nausea and vomiting induced by chemotherapy. The other classes of 5-HT receptors are very recently discovered and their potential as sites for therapeutic action awaits elucidation.

3.1.2 5-HT re-uptake inhibitors

As mentioned earlier one of the fates of 5-HT is to be re-uptaken into the presynaptic neuron. This removes 5-HT from the synaptic cleft. The primary pharmacological characteristic of 5-HT re-uptake inhibitors is to enhance serotonergic function by blocking this re-uptake. Selective serotonin re-uptake inhibitors (SSRIs e.g. Prozac and related compounds) are thought to alleviate depression by increasing 5-HT levels in the synaptic cleft and such drugs are an important strategy in the treatment of depression.

5-HT re-uptake inhibitors are typically tested for potency and selectivity by measuring their effect on p-chloroamphetamine (pCA)-induced '5-HT syndrome'. The 5-HT releasing property of pCA is dependent on prior active transport of the 5-HT re-uptake
system. Therefore the ability of drugs to inhibit the actions of pCA can be used as an index of \textit{in vivo} 5-HT uptake inhibition.\textsuperscript{222, 223} \textit{In vitro} systems can also be used, for example rat cortical synaptosomes and blood platelets.\textsuperscript{224}

There were a number of reasons that dictated the synthesis of these two potential 5-HT re-uptake inhibitors, namely, biological evaluation and the possibility of specifically labelling the resulting compounds, either with tritium or a suitable PET isotope.

\section*{3.2 RESULTS AND DISCUSSION}

\subsection*{3.2.1 Proposed Preparation of N-[[1-[(1-bromo-2-naphthalenyl)methyl]-4-piperidinyl]amino][carbonyl]-3-pyridinecarboxamide Derivatives}

\begin{align*}
\text{Y} &= \text{H or F} \\
\text{NBS} &= \text{N-Bromosuccinimide} \\
\text{NUP} &= \text{4-Nicotinoylureidopiperidine}
\end{align*}
The proposed preparation of these compounds is in three-stages. The first stage involves a classic aromatic electrophilic substitution reaction with bromine where the solvent (acetic acid) acts as a catalyst, and the alkyl group acts as an ortho-directing activator ensuring that the bromine enters at the desired 1-position on the naphthalene ring.

In 2-methylnaphthalene derivatives the methyl group at the 2-position activates ring A more than ring B (Figure 3.2 (i)) (though the presence of a substituent in a fused ring system affects all the rings, the effect is generally greatest on the ring to which it is attached). Therefore substitution would be expected to be greater at the positions activated by the methyl group (1 and 3) due to the ortho and para directing effect typically shown by this group in aromatic electrophilic substitution. However, substitution at the 3-position gives rise to an arene ion for which it is impossible to write a low-energy canonical form in which ring B has a complete sextet, and where the positive charge is situated on the methyl-substituted carbon (a tertiary carbocation), where it can best be stabilised by the methyl inductive effect. All we can write are forms like (Figure 3.2 (ii)), in which the sextet is intact, but the positive charge is not situated on the methyl-substituted carbon. In contrast, substitution at the 1-position gives rise to a more stable arene ion, for which two canonical forms can be written in which ring B is benzenoid (Figure 3.2 (iii and iv)), in one of which the positive charge is situated on the methyl-substituted carbon (Figure 3.2 (iii)). Therefore, substitution at the 1-position predominates (typically 98%). However, due to the active nature of naphthalene this aromatic electrophilic substitution reaction with bromine is best undertaken at low temperature to prevent any unwanted side reactions occurring, in particular labelling at the 4 and 6-positions.
Chapter 3 Synthesis & Attempted Tritiation of Two Potential 5-HT Re-Uptake Inhibitors

Figure 3.2 The arenium ion forms for aromatic electrophilic substitution with bromine at the 1 and 3 position for 2-methylnaphthalene derivatives.

The second stage of this synthesis (II) involves the use of N-bromosuccinimide (NBS) in a non-polar solvent (carbon tetrachloride), to brominate the methyl group of the 1-bromo-2-methylnaphthalene derivative via a free radical substitution reaction. NBS itself is a highly selective brominating agent in that it attacks only weak C-H bonds such as allylic and benzylic positions. The reaction requires the presence of free radical initiators, such as benzoyl peroxide and 1,1'-azobis-(cyclohexanecarbonitrile). Benzoyl peroxide has been used as a free radical initiator for a wide variety of reactions, in particular halogenations with bromine or NBS, while 1,1'-azobis-(cyclohexanecarbonitrile) is an efficient radical initiator for primary radical reactions.\(^{238}\)

Free radical substitution has been shown to be very sensitive to the presence of free radical initiators and inhibitors.\(^{239}\) The reaction may proceed (a) thermally, with an initiator providing the necessary free radical to begin the chains, or (b) photolytically, when bromine alone is sufficient and an added initiator is not needed. The mechanism of benzylic bromination is similar to that for allylic bromination of alkenes and involves abstraction of a benzylic hydrogen atom to generate an intermediate benzyl radical. The stabilised radical then reacts with Br\(_2\) to yield product and a bromine radical, which cycles back into the reaction to carry on the chain, as shown in Figure 3.3. The actual source of
Br₂ necessary for reaction with the benzyl radical is produced by a fast ionic reaction between NBS and the HBr liberated in the formation of the benzyl radical.

\[
R + Br^* + HBr \rightarrow ^N-Br + HBr
\]

**Intermediate benzyl radical**

\[
N=\text{Br} + \text{HBr} \rightarrow N-H + \text{Br}_2
\]

**Figure 3.3** Mechanism of benzylic bromination with \(N\)-bromosuccinimide.\(^{240}\)

Therefore, the function of the NBS is to provide a constant, but very low, ambient concentration of bromine and to use up the HBr liberated in step 1. Furthermore because the concentration of bromine is kept low, it helps to prevent any unwanted aromatic electrophilic substitution.

The final stage of this synthesis (III) involves an \(S_N2\) nucleophilic substitution reaction between an alkyl halide and a secondary amine in the presence of a polar non-hydroxylic solvent (DMF) and \(N,N\)-diisopropylethylamine (Hünig’s base). \(N,N\)-dimethylformamide is used as the reaction solvent because it increases the effectiveness of the nucleophile in the system, due to the fact that the nucleophile is very much less strongly solvated in DMF, than it would be in a polar hydroxylic solvent such as methanol (because of hydrogen bonding), and as such this allows an increase in the reaction rate. The \(N,N\)-diisopropylethylamine is present to remove the HBr generated as the
nucleophilic substitution reaction proceeds, and thus prevent formation of the unwanted quaternary salt of the desired product.

### 3.2.2 Discussion

The biological evaluation of these two 5-HT re-uptake inhibitors was performed at the MRC Cyclotron unit at Hammersmith Hospital, London. The subsequent results for \( N-[[1-[(1\text{-bromo-6-fluoro-2-naphthalenyl})\text{methyl}]\text{-4-piperidinyl}]\text{amino}]\text{carbonyl}]\text{-3-pyridinecarboxamide, and } N-[[1-[(1\text{-bromo-2-naphthalenyl})\text{methyl}]\text{-4-piperidinyl}]\text{amino}]\text{carbonyl}]\text{-3-pyridinecarboxamide show that at 1 nM they inhibit the binding of serotonin to the human 5-HT re-uptake site by 22.8% and 30.7% respectively, and at 10 nM by 70% and 59.3% respectively.}^{241} \) Tritium labelling attempts for these two compounds are discussed later in section 3.2.3, while experiments to replace the bromine in these two compounds with a suitable positron emitting isotope possibly via a tri-butyl tin derivative are still continuing.

Analysis of the NMR data in Figures 3.5 to 3.18, coupled to the use of NMR prediction programs\(^{242}\) and literature\(^{243}\) has enabled almost all proton and carbon signals to be assigned for the two synthesised 5-HT re-uptake inhibitors, as shown in Tables 3.2 and 3.4. The \( ^{13}C \) spectra obtained have all been broad-band proton-decoupled and as such no carbon-hydrogen couplings are observed. However, even under this broad-band hydrogen decoupling \( ^{13}C \) spectra do show couplings to other magnetically active nuclei, in particular to \( ^{19}F \) which has a 100% natural abundance and a nuclear spin \((I = \frac{1}{2})\). The effect of this heteroatom is to cause splitting to occur in the carbon spectrum due to the fact that the fluorine \((I = \frac{1}{2})\) can take up to two orientations with respect to the applied
field. The carbon atom therefore "sees" two different magnetic fields, and comes into resonance as a doublet. A further effect attributable to the presence of the fluorine atom is that carbon nuclei separated by 4 or more bonds from the $^{19}$F nuclei do not exhibit measurable long-range coupling constants unless the internuclear separation is relatively small. Therefore, for $N$-[[1-[(1-bromo-6-fluoro-2-naphthalenyl)methyl]-4-piperidinyl]amino]carbonyl]-3-pyridinecarboxamide the fluorine atom affects the ipso, alpha, beta, and gamma carbon nuclei resulting in observable carbon-fluorine coupling constants ranging from $J_{CF} = 247$ Hz to 4.5 Hz. Similar couplings are not observed for the bromine nucleus, because the higher halogens are quadrupolar nuclei, and as such have a very rapid relaxation pathway, so that the spin has relaxed before there is time for the coupling to develop.

Fluorine does not limit its coupling to carbon alone as it will also couple to protons, often complicating an already complex region of the $^1$H-NMR spectrum, especially for substituted aromatics. For example comparing Figures 3.5 and 3.12, the proton spectra of 1-bromo-2-methylnaphthalene and 1-bromo-2-methyl-6-fluoronaphthalene respectively, the effect of the fluorine can be clearly observed. In the first spectrum all six aromatic protons are clearly distinguishable along with their multiplicity and $J_{HH}$ coupling constants, while in the second spectrum the additional fluorine nucleus, apart from causing changes in some of the proton chemical shifts, also increases the multiplicity observed for some of the signals. For example, the protons at positions 7 and 8 become double doublets instead of doublets due to the additional $J_{HF}$ coupling which is of a different magnitude to the $J_{HH}$ couplings normally observed.

Spectral assignments for complex molecules are rarely possible using routine $^1$H, $^{13}$C and $^{13}$C Dept one-dimensional NMR spectroscopy alone. Therefore, it is customary
to use two-dimensional heteronuclear and homonuclear correlation NMR experiments to obtain extra structural information. Two dimensional heteronuclear experiments, in particular $^1$H/$^{13}$C correlations, are important due to the lack of multiplicity in the conventional decoupled $^{13}$C spectrum. Because of this, it is not always an easy matter to identify which carbon signals are which, while the use of chemical shift tables can only help in obtaining a good approximation to the position of any one peak - they can not be totally accurate. However, a two dimensional $^1$H/$^{13}$C correlation experiment yields cross signals for all protons and $^{13}$C nuclei which are connected by a $^{13}$C, $^1$H coupling over one bond. Therefore, the assignment of one member of a spin-coupled pair leads immediately to the assignment of the other.

Use of the homonuclear COSY (COrrelation SpectroscopY) pulse sequence generates a 2-D NMR spectrum in which the signals of a normal $^1$H-NMR spectrum are correlated with each other. Cross-peaks appear if spin coupling is present, thus the COSY sequence detects coupled pairs of protons. Since two or three bonds usually separate coupled protons, the connectivity and very often the chemical structure can be derived from the COSY spectrum. As such the COSY sequence is one of the most important and frequently used 2D NMR experiments. For example Figures 3.11 and 3.18 give the COSY NMR spectra of $N\text{-}[[1\text{-}[(1\text{-}bromo\text{-}2\text{-}naphthalenyl)methyl]4\text{-}piperidinyl]amino]carbonyl]3\text{-}pyridinecarboxamide$ in CDCl$_3$ and $N\text{-}[[1\text{-}[(1\text{-}bromo\text{-}6\text{-}fluoro\text{-}2\text{-}naphthalenyl)methyl]4\text{-}piperidinyl]amino]carbonyl]3\text{-}pyridinecarboxamide$ in CDCl$_3$ respectively. A number of coupled protons at the following positions can be identified:

- 7 (Naphthalene ring) couples to 8 (Naphthalene ring).
- 22 (Pyridine ring) couples to 23 (Pyridine ring).
- 23 (Pyridine ring) couples to 24 (Pyridine ring).
• 16 (Piperidine ring) couples to 17 (Amide proton).
• 14 and 15 (Piperidine ring axial and equatorial protons) couple to 16 (Piperidine ring).
• The expected couplings between the axial and equatorial protons at positions 12, 13, 14, and 15 are also observed.

Unfortunately due to the nature of the observed proton spectrum for Figure 3.18 no identifiable coupling is seen for the protons at positions 3 and 4 because they appear together as a double doublet. For Figure 3.11 no identifiable coupling is observed between protons at the following positions (3 & 4, 5 & 6, and 6 & 7), because again these signals are all very close to each other, and unfortunately the observed couplings overlap.

In summary, analysis of Figures 3.5 to 3.18, for the synthesis of the two 5-HT re-uptake inhibitors $N$-[[1-[(1-bromo-2-naphthalenyl)methyl]-4-piperidinyl]amino]carbonyl]-3-pyridinecarboxamide, and $N$-[[1-[(1-bromo-6-fluoro-2-naphthalenyl)methyl]-4-piperidinyl]amino]carbonyl]-3-pyridinecarboxamide, has enabled almost total proton and carbon NMR spectral assignments to be achieved for both compounds along with $J_{\text{H-H}}$ and $J_{\text{C-F}}$ couplings where observable, as shown in Tables 3.2 and 3.4. Unfortunately, due to the nature of the complexity of the observed spectra not all the quaternary carbons could be assigned with 100% certainty for both of the 5HT inhibitors, along with positions 3 and 5 for the $N$-[[1-[(1-bromo-2-naphthalenyl)methyl]-4-piperidinyl]amino]carbonyl]-3-pyridinecarboxamide derivative.

3.2.3 Attempted Tritiation Routes

Attempts to tritiate $N$-[[1-[(1-bromo-6-fluoro-2-naphthalenyl)methyl]-4-piperidinyl]amino]carbonyl]-3-pyridinecarboxamide by catalytic halogen-tritium
exchange at the 1-position using Pd/C (10%) proved to be unsuccessful. Furthermore attempts to perform a similar reaction on 1-bromo-2-methyl-6-fluoronaphthalene also failed. The reasons for these failures are unclear, although it appears from deuterium experiments, that the actual concentration of gas (deuterium or tritium) plays an important role in preventing competing reactions. At high concentrations of deuterium gas the reaction proceeds as expected, while at low concentrations in the presence of an inert carrier gas such as helium, unwanted exchange reactions occur preferentially.

Microwave-enhanced catalytic deuterodehalogenation of \( N-[[1-[(1-bromo-2-naphthalenyl)methyl]-4-piperidinyl]amino]carbonyl]3-pyridinecarboxamide \), has also been attempted, using deuterated potassium formate as the deuterium source, in the presence of both heterogeneous and homogeneous catalysts in DMSO. The microwave reaction conditions employed were those developed in house for the successful deuterodehalogenation of a variety of aromatic halides using deuterated metal formate salts in DMSO, in the presence of a metal catalyst (typically 10% Pd/C).

The actual experimental conditions employed were: 20 mg of \( N-[[1-[(1-bromo-2-naphthalenyl)methyl]-4-piperidinyl]amino]carbonyl]3-pyridinecarboxamide \), 10 mg of catalyst, 50 mg of dry deuterio-potassium formate \((\text{K}^+\text{CDOO}^-)\), and 1 ml of DMSO, were placed in a 25 ml pear shaped flask which was fitted with a pressure release stopper. The contents of the flask were frozen, before the flask was evacuated prior to the reaction taking place. After evacuation the contents were allowed to thaw before the flask was placed in a 400 ml Pyrex beaker and surrounded with vermiculite. The beaker was then placed in a Matsui M167BT microwave oven along with a further 50 ml beaker full of cold water. The instrument was then set on defrost for a period of 2 minutes. On completion of the time period the flask was allowed to cool for 3 minutes, the beaker of
water changed before the microwave settings were reset as above, and this procedure
repeated a further four times. [The beaker of water is present to absorb some of the
energy released in the event of an explosion and limit the extent of any damage].\textsuperscript{247} The
resultant reaction mixture was then treated to remove the catalyst employed and the
remaining solution lyophilised to concentrate any deuterated material. For all the
catalysts tried; 10\% palladium on carbon, palladium (II) acetylacetonate,
(bicyclo[2.2.1]hepta-2,5-diene)[bis(triphenylphosphine)] rhodium (I) PF\textsubscript{6}, and platinum
(II) acetylacetonate no discernible deuteration was observed under the described reaction
conditions. However, a review of the literature shows another viable avenue for
exploration, in that formate salts have also been used under thermal conditions in the
dehalogenation of organic halides in the presence of a variety of ruthenium and rhodium
complexes\textsuperscript{248} as well as the more usual Typically these reaction conditions
involve the use of solvents that are not particularly suited to the microwave conditions
such as alcohols and dioxane.

With the exception of catalytic halogen-tritium exchange on compounds of the
type I and II, other routes to the preparation of these tritium labelled compounds are
possible. For example catalysed exchange reactions with tritiated water should lead to a
generally labelled compound - particularly on the pyridine ring. Use of \textit{RuCl\textsubscript{3}} catalyst in
\textit{N,N}-dimethylformamide should lead to ortho labelling on the pyridine ring from the effect
of the amide functional group. The synthesis of similar unsaturated compounds such as
III or IV would allow the use of catalytic hydrogenation to yield specifically labelled
compounds of type I. Figure 3.4 shows a proposed reaction route to the synthesis of
compounds of type III. Should the label still be desired in the 1-position then reaction of
butyllithium with compounds of the type I or II to metallate the 1-position, followed by reaction with HTO should yield the desired tritiated compound.

![Diagram of chemical structures](image)

**Figure 3.4** Proposed synthesis for compounds of the type III.\[^{231,251}\]
3.3 EXPERIMENTAL

3.3.1 Synthesis of \( \text{N-[[[1-[(1-bromo-2-naphthalenyl)methyl]-4-piperidiny]amino]carbonyl]-3-pyridinecarboxamide} \)

A solution of bromine (840 mg, 270 \( \mu l \), 5% excess) in glacial acetic acid (5 ml) was added dropwise over a 15 minute period to a stirred, ice-cooled solution of 2-methyl-naphthalene (711 mg, 5 mmol) in glacial acetic acid (15 ml). The reaction was allowed to stand overnight, before being diluted with water (10 ml) and the product extracted with chloroform (3 \( \times \) 10ml). The organic extract was then washed with distilled water (5 \( \times \) 5ml), 5% aqueous sodium bicarbonate solution (2 \( \times \) 5ml), and finally a aqueous saturated sodium chloride solution (2 \( \times \) 5ml), before being dried over sodium sulphate for an hour. After drying the organic solution was decanted off, and the chloroform removed by rotary evaporation, to yield an oil, 645 mg (58.3%) of 1-bromo-2-methyl-naphthalene.

1-bromo-2-methyl-naphthalene (620 mg, 2.8 mmol), \( N \)-bromosuccinimide (500 mg, 2.8 mmol), benzoyl peroxide ("semi-micro spatula tip" \( \approx \) 1 mg), and 1,1'-azobis-(cyclohexanecarbonitrile) ("semi-micro spatula tip" \( \approx \) 1 mg) were placed in a 50 ml round bottomed flask to which 30 ml of carbon tetrachloride was added. The reaction mixture was then heated at reflux for 2.5 hours at which point \(^1\text{H}-\text{NMR} \) analysis showed over 90% complete reaction yielding 1-bromo-2-\( \alpha \)-bromomethyl-naphthalene. The solvent was then separated from the succinimide by-product and evaporated by rotary evaporation before a solution of 4-nicotinoylureidopiperidine (NUP) (803 mg, 3.23 mmol) and \( N,N \)-diisopropylethylamine (392 mg, 525 \( \mu l \), 3.03 mmol) in hot DMF (15 ml) was added in one portion with stirring to the residue. The mixture was stirred at ambient temperature
for 20 hours, before being diluted with water (30 ml) and hexane (15 ml). The precipitated product was collected by filtration and washed well with water (3 x 5 ml) and hexane (3 x 5 ml) before being recrystallised from ethanol and lyophilised to yield 986 mg (75.2%) of \( N\)-[[1-[(1-bromo-2-naphthalenyl)methyl]-4-piperidinyl]amino]carbonyl]-3-pyridinecarboxamide.

**Experimental Note:** The NBS should be pumped at 0.05 mm pressure over 2 days to remove traces of bromine, thus avoiding any ring substitution products.

### 3.3.1.1 Spectral assignment of 1-bromo-2-methylnaphthalene

![1-bromo-2-methylnaphthalene](image)

**Table 3.1** \(^1\)H and \(^{13}\)C NMR assignments for 1-bromo-2-methylnaphthalene.

<table>
<thead>
<tr>
<th>Position</th>
<th>(\delta^1)H (ppm)</th>
<th>Multiplicity</th>
<th>(J_{\text{H-H}}) (Hz)</th>
<th>(\delta^{13})C (ppm)</th>
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<td>—</td>
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<td>Singlet (3H)</td>
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Figure 3.5 $^1$H NMR spectrum of 1-bromo-2-methylnaphthalene in CDCl$_3$ (a comparison spectrum of authentic material is shown in Appendix B).

Figure 3.6 $^{13}$C NMR spectrum of 1-bromo-2-methylnaphthalene in CDCl$_3$. 
3.3.1.2 Spectral assignment of \( \text{N-}[[(1-\text{bromo-2-naphthalenyl})\text{methyl}-4-piperidinyl]amino]carbonyl]-3-pyridinecarboxamide \)

![Chemical structure](image)

\( \text{N-}[[(1-\text{bromo-2-naphthalenyl})\text{methyl}-4-piperidinyl]amino]carbonyl]-3-

pyridinecarboxamide

<table>
<thead>
<tr>
<th>Position</th>
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<th>( J_{\text{H-H}}) (Hz)</th>
<th>( \delta \text{ }^{13}\text{C}) (ppm)</th>
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Table 3.2 \(^1\text{H} \) and \(^{13}\text{C}\) NMR assignments for \( \text{N-}[[(1-\text{bromo-2-naphthalenyl})\text{methyl}-4-piperidinyl]amino]carbonyl]-3-

pyridinecarboxamide.
<table>
<thead>
<tr>
<th></th>
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<th>Assignment</th>
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<td>—</td>
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<td>—</td>
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<td>—</td>
<td>*</td>
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<td>Singlet (1H)</td>
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* = Unable to assign these quaternary carbons with total accuracy.

** = Unable to assign these aromatic carbons with total accuracy.

Figure 3.7 $^1$H NMR spectrum of $N$-[[1-((1-bromo-2-naphthalenyl)methyl)-4-piperidinyl]amino]carbonyl]-3-pyridinecarboxamide in CDCl$_3$. 
Figure 3.8 $^{13}$C NMR spectrum of $N$-[[1-[(1-bromo-2-naphthalenyl)methyl]-4-piperidinyl]amino]carbonyl]-3-pyridinecarboxamide in CDCl$_3$.

Figure 3.9 $^{13}$C DEPT NMR spectrum of $N$-[[1-[(1-bromo-2-naphthalenyl)methyl]-4-piperidinyl]amino]carbonyl]-3-pyridinecarboxamide in CDCl$_3$. 
Figure 3.10 $^1$H/$^{13}$C Correlation NMR spectrum of $N^1$-$[1-[(1-bromo-2-naphthalenyl)methyl]-4-piperidinyl]amino]carbonyl]-3$-pyridinecarboxamide in CDCl$_3$. 
Figure 3.11 $^1\text{H}/^1\text{H}$ Cosy NMR spectrum of $N$-[[1-[(1-bromo-2-naphthalenyl)methyl]-4-piperidinyl]amino]carbonyl]-3-pyridinecarboxamide in CDCl$_3$. 
3.3.2 Synthesis of N-[[1-[(1-bromo-6-fluoro-2-naphthalenyl)methyl]-4-piperidinyl]amino]carbonyl]-3-pyridinecarboxamide

A solution of bromine (263 mg, 85 μl, 5% excess) in glacial acetic acid (2 ml) was added dropwise over a 15 minute period to a stirred, ice-cooled solution of 2-methyl-6-fluoronaphthalene (250 mg, 1.56 mmol) in glacial acetic acid (10 ml). The reaction was allowed to stand overnight, before being diluted with water (10 ml) and the crystalline precipitate collected and first washed with a cold 1:1 mixture of glacial acetic acid and water (2 x 5 ml), and then with distilled water (2 x 5 ml). The compound was then recrystallised from ethanol:water (70:30) before being lyophilised to yield 229 mg (61.4%) of 1-bromo-2-methyl-6-fluoronaphthalene.

Powdered dry 1-bromo-2-methyl-6-fluoronaphthalene (200 mg, 0.84 mmol), N-bromosuccinimide (150 mg, 0.84 mmol), benzoyl peroxide ("semi-micro spatula tip" ≈ 1 mg), and 1,1'-azobis-(cyclohexanecarbonitrile) ("semi-micro spatula tip" ≈ 1 mg) were placed in a 25 ml round bottomed flask to which 15 ml of carbon tetrachloride was added. The reaction mixture was then heated at reflux for 2.5 hours at which point \(^1\)H-NMR analysis showed over 90% complete reaction yielding 1-bromo-2-α-bromomethyl-6-fluoronaphthalene. The solvent was then separated from the succinimide by-product and evaporated by rotary evaporation before a solution of 4-nicotinoylureidopiperidine (NUP) (248 mg, 1.0 mmol) and \(N,N\)-diisopropylethylamine (120 mg, 160 μl, 0.93 mmol) in hot DMF (10 ml) was added in one portion with stirring to the residue. The mixture was stirred at ambient temperature for 20 hours, before being diluted with water (30 ml) and hexane (15 ml). The precipitated product was collected by filtration and washed well with water (3 x 5 ml) and hexane (3 x 5 ml) before being recrystallised from ethanol and
lyophilised to yield 348 mg (85.7%) of \( N-[[(1-\{(1\text{-bromo-6-fluoro-2-naphthalenyl)}\text{methyl}\})-4\text{-piperidinyl}]\text{amino}]\text{carbonyl}\]-3\text{-pyridinecarboxamide}. 

**Experimental Note:** The NBS should be pumped at 0.05mm pressure over 2 days to remove traces of bromine, thus avoiding any ring substitution products.

### 3.3.2.1 Spectral assignment of 1-bromo-2-methyl-6-fluoronaphthalene

![1-bromo-2-methyl-6-fluoronaphthalene](image)

**Table 3.3** \( ^1\text{H} \) and \( ^{13}\text{C} \) NMR assignments for 1-bromo-2-methyl-6-fluoronaphthalene.

<table>
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<tr>
<th>Position</th>
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<th>Multiplicity</th>
<th>( J_{\text{H-H}} )</th>
<th>( \delta , ^{13}\text{C} ) (ppm)</th>
<th>( J_{\text{C-F}} )</th>
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<td>124.15</td>
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<td>—</td>
<td>—</td>
<td>—</td>
<td>135.45</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>7.29 - 7.31</td>
<td>Multiplet (1H)</td>
<td>—</td>
<td>129.96</td>
<td>—</td>
</tr>
<tr>
<td>4</td>
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</tr>
<tr>
<td>5</td>
<td>7.29 - 7.31</td>
<td>Multiplet (1H)</td>
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<td>6</td>
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<td>Singlet (3H)</td>
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<td>24.20</td>
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</table>

* = Unable to differentiate with total accuracy \( J_{\text{H-H}} \) couplings from \( J_{\text{H-F}} \) couplings.
Figure 3.12 $^1$H NMR spectrum of 1-bromo-2-methyl-6-fluoronaphthalene in CDCl$_3$.

Figure 3.13 $^{13}$C NMR spectrum of 1-bromo-2-methyl-6-fluoronaphthalene in CDCl$_3$. 
3.3.2.2 Spectral assignment of \( N-[[1-[(1-bromo-6-fluoro-2-naphthalenyl)methyl]-4-piperidinyl]amino]carbonyl]-3-pyridinecarboxamide \)

\[
\begin{align*}
\text{Table 3.4} & \quad \text{\( ^1 \text{H} \) and \( ^{13} \text{C} \) NMR assignments for \( N-[[1-[(1-bromo-6-fluoro-2-naphthalenyl)methyl]-4-piperidinyl]amino]carbonyl]-3-pyridinecarboxamide.} \\
\end{align*}
\]

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<th>Multiplicity</th>
<th>( J_{\text{H,H}} ) (Hz)</th>
<th>( \delta \text{( ^{13} \text{C} )} (ppm) )</th>
<th>( J_{\text{C,F}} ) (Hz)</th>
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<td>—</td>
</tr>
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* = Unable to assign these quaternary carbons with total accuracy.

**Figure 3.14** $^1$H NMR spectrum of $N$-[[1-[(1-bromo-6-fluoro-2-naphthalenyl)methyl]-4-piperidinyl][amino]carbonyl]-3-pyridinecarboxamide in CDCl$_3$. 
Figure 3.15 $^{13}$C NMR spectrum of $\text{N-[[[1-[(1-bromo-6-fluoro-2-naphthalenyl)]methyl]-4-piperidinyl]amino]carbonyl]-3-pyridinecarboxamide}$ in $\text{CDCl}_3$.

Figure 3.16 $^{13}$C DEPT NMR spectrum of $\text{N-[[[1-[(1-bromo-6-fluoro-2-naphthalenyl)]methyl]-4-piperidinyl]amino]carbonyl]-3-pyridinecarboxamide}$ in $\text{CDCl}_3$. 
Figure 3.17 $^1$H/$^{13}$C Correlation NMR spectrum of $N$-[[1-[(1-bromo-6-fluoro-2-naphthalenyl)methyl]-4-piperidinyl]amino]carbonyl]-3-pyridinecarboxamide in CDCl$_3$. 
Figure 3.18 \(^1\text{H}/^1\text{H} \) Cosy NMR spectrum of \(N^-[[1-[(1\text{-bromo-6-fluoro-2-naphthalenyl})\text{methyl}][4\text{-piperidinyl}][\text{amino}][\text{carbonyl}]]\text{3-pyridinecarboxamide in CDCl}_3\).
Chapter 4

Solid State Tritium NMR Spectroscopy

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4.1 INTRODUCTION TO SOLID STATE NMR SPECTROSCOPY

Nuclear magnetic resonance spectroscopy of solid materials is a considerably less advanced technique than the high resolution NMR spectroscopy of liquids. This is partly due to the development of Fourier transform NMR in the 1970’s, which focused applications on the structural analysis of dissolved molecules, while giving impetus to the development of methods and instrumentation. The more demanding requirements of solid state spectroscopy were kept in abeyance. Nevertheless, the very first NMR experiments were carried out on both liquids and solids.\textsuperscript{252,253}

Solid state NMR line widths are inherently broad by comparison with liquid or solution spectra, the main differences between the liquid and solid state being the time scale and geometry of molecular motions. These affect the NMR signal by modulating different interactions between the spins and their environment. The interactions are homo- and heteronuclear dipolar couplings, quadrupolar interactions, and the anisotropy of the chemical shift.

These couplings are also present in molecules in the liquid state, but in liquids rotational motion, which is fast compared to the NMR time scale, causes averaging of each Hamiltonian operator to reduce it down to its trace value. This eliminates all interactions except for chemical shift and scalar coupling, resulting in high-resolution spectra. These eliminated interactions are only observable on an intramolecular scale, as used in NOE experiments. In the solid state, the molecular motion is strongly reduced and therefore all the hamiltonian operators are non-zero and thus contribute to the line shape. For a given spin system the total Hamiltonian is composed of the following combinations:
\[ H = H_Z + H_{R.F.} + H_{CS} + H_Q + H_{SR} + H_D + H_J \]  

(Equation 4.1)

where, \( H_Z \) = Zeeman interaction, \( H_{R.F.} \) = spin interaction with the external r.f. field, \( H_{CS} \) = chemical shift, \( H_Q \) = quadrupolar interaction, \( H_{SR} \) = spin rotation, \( H_D \) = dipolar coupling, \( H_J \) = J-coupling.

The dominant operators in solid state NMR spectroscopy that are responsible for line broadening are \( H_D \), \( H_{CS} \), \( H_Q \). The relevant Hamiltonians are discussed in a review by Smith et al.\(^{254, 255}\)

4.2 DIPOLAR INTERACTION

The principal broadening mechanism in solid state NMR is due to dipolar coupling, which is caused by the interaction of two (or more) nuclear dipoles. All nuclei with spin \( I \neq 0 \) possess a nuclear magnetic dipole moment, thus the magnetic fields of moments \( \mu \) of nuclei in the neighbourhood produce local fields at the site of the nucleus under observation.\(^{256}\) The interaction between two nuclear dipoles \( \mu_j \) and \( \mu_k \) separated by a distance \( r_{jk} \) causes the eigenstates of the spin systems to be either stabilised or destabilised. The energy of the interaction is given by the expression:\(^{257}\)

\[ E = \frac{\mu_j \cdot \mu_k}{r_{jk}^4} \left( \frac{3(\mu_j \cdot r_{jk})(\mu_k \cdot r_{jk})}{r_{jk}^6} \right) \]  

(Equation 4.2)

where \( r_{jk} \) is the distance vector between the two nuclei.
For multiple, $N$, spins, the dipolar Hamiltonian can be expressed by the summation,

$$H_D = \frac{1}{2} \sum_{j=1}^{N} \sum_{k=1}^{N} \left\{ \frac{\mu_j \cdot \mu_k}{r_{jk}^3} - \frac{3}{2} \frac{\mu_j \cdot r_{jk}}{r_{jk}^5} \cdot \frac{\mu_k \cdot r_{jk}}{r_{jk}^5} \right\}$$

(Equation 4.3)

(The energy is halved so that each spin interaction is not counted twice and $j \neq k$).

Figure 4.1 below shows the situation for two spins, where $H_0$ is parallel to the $z$-axis.
(The orientation of the internuclear vector with respect to the magnetic field is characterised by polar co-ordinates).

![Figure 4.1 Dipole-Dipole interaction.](image)

Given that; $\mu = \gamma \cdot H \cdot I$ and $I = I_x \cdot x + I_y \cdot y + I_z \cdot z$, the Equation 4.3 can be expanded, in terms of polar co-ordinates. The resulting dipolar Hamiltonian can be written as a sum of six terms. The result is the dipole alphabet (the terms $I^\pm = I_x \pm iI_y$ are the raising and lowering operators and have the effect of causing a change up or down in the quantum number, $m$, of a spin),

$$H_D = \left( \frac{\gamma \cdot H \cdot I}{r^3} \right) (A + B + C + D + E + F)$$

(Equation 4.4)

$$\Lambda = I_{1z} \cdot I_{2z} (1 - 3 \cdot \cos^2 \theta)$$

(Equation 4.5)
The first term on the right hand side of Equation 4.4 is the dipolar coupling constant (not to be confused with the term D of the alphabet expansion).

\[ D = \frac{\gamma_1 \gamma_2 \hbar^2}{r^3} \]  

(Equation 4.11)

\( \hbar = h/2\pi, \) i.e. Planck’s constant \( \pm 2\pi \).

The spin interaction of each term in the dipole alphabet can be seen for a two-spin system in Figure 4.2.

**Figure 4.2** Spin interaction for two dipoles in a IS system.
The A term describes the static dipolar field produced at one spin due to the z-component of a magnetic moment of another nucleus. The precessional motion of a magnetic moment, $\mu_0$, at the Larmor frequency, due to the presence of $H_0$, results in time dependent $x$ and $y$ components of the dipolar field occurring at another nucleus, $I_k$. Therefore, the terms B-F describe coherences between the spin states caused by oscillating dipolar fields.\(^{259}\) In the heteronuclear AX case and for $H_2 \gg H_D$ only the A term is secular and contributes to a reduced Hamiltonian,

$$H_D = -\hbar (v_A \cdot I_{Ax} + v_x I_{Ix}) + D (I_{Ax} I_{Ix}) (1 - 3 \cos^2 \theta)$$  \hspace{1cm} (Equation 4.12)

($v$ is the resonant frequency for the spin).

The consequence of Equation 4.12 is that, the spectrum of spin $A$ will consist of two lines, each having a frequency given by:

$$v = v_A \pm \frac{1}{2} D (1 - 3 \cos^2 \theta)$$  \hspace{1cm} (Equation 4.13)

Thus, for a pair of isolated spins in a single crystal the spectrum of A (or X) will appear as two single lines with a separation of $D (3 \cos^2 \theta - 1)$ depending on the crystal orientation with respect to the external field. When the crystal is at an angle of $54^\circ 44'$ to the external field then $D (3 \cos^2 \theta - 1)$ becomes zero and a single line is observed. However, in the case of a powder all orientations are possible and these two lines form the "Pake Doublet",\(^{260}\) where the separation of the horns is equal to $D$, as shown in Figure 4.3.
In the homonuclear dipolar coupling case, the flip-flop (B) term is now secular and thus contributes to the dipolar Hamiltonian.\(^{258}\)

\[
\mathcal{H}_{D,\text{Hom}} = D \cdot (3 \cdot \cos^2 \theta - 1) \left[ I_{iz} \cdot I_{iz} - \frac{1}{4} (I_1^+ \cdot I_2^- + I_1^- \cdot I_2^+) \right] \tag{Equation 4.14}
\]

where \( I_{iz} \cdot I_{iz} = \frac{1}{4} (I_1^+ \cdot I_2^- + I_1^- \cdot I_2^+) \) can be re-written as \( \frac{1}{2} (3 \cdot I_{iz} \cdot I_{iz} - I_1 \cdot I_2) \). Thus, for a homonuclear two-spin system, the spin transitions will occur at,

\[
v = v_0 \pm \frac{3}{4} D \cdot (1 - 3 \cdot \cos^2 \theta) \tag{Equation 4.15}
\]

giving a doublet separation of \( \frac{3}{2} D \cdot (1 - 3 \cdot \cos^2 \theta) \).

As the dipolar coupling constant, \( D \), contains an internuclear separation term, \( r_{ij} \), the magnitude of dipolar broadening will also depend on whether the nuclear spins are abundant or dilute. An organic solid consists largely of C-H bonds and the close proximity and abundance of protons causes the \(^{13}\text{C}\) resonance to be dominated by heteronuclear dipolar coupling. A \(^{13}\text{C}-^{13}\text{C}\) homonuclear coupling will also be present but since the coupled nuclei are likely to be in different molecules (\( r_{12} \) is large) the interaction is much weaker.
4.3 CHEMICAL SHIFT ANISOTROPY

Another source of NMR line broadening in solid samples is chemical shift anisotropy (CSA). CSA arises from the non-spherical distribution of electrons around a nucleus. This variation in shielding can be described by a 2\textsuperscript{nd} rank tensor, where the value of the shielding constant for a particular direction, defines the electron density surrounding the nucleus with respect to the static field. A rigorous treatment of the shielding tensor can be found in the article by Anet and O'Leary.\textsuperscript{261,262}

For rapid and random rotation in the liquid state, the shielding experienced by the nucleus is described by a shielding constant $\sigma_{zz}$. The value of $\sigma_{zz}$ is derived from the averaging of the shielding tensor, due to the rapid molecular re-orientation, and is given by:\textsuperscript{258}

$$\sigma_{zz} = (1/3)(\sigma_{11} + \sigma_{12} + \sigma_{13})$$  \hspace{1cm} (Equation 4.16)

while the chemical shift part of the Hamiltonian is expressed as:

$$H_{\text{CS}} = -\gamma \hbar B_0 \sigma \cdot I$$  \hspace{1cm} (Equation 4.17)

$B_0 = \text{External magnetic field, } I = \text{Angular momentum of the nucleus}$

In the solid state, however, differing nuclear sites will be orientated in all possible directions, so there will be a distribution of local fields and, hence, a broadened line. However, if the solid sample is rotated at an angle frequency $\omega_r$ about an axis making an angle $\theta$ with respect to the magnetic field $B_0$ and angles $\chi_{11}, \chi_{12},$ and $\chi_{13}$ with respect to the principal axes of the sample then we obtain the following equation for the shielding constant.$^{258}$
\[ \sigma_{zz} = \frac{1}{2} (\sin^2 \theta + \cos^2 \theta - 1) \sum_p \sigma_{pp} \cos^2 \chi_p \] (Equation 4.18)

Therefore it can be seen that if \( \theta = 54^\circ 44' \), the same angle as for the dipole Hamiltonian, then Equation 4.18 reduces to the liquid state Equation 4.16.

### 4.4 Quadrupolar Interaction

For nuclei with spin \( >\frac{1}{2} \) the quadrupolar effect dominates interactions and the total spin hamiltonian can be largely described by:

\[ H = H_Z + H_Q \] (Equation 4.19)

The charge distribution for spin \( \frac{1}{2} \) nuclei is spherical, however for spin \( >\frac{1}{2} \) nuclei the charge distribution is spheroidal. The nucleus now possesses a quadrupole moment, \( Q \), the sign of which is dependent on whether the spheroid is prolate (positive \( Q \)) or oblate (negative \( Q \)) with respect to the axis of the nuclear spin. The quadrupolar hamiltonian for a spin \( I >\frac{1}{2} \) is expressed as:

\[ H_Q = I \cdot Q \cdot I \] (Equation 4.20)

where \( Q \), the quadrupole coupling tensor, fluctuates with molecular motion and \( H_Q \) induces transition between energy levels. An article by Taulelle\textsuperscript{263} gives a rigorous treatment of the quadrupolar effect.

### 4.5 Relaxation in Solids

The effect of restricted motion in the solid state has a quite marked effect on the nuclear relaxation times. Spin-spin relaxation tends to be very rapid due to large correlation times for the nucleus. The inverse effect is true for spin-lattice relaxation as
the lack of molecular motion reduces the spread and intensity of the random magnetic fields necessary to induce transitions.

### 4.6 METHODS OF LINE NARROWING

#### 4.6.1 Magic Angle Spinning

The rapid and random rotation of molecules in the liquid state tends to average out the line broadening due to the dipole-dipole interaction between nuclear spins, making possible the observation of many high resolution features in the solution NMR spectrum. For the solid state there is an experimental technique that under many circumstances has a similar effect - magic angle spinning (MAS). Magic-angle spinning (MAS) was developed by Andrew\(^2\) and Lowe\(^3\) as a method for suppressing the dipole-dipole interaction in solids. The effect of spinning the sample is analogous to the effect of rapid and random molecular rotation in a liquid sample. It is based on rotating a sample at the so-called magic angle of \(54.74^\circ\) for which the value of \((3\cos^2\theta - 1)\) (present in many of the above equations) is zero. Figure 4.4 shows the internuclear vector being rotated about the magic angle. By rotating at high speed \(\theta_m\) (the angle between the vector \(r_{jk}\) and the axis of rotation), becomes the magic angle, removing the appropriate interactions.

![Figure 4.4](image-url)  
**Figure 4.4** Internuclear vector rotating about the magic angle.
When a sample is spun at the magic angle the resonance will collapse to the isotropic shift value of the spin. However, if the spinning speed is less than the line width of the static solid resonance all the interactions are not completely removed and satellite transitions, known as spinning side bands, appear at multiples of the rotor speed.

It can be seen from Equations 4.12 and 4.18 that MAS can be used to remove the dipolar interaction and chemical shift anisotropy. The first order quadrupolar interaction also has \((3\cos^2\theta - 1)\) dependence for an axially symmetric molecule, and can thus be removed by MAS. However, if the site symmetry for the nucleus is less than axially symmetric then the centre band transition, broadened by the term \((\eta\sin^2\theta\cos2\phi)\), will only be partially averaged by MAS. The second order quadrupolar effect contains no \((3\cos^2\theta - 1)\) terms and thus even for axially symmetric molecules MAS will have only a small effect on the line width.

### 4.6.2 Double Rotation and Dynamic Angle Spinning

Higher order interactions of quadrupolar nuclei do not have a \(3\cos^2\theta - 1\) dependence in their Hamiltonian, thus, spinning at the dipolar magic angle only partially removes their interactions. A technique to remove second order quadrupolar effects has been developed by Samosen and Pines.\(^{266,267}\) This is known as double rotation. The double rotor (DOR) NMR makes use of two rotors, one inside the other, with individual rotational frequencies and relative angles to \(H_0\) (and each other). Dynamic angle spinning (DAS), which can achieve the same effect as DOR, was developed by Llor and Virlet.\(^{268}\) In this method a single rotor is placed at \(N\) discrete angles for a fraction of time, \(\kappa_i\), such that:
\[ \sum_{i}^{N} P_2(\cos \theta_i) \kappa_i = \sum_{i}^{N} P_4(\cos \theta_i) \kappa_i = 0 \]  
*(Equation 4.21)*

An infinite number of complementary angles satisfy the conditions of Equation 4.21 and have been calculated by Mueller et al. \(^{269}\) The simplest solution of Equation 4.21 is for two angles, where \( \kappa_1 = \kappa_2 \) such that \( \theta_1 = 37.38^\circ \) and \( \theta_2 = 79.19^\circ \).

### 4.6.3 Dipolar Decoupling

If the nucleus of interest is contained within a molecule where there is an abundance of a second spin, then a strong dipolar coupling will exist between them causing a large broadening of the line width. An example of this is the very large \(^{13}\)C line widths (c.a. 5,000 - 20,000Hz) obtained in organic solids from \(^{13}\)C-\(^{1}\)H coupling. Much of the linewidth is due to the dipole - dipole interaction of the \(^{13}\)C nuclear spin with the sea of abundant proton spins. The removal of heteronuclear dipolar coupling was first achieved by Pines et al.\(^{270,271}\) for the proton decoupled \(^{13}\)C spectra of adamantane. By applying irradiation at the proton resonance frequency the dipolar (and scalar) coupling is removed. While related to decoupling in the liquid state, decoupling in the solid state differs experimentally in that the r.f power in decoupling radiation must be several orders of magnitude higher in order for all the proton transitions to be saturated in the broad line proton NMR spectrum. When used in combination with MAS the line widths obtained can be compared with those of liquids.

This heteronuclear decoupling differs from homonuclear decoupling in which the linewidth, for example, of the proton resonance is to be reduced by suppressing the dipole - dipole interactions among the protons themselves. In this case the high powered decoupling irradiation would mask the resonance experiment itself. Therefore other
Chapter 4  Solid State Tritium NMR Spectroscopy

approaches have been developed, in particular, ultra-fast MAS (>20 kHz), or more commonly by pulse sequence manipulation which achieve decoupling among like spins, for example WaHuHa.\textsuperscript{272}

4.6.4 Cross Polarisation

Cross polarisation is an experimental technique developed by Pines, Gibby and Waugh\textsuperscript{271} for increasing the sensitivity in the NMR of sparse nuclear spins (such as natural abundance \textsuperscript{13}C). The basic aim of the method is to use the relatively strong spin polarisation of abundant spins such as protons to enhance the signal to noise of the rare spin. The experiment initially performed in liquids, using experiments such as INEPT,\textsuperscript{273,274} was applied to solids by Hartmann and Hahn.\textsuperscript{275} Polarisation transfer occurs through the abundant spin (with a lower spin temperature) being placed in contact with the rare spin (of higher spin temperature).

Establishing the cross polarisation transfer from the abundant spin to the rare spin is accomplished by having both r.f. fields present simultaneously under the Hartmann-Hahn condition\textsuperscript{275} shown in Equation 4.22,

\[ \gamma_A H_A = \gamma_R H_R \]  

(Equation 4.22)

where \(\gamma_A\) and \(\gamma_R\) are the magnetogyric ratios of the abundant and rare spins respectively. The quantity \(\gamma_A H_A\) is the precession frequency of abundant spin nuclear magnetic moments about the abundant spin r.f. field \(H_A\), and \(\gamma_R H_R\) is the precession frequency of rare spin nuclear magnetic moments about \(H_R\). As these frequencies are the same, it follows that there are components of the abundant and rare spin dipolar fields along the \(Z'\) axis that fluctuate at the same frequency, in effect acting like identical spins, therefore
enabling rapid polarisation transfer to occur by the flip-flop (B) term of the dipolar alphabet (Equation 4.6).

Additionally because the two spins are now in contact with each other and since the spin temperature of the abundant spin is much lower than that of the rarer spin, then 'heat' energy flows to equilibrate the system. Heat transfer takes the form of a transfer of spin polarisation from the rare spin to the abundant spin. The result of the dramatic spin temperature drop in the rare spin is to increase the nuclear spin magnetization and therefore its sensitivity. For example, in dipolar coupled proton (abundant) and carbon (rare) spins the effect of additional cross polarisation results in a 4-fold gain of $^{13}$C sensitivity compared to straight decoupling.

4.7 EXPERIMENTAL

4.7.1 Introduction

As highlighted in Chapter 1, the chemistry of tritium is virtually identical to that of hydrogen at low levels of substitution, while it's low natural abundance and the high sensitivity of the beta liquid scintillation counting method of detection make it an ideal label for proton and carbon sites. Deuterium, is also used, but the natural abundance is much higher and the available detection methods less sensitive. Deuterium is not a good candidate for high resolution solution-state NMR, as it has a spin of 1 and a low magnetogyric ratio. Tritium, on the other hand, is almost ideal, having a spin of $\frac{1}{2}$ and a magnetogyric ratio higher than that of hydrogen. The dangers from the radioactivity are minimised by adopting a double-containment and sealed tube approach. Consequently the technique is now widely used in the pharmaceutical industry where the relative ease
with which tritium-labelled compounds can be prepared and at high specific activities, are additional favourable factors.

Despite its radioactivity tritium has several features in common with $^{13}$C. Both nuclei having a nuclear spin of $\frac{1}{2}$ and natural abundance levels that are very low, (particularly for $^3$H). The development of $^{13}$C-NMR spectroscopy of solids, over the last 20 years, has seen its evolution into a very powerful technique, with applications covering a wide range of interests. Therefore, the possibility exists of developing $^3$H-NMR spectroscopy of solids in an analogous manner, providing the rigorous safety requirements can be met. Moreover, with the observed high sensitivity of solution-state tritium NMR spectroscopy, solid-state detection should be possible at acceptable levels of radioactivity.

Rapid molecular reorientation in the liquid state is the main reason why the intrinsic line widths of solution NMR spectra are so narrow. In contrast, in most solids, molecular reorientation is not sufficiently fast to average the dipolar spin interactions, consequently broad lines are obtained. These are usually narrowed by a combination of techniques- magic angle spinning, high power proton decoupling, cross polarisation and multiple-pulse sequences of which the former is probably the most important, all of which have been discussed in the sections above. Low power proton decoupling has been regularly used in obtaining $^3$H NMR solution spectra, but the small differences in the $^1$H and $^3$H frequencies (~6%) pose a problem for the solid state. A similar situation exists, and has been recently been solved, in the case of $^{19}$F-NMR spectroscopy,\textsuperscript{276,277} which has a Larmor frequency about the same value below protium, suggesting that this difficulty can be overcome. The need to decouple $^1$H-$^3$H interactions can, of course, be avoided by employing the appropriate deuterated compounds,\textsuperscript{278} although it is not always practicable to do so.
Tritium like $^{13}$C is (normally) isotopically dilute, without homonuclear dipole-dipole interactions, but, unlike $^{13}$C, tritium is a very sensitive nucleus (1.06 times as sensitive as protium, at constant field) and cross-polarisation is not required, to enhance the signal. Furthermore tritium like $^{13}$C, shows heteronuclear dipole-dipole interaction with protium, but, in this case, the frequencies are close and high-power dipolar decoupling presents severe technical difficulties.

In order to produce substantial narrowing of the broad lines expected sample spinning rates must be of the same order as the spread of the spectral frequencies. It is therefore fortunate that recent improvements in rotor design now make it possible to do this with much greater levels of confidence than hitherto. Nevertheless with the proposed use of radioactive materials, it is necessary to ensure that in the event of a breakage the radioactivity is contained and contamination minimised.

The system evaluated was a second-generation commercial MAS probe, Bruker 4mm double air bearing probe for a narrow-bore cryomagnet. The tuning circuit of the High-frequency channel had been modified to allow it to be adjusted up to 320 MHz, but it was, otherwise, standard. The rotors were plain yttrium stabilised zirconia cylinders with finned, “Kel-F” turbine-caps. (The standard rotors were rated safe, up to 12 kHz and a later, heavy-wall version, up to 20 kHz). In operation, a large volume of air was forced through the two air bearings and the turbine jets in the stator, to produce rotation rates of up to 17.5 kHz. The bottom of the probe projected below the magnet base. The top of the probe was extended by a “chimney”, to the top of the magnet bore, so that samples could be loaded without dismantling the equipment, and the air escaped at both ends.

Before a magic-angle spinning experiment on tritiated material could be attempted, it was necessary to demonstrate that the radioactive material could be contained, in the event of rotor breakage. Any powdered material, released by such
breakage, would be entrained by the air system and expelled from the top or bottom of the probe. To satisfy the safety requirement, the top and bottom of the probe assembly were shrouded and connected to a “High Efficiency Particulate Arrestance” filter (BS 3928), with semi-rigid tubing. The filter was attached to a two-stage, centrifugal exhauster, which was capable of maintaining some 10 cm water gauge vacuum, at the probe outlets, at the maximum flow-rate of the system.

### 4.7.2 Preliminary MAS Experiments

Initial proton experiments by Jones and Bloxsidge,\textsuperscript{279} using carefully dried yttrium stabilised zirconia rotors, dried zeolite powders and the empty stator, has demonstrated that the probe itself gives a featureless proton background signal, some 20 ppm wide. Temperature control, at high rotation speeds, was also examined using a sample of potassium bromide, moistened with ethylene glycol, from which the proton chemical shift difference of the glycol signals indicated that the sample could be up to eight degrees Kelvin warmer than the indicated temperature of the bearing gas, at 10,000 Hz rotation. Drive gas normally enters the probe at 293K.

To avoid the line broadening due to dipolar coupling to abundant protons, experiments were carried out on "per-deuterated" materials, as the effects of deuterium dipolar coupling are relatively small and susceptible to removal at modest spinning rates. These dilute, residual, proton signals, from the deuterated compounds, provided the model for the later tritium experiments.

The per-deuterated compounds chosen included dimethyl sulphoxide (99.8% Merck, Sharpe and Dohme) and sodium d\textsubscript{3}-acetate (Aldrich). The proton residue signals were readily detected, spinning on the bearing air only. Rotation of the samples at 10
kHz, at the “magic angle” gave linewidths at half height of the order of 110Hz for the dimethyl sulphoxide sample run at 268K, and 150Hz for the sodium d₃-acetate. These widths seem to be typical of “high resolution” proton signals, from the solid.

A further series of experiments, examining the single-line, proton residue signal from commercial (Aldrich) sodium d₃-acetate, at different spinning speeds and different levels of deuteration were then undertaken, the results of which indicated that linewidths of the order of 150 Hz were obtained at deuteration levels above 95%, broadening significantly at higher proton levels as the proton-proton distances in the sample began to decrease. The linewidths stayed constant at all spinning speeds above 1000 Hz, but the dipolar coupling sidebands were still just visible at a rate of 10,000 Hz. At this speed the first sidebands had an intensity of less than 10% of the main signal and were well outside the normal proton chemical shift range.

### 4.7.3 Tritium MAS Experiments

These ³H-NMR-MAS experiments, employed a Bruker AC 300 spectrometer, which is predominantly used for solution work, a commercial MAS probe modified for tritium observation, and some necessary safety precautions to contain the sample in the event of rotor breakage (as described previously). Details of the connection and operation of the MAS probe can be found in Appendix C. Typical operating conditions using a 4mm heavy walled yttrium stabilised zirconia rotor were as follows:

- 320.13MHz frequency with a 35° pulse.
- Magic Angle Spinning rate of 250 Hz - 15 kHz.
- Two second repetition rate without dipolar decoupling or cross polarisation.
- Samples referenced to external hexamethyl disiloxane.
Each rotor contained approximately 50 mg of material (substrate plus inert support, usually zeolite), with a total radioactivity in the range 8 - 30 mCi. For convenience the samples were run overnight. The variation in bulk magnetic susceptibility from sample to sample, with the different substrates used, makes external referencing rather approximate. A better reference could be obtained by re-running each sample, or an inactive blank, with some hexamethyl disiloxane added, and observing the proton signal. However, in the case of these samples, where assigned solution spectra are available, the most convenient approach was to use the solution data to set the scale in the solid spectrum. Finally the resulting FID's were processed with an optimal Lorentzian/Gaussian, resolution enhancement function for an optimum signal to noise ratio.

The $^3$H-NMR spectra of all the specifically labelled compounds given in Table 4.1 with their corresponding structures shown in Figure 4.5, show a range of linewidths at half height of between 42 Hz for $[^G\cdot^3H]\alpha$-methyldihydrocinnamic acid up to 1200 Hz for $[^\beta\cdot^3H]\gamma$-oxo-$[1,1'-$biphenyl]-$4$-butanoic acid. The specific labelling methods employed for all the compounds listed are outlined in Appendix D, with the exception of L-phenyl-$[2,3\cdot^3H]$-alanine and L-[sidechain 2,3-$^3H$]-tyrosine which were gifts from Amersham International and benzoyl acetone which was prepared by Dr J-M Barthez.280
Table 4.1 Linewidths and radioactivities of the compounds used in these $^3$H-MAS-NMR studies

<table>
<thead>
<tr>
<th>Compound</th>
<th>Linewidth at half-height and 15kHz Rotation</th>
<th>Activity (mCi)</th>
<th>Specific activity (Ci/mmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$[^{2}\text{H}]\alpha$-methylidihydrocinnamic acid</td>
<td>42 Hz</td>
<td>15.6</td>
<td>0.19</td>
</tr>
<tr>
<td>[methyl-$^{3}\text{H}$] 1,4-diacetylbenzene</td>
<td>466 Hz</td>
<td>14.5</td>
<td>0.24</td>
</tr>
<tr>
<td>[Side-chain-$^{2}\text{H}$] 4-[6-methoxy-2-naphthyl]-2-butanol</td>
<td>745 Hz</td>
<td>10.3</td>
<td>0.23</td>
</tr>
<tr>
<td>[5,6-$^{2}\text{H}$]-norbornane-2,3-dicarboxylic anhydride</td>
<td>651 Hz</td>
<td>10.7</td>
<td>0.37</td>
</tr>
<tr>
<td>[2,3-$^{3}\text{H}$] succinic acid</td>
<td>620 Hz</td>
<td>8.1</td>
<td>0.06</td>
</tr>
<tr>
<td>$[^{3}\text{H}]\gamma$-oxo-[1,1'-biphenyl]-4-butanoic acid</td>
<td>1200 Hz</td>
<td>7.6</td>
<td>0.11</td>
</tr>
<tr>
<td>3-acetyl-2,5-dimethyl Pyrrole</td>
<td>412 Hz</td>
<td>9.7</td>
<td>0.15</td>
</tr>
<tr>
<td>[2,5-$^{2}\text{H}$]-methylsuccinic acid</td>
<td>435 Hz</td>
<td>14.2</td>
<td>Not known</td>
</tr>
<tr>
<td>L-phenyl-[2,3-$^{3}\text{H}$]-alanine</td>
<td>473 Hz</td>
<td>15</td>
<td>44</td>
</tr>
<tr>
<td>L-[Side-chain 2,3-$^{3}\text{H}$]-tyrosine</td>
<td>Not discernible</td>
<td>15</td>
<td>44</td>
</tr>
<tr>
<td>Benzoylacetone</td>
<td>71 Hz</td>
<td>15</td>
<td>0.02</td>
</tr>
</tbody>
</table>
Figure 4.5 The structures and positions of the tritium label for all compounds used in these $^3$H-MAS-NMR studies.
Figure 4.6 $^3$H-NMR ($^1$H-Decoupled) Spectrum of [G-$^3$H]$\alpha$-methylidihydrocinnamic acid in CDCl$_3$.

Figure 4.7 $^3$H-MAS-NMR of [G-$^3$H]$\alpha$-methylidihydrocinnamic acid on zeolite at a rotation speed of 15 kHz.
Figure 4.8 $^3$H-MAS-NMR of [G-$^3$H]α-methyldihydrocinnamic acid on zeolite at a rotation speed of 10 kHz.

Figure 4.9 $^3$H-MAS-NMR of [G-$^3$H]α-methyldihydrocinnamic acid on zeolite at a rotation speed of 5 kHz.

Figure 4.10 $^3$H-MAS-NMR of [G-$^3$H]α-methyldihydrocinnamic acid on zeolite at a rotation speed of 250 Hz.
Figure 4.11 $^3$H-NMR ($^1$H-Decoupled) spectrum of [methyl-$^3$H]1,4-diacetylbenzene in CDCl$_3$.

Figure 4.12 $^3$H-MAS-NMR spectrum of [methyl-$^3$H]1,4-diacetylbenzene on zeolite at a rotation speed of 15 kHz.
Figure 4.13 \(^3\text{H}-\text{MAS-NMR} \) spectrum of \([\text{methyl-}^3\text{H}] 1,4\text{-diacetylbenezene on powdered sodium chloride at a rotation speed of 15 kHz.}\)

Figure 4.14 \(^3\text{H}-\text{MAS-NMR} \) spectrum of \([\text{methyl-}^3\text{H}] 1,4\text{-diacetylbenezene on powdered sodium chloride at a rotation speed of 10 kHz.}\)
Figure 4.15 $^3$H-MAS-NMR spectrum of [methyl-$^3$H] 1,4-diacetylbenzene on powdered sodium chloride at a rotation speed of 5 kHz.

Figure 4.16 $^3$H-MAS-NMR spectrum of [methyl-$^3$H] 1,4-diacetylbenzene on powdered sodium chloride at a rotation speed of 250 Hz.
Figure 4.17 $^3$H-NMR ($^1$H-Decoupled) spectrum of [Side-chain-$^3$H] (4-[6-methoxy-2-naphthyl]-2-butanoine) in CDCl$_3$.

Figure 4.18 $^3$H-MAS-NMR spectrum of [Side-chain-$^3$H] (4-[6-methoxy-2-naphthyl]-2-butanoine) on powdered sodium chloride at a rotation speed of 15 kHz.

Figure 4.19 $^3$H-MAS-NMR spectrum of [Side-chain-$^3$H] (4-[6-methoxy-2-naphthyl]-2-butanoine) on zeolite at a rotation speed of 15 kHz.
Figure 4.20 $^3$H-NMR ($^1$H-Decoupled) spectrum of [5,6-$^3$H]-norbornane-2,3-dicarboxylic anhydride in CDCl$_3$.

Figure 4.21 $^3$H-MAS-NMR spectrum of [5,6-$^3$H]-norbornane-2,3-dicarboxylic anhydride on zeolite at a rotation speed of 15 kHz.
Figure 4.22 $^3$H-NMR ($^1$H-Decoupled) spectrum of [2,3-$^3$H] succinic acid in CDCl$_3$.

Figure 4.23 $^3$H-MAS-NMR spectrum of [2,3-$^3$H] succinic acid on zeolite at a rotation speed of 15 kHz.
Figure 4.24 $^3$H-NMR ($^1$H-Decoupled) spectrum of $[\beta-^3\text{H}]$-$\gamma$-oxo-[1,1'-biphenyl]-4-butanoic acid in CDCl$_3$.

Figure 4.25 $^3$H-MAS-NMR spectrum of $[\beta-^3\text{H}]$-$\gamma$-oxo-[1,1'-biphenyl]-4-butanoic acid on zeolite at a rotation speed of 15 kHz.
Figure 4.26 $^3$H-NMR ($^1$H Decoupled) spectrum of [3-acetyl-$^3$H]-2,5-dimethyl pyrrole in CDCl$_3$.

Figure 4.27 $^3$H-MAS-NMR spectrum of [3-acetyl-$^3$H]-2,5-dimethyl pyrrole on zeolite at a rotation speed of 15 kHz.
Figure 4.28 $^3$H-NMR ($^1$H-Decoupled) spectrum of $[2,5-^3$H]-methylsuccinic acid in CDC$_3$.

Figure 4.29 $^3$H-MAS-NMR spectrum of $[2,5-^3$H]-methylsuccinic acid on zeolite at a rotation speed of 15 kHz.
Figure 4.30  $^3$H-NMR ($^1$H-Decoupled) spectrum of L-phenyl [2,3-$^3$H] alanine in CDC$_3$.

Figure 4.31  $^3$H-MAS-NMR spectrum of L-phenyl [2,3-$^3$H] alanine on zeolite at a rotation speed of 15 kHz.
Figure 4.32 $^3$H-NMR ($^1$H-Decoupled) spectrum of L-[Side-chain 2,3-$^3$H]-tyrosine in CDC$_3$.

Figure 4.33 $^3$H-MAS-NMR spectrum of L-[Side-chain 2,3-$^3$H]-tyrosine on zeolite at a rotation speed of 15 kHz.
Chapter 4  Solid State Tritium NMR Spectroscopy

Figure 4.34  $^3\text{H}$-NMR ($^1\text{H}$-Decoupled) spectrum of benzoyl acetone in CDCl$_3$.

Figure 4.35  $^3\text{H}$-MAS-NMR spectrum of benzoyl acetone on zeolite at a rotation speed of 15 kHz.
4.8 DISCUSSION

Techniques for obtaining the NMR spectra of solids, developed slowly from the implementation of magic angle spinning in 1958 and have had an immense effect on a wide range of chemical problems in the last decade. In particular, when combined with cross polarisation\(^{271}\) and high power proton decoupling,\(^{281}\) high quality, high resolution spectra can now be routinely obtained from samples containing "dilute" spin I = \(\frac{1}{2}\) nuclei such as \(^{13}\)C, \(^{15}\)N and \(^{31}\)P. Sample spinning techniques including variable angle,\(^{282}\) dynamic angle\(^{269}\) and double axis rotation\(^{268}\) methods are now further extending solid state NMR into areas involving quadrupolar nuclei such as \(^{17}\)O, \(^{23}\)Na, and \(^{27}\)Al.

One of the recent main applications of \(^3\)H NMR spectroscopy has been in the area of catalysis, both homogenous and heterogeneous. The development by Myasaedov and co-workers of solid state catalysed reactions\(^{283,284,285}\) (hydrogen exchange, hydrogenation and dehalogenation) and the increasing use of polymer supported catalysts points to the need for a complementary technique to \(^{13}\)C NMR spectroscopy of solids so that hydrogen sites, as well as carbon, can be investigated.

Figures 4.6 to 4.35, show that it is possible to obtain \(^3\)H NMR spectra of solid tritiated compounds supported on inert materials such as sodium chloride or zeolite, in a safe and routine manner for a range of different compounds. (Although for Figures 4.15 and 4.16 it is clear that the spinning rate was too slow). In particular these results show that generally labelled compounds such as \([G-^3\text{H}]\alpha\text{-methyl}\text{dihydrocinnamic acid}\) can be analysed, where exchange into the methyl group of the precursor and the product, as well as addition across the double bond are clearly visible in both the solution (Figure 4.6) and MAS (Figure 4.7) spectra. Compounds such as \([2,3-^3\text{H}]1,2\text{-propane dicarboxylic acid}, \text{L-phenyl-[2,3-}^3\text{H]-alanine, and L-[sidechain \text{2,3-}^3\text{H]-tryosine indicate that even with}\)
increased linewidths the two labelled sites of the compound are discernible. Benzoyl acetone is shown, as an example because of the interconversion between its keto and enol forms (tautomerism). In fact being a $\beta$-diketone, the enol form is highly stabilised by resonance delocalisation of the negative charge over both neighbouring carbonyl groups, while also being stabilised by an intramolecular hydrogen bond between the formed hydroxyl and the carbonyl group, essentially forming a stabilising 6-membered ring. Therefore it was interesting to observe that this keto-enol tautomerism was observable in both the solution state (Figure 4.34) and the solid state (Figure 4.35). Overall the relatively sharp signals obtained for the majority of these compounds suggests that a whole range of tritiated compounds may be analysed in this manner.

As a result of chemical shift anisotropy effects, solid state NMR signals are much broader than in solution and are usually narrowed by a combination of magic angle sample spinning at high speed, high power dipolar decoupling of protons, and cross polarisation. Although for these experiments there were no facilities available for the use of cross polarisation or high power proton decoupling, Figures 4.7 to 4.10, and Figures 4.13 to 4.16, illustrate the dramatic narrowing of line widths that are observed as the spinning speeds of the samples are increased from 250 Hz, through 5 and 10 kHz up to 15 kHz for [G-$^3$H]-$\alpha$-methylidihydrocinnamic acid and [methyl-$^3$H]1,4-diacylbenzene respectively.

However, one would expect a significant improvement in line widths for $^3$H-NMR MAS samples once the problem of decoupling the $^3$H (320MHz) and $^1$H (300MHz) signals is resolved. This small 20 MHz frequency difference (~6%) compared to the four-fold difference between $^{13}$C and $^1$H frequencies (75.3MHz and 300Mhz respectively), and the much greater decoupling power necessary for solids than liquids makes the design of a special probe necessary, possibly along the lines used for $^{19}$F. Because the $^1$H and $^{19}$F
frequencies are also relatively close (within 6% - similar to $^1\text{H}$ and $^3\text{H}$), high power proton decoupling has not been routinely available, and solid state fluorine NMR has usually required the use of magic angle spinning with multiple pulse sequences (CRAMPS), which are somewhat technically challenging.

Recent work by the group of Harris and co-workers has demonstrated the influence high power proton decoupling can have on $^{19}\text{F}$ MAS NMR by significantly narrowing the signal line widths. Therefore, by analogy, one might also anticipate a similar effect for $^3\text{H}$ MAS NMR once the problem of high power proton decoupling is solved for this nucleus in the solid state. In the meantime the problem can be by-passed by either (a) using compounds for which no decoupling is required, which is rather restrictive or (b) replacement of the hydrogens by deuterium, which has been shown to give substantial improvements in the resolution of $^{13}\text{C}$ MAS spectra of paramagnetic lanthanide acetates and TEMPOL free radical.

Magic-angle NMR studies on the proton residue signal from various "perdeuterated" compounds by Jones and Bloxsidge established that high resolution proton signals could be obtained from this "dilute" solid, at reasonable magic-angle-rotation rates (10 kHz). This discovery then led the group to inspect the solid state tritium spectra of "perdeuterated" compounds under similar conditions. Perdeuterated substrates were used, to avoid the direct and indirect broadening effects of proton dipolar couplings. 99.8% d$_5$-DMSO, easily labelled with tritium and resistant to radiolytic decay, has found wide use in setting up solution-state tritium NMR spectra. Tritiated (ca 0.1% isotopic abundance) d$_5$-DMSO (labelled by the method of Jones and co-workers), was initially used (liquid) to locate a tritium signal and set up the 90° pulse for these early $^3\text{H}$-MAS-NMR experiments by Jones and Bloxsidge. This preliminary tritium MAS study on frozen tritiated d6-DMSO demonstrated good detectability with the standard AC-300
amplifiers and yielded a half height signal ca 300 Hz wide with a good signal-to-noise ratio. Other tritiated perdeuterated samples including sodium d₃-acetate, sodium d₂-succinate, and d₆-benzoic acid were also analysed by ³H-MAS-NMR, resulting in linewidths of ca 150 – 200 Hz for the first two samples, while d₆-benzoic acid gave a ³H-MAS spectrum with two broad humps.

Comparisons of the results shown in figures 4.6 to 4.35 of dilute tritium labelled MAS spectra of protonated compounds, to those obtained from the studies on dilute MAS spectra of “perdeuterated” compounds is favourable when you take into account a number of mitigating factors.

1. The line broadening effect of tritium-proton dipolar coupling, due to the absence of any high power-proton decoupling.

2. The fact that the magic angle is located by hand-eye co-ordination, using the quadrupolar coupling sidebands in the ⁷⁹Br signal from solid potassium bromide. Any small variation in magic angle setting at this stage, could lead to a greater line broadening in the sample.

3. The Unitcell crystal packing of the solid could affect the observed ³H-MAS-NMR spectrum. For example if there were two different possible crystal packing orientations, then one would expect to see two different MAS NMR signals, which may overlap if close enough.

4. Significant mobility of the tritiated sites in the solid state, could lead to pseudo random orientation of the crystal structure (effectively mirroring the solution state), and therefore result in narrower lines in the MAS-NMR spectrum.

5. It has been shown that deuterium substitution can be used to reduce the linewidth of residual protons, because the average ¹H-¹H distance is increased and the lower
magnetogyric ratio reduces the dipolar couplings, resulting in the linewidths being almost an order of magnitude narrower than the corresponding protonated compound.

In conclusion the results obtained show that it is possible to obtain tritium MAS-NMR spectra of solid tritiated compounds in a safe and routine manner. Since the chemical shift anisotropy of $^3$H should be virtually the same as for $^1$H, it can be envisaged, that with the help of the NMR industry one might expect significant rapid advancements in the technique of $^3$H-MAS-NMR as was the case for $^3$H solution NMR many years ago. The relatively sharp signals obtained on a Bruker AC 300 spectrometer, (predominantly used for solution work), and a commercial MAS probe modified for tritium observation suggests the following:-

- In many of the applications envisaged tritiated compounds at low isotopic abundance (<1%) will be employed, consequently there will be no $^1$H-$^3$H coupling. However $^1$H-$^3$H coupling will be present and it is this problem of high power dipolar decoupling of protons from tritons that should be considered a priority in order to gain significant reduction in $^3$H-MAS-NMR linewidths

- The application of some of the current improvements in solid state NMR hardware would also be expected to offer a significant reduction in the currently observed linewidths.

With these improvements one would expect the technique of $^3$H-MAS-NMR to become widely used in the field of tritium chemistry, allowing the analysis of many more species of tritiated compounds especially labelled polymers.
Appendix A

Additional Experimental Information for the Preparation of Tritiated Methyl Iodide
The tritiated butane generated on the gas line was removed once any residual tritium gas had been removed by an appropriate “clean up” reaction, by absorbing it onto activated carbon from which point it was disposed of to drains by aqueous washing with copious quantities of iced water. The solubility of butane in water is $2.687 \times 10^{-5}$ M dm$^3$ at 293K.

A controlled flow rate dry nitrogen was achieved by passing the fume hood supply nitrogen through a combination of two needle valves and two drying agents (an Aldrich “Dririte” column and concentrated sulphuric acid), before finally passing through a gas flow meter to confirm the flow rate.

The dry ether used in the generation of tritiated methanol described previously was prepared as follows. High-grade sodium dried diethyl ether of analytical reagent quality was used as a starting point. Approximately 250 ml of this reagent was placed in a clean dry clear 250 ml reagent bottle, to which was added some sodium wire from the sodium press (CARE). The bottle with a loose fitting top was placed in a cool dry, dark environment, sealed with a calcium chloride guard tube, and left overnight.

If on the following day, no bubbles of hydrogen rise from the sodium wire in the ether, and it still possesses a bright surface, the ether is dry and ready for use. However it should still be stored in the same dark environment so as to minimise the formation of unwanted peroxide as far as possible. If however the surface of the sodium wire is badly attacked due to insufficient drying, the ether must be filtered through a fluted filter paper into another clean, dry reagent bottle, and the treatment with sodium wire repeated. The absolute diethyl ether thus prepared is now suitable for use, providing the surface of the sodium wire remains bright. If this is the case then a small quantity for use should be filtered through an alumina column (to remove
any peroxide) into a suitable dry flask, into which a small quantity of commercial LiAlH$_4$ may be placed to maintain the dryness of the ether.
Appendix B

Comparison $^1$H NMR Spectrum of 1-bromo-2-methylnaphthalene
Figure B.1  Comparison $^1$H NMR Spectrum of 1-bromo-2-methylnaphthalene.
Appendix C

Connecting the MAS Probe
CONNECTING THE MAS PROBE

Before attempting to remove the narrow band $^1$H/$^{13}$C probe, it was necessary to ensure that the variable temperature unit’s gas flow had been switched off, and that the unit itself was also off. Next at the Bruker AC 300 console the Decoupler and the Pulser were disabled by typing “PO” and “PR” respectively. Once this had been done, then the variable temperature air hose, the heater coil, and the thermocouple (CARE), were removed from the base of the probe, and the three coaxial leads at the side of the probe disconnected.

The two gold plated screws were now carefully loosened, while supporting the probe and taking care not to disturb any of the other probes settings and screws. Once these two screws had been sufficiently unfastened, the probe was gently lowered from the magnet casing, taking care to protect the ceramic top, before transferring the probe to its probe box.

With the $^1$H/$^{13}$C probe safely removed, the MAS probe was inserted carefully into the magnet, ensuring that all the black markers are aligned, before the gold-plated screws were gently screwed into place using the specialist trimmer tool until they were moderately tight. The probe range setting rod (a silvery-grey cylindrical knob), in the base of the probe was pulled right down (for the $^3$H setting), and the sample insertion chimney inserted into the top of the magnet and seated at the top of the MAS probe.

The “MAS unit air” tap was turned on, slowly, followed by the Oscilloscope and the “MAS-DB Pneumatic unit”. The “MAS-DB Pneumatic unit” was cleared of any loose dust by turning the air control switch to “EJECT” and “INSERT”, after which it was switched back to “OFF”. The “MAS-DB Pneumatic unit” connector was
now attached to the probe, taking care to align all the connectors carefully, before tightening the locking ring, after which the "spinning optics" lead was plugged into the little, three-pin connector at the left of the probe side plate. With the amplifiers set in the usual $^{13}$C positions, the lock and $^1$H leads were tucked out of the way, while the "PREAMP 2 BB" lead was connected to BB, on the probe. A 50 Ohm terminator was placed on the $^1$H/$^3$H connector on the probe, after which the blue, crossed-diode box was connected between the red plug and the "TRANSM-F1" socket, on the side of the preamp rack.

It was necessary to ensure that the KBr test sample was clean and that there was a half circle of black marker round the bottom edge of the rotor. The air control was turned to INSERT, the sleeve opened, at the top of the Chimney, and the rotor inserted. THE ROTOR CAP MUST BE AT THE TOP. The air control was switched to BEARING and put on about 0.2-0.3 bar pressure. When the rotor starts spinning, there should be a readout of a few tens of Hertz, in the SPINING RATE window and a square-wave, on the Oscilloscope. Occasionally the rotor did not "take off" with bearing air only, in which case the air control was turned to DRIVE and pressure applied, very gently. A very small amount of drive-air would push the spinning rate up to 2000 Hz. The air control was left in the DRIVE position, as long as the rotor was spinning, and only turned back to BEARING when shutting-down, with drive pressure at zero and bearing pressure low.

The oscilloscope time-base was adjusted to display the higher frequency, before winding up the air-bearing to 1 bar and increasing the drive pressure gently, to take the spinning-rate up to 6000 Hz. The BEARING PRESSURE MONITOR would come out with a 'loud pop', as the bearing pressure was increased and an internal, spinning-rate monitor would click, as the rate goes above 5000 Hz. If the spinning-
rate drops subsequently below 5000 Hz, the drive-air cuts off and the rotor will have to be restarted, as above.

By typing "SOLV (Rtn)A" at the Bruker AC 300 console, the field was set to the standard acetone value. After the macro had finished, “RSH KBRSHIM(Rtn)” was entered to set the line-shape parameters, followed by “RJ KBRJOB(Rtn)” and “PJ KBRJOB(Rtn)”, to set the parameters for the “Magic Angle”. “SWEEP OFF”, on the keypad was selected, to allow unlocked working, and “(Ctrl)-L”, was typed as necessary in order to remove the green, lock-display signal. “GS(Rtn)” was then entered to display the $^{79}$Br, solid-state FID, followed by “(Ctrl)-Y” to separate the components of this FID, so the fine structure could be observed. Very slight movement of the gold-plated Vernier screw, on the base of the probe, allowed adjustment of the observed comb of sidebands to be made longer or shorter in this FID. The Vernier screw was adjusted to obtain a maximum length of the comb (there should be lines visible right to the end of the two FIDs). **NOTE:** the final adjustment of the Vernier screw was made in a clockwise direction. Finally “(Ctrl)-Y” was typed, to put the FID back together again, and “(Ctrl)-H” entered in order to stop the pulser.

The drive air pressure was then slowly reduced, until it was off (the spinning-rate followed it down to a few hundred Hz). The air control was then switched to BEARING and the bearing air pressure was also slowly reduced to zero, at which point the air control was switched to OFF. Before turning the air control back to EJECT in order to remove the sample, the sleeve on the sample insertion chimney was opened, to allow the sample to be ejected into the saucer around the aperture. Once the sample had been ejected, the air control was again switched back to OFF, and the KBr sample put away.
With the fine adjustments made to the magic angle the preamplifier was changed to the 320 MHz tritium unit, and the “PREAMP 2 SELECTIVE” lead was connected to the "^3H" socket on the MAS probe. With the 50 Ohm terminator now placed on the “BB” connector. At this stage the approved containment safety apparatus was connected to the MAS probe. Firstly the “High Efficiency Particulate Arrestance” filter (BS3928) was connected to the top of the sample insertion chimney, by means of semi-rigid tubing connected to an aluminium bell, which fits into the rim of the saucer. A loop of string was then tied around the tubing and it was attached to the magnet, so to help support the weight of this section. Secondly, a Perspex plate attached to tubing from the filter was fitted into the Perspex housing, round the base of the probe, and fixed into place by means of a brass thumbscrew. Then one end of a double coil of PVC tubing attached to a Dural rod with a small piece of rubber tubing on it was fitted snugly into the hole in the Perspex baseplate. The longer coil of PVC tubing was then attached to the spigot on the aluminium bell. A 500 ml beaker of water was placed at the foot of the magnet support-leg. Into this was placed the two free ends of the PVC tubing attached to the Dural rod, which was then secured to the magnet support-leg with tape, so that the two tubes were held vertically, as manometers. Finally the centrifugal exhauster was switched on, at which point it was noted that air was being sucked in through the various holes in the probe base. These holes were sealed, along with the bottom edges of the Perspex box shielding the probe, with adhesive tape, so that the manometers showed about 10 cm of water.

The prepared tritium rotor was checked to ensure that it was clean, properly plugged and that it had a half-circle of black marker, round the base. The air control was then switched to INSERT, the aluminium bell was removed, the sleeve opened, and the sample dropped down the chimney with the finned cap facing upwards. The
aluminium bell was then replaced and the vacuum checked, before the rotor speed was increased up to 5 kHz, as outlined earlier, before being allowed to settle at this speed for a few minutes.

"RJ H3MASJOB(Rtn), PJ H3MASJOB(Rtn), ZE(Rtn)", were then entered at the console, to initiate the $^3$H-MAS job files. Meanwhile the bearing air pressure was increased up to 1.5 bar, followed by gentle increases in the drive pressure, allowing the rotor speed to catch up with each increase, until 10 kHz was reached. At which point the system was allowed to settle, again for another few minutes. Following the same procedure as before, the bearing pressure was increased to 2 bar, with further gentle increases in the drive pressure to push the rotor speed up to 12.5 kHz. When once again the system was allowed to equilibrate for a few minutes. Finally the bearing pressure was increased to 2.5 bar, with slow increases in the drive pressure to push the rotor speed up to 15 kHz. Once the rotor was spinning stably, at 15 kHz, "RGA;ZE;GO(Rtn)", was typed at the console in the usual way, to initiate collection of a tritium MAS spectrum.

At the end of the experiment, the sample was removed in the way outlined above, before the probe was disconnected, following the above outline in reverse, and replaced with the $^1$H/$^{13}$C probe.
Appendix D

Tritiation of Compounds for Study by Solid State Tritium NMR Spectroscopy

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D.1 INTRODUCTION

Section 1.5 in chapter 1 already summarises the three main labelling methods used for the incorporation of tritium into a variety of compounds. These being; isotope exchange reactions, direct chemical synthesis, and biochemical methods. Examples of the first two of these will be outlined later on.

D.2 PALLADIUM CATALYSED REDUCTION OF UNSATURATED INTERMEDIATES

Direct chemical synthesis, in particular the catalytic reduction of unsaturated intermediates in solution with tritium gas is one of the most precise and reliable ways of labelling a compound with tritium. However, the principle disadvantage of this catalytic reduction route is that there may be a tendency for the tritium to be attached to carbon atoms other than those of the unsaturated bond being reduced, this effect was only observed in the labelling of \([G-^2H]-\alpha\)-methyldihydrocinnamic acid. The compounds labelled in this manner from suitable unsaturated precursors are:

- \([5,6-^3H]\)-norbornane-2,3-dicarboxylic anhydride.
- \([G-^3H]\)-\(\alpha\)-methyldihydrocinnamic acid.
- \([2,3-^3H]\)-succinic acid.
- \([2,5-^3H]\)-methylsuccinic acid.

Only one of the four compounds listed previously (\([5,6-^3H]\)-norbornane-2,3-dicarboxylic anhydride) required the synthesis of an appropriate intermediate - \([5,6]\)-norbornene-2,3-dicarboxylic anhydride. The remaining three already having suitable
Appendix D Tritiation of Compounds for Study by Solid State Tritium NMR Spectroscopy

Commercially available unsaturated intermediates on the shelf, these being \( \alpha \)-methylcinnamic acid, maleic acid, and itaconic acid respectively.

D.2.1 Preparation of [5,6]-norbornene-2,3-dicarboxylic anhydride

[5,6]-norbornene-2,3-dicarboxylic anhydride may be prepared by the Diels-Alder reaction between cyclopentadiene and maleic anhydride as shown below in Figure D.1.292

\[
\begin{align*}
\text{HEAT} & \quad \text{cyclopentadiene} + \text{maleic anhydride} \\
& \quad \xrightarrow{\text{Reaction}} \quad [5,6]-\text{norbornene-2,3-dicarboxylic anhydride}
\end{align*}
\]

Figure D.1 Preparation of [5,6]-norbornene-2,3-dicarboxylic anhydride.

To a 25ml round bottom flask fitted with a vigreux column and appropriate distillation apparatus was added dicyclopentadiene (7 ml) and a piece of iron wire. The flask was then heated to a temperature of 170°C, and the clear monomer distillate (boiling point 40-43°C) was collected (approximately 1-2 ml). Powdered maleic anhydride (1 g, 10.2 mmol) was dissolved in ethyl acetate (5 ml) in a small conical flask with warming. To this solution was added petroleum ether (5 ml, 60-80°C fraction), and the resulting solution was placed in an ice bath to cool thoroughly. To this cool solution freshly distilled cyclopentadiene (1 ml 11.6 mmol) was added with swirling and cooling. The resulting mixture was then heated gently until everything re-dissolved, before being left to crystallise out. The resultant adduct was subsequently recrystallised from petroleum ether (80-100°C), yielding [5,6]-norbornene-2,3-dicarboxylic anhydride (1.58 g, 94.4%). The identity of the product was confirmed by NMR spectroscopy and NMR prediction programs.242
D.2.2 General Procedure for the Reduction of Unsaturated Intermediates with Tritium Gas

Following the tritium gas line procedure outlined in sections 2.5.3 and 2.5.4, the already prepared or available unsaturated intermediates outlined above were used in various tritium gas line “clean up” reactions detailed in figure D.2.

The unsaturated substrate (10 - 20 mg) and 10% palladium on carbon catalyst (10 - 20 mg) were placed in a 3 ml gas line flask to which was added 2 ml of a suitable solvent (typically chloroform) and a small glass encased magnetic stirrer bar. The reaction vessel was then attached to the gas line following the normal gas line procedure as detailed in section 2.5.4, before taps B and C were opened, to allow the residual tritium gas to come into contact with the reaction solution, allowing the “clean up” reaction to proceed.

After 24-48 hours the reaction flask was removed from the gas line, again following normal gas line procedure. The reaction solution was now diluted with a further 5 ml of solvent before being passed through a small Celite filter in a Pasteur pipette to remove the catalyst. The resultant filtrate was then analysed by liquid scintillation counting to determine the samples activity, before undergoing $^3$H-NMR analysis to check the position of the label. Table D.1 outlines the position of the label in each compound with their corresponding radioactivities.
Figure D.2 Reaction summary of the reduction of unsaturated intermediates reactions performed using tritium gas and 10% Pd/C.
Table D.1  Radioactivities and position of $^3$H label of the resulting saturated compounds.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Position of label</th>
<th>Activity (mCi)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[5,6-$^3$H]-norbornane-2,3-dicarboxylic anhydride</td>
<td>$\delta = 1.62$ppm, Clean insertion about the double bond.</td>
<td>55.6</td>
</tr>
<tr>
<td>[G-$^3$H]-α-methyldihydro-cinnamic acid</td>
<td>$\delta = 2.97, 2.65, 2.04, &amp; 1.07$ppm, Insertion about the double bond, along with migration to the methyl group in the reduced and unreduced compound.</td>
<td>17.6</td>
</tr>
<tr>
<td>[2,3-$^3$H]-succinic acid</td>
<td>$\delta = 2.46$ppm, Clean insertion about the double bond.</td>
<td>8.1</td>
</tr>
<tr>
<td>[2,3-$^3$H]-1,2-propane dicarboxylic acid</td>
<td>$\delta = 2.78 &amp; 1.17$ppm, Clean insertion about the double bond.</td>
<td>42.7</td>
</tr>
</tbody>
</table>

D.3  REGIOSPECIFIC TRITIATION OF VARIOUS SUBSTRATES USING A HOMOGENEOUS RUTHERNIUM TRICHLORIDE CATALYST

Hydrogen isotope exchange reactions in the presence of tritiated solvents and a heterogeneous or homogeneous metal catalyst are widely used labelling procedures, and as such have already been discussed in chapter 1, section 1.5.1.4. Of these many labelling routes available, it is the regiospecific catalysts that are seeing greater use. In particular, recent studies have shown that rhodium and ruthenium trichloride homogeneous catalysts are able to catalyse the ortho-exchange labelling of aromatic acids, amides, aralkylamines, and anilides, with high regioselectivity. 65, 66, 67 The
mechanism of this regiospecific labelling has been hypothesised to proceed via a cyclometallation reaction involving the formation of a five-membered ring\textsuperscript{66, 71} as illustrated in Chapter 1, Figure 1.5. The compounds labelled in this manner were:

- [Methyl-\textsuperscript{3H}]-1,4-diacetylbenzene.
- [Side-chain-\textsuperscript{3H}]-4-[6-methoxy-2-naphthyl]-2-butanone.
- [3-acetyl-\textsuperscript{3H}]-2,5-dimethylpyrrole.

### D.3.1 General Tritiation Procedure for Labelling Using Ruthenium Trichloride

The catalyst ruthenium trichloride (RuCl\textsubscript{3}.3H\textsubscript{2}O, 0.025-0.035 mmol, 6.5-9.1 mg) and substrate (0.025-0.035 mmol) were placed in a 1 ml vial, and dissolved in 200 \( \mu \)l of anhydrous dimethylformamide before being transferred to a 1 ml capacity thick glass walled reaction tube. A further 150-200 \( \mu \)l of anhydrous dimethylformamide is used to flush the 1 ml vial employed, and this was also transferred to the reaction tube to ensure maximum transfer of the reactants. Tritiated water 10 \( \mu \)l (5 Ci/ml) was added and a rubber septum placed in the top of the reaction tube. The tube was then subsequently cooled in liquid nitrogen, evacuated, and flame sealed. On thawing of the reaction solution, the tube was placed in a thermostatic oil bath at 118°C for between 18-24 hours. On completion, the tube was removed from the oil bath and again frozen in liquid nitrogen, before the tube was opened and the substrate extracted from the reaction solution.

Isolation of the desired substrate was achieved in the following manner. The reaction tube contents were poured into ethyl acetate (15 ml), and washed successively with sodium bicarbonate (2 x 2 ml), hydrochloric acid (3M, 2 x 2 ml), and water (3 x 2 ml). In each case, the 2 ml fractions were re-extracted with 10 ml of
ethyl acetate, and this subsequent extract added to the ethyl acetate fraction containing the desired labelled substrate. This latter fraction was then dried over anhydrous sodium sulphate, filtered, before being gently rotary evaporated to yield the labelled compound. The resultant material was then analysed by liquid scintillation counting to determine the samples activity, before undergoing $^3$H-NMR analysis to check the position of the label. Figure D.3 and Table D.2 outline the reaction conditions employed and illustrate the position of the label in each compound with their corresponding activities.

Note: These exchange reactions were done in duplicate to ensure enough active material was produced to allow a good $^3$H-MAS NMR spectrum to be obtained.

* - Position of tritium label.

Figure D.3 Reaction summary for the exchange labelling procedure of compounds using Ruthenium trichloride.
Table D.2 Radioactivities and position of $^3$H label of the resulting Ruthenium trichloride exchange labelled compounds.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Position of label</th>
<th>Activity (mCi)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Methyl-$^3$H]-1,4-diacetylbenzene</td>
<td>$\delta = 2.72$ppm, - Clean exchange at the methyl groups.</td>
<td>17.2</td>
</tr>
<tr>
<td>[Side-chain-$^3$H]-4-[6-methoxy-2-naphthyl]-2-butanone</td>
<td>$\delta = 2.87, 2.16$ppm, - Clean exchange into the methyl and methylene groups alpha to the keto moiety.</td>
<td>12.4</td>
</tr>
<tr>
<td>[3-acetyl-$^3$H]-2,5-dimethylpyrrole</td>
<td>$\delta = 2.71$ppm, - Clean exchange into the methyl of the acetyl moiety. $\delta = 2.54$ppm, - Unknown impurity.</td>
<td>10.9</td>
</tr>
</tbody>
</table>

D.4 Base Catalysed Exchange Procedure for the Labelling of $\gamma$-oxo-[1,1'-biphenyl]-4-butanoic acid.

Homogeneous base catalysed hydrogen isotope exchange reactions are useful methods for exchanging protons in the alpha position to a keto- or oxo-group as shown by the proposed mechanism in Figure D.4.

![Figure D.4](image-url)
D.4.1 Tritiation Procedure for Labelling γ-oxo-[1,1'-biphenyl]-4-butanoic acid using Base Catalysed Tritium Exchange.

The catalyst dry powdered potassium hydroxide, (0.054 mmol, 3.1 mg) and γ-oxo-[1,1'-biphenyl]-4-butanoic Acid (0.035 mmol, 9mg) were placed in a 1 ml vial, and dissolved in 200 µl of anhydrous dimethylformamide before being transferred to a 1 ml capacity thick glass walled reaction tube. A further 150-200 µl of anhydrous dimethylformamide is used to flush the 1 ml vial employed, and this was also transferred to the reaction tube to ensure maximum transfer of the reactants. Tritiated water 10 µl (5 Ci/ml) was added and a rubber septum placed in the top of the reaction tube. The tube was then subsequently cooled in liquid nitrogen, evacuated, and flame sealed. On thawing of the reaction solution, the tube was placed in a thermostatic oil bath at 118°C for between 18-24 hours. On completion, the tube was removed from the oil bath and again frozen in liquid nitrogen, before the tube was opened and the substrate extracted from the reaction solution.

Isolation of the desired substrate was achieved in the following manner. The reaction tube contents were poured into ethyl acetate (15 ml), and washed successively with and water (5 x 5 ml). In each case, the 5 ml fractions were re-extracted with 5 ml of ethyl acetate, and this subsequent extract added to the ethyl acetate fraction containing the desired labelled substrate. This latter fraction was then dried over anhydrous sodium sulphate, filtered, before being gently rotary evaporated to yield the labelled compound. The resultant material was then analysed by liquid scintillation counting to determine the samples activity, before undergoing $^3$H-NMR analysis to check the position of the label. Figure D.5 and Table D.3 outline the
reaction conditions employed and illustrate the position of the label in each compound with their corresponding activities.

Note: These exchange reactions were done in duplicate to ensure enough active material was produced to allow a good $^3$H-MAS NMR spectrum to be obtained.

\[
\begin{align*}
\text{OH} & \quad \rightarrow \quad \text{OH} \\
\text{HTO} & \quad \KOH/\text{DMF} \\
\text{gamma-oxo}[1,1'-\text{biphenyl}]-4\text{-butanoic acid} & \quad \rightarrow \quad \text{[Beta-$^3$H]-}\text{gamma-oxo}[1,1'-\text{biphenyl}]-4\text{-butanoic acid} \\
\end{align*}
\]

$^*\quad$ - Position of tritium label.

**Figure D.5** Reaction summary for the base catalysed exchange labelling procedure of $\gamma$-oxo-[1,1'-biphenyl]-4-butanoic acid.

**Table D.3** Radioactivities and position of $^3$H label of the resulting base catalysed exchange of $\gamma$-oxo-[1,1'-biphenyl]-4-butanoic acid.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Position of label</th>
<th>Activity (mCi)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$[\beta-^3$H$]-\gamma$-oxo-[1,1'-$^\prime$-biphenyl]-4-butanoic acid.</td>
<td>$\delta = 2.86$ppm, - Clean exchange at the methylene group alpha to the oxo moiety.</td>
<td>7.9</td>
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