

**SCHOOL OF CHEMISTRY**

**CARDIFF UNIVERSITY**



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**New methods for rapid H/D exchange and  
related processing using microwave  
irradiation**

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Thesis submitted for the degree of Doctor of Philosophy by:

**Gabriele Banci**

## **Declaration**

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...and to Laura just for being beside me, “....che solo amore e luce hai per confine...”.

## **Abstract**

Microwave mediated H/D exchanges have been studied in this thesis. The purpose of these studies is to validate an efficient method for replacing hydrogen by deuterium in molecules with biological and pharmaceutical activity. As a result of this, such deuterated compounds would be helpful during the investigation of pharmacological and metabolic studies.

At the beginning of this research, aniline derivatives were mainly employed as substrates. Their importance in drug synthesis makes the corresponding labelled compounds useful as building blocks. During these studies a Pt catalyst was employed. As a consequence of its strong efficiency, fully deuterated compounds were obtained in many cases.

In the first part of this research, yields were based on ratios between non-deuterated and deuterated original compounds. However, due to solubility problems and metal-complex formation, this parameter was found to vary considerably. Therefore, an analytical method, involving introduction of a non-deuterated acetyl group to the labelled substrate was employed, so that the acetyl group could be regarded as an internal reference for  $^1\text{H}$ NMR integration, allowing quantification of deuterium incorporation at all positions in the molecular structure. Kinetic experiments have also enabled the best conditions to be defined for the exchange reactions. Lastly, as a consequence of a reductive process,  $\text{Pt}^0$  has been recognized as a serious problem for calculating final yield. An appropriate method for eliminating it from the solutions has therefore been applied.

A valid Pt mediated H/D mechanism has been proposed, based on suggestions from the literature that Pt can move around both aromatic rings and alkyl side chains. To try to get evidence for such a mechanism, different alkylanilines have been used, giving useful information regarding metal activity, particularly with respect to steric aspects.

In the last part of the work, as a consequence of its importance in pharmaceutical chemistry, heterocyclic derivatives have been investigated. Satisfactory results have permitted the method to be exploited for some relevant drugs.

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## Abbreviations

<b>CD<sub>3</sub>OD</b>	Methanol deuterated
<b>DCI</b>	Deuterium chloride
<b>DCM</b>	Dichloromethane
<b>dd.</b>	Doublet of doublets
<b>DMSO</b>	Dimethyl sulfoxide
<b>dt</b>	Doublet of triplets
<b>EI</b>	Electronic ionization
<b>eq.</b>	Equivalent
<b>Et</b>	Ethyl
<b>Et<sub>2</sub>O</b>	Diethylether
<b>EtOAc</b>	Ethyl acetate
<b>h</b>	Hour(s)
<b>HRMS</b>	High Resolution Mass Spectrometry
<b>Hz</b>	Hertz
<b>IR</b>	Infra Red
<b><i>J</i></b>	Coupling constant (in Hz)
<b>LRMS</b>	Low Resolution Mass Spectrometry
<b>m</b>	Multiplet
<b>Me</b>	Methyl
<b>MeOH</b>	Methanol
<b>min</b>	Minute(s)
<b>mmol</b>	Millimole(s)
<b>mp</b>	Melting point
<b>Mr</b>	Molecular weight
<b>M<sub>w</sub></b>	Microwave irradiation

<b>N.A.</b>	Not applicable
<b>NMR</b>	Nuclear Magnetic Resonance
<b>q</b>	quartet
<b>R</b>	Unspecified aryl substituent
<b>R.T.</b>	Room temperature
<b>s</b>	Singlet
<b>sec</b>	Second(s)
<b>t</b>	Triplet

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# Chapter 1

## Introduction

1.1 1.1 History

1.2 Deuterium

1.3 Drug metabolism

1.4 Tracer studies

1.5 Internal standard

1.6 LC-IDMS

1.7 H/D studies

1.8 Heterogeneous H/D reactions

1.9 Homogeneous H/D reactions

1.10 H/D microwave-assisted reactions

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### 1.1 History

Isotopes are known as variants of chemical elements (e.g., carbon C or hydrogen H). They present the same atomic number (so they have same number of protons in their nuclei) but a different mass number (resulting from a different number of neutrons). So, each isotope presents a particular molecular weight which, as we will see later, allows us to distinguish them. Radioactive species among them were also discovered and characterized by a precise decay over time (known as half-life). Investigating such processes in 1912, Frederick Soddy<sup>1</sup> discovered forty new species between uranium and lead, not yet found in the periodic table, and then described as radioelements (i.e. radioactive element). Furthermore, he stated that such specific decays (called *alpha* and *beta* according to the specific particles produced) created different chemical elements<sup>2-3</sup> despite their different radioactive properties. As a consequence of this, he proposed that more than one of these species could occupy the same place on the periodic table. For this reason, he also proposed the term isotope, which arises from Greek meaning “*at the same place*”.<sup>4</sup>

Isotopes not involved in any decay process were also discovered by Thomson in 1913. By investigating positive ions<sup>5-6</sup>, which were channelled streams of neon deflected by magnetic and electric fields, Thomson was able to show two different paths on a photographic plate. He proposed that some neon atoms could have a higher mass than all the others. Lastly, the different molecular isotopic weight was successfully determined by Aston while separating numerous isotopes using mass spectrometry.<sup>7</sup>

Nowadays, it is established that some isotopes, such as deuterium, have such a long decaying process (more than 250 million years) that they are described as stable.

## 1.2 Deuterium

Hydrogen (H) can exist in three different isotopic forms known as protium ( $^1\text{H}$ ), deuterium ( $^2\text{H/D}$ ) and tritium ( $^3\text{H/T}$ ). The superscript number indicates the sum of protons (1) and neutrons (0, 1, 2) in each nucleus.

Deuterium, whose name arises from the Greek for “*second*”, was introduced in chemistry by the Nobel Prize winner Harold Urey in 1931. As a result of a slow distillation process from five litres of cryogenically liquid hydrogen, Urey was able to recover one millilitre of what was then, spectroscopically identified as pure deuterium.<sup>8-9</sup>

However, the significant importance for the use of this isotope arose from the introduction of “heavy water” (deuterium oxide,  $\text{D}_2\text{O}$ ). Since it became a relevant component for nuclear programs, as the neutron moderator<sup>10</sup> (a means able to reduce the speed of neutrons and so to control any nuclear chain reaction), the interest surrounding its production increased. After its isolation by Lewis via electrolysis<sup>11</sup>, many other procedures were developed, in particular, the Girdler sulfide process<sup>12</sup> proved to be a relevant industrial system.<sup>13</sup> This process was mainly based on H/D exchange between *hydrogen-deuterium sulfide* (HDS) and an initial  $\text{D}_2\text{O}$  at two different sets of temperature (usually at 30 °C and at 130 °C). A series of different exchanges allowed water to be progressively enriched with deuterium. The most relevant aspect is that such isotopes are not chemically identical. As a consequence of their different masses, deuterium and hydrogen show two different covalent bonds in energetic terms. Specific bonds, such as carbon-hydrogen (C-H), will appear less stable than carbon-deuterium (C-D) and so will require less energy to be broken. In further support, the energy necessary to stretch and bend these two bonds is substantially different. In infrared spectroscopy technique, the frequency at which C-D vibrates is lower than C-H (2200  $\text{cm}^{-1}$  against 3000  $\text{cm}^{-1}$ ). This aspect is also perfectly coherent with Hooke’s law which claims a reduction of such vibration frequency with the increasing of atomic mass.<sup>14</sup> The same model leads to the prediction of a lower zero-point energy for C–D than for C–H bonds; it is this which gives the former slightly greater strength.

Lastly, the considerations discussed previously have seen a wide and relevant application in organic chemistry. In particular, deuterium played a considerable role in reaction mechanism

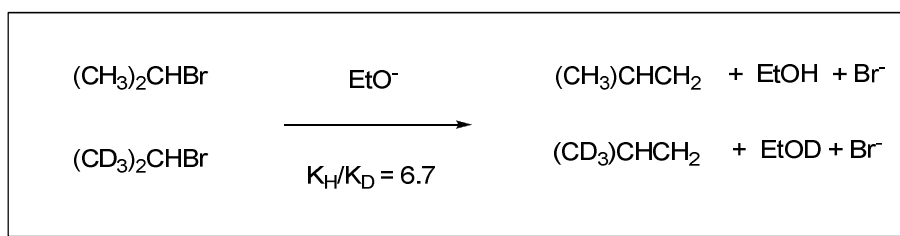
investigations. In general, any isotopic substitutions in an organic molecule could significantly influence the corresponding reaction rates. Due to the fact that deuterium has twice the mass of hydrogen, its consequent effect should be particularly pronounced. This is better described in organic chemistry by the relation shown below (Scheme 1.1), defined as *kinetic isotope effect* (KIE). So, deuterium introduced in replacement of hydrogen could considerably slow down many specific chemical reaction rates. This aspect is particularly relevant when substitution regards the breaking bond of a rate determining step. In such specific cases the general kinetic isotope effect (KIE) is also called primary kinetic isotope effect (PKIE).

$$\text{KIE} = \frac{\text{Rate with substrate containing } ^1\text{H}}{\text{Rate with substrate containing } ^2\text{H}}$$

$K_H$  ← Rate with substrate containing  $^1\text{H}$   
 $K_D$  ← Rate with substrate containing  $^2\text{H}$

**Scheme 1.1.** Kinetic isotope effect relation.

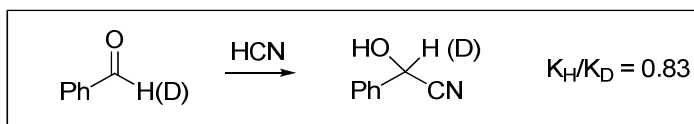
Thus, the relation described above allows rate changes to be quantified. Furthermore, when referring to PKIE, the value of this ratio is significantly high. (Scheme 1.2).



**Scheme 1.2.** Primary kinetic isotope effect example.

As indicated in Scheme 1.2, KIE is around 7. Since an elimination reaction is above described, one of deuteriums replaced in methyl groups is eliminated in forming an unsaturated bond. As above explained, the different reactivity here shows a subsequent change in reaction rate. As a result of this, the corresponding hydrogens and their bonds were recognized as key roles of rate determining step. On the other hand, according to the specific reaction investigated, entirely different results could be obtained in KIE terms. When

deuterium is introduced in a position not involved in any eventual breaking bond, KIE is then defined as secondary kinetic isotope effect (SKIE) (Scheme 1.3).



**Scheme 1.3.**Secondary kinetic isotope effect example.

As reported in the Scheme above, KIE is here considerably lower. As a natural conclusion, the reaction mechanism of nucleophilic addition here does not involve such substituted hydrogens and its bond.

In summary, a general description of deuterium and its relevant uses in organic chemistry have been provided. As previously mentioned, this isotope can have many different applications in various fields. However, it is fundamental for the aims of this project to focus the attention on the pharmacological context. For this reason, the next pages will offer a short explanation of its use in this field.

### 1.3 Drug metabolism

In order to obtain the approval for every drug by the FDA (*food and drug administration*, for U.S. trading) and EMEA (*European medicines agency*, in a European context), all pharmaceutical industries need to report a clear documentation of their metabolic pathways both in the human and animal body.<sup>15-16</sup>

Metabolism plays a crucial role in drug discovery and development as a part of Pharmacokinetic (PK) studies. Defined as a discipline dedicated to optimizing the biological drug properties, PK attention is also focused on other pharmacological aspects such as absorption, distribution and excretion (in metabolism, these are recognized as ADME). All the parameters previously mentioned are fundamental for defining the intensity and time course of pharmacological (therapeutic and toxic) drug effect.<sup>17-18</sup>

Additionally, metabolic studies should be able to state metabolite's eventual biological activities. As recently reported in some papers<sup>19-21</sup>, some metabolites often show toxic and carcinogenic effects which, obviously, is in contrast with various therapeutic applications. On the other hand, some of them have been recognized as provided pharmacological properties

for other therapeutic fields.<sup>22-23</sup> Moreover, important interactions between drugs and endogenous molecules have been also revealed, in some cases avoiding serious health consequences.<sup>24</sup>

The use of stable isotopes was considered really helpful for such studies. Although the introduction of isotope techniques in this field occurred as late as 1970s and 1980s, the implications of such labelled compound studies through mass spectrometry were already defined. In particular, following Aston's experiment, seen previously, their application as metabolic tracers strictly followed mass spectrometry's evolution.<sup>25</sup> As will be shown in the next pages, the combination of gas-chromatography with mass spectrometry (GC-MS) in a unique system gave a strong impulse to the development of stable isotope applications. The introduction of electrospray ionization inside the mass spectrometry context also represented a great innovation, allowing most of the samples to be analyzed easily on mass analyzer.<sup>26-28</sup>

Due to its non-radioactive property and low cost, deuterium was the first isotope to be used for this purpose. Moreover, the extreme sensitivity of mass spectroscopy equipment<sup>29</sup> allowed later other isotopic species (*e.g.*,  $^{15}\text{N}$ ) to also be used and investigated in such metabolic studies.<sup>30</sup>

The first analysis of stable isotope applications reported by Baille *et al.*<sup>31</sup> and Brown *et al.*<sup>32-33</sup> were crucial for defining their real efficiency. In addition, Pons<sup>34</sup> and, later, Abramson<sup>35</sup> reported results of these techniques in particular contexts, such as paediatric and pregnancy cases. Due to their non-radioactivity, stable isotopes enable the applications *in vivo* without any significant consequences to health. Therefore, the use of isotopes in bio-analysis has always been seen as an interesting model to investigate, since its introduction in metabolic studies.

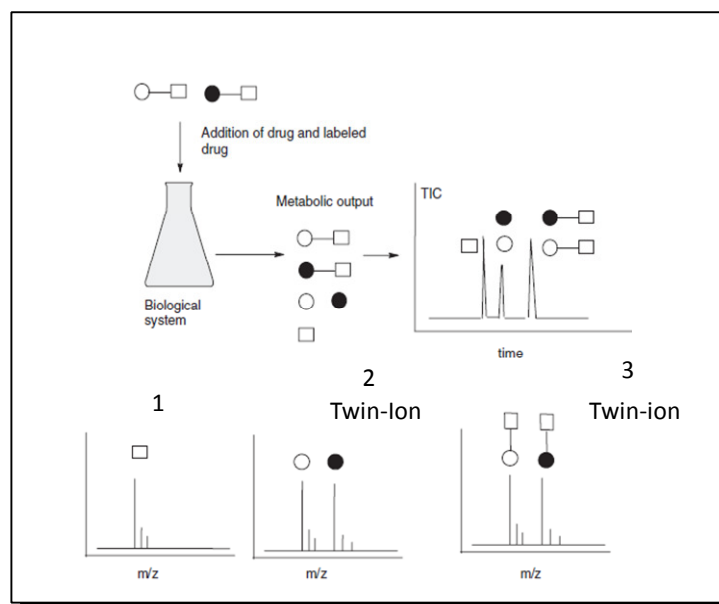
## 1.4 Tracer studies

As already stated, the first real isotope application in metabolic studies was only introduced in the 1970s. Knapp *et al.*<sup>36</sup> later introduced a technique known as *twin-ion*. In order to identify the metabolites of *nortryptiline*, a second-generation of tricyclic antidepressant, Knapp decided to administer the same amounts of labelled and unlabelled drug to subjects for an *in vivo* assay.

In Figure 1.1 a general example of this procedure has been reported. In order to simplify the main aspects, the theoretical administered drug is represented by two parts; the unlabelled



species by two white keys ( $\circ$ - $\square$ ), and the corresponding labelled species with a black key ( $\bullet$ ), which represents the deuterated moiety, and a white key ( $\square$ ). Lastly, a gas chromatography-mass spectrometry analysis on different pools (liver, plasma, urine etc.) has been used for identifying the *parent drug* (the original drug, labelled or unlabelled, in Figure 1.1 represented by  $\circ$ - $\square$  or  $\bullet$ - $\square$ ) and its eventual metabolites.



**Figure 1.1.** Example of twin-ion technique for metabolite identification using labelled and non labelled drugs in a biological system.<sup>37</sup>

As discussed, deuterium has been used as a labelling isotope. Although Brazier *et al.*<sup>38</sup> employed isotopes like  $^{15}\text{N}$  and  $^{13}\text{C}$  in the same technique; deuterium was often seen as the favourite choice for labelling compounds. However, the synthesis of label drug is still nowadays technically complicated and, overall, costly.<sup>39</sup> As a consequence of this, at the beginning of this research, deuterium was only introduced in some target sites of molecular structure. The core of this technique was effectively to identify these labelled sites after metabolic process. Due to their different masses, labelled ( $\bullet$ - $\square$ ) and non-labelled ( $\circ$ - $\square$ ) drugs give the characteristic *twin-ion* mass spectrum (mass spectra 3 in Figure 1.1). Such particular ion clusters separated by a precise mass difference confirms undoubtedly the metabolic presence. However, this technique presents considerable limitation. As a consequence of metabolic process, the labelled site could be clipped, shearing the molecule in two labelled and unlabelled parts. So, two different results are likely to occur and they are

reported in mass spectra 1 and 2. In this last one the *twin-ion* is still present while in mass spectra 1 only one peak can be spotted. Due to the lack of its labelled *twin*, this fraction here cannot be definitely recognized as a metabolite.

Although Weidolf,<sup>40</sup> Akwa<sup>41</sup> and Morfin<sup>42</sup>, were able to define omeoprazole and steroid metabolites using this technique, due to the limits described above, no relevant developments were here introduced. Hence, the need to recover fully or almost fully labelled compounds became fundamental. Due to their increasing mass, such new species could guarantee an efficient resolution from unlabelled *parent drug* on mass spectra. Subsequently to this, and in order to easily identify all metabolites involved, a high deuterium distribution on all molecular surfaces was seriously recommended. Furthermore, for the financial costs highlighted, it was equally apparent that the scientific attention should focus on specific deuterium exchange. As it will be described in the next pages, the studies on H/D exchange resulted in a development of a characteristic quantitative analytical technique based on *internal standard* use.

## 1.5 Internal standard

In order to improve the quantitative measurements of analytes in biological system, the concept of *internal standard* was considered extremely important. As principal definition, internal standard referred to a compound added in a constant amount for quantifying an otherwise unknown sample. For this aim, its initial role was on setting up an appropriate calibration curve. As will be explained later, a set of different mixture or "batches" composed by such constant amounts of internal standard and variable known amounts of analyte, were run in a *liquid chromatography-mass spectrometry* system (LC-MS). Thus, a series of plots taken from specific area ratios between the analyte and the internal standard signals on the chromatogram were recorded informing the curve, in particular it was crucial extrapolating a linear relationship between them. Immediately after its extraction from a specific biological system, the analyte was injected again on LC-MS in addition to the usual amount of the internal standard. As will be demonstrated in the example, the just mentioned linear relationship turns out to be very helpful in calculating the specific analyte's concentration.

In order to understand the role played by labelled compounds in such specific analysis, it is also necessary to report some relevant information. In 1940, Rittenberg *et al.*<sup>43</sup> produced a valid technique to quantify endogenous compounds, such as amino acids and fatty palmitic acid,

using mainly  $^{15}\text{N}$  and deuterated analogues. Introducing for the first time the concept of internal standard, Rittenberg also proposed to define stable isotopes as “ideal” internal standard. This was due principally to their identical physical and chemical properties to the unlabelled analogues. So, the same retention time shown in LC-MS allows the area, below to the chromatogram peak, to be easily calculated by an integration process. On the other hand, because of their different molecular weight, labelled and unlabelled species could be successfully differentiated by mass spectrometry.

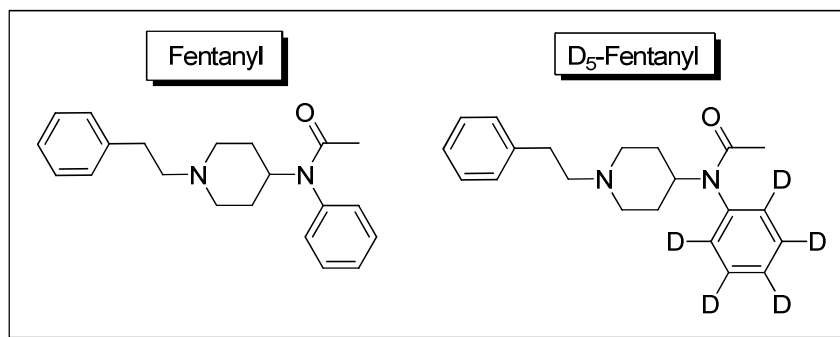
These last aspects also highlight another relevant advantage in using stable isotopes. Through the internal standard addition, the *matrix effect* is considerably reduced.<sup>44-46</sup> This term describes the general problems caused by unknown species co-eluted with the sample during the chromatography purification. Due to their difficulty to be recorded by mass, such species could disrupt the quantification analysis. Through this addition instead, the concentration of the sample is increased so that such effect is finally minimized. As a result of this, the result recorded with the *internal standard* appears in general much more reliable than without it. As evidence of the importance of such a technique in bioanalysis field, Grey *et al.*<sup>47</sup> set up a method which quantified *paraquat* and *diquat*, two desiccant and defoliant industrial products. Due to the aforementioned matrix effect, such compounds could not be properly measured in environmental water and vegetarian matrices. Since here  $^2\text{H}$  corresponding labelled compounds were used as internal standard inside a LC-IDMS procedure (see next paragraph), more accurate results have instead been obtained.

## 1.6 Liquid chromatography-isotope-dilution tandem mass spectrometry (LC-IDMS)

As previously discussed, liquid chromatography is currently the most favourable purification technique when an internal standard is used. The introduction of this process was fundamental for developing *liquid chromatography-isotope dilution tandem mass spectrometry* (LC-IDMS). As indicated by this name, such technique is based on the principle of isotope dilution analysis, formulated by Rittenberg during his studies on the internal standards. Although considerable results were already obtained by the advent of gas chromatography-mass spectrometry (GC-MS)<sup>48-49</sup>, this technique received the greatest expansion in its development from liquid chromatography introduction.<sup>39</sup> Such system allowed some non-polar and non-thermal stable molecules to be purified and, as a result, analyzed. As already

mentioned at the beginning of this chapter, for similar reasons, *electrospray ionization* (ESI) was also essential for improving mass analysis.<sup>26</sup> As a consequence of such important changes, LC-IDMS gave the opportunity to investigate a wide range of drugs in biological system.<sup>47, 50-54</sup>

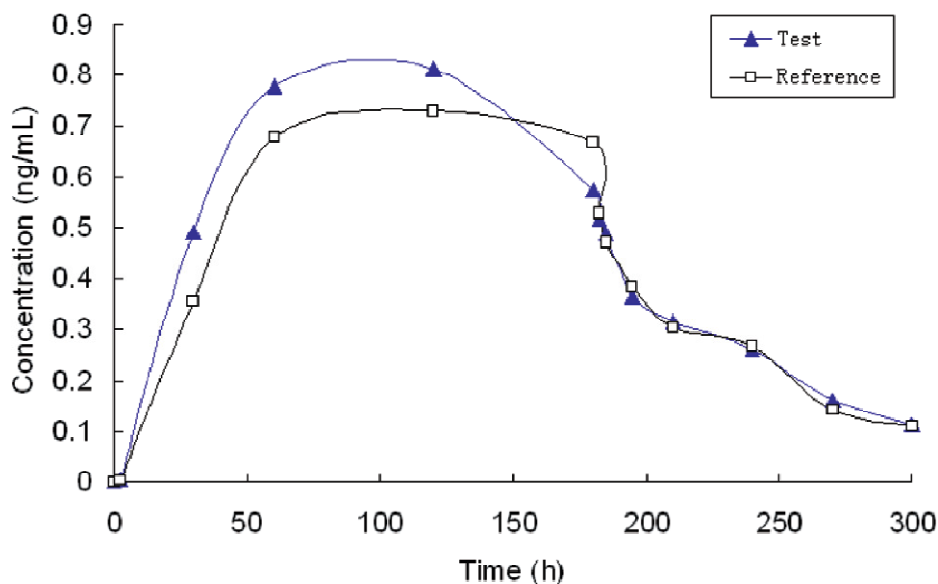
In order to offer an example of such technique application, an interesting bio-analytical study has been reported. Chau *et al.*<sup>55</sup> described a quantitative analysis of a potent narcotic analgesic known as *fentanyl*. The aim of this research was to measure the weekly variations of such drug concentrations in human plasma. As internal standard, a D<sub>5</sub>-analogue has been used. (Fig. 1.2). As stated in the last paragraph, such labelled compound has been used mainly for defining a calibration curve.



**Figure 1.2** Fentanyl and its D<sub>5</sub>-analogue used for LC-IDMS analysis.

In particular, in such case,  $y=2.32x+0.00883$  has been found as the function act to describe this curve. Specifically,  $y$  here represents the peak area ratios between *fentanyl* and its deuterated analog (*internal standard*), while  $x$  is instead related to the concentrations of *fentanyl*.

After the drug administration, fentanyl has been regularly extracted from blood in a range of 300 hours. The compound has been injected in LC-MS system with the additional constant amount of internal standard. Finally, the peak area ratios obtained were used in the relation examined above to increase the corresponding fentanyl concentration. In conclusion, according to the different range of time, a graphic of *fentanyl* concentration was realized. Furthermore, the experiment set up has involved two different trans-dermal *fentanyl* formulations. In Figure 1.3 the result for both has been reported. In conclusion, a series of information regarding this drug in human plasma, such as its emi-life and elimination, has been defined by LC-IDMS system.



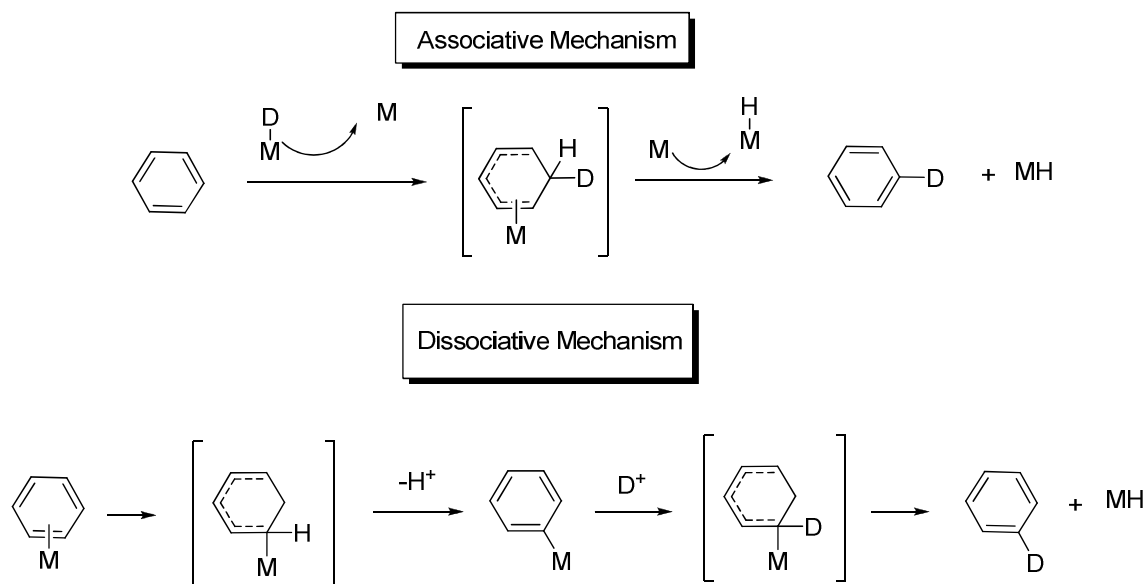
**Figure 1.3.** Concentration-time curve obtained after LC-IDMS analysis. Two different doses of *Fentanyl* have been administered to around 20 patients.<sup>55</sup>

The work reported above is only one of many examples in literature. Yiung *et al.*<sup>56</sup>, for example, were able to measure *Nudalarin-R*, a hepatotoxic cyclic pentapeptide, in human blood. Liu *et al.*<sup>57</sup> instead established the concentration of neurotransmitter acetylcholine in cerebral extra-fluids. In order to show the expansion that this technique has recently had, Dai *et al.*<sup>58</sup> investigated the amount of urea present in milk, an indicator of nutritious status for lactating animals. Similar investigations were developed by Koh *et al.*<sup>59</sup> in an attempt to determine melamine, a toxic organic base, found inside milk powder. Furthermore, in microbiology Dong *et al.*<sup>60</sup> defined the bacterial virus *Lambda* through LC-IDMS. Lastly, Bao *et al.*<sup>61</sup> quantified Sarin and other organophosphorus nerve agents, a highly toxic chemical category against cholinesterase (ChE), in rat plasma.

## 1.7 Hydrogen/deuterium (H/D) studies

An important consideration arises from LC-IDMS. As already seen in *fentanyl's* case, deuterium has been introduced in only one aromatic moiety. However, the deuterium incorporation here is so high that any form of *cross-contamination*<sup>62</sup>, which could disrupt the fundamental isotope ratio, can be avoided. As cited above, strategies for obtaining homogeneous deuterium distribution throughout the chemical structure have been thought to be extremely relevant. Consequently, the necessity to define a highly efficient H/D exchange is vital.

For the purposes of this project, it was fundamental to focus the attention on microwave (Mw) H/D exchange studies. As will be explained in the next pages, its particular heating system could guarantee the development and validation of a rapid and efficient H/D method. Moreover, initial bibliographic research of such background studies was equally necessary. Following such literature research, it was considered relevant initially to investigate the role of transition metal in heterogeneous and/or homogeneous catalysis. In order to give a clear explanation of both heterogeneous and homogeneous meaning a quick description will be given in the next few pages. Furthermore, since interchange reaction mechanisms are fundamental for understanding the metal efficiency in such context, two different kinds of H/D exchanges have been proposed. (Scheme 1.4).<sup>63</sup>



**Scheme 1.4.** Description of *associative* and *dissociative* mechanisms.

Although the general mechanisms proposed above arise from ligand substitution reactions, the experimental data recovered during this project will permit to propose new mechanistic considerations. Anyway, in inorganic chemistry these two different concepts are substantially distinguished by kinetic parameters. In particular, in *associative* mechanism the reaction rate depends on the entering group (deuterium in Scheme 1.4). On the other hand, in a *dissociative* mechanism, the rate will be fundamentally determined by leaving group. According to the metal used, a different percentage of both of these two theoretical mechanisms will be involved in the exchange. An example of this is rhodium which is thought to be characterized by both mechanisms<sup>64</sup> while in palladium *associative* mechanism is predominant. For the

importance that it will have in the development of this project, it is also very relevant to focus our studies on platinum and its chemistry. In particular, such metal is thought to catalyze the exchange according to a prevalent dissociative mechanism.

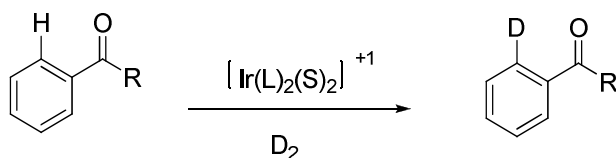
## 1.8 Heterogeneous H/D reactions

As in all reactions where a metal catalyst is in a different phase from reagents and solvent, *heterogeneous* H/D exchanges present some benefits. In particular, they do not require specific treatment to separate the catalyst from the product; a regular filtration is often enough for this purpose. In addition, since side products have rarely been reported, no particular purification process is necessary.<sup>65</sup> In the 1980s, interesting results were obtained in such context by use of rhodium.<sup>66-68</sup> Furthermore, *Lockley et al.*<sup>69</sup> were able to achieve *ortho*-selective deuteration on pyridine, quinoline and phthalazine derivatives by use of ruthenium under D<sub>2</sub> pressure. In addition, nickel was often considered as a useful metal catalyst in heterogeneous H/D exchange. Introduced by *Errede et al.*<sup>70</sup> in 1954, it has been used often for aromatic deuterations.

## 1.9 Homogeneous H/D reactions

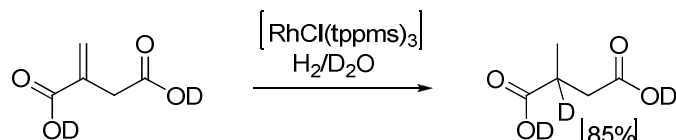
As a consequence of using metal catalyst soluble in solvent reaction, *homogeneous* reactions deserve some specific considerations. Firstly, they can benefit from milder conditions and secondly, as a consequence, they can also present considerable tolerance towards different functional groups.

The most relevant example of homogeneous H/D exchange is represented by cationic iridium complexes.<sup>71</sup> Due to their efficiency in activating C-H bonds, these species have been studied deeply. In particular, their characteristic *ortho*-deuteration on different aryl ketones and acetanilide has been reported.<sup>72-73</sup> (Scheme 1.5).



**Scheme 1.5.** Ortho-deuteration for acetamide under cation iridium (+1) catalysis.

Other similar metals, such as rhodium, have never been considered as efficient as iridium in H/D exchange. The only cases reported involve rhodium-olefin complex. Here, Brookart *et al.*<sup>74</sup> were able to obtain high deuterium incorporation from aniline and cyclopentene. Lastly, Joo *et al.*<sup>75</sup> measured an H/D exchange on C2 position of itaconic acid during its double bond reduction. (Scheme 1.6). The reaction has been set up with rhodium phosphate complexes as catalyst, soluble in D<sub>2</sub>O solvent.



**Scheme 1.6.** Selective deuteration on C2 position of itaconic acid catalyzed by rhodium salt.

Other relevant results were obtained from ruthenium catalysis. Mastubara *et al.*<sup>76</sup> recovered deuterated alkalones from alkenol substrate in deuterium oxide, while Lockley and Hesk<sup>69</sup> confirmed the important role played by such a catalyst in homogeneous H/D exchange for alcohols, cyclic and aromatic substrates.

To conclude this paragraph, as previously mentioned, one of the targets of this project is also studying the real efficiency of microwave in H/D exchange. Chappelle *et al.*<sup>77</sup> have described this aspect, reporting in particular that using an iridium catalyst, microwave irradiation improved the exchange reactions both in terms of time and deuterium incorporation. In light of this, the next pages will examine microwave heating in H/D studies.

## 1.10 Hydrogen/deuterium (H/D) microwave-assisted-reactions

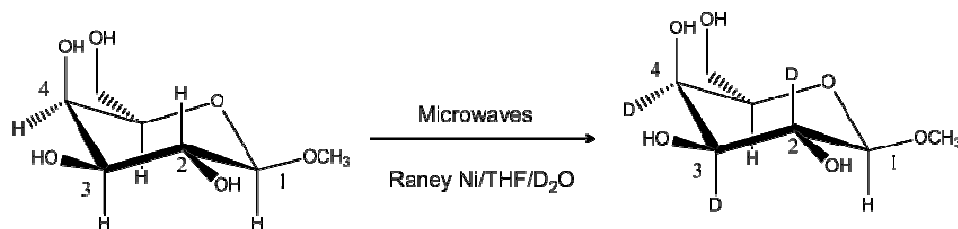
Nowadays, microwave-assisted reactions in organic synthesis represent a common way for obtaining products in high yield in a short time.<sup>78-79</sup> Many reviews describe efforts on the use of this technique for the improvement of the hydrogen/deuterium (H/D) exchange. It has been discussed that the energy released by a MW magnetron, directed to the body of a solution, is able to activate the C-H bond, thus promoting H/D exchange.<sup>80</sup>

Past studies have also revealed the important role played by solvent and catalyst.<sup>81</sup> It is common knowledge that microwave heating involves the physical properties of the chosen



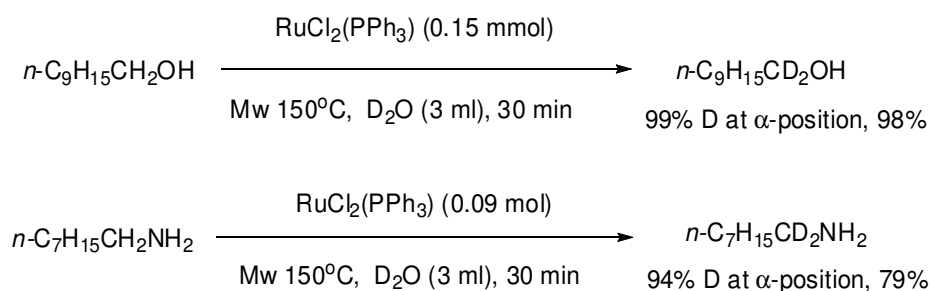
solvent and in particular its dielectric constant ( $\epsilon$ ).<sup>82</sup> Deuterium oxide was always considered the first choice in this instance, both because of its high polarity and for the chance of creating a deuterium enriched chemical environment. The synthesis of deuterium labelled compounds using microwave irradiation has also been very successful with a great variety of conditions and catalysts.<sup>83</sup>

Cioffi *et al.*<sup>84</sup> have applied all of these considerations for introducing deuterium in a stereospecific fashion on carbohydrate substrates. For this purpose Raney Nickel<sup>®</sup> was used as catalyst producing interesting results as shown on Scheme 1.7.



**Scheme 1.7.** Effect of MW process on 1-*O*-methyl- $\beta$ -D-galactopyranoside; only some stereospecific positions were deuterated.<sup>84</sup>

The specific activity of metal catalysis was confirmed by Masaaki *et al.*<sup>85</sup> by an investigation on primary alcohols and amines using a ruthenium catalyst. As shown in Scheme 1.8 the products are reported being deuterated on  $\alpha$ -carbon positions:

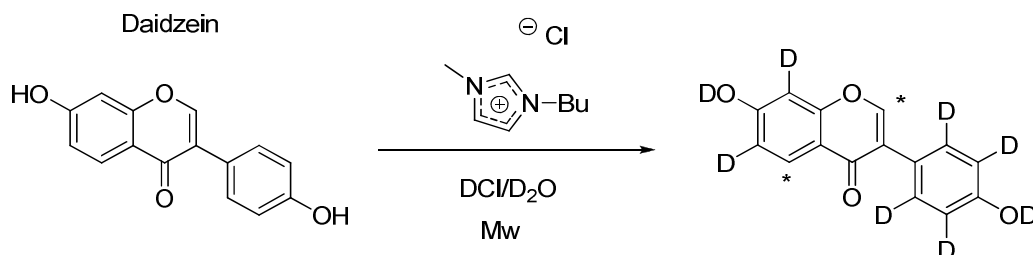


**Scheme 1.8.** Deuteration of primary alcohols and amines on  $\alpha$ -positions.<sup>85</sup>

The mechanism proposed by Masaaki *et al.*<sup>85</sup> has suggested how the metal catalyst is involved in this process of  $\alpha$ -deuteration. The particular catalytic effect was compared with that for acid catalyzed reactions.

Ullastiina *et al.*<sup>86-87</sup> reported a study on polyphenolic deuterations. These compounds represent interesting substrates in the pharmaceutical field in order to prevent hormone-

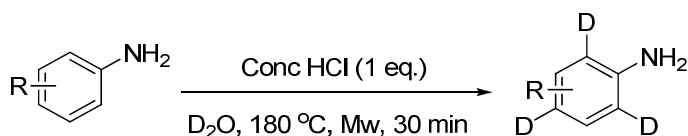
dependent diseases. Under microwave irradiation, using an ionic liquid to increase the solubility, deuterium was incorporated at multiple positions into one of the substrates (Daidzein), as reported in Scheme 1.9.



**Scheme 1.9.**Deuteration of Daidzein with a high level of deuterium incorporated. <sup>86</sup>

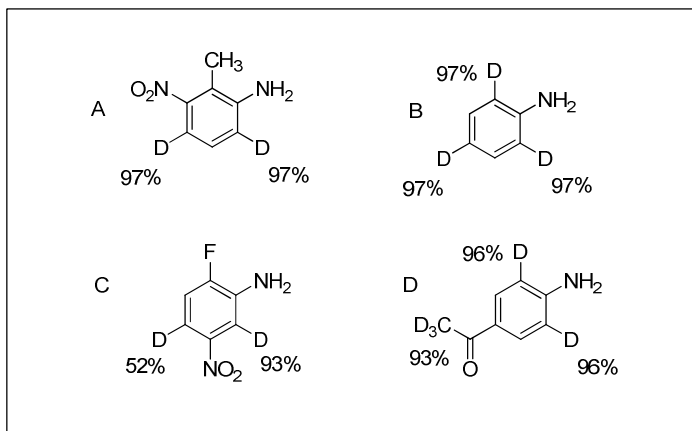
Despite the high level of deuteration,  $D^+$  was introduced only on the positions that are relatively active for the electrophilic aromatic substitution. For this reason, due to the inactivation inherent within the chromone, the two positions indicated by the asterisks were not exchanged at all.

Furthermore, in order to confirm the tendency shown by acid catalysed deuterations, Martins *et al.*<sup>88</sup> carried out a study on different aniline substrates in the presence of hydrochloric acid (HCl) (Scheme 1.10).



**Scheme 1.10.**Procedure for deuterating anilines under acid catalysed conditions. <sup>89</sup>

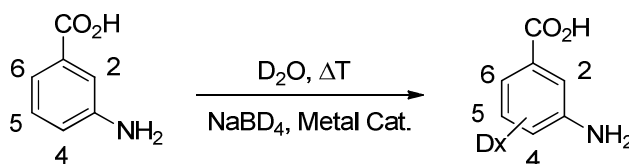
The environment created by HCl led to an excess of deuteronium ions which encourages the deuteration by an electrophilic aromatic substitution pathway. Some results obtained on different substituted anilines are shown in Scheme 1.11.



**Scheme 1.11.** Some anilines obtained through acid catalysed deuteration reactions.

In this case deuterium is incorporated according to the rules of electrophilic aromatic substitution. Notably, in the context of H/D exchange, the electronic influence of the amino group is more pronounced than the other functional groups on the aromatic ring. The importance of metal catalysts in these processes was supported by a consistent investigation by Aztrodt *et al.*<sup>90</sup> Using microwave irradiation and a transition metal catalyst, deuterium was also introduced at non-activated aromatic positions (Table 1.1).

**Table 1.1** Anilines obtained through metal catalysed reactions



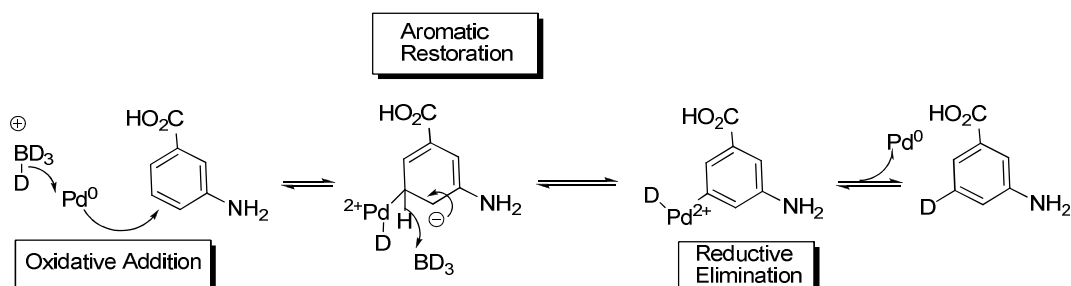
Entry	Catalyst	T <sup>o</sup> C	time	C2 <sup>a</sup>	C4 <sup>a</sup>	C5 <sup>a</sup>	C6 <sup>a</sup>	D <sub>max</sub> <sup>b</sup>	Yield
1	Pd/C	150	2h	88%	76%	45%	11%	D <sub>2</sub>	61%
2	Pd/C	150 Mw	2h	97%	96%	95%	53%	D <sub>4</sub>	57%
3	RhCl <sub>3</sub>	150	2h	92%	93%	89%	12%	D <sub>3</sub>	65%
4	RhCl <sub>3</sub>	150 Mw	2h	91%	92%	88%	12%	D <sub>3</sub>	67%

<sup>a</sup>Measured by <sup>1</sup>H NMR spectroscopic analysis; MW refers to microwave heating

<sup>b</sup>Measured by LC-MS.

These findings establish the importance of microwave heating on H/D exchange. In particular entries 1-2 demonstrate different deuterium incorporation can be promoted at relatively inactivated positions (C5 and C6). On the other hand, for a rhodium (Rh) based catalyst, the last two examples in entry 3 and 4 show no differences between the two different heating methods for such an exchange.

Furthermore, according to the review,  $\text{NaBD}_4$  could be used as a means to activate the Pd catalyst. In this case the considerations regarding *associative/dissociative* mechanism seems not valid. Since in such reaction is not involved any ligand substitutions ( $\text{Pd}^0$  does not present any attached ligands), an alternative mechanisms needed to be proposed. Hypothetically, it could be suggested that an intermediate, obtained through *oxidative addition*, links on its surface both  $\text{D}^-$  and to the aromatic substrates, allowing the subsequent electrophilic substitution (Scheme 1.12). The concept of *oxidative addition/reductive elimination* will be mentioned again in the next chapters when Pt behaviour will be described.



**Scheme 1.12.** Mechanism for deuterating the aromatic substrates. The intermediate is formed through *oxidative addition*. The final product is instead obtained through *reductive elimination*.

Currently, microwave-assisted deuteration processes represent a quite complex branch of research. The general overview just reported indicates most of these difficulties, which include such issues: harsh experimental conditions, variety of the choice of the catalyst and, last but not least, how to obtain a good deuterium incorporation in all structural positions.

## 1.11 Aims and Objectives

A general overview of deuterium importance in bio analysis has been provided. As one of the aims of this project, a rapid method for improving H/D exchange will be investigated and then validated in the next chapters. S

Such method will be then expanded to a series of different substrates with relevance in biology and pharmacology. In particular, starting materials involved in drug synthesis will be specifically considered. As a result of this, their use could have a relevant role in defining fully deuterated syntheses for biological active compounds.

## *Chapter 2*

# *Validating a new procedure for microwave-assisted H/D exchange*

**2.1 Introduction**

**2.2 First attempt of process validation**

**2.3 Deuterated compound analysis**

**2.5 Acid effect on metal catalyst**

**2.4 Calculation of deuteration yields**

**2.6 Effect of the amount of catalyst**

**2.7 Kinetic experiments**

**2.8 Conclusions**

### **2.1 Introduction**

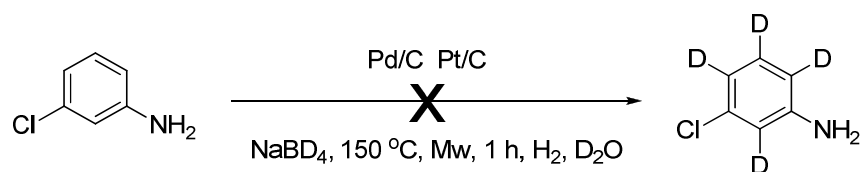
In the previous chapter a general overview of isotopes, deuterium and their applications and studies has been provided. In this chapter, the research for reaching a valid and efficient deuteration process will be described. As already mentioned, this experimental approach will be focused on microwave irradiation effect on H/D exchange. Parallel to these studies an analytical investigation will also be developed. In particular, *nuclear magnetic resonance* (NMR) and *mass spectrometry* aspects will be investigated. Since a clear form to present the results is necessary, a precise way to show them has been proposed. So, labelled molecules will be drawn with the corresponding deuterium percentage on all positions. When such percentages are not required to be reported, the molecules will instead be presented with deuterium in replace of hydrogen. This substitution will occur only when deuterium incorporation surpasses the limit of 50%. In addition, when the alkyl group is analyzed, the percentage of exchange will be referred to a singular C-D bond. In order to indicate its characterization in the experimental section, the acetylated compound number will be indicated by adding a letter to the corresponding aniline substrate number.

## 2.2 First attempt of process validation

After having defined the importance of labelled compounds in bio-analytical fields, a new method for obtaining deuterated molecules was established. As a first strategy, it was thought to recover labelled compounds from their labelled starting materials in their corresponding synthesis. McNeil *et al.*<sup>91</sup> reported detailed studies whereby <sup>14</sup>C was introduced into an antimalari drug during its synthesis. Following this example, and with the target of recovering fully deuterated compounds, it was thought to investigate small molecules directly involved in these synthetic processes. Consequently, in light of previous reports, anilines were considered ideal substrates to test the aims of this project. In addition, as will be explained in the next pages, these compounds are particularly appropriate in order to quantify the H/D exchange at all their positions.

So, following the considerations reported in McNeill's work<sup>91</sup>, 3-chloroaniline was chosen as example to explore.

Starting from Atzrodt's fundamental work<sup>90</sup>, already mentioned in the introduction, an initial reaction consisting of a mix of Pd/C (10 mol%, 10% on carbon), Pt/C (10 mol%, 6% on carbon), NaBD<sub>4</sub> (10 mol%, 98% D) in D<sub>2</sub>O was stirred at 150 °C for 1 h. (Scheme 2.1).

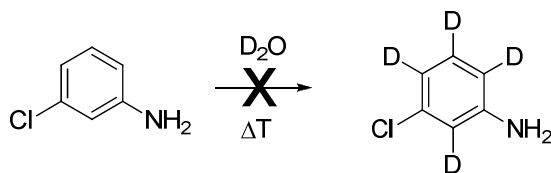


Scheme 2.1. Atzrodt's procedure using 3-chloroaniline.

As Atzrodt suggested in his investigations, the mixture of Pd/Pt/C represents a reasonably good way for increasing the efficiency of deuteration. In addition, hydrogen atmosphere was also used in order to liberate D<sub>2</sub> as a consequence of its reaction with NaBD<sub>4</sub>. In fact Sajiki *et al.*<sup>92-93</sup> have proposed that D<sub>2</sub> could be directly involved in H/D exchanges. Even though this hypothesis seems perfectly adaptable for unsaturated hydrocarbons, deuterohalogenation of aryl chlorides or bromides, benzil positions<sup>94</sup>, opening of epoxide rings and reductive methods<sup>95-96</sup>, the recommended process proved more difficult when applied to aromatic compounds. A comparison with the experiment reported in Tab 1.1 gives the opportunity to consider some relevant aspects. In this case some considerable results have been obtained. As a first possible explanation, the shorter time of reaction here (1 h against 2 h on Table 1.1)

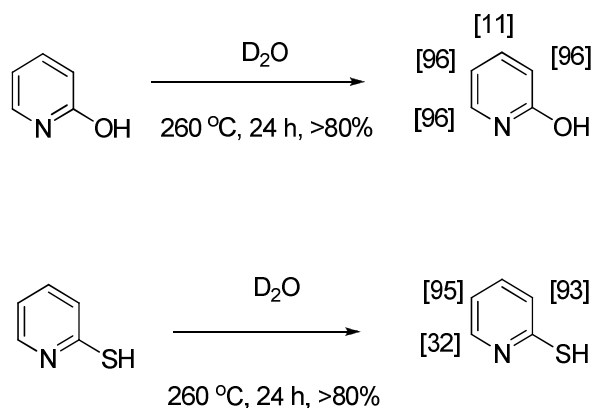
could have had a negative influence on the final result. Otherwise, following the mechanistic suggestion proposed in the last chapter, hydrogen atmosphere could have been exchanged with deuterium on NaBD<sub>4</sub>. As a consequence of a reduced deuterium concentration, the H/D exchange on the aromatic system could not have been developed as expected.

Furthermore, after the extraction in *diethyl-ether* (Et<sub>2</sub>O), only 3 mg of product from 300 mg of 3-chloroaniline were obtained. Arguably, due to solubility problems, much of the crude product was lost in the aqueous phase. Although deuterium oxide acts mainly as a solvent here, it also represents an important deuterium source. As a consequence, it should play an active role in H/D exchange. So, it was thought relevant to investigate its efficiency. For this reason a reaction using only D<sub>2</sub>O was set up (Scheme 2.2).

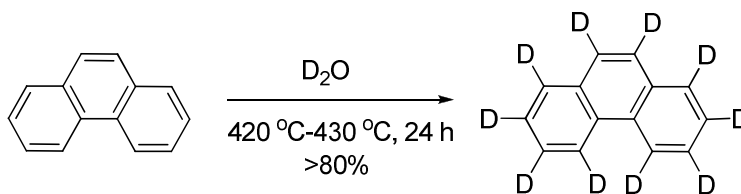


**Scheme 2.2.** Attempted deuteration of 3-chloroaniline using only deuterium oxide..

Although no relevant H/D exchanges were recorded in the experiment above, the result was nevertheless coherent with the research present in the literature. Most of the examples recovered required very harsh conditions. Werstiuk and Ju<sup>97</sup>, for example, were able to deuterate pyridine derivatives with remarkable yields (>80%) and high levels of deuteration (Scheme 2.3). Junk and Catalo<sup>98</sup> achieved almost complete deuteration of phenanthrene at 380°C-430°C (Scheme 2.4).

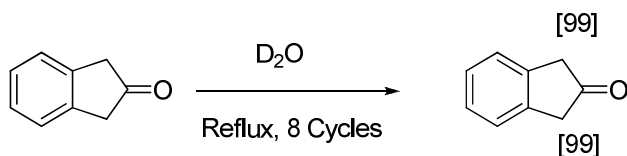


**Scheme 2.3.** Werstiuk and Ju's experiments on pyridine derivatives and their levels of deuteration obtained.<sup>97</sup>



**Scheme 2.4.**Junk and Catalo's experiment on phenanthracene.<sup>98</sup>

Conversely, Edlund and Berson<sup>99</sup> were able to establish a method without such extreme conditions. [1, 1, 3, 3-tetradeutero]-2-Indanone (Scheme 2.5), was obtained through repeated heating cycles of the starting material in D<sub>2</sub>O under reflux, giving a consistently high percentage of deuteration regioselectivity on the  $\alpha$ -carbon positions.



**Scheme 2.5.**Edlund and Berson's experiment obtaining [1,1,3,3-tetradeutero]-2-indanone.<sup>99</sup>

This final example also demonstrates how the use of deuterium oxide will only work efficiently under these conditions for exchange at acidic positions. Starting from these considerations, base catalysis was thought to give a relevant contribution in such cases. Many studies report considerable results for ketons<sup>100</sup>, esters<sup>101</sup>, aldehydes<sup>102</sup> and carboxylic acids<sup>103</sup> via a mechanism involving keto-enol equilibria.<sup>71</sup> However, as we will see shortly, for different reasons connected with solubility and metal activation, such conditions were not considered suitable for this project.

In view of the bibliographic considerations, extreme conditions of temperature and time were undoubtedly fundamental parameters. In order to establish the real effectiveness of microwave irradiation, a conventionally heated reaction (reflux at 100 °C for 24 hours) was set up in parallel with an analogue microwave-assisted reaction. However, due to the high pressure control associated with the microwave synthesis instrument, this device is able to perform reactions at temperatures over 200 °C and consequently above the solvent boiling point. As a result of this, in this trial the reaction conditions were considerably different. This fundamental microwave aspect was relevant for a number of reasons. Firstly, it enabled a quick and practical method for deuteration to be tested under controlled conditions at high reaction temperatures using commercial instruments. Secondly, it allowed H/D exchange



experiments to be performed under conditions little explored using conventional heating. As a consequence of these considerations, the first microwave reaction was set up at 180 °C for one hour in the absence of any additional catalyst to establish the scope of this process. (Table 2.1).

**Table 2.1.** H/D exchange of 3-chloroaniline in D<sub>2</sub>O using conventional and microwave heating.

Entry	Heating	Time	Temperature °C	<i>m/z</i> peak <sup>a</sup>	D <sub>max</sub>
1	Conventional	24 h	100	127	<i>d</i> <sub>0</sub>
2	Microwave	1 h	180	127	<i>d</i> <sub>0</sub>

<sup>a</sup>*m/z* refers to dominant peak observed by mass spectrometry.

Additionally, in order to circumvent the problems connected with the solubility of the products in *diethyl ether* (Et<sub>2</sub>O), *dichloromethane* (DCM) was chosen as a more efficient solvent for *the work up*.

These two attempts revealed no substantial deuteration of the substrate. Mass spectrometry in fact showed only one peak, corresponding to the starting material (M<sub>r</sub>=127). For this reason the reactions were stopped and the corresponding yields were not calculated.

This result confirmed once again the importance of metal catalysts in such experiments. However, parallel to metal catalysis, acidic catalysis H/D exchange was also investigated. As we will see shortly, the pH will influence metal activity and final results. In particular, two different kinds of such acids could be described. Strong Brønsted acids, such as *deuterium sulfuric acid* (D<sub>2</sub>SO<sub>4</sub>), or, alternatively, Lewis acid in combination with a deuterium source could be used. Wahala *et al.*<sup>104</sup> demonstrated that combining both methods could result in a high level of deuteration at only the activated position. Substrates as flavonoids, isoflavonoids and lignans were deuterated at temperatures between 20 and 50°C. However, in order to improve the level of exchange, the same authors decided to increase the reaction time (reaching 20 hours). As a result of this, enterolactone, one of the substrates chosen for this investigation, was almost fully deuterated (99%) in all of its *active* and *inactive* positions.<sup>105</sup> Microwave irradiation gave a considerable contribution to such acidic catalyzed H/D

exchange development. This was due in principle to the reduction of reaction time and, on the other hand, into preserving an identical level of deuteration.<sup>106-107</sup>

Atzrodt *et al.*<sup>71</sup> have previously highlighted the interesting role played by CF<sub>3</sub>COOD, D<sub>2</sub>SO<sub>4</sub>, AcOD and DCl on H/D exchange. However, in the introduction, it was already described how difficult it was exchanging at less active aromatic positions. With the prospect of performing synergism between acidic and metal catalysis in our reactions, it was considered relevant to develop an accurate study of acidic efficiency. For the initial trials deuterium chloride (DCl) was chosen as a reasonably cheap product in the quantities needed for this project. Hence, a series of experiments was developed, dedicated to establishing the effect of this acid on deuteration. The first step of this investigation involved measuring the variability of the *m/z* values of molecular ions in the mass spectra according to different quantities of DCl added (Table 2.2). Lastly, in order to accelerate the experimental data recovering, it was considered helpful to set up reactions in a time range of 30 minutes.

**Table 2.2.** Results for H/D exchange of 3-chloroaniline using variable equivalents of DCl over time.

Entry	Heating	Time (min)	T °C	DCl (Eq.)	<i>m/z</i> <sup>a</sup>	<i>D</i> <sub>max</sub>
1	Mw	30	180	1	130	<i>d</i> <sub>3</sub>
2	Mw	30	180	2	130	<i>d</i> <sub>3</sub>
3	Mw	30	180	3	130	<i>d</i> <sub>3</sub>
4	Mw	30	180	4	130	<i>d</i> <sub>3</sub>

<sup>a</sup>Analysis developed on DCM as solvent.

<sup>b</sup>*m/z* refers to the dominant peak observed by mass spectrometry of the crude reaction mixture.

As Table 2.2 shows, in all of these experiments a considerable deuteration of 3-chloroaniline occurred. This was confirmed by mass spectra, where the main peak corresponded to the trideuterio structure. So, the next step was to explore the effect of DCl over different reaction times. After having seen the results on Table 2.2, it was decided to use four equivalents of DCl for this investigation. After also having reported difficulties in solving 3-chloroaniline, this decision was necessary to guarantee better solubility of this substrate in deuterium oxide (Table 2.3). Furthermore, for the similar reason reported above, the reaction times chosen for this second trial were relatively short. This aspect gave us the opportunity again to accelerate the investigation and, on the other hand, to obtain considerable information on time dependant acidic trend. In particular, a first look of the results recorded showed that time is a parameter strictly connected with H/D exchange. As we will see later, the necessity to

clarify this relationship will force us to set up a series of kinetic experiments which will further clarify this aspect.

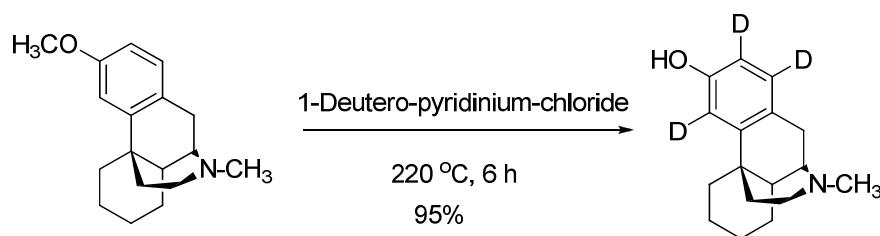
**Table 2.3.** Results of reactions set up at different times with identical DCl equivalents.

Entry <sup>a</sup>	Heating	Time (min)	T °C	DCl (Eq.)	m/z <sup>b</sup>	D <sub>max</sub>
1	Mw	10	180	4	130	d <sub>3</sub>
2	Mw	20	180	4	130	d <sub>3</sub>
3	Mw	30	180	4	130	d <sub>3</sub>
4	Mw	40	180	4	131	d <sub>4</sub>

<sup>a</sup>Analysis developed on dichloromethane as solvent

<sup>b</sup>m/z refers to dominant peak observed on mass spectra.

In addition, Heinkele *et al.*<sup>108</sup> were able to completely deuterate dextrometethorphan using a 50 to 100 fold excess of 1-deuterio-pyridinium-chloride under extreme conditions. (Scheme 2.6). As a result of this, the impressive efficiency of microwave irradiation in such field has been one more time confirmed.



**Scheme 2.6.** Heinkele's H/D exchange reaction.

This experiment is correlated with the results reported in Table 2.3. The most relevant product (3-d<sub>4</sub>-chloroaniline) has been recorded only in Entry 4. As a consequence of this, an appropriate balance time/acid has been thought to be an important parameter in H/D exchange. In addition, this last consideration indicates that the fourth, less active position on 3-chloroaniline could be deuterated only under appropriate conditions.

In conclusion, it has been demonstrated that good deuterium incorporation could be attained using reasonable experimental parameters, such as appropriate temperatures and even short time-scales, and only 4 eq. of DCl as an acidic environment. Furthermore, the considerations taken from Heinkele and the results shown in Table 2.2 and 2.3 confirm that DCl would be a good point from which to begin validating an efficient method.

## 2.3 Deuterated compounds analysis

### 2.3.1 Mass spectrometry

In general, deuteration consists of a series of H/D exchanges that could have different effects on every molecule present in solution. As a result, the mixture will show a set of deuterated species characterized by different levels of isotopic incorporation and molecular weight. Mass spectrometry allows the distinction of all these species during the analysis and the consequent spectra will display the typical *cluster* for the deuterated compounds.

In Fig 2.1 the mass spectrum of 3-*d*<sub>3</sub>-chloro-aniline obtained from Entry 4 Table 2.2. is displayed. The cluster linked to the substrate (main peak 130) is indicated. This peak is present with other strict signals beside which represent the isotopic species mentioned above. Lastly, other clusters arising from fragments are reported and easily distinguishable because of their characteristic shapes.

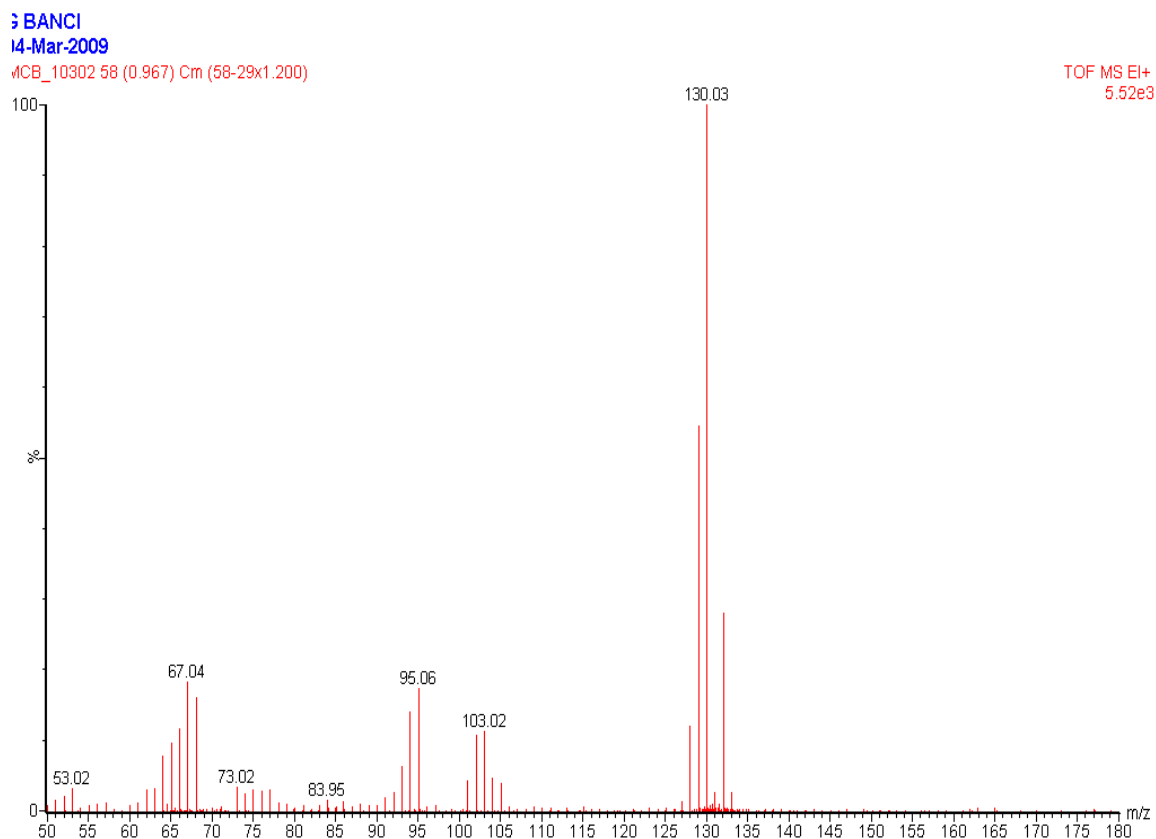


Fig 2.1. Mass spectra of 3-*d*<sub>3</sub>-chloroaniline. Clusters are indicated with their characteristic shapes.

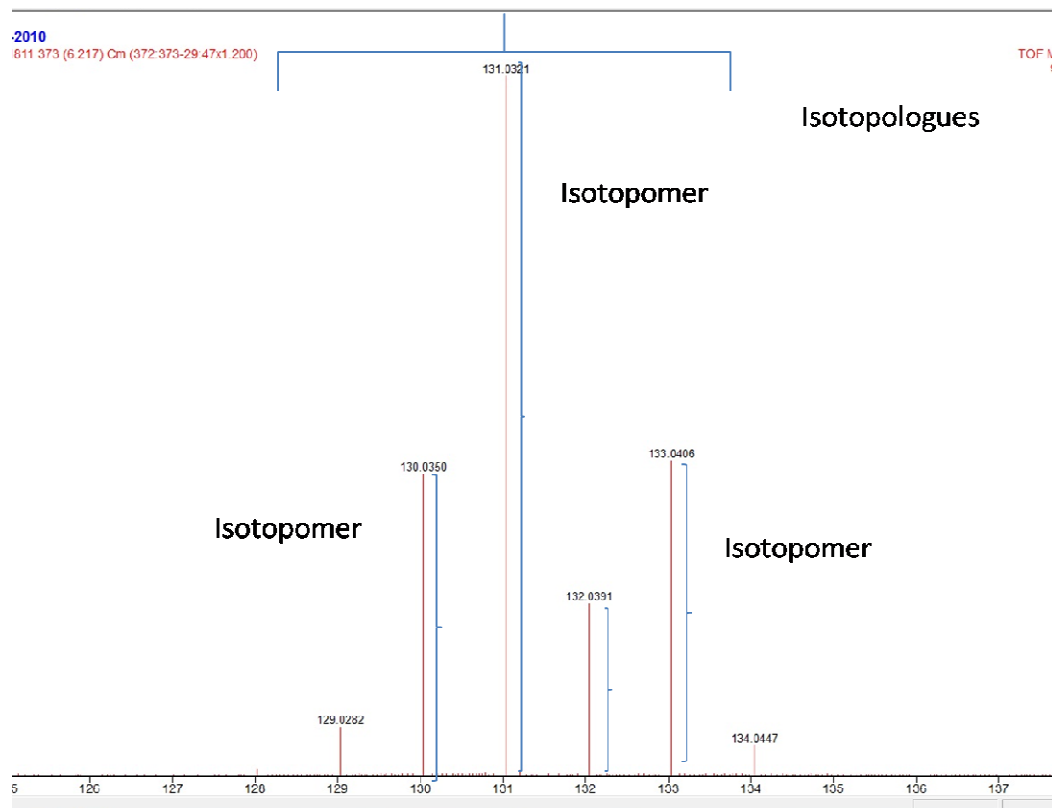
In addition, a cluster includes molecules with the same chemical structure but different isotopic incorporation. Such species are better known as *isotopologues*. On the other hand, a single peak represents several different molecules with the same structure and total isotopic incorporation (meaning same molecular weight and  $m/z$ ) but different deuterium distribution. Such species are instead called *isotopomers* (Scheme 2.7).

Isotopologues				
Isotopomers	 MW 128	 MW 128	 MW 128	 MW 128
Isotopomers	 MW 129	 MW 129	 MW 129	 MW 129
Isotopomers	 MW 130	 MW 130	 MW 130	 MW 130

Scheme 2.7. Example of *isotopologues* and *isotopomers*.

In order to clarify this concept, in Fig 2.2 the specific cluster relating to Entry 4 of table 2.3 has been reported. The position of *isotopologues* and *isotopomers* is once again indicated.

As mentioned before and reported in Table 2.3 and 2.4, all the mass spectrometric analyses were conducted with DCM solutions. The choice of this solvent was suggested when *scrambling process* arose as a relevant problem for the correct spectrum interpretation. Since it was thought that the amino group on the 3-chloroaniline could be partially or completely involved in eventual H/D exchange during the spectrometric run, methanol and other protic solvents were definitely abandoned. This decision was made in order to obtain a spectrum able to describe the reality of the solution investigated. Since DCM guaranteed any exchange on the most activated position, such solvent was seen as the best choice.



**Fig 2.2.** Cluster with *isotopologues* and *isotopomers* indicated.

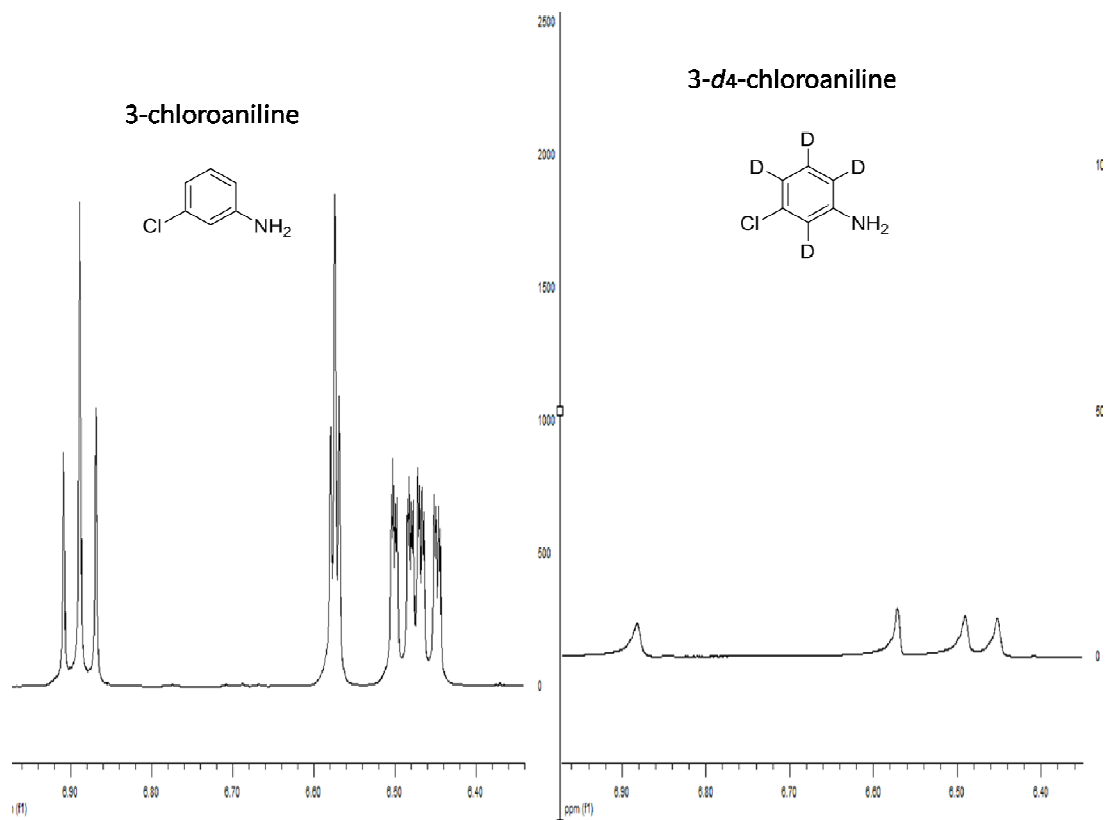
Usually the two definitions (of *isotopologues* and *isotopomers*) are quite pertinent in metabolic studies.<sup>109</sup> A narrow isotope cluster (rich in *isotopomers* and poor of *isotopologues*) could guarantee both a good level of H/D exchange and a low level of  $M_0$  (non deuterated species). Furthermore, this aspect has already been treated in the last chapter when such compounds have been used as internal standard for LC-MS/MS investigations.

For the aim of this project, mass spectrometry presents serious limits for a complete deuterium analysis. As already described above, we can only have an idea of deuterium incorporation from the narrow isotope cluster. Moreover, while this technique allows us to measure the level of H/D exchange through the molecular weight reported on the main peak, it does not reveal anything about where the deuterium has been introduced and in which percentage at each location.

### 2.3.2 $^1\text{H}$ -NMR

Since mass spectrometry could not provide the detailed information required, another analytical method needed to be investigated. Although in the previous chapter IR has been presented as a theoretical technique to distinguish labelled and unlabelled compounds, the analytical information recovered from it was not as valid as  $^1\text{H}$ -NMR. As a result of its analytical importance in organic chemistry, the main interest was inevitably based on nuclear magnetic resonance.

However, establishing deuterium incorporation on every single position of a chemical structure was not easy. A comparison between a  $^1\text{H}$ -NMR signals for a proton and its corresponding deuterated analogue reported in Figure 2.3 highlights strong difference in intensity and in shape.



**Fig.2.3.** Comparison between  $^1\text{H}$ -NMR signals in 3-chloroaniline and 3- $d_4$ -chloroaniline.

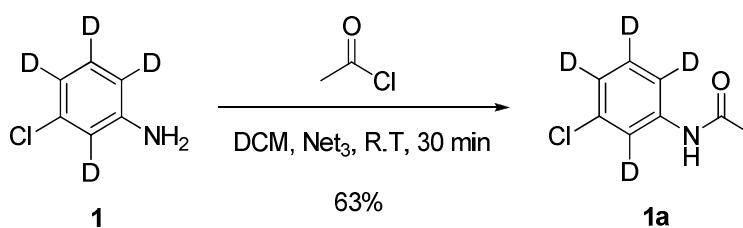
The signals arising from labelled compound (spectrum *on the right*) are due to the remaining protons still present in the molecule. However, because the level of deuterium incorporation is here high, their intensity is so low that no particular coupling can be spotted. The

integration reveals the ratios of the peaks areas, permitting only to understand which positions are relatively more deuterated than others. As it will be explained shortly, the use of  $^1\text{H-NMR}$  in such project requires a clearly defined proton 1. As a result of this, deuterium incorporation can be easily established at every position of the product.

In literature, much research involves addition of a known amount of chemical or solvent to the  $^1\text{H-NMR}$  samples in order to act as a standard for measuring the absolute isotopic amount of component in the mixture. Guo *et al.*<sup>110</sup> reported that 1,4-dioxane was added for their purpose. On studying aromatic substrates this solvent was chosen because of its intensity, the absence of coupling giving a singlet and finally, the chemical shift, which is quite far from the aromatic region. Derdau *et al.*<sup>90</sup>, instead, preferred tartaric acid for their deuterium analysis.

At the beginning of this project, it was decided that a similar procedure would be followed. Acetic anhydride ( $\text{Ac}_2\text{O}$ ) was used for this aim, but immediately all the difficulties in adding a strictly precise amount of this compound into the  $^1\text{H-NMR}$  samples became clear.

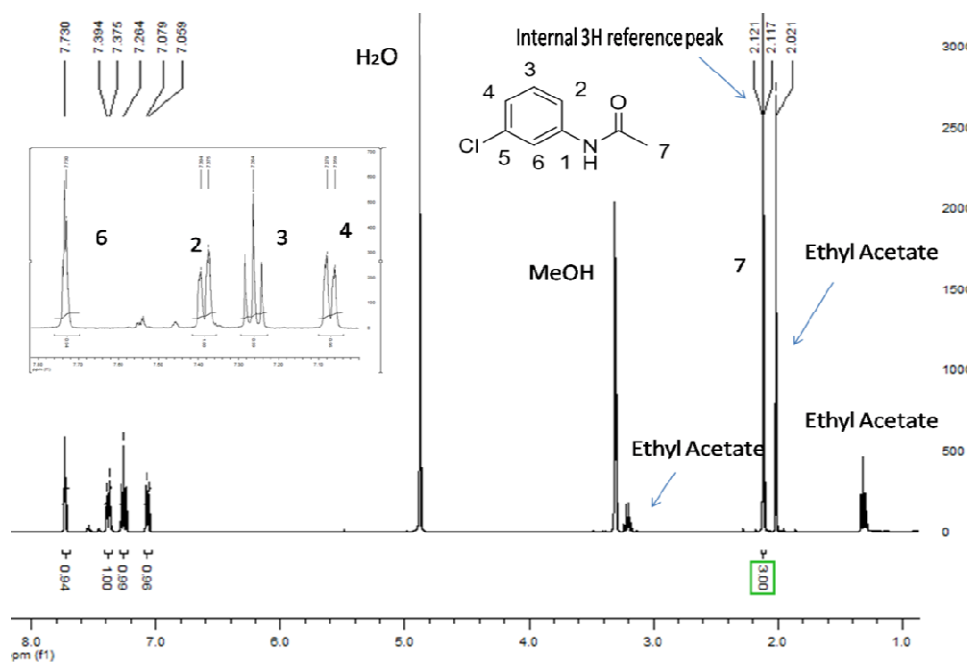
For this reason, it was thought to introduce a proton containing functional group directly to the molecule just deuterated, so that the integration values for the deuterated locations could be compared to those for protons of the added group, thereby providing an internal reference. The acetyl group (Ac) was considered to be the best choice and, consequently, an acetylation reaction was carried out on 3-chloroaniline (Scheme 2.8).



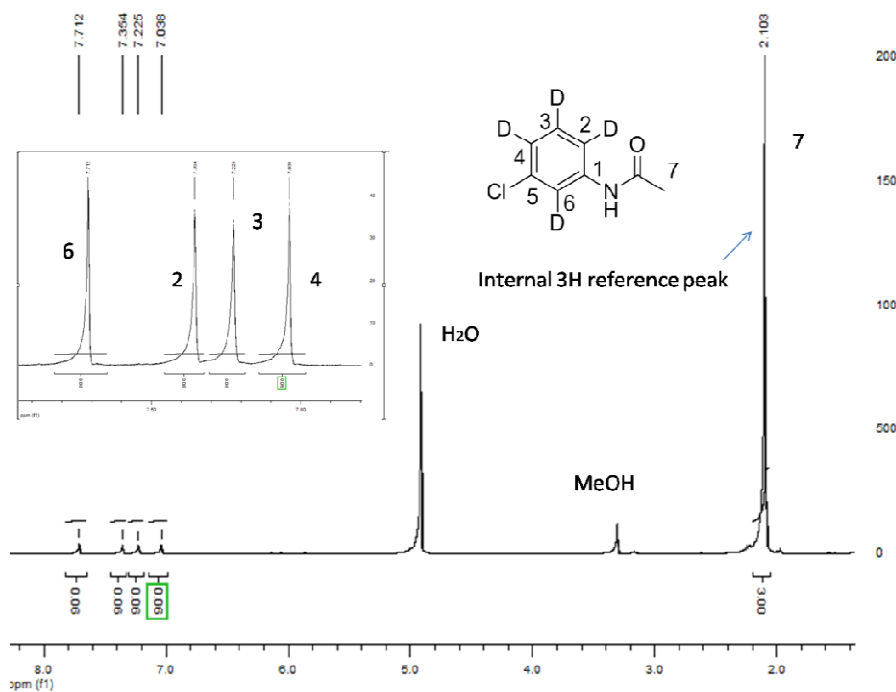
Scheme 2.8. General procedure for acetylation of deuterated anilines.

As shown in Scheme 2.8, the methyl group (Me) is not deuterated at all, so it provides the reference against which other integrations can be compared (Fig 2.4 and 2.5).





**Fig.2.4**  $^1\text{H-NMR}$  for *N*-(3-chlorophenyl)acetamide. As a result of chromatographic process (petroleum ether/ethylacetate 2:1), traces of ethylacetate (EtoAc) are present.



**Fig. 2.5.**  $^1\text{H-NMR}$  for *N*-(2,3,4,6-tetradeutero-3-chlorophenyl)acetamide. The signals on aromatic region present no coupling due to the high level of deuterium incorporated (94% each).

With the additional acetyl group it was possible to calculate the deuteration percentage of different hydrogen environments by integrating each peak against the integration of the acetyl group (peak number 7).

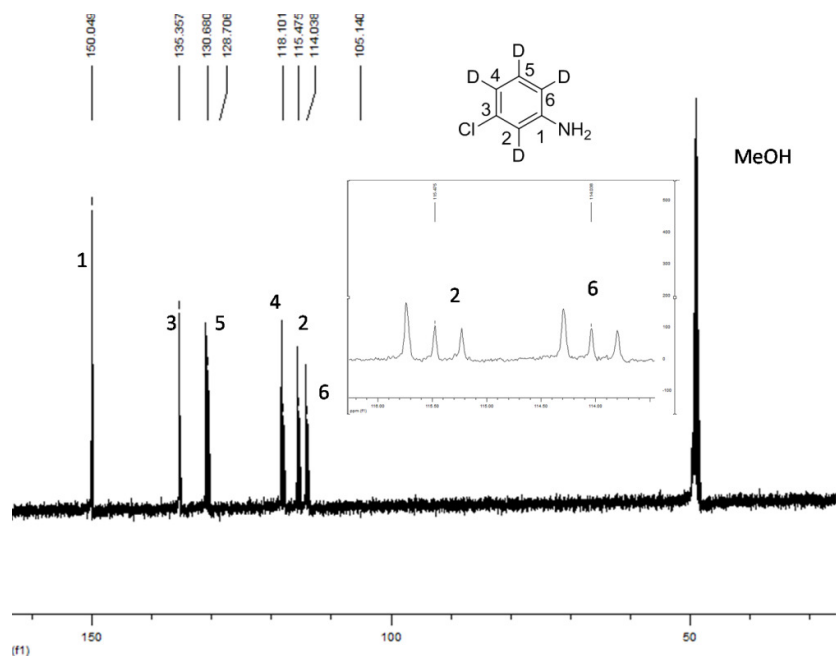
A very simple calculation was needed to obtain such percentages. For example, a comparison between peak 6 ( $\delta = 7.71$ ) in Fig 2.5 and peak 6 ( $\delta = 7.73$ ) in the Fig 2.4 highlights a considerable difference in their corresponding integrated area. In labelled case, 0.06H versus the methyl group (peak number 7) was recovered. This hydrogen amount opposes the true value of 1.00 H in unlabelled analogue. An easy subtraction can finally quantify deuterium as 0.94 of 1.00. As a result, 94% of deuterium is present in this position. The same considerations can be extended to all other environments to give the deuteration level of each active hydrogen position.

In conclusion, a consideration regarding the deuterated solvent chosen regularly for this analysis needs to be faced. Solubility problems sometimes necessitated a change of solvent, and *d*<sub>4</sub>-methanol-deuterated (CD<sub>3</sub>OD) was seen as the best choice. As will be clear following the reading of the next chapters, all of the substrates investigated in this work were aromatic compounds. Because of the necessity of having an isolated signal in the aromatic area in the NMR spectrum, *d*-chloroform (CDCl<sub>3</sub>) was found not to be very useful for this purpose. Since its typical chemical shift signal ( $\delta = 7.26$ ) is in the aromatic region, hence it may be difficult to obtain accurate data. Similar considerations can be advanced for *d*<sub>6</sub>-dimethylsulphoxyde (DMSO); in this case the solvent shows a peak ( $\delta = 2.50$ ) in that is a very close to the acetyl signal ( $\delta = 2.10$ ). That could cause serious difficulties for establishing the integration value of 3H signal of the acetyl group and disrupt the rest of integration process. As a consequence of these considerations, methanol was judged the best option. Its typical chemical shift signals ( $\delta = 3.31$  for solvent and  $\delta = 4.92$  for water) reduce the integration problems in the NMR experiments. That leads to a more reliable and accurate deuterium incorporation figure for every single position of the compound explored.

### **2.3.3 <sup>13</sup>C-NMR**

Strong deuterium incorporation can also cause considerable changes on the <sup>13</sup>C-NMR spectrum. In Fig 2.6 the corresponding <sup>13</sup>C-NMR spectrum for 3-*d*<sub>4</sub>-chloroaniline is shown. The difference from the corresponding non-deuterated spectrum is significant. Due to a different resonance frequency, deuterium is not involved in any form of decoupling process.

As a result of this,  $^{13}\text{C}$ -NMR records every carbon bonded to a deuterium (C-D) as a multiplicity signal. Since deuterium's spin is 1, the corresponding C-D coupling ( $J$ ) results in a particular *triplet* with the ratio of intensity recorded equal to 1:1:1.



**Fig.2.6.**  $^{13}\text{C}$ -NMR spectrum for  $d_4$ -3-chloroaniline. The C-D peaks are now triplet with a  $J_{c-d}$  around 25.5 Hz. The specific C-D triplets for 2 and 6 carbons have been expanded.

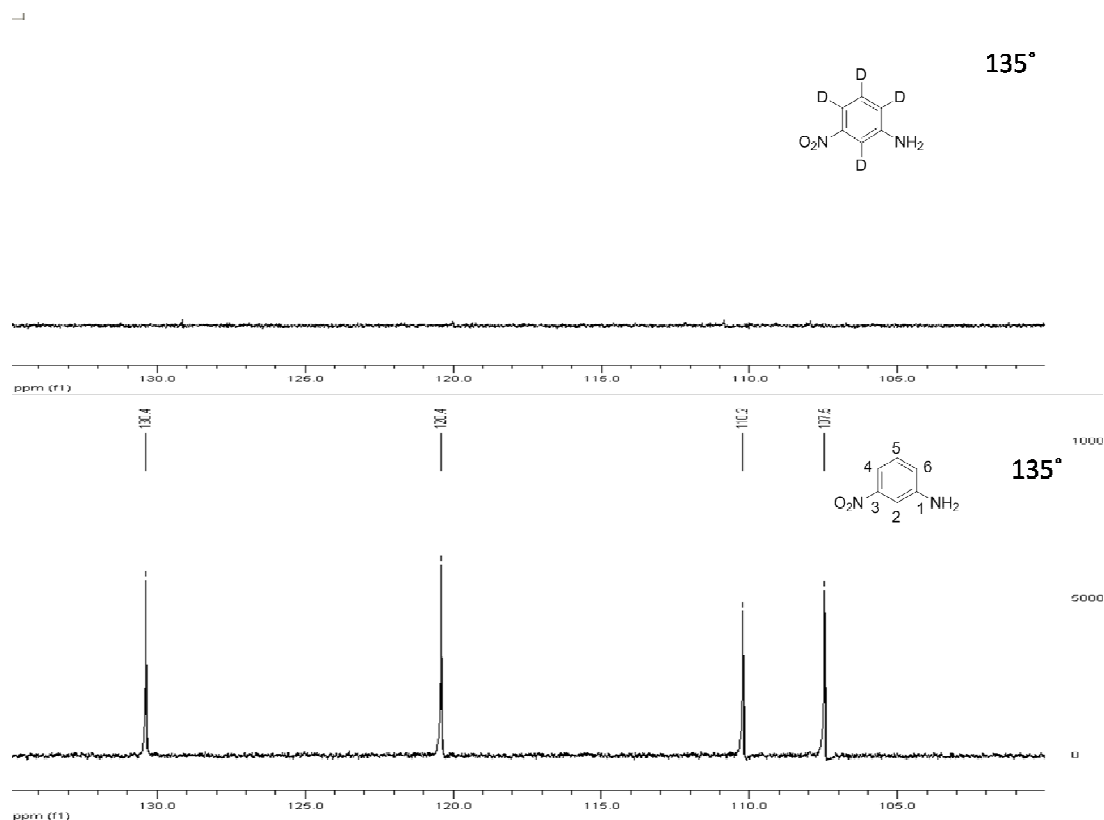
However, in the spectrum above, the intensity of the C-D peaks seems not to deviate from the regular ratios 1:1:1. Notably, the last signal on the *left* seems to be more intense than all the others. That is as a result of an overlapping with the non-deuterated isotopologues, which gives a singlet peak due to  $^{13}\text{C}$ -H decoupling.

As Dziembowska *et al.*<sup>111</sup> have described in their studies, deuterium chemical shift is moved from the natural C-H chemical shift, and it is also measured by referring to the central peak of the triplet.

Finally, it is important to highlight that these peaks are observable only if the concentration of deuterated product is particularly high. Due to *splitting*, the C-D signal's intensity could be quite low. As a consequence of this, for low concentrations it is very difficult to see any relevant C-D signals.

### 2.3.4 DEPT (distorsionless enhancement polar transfer)

This NMR technique is usually used for establishing the environment around each carbon atom and in particular for calculating the number of Hs bonded to it. The key of this experiment is based on *polarization transfer* through which  $^{13}\text{C}$  signal intensity has the chance to be varied. The concept of this process is directly connected with *gyromagnetic ratio*  $\gamma$  parameter. As a way to indicate specific resonance frequencies, *gyromagnetic ratio*  $\gamma$  is responsible for NMR intensity signal. So, *polarization transfer* occurs from one nucleus with a larger parameter  $\gamma$  (meaning high resonance frequency), such as a proton, to a smaller one, such as a  $^{13}\text{C}$ . As we will see shortly, this experiment involves the use of an additional pulse. As a result of this, a selective inversion of one of proton transition inside a carbon-hydrogen coupling system occurs. According to its degree, such pulse causes considerable change on  $^{13}\text{C}$  signal intensity. Three different pulse sequences are usually used with regards three different impulses being sent at specific angles ( $45^\circ$ ,  $90^\circ$  and  $135^\circ$ ). As mentioned above, carbon environment can be established. In particular, CH,  $\text{CH}_2$  and  $\text{CH}_3$  bonds became easily distinguishable. While at  $45^\circ$  all of these intensities are preserved, at  $90^\circ$   $\text{CH}_2$  and  $\text{CH}_3$  are instead equal to zero. As a consequence, while CH can be initially recognized,  $\text{CH}_2$  and  $\text{CH}_3$  need to wait the last pulse at  $135^\circ$ . Here  $\text{CH}_2$  intensity becomes negative and distinguishable from  $\text{CH}_3$ . Lastly, due to the hydrogen absence, quaternary carbons are instead not recorded with this technique. Since the experiment described until now is set up according to proton parameters, having deuterium different resonance frequency, it cannot be involved in any polarization transfer. As a consequence of this, generic C-D bond can be considered equal to quaternary carbon and so not be recorded on the spectrum. However, if peaks are still present, they are directly due to the unexchanged hydrogens after the deuteration process. (Fig 2.7).



**Fig.2.7.** DEPT-NMR for 3-*d*<sub>4</sub>-nitroaniline. A corresponding DEPT-NMR for non-deuterated compound is also reported. No signals are recorded in the labelled substrates.

As Fig 2.7 shows, only signals arisen from non-deuterated species are displayed in this spectrum. In conclusion, the complete disappearance of these signals in this kind of experiment would confirm complete deuteration.

## 2.4 Calculation of the deuteration yield

At this point a description of how yield calculations are conducted is necessary. Firstly, an average molecular weight ( $Mr'$ ) is determined. (Scheme 2.9).

$$Mr' = Mr(\text{starting material}) + 1.001 (D2\% + D3\% + D4 + D5\% + D6\%)$$

$\swarrow$ 
 $\underbrace{\hspace{15em}}$

**Mr(D)-Mr(H)**
**Levels of Deuteriations**

**Scheme 2.9.** Calculations of the average molecular weight ( $Mr'$ ) of a deuteration product mixture.

Using the value of  $Mr'$  obtained above, the percentage yield is calculated in the usual manner. (Scheme 2.10).

$$\text{mmol}' = \frac{\text{Mr}'}{\text{Mass of Recovered Product}}$$
$$\text{yield} = \frac{\text{mmol}'}{\text{mmol starting material}} \times 100$$

Scheme 2.10. Calculations for mmol and yield.

## 2.5 Acid effect on metal catalysis

As will be explained shortly, this project has been based on *homogenous catalysis*. As described in the introduction, the advantages from such catalysis are considerable. In particular, the results appear to be much more impressive than the ones in heterogeneous case. The key role played by deuterium oxide in microwave irradiation system has been already highlighted. Its importance as the main deuterium source was equally fundamental. As a consequence of this, the scientific interest for H/D exchange was focused mainly on the aqueous phase. Following this conclusion, appropriate conditions and metal catalyst were necessary to be stated. In such research bibliographic investigations gave an important contribution. In particular, the work reported by one of the first chemists to investigate H/D exchanges, John Garnett<sup>112</sup>, who succeeded in achieving great recognition in this field was significant.

Initially used by Garnett as a means of investigation for reaction mechanisms in *heterogeneous catalysis*, *homogeneous catalysis* earned more and more importance when it revealed to be a real valid method for H/D exchange. Furthermore, Pt<sup>2+</sup> and Pt<sup>4+</sup> salts, which until then were used as catalysts for completely different reactions, such as hydrogenation and oxidations<sup>113</sup>, were regularly employed.

In particular, H<sub>2</sub>PtCl<sub>6</sub><sup>112</sup> was the first one to be explored. Kinetic studies showed that H<sub>2</sub>PtCl<sub>6</sub> was not involved in any role as a catalyst because the rate of exchange did not follow its

concentration's variations. Different conclusions were reached for the salt  $K_2PtCl_4$ . New kinetic studies showed a high dependence of H/D exchange on the amount of catalyst used. Although more soluble than  $K_2PtCl_4$  in  $D_2O$ ,  $Na_2PtCl_4$  was a second choice in these trials because its level of hygroscopicity was considered unsuitable for such experiments.

Focusing all the attention on  $K_2PtCl_4$  it was apparent that the acidic conditions had a great influence on catalyst's efficiency. Garnett, established that a high HCl concentration was needed to avoid a strong precipitation of  $Pt^0$  from  $[PtCl_4]^{2-}$ . This consideration was also confirmed by  $H_2PtCl_6$ , which generated  $Pt^0$  unless HCl was present in strong excess. This last condition was also fundamental for the success of the experiment. Catalysis by  $Pt^0$  was in fact inhibited by acids still present in solution; therefore, if  $Pt^0$  was formed the metal could no longer be used as a catalyst. Subsequently, the H/D exchange could not proceed and deuterium incorporation recovered would be very low. The conclusions reached by Garnett were coherent with that one recorded in Tab 2.2. So, the acidic influence studies on H/D exchange helped us to define an efficient method.

Furthermore, since all Garnett's experiments were conducted under conventional heating, an investigation into DCl and  $K_2PtCl_4$  under microwave irradiation was natural and essential.

Choosing an appropriate substrate for this attempt was also deemed imperative. At the beginning of this study, aniline was seen as the best choice for establishing the efficiency of deuteration without any other electronic influence than the amine group.

Garnett's experiments were set up at 130 °C for over 168 hours. Considering the typical performance of microwave to reach high temperatures in a short time<sup>107</sup>, standard parameters of time/temperature were selected as 2h at 200 °C.

The same standard amount of  $K_2PtCl_4$  applied by Garnett in his experiments (20 mol %, respect the amount of the substrate explored) was also applied (Table 2.4).

Table 2.4. Set of experiments with different DCl amounts.

Entry	N <sup>o</sup> <sup>c</sup>	DCl	K <sub>2</sub> PtCl <sub>4</sub>	D2 <sup>a</sup>	D3 <sup>a</sup>	D4 <sup>a</sup>	D5 <sup>a</sup>	D6 <sup>a</sup>	Yield <sup>b</sup>
1	N.A	0 (eq.)	20 mol%	95%	45%	95%	45%	95%	46%
2	N.A	2 (eq.)	20 mol%	91%	91%	91%	91%	91%	90%
3	2a	4 (eq.)	20 mol%	91%	91%	91%	91%	91%	44%
4	2c	4 (eq.)	2 mol%	91%	25%	91%	25%	91%	80%

<sup>a</sup> Deuterium incorporation calculated followed acetylation.

<sup>b</sup> Yield calculated as in Scheme 2.10.

<sup>c</sup> Structure number assigned to the deuterated aniline intermediate in the particular experiment, as correlated in the experimental section.

As described above, in Entry 1 the absence of DCl caused relevant Pt<sup>0</sup> precipitation. So, in this case, metal catalysis has been replaced by acidic catalysis resulting in H/D exchange occurring prevalently on the active aromatic positions. 45% on D3/D5 could be due to a partial contribution of metal catalyst before Pt<sup>0</sup> precipitation occurring. Furthermore, such metal formation here has been confirmed by a black powder on the bottom of the vial. Therefore, a filtration process could permit an efficient separation of this metal from aqueous phase. On the other hand, in Entry 2 this work appeared to be more complicated. Due to a reduced precipitation process caused by acidic presence, Pt<sup>0</sup> tended to not be accumulated on the bottom of the vial. As a consequence of this, some traces could be suspended in solution and here hardly eliminated. In addition, due to the very small size of Pt<sup>0</sup> particle, no accurate filtration process could guarantee the complete elimination of the metal from aqueous phase. Lastly, because of its considerable molecular weight, its presence could disrupt the weight of the final crude recorded. Consequently, the yield could not be dependable. This problem will be faced again when other experiments show excessively high yields. In Entry 3 (**2a**) the balance between DCl and catalyst respects perfectly Garnett's forecast and so, in this case, no Pt<sup>0</sup> precipitation was observed. For this reason the yield was also considered much more reliable than the previous experiments. Due to maybe a partial catalyst extraction in organic phase, the result in Entry 4 (**2c**) shows that a better overall yield was achieved than in Entry 3, while substantial level of deuterium exchange was also observed, with only one tenth of



amount of catalyst. Lastly, as a consequence of the cost of this catalyst, a detailed study on its quantity/efficiency relationship was also necessary in order to work within financial restraints.

## 2.6 Effect of the amount of catalyst

While the result in Entry 4 of Table 2.4 encouraged a reconsideration of the influence that different catalytic amounts could have on the level of deuterium exchange, at the same time it was thought that this new area of investigation could be used for studying H/D exchange on an alkyl chain. Garnett reported many helpful studies<sup>114-115</sup> for advancing the hypotheses of the mechanisms of such reactions.

Simple alkylbenzenes provided useful targets for this purpose. These substrates permit the opportunity to study on a part of hydrocarbons which is activated differently from the aromatic systems. For this purpose, 4-butylaniline was chosen. This molecule presents a chain of four carbons with different reactivity. The alkyl group, in *para* relationship with amino group, allows analysis of the electronic effect of two electron donor groups (amino and alkyl groups) on the four remaining positions of the aromatic ring. The experiment is indicated in Table 2.5 show the series of experiments set up.

**Table 2.5.** Set of experiments with different catalyst amounts.

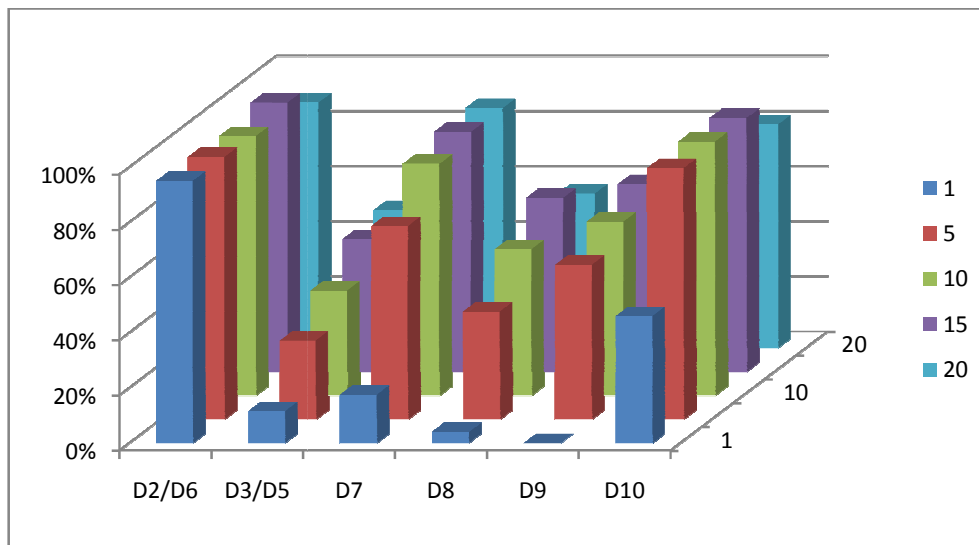
Entry	N <sup>oc</sup>	K <sub>2</sub> PtCl <sub>4</sub>	D2/D6	D3/D5	D7	D8	D9	D10	Yield <sup>a</sup>
1	N.A	1 mol%	95%	12%	18%	4%	0%	46%	99%
2	N.A	5 mol%	95%	29%	70%	39%	56%	91%	99%
3	N.A	10 mol%	94%	38%	84%	53%	63%	92%	99%
4	N.A	15 mol%	98%	48%	87%	63%	68%	92%	99%
5	3 <sup>b</sup>	20 mol%	89%	43%	87%	56%	61%	81%	50%

<sup>a</sup>Yield calculated following acetylation.

<sup>b</sup>This experiment is one reported in the experimental section.

<sup>c</sup>Structure number assigned to the deuterated aniline intermediate in the particular experiment, as correlated in the experimental section

The results obtained are better displayed in a graph reported in Scheme 2.11. The graph below joins simultaneously catalyst amount, positions and H/D percentage exchange.



Scheme 2.11. Variations of the level of deuteration with different amounts of  $K_2PtCl_4$ .

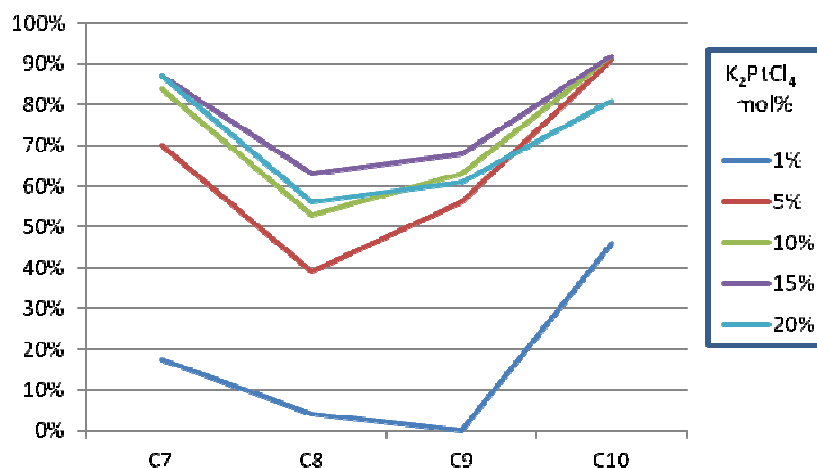
Some considerations arise from these studies. Firstly, the yields reported are considerably high. Although one reasonable explanation could be that one already reported for Entry 4(2c) in Table 2.4, the yield in Entry 5 (3) in Table 2.5 may be also depended on the tendency for complex formation. In this case Pt could interact with the basic amino group, perhaps playing a role as a Lewis acid, which could reduce the amount extracted into the organic phase during the the work up. So, this tendency seems directly proportional with the increasing catalytic amount. This aspect will be also faced in the next chapter. Lastly, although the result in Entry 4 appears to be much better than Entry 5, for the reason reported above, the yield in this last case seems much more reliable. As a consequence of this, 20% mol of  $K_2PtCl_4$  will be seen the best catalytic conditions for our H/D exchange.

The considerable decrease of deuterium incorporation in Entry 5 (3) for positions D2, D6 and D10 (Table 2.5) could be explained in term of proton displacing deuterium. The excess of catalytic amount here allows the H/D process to carry on in an environment no longer deuterium rich because it was already consumed during the exchange itself. This could be explained by the deuterium on the substrate being displaced by proton and subsequently reducing the amount of deuterium observed.

Last considerations regard once more these last positions mentioned above. It is clear from the results in Table 2.5 that amine group has undoubtedly the greatest effect on the aromatic ring. *Ortho* positions (D2 and D6) are strongly activated while *meta* needed a significant

amount of catalyst to present considerable deuterium incorporation. The same conclusion could be made for D10 position. As will be shown in chapter 4, this carbon atom represents the most active position on the alkyl chain. This may be due to steric reasons and consequently to the ability of the catalyst to interact here much more easily than on all the other alkyl positions.

This aspect could be supported by the way in which deuterium was incorporated on alkyl chain itself. At every catalytic amount D10 appears always to be more deuterated than all the others. In Entry 5 (3) (Table 2.5) instead D7 overtakes D10. As mentioned some pages before, a reverse exchange could have occurred here again. As proof of this, sensitive changes are also observable on activated D2/D6. So, on all such positions it was thought that a new exchange process could permit hydrogen replacing deuterium. Steric effect could also clarify some aspects. In particular, D7 reduced activation could be explained in term of  $K_2PtCl_4$  difficulties to interact properly with this carbon atom. As a consequence of this, the reverse exchange cannot occur and the level of incorporated deuterium does not change significantly. For the aim of this project, in this instance, it is also important to highlight this particular exchange pathway along the alky chain. (Scheme 2.12).



**Scheme 2.12.** Level of deuteration for different catalytic amounts on alky chain.

This scheme confirms some of the aspects faced previously. Firstly, the level of deuterium introduced is changeable, moving from 1% to 5% of  $K_2PtCl_4$ . The most active position (D10) is also more undergone to a reverse exchange and apparently this process is evident only with a 20% amount of Platinum catalyst.

Finally, as further proof of the role played by steric effect, the internal positions are always much less deuterated than the external ones. This last conclusion represents an unexpected observation will need to be investigated in more depth during the mechanistic studies on H/D exchange in chapter 4.

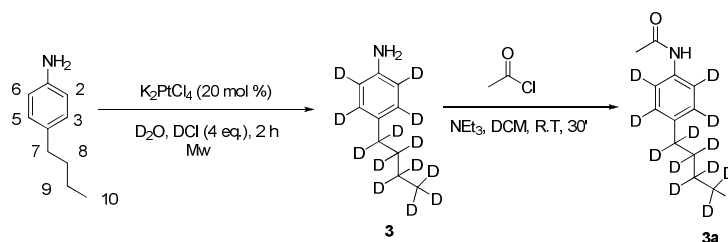
## 2.7 Kinetic Experiments

### 2.7.1 Temperature

Even though the *homogeneous catalysis* usually involves milder conditions than *heterogeneous catalysis*<sup>116</sup> Garnett and others<sup>117-118</sup> recorded H/D exchanges as reactions in need of high temperature and extended time parameters even with the homogeneous catalysts.

For this reason another series of experiments were set up in order to establish the contribution made by temperature to the deuteration. Due to reasons connected with Pt<sup>0</sup> precipitation and consequently yield reliability, Entry 5(3) in Table 2.5 was chosen as a good point for starting this investigation. So, reaction conditions as time, catalytic amount and DCl equivalents were preserved, while temperature instead was treated as variable parameter. Consequently, as in previous experiments, 4-butylaniline was chosen as the substrate (Table 2.6).

**Table 2.6.** Set of experiments with different temperatures.

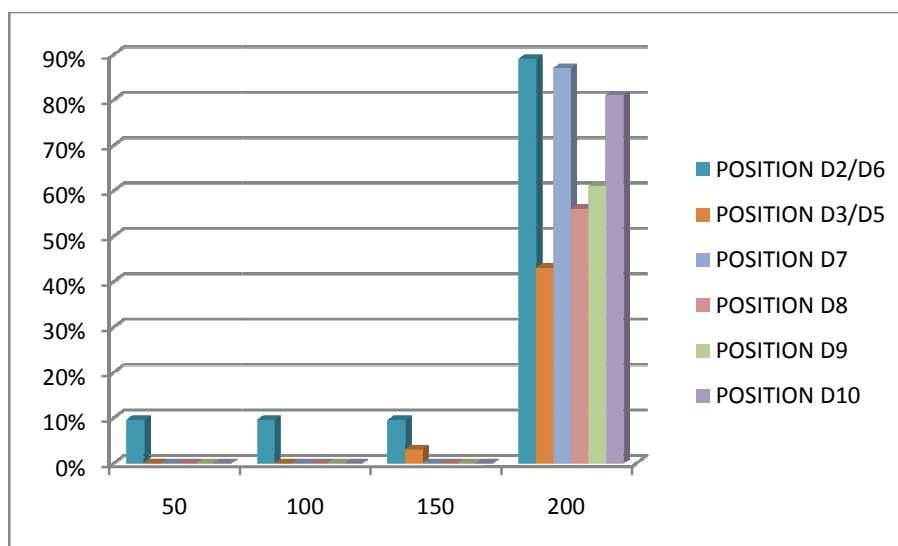


Entry	N <sup>o</sup> <sup>b</sup>	T °C	D2/D6	D3/D5	D7	D8	D9	D10	Yield <sup>a</sup>
1	N.A	50	10%	0%	0%	0%	0%	0%	-
2	N.A	100	10%	0%	0%	0%	0%	0%	-
3	N.A	150	10%	3%	0%	0%	0%	0%	-
4	3	200	89%	43%	87%	56%	61%	81%	50%

<sup>a</sup> Yield calculated following acetylation.

<sup>b</sup> Structure number assigned to the deuterated aniline intermediate in the particular experiment, as correlated in the experimental section

The corresponding graph recovered from these results clearly shows the most interesting aspect of this investigation (Scheme 2.13).



**Scheme 2.13.** Variation on deuteration level according to different temperatures of  $K_2PtCl_4$ .

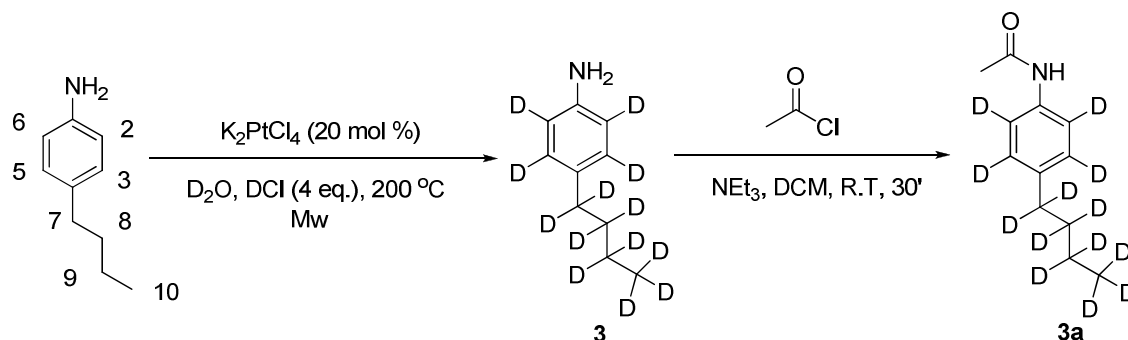
According to this graph, the effect of temperature on all positions is significantly important. Certainly, 200°C seems the best parameter to be used.

## 2.7.2 Time

As previously seen in Garnett's experiments, the attempts set up were developed in a range of times, from days to sometimes weeks. Due to different heating dispersion forms, it is reasonable to think that the reactions investigated by Garnett could actually be set up at a lower temperature. As a direct consequence of the conventional heating, the temperature used for these experiments was only rarely that one expected. So, not only could the final result not be considered completely reliable but the corresponding reaction also resulted in very long processes. The strict correlation existing between time and temperature has been interestingly studied by Sabot *et al.*<sup>119</sup> Intending to set up deuteration reactions at room temperature, they were able to show how much these conditions influenced the corresponding reaction times. These in fact became particularly long, passing from twelve hours to 3 days. As already observed, microwave system is instead able to reduce reaction-time. As mentioned in the introduction, the heating by microwave irradiation occurs directly on the core of the

solution, avoiding any form of energetic dispersion. As a result of this, the temperature achievable by microwave could be approximately close to that onereally expected. In using a device with these characteristics, it was considered relevant to investigate the variations of H/D exchange under different time conditions. Furthermore, as it is quite unusual to find microwave assisted methods which last for more than 2 hours, this parameter was seen as the upper limit. The results of these experiments are reported in Table 2.7.

Table 2.7. Effect of different reaction times.



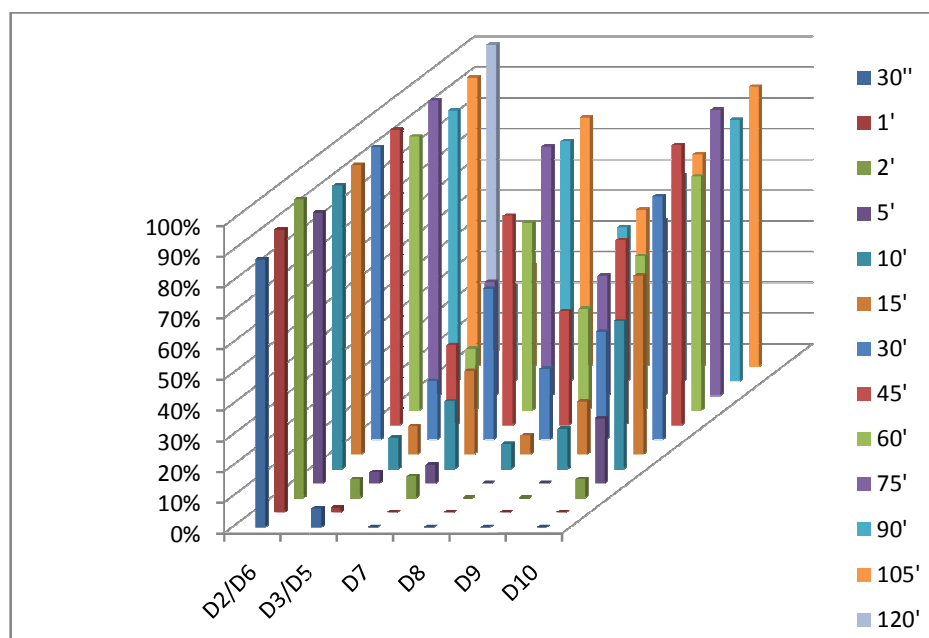
Entry	N <sup>o</sup> <sup>b</sup>	Time	D2/D6	D3/D5	D7	D8	D9	D10	YIELD <sup>a</sup>
1	N.A	30''	87%	6%	0%	0%	0%	0%	98%
2	N.A	1'	92%	2%	0%	0%	0%	0%	98%
3	N.A	2'	97%	6%	7%	0%	0%	6%	94%
4	N.A	5'	88%	3.5%	6%	0%	0%	21%	-
5	N.A	10'	92%	10%	22%	8%	13%	48%	98%
6	N.A	15'	94%	9%	27%	6%	17%	58%	-
7	N.A	30'	95%	19%	49%	23%	35%	79%	64%
8	N.A	45'	96%	26%	68%	37%	60%	91%	93%
9	N.A	60'	89%	20%	61%	33%	50%	76%	82%
10	N.A	75'	96%	37%	81%	39%	57%	93%	70%
11	N.A	90'	88%	31%	78%	50%	67%	85%	99%
12	N.A	105'	94%	33%	81%	51%	69%	91%	99%
13	3	120'	89%	43%	87%	56%	61%	81%	50%

<sup>a</sup> Yield calculated following acetylation.

<sup>b</sup> Structure number assigned to the deuterated aniline intermediate in the particular experiment, as correlated in the experimental section

Even though the Hs in *ortho* are able to be exchanged easily after only 30'', the rest of the positions need to reach 75' before reaching a considerable deuterium level. As a consequence of this, *ortho* positions seem to be completely independent from time. Furthermore, as mentioned before, D10 is also an activated position. Its low exchange in the first steps of this investigation (from Entry 1 to Entry 5) is maybe due to the short time which did not enable the catalyst to reach this position. As it will be explained in Chapter 4, it is reasonable to suggest that  $K_2PtCl_4$  could start its H/D exchange from the aromatic moiety and then move to the alkyl chain beside. Lastly, following the considerations developed previously, the reverse exchange seems to influence the results for each experiment. In particular in Entry 9, 11 and 13 there is considerable reduction of deuterium incorporation. The yield in Entry 13 (**3**) could also be explained again as a result of platinum complex formation which is apparently a not so relevant problem for all the other attempts.

The considerations just reported above are better represented in the graph below. (Scheme 2.14).



**Scheme 2.14.** Variation on deuteration level according to different reaction-times.

As a result of this, in order to obtain relevant deuterium incorporation in most of the molecular structure investigated, time represents another crucial parameter.

## 2.8 Conclusion

In conclusion of this investigation, the right conditions for each parameter were established. Table 2.8 reports the best conditions found for validating a microwave-assisted deuteration method.

**Table 2.8.** Best parameters found during the validation process.

<b>Time</b>	<b>Temperature</b>	<b>Solvent</b>	<b>Acid</b>	<b>Catalyst</b>
<b>2 h</b>	200 °C	D <sub>2</sub> O	DCl (4 eq.)	K <sub>2</sub> PtCl <sub>4</sub> (20% mol)

A complex analytical system for deuterated compounds was perfected. The system presents a rigorous way for establishing the deuterium incorporation with accuracy and precision. A series of technical parameters for microwave-assisted reactions were studied thoroughly. The conditions chosen at the end of these investigations offer the best balance between cost and time. It was also demonstrated that the system just validated offers a more reliable yield, and a reduction in the risk of metal precipitation.



# Chapter 3

## New aromatic substrates for H/D exchange

### 3.1 Introduction

### 3.2 Methoxyanilines

### 3.3 4-Fluoroaniline

### 3.4 (Trifluoromethyl)anilines

### 3.5 Nitroanilines

### 3.6 Platinum ligand

### 3.7 3-Chloroanilines

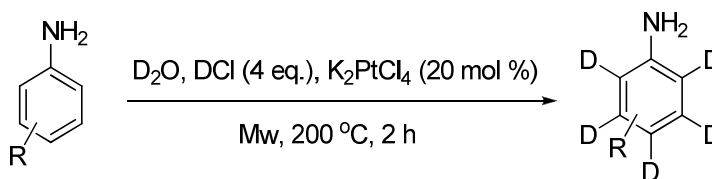
### 3.8 Chloroanilines

### 3.9 Conclusions

## 3.1 Introduction

The microwave assisted system validated in Chapter 2 showed the first results only from a small set of aniline compounds. As was discussed there, aniline derivatives were selected for their importance as building blocks in drug synthesis.<sup>120</sup> Chosen already by Garnett as a good way of investigation, these substrates were also able to provide the basis for understanding and improving the validated method.

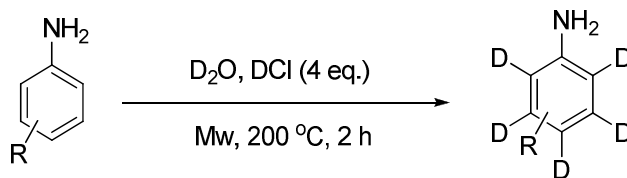
In order to appreciate the efficiency of this process a much larger range of similar aniline substrates needed to be explored (Scheme 3.1). Clearly, it is relevant to report the corresponding level of H/D exchanges and yields for every single substrate investigated.



Scheme 3.1. Platinum-catalysed deuteration of a range of aniline derivatives.

However, Scheme 3.1 shows only the first deuteration step of the process. The following acetylation step is omitted, even though its importance in establishing the yield and deuterium incorporation is fundamental. As mentioned in the introduction of Chapter 2, such compounds will be reported in the experimental data with a letter associated to their corresponding aniline substrate numbers.

Occasionally it will be necessary to compare catalytic data generated by Pt catalyst also necessary to compare the metal catalytic results, described in the scheme above, with that generated by DCl the correspondent acidic catalytic type (Scheme 3.2).



**Scheme 3.2.** Acid-catalysed deuteration of a range of aniline derivatives.

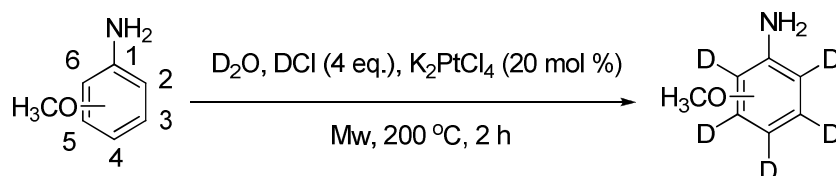
This aspect allowed the clarification of the role played by catalyst and by acid on some of this set of molecules and at the same time to advance the possible mechanism of reaction for H/D exchange.

Lastly, in Chapter 2, Pt<sup>0</sup> precipitation revealed itself to be a serious inconvenience when trying to obtain an accurate final yield. For this reason this problem will be faced specifically in the next pages and a practical way to reduce its negative effect will be proposed.

### 3.2 Methoxyanilines

Methoxyanilines were chosen as the first group to be explored. Their importance in drug design and synthesis is reasonably known and developed. For example, Favoret *al.*<sup>121</sup> used 3-methoxyaniline as a building block for 4-arylpiperazine derivatives. Actually, arylpiperazine template is still currently considered relevant according to a pharmacological point of view for its interesting biological activity.

In order to have a reasonable view of deuterium incorporation on their aromatic ring, all methoxyanilines were investigated. (Table 3.1).

**Table 3.1.** Methoxyanilines deuteration results.

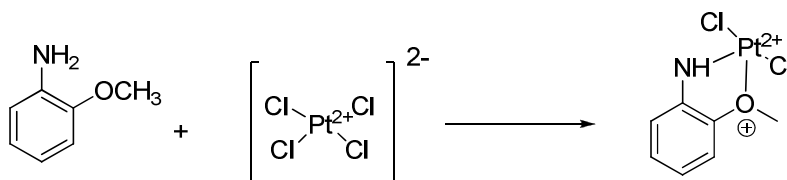
Entry	N <sup>oc</sup>	OCH <sub>3</sub>	D1 <sup>a</sup>	D2 <sup>a</sup>	D3 <sup>a</sup>	D4 <sup>a</sup>	D5 <sup>a</sup>	D6 <sup>a</sup>	OCD <sub>3</sub> <sup>a</sup>	Yield <sup>b</sup>
1	4	Para	-	93%	91%	-	91%	93%	-	11%
2	5	Meta	-	93%	-	88%	81%	81%	-	44%
3 <sup>122</sup>	N.A	Ortho	-	-	-	-	-	-	-	-

<sup>a</sup> Deuterium incorporation calculating through acetylation.

<sup>b</sup> Yield calculated through the relation on Chapter 2.

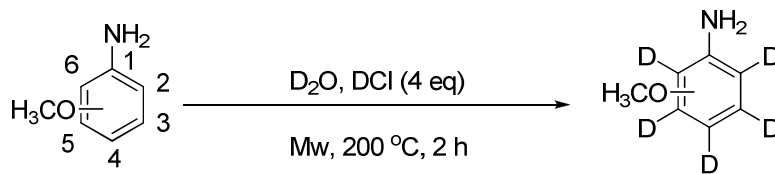
<sup>c</sup> Structure number assigned to the deuterated aniline intermediate in the particular experiment, as correlated in the experimental section

As shown in Table 3.1, the yields reported appear dramatically low. Entry 3<sup>122</sup> experiment in particular requires some deeper analysis. In this case no product was recovered. As a consequence of this, it was thought that an interaction between catalyst and oxygen/nitrogen could have influenced the final result of this experiment. (Scheme 3.3).



**Scheme 3.3.** Reaction where the complex between 2-methoxyaniline and platinum tetrachloride anion is formed.

Generally, Pt shows a well-known tendency to form metal complexes. Starting from *cisplatin*, many of its analogues are currently successfully applied to the chemotherapeutic field.<sup>123-124</sup> Their properties to link DNA are in fact used nowadays in cancer treatments. In this case [Pt(Cl)<sub>4</sub>]<sup>2-</sup> presents interesting abilities to interact with heteroatoms.<sup>125</sup> Possibly due to the high concentration of chlorine, the central metal could act as electrophilic species. Consequently the data suggest a nucleophilic interaction between either nitrogen/oxygen from the substrate and the Pt of the catalyst. A comparison between this metal-catalyzed result and a corresponding acidic process<sup>126</sup> could confirm this hypothesis. (Table 3.2)

**Table 3.2** Methoxyanilines deuteration under acidic catalyzed conditions.

Entry	OCH <sub>3</sub>	D1 <sup>a</sup>	D2 <sup>a</sup>	D3 <sup>a</sup>	D4 <sup>a</sup>	D5 <sup>a</sup>	D6 <sup>a</sup>	OCD <sub>3</sub> <sup>a</sup>	Yield <sup>b</sup>
<b>1</b> <sup>126</sup>	Para	-	90%	89%	-	89%	90%	-	21%
<b>2</b> <sup>126</sup>	Meta	-	94%	-	96%	2%	96%	-	31%
<b>3</b> <sup>126</sup>	Ortho	-	-	94%	95%	95%	96%	-	58%

<sup>a</sup>Deuterium incorporation calculating through acetylation.

<sup>b</sup>Yield calculated through the relation on Chapter 2.

Pt efficiency in these set of experiments has been shown by a direct comparison of D5 exchange in **5** (Table 3.1) and entry 2 in Table 3.2. The D5 position is inactive position because of the two electron donor groups are *meta* to D5. In these two trials the difference of deuterium incorporation is impressive (2% against 81%). This observation highlights As a result of this, the enormous advantage of using Pt metal catalyst over conventional acid catalyst; the opportunity in fact to obtain H/D exchange even in inactivated positions permitted us to obtain almost fully deuterated compounds. The yield reported in Entry 3<sup>122</sup> (Table 3.2) confirms that platinum considerably influences the final result. The hypothetical complex described in Scheme 3.4 could be significant here.

Similar considerations are also suggested from Entry 1(**4**)(Table 3.1). The corresponding acidic and metal yields are both significantly low. Even after the basification of the aqueous solution. In fact, only a few mg of crude product were collected in organic phase in both of the experiments. This result could have also occurred as a consequence of solubility problems in deuterium oxide.

In the case of 3-methoxyaniline case (**5** in Table 3.1 and Entry 2 in Table 3.2) some points need to be clarified. Thioglycolic acid ligand was taken into consideration when calculating the final yield. However, in this case no improvement in yield was observed.

Additionally, the interaction between Pt and oxygen was also studied. Essentially, in all of these attempts the catalyst was not able to obtain a major deuteration on the three hydrogens of the methoxy group. It was assumed that the Pt would form a stable complex with the oxygen. Anderson *et al.*<sup>127</sup> have accurately studied the right conditions for forming such complex. Therefore, it was thought that oxygen could play the role of the electron-donor through the help of methyl group which could increase its nucleophilicity. The strongly

Electron withdrawing chloride ligand attached to the Pt helps to reduce the electronic repulsions between the electron of oxygen atom and the metal *d* orbitals. These lone electron pairs here could be pushed mostly towards halogen atoms allowing platinum to become so electronpositive to interact with oxygen species. As a result of this Pt is able to form a stable bond and consequently a stable metal-organic complex. It is necessary to highlight the importance of oxidation state IV for platinum. In this state the electrons on *d* orbitals are closer to the nucleus for electrostatic attraction and subsequently the electronic repulsion is lower. That allows the stabilization of the system as explained in the scheme below. (Scheme 3.4).



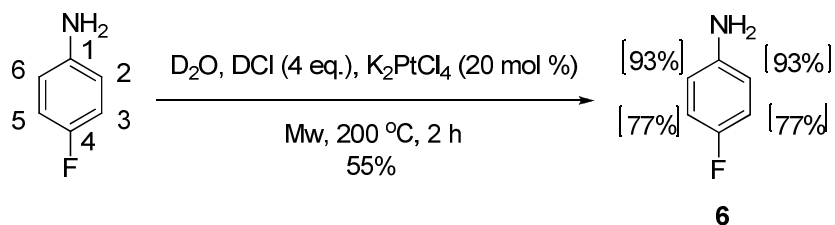
**Scheme 3.4.** Hypothetical complex formed between platinum (IV) catalyst and oxygen of methoxy group.

In scheme 3.6 platinum (IV) was reported as species obtained by *oxidative addition* by DCl. This aspect will be explored in the next few pages when a mechanism for Pt<sup>0</sup> precipitation will be proposed.

All these considerations also suggest that kinetic factors could be involved in the catalytic mechanism. The level of deuterium introduced on to the aromatic rings (Table 3.1) presents an homogeneous distribution. The Methoxy group seems not to have any particular effect on the H/D exchanges. In this sense Pt could be thought of as a catalyst, which is able to activate all positions without any particular difficulty. Although this aspect will be explored in the next chapter, it is quite relevant to observe that the complex stabilities shown in Scheme 3.6 seem to respect the electrophilic aromatic substitution. It is also relevant to specify that no specific H/D mechanism has been proposed until now. In Chapter 4, these considerations will be addressed there particularly in the light of Garnett's discoveries. However, here, Pt(IV) seems to play a relevant role.

### 3.3 4-Fluoroaniline

The idea reported above gave the suggestion to investigate a substrate where the substituent could not present the difficulties faced before. Fluoroaniline is widely used in drug synthesis<sup>128</sup> and it was chosen for this purpose. In this sense 4-fluoroaniline derivative is particularly interesting for the considerations about complex stability just proposed above. The scheme below (Scheme 3.5) reports the result obtained through this experiment.

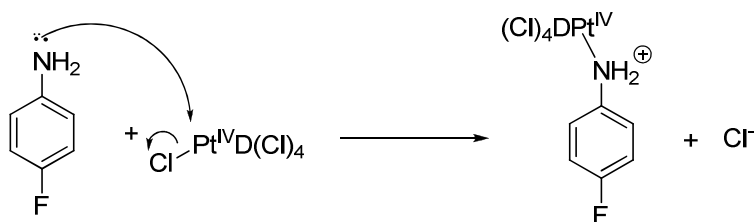


**Scheme 3.5.** 4-Fluoroaniline deuteration experiment.

As Scheme 3.5 shows, **6** shows all aromatic positions deuterated in a high percentage. However, C3 and C5 present a lower percentage. As a result of *ortho* inactivation by fluorine's electron-withdrawing inductive effect, these two positions offer a comparison with this result of the correspondent aniline explored in Chapter 2. Here the level of incorporated deuterium is considerably higher (92%), hydrogen in C6 is not able to apply a similar effect on the correspondent *ortho* carbons. Consequently the electron density here for 4-fluoroaniline is importantly reduced, giving the right explanation for the result just reported.

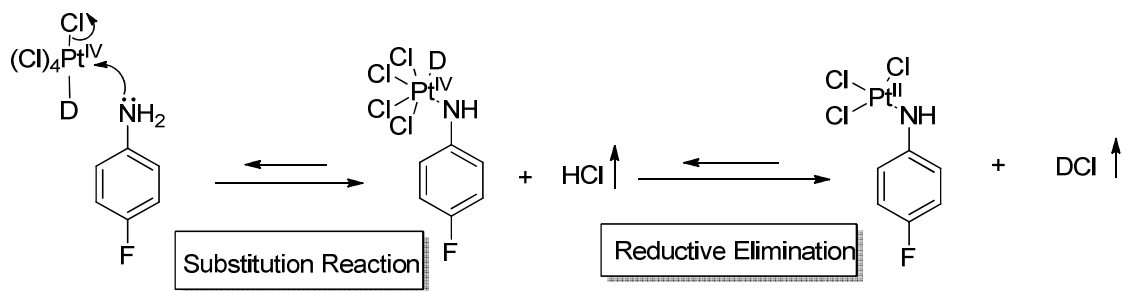
The interesting aspect of this experiment is nevertheless represented by the yield. While in the last paragraph it was suggested that the presence of Pt-O complex could have influenced the extraction of methoxyanilines in organic phase, here this consideration could not be considered valid anymore.

Many examples in the literature deal with the tendency of Pt to form complex with nitrogen in aniline substrates.<sup>129-130</sup> Consequently, it could be relevant to suggest a hypothetical complex is formed in solution between catalyst and fluoroaniline. (Scheme 3.6).



**Scheme 3.6.** Pt-4-fluoroaniline complex formation.

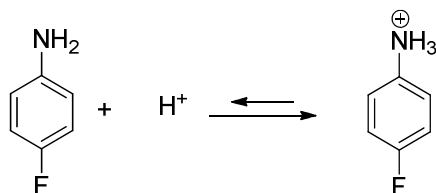
As reported above, here the species of Pt(IV) formed through *oxidative addition* was yet again considered to be the most active in this process. A substitution reaction could occur therefore, through such species and basic nitrogen on the aniline; favoured also by harsh experimental conditions. It was additionally proposed that a further *reductive elimination* could follow this process according to the scheme below (Scheme 3.7).



**Scheme 3.7.** Reductive elimination for Pt-4-fluoroaniline complex. An intermediated reaction substitution has been also occurred.

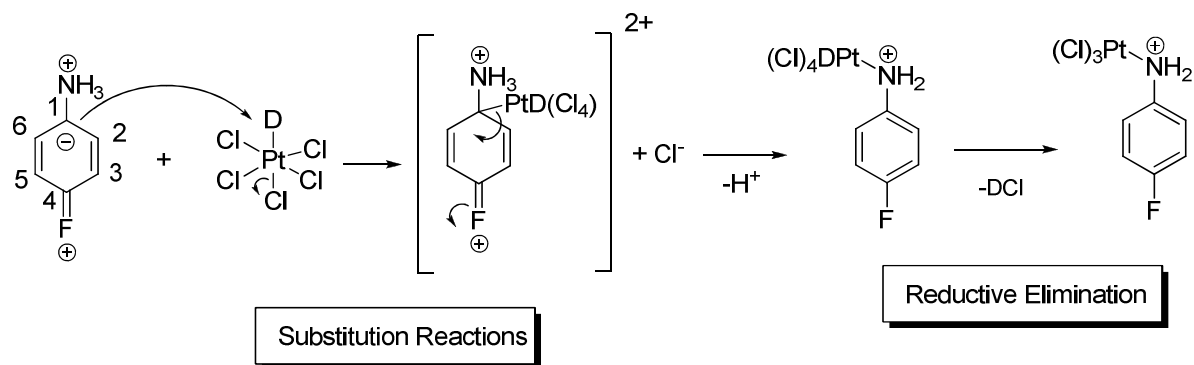
Reasonably, the heating could favour the acid chloride (HCl)/deuterium chloride (DCl) elimination and subsequently move the equilibrium towards the right.

Furthermore, it is relevant to reflect upon the behaviour of such a substrate under strong acidic conditions. This compound presents a  $pK_a$  of 4.66. This value permits keeping the aniline in a protonated state (Scheme 3.8).



**Scheme 3.8.** 4-Fluoroaniline formation. The equilibrium is shifted on the right due to excess of acid in solution.

Even though this aspect seems not to cause any modification on *ortho* activation, a very good level of deuterium incorporation is still present. As a consequence of this, it was thought that instead fluorine could be directly involved in some sort of conjugation process. As halogen, this atom could overlap its 2p orbital with 2p carbon orbital. In particular, as the resonance form shown below, such interaction could occur through an empty  $sp^2$  orbital offered in carbon in 4. (Scheme 3.9).



**Scheme 3.9.**Complex formed during the reaction ongoing.

As a result of this, position 1 is activated. So Pt(IV) could be seen as Lewis acid or possibly as a species with its nucleus positively charged due to five electron-withdrawing ligands. The interaction shown in the Scheme 3.9 brings to obtain the final complex.

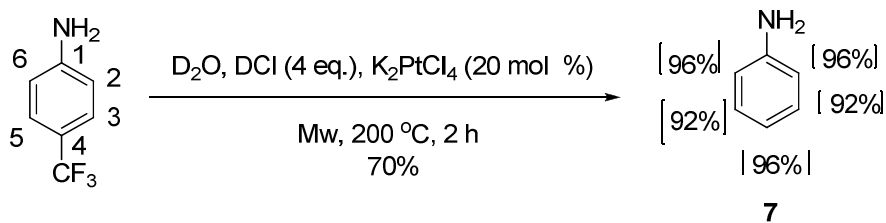
Although the presence of these complexes was not proved, this hypothesis could be helpful in finally explaining the yields obtained for the methoxyanilines experiments. The process reported above and the interaction Pt-O could give a double contribution and reduce dramatically the yield.

### 3.4 (Trifluoromethyl)anilines

The considerations recovered from the last paragraph moved the interest towards different compounds. It was considered fundamental to expand this results to different substrates. For this purpose (trifluoromethyl)anilines were retained as the fittest examples. These compounds are important for their use in drug design and synthesis.<sup>131</sup> Moreover trifluoromethyl electron-withdrawing group ( $CF_3$ ) could allow suggesting a series of interesting consequences. Firstly it could be shown in which way such substituent could influence the H/D exchange, secondly it could be investigated how it could influence the final yield.

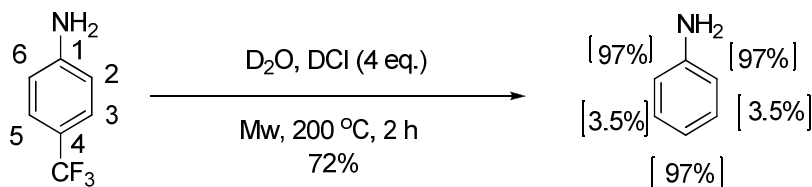


So 4-(trifluoromethyl)aniline was the first compound explored. The result obtained is quite unusual and it is reported in Scheme 3.10.



**Scheme 3.10.**4-(Trifluoromethyl)aniline experiment. Aniline is the unexpected final product.

Many hypotheses were advanced to explain this strange result. For this purpose, it was necessary to make a comparison with the corresponding acidic-catalyzed reaction.<sup>126</sup>(Scheme 3.11).

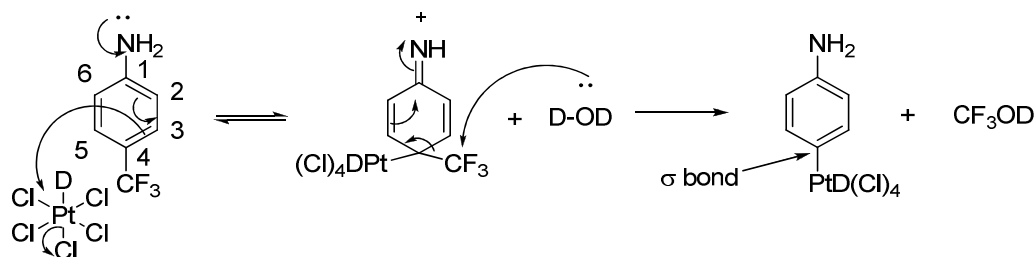


**Scheme 3.11.**4-(Trifluoromethyl)aniline experiment under acidic catalyzed condition. Aniline is still the final product.

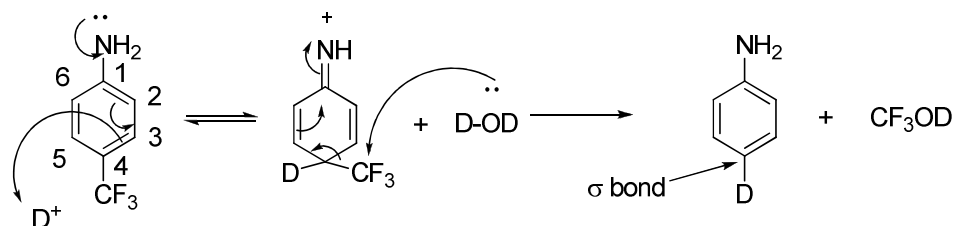
The data reported above show interesting aspects. In both of the experiments, *ortho* and *para* positions were deuterated with approximately the same percentage. In *meta* instead any relevant exchange was observed under acidic catalysis. Finally, the yields for both are quite similar. After these conclusions two reasonable mechanisms were proposed for explaining the final aniline formation.

### 3.4.1 Trifluoro-carbocation ( $\text{CF}_3^+$ ) elimination

In this reaction a trifluorocarocation ( $\text{CF}_3^+$ ) was thought to be the key of this mechanism. As a very good leaving group, it could also enable the consequent electrophilic aromatic substitution. The electron withdrawing effect generated by this substituent could also activate the correspondent carbon (position 4) on the aromatic ring and allowing the attack by both  $\text{D}^+$  and Pt-catalyst. (Scheme 3.12 and 3.13).

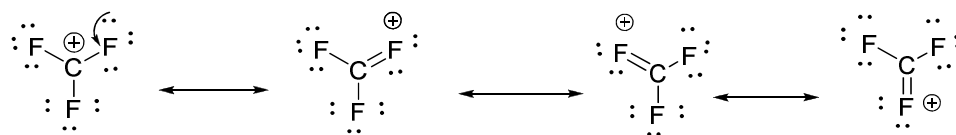


**Scheme 3.12.** Pt attack to position 4 on 4-(trifluoromethyl)aniline. The final result is the formation of one  $\sigma$  bond between metal and carbon.



**Scheme 3.13.** deuterium ion attack to 4 position on 4-(trifluoromethyl)aniline. The reaction is a typical electrophilic aromatic substitution.

Even though in literature there are no particular references to confirm this mechanism, Olah *et al.*<sup>132</sup> reported a study about trihalomethyl carbocations' stability in which their extreme reactivity was reported.  $\text{CF}_3^+$  in this context results the most reactive species due to the highest electronegativity of the three fluorine atoms present. However, a conjugation of a lone pair on one of them could stabilise the positive charge on carbon atom and allow the reduction of energy activation. (Scheme 3.14). Add to that the extreme condition reproduced in the microwave reaction; all the processes described above show promise

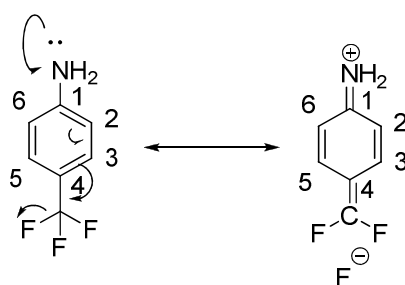


**Scheme 3.14.** Resonance forms of trifluoro carbocations. In three of them the positive charge is delocalized through electronic conjugation.

3.4.2 CO<sub>2</sub> elimination

Even though the proposed mechanism below appears reasonable, the presence of trifluorocarbenium ion remains controversial. For this reason another explanation, supported by more literature references, was advanced.

The amine group plays a fundamental role for C-F activation.<sup>133</sup> This bond is usually very difficult to involve in every step of reactions. However Liddle *et al.*<sup>134</sup> reported a study where this bond was able to take part in the synthetic process. The considerations recovered from this paper were used to advance hypotheses for 4-(trifluoromethyl)aniline stability. (Scheme 3.15).

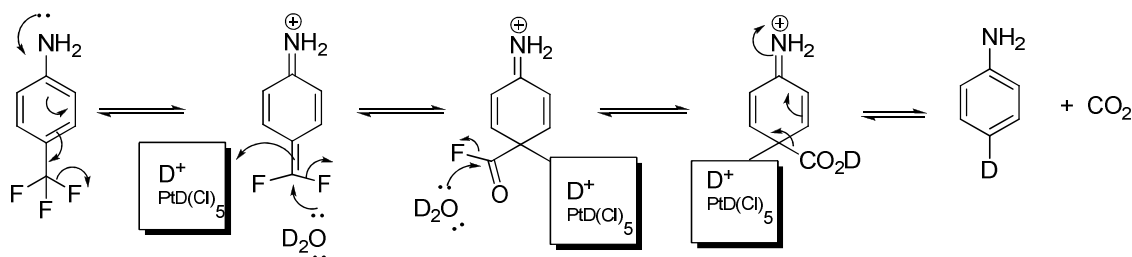


**Scheme 3.15.** Resonance forms of 4-(trifluoromethyl)aniline.

As a consequence of *hyperconjugation* interaction, C-F bond might be weakened. Starting from this consideration, Liddle *et al.* obtained relevant results in using inorganic base such as KOH and NaOH. As possible explanation, the metal cation here played a fundamental role catching fluoride and stabilizing the ionic resonance form (*on the right* of scheme 3.15). In strong acidic media something similar was observed. As a consequence of heating system, hydrofluoride (HF) could be in fact eliminated resulting in a similar process described above.

So, a difluoridemethyl (CF<sub>2</sub>) species which is easily attacked by any weak nucleophile species present in solution. The presence of this activated part is so beneficial that many researchers, such as Qiao *et al.*<sup>135</sup>, have adopted it for C-F activation and heterocycles synthesis.

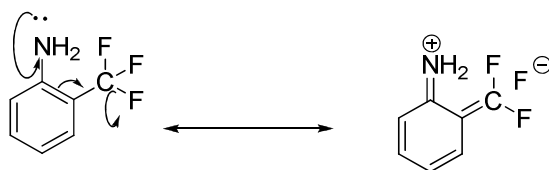
In our case deuterium oxide represents the only nucleophilic species able to attack (CF<sub>2</sub>). Moreover, the necessity of reforming the aromatic system brings the reaction to give the correspondent aniline. The Scheme 3.16 describes the main points of this theoretical mechanism.



**Scheme 3.16.** Deuterium introduction in replacement of  $\text{CF}_3$ . It is shown the attack directly by  $\text{D}^+$  or by  $\text{Pt(IV)}$ . Carbon-dioxide is eliminated at the end of this process.

The procedure finally could be favoured by carbon dioxide ( $\text{CO}_2$ ) elimination under the high temperatures used for the reaction.

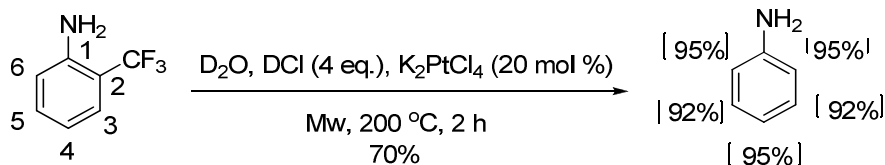
The hypothesis reported above need to be confirmed by further experimental investigations. So, similar substrates, such as 2-(trifluoromethyl)aniline, shows some analogies with the 4-substituted one. The electron-withdrawing substituent in fact is in a position that an electron-donor conjugation can be proposed again. (Scheme 3.17).



**Scheme 3.17.** Resonance forms of 2-(trifluoromethyl)aniline. Again fluoride could be the key to fix the equilibrium.

The resonance forms described in Scheme 3.17 were already investigated by Strekowski *et al.*<sup>136</sup> who applied similar considerations to synthesize heteroaromatic rings. Although an *imine-difluoridemethyl* species has been here obtained by ionization and then fluoride elimination, the consequent nucleophilic attack has resulted identical to Scheme 3.16. As described above, the high temperature and the consequent HF elimination could stabilize here the ionic resonance form.

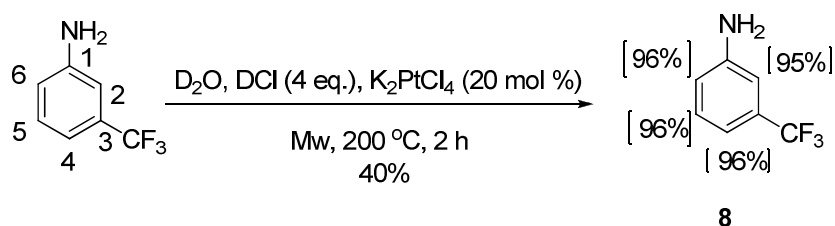
So, 2-(trifluoromethyl)aniline was explored from H/D exchange point of view.<sup>122</sup> (Scheme 3.18).



**Scheme 3.18.** 2-(Trifluoromethyl)aniline experiment. It is shown the final product which is again a deuterated aniline.

As the scheme above illustrates, aniline is again the final product of the experiment. Furthermore, the mechanism involved could be the same proposed for 4-(trifluoromethyl)aniline. (Scheme 3.16).

After these last results, it was thought that the position of CF<sub>3</sub> on the aromatic ring was fundamental for activating C-F bond and consequently to eliminate this substituent. However, in order to confirm this, it was finally necessary to investigate 3-(trifluoromethyl)aniline which was the last substrate not yet explored. (Scheme 3.19).



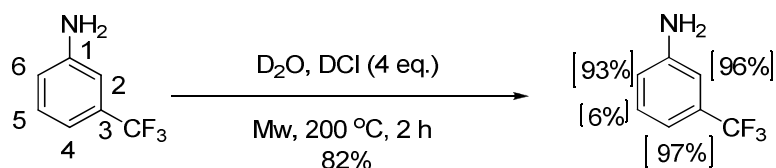
**Scheme 3.19.** 3-(Trifluoromethyl)aniline experiment. In this case CF<sub>3</sub> has been preserved maybe because the presence on *meta* does not allow the right activation on carbon 3.

As shown above, in this case the electron-withdrawing group was not eliminated. The clearest explanation of this result could be due to a different activation of correspondent carbon 3 on aromatic ring mediated by trifluoromethyl group.

Furthermore, as reported above, it is quite hard to expect any hyperconjugation here. Due to its instability, the ionic resonance form was not formed. In conclusion, although CF<sub>3</sub> was preserved, its influence seems not to be so relevant in H/D exchange.

As reported above the metal catalyzed reaction is able to deuterate all the aromatic positions with high efficiency.

The corresponding acidic-catalyzed experiment<sup>126</sup> was set up to show once again the efficiency of this catalyst and at the same time to display its influence on the final yield. (Scheme 3.20).

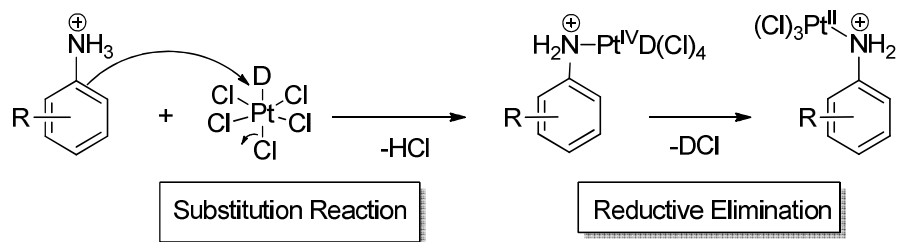


**Scheme 3.20.** 3-(Trifluoromethyl)aniline acidic catalyzed experiment.

As Scheme 3.20 shows, position 5 was modestly exchanged. Otherwise the yield reported is considerable high. As suggested before,  $\text{CF}_3$  in *meta* position could not permit any relevant hyperconjugation interaction. As a result of this, the electronic lone pair on amine group is still available for interacting with Pt and forming corresponding complex. As mentioned above, the proposed complex could be the main reason for the low yield.

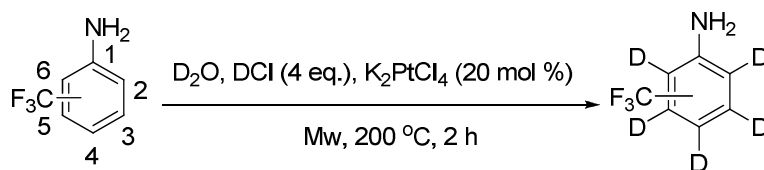
On the other hand, the result obtained in acidic-catalyzed reaction reveals a very high yield.

It is clear after that metal catalyst interacts in some way with this kind of anilines. As already stated in the first pages of this chapter, Pt has always shown a tendency to form stable bonds with heteroatoms and in particular with nitrogen. Moreover, as mentioned earlier, Pt(IV) could play a role as electrophilic species. Following this consideration, it was thought that this metal species could attack the aromatic ring on the more activated, hence electron-rich positions. It was also suggested that carbon bonded nitrogen (position 1) could not be excluded from this process. According to the nature of the substituent present on the ring, this position could result more or less activated; so the importance of this specific electrophilic attack is fundamental. The proximity of nitrogen could in effect generate a stable bond with Pt and avoid the extraction of consequent complex formed in the organic phase, eventually reducing the final yield. (Scheme 3.21).



**Scheme 3.21.** Pt-aniline complex formation. Arisen from the yield obtained from trifluoromethylanilines, this mechanism shows the hypothetical electrophilic attack by the Pt catalyst.

Lastly, the proposed mechanism explains the yield's obtained for (trifluoromethyl)anilines. Table 3.3 shows a summary of these results.

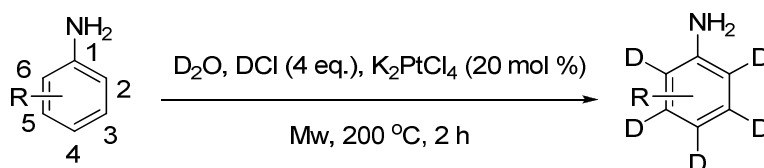
**Table 3.3.** (Trifluoromethyl)anilines yields.

Entry	N <sup>o</sup> <sup>a</sup>	CF <sub>3</sub> Position	Yield
1	N.A	Ortho	70%
2	8	Meta	40%
3	7	Para	70%

<sup>a</sup>Structure number assigned to the deuterated aniline intermediate in the particular experiment, as correlated in the experimental section

Entry 1 and 3(7) present interesting yields. These results could be due to a different interaction with catalyst according to the mechanism explained before. In this case position 1 could be deactivated by the electron-withdrawing effect of CF<sub>3</sub>. As a result, this carbon became too electron-poor to be attacked by Pt. 3-(trifluoromethyl)aniline shows a lower yield which is likely due to a reduced deactivation of position 1. The effect of this substituent in *meta* allows one to obtain a carbon more prone to interact with the catalyst and, consequently, revealing a reasonable explanation the final result reported.

The positive aspect of the hypothesis just proposed is to extend these considerations to the other substrates just explored. (Table 3.4).

**Table 3.4.** Summary of aniline yields.

Entry	N <sup>o</sup> <sup>a</sup>	R substituent	Yield
1	N.A	<i>o</i> -OCH <sub>3</sub>	-
2	5	<i>m</i> -OCH <sub>3</sub>	44%
3	4	<i>p</i> -OCH <sub>3</sub>	11%
4	6	<i>p</i> -F	55%

<sup>a</sup>Structure number assigned to the deuterated aniline intermediate in the particular experiment, as correlated in the experimental section.

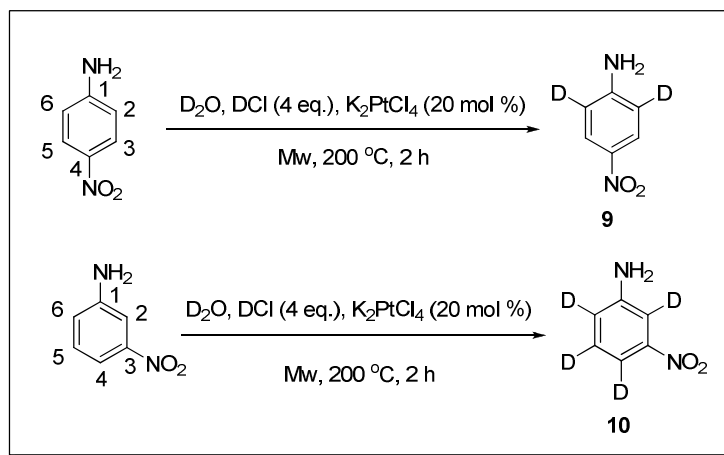
The results reported in Table 3.4 follow the guideline established before. Entry 1 presents no yield, maybe due to the combination of these effects. Adding to the activation on carbon 1 made by an electron-donor group (OCH<sub>3</sub>) towards electrophilic attack, it is necessary to consider the hypothetical chelating effect that oxygen and nitrogen could exercise on Pt. The consequence is finally a stable metal complex. This last aspect cannot be found in Entry 3 (**4**). The methoxy group is so far from the amine one that any similar effect on the metal cannot be realized. Consequently in this case the yield is only a little bit higher. Entry 2 (**5**) shows the best result in this group. This is possibly due to a reduced effect by substituent on *meta*. Carbon 1 is less electron-rich, as a result the complex formation is more unlikely and the yield is considerably increased (See Scheme 3.11). Lastly, in Entry 4 (**6**), fluoro conjugation allows the increase of electro density on carbon 1. Although this element presents the same Hammett  $\sigma$  value of hydrogen (Table 2.5, Entry 3), that means a similar electronic effect on the aromatic ring, the final result is nevertheless slightly different (44% compared to 55%). In this case the elevation of the halogen electronegativity is responsible for generating the final balance of yield to around 50%.

### 3.5 Nitroanilines

After reaching all of these conclusions for different substituents of aniline derivatives, a further investigation regarding in particular the electron-withdrawing groups was necessary. For this purpose, nitroanilines were the last kind of such compounds to be explored.<sup>137</sup>

As shown below, the H/D exchanges here suggest some interesting aspects, in particular arising from the comparison with the result obtained before.

Scheme 3.22 shows the nitroanilines used and their correspondent reactions set up.



**Scheme 3.22.** Experiments set up for nitroanilines exploration.



The correspondent table (Table 3.5) reassumes the results obtained and the consequent yield for both.

**Table 3.5.** Summary of nitroanilines results.

Entry	N <sup>o</sup> c	NO <sub>2</sub>	D1 <sup>a</sup>	D2 <sup>a</sup>	D3 <sup>a</sup>	D4 <sup>a</sup>	D5 <sup>a</sup>	D6 <sup>a</sup>	Yield <sup>b</sup>
<b>1</b>	<b>9</b>	Para	-	93%	33%	-	33%	93%	23%
<b>2</b>	<b>10</b>	Meta	-	94%	-	94%	94%	94%	86%

<sup>a</sup> Deuterium incorporation calculating through acetylation.

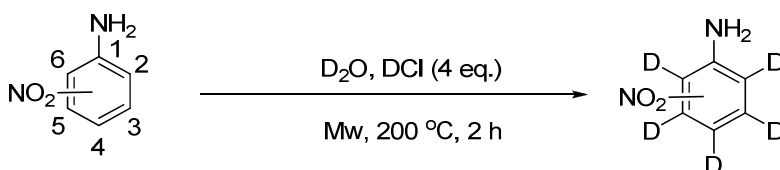
<sup>b</sup> Yield calculated through the relation on Chapter 2.

<sup>c</sup> Structure number assigned to the deuterated aniline intermediate in the particular experiment, as correlated in the experimental section

In Entry 1 (**9**) the most relevant aspect is not linked to the distribution of deuterium, which respects perfectly the electrophilic aromatic substitution, but it is instead the final yield recorded. Conversely, in Entry 2 (**10**), in addition to the good deuteration in all positions, the yield is considerably higher. The result just shown is consequently different from CF<sub>3</sub> anilines. Here in fact *meta* derivative seems to be the best substrate for the balance between yield and H/D exchange.

Some hypotheses also can be proposed only by comparing the results just obtained with that from acidic-catalyzed reactions.<sup>126</sup> (Table 3.6).

**Table 3.6.** Summary of nitroanilines results under acidic catalyzed conditions.



Entry	NO <sub>2</sub>	D1 <sup>a</sup>	D2 <sup>a</sup>	D3 <sup>a</sup>	D4 <sup>a</sup>	D5 <sup>a</sup>	D6 <sup>a</sup>	Yield <sup>b</sup>
<b>1</b>	Para	-	97%	0%	-	0%	97%	78%
<b>2</b>	Meta	-	95%	-	95%	0%	95%	96%

<sup>a</sup> Deuterium incorporation calculating through acetylation.

<sup>b</sup> Yield calculated through the relation on Chapter 2.

The data shown above reflect again the electrophilic aromatic substitution ongoing. Both of the experiments in fact display the completely absence of any H/D exchange on their correspondent *meta* inactivated positions. The difference of deuterium incorporated between acidic and catalyzed reactions could be due to a continuous exchange process on activated

positions mediated by platinum catalyst. Hydrogen finally could replace deuterium at the end of this process.

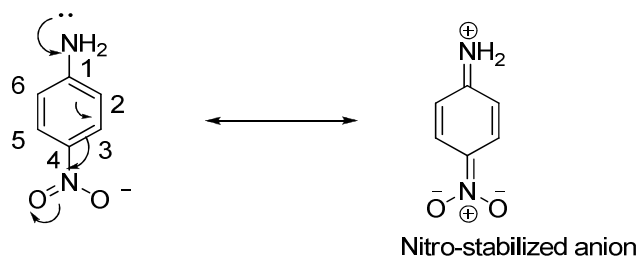
However the most interesting aspect of these last results is represented by final yields. In particular a direct comparison between **9** and Entry 1 in Table 3.6 could establish the important role played by Pt in this case.

The consequent hypothesis proposed to explain this result regards mainly the effect that nitro group could play according to the position on the aromatic ring.

Considering the further presence of an amino group here, it was suggested again that an aromatic resonance form could explain the activation for the electrophilic attack.

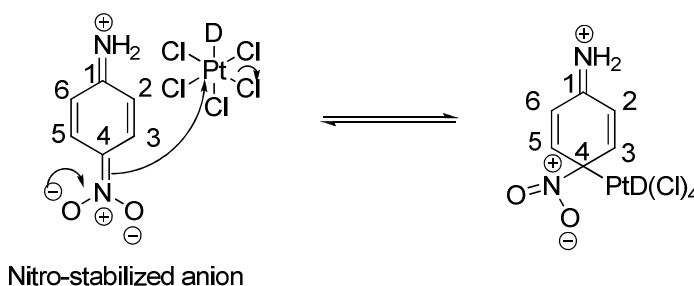
Following the mechanism proposed for alkylation of nitroalkanes<sup>14</sup>, it was thought that a similar procedure could further happened for nitroaniline.

Starting from *para*-derivative, a correspondent nitro-stabilized anion could be formed through the conjugation of 2p orbital with the aromatic system. (Scheme 3.23).



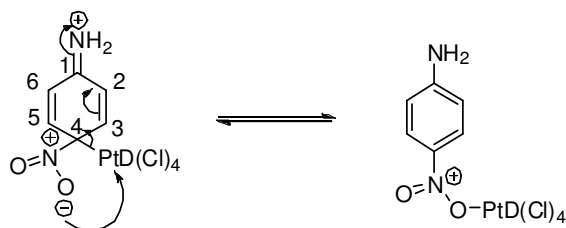
**Scheme 3.23.** Mechanism through which a nitro stabilized anion is formed.

The anion illustrated above could activate position 4 towards any electrophilic species. Consequently, the platinum (IV), whose structure was already shown, could be attacked on this carbon according the scheme below. (Scheme 3.24).



**Scheme 3.24.** Pt catalyst (IV) attacks the nitro-anion on position 4. The product obtained is quickly removed to reform the aromatic system.

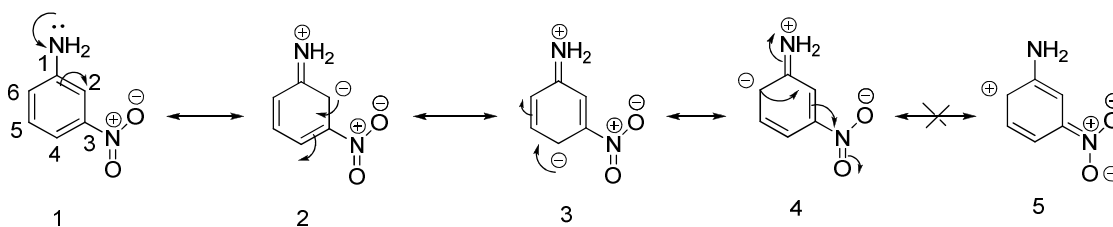
The product just obtained by the electrophilic attack is removed for reforming the aromatic system. This could be favoured by an oxygen bond formation. (Scheme 3.25).



**Scheme 3.25.** Pt catalyst (IV) attacks the nitro-anion on position 4. The product obtained is quickly removed to reform the aromatic system.

As already explained for 2-methoxyanilines, here a chelating action, mediated by oxygen and nitrogen, could entrap Pt and form a stable complex to cause consequently a reduction of the yield. (Table 3.5).

The considerations stated until now could be confirmed by the 3-nitroaniline experiment. In this case the result (**10** in Table 3.5) shows strong differences from the correspondent *para* derivative. All the positions were highly and homogeneously deuterated. An analysis of the conjugation process could also explain why such a result was achieved. (Scheme 3.26).



**Scheme 3.26.** Resonance forms for 3-nitroaniline. Resonance number 5 is prohibited because any conjugation can be formed.

As scheme 3.26 shows, resonance 5 is prohibited due to absence of elements of conjugation. As a result of this, any Pt complex could be formed according to the procedure described in Scheme 3.27 and consequently the yield recovered is very high (86%, Entry 2 Table 3.5).

### 3.6 Platinum ligand

All the conclusions reached in the previous paragraphs show that the platinum complexes represent serious problems for the aims of this project. Until now unreliable and low yields were obtained and a method for improving them seems necessary.

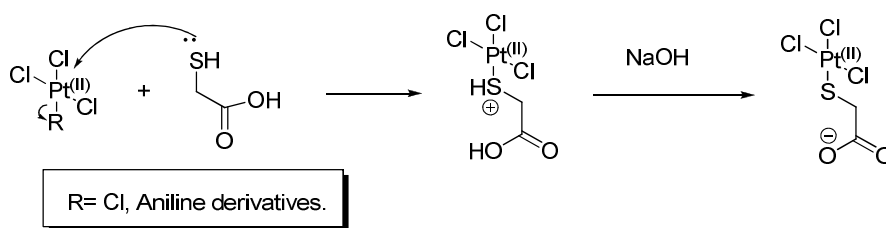
Another aspect also appears relevant. Nowadays, some studies are dedicated to showing the efficiency of deuterium-containing drugs in pharmacology and chemotherapy.<sup>138</sup> As a

consequence of this, the interest towards to our method for recovering deuterated compounds with biological and pharmaceutical activities could be reduced by the presence of toxic Pt.

Even though recent research was able to synthesize platinum compounds with strong anti-tumoral activity and low toxicity<sup>139</sup>, the general knowledge that platinum can be seriously toxic for the human body<sup>140</sup> is agreed. According to many studies in this field, platinum (II) complexes are able to bind sulfhydryl (SH) group of proteins and enzymes causing their deactivation.<sup>141</sup> The consequences of such interactions are mainly based on nephro and gastrointestinal toxicity.

Starting from the binding between Pt(II) and a series of endogenous thiols, such as L-glutathione (GSH) and L-cysteine, kinetic studies were developed for establishing its characteristics. These revealed a very intensive interaction. Hagrama *et al.*<sup>142</sup> stated that this interaction is so intensive in human cancer cells that the bioavailability of Pt-drugs is strongly reduced as well as its chemotherapeutic effect. Petrovic *et al.*<sup>143-144</sup> were also able to investigate the main aspects of this interaction according to kinetic and nucleophilic studies. Furthermore, the size of thiol used was addressed as fundamental. Due to steric effect, big ligands of this kind were unable to attack Pt.

For this reason, thioglycolic acid was chosen as thiol derivatives to explore. As nucleophilic ligand with consequently a strong affinity towards Pt, the molecule could be also seen as a way to entrap the metal (Scheme 3.27).



**Scheme 3.27.** Nucleophilic attack by thioglycolic acid. The following basification allows the left the metal complex in aqueous solution.

Pt shows a strong affinity towards sulphur. As a result of substitution reaction, R is got replaced by thiol. In particular, as Scheme 3.27 shows, aniline derivatives could be recovered from the work up through an appropriate basification (DCM).

Such considerations are valid for Pt (II). It was assumed in previous pages of this chapter that  $[\text{Pt}(\text{Cl})_4]^{2-}$ , the anionic species from the correspondent potassium salt, could be react with DCl according to the scheme below. (Scheme 3.28).

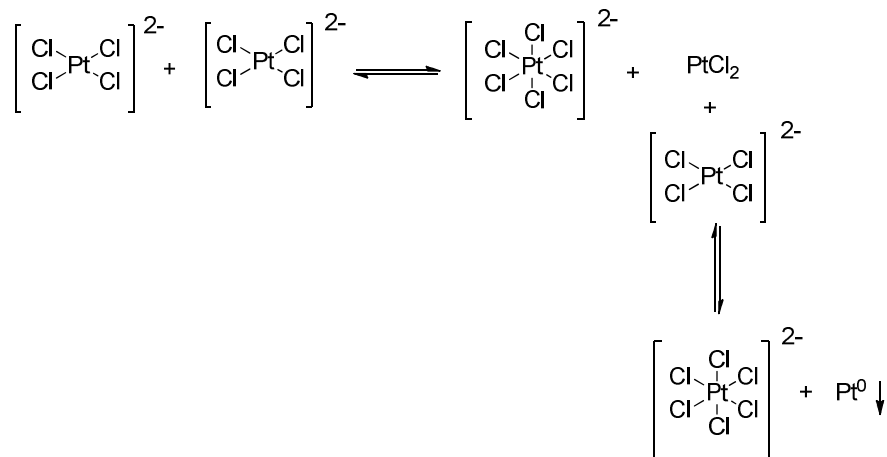


**Scheme 3.28.** Oxidative addition reaction set up through DCl. The excess of acid could increase the number of oxidation (+2) of most of catalytic molecules and avoid the black platinum precipitation.

According to Scheme 3.28, the Pt(IV) complex obtained could avoid black platinum precipitation. The high excess of acid could be able to oxidate all Pt(II) species present in solution. The species just formed should also not be able to disproportionate and thus, to produce Pt(0).

Moreover many kinetic studies stated that this complex Pt(IV) performs slower reactions, such as ligand substitution, than the correspondent Pt(II).<sup>145</sup> According to Elding *et al.*<sup>146</sup> thiol groups are directly involved in reductive process from Pt(IV) to Pt(II). So, as a reductive agent, thioglycolic acid could play another additive role. The double effect of this compound for the aims of the project was considered relevant. A direct quenching at the end of reaction and a strong effect with a reasonable excess of thiol (4 equivalent, referred to catalytic amount) could guarantee the removal of Pt(II) from the aqueous solution.

As already stated in the last chapter, black Platinum (0) could precipitate as a result of disproportionate process among two  $[\text{PtCl}_4]^{2-}$  catalytic molecules. (Scheme 3.29).



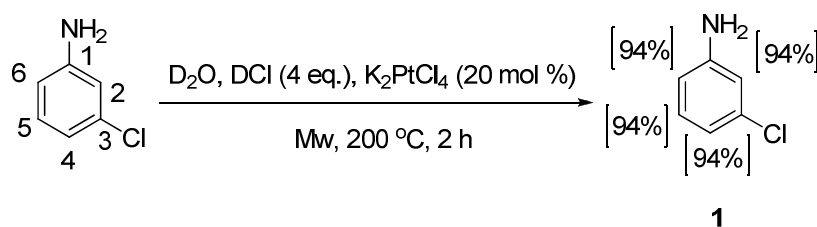
**Scheme 3.29.** Dismutative process among the catalytic molecules. The final products are black platinum and  $[\text{Pt}^{(IV)}(\text{Cl})_6]^{2-}$ .

### 3.7 3-Chloroanilines

In order to quantify  $\text{Pt}^0$  present in our aqueous solution, we decided to control its amount by *atomic absorption spectrometry*, probably the most common technique to evaluate the concentration of metals in solution. In Table 3.7 are shown the absorbance for precise amounts of  $\text{Pt}^0$  concentrations, evaluated with a calibration curve.

However, the core of our experiment was to calculate the corresponding absorbance value for 3-chloroaniline. This substrate was chosen for investigating the effect of thioglycolic acid in removing the metal. Table 3.7 describes the characteristics of this final trial.

**Table 3.7.** Absorbance (abs) results for two 3-chloroaniline.



Entry	N <sup>o</sup> a	Thioglycolic acid	Abs	ppm	µg	Yield
1	1	Yes	0.025	52	15	96%
2	N.A	No	0.069	230	66	95%

<sup>a</sup>Structure number assigned to the deuterated aniline intermediate in the particular experiment, as correlated in the experimental section

The absorbances consequently recovered for both of these two experiments and the correspondent *ppm* values, permit to know the amount of Pt inside. Even though in Entry 2 the platinum recorded is already low enough for not representing a serious problem for the reliability of the yield, the data reported in Table 3.12 show without any doubt the strong efficiency of thioglycolic acid in removing this metal.

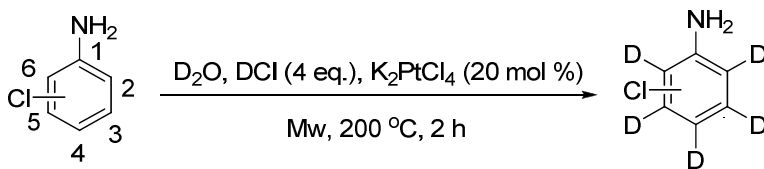
### 3.8 Chloroanilines

The results obtained for 3-chloroaniline inevitably focused the interest towards all the other substrates of this series. The data obtained are summarized in Table 3.8.

Unfortunately, because of lack of time in the last part of the project, we have not been able to apply such method of the platinum elimination excess with to the previously synthesised deuterated products. The comparison between *p*-chloro and *p*-fluoroaniline results, would

have been especially meaningful but, anyway, in the next chapters we will demonstrate the usefulness of this method with a number of other different substrates.

**Table 3.8** Summary of chloroanilines substrates.



Entry	N <sup>o</sup> c	Cl	D1 <sup>a</sup>	D2 <sup>a</sup>	D3 <sup>a</sup>	D4 <sup>a</sup>	D5 <sup>a</sup>	D6 <sup>a</sup>	Yield <sup>b</sup>
1	11	Para	-	95%	41%	-	95%	41%	86%
2	1	Meta	-	94%	-	94%	94%	94%	96%
3	12	Ortho	-	-	32%	92%	92%	92%	92%

<sup>a</sup>Deuterium incorporation calculating through acetylation.

<sup>b</sup>Yield calculated through the relation on Chapter 2.

<sup>c</sup>Structure number assigned to the deuterated aniline intermediate in the particular experiment, as correlated in the experimental

### 3.9 Conclusions

A first set of molecules was investigated by the method validated in Chapter 2. For this purpose aniline derivatives were chosen for the fact they have a large application on drug synthesis. However many difficulties arose during these tests, in particular during the yield recording. A valid explanation was advanced to try to justify every single experiment. So interactions between platinum catalyst and different aniline substrates were suggested. Such interactions can change quite differently according to the chemical and electronic properties of the compound explored. In 4-(trifluoromethyl)anilines case an unexpected product was obtained. Explanations which were suggested to clarify this process were shown to be quite reasonable. One in particular was confirmed by both the results of the acidic catalyzed and 2-(trifluoromethyl)anilines. In order to avoid any doubt regarding the yields' reliability, a Pt concentration was monitored by atomic absorption spectroscopy. The data obtained confirmed the purity of deuterium compound recovered and consequently the reproducibility of our method to many other substrates

# *Chapter 4*

## *Alkylanilines for H/D exchange*

### **4.1 Introduction**

### **4.2 Garnett's H/D exchange mechanism**

### **4.3 Pt(II)/Pt(IV) redox cycle**

### **4.4 Methylanilines**

### **4.5 Ethylanilines**

### **4.6 Propylanilines**

### **4.7 Butylanilines**

### **4.8 1-Phenylpiperazine**

### **4.9 Conclusion**

## **4.1 Introduction**

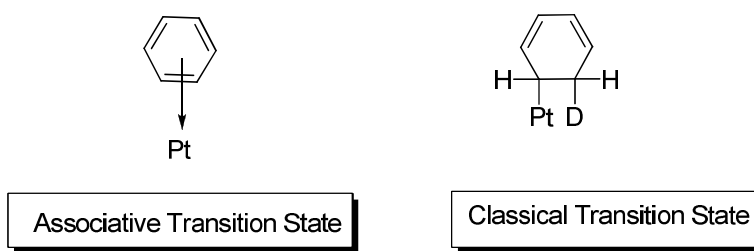
In the last chapter, a report of the initial H/D exchange results was presented. Some considerations of Pt-interactions and metal-complex formations were also faced. However, the conclusions recovered were useful for explaining yields rather than suggesting mechanistic H/D process. For this reason, in view of the results reported in previous chapters and in comparison with the corresponding acid catalyzed trials, a study of Pt role in H/D exchange was considered crucial. Although in the last chapter some mechanistic aspects were offered, here this part they will be expanded. Following bibliographic research<sup>147</sup> and the experimental data recovered a theoretical mechanism will be proposed. Additionally, a set of new anilines will be explored. In accordance with Garnett's studies on effective substrates, alkyl-derivatives will be used. Subsequently, a considerable evaluation of Pt mediated H/D exchanges both on the aromatic rings and on the alkyl chains will be established.

## **4.2 Garnett's H/D exchange mechanism**

In Chapter 2 Garnett's studies have been considered as the key reference for this project. In order to propose a reliable H/D mechanistic exchange, his results have been meticulously studied. However, in light of the results recovered during this project, some further considerations have been introduced. This will be seen later when this conclusion are adapted to a hypothetical H/D exchange mechanism on the alkyl chain.



In 1971, Garnett published a review<sup>148</sup> where platinum was classified as an important metal for regulating H/D exchange. However, classical associative and dissociative mechanisms already described in Chapter 1 (Scheme 1.4) showed some unclear points for explaining H/D aromatic exchange. In particular, relevant chemical considerations, such as ionisation potential and delocalised systems, resulted in Garnett's new alternative research. Since no loss of energy resonance was recorded, associative context was also excluded from playing a relevant role in this process. Garnett and Hodges<sup>149</sup> also established the important role played by delocalized molecular orbitals in this process. Finally, introducing a  $\pi$ -complex concept where  $\pi$ - electrons were preserved. (Scheme 4.1).



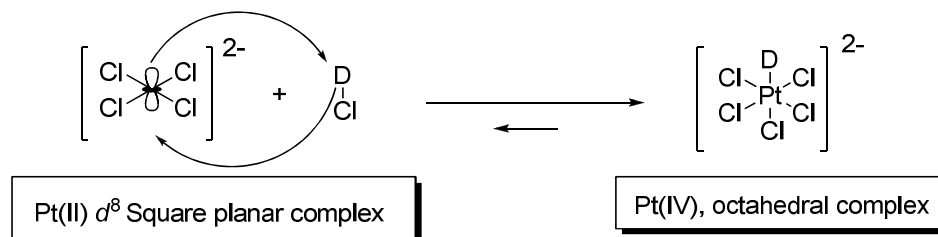
**Scheme 4.1.** Associative  $\pi$ -complex and classical associative intermediates. The arrow in  $\pi$ -complex indicates the net charge flow.

In this particular case, C-H is considered a double-electron donor while the metal an acceptor through its empty  $d$  orbitals. Even though this definition usually referred to metal-alkyl H/D exchanges, some interesting aspects could be moved to the aromatic context. Due to  $\pi$ -electron-rich cloud and the metal empty orbitals, a  $\pi$ -intermediate was considered reliable. As will be considered later, the formation of such a complex could be also explained in terms of *bonding orbital* overlapping. Consequently, the complex shows a net charge flow in direction of the metal.

### 4.3 Pt(II)/Pt(IV) redox cycle

Platinum tetrachloride anion  $[\text{Pt}(\text{Cl})_4]^{2-}$  appears to be a stable species in an aqueous solution. Like most of  $d^8$  transition metal complexes, this species is characterized by one typical square planar conformation, which also becomes extremely stable through geometrical and steric factors. Moreover, according to the corresponding electronic configuration, platinum is surrounded by only 16 electrons. In contrast to the 18 electron rule which is valid for most of

the transition metals, the catalytic species explored here shows a considerable stability toward this oxidation state. Lastly, as mentioned above, the empty orbital bondings available here are responsible for  $\pi$ -electrons overlapping and the corresponding  $\pi$ -complex formation. However, under the strong acidic conditions set up for the experiments, one of these empty orbitals could fill in through *oxidative addition* reaction, whose general aspects were already highlighted in Chapter 2. (Scheme 4.2).

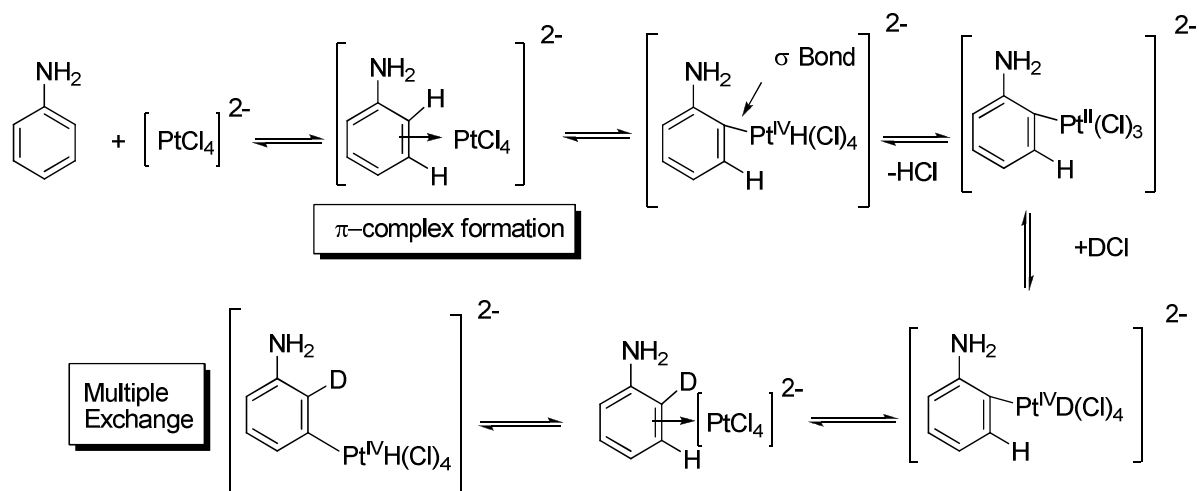


**Scheme 4.2.** Oxidative addition's scheme. At the centre of platinum tetrachloride an empty  $d$  orbital is reported where DCl attacks.

The final product of this process is a Pt(IV) octahedral complex. As explained in Chapter 3, this species is likely to not reduce  $\text{Pt}^{2+}$  to  $\text{Pt}^0$ . Despite the acidic excess, some traces of original Pt tetrachloride could still be reasonably present in solution. According to Garnett's considerations, this catalyst could still regulate the H/D process. So, as will be explained shortly, the general mechanism could be seen as a consequence of Pt(II)/Pt(IV) redox cycle.<sup>150-151</sup>

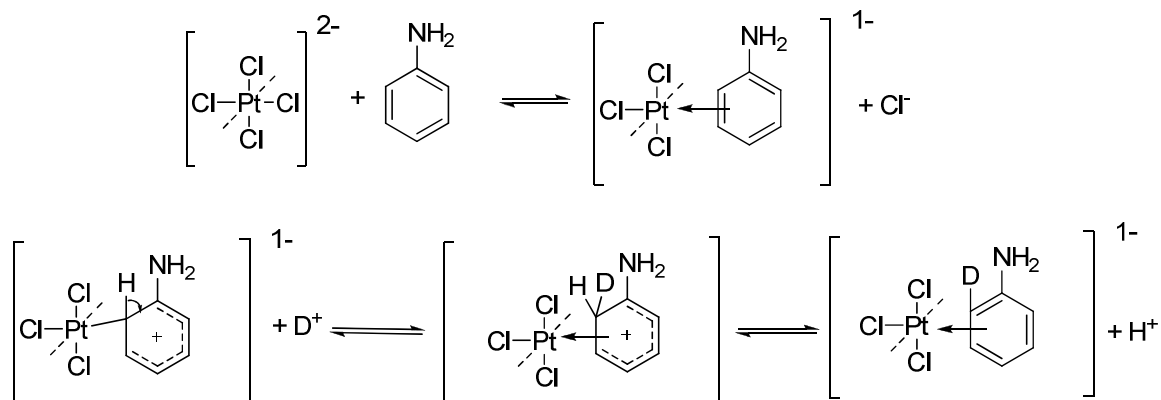
In order to clarify the hypothetical H/D mechanism on the aromatic ring, aniline has been chosen as the best initial substrate. As a first step of this investigation, this compound was able to exclude all substituent influences from these processes.

During his research, Garnett was able to establish that activating and deactivating substituents on the aromatic ring did not influence the rate of exchange. This was later confirmed by the work of Kanski and Kanska.<sup>152-153</sup> Ultimately, this aspect was also coherent with a dissociative mechanism for platinum as already discussed in Chapter 1. Thus, after having joined this last consideration to the  $\pi$ -complex concept, a new mechanism was subsequently proposed. (Scheme 4.3).



**Scheme 4.3.** Dissociative mechanism with a reversible  $\pi$ - $\sigma$  conversion.

The scheme above describes the main aspects of H/D process proposed by Garnett. In addition, he also suggested a mechanism where Pt(II) was the only metallic species involved (scheme 4.4).



**Scheme 4.4.** General description of the hypothetical H/D aromatic exchange mechanism where Pt (II) played a significant role in the process. As shown, the process is also based on only  $\pi$ -bond.

As will be discussed later, the mechanism on Scheme 4.3 was particularly adaptable for the alkyl chain experimental data. Moreover, as is shown, the processes in both Scheme 4.3 and 4.4 ultimately cause one  $\sigma$ -bond between Pt and phenyl ring. This final metal intermediate seems to be one of the best explanations for *multiple* H/D exchanges, which assumes that every hydrocarbon undergoes a continuous series of such exchanges during the reaction time. Specifically, H/D exchange could occur as a result of carbon activation mediated by Pt. In conclusion, this mechanism seems to be based on a reversible C-H bond-cleavage and C-D

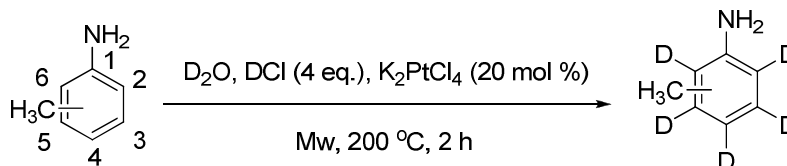
bond-formation which occurs when the hydrocarbon molecule is on the coordination sphere of Pt.<sup>154</sup>

## 4.4 Methylanilines

### 4.4.1 Aromatic H/D exchange

As established in the introduction of this chapter, alkylanilines represent the best substrates for alkyl H/D exchange mechanism. At the beginning of this investigation a series of methylaniline derivatives was chosen because of their small side chain. (Scheme 4.5).

**Table 4.1** Methylanilines deuterations results.



Entry	N <sup>o</sup> c	-CH <sub>3</sub>	D1 <sup>a</sup>	D2 <sup>a</sup>	D3 <sup>a</sup>	D4 <sup>a</sup>	D5 <sup>a</sup>	D6 <sup>a</sup>	CD <sub>3</sub> <sup>a</sup>	Yield <sup>b</sup>
1	13	Para	-	94%	63%	-	63%	94%	92%	85%
2	14	Meta	-	89%	-	89%	46%	91%	45%	97%
3	15	Ortho	-	-	46%	92%	93%	91%	77%	85%

<sup>a</sup>Deuterium incorporation calculating through acetylation.

<sup>b</sup>Yield calculated through the relation on Chapter 2.

<sup>c</sup>Structure number assigned to the deuterated aniline intermediate in the particular experiment, as correlated in the experimental section

Table 4.1 reports the relevant results both for the deuteration levels and for the yields. The interesting difference between D2 and D3 in Entry 1 (**13**) is due to the stronger influence that amino group played on the aromatic electronic density distribution. However, the consistent level of deuterium recorded on D3 can only be explained by the inductive electron-donor effect of methyl group on this position. While, in Entry 2(**14**) the H/D exchanges respect uniformly the electrophilic aromatic substitution rules. Here, D5 carbon is strongly deactivated by the contributions of both substituents.

The most pertinent aspect in this set of experiments regards the data in Entry 3 (**15**). D3 position shows very low deuterium incorporation (46%). This value is clearly contrasting with the opposite D5 where the H/D exchange is instead successfully high. Considering that both receive the same electronic activation, other factors must be involved. Naturally, the

attention was directed towards steric aspects. Starting from this, Garnett introduced the specific concept of *ortho steric* effect.<sup>148</sup> Following the mechanistic scheme in 4.3, Pt, and in particular Pt(IV), which is undoubtedly the most sizeable platinum species involved, could face relevant difficulties in introducing itself in D3. In the light of H/D results in D5, this catalyst further confirms its efficiency to work on both activated and deactivated aromatic positions. Accordingly, *ortho steric* effect remains the single factor responsible for the low percentage of deuteration in D3. This aspect will be investigated in the next experiments when larger alkyl chains will be used. Lastly, this conclusion also highlights another relevant point. The shifting movement on the aromatic ring could cause the catalyst to interact with its substituents. Therefore, some aspects of this interaction will be faced in the next pages and a H/D mechanism for the corresponding alkyl chain then proposed.

#### 4.4.2 Methyl H/D exchange

After having seen the results in Table 4.1, a further mechanistic investigation is necessary. The *ortho steric* effect is not the only aspect to arise from these experiments. The different percentages of deuteration recorded for the methyl groups are also significant. In order to summarize the results from this new point, a summary of deuterium incorporations was reported. (Table 4.2).

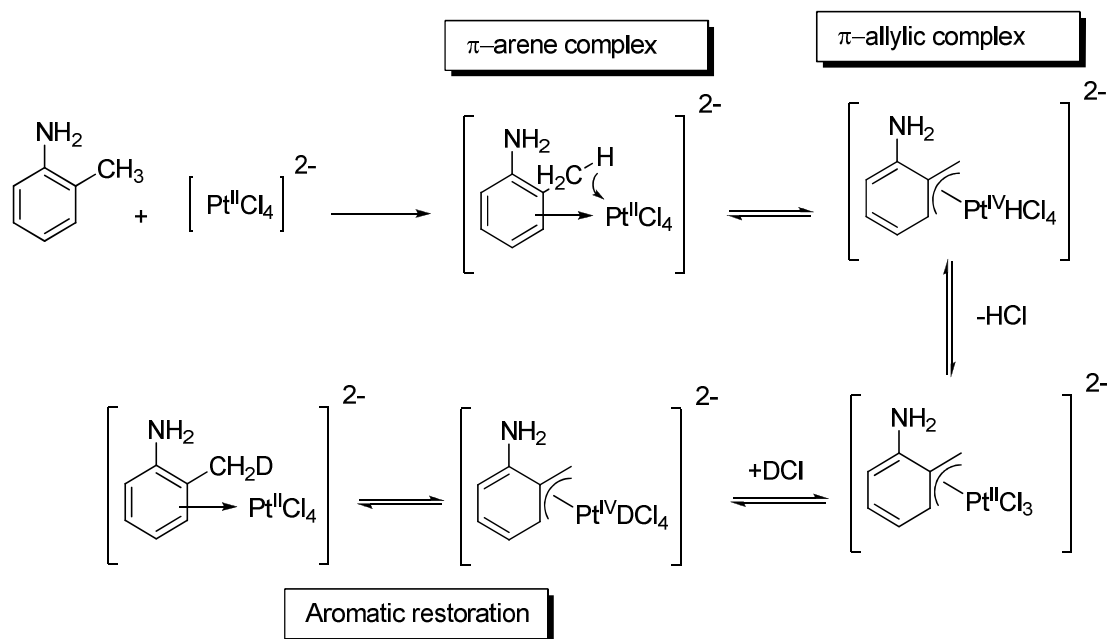
**Table 4.2** Summary of methyl exchange for methylanilines.

Entry	N <sup>oa</sup>	-CH <sub>3</sub>	CD <sub>3</sub>
1	13	Para	92%
2	14	Meta	45%
3	15	Ortho	77%

<sup>a</sup>Structure number assigned to the deuterated aniline intermediate in the particular experiment, as correlated in the experimental

Garnett *et al.*<sup>63</sup> were able to establish that H/D mechanism exchanges on alkyl chain showed common aspects with the aromatic context. Furthermore, they observed that alkyl chains on alkylbenzenes were more reactive than the corresponding saturated aliphatic ones. The easiest explanation could be the  $\pi$ -electronic cloud presence which could activate the alkyl chain through a conjugation and hyperconjugation process. For this reason, they proposed an alternative alkyl dissociative  $\pi$ -complex in competition with that one in Scheme 4.3. This

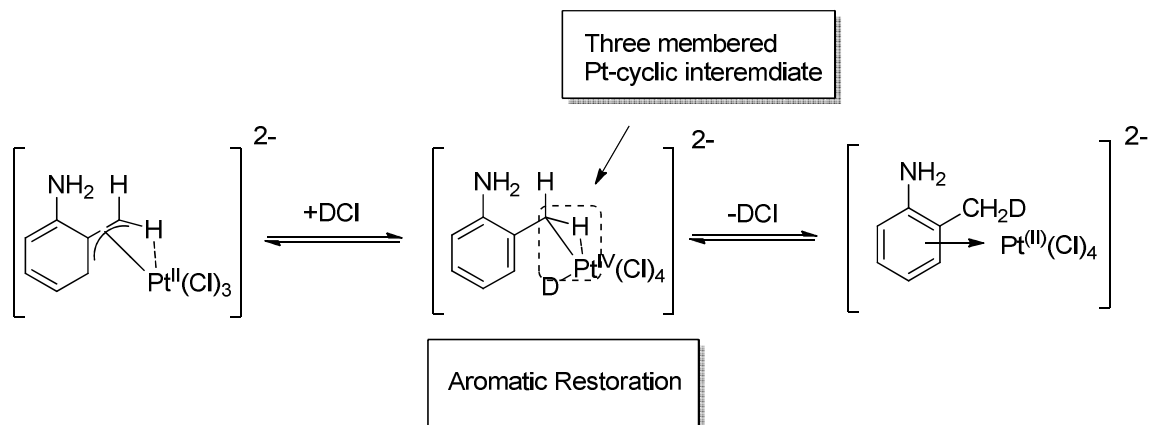
mechanism deserves a clear description. In particular, for this aim a  $\eta^3$ -allylplatinum complex as intermediate needs to be introduced. As Khusnutdinova *et al.*<sup>155</sup> have established, these complexes are generally formed as a consequence of an allylic C-H deprotonation. Coherently with what was assumed in scheme 4.3 the process could be characterized by a hydride migration. Since the Pt acts as an electrophilic species, hydride could be in fact forced to move in the direction of the metal, forming here a  $\sigma$ -bond. Although in the allylic metal complex studies where both cyclic and acyclic unsaturated systems were often investigated, aromatic rings were never considered as valid substrates. For this reason, the relevant work developed by Driver *et al.*<sup>156</sup> was fundamental for expanding this aspect to the aromatic context. After highlighting once again the importance of  $\pi$  electrons on activating the aliphatic hydrogen, they thought that the key to this process was based on a reversible equilibrium between a  $\pi$ -arene complex and a  $\pi$ -allylic one. (Scheme 4.5).



**Scheme 4.5.** Mechanism for methyl H/D exchange. The equilibrium between  $\pi$ -arene and  $\pi$ -allylic complex is shown.

For the current purpose, the behaviour of platinum towards the methyl group needs to be specified. In the  $\eta^3$ -allylic complex, Pt (II) has been synthetically transformed in Pt (IV) by DCl addition. However, this process could appear to be more complicated than actually described. For this reason, many studies were developed in order to define this aspect. Garnett and Hodges<sup>149, 157</sup> suggested that a  $\sigma$ -complex could be involved, whereas other

research elaborated on different conclusions. In particular, Shestakov *et al.*<sup>158</sup> gave an important contribution in the understanding of such interactions. Their research reports an interesting explanation about H/D exchange on alky chain. The core of their conclusions is based on the presence of C-H $\cdots$ Pt bond, through which deuterium can be introduced in the methyl group. (Scheme 4.6).

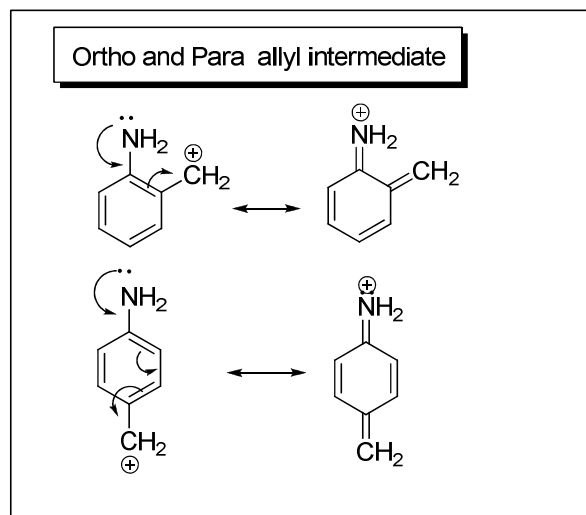


**Scheme 4.6.** General description of H/D exchange on methyl group for 2-methylaniline. The hydrogen bond and the corresponding Pt three membered cyclic intermediate are reported.

Such interaction is favoured on Pt(II) complex<sup>159</sup>, where apparently an empty *d* orbital metal can act as a hydrogen-bond acceptor. This interaction could direct platinum towards methylic carbon instead of toward the aromatic one. As previously described, *ortho* steric effect on the aromatic ring gives an important contribution to direct the result toward this direction. In addition, the electronic activation could play a relevant role. After having formed a  $\sigma$ -bond here, Albinati *et al.*<sup>160</sup> suggest that the system could be considered as a two electrons three-centre bond (see Scheme 4.6). The consequent C-H activation could finally favour the exchange here and concertedly bring back Pt (IV) to Pt (II).

The process on the methyl group just described could be repeated cyclically until the deuterium incorporation reported in Table 4.2 is reached.

Lastly, considerations regarding H/D exchange on methyl group according to its aromatic position are necessary. In view of the process described in Scheme 4.6 and the results reported in Table 4.2, the different allyl intermediate stability seems to play a key role. In Scheme 4.7 these different stabilities have been shown. It appears evident that *ortho* and *para* methyl substituted species can form corresponding, more stable allylic system than *meta* analogues. As a consequence of this, kinetic and energetic parameters could undoubtedly favour methyl H/D exchange on *ortho* and *para* positions.



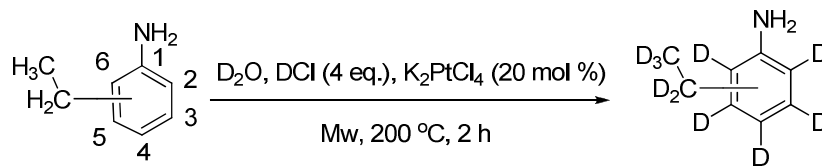
**Scheme 4.7.** Methylanilines allyl intermediates. *Ortho* and *para* have the opportunity to delocalize the positive charge better than *meta*.

## 4.5 Ethylanilines

### 4.5.1 Aromatic H/D exchange

An investigation of a new set of compounds was essential for accumulating further experimental data. In order to confirm the theoretical mechanisms described until now. So, being the natural sequence of methylanilines, because of only one carbon more present in their alkyl chain, ethylanilines were consequently explored. (Table 4.3).

**Table 4.3** Ethylanilines deuterations results



Entry	N <sup>oc</sup>	-Et	D1 <sup>a</sup>	D2 <sup>a</sup>	D3 <sup>a</sup>	D4 <sup>a</sup>	D5 <sup>a</sup>	D6 <sup>a</sup>	CD <sub>2</sub> <sup>a</sup>	CD <sub>3</sub>	Yield <sup>b</sup>
1	16	Para	-	87%	65%	-	65%	87%	91%	92%	56%
2	17	Meta	-	92%	-	92%	92%	92%	89%	90%	81%
3	18	Ortho	-	-	23%	92%	92%	92%	82%	92%	75%

<sup>a</sup> Deuterium incorporation calculating through acetylation.

<sup>b</sup> Yield calculated through the relation in Chapter 2.

<sup>c</sup> Structure number assigned to the deuterated aniline intermediate in the particular experiment, as correlated in the experimental

As is shown, the ethyl chain has been fully deuterated in all the experiments set up.



In Entry 1(**16**), D2 and D6 show exchange percentages slightly lower than the other substrates. As already mentioned in previous chapters, in the most active positions a further exchange process could have occurred and have replaced hydrogen instead of the corresponding isotope. However, alternative explanation could be proposed. Although here the activation by amino group is relevant, the competitive inductive effect by ethyl group could have caused an incomplete activation for these positions.

D3 and D5 results are indicative of this. The aromatic deuterium incorporation could once again be a direct consequence of strong competition between these two substituents. Therefore, the final electron activation could explain the final H/D exchange. The same consideration could be proposed in Entry 2 (**17**) for D5. Despite these substituents being on *meta* relationship, exchange results are as equally activated as the others. Therefore, the deuterium exchanged here is considerably high. One possible explanation could be connected with the mechanism proposed previously (Scheme 4.3). Furthermore, Pt could be easily exchanged here due to the fact that any important steric hindrances are present.

As already established in methyl H/D exchange, the steric contribution in such a mechanism is strongly relevant. In this set of trials more evidence of this aspect was observed. In particular, in Entry 3 (**18**), D3 position shows a level of deuterium surprisingly lower than in the corresponding methyl experiment. (23% against 46%, Entry 3, Table 4.1).

#### 4.5.2 Ethyl chain H/D exchange

The H/D exchange mechanism on the methyl chain described in the last paragraph could be further expanded here. In each of the three experiments, ethyl chain shows high deuterium incorporation. In order to summarize the results obtained Table 4.4 illustrates this exchange. Since some slight differences amongst each entry are observed, some considerations will be offered in this section.

**Table 4.4** Summary of ethyl exchange for methylanilines.

Entry	N <sup>0a</sup>	-Et	CD <sub>2</sub>	CD <sub>3</sub>
<b>1</b>	<b>16</b>	Para	91%	92%
<b>2</b>	<b>17</b>	Meta	89%	90%
<b>3</b>	<b>18</b>	Ortho	82%	93%

<sup>a</sup>Structure number assigned to the deuterated aniline intermediate in the particular experiment, as correlated in the experimental section.

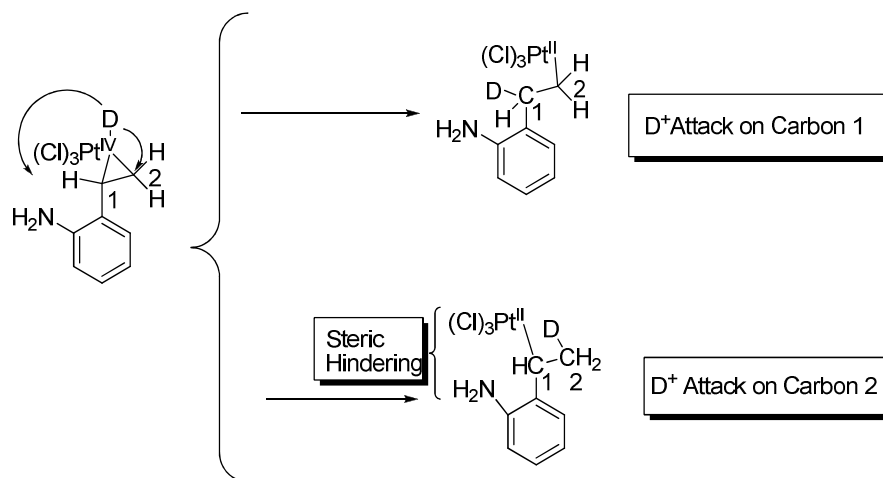
So the suggestions reported by Garnett and Hodges<sup>115, 157, 161</sup> during the 60s and 70s need to be expanded in the light of new organometallic discoveries.

Mustafin *et al.*<sup>162-164</sup> have studied the initial asymmetric deuterium distribution on the two ethyl carbons. In particular, they focused their attention on Pt-H shifting on this alkyl chain. Their kinetic studies on how hydrogen can move from carbon to platinum shows interesting conclusion. Overall, they established that the 1,2 shifting process could occur more slowly than Pt dissociation or 1,1 shifting process itself.

Although Flood<sup>165</sup> and later, Jones<sup>166</sup> were able to investigate this deuterium migration aspect on rhodium (Rh) catalysis, only few recent examples in the literature offer the chance to investigate the Pt context.

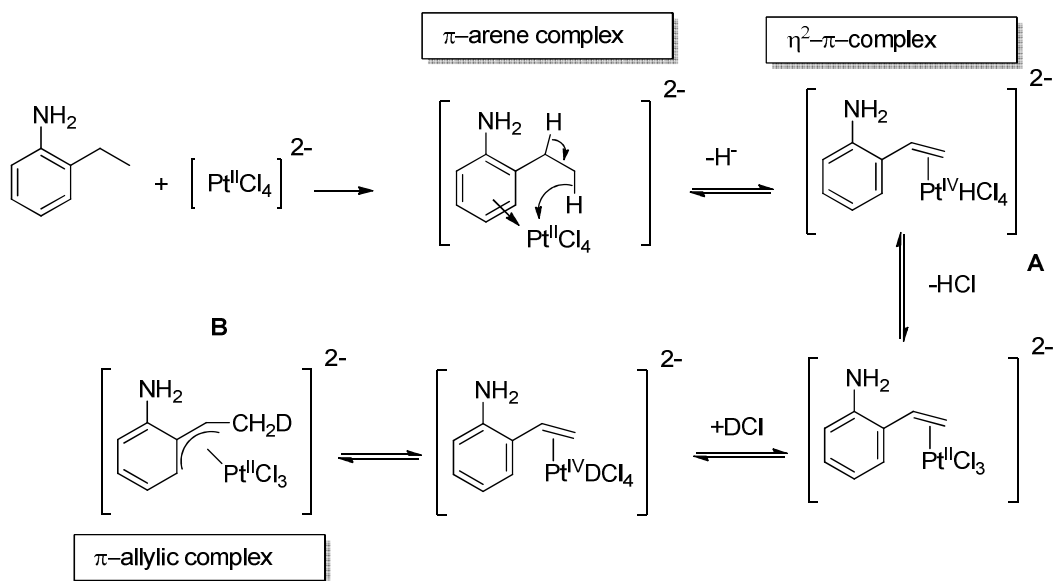
Initially it was thought that Pt (II) and Pt (IV) spatial conformations could bring chloro ligands and ethyl hydrogen closer. Furthermore, due to the drastic conditions inside the microwave, hydrochloric acid could be eliminated. Subsequently, a new three-member cyclic intermediate could then be formed (Scheme 4.10). Although mechanisms like this are reported in literature mainly for Pt (II)<sup>167</sup>, it is also reasonable to suggest that it could occur for Pt (IV) as well. So, DCl addition could play a key role. As a consequence of conformational changing, the chloro ligands could be in the right position for interacting with the alkyl hydrogens.

After the cyclic intermediate formation, deuterium ( $D^+$ ) could move to the ethyl chain according to two possible directions. The favourite orientation could be determined by steric factors, establishing the final distribution of deuterium. In Scheme 4.8 a specific description of ethyl context is provided. As shown in Table 4.4, deuterium is  $\gamma$  incorporated on this chain in all of the molecules explored. The steric aspect is maybe not so relevant for 3 and 4 ethylanilines. The importance of the steric factor instead becomes relevant for the 2-ethylaniline result. As previously mentioned, the final exchange could be directly connected with the amine group present beside. Because of this obstacle, the  $D^+$  attack on carbon 1 is undoubtedly favourable. In this way Pt(II) complex is shifted on carbon 2 and free to carry on the exchange. As a result of this mechanism, the percentage in this last position is slightly higher than the first one.



**Scheme 4.8.** Initial mechanism proposed. Two possible orientations are present in Pt-cyclic intermediate. Both attacks can cause H/D exchange on carbon 1 and carbon 2.

However, the proposed H/D methyl chain exchange in the last paragraphs could represent a good referential point for a reasonable and logical application on the ethyl group. For this reason, another mechanism has been coherently considered. Once again, Driver *et al.*<sup>156</sup> contributed greatly to these studies. After they established that Pt interaction starts through  $\pi$ -electronic cloud, they proposed a mechanism substantially suitable for the results reported here. In order to justify the particular deuterium distribution, they noted that specific alkyl chain conformation could play a key role. (Scheme 4.9).



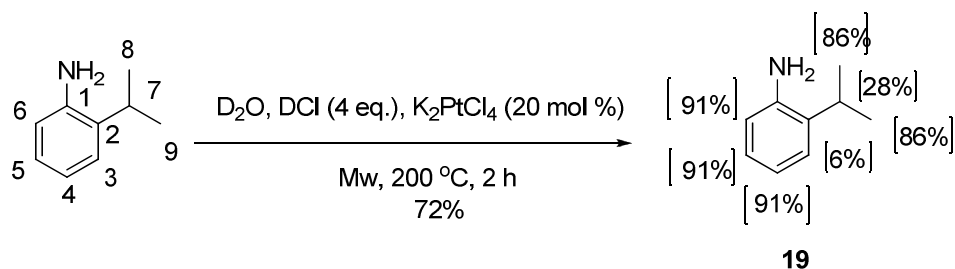
**Scheme 4.9.** General description of ethyl chain H/D exchange. The particular conformation of ethyl chain could allow hydride on  $CH_3$  to interact with the catalyst and then to form a subsequent allyl system.

Following what was assumed before and synthesized in Scheme 4.6, a C-H interaction with the catalyst could cause a hydride migration from CH<sub>3</sub>. As a consequence of this, a  $\eta^2$ - $\pi$ -complex is obtained (**A**). Due to its conjugation with the aromatic ring, this system could be energetically favoured. In addition, as a consequence of steric reasons explained in Scheme 4.7, deuterium could be introduced in ethyl CH<sub>3</sub> allowing a subsequent allyl system to be formed (**B**). Then the process could follow the pathway described for methylaniline context. Furthermore, the lowest deuterium incorporation in **18** D<sub>3</sub> (23%, Table 4.3) could be explained one more time in terms of an increasing *ortho* steric effect as a consequence of a larger alkyl chain.

As is clear from Table.4.4, deuterium distribution is homogeneous on the ethyl chain. Although in **18** methylene (CH<sub>2</sub>) has been exchanged less than methyl due, probably, again to steric reasons, deuterium distribution generally shows no particular differences between these two carbons. As possible explanation, a competition between CH<sub>2</sub> and CH<sub>3</sub> could occur. As will be seen later, two different allyl intermediates could be formed resulting in two different H/D exchange on this alkyl chain. This aspect could be also clarified by Collman *et al.*<sup>168</sup> studies. Here a certain tendency of forming the most hindered  $\eta^3$ -allylmetal chloride intermediate is established. As a consequence of this, it is reasonable to assume that a competition could occur providing the explanation of the homogeneous deuterium distribution on the ethyl chain. Finally, any relevant differences are also recorded for the ethyl chain on different aromatic positions. The most reasonable explanation could be in replacing hydrogen with methyl group, the corresponding allyl intermediates could be more stable than that of the methyl context. So, as a result of more important *inductive electron donor effect*, the considerations reported in Scheme 4.8 are not so relevant in this case.

#### 4.6 Propylanilines

The considerations described above enable us to expand the proposed mechanism to a further series of alkylanilines. For this purpose, propylanilines were considered the natural follow up of ethylanilines. In the light of steric conclusions reached before, a proper substituent in 2 position was chosen. In order to create the maximum hindrance, 2-isopropylaniline was explored. (Scheme 4.10) Later, *n*-propyl chain will also be investigated and useful considerations will be finally recovered from these results. In addition, such results will show a sound consistency towards the mechanism until now described.



**Scheme 4.10.** H/D exchange results for 2-isopropylaniline.

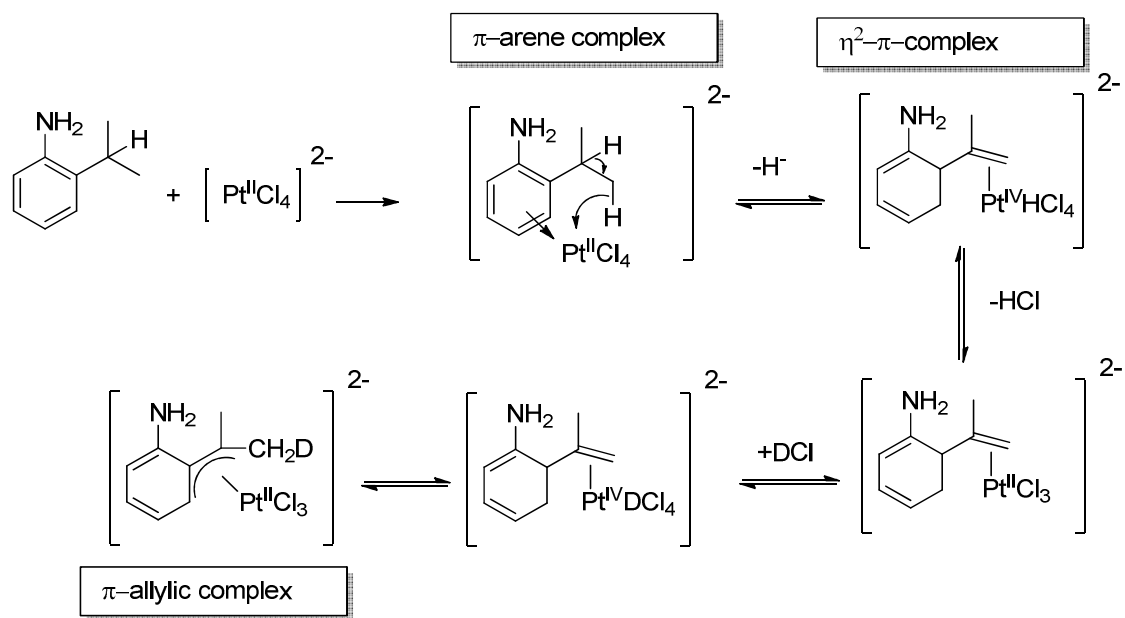
The data recorded in this first experiment confirm a certain correlation between alkyl size and Pt activity. In Table 4.6 a summary of the H/D exchange in D3 is reported. So, it is initially evident that the level of deuterium introduced here is reduced proportionally with the size of the alkyl chain. From this table it appears quite clear that steric effect has a strong influence on such general processes.

**Table 4.5.** Summary of D3 exchanged for 2-alkyl-substituted-anilines.

Entry	N <sup>0a</sup>	2-Alkyl-substituent	D3
1	15	Me	46%
2	18	Ethyl	23%
3	19	Isopropyl	6%

<sup>a</sup>Structure number assigned to the deuterated aniline intermediate in the particular experiment, as correlated in the experimental section

Additionally, it is relevant to present some mechanistic considerations about isopropyl H/D exchange. After having considered the structural similarities with ethyl chain, a similar process has been proposed. In Scheme 4.11, a mechanism has been drawn according to the guidelines already defined in Scheme 4.5 and 4.9.

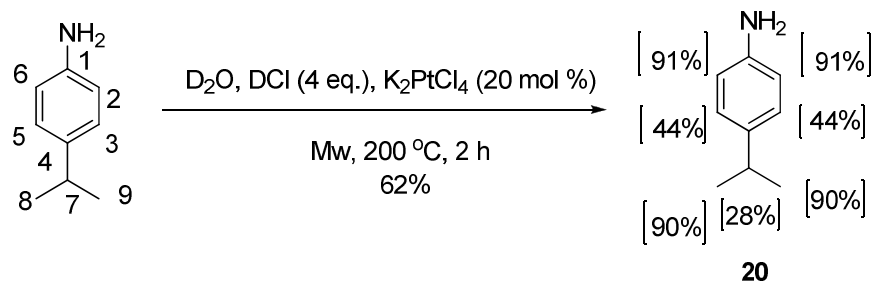


**Scheme 4.11.** Scheme of H/D exchange on 2-isopropyl chain. .

In particular, here D7 shows very low deuterium incorporation. Furthermore, as a consequence of a free rotation process, both of two methyl groups (8 and 9 in scheme 4.12) on the isopropyl chain could attain an identical steric influence by the amino group. Therefore, both of these positions are finally, equally involved in the H/D exchange. As shown in Scheme 4.13, isopropyl chain can be thought of as an ethyl chain, where one hydrogen was replaced by a methyl group. As a result of this, the consequent general system became more hindering. This effect results in a very low percentage of deuterium incorporation in D3 position (6%). Similar considerations could be also proposed for D7 (28%). In conclusion, it appears that space is a fundamental aspect for catalyst to attack the alkyl chain.

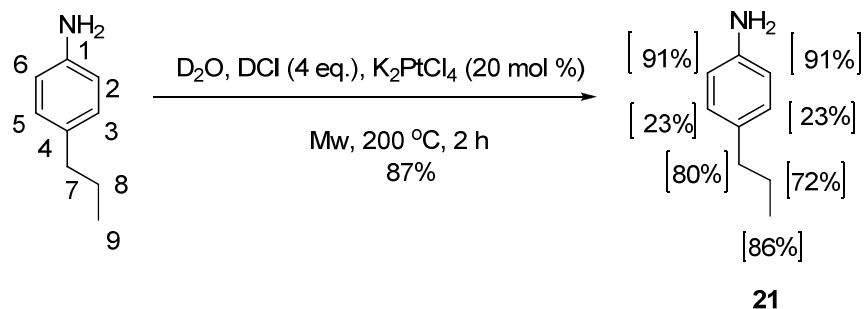
Then it was decided to explore 4-propyl substrates. Here *iso* and *normal* propyl groups (Scheme 4.14 and 4.15) were also investigated. As will be treated in the next pages, these two compounds gave the opportunities to study the contribution that steric and electronic-donor inductive effect have on the H/D exchange.

The results for 4-isopropylaniline were reported in Scheme 4.14.



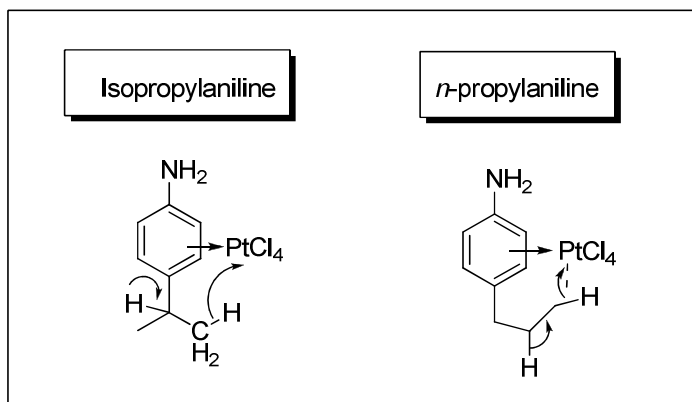
Scheme 4.12. Scheme of H/D exchange on 4-isopropylaniline.

Below 4-*n*-propylaniline H/D exchange is reported. (Scheme 4.13).

Scheme 4.13. General scheme of H/D exchange on 4-*n*-propylaniline.

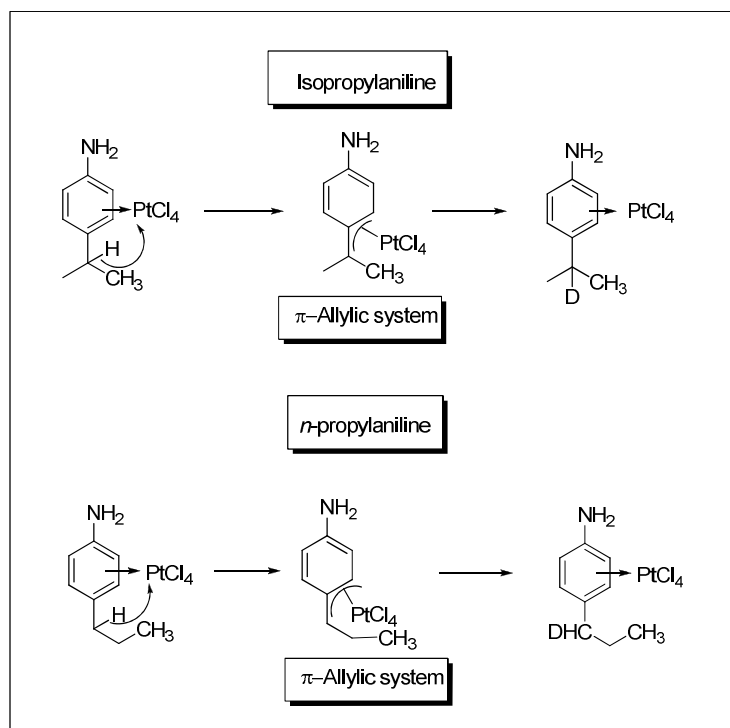
Many considerations can be claimed from these two experiments.

Firstly, the H/D exchange on D3 and D5 positions. Although the two positions should reveal the same electron activation in both experiments, the deuterium incorporation is significantly different. It is also interesting that the most bulky isopropyl species displays the highest level of deuterium on it. Since these two alkyl groups have similar chemical properties, it was not logical to propose a different electronic activation here. So, reasonably, this aspect could be explained in terms of their specific alkyl conformation. It was thought that in *n*-propyl case, a methyl group could get closer to the aromatic D3 and D5 positions than to the isopropyl one. Thus, the exchange in the *normal* chain could be more favoured than to the *iso* one. (Scheme 4.14). So, spatial availability could be again a relevant factor to explain this aspect. In addition, in both of these cases, deuterium will be subtracted from D3 and D5 positions in the aromatic ring.



**Scheme 4.14.** Different alkyl conformation could allow a different catalytic interaction.

In order to explain the particular deuterium distribution on *n*-propyl chain, where deuterium has been mainly exchanged on D7 and D9 positions, another alternative mechanism has been considered. This mechanism could be identical with what is assumed in the ethyl context. There, referring to a competition between CH<sub>2</sub> and CH<sub>3</sub> in allyl intermediate formation. So, although methylene species are less reactive in forming allyl system than methyl group<sup>168</sup>, one of their hydrogens could nevertheless be close enough to the catalyst to create competition with CH<sub>3</sub> group. (Scheme 4.15).



**Scheme 4.15.** Scheme to show the hypothetical mechanism for deuterating the propyl chain.



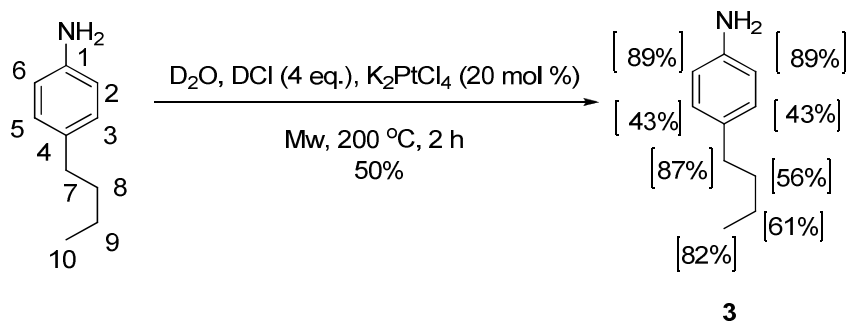
Following this description, D8 could undergo a similar exchange. However, the steric hindrance could reduce the H/D efficiency here causing the final result reported in Scheme 4.13 (72% against 80% in D7 and 86% in D9).

As will be revealed in the next paragraph, this mechanism could be in part confirmed by the kinetic studies already developed in Chapter 2. Although in this case *n*-butylaniline was the subject of that investigation, the results recovered there were linear with that one reported here. In particular, it was relevant to observe that in both of these cases the internal alkyl positions were less deuterated than the external ones.

## 4.7 Butylanilines

The conclusions achieved until now give a substantial understanding of the hypothetical mechanisms involved in H/D exchange. The next set of experiments should further confirm this knowledge. However, due to cost and time limitations, the range of these compounds has been restricted. Only two species of 4-butylanilines were investigated in this attempt.

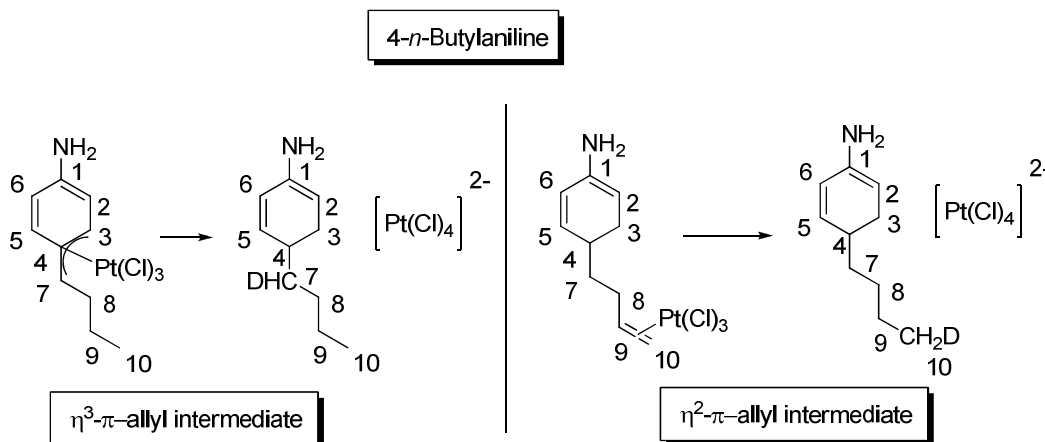
In order to expand the considerations on 4-*n*-propylaniline, 4-*n*-butylaniline was undoubtedly very helpful. The results for this substrates show a perfect linearity with the data recovered until now (Scheme 4.16).



**Scheme 4.16.** Scheme of H/D exchange mechanism on 4-*n*-butylaniline.

Remarkably, the deuterium incorporation in D3 and D5 is identical to that one in 4-isopropyl context. (Scheme 4.14). As discussed there, the alkyl conformation could once again play a key role. However, it was thought that the major size of this chain could create some difficulties for methyl group in D10 to interact with the catalyst. Otherwise, the electron-donor action could also expand the influence to the *ortho* aromatic positions. Due to this strong competition, the exchange in D3 and in D5 could be expected similar to *n*-

propylaniline context.(Scheme 4.13).In order to reach accurate conclusion, it was relevant to investigate the particular deuterium distribution on this alkyl chain. Differently from the other experiments, here D7 is more deuterated than D10. Once again, one of the most reasonable explanations could be a strong competition between the two intermediates reported in Scheme 4.17.

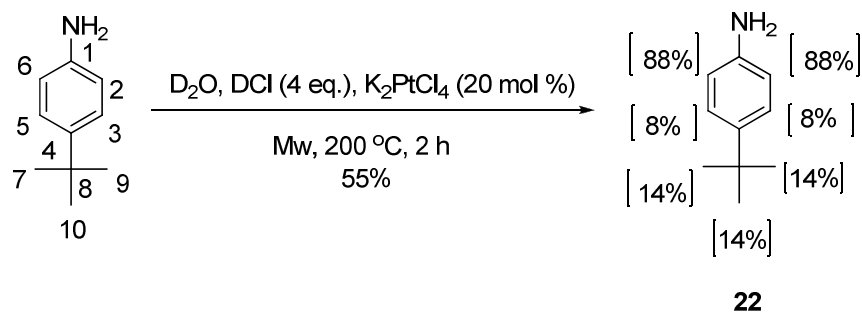


**Scheme 4.17.** Two intermediates forming. As a result of steric hindering, the structure on the left is favoured.

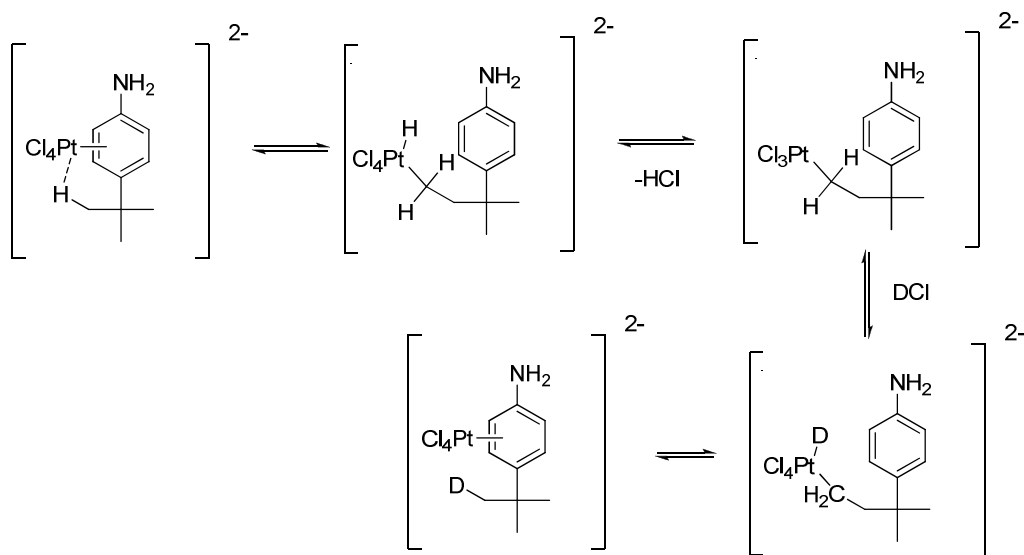
As proof of this tendency to arise from kinetic experiments. As already discussed in Chapter 2, H/D exchange in 2 *n*-butylaniline was already investigated. An experiment with different reaction times has been set up (Table 2.7). So, after 2', D7 was more deuterated than D10 (7% against 6%) showing that the corresponding intermediate (*on the left* in Scheme 4.17) could be maybe kinetically favoured. Therefore, this set of experiments confirms that this process starts definitely from the aromatic ring (all these positions are in fact deuterated first). The increase of time also allows  $\eta^2\text{-}\pi\text{-intermediate}$  favouring H/D exchange in D10 to stabilize. So, the interaction of methyl group in  $\pi\text{-platinum}$  complex could be relevant. As shown in Table 2.7, deuterium incorporation passes from 91% at 105' to 82% due to a reverse exchange which again introduces hydrogen. In conclusion, methyl group in D10 appears to be still directly involved in H/D exchange. Furthermore, in the light of the results in D3 and D5, it is reasonable to think that deuterium incorporation here depends strongly on the inductive electron donor effect mediated by *n*-butyl chain.

Lastly, 4-*tert*-butylaniline was also a good point for other relevant considerations. In particular, the *tert*-butyl chain can confirm the mechanism proposed. In the absence of hydrogen for forming a conjugated system, the eventual alkyl H/D exchange could be due to

a regular catalyst shifting from D5/D3 to D7/D9. Although Scheme 4.18 shows a homogeneous deuterium distribution in all CH<sub>3</sub> of *tert*-butyl chain, in the light of what assumed above, deuterium could only be drawn in D7 and D9 position as described in Scheme 4.19.



**Scheme 4.18.** Description of H/D exchange results for 4-*tert*-butyl-aniline.



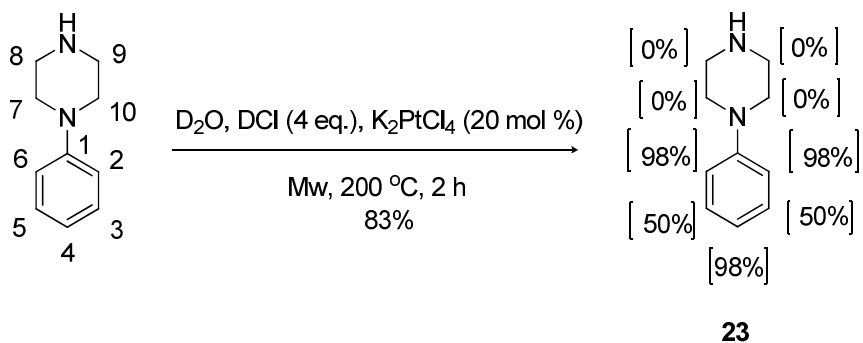
**Scheme 4.19.** Hypothetical mechanism for deuterating D7 and D9 positions.

In conclusion, steric effect in combination with *tert*-butyl chain electron donor could be responsible for the low deuterium level in D3 and D5.

## 4.8 1-Phenylpiperazine

The last experiment gave the opportunity to further expand this investigation. 1-Phenylpiperazine was chosen for a new particular branch of studies. In certain aspects, this substrate respects many 4-*tert*-butylaniline characteristics. Although a quaternary carbon was replaced by a tertiary amine, the absence of hydrogen confirms once again its importance in the general H/D alkyl exchange. However, the context here is appreciably different because of the presence of one heteroatom between aromatic ring and alkyl chain.

The results recorded for this final experiment were fundamental for establishing accurate conclusions. (Scheme 4.20).



**Scheme 4.20.** Description of H/D exchange results for 1-phenylpiperazine.

D5/D3 positions show a considerable lower deuterium incorporation than compound **2a**. As a reasonable explanation, piperazine could have a stronger electron donor effect than amine. So, *ortho/para* positions could receive a more relevant activation than *meta*.

However, the most interesting consideration concerns the  $\pi$ -allyl system formation proposed until now. Since this intermediate seems difficult to form, no H/D exchange is observed in piperazine moiety. Perhaps the extreme acidic conditions could result in nitrogen protonation. In view of the results obtained until now, it is also necessary to expand some considerations already made in Chapter 3. Although in that case hetero-complex formation mediated by Pt(IV) was noted, we reached similar conclusions. In both case in fact, the metal is not allowed to move further on the alkyl chain. In particular, in methoxyaniline context, it was shown that methyl group could not be exchanged at all. As a possible explanation, oxygen could be protonated as well, forbidding the formation of any  $\pi$ -allyl system.

In conclusion, both of these processes could equally take part during the H/D exchange. Amino and methoxygroup could in fact act as nucleophile/base causing complex/acid conjugation species formation.

## **4.9 Conclusions**

Through a series of bibliographic and experimental data, an initial H/D aromatic and alkyl exchange mechanism was studied and then proposed. Starting from a set of methylanilines, this mechanism was then successfully applied to a progressive series of alkylaniline derivatives. These final results were also able to highlight new aspects of H/D exchange. In particular it was established that steric hindrance strongly influenced the deuterium distribution. While an equilibrium between Pt(IV)/Pt(II) was presumed to be the key species in the validated conditions, the  $\pi$ -complex was also a fundamental part involved in such a process. Additionally, a proposed allyl intermediate was suggested. The existence of this species was then directly linked to hydride replacement on the corresponding alkyl chains.

In the end, it was fundamental to highlight that the process studied above could have its natural application to many different substrates, including the ones already described. So, the conclusions achieved here will be generally and synthetically recalled in the next chapters where they will be related to the specific contextual considerations of the other compounds explored there.

# *Chapter 5*

## *Heterocycles for H/D exchange*

### **5.1 Introduction**

### **5.2 Simple aminopyridines**

### **5.3 Methyl and amino pyridines**

### **5.4 Quinolines**

### **5.5 2-Methylindole**

### **5.6 Conclusions**

## **5.1 Introduction**

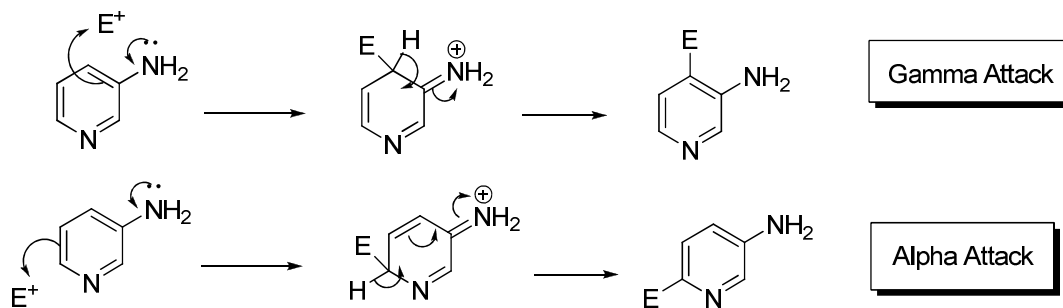
In this chapter heterocyclic molecules will be explored. It was considered fundamental to validate our method on this new set of molecules. Nowadays their presence in pharmaceutical drugs is commonplace and obviously their synthesis<sup>169</sup> is correspondingly quite developed. As a consequence, this type of compounds seem to be particularly relevant for the aims of this project. However, the different reactivity of heterocyclic compounds towards electrophilic aromatic substitution meant that the method for analyzing the labelled compounds described previously needed to be changed. In addition, the H/D mechanisms described in Chapter 4 will be directly applied here.

As explained in Chapter 2, acetylation was a fundamental process to enable the precise deuterium incorporation in all structural positions to be recovered. However, the reaction defined in Chapter 2 was no longer considered suitable for the heterocyclic substrates to be investigated here. Furthermore, as will be explained during the development of this chapter, steric and electronic parameters could influence the reactivity of every specific molecule explored differently. Consequently, in some cases appropriate reactions for measuring the H/D exchanges will be specifically introduced.

## 5.2 Simple aminopyridines

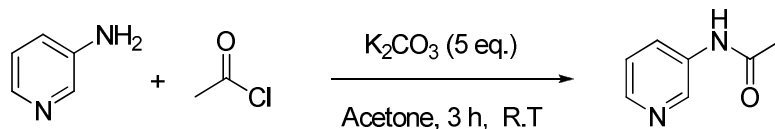
Pyridines are very common moieties for biological compounds and drugs.<sup>170</sup> Described initially by Barnes *et al.*<sup>171</sup> as analogues of nitrobenzene derivatives, these compounds indeed show similar electron distribution. However, due to the electron-withdrawing effect of the pyridine nitrogen, *highest occupied molecular orbital* (HOMO) and *lowest unoccupied molecular orbital* (LUMO) energetic levels will be lower than corresponding benzene-analogues. As arisen from their definition, HOMO and LUMO will be necessarily involved in electrophilic and in nucleophilic attacks respectively. In particular, being HOMO energetically lower than LUMO, the reactivity towards electrophilic aromatic substitution is strongly reduced.

For this reason, it was necessary to choose a set of pyridine derivatives with electron-donating groups, so that their reactivity towards electrophilic aromatic substitution would have been enhanced. Aminopyridines were seen as appropriate substrates for this purpose. Supplying a *non-bonding* lone pair, amine nitrogen allows HOMO energetic level to be increased. As shown in Scheme 5.1, an eventual conjugation with  $\pi$ -electron system permits a strong activation on *alpha* and *gamma* positions prevalently. In particular, these two positions will become electron rich, permitting mainly the electrophilic attack ( $E^+$ ) taking place on the pyridine ring (Scheme 5.1).



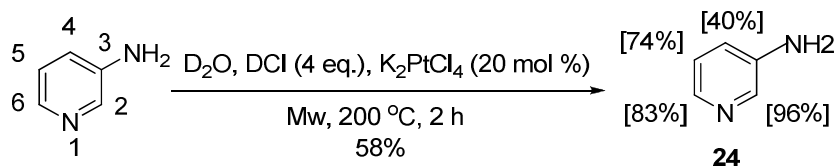
**Scheme 5.1.** Attack by an electrophilic species ( $E^+$ ) on the pyridine ring of an aminopyridine is favoured at the positions adjacent (*gamma*) or opposite (*alpha*) to the amino group.

The general basicity of the aminopyridines is considerably higher than the corresponding anilines. For example, the  $pK_a$  of the conjugate acid of 3-aminopyridine is 6.16, whereas the  $pK_a$  of the conjugate acid of aniline is 4.6. In Scheme 5.2 the 3-aminopyridine case was reported. According to the work of Elemans *et al.*<sup>172</sup>, an excess of a stronger base ( $K_2CO_3$ ,  $pK_a$  of conjugate acid = 10.33, 5 eq.) was definitely necessary for acetylating the amino group present. (Scheme 5.2).



Scheme 5.2. Acetylation of 3-aminopyridine.

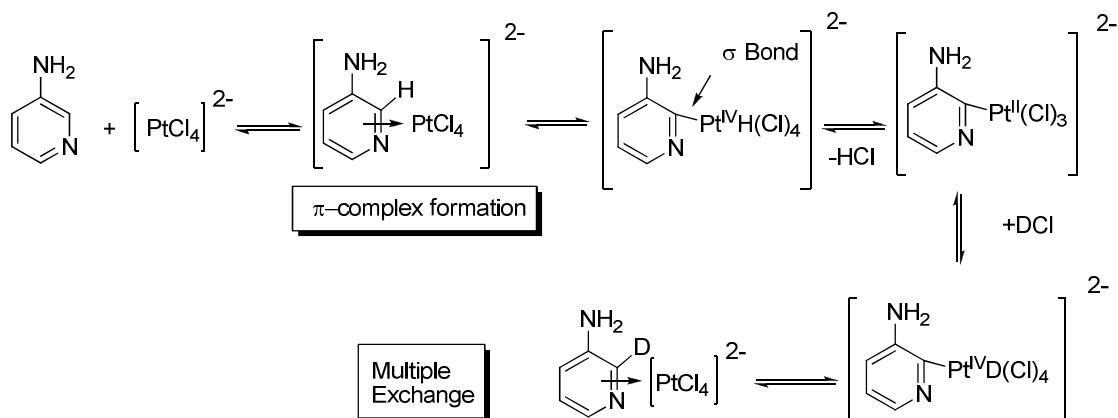
This method would be used as part of the procedure for measuring the H/D exchange in the deuteration process. The actual process for deuteration is shown in Scheme 5.3. 3-Aminopyridine was isolated from the mixture in 58% yield and the levels of deuterium incorporation at the different positions (obtained by measuring the  $^1\text{H}$  NMR spectrum of the acetylation product) are also shown in Scheme 5.3.



Scheme 5.3. Deuteration results for 3-aminopyridine.

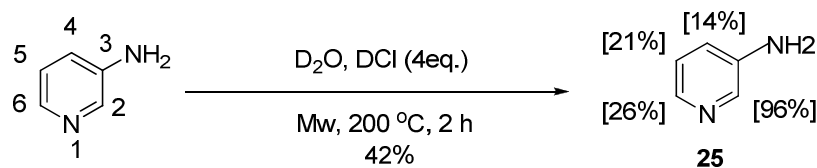
The results reported above appear rather odd. Despite the amino group activation on adjacent and opposite positions, only the hydrogens on positions 2 and 6 were almost completely exchanged, while position 5 (which is neither adjacent nor opposite to the amino group) was exchanged to a greater extent than position 4, which is adjacent to the amino group. In the literature, there are other examples of electrophilic aromatic substitution reactions of 3-aminopyridine that have shown similar characteristics. For example, Ammer and Bach<sup>173</sup> studied a new total synthesis of the thiopeptides amythiamicin C and D starting from a 2,6-dibromo-3-iodopyridine intermediate. In order to obtain this intermediate, the initial step was bromination of 3-aminopyridine. The reported result showed that two bromine atoms were introduced exclusively at the pyridine positions 2 and 6. However, Canibano *et al.*<sup>174</sup> reported that monobromination can often be obtained with only 2 equivalents of *N*-bromosuccinimide. Logically, as displayed in Scheme 5.3, the level of H/D exchange in these two positions is also considerably high. Additionally, the different deuterium incorporation between them is also extremely relevant. Maybe due to a different electronic distribution, electrophilic attack in 2 seems particularly preferred. As explained in Chapter 4, platinum catalyst could shift along the aromatic ring through a reversible  $\pi$ - $\sigma$  conversion. So, a similar mechanism has been here proposed for the heterocyclic context. (Scheme 5.4).



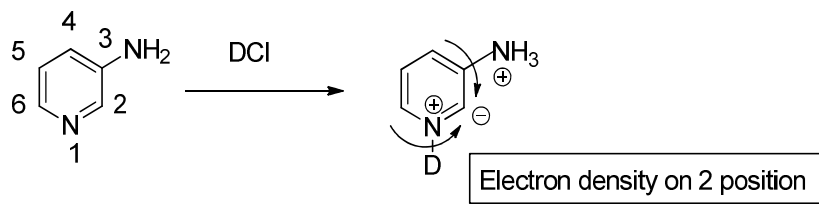


The process is also supposed to carry on all the rest of aromatic carbons.

In order to expand on our own investigation, a H/D exchange reaction of 3-aminopyridine was carried out without the chloroplatinate as a catalyst. The results are indicated in Scheme 5.5.



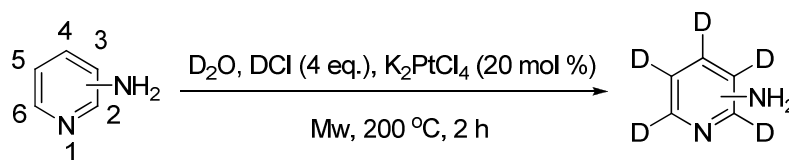
The result showed the same trend in reactivity as had been shown in the presence of a Pt catalyst, but the level of exchange was much lower for most of the positions, again showing the better efficiency of the platinum catalyst in such experiments. The exception was position 2, which showed virtually the same level of incorporation of deuterium as in the Pt catalyzed reaction, showing that this position is particularly strongly activated. In order to explain this particular result, Zoltewicz *et al.*<sup>175</sup> works were relevant. Having studied the inversion of reactivity positional order in pyridine, they were able to establish interesting aspects. As a consequence maybe of electronic disruption on the aromatic ring under such acidic conditions, 2,6 carbons were spotted as main H/D exchangeable positions. However, in our specific case, 2 position results to be more activated than 6. This could be reasonable explained as a consequence of an increased electron density here due to its strict closeness of both protonated nitrogen. (Scheme 5.6).



**Scheme 5.6.** Description of possible electronic disruption under acidic conditions. The arrows indicate the theoretical  $\pi$ -electron cloud flow.

Since these last results, it was logical to extend the investigation to similar substrates, for example 2- and 4- aminopyridines. The results obtained in the presence of a Pt catalyst are shown in Table 5.1.

**Table 5.1.2** and 4-Aminopyridine H/D exchange.



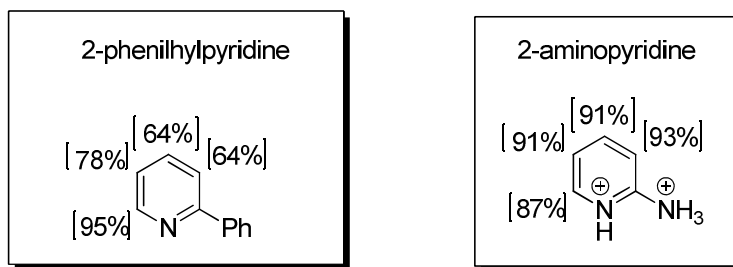
Entry	N <sup>oc</sup>	-NH <sub>2</sub>	D1 <sup>a</sup>	D2 <sup>a</sup>	D3 <sup>a</sup>	D4 <sup>a</sup>	D5 <sup>a</sup>	D6 <sup>a</sup>	Yield <sup>b</sup>
1	26	2	-	-	93%	91%	91%	87%	79%
2	27	4	-	73%	92%	-	92%	73%	23%

<sup>a</sup>Deuterium incorporation calculating through acetylation.

<sup>b</sup>Yield calculated through the relation on chapter 2.

<sup>c</sup>Structure number assigned to the deuterated aniline intermediate in the particular experiment, as correlated in the experimental section

In **26** (Entry 1, Table 5.1) deuterium has occurred at all positions at a high level. Following the considerations described above, it could be useful to consider once more time that acid could have disrupted the electronic distribution. In order to confirm this point, Werstiuk *et al.*<sup>97</sup> studies resulted to be helpful. In their research, 2-phenylpyridine has been investigated as a substrate for H/D exchange. Considering phenyl an electron donor substituent similar to amine group, it was thought useful to compare these results with that ones in **26**. (Scheme 5.7). Although the experiment set up there was based on only deuterium oxide under drastic conditions, strict analogies were arisen from it. Positions 4 and 5 seem in fact receiving an almost equal deuteration, but position 5 is increased. This last aspect is maybe a consequence of phenyl activation on its opposite position. In our context instead, the acidic conditions could have caused a considerable electronic disruption, resulting in the level of exchange reported in Table 5.1.



**Scheme 5.7.** Deuteration of 2- phenylpyridine and 2-aminopyridine. 2-aminopyridine has been also presented in protonated form as a result of acidic conditions.

In addition, the slightly lower level of exchange at positions 6 presumably could reflect the lower electron density at that position, but the experiment is not sufficiently discriminating to show up any effect at the 4-position, which should also have lower electron density. After having referred to kinetic experiments reported in Chapter 2, it was also thought that on position 6 reverse exchange could occur allowing hydrogen to be introduced back. Coherently with Wierstuk's result, this position could be finally considered particularly active. To finish, a comparison of deuterium incorporation between these last two experiments shows once more time the impressive efficiency in H/D exchange obtained through Pt catalyst.

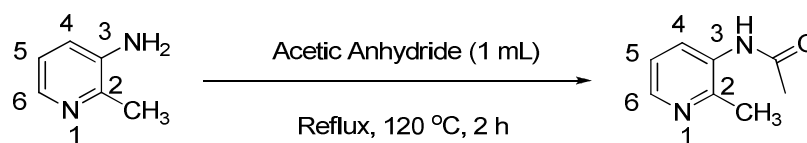
This conclusion is also confirmed by the results with the symmetrical 4-aminopyridine (**27**). The adjacent activated positions (positions 3 and 5) are exchanged to a greater extent than the corresponding less active 2 and 6 positions, but the level of deuterium incorporation is still considerable also at these positions, which is a great advantage in the use of the Pt catalyst. As a further confirmation of what assume above, the electronic density could be shared between alpha and beta carbons. In addition, it should be noted that the recovery of product from the exchange reaction of 4-aminopyridine was very low. A similar observation (yield 26%) was made by a colleague for the reaction under Pt-free conditions.<sup>126</sup> It is likely that this substrate is particularly soluble in water and consequently quite hard to extract into organic phase. This is a point that should be addressed if such a highly soluble compound is to be exchanged in future.

### 5.3 Methyl and amino pyridines

As a consequence of the results recovered above, it was necessary to develop a further investigation of similar substrates. Pyridines incorporating both methyl and amino groups

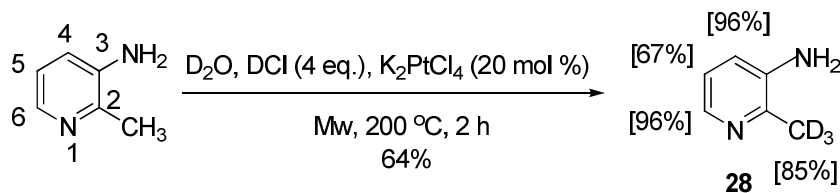
were chosen for this purpose. Their study gave the opportunity to evaluate the influence that a methyl group generates on the pyridine ring for H/D exchange. Furthermore, it was relevant to compare how the level of deuterium incorporation on this alkyl chain would be affected by its different positions on the ring.

2-Methyl-3-aminopyridine (**28**) was the first compound investigated in this set of experiments. Although for this substrate and most of all the others in this paragraph the H/D exchange measurement described in Scheme 5.2 will be preserved, an alternative way to introduce the acetyl group has been explored. As it will be seen, this reaction will result useful for acetylating 4-amino-2-methylpyridine. In particular, studies by El-Shishtawy *et al.*<sup>176</sup> were helpful for defining this appropriate method for this purpose. (Scheme 5.8).



**Scheme 5.8.** Acetylation process using acetic anhydride under reflux.

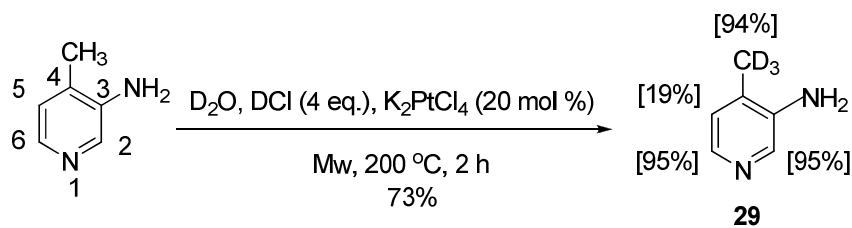
The results obtained here are reported below. (Scheme 5.9).



**Scheme 5.9.** Deuteration results for 2-methyl-3-aminopyridine.

Even though the amino group controls the regioselectivity, as confirmed by the strong deuteration at the opposite and adjacent positions (4 and 6), the methyl group influences, interestingly, the activation in 4. Comparing this result with **24** on Scheme 5.3, the H/D exchange was strongly increased, passing from 40% to 96%. Coherently with the consideration above proposed, it was thought that methyl group could forced the electron density towards to the rest of aromatic carbons. As a consequence of this, the most of electron density could be concentrated around nitrogen ions that means on 4 and 6 positions. As a result of this, deuterium distribution appears to respect the electrophilic aromatic substitution activation.

4-Methyl-3-aminopyridine (**29**) was next studied and the results are reported in Scheme 5.10.

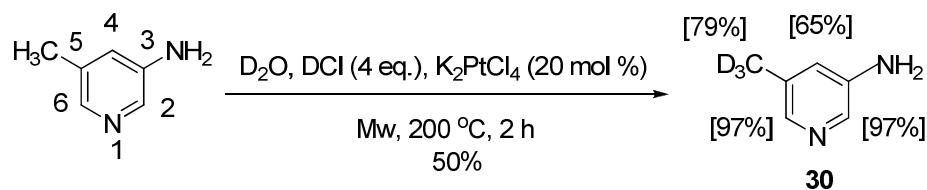


**Scheme 5.10.** Deuteration results for 4-methyl-3-aminopyridine.

In this experiment again the amino group seems to direct the H/D exchange. As active positions, the 2/6 positions present high percentages of deuterium incorporation while at the 5 position the level of exchange was much less. As proposed before, methyl group could have increased the electron density particularly on position 5 and 6. However, the deuterium incorporation recorded on 5 is indicative of a considerable ortho steric effect. The reduced chance for platinum to attack carbon 5 could have induced it then to prefer positions 6 and 4 (thereby involving the methyl group) and, at the same time, increasing the likelihood of exchange at those positions.

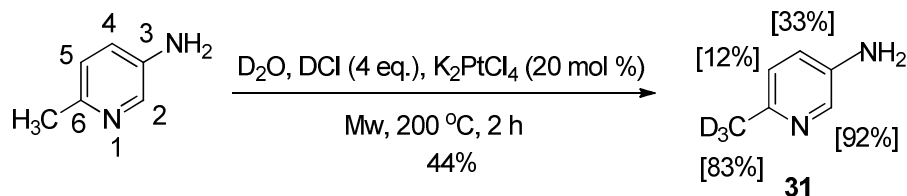
Finally, the exchange percentage recorded for the methyl group is higher than (**28**) (Scheme 5.9). Maybe due to the activation in 4, the corresponding alkyl chain shows an intense H/D exchange (94%). However, according the results reported in Scheme 5.3, the 4-position of 3-aminopyridine itself does not seem really activated for H/D exchange (40%). A possible explanation here could arise from the consideration already set up in Chapter 4. The H/D mechanism there proposed involved a  $\eta^3$ -allylplatinum intermediates. As mentioned there, platinum could be forced to move towards alkyl chain due to strong steric reasons. As a consequence of this, deuterium incorporation results in favouring methyl group instead of 5 positions. So, the final balance appears to be completely different than in Scheme 5.3.

In the case of 5-methyl-3-aminopyridine (**30**, Scheme 5.11), the level of exchange at the methyl group is lower (79%) than that for the methyl group of 4-methyl-3-aminopyridine (94%). As a completely different situation, here the steric reason maybe could have played a not so relevant role. Firstly, the H/D exchange on methyl group is perfectly coherent with **24**. Secondly, in Scheme 5.3, position 4 resulted to be the less activated (40%). According to the  $\pi$ -electronic disruption, methyl group could have forced the electron density towards to 4 position, causing consequently the exchange. Furthermore, the two adjacent electron-donor groups could have increased its reactivity until to reach the level of 65%. As a result of this, the electron activation here seems to have played a key role in comparison of what assumed in 4-methyl-3-aminopyridine context.



Scheme 5.11. Deuteration results for 5-methyl-3-aminopyridine.

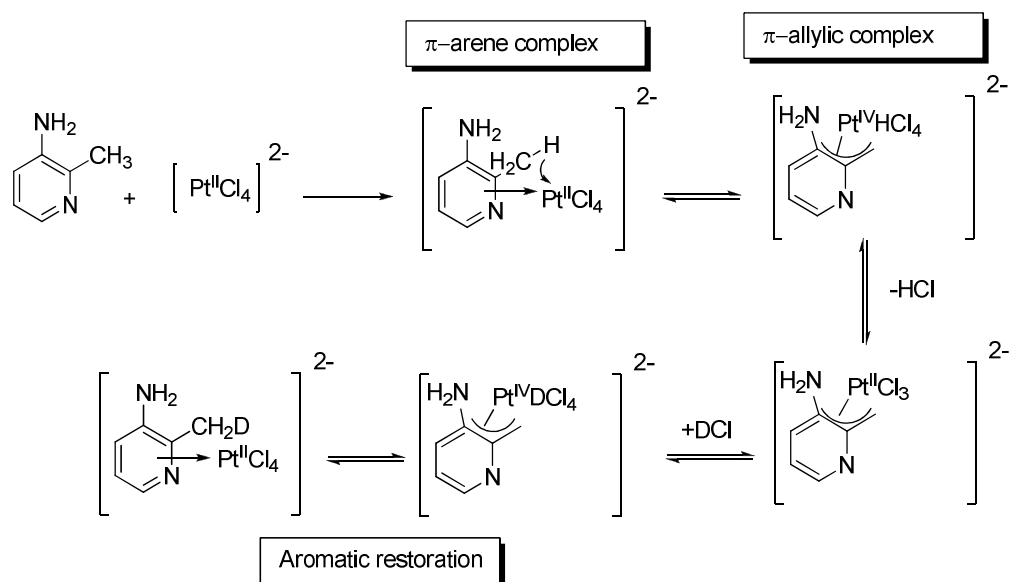
A slightly different situation was faced with 6-methyl-3-aminopyridine. (Scheme 5.12).



Scheme 5.12. Deuteration results for 6-methyl-3-aminopyridine.

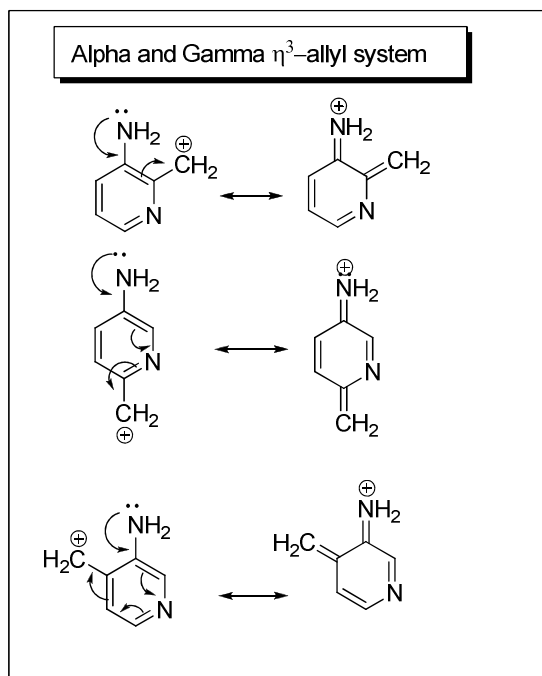
The percentage recorded in position 4 is consistent with the corresponding 3-aminopyridine. As already established above, this position seems weakly activated. In particular, the methyl group seems to be able to influence its level of activation depending on its position on the pyridine ring. For this reason, a possible explanation of the reduced deuteration at the 4-position of **31** (33% against the 40% for 3-aminopyridine) could be due to the deactivating presence of the alkyl group at position 6. At position 5, a combination of steric effect and electronic deactivation, mediated by the amino group, is responsible for the low level of exchange (12%). The strong activation already mentioned on position 2 of the last examples is further supported here by the high level of deuteration reported (92%).

As mentioned before, all the considerations about methyl exchange could be explained in terms of  $\eta^3$ -allyl system. (Scheme 5.13). Coherently on what assumed on Chapter 4, a similar process has been applied on heterocyclic context.



**Scheme 5.13.** H/D mechanism for deuteration results for 2-methyl-3-aminopyridine.

The scheme above describes the hypothetical H/D mechanism for 2-methyl-3-aminopyridine. As shown also in Scheme 5.14, amino group could help to stabilize the allyl system formed here as a consequence of hydride migration.



**Scheme 5.14.** Different allyl system stability shown by resonance forms.

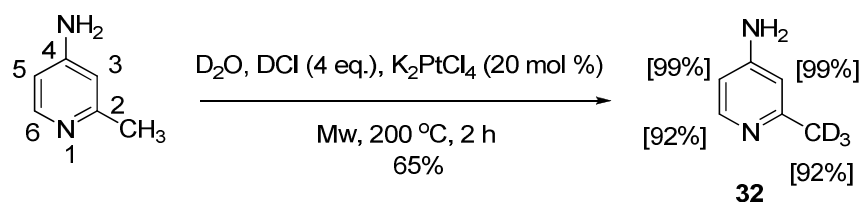
To conclude, Table 5.2 shows an indicative summary for this process in all the other substrates used. Although the exchange appears to follow the electron activation on the aromatic ring, it seems to mirror the results obtained on methylanilines. (Table 4.2). As established above, 2 carbon is the most active position in 3-aminopyridine case. Probably due to steric effect caused by amino group beside, in **28** the exchange here is less than expected. Entry 2 (**29**) and 3 (**30**) could be instead strictly connected. In both case, the *ortho* steric effect could cause an increase of deuterium incorporation in adjacent methyl group. Finally, in Entry 4 (**31**) methyl group exchange shows a perfect coherence with the corresponding result reported in Scheme 5.3.

**Table 5.2.** Methyl group H/D exchange on 3-aminopyridines.

Entry	N <sup>oa</sup>	-NH <sub>2</sub>	-CH <sub>3</sub>	H/D
<b>1</b>	<b>28</b>	3	2	85%
<b>2</b>	<b>29</b>	3	4	94%
<b>3</b>	<b>30</b>	3	5	79%
<b>4</b>	<b>31</b>	3	6	83%

<sup>a</sup>Structure number assigned to the deuterated aniline intermediate in the particular experiment, as correlated in the experimental section

The last substrate investigated in this section of work was 2-methyl-4-aminopyridine, which allowed us to study the H/D exchange when the amino group occupies a different position. (Scheme 5.15).



**Scheme 5.15.** Deuteration results for 2-methyl-4-aminopyridine.

The methyl group here seems to be having the same effect on each of the adjacent and opposite (3/5) positions. However, the results obtained are quite interesting, particularly when compared with that on **27** (Table 5.1). As already mentioned, methyl group could have forced the electron density towards the aromatic carbons. Lastly, following the considerations above, position 2 could have been equally exchange as a consequence of its strong activation.

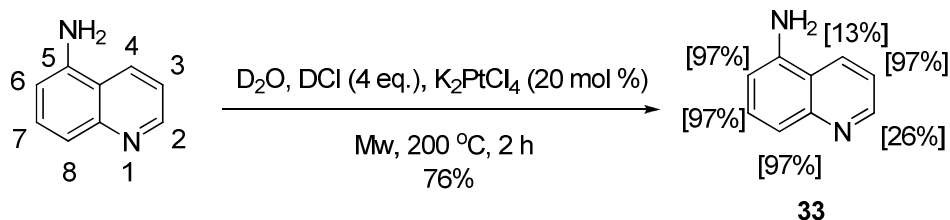


## 5.4 Quinoline

Following the investigation of relevant heterocyclic substrates, quinoline derivatives were seen as another fundamental part of this research. Many alkaloids of these species, which provide pharmaceutical properties, are observed in nature.<sup>177</sup> Other synthetic drugs are commonly used in different therapeutic fields, even though their most important application is undoubtedly regards the antibiotic care against malaria.<sup>178</sup>

5-Aminoquinoline (**33**) was the first molecule to be investigated. In order to find the most valid acetylation method for this compound, a series of attempts were made. Eventually, the reaction already defined in Scheme 5.2 was chosen as the best *N*-acetylation process.

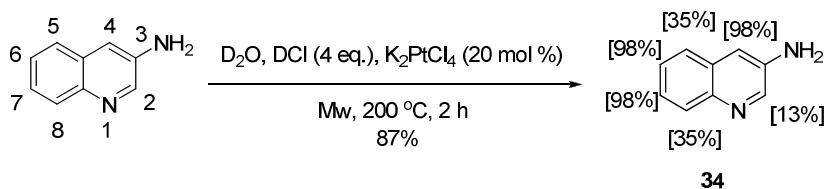
In this experiment the results obtained were found to be in line with the general quinoline characteristics. (Scheme 5.16).



**Scheme 5.16.**Deuteration process for-5-aminoquinoline reaction.

As heterocyclic fused rings, quinolines can be studied according to their two different components. The substrate investigated can be considered to be sharing a molecule of benzene and one of pyridine. The results obtained in this experiment are perfectly consistent with the expectation based on this approach. As seen in the case of aniline (see Chapter 2), Pt is able to deuterate homogeneously all the positions on this aromatic system. Conversely, on the pyridine part, as a consequence of the inactivation towards general electrophilic substitutions, only position 3 was efficiently exchanged. The small difference between positions 2 (26%) and 4 (13%) could be due to hindrance at position 4.

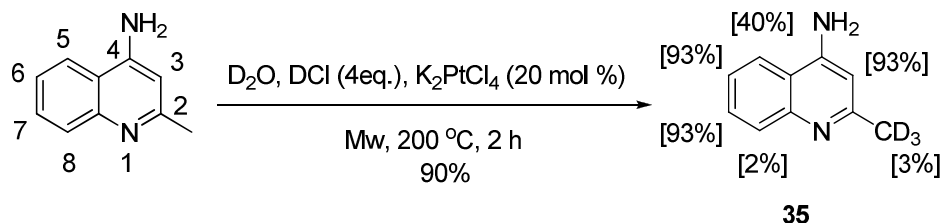
Different considerations can be reached instead with the next compound. Preserving the method for H/D exchange measurement, 3-aminoquinoline (**34**) has been investigated. The results obtained are reported in Scheme 5.17.



**Scheme 5.17.**Deuteration process for-3-aminoquinoline reaction.

Maybe as a consequence of the hindering effect, the deuterations on position 5 and 8 are considerably low. Since they show the same exchange percentage, it was logical to deduce that the amino group does not play any particular role, although Garman *et al.*<sup>179</sup> showed that in this substrate positions 2 and 4 have the same activation towards H/D exchange, D4, which ought to be more hindered, presents considerably higher deuterium incorporation than D2. Additionally, because Garman's experiments were set up in neutral solution, it was natural to presume that the acidic condition could have, in similar way described above, influenced the electronic activation. Consequently, D4 could be the favourite position because it is farther from the nitrogen atom than D2.

To finish this set, 2-methyl-4-aminoquinoline (**35**) was investigated (Scheme 5.18). Furthermore, due to its structural similarities with 4-amino-2-methylpyridine (**32**), it was thought to use the same acetylation method reported in Scheme 5.8.



**Scheme 5.18.**Deuteration process for 2-methyl-4-aminoquinoline reaction.

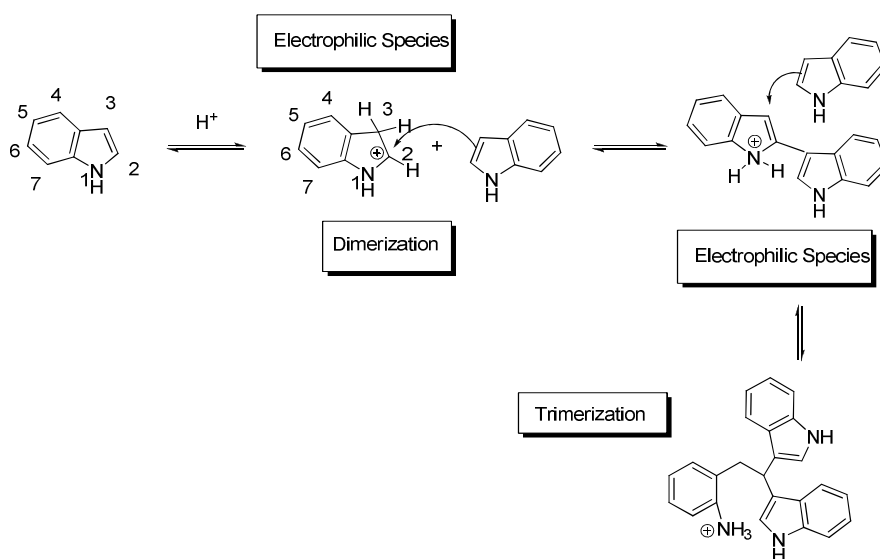
This experiment reflects the common conclusions already discussed above. Positions 5 and 8 are characterized by a consistent steric hindrance which could have caused the low level of deuteration developed. Moreover, the different amino group position here could have played a crucial role. As a consequence of all of this, the final exchange in D8 is remarkably low. In addition, in order to find an explanation for the result reported on methyl group, an appropriate bibliographic research was necessary. No relevant examples were found for nucleophilic and electrophilic attack on position 2, hence, it was suggested that carbon on position 2 presents an almost complete inactivation here. As already mentioned above, the exchange of alkyl substituent is directly connected with the activation of the corresponding aromatic position. Consequently, low deuterium incorporation was observed.

The last observation regards the exchange in position 3 on this molecule, which still preserves a very good reactivity, confirmed by the high level of H/D exchange, consistently with the results reported in Entry 2 (**27**) Table 5.1.

## 5.5 2-Methylindole

Nowadays, the importance of indoles in biological and pharmaceutical fields is well recognized. Many alkaloids and proteins incorporate an indole unit as a primary component<sup>180</sup> and recent drug syntheses, in particular indomethacin and its derivatives<sup>181</sup>, were based on it as a building block.<sup>182</sup> In light of this, it was thought useful, for this project, to validate our method for such a compound and its analogues.

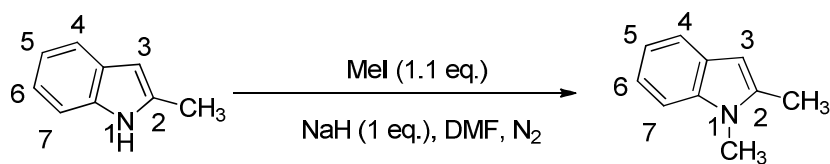
However, the literature indicates that indole is susceptible to di- and trimerization processes.<sup>183-184</sup> In fact, as reported by Quarton *et al.*<sup>185</sup> this event is quite common under strongly acidic conditions and involves mainly the 3 and 2 indole positions (Scheme 5.19).



**Scheme 5.19.** Dimerization and trimerization processes for indole.

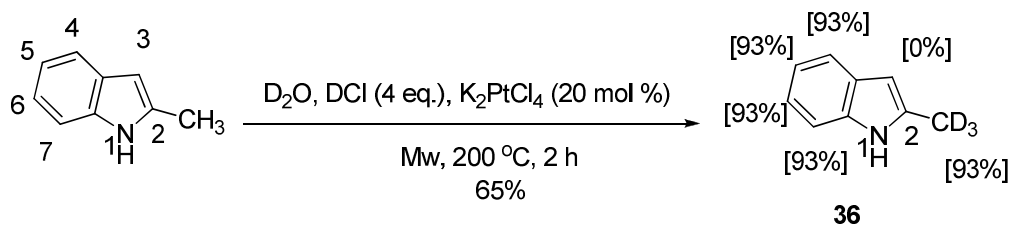
As a consequence of the problems arisen from such substrates, in particular connected with the solubility of the obtained salts, 2-methylindole was chosen for study. Of course, the presence of a 2-alkyl chain generates some steric hindrance, which causes problems for acetylating on the *N*-position. For this reason an alternative method was needed.

In view of these problems, it was decided to follow the procedure described by Seefeld *et al.*<sup>186</sup> where *N*-methylation was realized on the indole derivative. (Scheme 5.20), rather than acetylation.



**Scheme 5.20.** 1,2-Dimethyl-1H-indole preparation.

In order to define a valid method for measuring the H/D exchange, the corresponding deuteration process was set up on this substrate. (Scheme 5.21).



**Scheme 5.21.** Deuteration process for 2-methyl-1H-indole reaction.

The results shown above appear consistent, with a very high degree of exchange, at all positions except position 3. Here, an intense H/D exchange was expected due to the particular indole characteristic. Actually, in this case, no exchange was observed. It seems highly unlikely that this position was completely deactivated towards exchange, since many examples in the literature confirm that this position is still activated toward electrophilic attack. In particular, Rajesh *et al.*<sup>187</sup> were able to carry out nitration at position 3 of 2-methylindole. This experiment is confirmation that the 3-position is still activated in the presence of the 2-methyl group, so the deuterium exchange result needs an alternative explanation. One possible explanation involves steric hindrance. In other words, due to the presence of a methyl and a benzene group, Pt could have insufficient space for interacting with the hydrogen on position 3. However, steric hindrance in other compounds does not appear to have been severe enough to have caused such a major effect in other compounds, and this explanation is probably not very likely. An alternative explanation might be that this position is so highly activated that as a result of contact with water during the work up the exchange process is reversed. The strongly acidic conditions prevailing in the mixture may be sufficient to allow complete exchange at such a highly activated position even without the presence of the catalyst.

## 5.6 Conclusions

The experiments set up in this chapter have shown in general very good results both in terms of yields and for H/D exchange. Moreover, all the compounds chosen in this section show the right compromise between cost efficiency and chemical interest, so that they are relevant to and involved in several drug syntheses or biochemical processes. Here, the indolic context deserves great consideration, as the supposed activate 3 position has not been deuterated at

all. In conclusion, the results obtained could be crucial for the aims of this project, allowing us the opportunity to plan future labelled drug synthesis.

# Chapter 6

## Drugs for H/D exchange

### 6.1 Introduction

### 6.2 Harmane

### 6.3 Lidocaine

### 6.4 2-(Piperazin-1-yl)quinoline

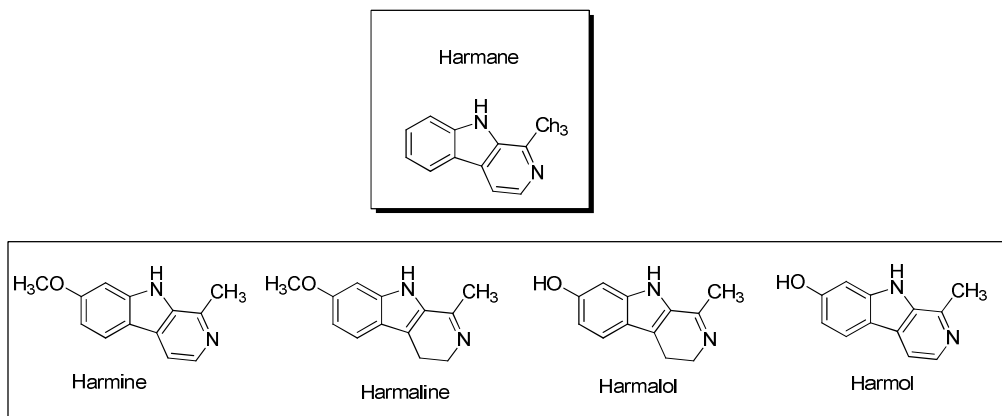
### 6.5 Conclusions

## 6.1 Introduction

In this final chapter, a drug investigation will be developed. The possibility of expanding our H/D exchange method to pharmaceutical compounds was one of the main priorities of this project. It was hoped that use of the procedure would allow fully deuterated drugs to be recovered and their metabolic studies in human and animal bodies developed (see Chapter 1). Consequently, such compounds were to be subjected to our H/D exchange process and in the next few pages we report the results.

## 6.2 Harmane

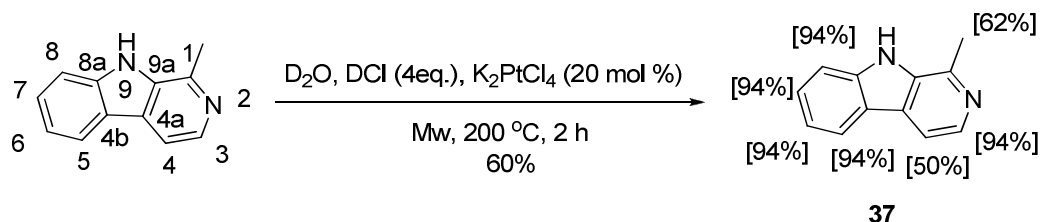
Chemically known as 1-methyl-9*H*-pyrido[3,4-*b*]indole, harmane is recognised as one of the five  $\beta$ -carboline alkaloids of the *Peganum Harmala* plant (Scheme 6.1). Harmane is also a common compound found in different foods; from beef to sardines.<sup>188</sup>



Scheme 6.1  $\beta$ -Carboline alkaloids of the plant *Peganum Harmala*.

It has long been acknowledged that all of these compounds showed pharmacological properties. In particular antitumor<sup>189</sup>, analgesic<sup>190</sup> and vasorelaxant<sup>191</sup> effects have been reported in the literature. However, their role as inverse agonists on the  $\gamma$ -aminobutyric acid (GABA) receptor has been much more defined. As investigated by Chapouthier *et al.*<sup>192</sup>, this specific interaction caused a diametrically opposite effect to that of the already well-known anxiolytic benzodiazepine.

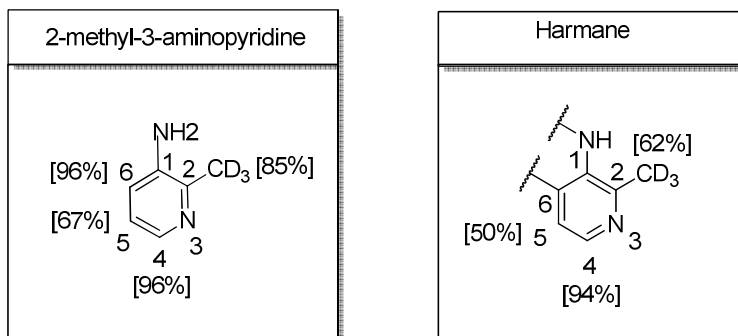
Due to cost and time restrictions, only harmane was investigated among these compounds. Despite this, in light of its structural similarities, it could be reasonable to consider the other  $\beta$ -carboline alkaloids suitable for experiments identical to that undertaken with harmane (Scheme 6.2, Table 6.1). As a consequence, this last aspect could be interpreted as a further confirmation of our H/D method efficiency even in this new branch of investigation.



**Scheme 6.2** Harmane results for H/D exchange.

Additionally, for calculating the H/D exchange, it was necessary to set up the reaction already described in the context of indole (Chapter 5, Scheme 5.13). In particular, as a consequence of methyl group hindrance, iodomethane was undoubtedly a better electrophilic species than acetyl chloride in attacking the *N*-atom.

The benzene moiety is more activated towards electrophilic attack than pyridine ring; so the deuterium incorporation recorded here is a further confirmation. Interestingly, position 3 seems strongly activated as well. Since Ponce *et al.*<sup>193</sup> were able to brominate  $\beta$ -carboline alkaloids at position 3, it was natural to deduce that this carbon could easily be involved in other electrophilic processes. Additionally, a comparison with the results obtained with 2-methyl-3-aminopyridine was helpful. (Scheme 6.3).



**Scheme 6.3** Comparison of H/D exchange between 2-methyl-3-aminopyridine and harmane.

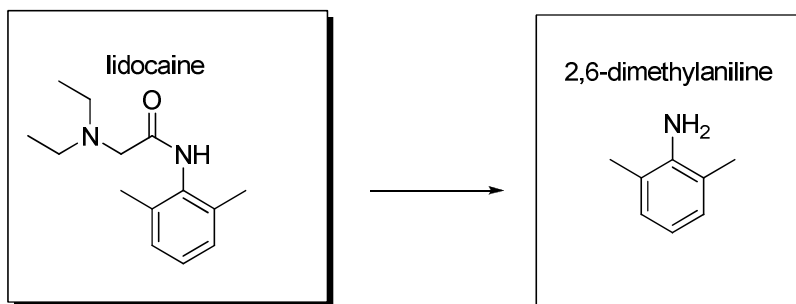
The scheme above highlights the relevant structural similarities between the two compounds. As a result of this, the corresponding positions could be similarly activated, consequently causing similar deuterium incorporation. Furthermore, the slight difference shown in C5 could be due to a more substantial steric effect in the Harmane case. Finally, the same conclusion could be proposed for deuterium exchange on the two corresponding methyl groups.

### 6.3 Lidocaine

Pharmacologically defined as an amine derivative of cocaine<sup>194</sup>, lidocaine has been known since 1948 as a local anaesthetic drug. Other different pharmacological properties were soon discovered, such as its strong antiarrhythmic effect in 1960, and later, in 1970, its anticonvulsant action.<sup>195</sup> Nowadays, although there are still many unresolved questions about its mechanism of action, it is assumed that a voltage-gated sodium-channel is involved in all of these processes. As recently reported by Meng *et al.*<sup>196</sup>, lidocaine is able to influence the cationic currents ruled by these channels, having clear consequences on the action potential in neuronal and muscle cells.

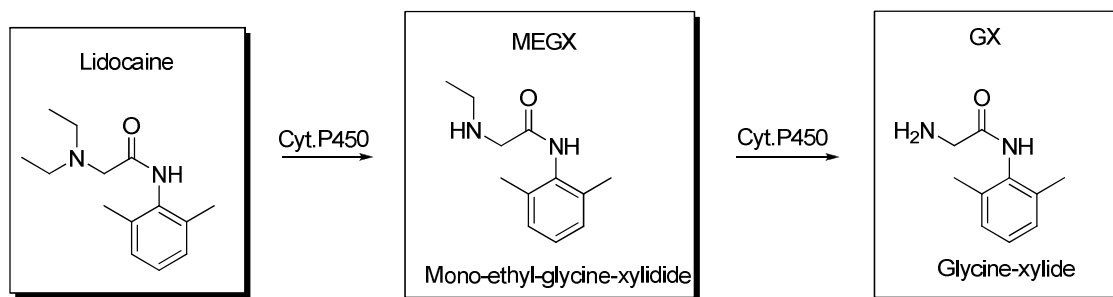
This drug presents a structure that contrasts with the standards set up for this project. Chemically indicated as 2-(diethylamino)-*N*-(2,6-dimethylphenyl)acetamide, lidocaine is an acetamide derivative (Scheme 6.4). Due to the acidic excess and the drastic temperature conditions, which could cause unwanted hydrolysis reactions, no molecules like these were explored until now. However, the particular metabolic pathway followed by this drug in the human body allowed the corresponding aniline as substrate for H/D exchange studies to be used (Scheme 6.4).





Scheme 6.4 Lidocaine and its corresponding aniline.

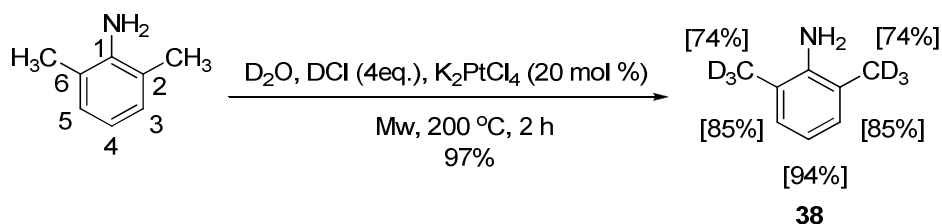
In particular, according to the experimental data, lidocaine is metabolized by cytochrome P450 in the liver to its most important metabolite, monoethylglycinexylidide (MEGX), which is considered truly responsible for pharmacological activities<sup>197</sup>; and then, through a N-desethylation process, to glycinexylidide (GX) (Scheme 6.5).



Scheme 6.5 Lidocaine metabolism and its metabolite.

As shown in the scheme above, the aromatic structure is preserved in both of the metabolites. As a consequence of this, the deuterated aniline, reported in Scheme 6.4, could allow a regular bioanalytical investigation along all metabolic pathways.

For this reason, 2,6-dimethylaniline was explored according to our usual method (Scheme 6.6).

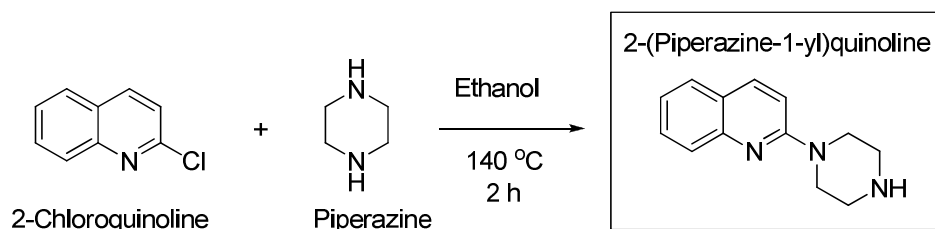


Scheme 6.6 2,6-Dimethylaniline results for H/D exchange.

The results reported above show that 2,6-dimethylaniline's aromatic ring could be almost completely deuterated. A direct comparison with the 2-methylaniline explored in Chapter 4 highlights a slight difference at the corresponding 3 and 5 positions (85% against 63% reported in Table 4.1). There, the steric hindrance caused by the methyl group restricts the level of deuteration at 3 positions. Here instead methyl groups in the para position seems to overtake steric effect. Due to symmetrical reason finally, the H/D exchange on both methyl groups is identical.

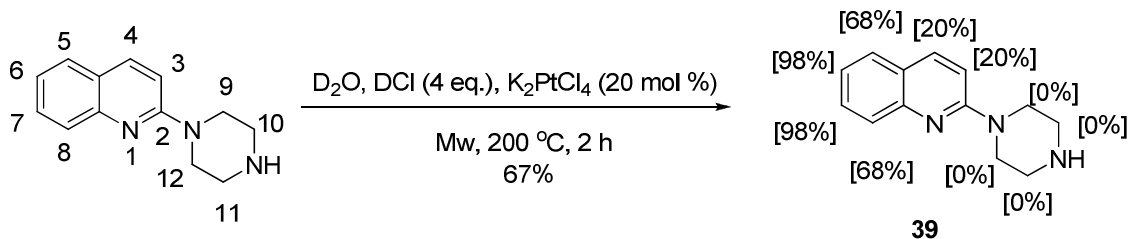
#### 6.4 2-(Piperazin-1-yl)quinoline

Following the results obtained from harmine, it was decided to carry on the investigation of other similar substrates. The bibliographic research focused its attention on studies by Asagarasu *et al.*<sup>198</sup> The aim of this research was to find valid compounds to treat and manage irritable bowel syndrome (IBS). Clinical investigations have proved that this disease is usually caused by two different serotonin receptors (subtype 1 (5-HT<sub>1A</sub>) and subtype 3 (5-HT<sub>3</sub>).<sup>199-200</sup> Pharmacological studies have also shown that using antagonist molecules for one (5-HT<sub>1A</sub>) and agonist molecules for the other (5-HT<sub>3</sub>) could be a fine therapeutic way for defeating the disease. In the light of pharmacophoric considerations, an accurate synthesis of different aryl-piperazine derivatives was developed. Due to time restrictions, only one of these substrates was chosen as a candidate to be explored in our H/D exchange process. Moreover, because of cost reasons, 2-(piperazin-1-yl)quinoline was easily synthesized following the procedure shown in Scheme 6.7.



**Scheme 6.7** Synthesis of 2-(piperazin-1-yl)quinoline.

Using this substrate, it was possible to record the results reported below (Scheme 6.8, Table 6.3).



Scheme 6.8 2-(piperazin-1-yl)quinoline H/D exchange.

A statement by Chambers *et al.*<sup>201</sup> relating to selective quinoline fluorination was relevant in order to explain the particular deuterium distribution recorded. Firstly, the acidic media is able to set up a real electrophilic aromatic substitution, which is also helpful for understanding the H/D exchange.<sup>202</sup> Following these considerations, and the order of reactivity towards electrophiles in quinoline established by Katritzky *et al.*<sup>203</sup>, the results above are quite controversial. Being more activated than 6 and 7, positions 5 and 8 should present the highest deuterium incorporation recorded. Maybe due to steric hindrance, attachment of the catalyst at the 5 and 8 positions is inhibited, forcing it to move to the 6 and 7 positions where, without any steric contribution, it can set up an intense H/D exchange. In addition, as previously mentioned, the heterocyclic ring shows lower reactivity towards electrophilic attack; consequently only 20% of exchange for both of its carbons was recorded. Lastly, the total absence of any deuteration process on the piperazine ring is a further confirmation of what was already stated in Chapter 4 (Scheme 4.22). In particular, as mentioned there, the presence of a nitrogen atom represents a sort of barrier for the catalyst, which is consequently not able to interact with the neighbouring carbons.

## 6.5 Conclusions

The results obtained here have shown that the H/D exchange process might be expanded successfully to different kinds of drugs. Additionally, the experiments confirm that this method could have certain relevance in the future. In particular, the results recorded above show the possibilities to obtain fully deuterated drugs, which could represent a fundamental aspect for improving bioanalytical investigations. Although only three biologically active compounds were used in the work reported in this chapter, their considerable final H/D exchange and yield could encourage further studies on a much larger range of compounds with a larger variety of pharmacological activities.

# Chapter 7

## Experimental

### 7.1 Experimental technique

### 7.2 General procedure

### 7.3 Specific experimental procedure

## 7.1 Experimental technique

Starting materials and solvents were purchased from commercial suppliers and they were all used without further purification. Column chromatography was carried out using Merck Kieselgel 60 H silica on Matrex Silica 60. Analytical thin layer chromatography was carried out using aluminium-backed plates coated with Merck Kieselgel 60 GF<sub>254</sub> that were visualised under UV light (at 254 and/or 360 nm) and/or potassium permanganate stain. Melting points (mp) were determined on Kofler hot stage apparatus and are uncorrected. Infra-red (IR) spectra were recorded in the range 4000-600 cm<sup>-1</sup> on a Perkin-Elmer series FTIR spectrometer using KBr plates for most of all samples and nujol mull. Nuclear magnetic resonance (NMR) spectra were recorded in CD<sub>3</sub>OD (unless otherwise stated) using an Avance Bruker DPX 400 instrument (400 MHz) or an Avance Bruker DPX 500 (500 MHz), with <sup>13</sup>C recorded at 100 MHz and 125 MHz respectively and reported in ppm; *J* values were recorded in Hz and multiplicities were expressed by the usual convention (s=singlet (seen as singular line in the proton decoupled spectra), d= doublet, dd= doublet of doublets, dt= doublet of triplets, t= triplet, q= quartet, p= pentlet, sept= septlet, m= multiplet). Low resolution mass spectrometric data were determined using a Fisons VG Platform II instrument, High resolution mass spectrometric data were obtained on a Waters Q-TOF micromass spectrometer. *Under vacuum* refers to evaporation at reduced pressure using a rotary evaporator and diaphragm pump, followed by removal of trace volatiles using a vacuum (oil) pump. Microwave experiments were developed in a CEM Discover microwave

synthesizer at the temperature and initial power stated, with modulation of power to maintain reaction temperature as monitored by the in-built IR sensor.

## **7.2 General experimental procedures**

### **7.2.1 General procedures A: Deuteration using $K_2PtCl_4$**

Different substituted derivatives of aniline (1 eq.) in  $D_2O$  (1 mL) were added to a stirred solution of  $K_2PtCl_4$  (20 mol %) and DCl (35%) (4 eq.) in  $D_2O$  (3 mL). The mixture was irradiated at 200 °C (initial power 300 W, pressure 150 psi) in a sealed vessel for 2 hours. Then the solution was neutralized by the addition of NaOH (1M, 10 mL) and extracted with DCM. The organic extracts were combined, dried over  $MgSO_4$ , filtered and evaporated under vacuum to afford the product.

### **7.2.2 General procedures B: Deuterium substituted anilines acetylation**

Acetyl chloride (1.1 eq.) was added to a stirred, cooled (ice bath) solution of deuterated amino compound (1 eq.) in DCM (5 mL), under a  $N_2$  atmosphere. The mixture was warmed to room temperature (R.T) and stirred for 30 min. A further portion of acetyl chloride (1.1 eq.) was added and the solution stirred for another 30 min. Hydrochloric acid (1M, 10 mL) was added and the organic layer was washed with hydrochloric acid (1M), dried over  $MgSO_4$ , and evaporated under vacuum to afford the product.

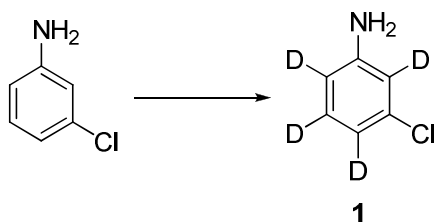
### **7.2.3 General procedures C: Alternative acetylation of deuterated aminopyridines**

The aminopyridine (1 eq.) was dissolved in acetone (15 mL), and  $K_2CO_3$  (5 eq.) was added, followed by the dropwise addition of acetyl chloride (3 eq.) in acetone (5 mL). The reaction mixture was stirred for 3 h. The solution was quenched by water, then the solvent was

eliminated *in vacuo* and then the crude product was extracted with DCM. The organic phase was dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and evaporated *in vacuo* to give the product which was dried at high vacuum

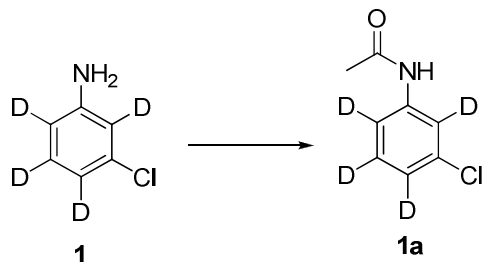
### 7.3 Specific experimental procedures

#### 2,4,5,6-Tetradeutero-3-chloroaniline (1)<sup>204</sup>

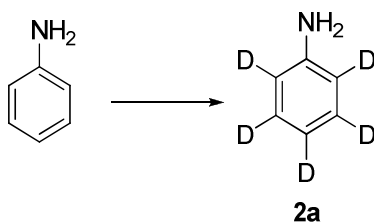


A solution of 3-chloroaniline (300 mg, 250  $\mu$ L, 2.3 mmol, 1 eq.) was added to a stirred solution of K<sub>2</sub>PtCl<sub>4</sub> (190 mg, 0.46 mmol, 20 mol %) and DCl (35%) (750  $\mu$ L, 9.2 mmol, 4 eq.) in D<sub>2</sub>O (3 mL) and reacted according to the general procedure A. To the solution thioglycolic acid (127  $\mu$ L, 1.84 mmol, 4 eq. referred to the catalyst amount) was added and the mixture was stirred for 30 min, the rest of the the work up followed the general procedure A to give the *title compound* as a brown oil (291 mg, 96%): Found M<sup>+</sup>, 133.0401. C<sub>6</sub>H<sub>2</sub>D<sub>4</sub>ClN [M<sup>+</sup>] requires 133.0401;  $\nu_{\max}$  (KBr) 3437, 3240, 3365, 3224, 1619, 1574, 1432, 1403, 1376, 1297, and 925;  $\delta_{\text{H}}$  (500 MHz; CD<sub>3</sub>OD) <sup>a</sup>6.88 (0.06H, s), <sup>a</sup>6.57(0.06H, s), <sup>a</sup>6.49(0.06H, s), <sup>a</sup>6.45(0.06H, s),  $\delta_{\text{C}}$ (150 MHz, CD<sub>3</sub>OD) 150.4 (s, C-NH<sub>2</sub>), 135.6 (s, C-Cl), 130.8 (t, C-D,  $J_{\text{C-D}}$  24), 117.9 (t, C-D,  $J_{\text{C-D}}$  25), 115.6 (t, C-D,  $J_{\text{C-D}}$  24), 114.1 (t, C-D,  $J_{\text{C-D}}$  24);  $m/z$  (EI) 133 (50%).

<sup>a</sup>Signals arise from the presence of isotopologues.

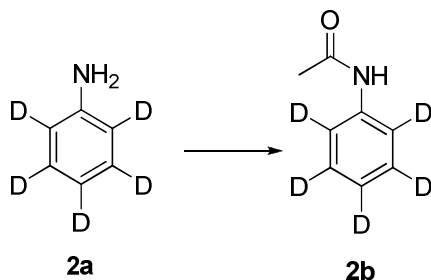
**N-(2,4,5,6-Tetradeutero-3-chlorophenyl)acetamide (1a)**

Acetyl chloride (106.2 $\mu$ L, 1.49 mmol, 1.1 eq.) was added to a stirred solution of *d*<sub>4</sub>-3-chloroaniline (**1**) (178 mg, 1.36 mmol, 1 eq.) in DCM (5 mL) and NEt<sub>3</sub> on ice (419.7 $\mu$ L, 2.9 mmol, 2.2 eq.) under N<sub>2</sub> atmosphere and reacted according to the general procedure **B**. Purification by chromatography column, eluting with petroleum ether/ ethyl acetate (2/1) gave the *title compound* as a brown powder (150 mg, 63 %): Found M<sup>+</sup>, 173.0540. C<sub>8</sub>H<sub>4</sub>D<sub>4</sub>ClNO [M<sup>+</sup>] requires 173.0545; mp 59-62 °C;  $\nu_{\max}$  (KBr) 3293, 3248, 3165, 3081, 2926, 2384, 1669, 1568, 1518, 1391; 1342, and 1303,  $\delta_{\text{H}}$ (400 MHz; CD<sub>3</sub>OD) 7.58 (0.06H, s), 7.25 (0.06H, s), 7.12 (0.06H, s), 6.93 (0.06H, s), 2.00(3H, s);  $\delta_{\text{C}}$  (150 MHz, CD<sub>3</sub>OD) 171.8 (s, C=O), 141.2 (s, C-NH), 135.3 (s, C-Cl), 130.5(t, C-D, *J*<sub>C-D</sub> 25), 124.5 (t, C-D, *J*<sub>C-D</sub> 25), 120.6 (t, C-D, *J*<sub>C-D</sub> 26), 118.6 (t, C-D, *J*<sub>C-D</sub> 26), 23.9 (s, CH<sub>3</sub>); *m/z* (EI) 173 (M<sup>+</sup>, 20%).

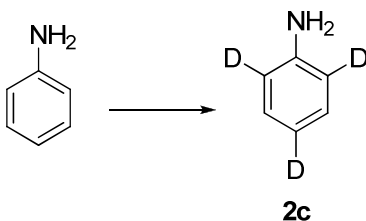
**2,3,4,5,6-Pentadeuteroaniline (2a)**<sup>205</sup>

A solution of aniline (300 mg, 294 $\mu$ L, 0.31 mmol, 1 eq.) in D<sub>2</sub>O (1 mL) was added to a stirred solution of K<sub>2</sub>PtCl<sub>4</sub> (25 mg, 0.64 mmol, 20 mol %) and DCl (35%) (1.02mL, 1.2 mmol, 4 eq.) in D<sub>2</sub>O (3 mL) and reacted according to the general procedures **A** to give the *title compound* as a brown oil (137 mg, 44%): Found M<sup>+</sup>, 98.0892. C<sub>6</sub>H<sub>2</sub>D<sub>5</sub>N [M<sup>+</sup>] requires 98.0891;  $\nu_{\max}$ (KBr) 3357, 3222, 3051, 2513, 1619, 1581, 1474, 1451 and 1261;  $\delta_{\text{H}}$  (400 MHz; CD<sub>3</sub>OD) <sup>a</sup>7.10(0.18H, s), <sup>a</sup>6.72 (0.27H, s);  $\delta_{\text{C}}$ (100 MHz, CD<sub>3</sub>OD) 147.2 (s, C-NH<sub>2</sub>), 128.4(t, C-D, *J*<sub>C-D</sub> 24), 118.4 (t, C-D, *J*<sub>C-D</sub> 25), 115.4 (t, C-D, *J*<sub>C-D</sub> 27); *m/z* (EI) 98 (M<sup>+</sup>, 100%).

<sup>a</sup>Signals arise from the presence of isotopologues.

**N-(2,3,4,5,6-Pentadeuterophenyl)acetamide (2b)**

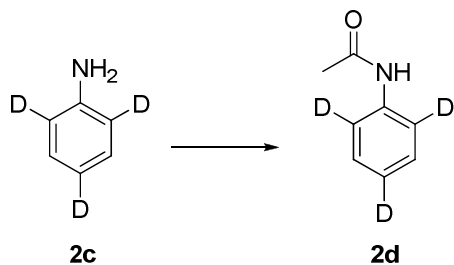
Acetyl chloride (125  $\mu\text{L}$ , 1.76 mmol, 1.1 eq.) was added to a stirred solution of *d*<sub>5</sub>-aniline (**2a**) (161 mg, 1.6 mmol, 1 eq.) in DCM (5 mL) and NEt<sub>3</sub> on ice (493.8  $\mu\text{L}$ , 3.52 mmol, 2.2 eq.) under N<sub>2</sub> atmosphere and reacted according to the general procedure **B**. Purification by chromatography column, eluting with petroleum ether/ethyl acetate (3/1) gave the *title compound* as a brown powder (97 mg, 43%): Found M<sup>+</sup>, 140.1002. C<sub>8</sub>H<sub>4</sub>D<sub>5</sub>NO [M<sup>+</sup>] requires 140.0998, mp 108-112°C;  $\nu_{\text{max}}$  (KBr) 3293, 3249, 3164, 3090, 2926, 2851, 2434, 1641, 1591; 1469, and 1382;  $\delta_{\text{H}}$ (400 MHz; CD<sub>3</sub>OD) 7.49 (0.18H, s), 7.15 (0.18H, s), 6.94 (0.09H, s), 1.98 (3H, s);  $\delta_{\text{C}}$  (100 MHz, CD<sub>3</sub>OD) 172.1 (s, C=O), 140.1 (s, C-NH), 129.7 (t, C-D,  $J_{\text{C-D}}$  25), 125.1 (t, C-D,  $J_{\text{C-D}}$  25), 121.2 (t, C-D,  $J_{\text{C-D}}$  25), 24.3 (s, CH<sub>3</sub>); *m/z* (EI) 140 (M<sup>+</sup>, 30%).

**2,4,6-Trideuteroaniline (2c)**<sup>204, 206</sup>

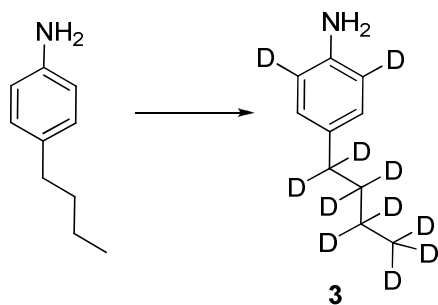
A solution of aniline (300 mg, 294  $\mu\text{L}$ , 0.31 mmol, 1 eq.) in D<sub>2</sub>O (1 mL) was added to a stirred solution of K<sub>2</sub>PtCl<sub>4</sub> (25 mg, 0.062 mmol, 2 mol %) and DCl (35%) (102  $\mu\text{L}$ , 1.24 mmol, 0.38 eq.) in D<sub>2</sub>O (3 mL) and reacted according to the general procedure **A** to give the *title compound* as brown oil (245 mg, 80%); Found M<sup>+</sup>, 96.0764. C<sub>6</sub>H<sub>4</sub>D<sub>3</sub>N [M<sup>+</sup>] requires 96.0767;  $\nu_{\text{max}}$ (KBr) 3357, 3222, 3051, 2513, 1619, 1581, 1474, 1451 and 1261;  $\delta_{\text{H}}$  (400 MHz; CD<sub>3</sub>OD) <sup>a</sup>7.10(1.95H, s), <sup>a</sup>6.72 (0.27H, m);  $\delta_{\text{C}}$ (100 MHz, CD<sub>3</sub>OD) 147.2 (s, C-NH<sub>2</sub>), 128.4(s, C-H), 118.4 (t, C-D,  $J_{\text{C-D}}$  25), 115.4 (t, C-D,  $J_{\text{C-D}}$  26); *m/z* (EI) 96 (M<sup>+</sup>, 100%).

<sup>a</sup>Signals arise from the presence of isotopologues.



**N-(2,4,6-Trideuterophenyl)acetamide (2d)**

Acetyl chloride (79.8  $\mu\text{L}$ , 1.12 mmol, 1.1 eq.) was added to a stirred solution of *d*<sub>3</sub>-aniline (2c) (100 mg, 1.02 mmol, 1 eq.) in DCM (5 mL) and NEt<sub>3</sub> on ice (314.7  $\mu\text{L}$ , 2.24 mmol, 2.2 eq.) under N<sub>2</sub> atmosphere and reacted according to the general procedure B. Purification by chromatography column, eluting with petroleum ether/ethyl acetate (3/1) gave the *title compound* as brown powder (93 mg, 66 %): Found M<sup>+</sup>, 138.0868. C<sub>8</sub>H<sub>6</sub>D<sub>3</sub>NO[M<sup>+</sup>] requires 138.0872; mp 103-108 °C;  $\nu_{\text{max}}$  (KBr) 3293, 3254, 3176, 3046, 2913, 2420, 1644; 1858, 1644, 1578, 1527, 1469 and 1382,  $\delta_{\text{H}}$  (400 MHz; CD<sub>3</sub>OD, 50°C) <sup>a</sup>7.49 (0.15H, d, *J*<sub>H-H</sub> 8.54), <sup>a</sup>7.16 (1.95H, s), <sup>a</sup>6.94 (0.09H, d, *J*<sub>H-H</sub> 7.41 ), 1.98 (3H, s);  $\delta_{\text{C}}$  (100 MHz, CD<sub>3</sub>OD) 172.1 (s, C=O), 140.1 (s, C-NH), 129.7 (s, C-H), 125.1 (t, C-D, *J*<sub>C-D</sub> 25), 121.2 (t, C-D, *J*<sub>C-D</sub> 25), 24.3 (s, CH<sub>3</sub>); *m/z* (EI) 138 (M<sup>+</sup>, 95%).

**2,6-Dideutero-4-(1,1,2,2,3,3,4,4,4-nonadeuterobutyl)aniline (3)**

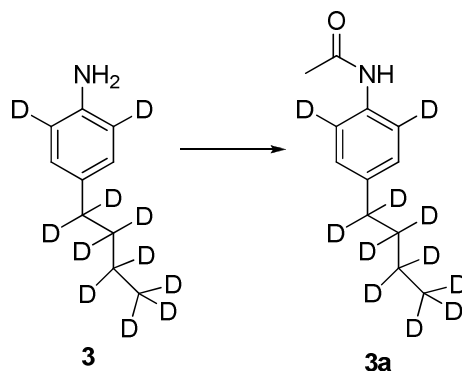
A solution of 4-*n*-butylaniline (300 mg, 317  $\mu\text{L}$ , 2.01 mmol, 1 eq.) was added to a stirred solution of K<sub>2</sub>PtCl<sub>4</sub> (166 mg, 0.40 mmol, 20 mol %) and DCI (35%) (662  $\mu\text{L}$ , 8.04 mmol, 4 eq.) in D<sub>2</sub>O (3 mL) and reacted according to the general procedure A. To the solution thioglycolic acid (120  $\mu\text{L}$ , 1.6 mmol, 4 eq. referred to the catalyst amount) was added and the mixture was stirred for 30 min, the rest of the the work up follows the general procedure A to give the *title compound* as a brown oil (160 mg, 50%): Found M<sup>+</sup>, 160.1895. C<sub>10</sub>H<sub>4</sub>D<sub>11</sub>N [M<sup>+</sup>] requires 160.1895;  $\nu_{\text{max}}$  (KBr) 3444, 3349, 3214, 3019, 2901, 1619, 1499,

1462, 1442, 1300 and 1256;  $\delta_{\text{H}}$  (400 MHz; CD<sub>3</sub>OD) <sup>a</sup>6.91(1.13H, s), <sup>a</sup>6.66(0.22H, m), <sup>a</sup>2.44(0.26H, s), <sup>a</sup>1.47 (0.88H, m), <sup>a</sup>1.26 (0.78H, m), <sup>a</sup>0.86 (0.54H, m);  $\delta_{\text{C}}$ (100 MHz, CD<sub>3</sub>OD) 145.9(s, C-NH<sub>2</sub>), 134.1(s, C-C), 129.9 (s, C-H), 117.0(m, C-D), 35.8(m, CD<sub>2</sub>) , 35.2 (m, CD<sub>2</sub>), 23.3 (m, CD<sub>2</sub>), 14.3 (m, CD<sub>3</sub>);  $m/z$  (EI) 160 (M<sup>+</sup>, 80%).

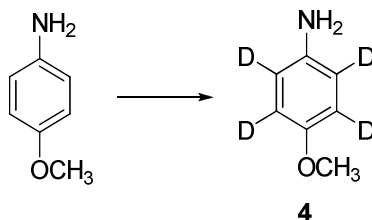
<sup>a</sup>Signals arise from the presence of isotopologues.

### **N-[2,6-Dideutero-4-(1,1,2,2,3,3,4,4,4-nonadeuterobutyl)phenyl]acetamide**

#### **(3a)**

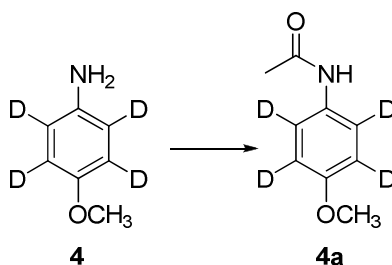


Acetyl chloride (63.4 $\mu$ L, 0.88 mmol, 1.1 eq.) was added to a stirred solution of *d*<sub>11</sub>-4-*n*-butylaniline (**3**) (130 mg, 0.89 mmol, 1 eq.) in DCM (10 mL) and NEt<sub>3</sub> on ice (246.9 $\mu$ L, 1.77 mmol, 2.2 eq.) under N<sub>2</sub> atmosphere and reacted according to the general procedure **B** to give the *title compound* as a colourless powder (59 mg, 36%): Found M<sup>+</sup>, 202.1997. C<sub>12</sub>H<sub>6</sub>D<sub>11</sub>NO [M<sup>+</sup>] requires 202.2001; mp 101-105 °C;  $\nu_{\text{max}}$  (KBr) 3267, 3124, 2916, 2930, 2854, 1660, 1514, 1451, 1371 and 1291;  $\delta_{\text{H}}$  (400 MHz; CD<sub>3</sub>OD) 7.43 (0.22H, m), 7.11 (1.13H, s), 2.53 (0.26H, m), 2.10 (3H, s), 1.53 (0.88H, s), 1.29 (0.78H, m), 0.88 (0.54H, m);  $\delta_{\text{C}}$  (150 MHz, CD<sub>3</sub>OD) 171.5 (s, C=O), 139.9 (s, C-NH), 137.3(s, C-C), 129.5(s, C-H), 121.3(m, C-D), 36.0 (m, CD<sub>2</sub>), 34.9 (m, CD<sub>2</sub>), 23.7 (s, CH<sub>3</sub>) 23.3 (m, CD<sub>2</sub>), 14.2 (m, CD<sub>3</sub>);  $m/z$  (EI) 202 (M<sup>+</sup>, 85%).

**2,3,5,6 Tetradeutero-4-methoxyaniline (4)**

4-Methoxyaniline (300 mg, 2.43 mmol, 1 eq.) was added to a stirred solution of  $K_2PtCl_4$  (199 mg, 0.48 mmol, 20 mol %) and DCl (35%) (802  $\mu$ L, 9.72 mmol, 4 eq.) in  $D_2O$  (3 mL) and reacted according to the general procedure **A** to give the *title compound* as a black powder (34 mg, 11 %): Found  $M^{++}$ , 127.0931.  $C_7H_5D_4NO$  [ $M^{++}$ ] requires 127.0935, mp 56-60°C;  $\nu_{max}$ (neat) 2961, 2922, 2850, 2255, 2059, 1574, 1552, 1455 and 1412;  $\delta_H$  (400 MHz;  $CD_3OD$ )  $^a$ 6.63 (0.09H, s),  $^a$ 6.52 (0.07H, s),  $^a$ 3.61 (2.93H, s);  $\delta_C$  (100 MHz,  $CD_3OD$ ) 154.9 (s, C-NH<sub>2</sub>), 141.9 (s, C-O), 118.6 (t, C-D,  $J_{C-D}$  25), 116.1 (t, C-D,  $J_{C-D}$  24), 56.5 (s, O-CH<sub>3</sub>);  $m/z$  (EI) 127 ( $M^{++}$ , 90%).

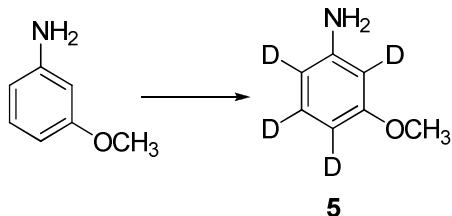
<sup>a</sup>Signals arise from the presence of isotopologues.

**N-[2,3,5,6-Tetradeutero-4-methoxyphenyl]acetamide (4a)**

Acetyl chloride (14.2  $\mu$ L, 0.22 mmol, 1.1 eq.) was added to a stirred solution of *d*<sub>4</sub>-4-anisidine (**4**) (34 mg, 0.2 mmol, 1 eq.) in DCM (5 mL) and  $NEt_3$  on ice (61.72  $\mu$ L, 0.44 mmol, 2.2 eq.) under  $N_2$  atmosphere and reacted according to the general procedure **B**. Purification by chromatography column, eluting with petroleum ether/ethyl acetate (2/1) gave the *title compound* as a colourless powder (11 mg, 32%): Found  $M^{++}$ , 169.1040.  $C_9H_7D_4NO_2$  [ $M^{++}$ ] requires 169.1041, mp 137-139 °C;  $\nu_{max}$  (neat) 3272, 3165, 3097, 2962, 2383, 1762, 1654, 1594, 1513, 1414 and 1261;  $\delta_H$  (400 MHz;  $CD_3OD$ ) 7.38 (0.07H, s), 6.76 (0.09H, s), 3.61 (2.93H, s), 1.99 (3.00 H, s);  $\delta_C$  (150 MHz,  $CD_3OD$ ) 171.3 (s, C=O), 157.8 (s, C-NH), 132.7 (s,

C-O), 122.9 (t, C-D,  $J_{C-D}$  25), 125.6 (t, C-D,  $J_{C-D}$  24), 55.9 (s, O-CH<sub>3</sub>), 23.6 (s, CH<sub>3</sub>);  $m/z$  (EI) 169 ( $M^{+}$ , 60%).

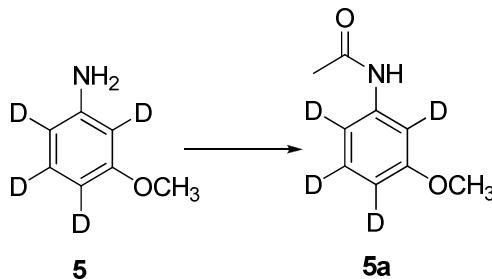
### **2,4,5,6-Tetradeutero-3-methoxyaniline (5)**



A solution of 3-methoxyaniline (300 mg, 273  $\mu$ L, 2.4 mmol, 1 eq.) was added to a stirred solution of K<sub>2</sub>PtCl<sub>4</sub> (199 mg, 0.48 mmol, 20 mol %) and DCl (35%) (790  $\mu$ L, 9.6 mmol, 4 eq.) in D<sub>2</sub>O (3 mL) and reacted according to the general procedure A. To the solution, thioglycolic acid (133  $\mu$ L, 1.92 mmol, 4 eq. referred to the catalyst amount) was added and left under stirring for half an hour; the rest of the work up followed the general procedure A to give the *title compound* as a brown oil (135 mg, 44%): Found  $M^{+}$  127.0935. C<sub>7</sub>H<sub>5</sub>D<sub>4</sub>NO [ $M^{+}$ ] requires 127.0936;  $\nu_{\max}$  (neat) 3367, 2941, 2834, 2053, 1859, 1589, 1463, 1300 and 1173;  $\delta_{\text{H}}$  (400 MHz; CD<sub>3</sub>OD) <sup>a</sup>6.97(0.19H, s), <sup>a</sup>6.35 (0.12H, s), <sup>a</sup>6.22 (0.26H, s), 3.67 (2.79H, s);  $\delta_{\text{C}}$  (100 MHz, CD<sub>3</sub>OD) 159.5 (s, C-O), 150.2 (s, C-NH<sub>2</sub>), 131.4(m, C-D), 109.0 (m, C-D), 107.0 (m, C-D), 104.3 (m, C-D), 55.9 (s, O-CH<sub>3</sub>);  $m/z$  (EI) 112 ( $M^{+}$ - CH<sub>3</sub>, 100%).

<sup>a</sup>Signals arise from the presence of isotopologues.

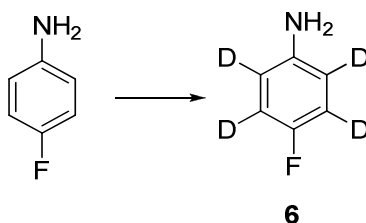
### **N-[2,4,5,6-Tetradeutero-3-methoxyphenyl]acetamide (5a)**



Acetyl chloride (79.3  $\mu$ L, 1.12 mmol, 1.1 eq.) was added to a stirred solution of *d*<sub>4</sub>-3-methoxyaniline (**5**) (125 mg, 1.01 mmol, 1 eq.) in DCM (5 mL) and NEt<sub>3</sub> on ice (311  $\mu$ L, 2.22 mmol, 2.2 eq.) under N<sub>2</sub> atmosphere and reacted according to the general procedure B.

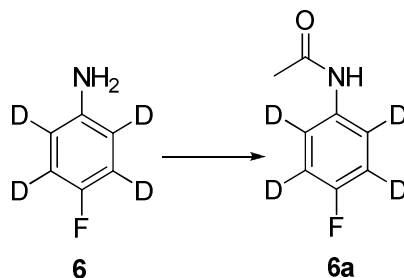
Purification by chromatography column, eluting with petroleum ether/ ethyl acetate (2/1) gave the *title compound* as a brown oil (28 mg, 21 %): Found  $M^{+}$ , 169.1038.  $C_9H_7D_4NO_2$  [ $M^{+}$ ] requires 169.1041;  $\nu_{max}$  (KBr) 3302, 3251, 3165, 3103, 2932, 1661, 1575, 1520, 1377, 1269, and 1117;  $\delta_H$  (400 MHz;  $CD_3OD$ ): 7.25 (0.07H, s), 7.17 (0.19H, s), 7.02 (0.19H, s), 6.64 (0.19, s), 3.75 (2.79H, s), 2.09 (3H, s);  $\delta_C$  (150 MHz,  $CD_3OD$ ) 170.2 (s, C=O), 160.04(s, C-NH), 139.5 (s, C-O), 128.8 (m, C-D), 111.7 (m, C-D), 110.01 (m, C-D), 109.1 (m, C-D), 54.1 (s, O-CH<sub>3</sub>), 22.4 (s, CH<sub>3</sub>);  $m/z$  (EI) 169 ( $M^{+}$ , 55%).

### **2,3,5,6-Tetradeutero-4-fluoroaniline (6)**

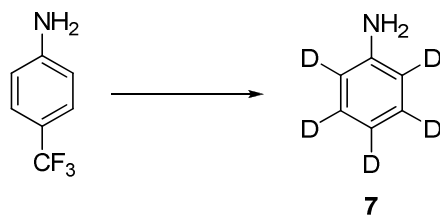


4-Fluoroaniline (300 mg, 2.7 mmol, 1 eq.) in  $D_2O$  (1 mL) was added to a stirred solution of  $K_2PtCl_4$  (224 mg, 0.54 mmol, 20 mol %) and DCl (35%) (890  $\mu$ L, 10.8 mmol, 4 eq.) in  $D_2O$  (3 mL) and reaction was carried out according to the general procedure A to give the *title compound* as a yellow oil (170 mg, 55%): Found  $M^{+}$ , 115.0731.  $C_6H_2D_4NF$  [ $M^{+}$ ] requires 115.0735;  $\nu_{max}$ (neat) 3436, 3361, 3325, 2963, 2922, 2856, 1623, 1446, 1117 and 803;  $\delta_H$  (400 MHz; DMSO) <sup>a</sup>6.83 (0.23H, m), <sup>a</sup> 6.52 (0.07H, m), <sup>a</sup>2.55 (2H, s);  $\delta_C$  (100 MHz, DMSO) 154.6 (d, C-F,  $J_{C-F}$  230), 145.3 (s, C-NH<sub>2</sub>), 115.4 (m, C-D), 114.9 (m, C-D);  $m/z$  (EI) 115 ( $M^{+}$ , 100%).

<sup>a</sup>Signals arise from the presence of isotopologues.

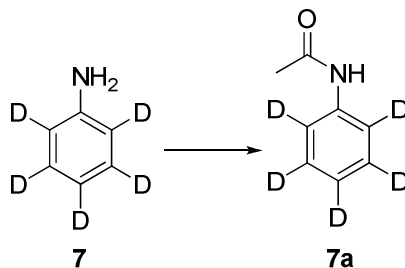
**N-[2,3,5,6-Tetradeutero-4-fluorophenyl]acetamide (6a)**

Acetyl chloride (84.6  $\mu\text{L}$ , 1.19 mmol, 1.1 eq.) was added to a cooled (ice bath) stirred solution of *d*<sub>4</sub>-4-fluoroaniline (**6**) (170 mg, 1.11 mmol, 1 eq.) and  $\text{NEt}_3$  on ice (333  $\mu\text{L}$ , 2.38 mmol, 2.2 eq.) in DCM (5 mL) under  $\text{N}_2$  atmosphere and reacted according to the general procedures **B** to give the *title compound* as a brown powder (154 mg, 90%): Found  $\text{M}^+$ , 157.0839.  $\text{C}_8\text{H}_4\text{D}_4\text{NOF}$  [ $\text{M}^+$ ] requires 157.0841, mp 131-136  $^\circ\text{C}$ ;  $\nu_{\text{max}}$  (neat) 2963, 1660, 1412, 1260, 1023, 799;  $\delta_{\text{H}}$  (400 MHz;  $\text{CD}_3\text{OD}$ , 50 $^\circ\text{C}$ ) 7.40 (0.07H, m), 6.90 (0.23H, m) 1.95 (3H, s);  $\delta_{\text{C}}$  (100 MHz,  $\text{CD}_3\text{OD}$ ) 170.1 (s, C=O), 159.1 (d, C-F,  $J_{\text{C-F}}$  230), 134.6 (s, C-NH), 121.1 (m, C-D), 114.7 (m, C-D), 24.09 (s,  $\text{CH}_3$ );  $m/z$  (EI) 157 ( $\text{M}^+$ , 90%).

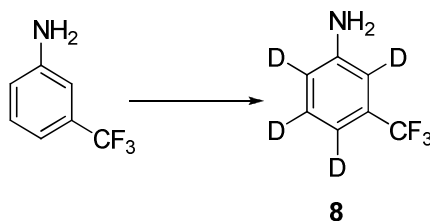
**2,3,4,5,6-Pentadeuteroaniline (7)**<sup>205</sup>

A solution of 4-(trifluoromethyl)aniline (300 mg, 231  $\mu\text{L}$ , 1.8 mmol, 1 eq.) in  $\text{D}_2\text{O}$  (1 mL) was added to a stirred solution of  $\text{K}_2\text{PtCl}_4$  (154 mg, 0.37 mmol, 20 mol %) and DCl (35%) (612  $\mu\text{L}$ , 7.44 mmol, 4 eq.) in  $\text{D}_2\text{O}$  (3 mL) and reacted according to the general procedure **A** to give the *title compound* as an orange oil (126 mg, 70%): Found  $\text{M}^+$ , 98.0892.  $\text{C}_6\text{H}_2\text{D}_5\text{N}$  [ $\text{M}^+$ ] requires 98.0892;  $\nu_{\text{max}}$  (neat) 3493, 3399, 3227, 2962, 2962, 2924, 2850, 1627, 1328 and 1262;  $\delta_{\text{H}}$  (400 MHz;  $\text{CD}_3\text{OD}$ ) <sup>a</sup>7.10(0.16H, s), <sup>a</sup>6.72 (0.08H, s), <sup>a</sup>6.51 (0.04H, s);  $\delta_{\text{C}}$  (100 MHz,  $\text{CD}_3\text{OD}$ ) 148.7 (s, C-NH<sub>2</sub>), 128.4(t, C-D,  $J_{\text{C-D}}$  24), 117.5 (t, C-D,  $J_{\text{C-D}}$  25), 114.9 (t, C-D,  $J_{\text{C-D}}$  25);  $m/z$  (EI) 98 ( $\text{M}^+$ , 100%).

<sup>a</sup>Signals arise from the presence of isotopologues.

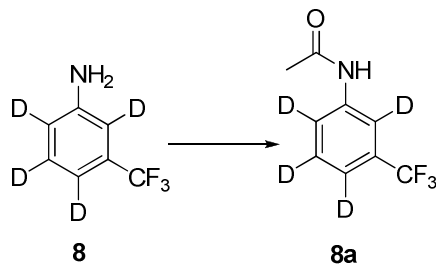
**N-[2,3,4,5,6-Pentadeuterophenyl]acetamide (7a)**

Acetyl chloride (57.9  $\mu\text{L}$ , 0.77 mmol, 1.1 eq.) was added to a stirred solution of  $d_5$ -aniline (**7**) (120 mg, 0.07 mmol, 1 eq.) in DCM (5 mL) and  $\text{NEt}_3$  on ice (225  $\mu\text{L}$ , 1.54 mmol, 2.2 eq.) under  $\text{N}_2$  atmosphere and reacted according to the general procedure **B** to give the *title compound* as a brown powder (128 mg, 84%): Found  $\text{M}^{+\cdot}$ , 140.0998.  $\text{C}_8\text{H}_4\text{D}_5\text{NO}$  [ $\text{M}^{+\cdot}$ ] requires 140.0998, mp 108-111  $^\circ\text{C}$ ;  $\nu_{\text{max}}$  (neat) 3293, 2963, 2420, 2348, 1640; 1565, 1473, 1328 and 1261,  $\delta_{\text{H}}$ (400 MHz;  $\text{CD}_3\text{OD}$ , 50 $^\circ\text{C}$ ) 7.49 (0.07H, s), 7.16 (0.08H, s), 6.93 (0.04H, s), 1.96 (3H, s);  $\delta_{\text{C}}$  (100 MHz,  $\text{CD}_3\text{OD}$ ) 172.07 (s, C=O), 140.1 (s, C-NH), 129.7 (t, C-D,  $J_{\text{C-D}}$  24), 125.1 (t, C-D,  $J_{\text{C-D}}$  25), 121.2 (t, C-D,  $J_{\text{C-D}}$  25), 24.3 (s,  $\text{CH}_3$ );  $m/z$  (EI) 140 ( $\text{M}^{+\cdot}$ , 80%).

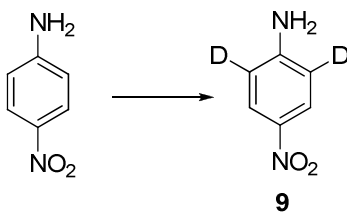
**2,4,5,6-Tetradeutero-3-(trifluoromethyl)aniline (8)**

A solution of 3-(trifluoromethyl)aniline (300 mg, 231  $\mu\text{L}$ , 1.86 mmol, 1 eq.) in  $\text{D}_2\text{O}$  (1 mL) was added to a stirred solution of  $\text{K}_2\text{PtCl}_4$  (154 mg, 0.37 mmol, 20 mol %) and  $\text{DCI}$  (35%) (612  $\mu\text{L}$ , 7.44 mmol, 4 eq.) in  $\text{D}_2\text{O}$  (3 mL) and reacted according to the general procedure **A** to give the *title compound* as a brown oil (125 mg, 40%); Found  $\text{M}^{+\cdot}$ , 165.0703.  $\text{C}_7\text{H}_2\text{D}_4\text{F}_3\text{N}$  [ $\text{M}^{+\cdot}$ ] requires 165.0700;  $\nu_{\text{max}}$  (KBr) 3447, 3390, 3229, 1624, 1584, 1619, 1581, 1479, and 1426;  $\delta_{\text{H}}$  (400 MHz;  $\text{CD}_3\text{OD}$ )  $^a$ 7.23(0.04H, s),  $^a$ 6.94 (0.04H, s),  $^a$ 6.75 (0.04H, s),  $^a$ 6.74 (0.04H, s);  $\delta_{\text{C}}$ (100 MHz,  $\text{CD}_3\text{OD}$ ) 150.1 (s, C-NH $_2$ ), 132.7.4 (m, C-CF $_3$ ), 130.6 (t, C-D,  $J_{\text{C-D}}$  24), 126.3 (m, CF $_3$ ), 119.2 (t, C-D,  $J_{\text{C-D}}$  25), 114.7 (t, C-D,  $J_{\text{C-D}}$  26), 112.2 (t, C-D,  $J_{\text{C-D}}$  25);  $m/z$  (EI) 165 ( $\text{M}^{+\cdot}$ , 100%).

$^a$ Signals arise from the presence of isotopologues.

**N-[2,4,5,6-Tetradeutero-3-(trifluoromethyl)phenyl]acetamide (8a)**

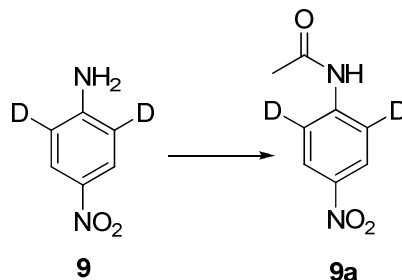
Acetyl chloride (109  $\mu\text{L}$ , 1.53 mmol, 1.1 eq.) was added to a stirred solution of  $d_4$ -3-(trifluoromethyl)aniline (**8**) (230 mg, 1.39 mmol, 1 eq.) in DCM (5 mL) and  $\text{NEt}_3$  on ice (429  $\mu\text{L}$ , 3.06 mmol, 2.2 eq.) under  $\text{N}_2$  atmosphere and reacted according to the general procedure **B**. Purification by chromatography column, eluting with petroleum ether/ethyl acetate (3/1) gave the *title compound* as a colourless powder (225 mg, 78 %): Found  $\text{M}^+$ , 207.0805  $\text{C}_9\text{H}_4\text{D}_4\text{F}_3\text{NO}$  [ $\text{M}^+$ ] requires 207.0809, mp 95-101 $^\circ\text{C}$ ;  $\nu_{\text{max}}$  (KBr) 3304, 3265, 3129, 3070, 2962, 2972, 1662, 1616, 1580, 1535; 1411, and 1284,  $\delta_{\text{H}}$  (400 MHz;  $\text{CD}_3\text{OD}$ , 50 $^\circ\text{C}$ ) 7.87 (0.04H,s), 7.57 (0.04H,s), 7.29 (0.04H,s), 7.18 (0.04H,s), 2.01 (3H, s);  $\delta_{\text{C}}$  (100 MHz,  $\text{CD}_3\text{OD}$ ) 172.3 (s, C=O), 141.1 (s, C-NH), 132.3(m, C-CF<sub>3</sub>), 130.04 (t, C-D,  $J_{\text{C-D}}$  25), 127.3 (m, CF<sub>3</sub>), 124.01 (t, C-D,  $J_{\text{C-D}}$  27), 121.3 (t, C-D,  $J_{\text{C-D}}$  24), 117.4 (t, C-D,  $J_{\text{C-D}}$  27), 24.3 (s, CH<sub>3</sub>);  $m/z$  (EI) 207 ( $\text{M}^+$ , 20%).

**2,6-Dideutero-4-nitroaniline (9)**<sup>207</sup>

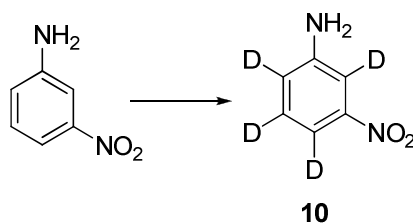
4-Nitroaniline (300 mg, 2.17 mmol, 1 eq.) was added to a stirred solution of  $\text{K}_2\text{PtCl}_4$  (180 mg, 0.43 mmol, 20 mol %) and DCl (35%) (714  $\mu\text{L}$ , 8.68 mmol, 4 eq.) in  $\text{D}_2\text{O}$  (3 mL) and reacted according to the general procedure **A** to give the *title compound* as a brown powder (70 mg, 23%): Found  $\text{M}^+$ , 140.0554.  $\text{C}_6\text{H}_4\text{D}_2\text{N}_2\text{O}_2$  [ $\text{M}^+$ ] requires 140.0555; mp 124-128  $^\circ\text{C}$ ;  $\nu_{\text{max}}$  (KBr) 3482, 3455, 3362, 2608, 2505, 2445, 1631, 1577 and 1469;  $\delta_{\text{H}}$  (400 MHz;  $\text{CD}_3\text{OD}$ ) <sup>a</sup>7.87(1.34H, s), <sup>a</sup>6.51(0.14H, d,  $J_{\text{H-H}}$  9.62);  $\delta_{\text{C}}$  (100 MHz,  $\text{CD}_3\text{OD}$ ) 157.5 (s, C-NO<sub>2</sub>), 150.5(s, C-NH<sub>2</sub>), 127.6(s, C-H), 113.8 (t, C-D,  $J_{\text{C-D}}$  24);  $m/z$  (EI) 140 ( $\text{M}^+$ , 100%).

<sup>a</sup>Signals arise from the presence of isotopologues.



**N-[2,6-Dideutero-4-nitrophenyl]acetamide (9a)**

Acetyl chloride (68  $\mu\text{L}$ , 0.9 mmol, 1.1 eq.) was added to a stirred solution of *d*<sub>4</sub>-4-nitroaniline (124 mg, 0.87 mmol, 1 eq.) in DCM (5 mL) and NEt<sub>3</sub> on ice (268  $\mu\text{L}$ , 1.91 mmol, 2.2 eq.) under N<sub>2</sub> atmosphere and reacted according to the general procedure **B**. Purification of chromatography column, eluting with petroleum ether/ ethyl acetate (1/1) gave the *title compound* as a brow powder (56 mg, 34%): Found M<sup>+</sup>, 182.0655. C<sub>8</sub>H<sub>6</sub>D<sub>2</sub>N<sub>2</sub>O<sub>3</sub> [M<sup>+</sup>] requires 182.0660, mp>200°C;  $\nu_{\text{max}}$  (KBr) 3271, 3228, 3168, 3097, 2385, 1846, 1682, 1611, 1558, 1502, 1451, 1345 and 1276;  $\delta_{\text{H}}$  (400 MHz; CD<sub>3</sub>OD, 50°C) 8.07 (1.34H, s), 7.67 (0.14H, d, *J*<sub>H-H</sub> 9.62), 2.06 (3H, s);  $\delta_{\text{C}}$  (125 MHz, CD<sub>3</sub>OD) 172.9 (s, C=O), 146.5 (s, C-NH), 144.9(s, C-NO<sub>2</sub>), 126.1(s, C-H), 120.1(t, C-D, *J*<sub>C-D</sub> 25), 24.0 (s, CH<sub>3</sub>); *m/z* (EI) 182 (M<sup>+</sup>, 50%).

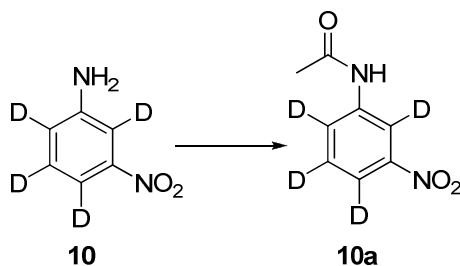
**2,4,5,6-Tetradeutero-3-nitroaniline (10)**

3-Nitroaniline (300 mg, 2.17 mmol, 1 eq.) was added to a stirred solution of K<sub>2</sub>PtCl<sub>4</sub> (180 mg, 0.43 mmol, 20 mol %) and DCl (35%) (714  $\mu\text{L}$ , 8.68 mmol, 4 eq.) in D<sub>2</sub>O (3 mL) and reacted according to the general procedure **A** to give the *title compound* as a yellow powder (264 mg, 86%): Found M<sup>+</sup>, 142.0676. C<sub>6</sub>H<sub>2</sub>D<sub>3</sub>N [M<sup>+</sup>] requires 142.0680; mp 110-116 °C;  $\nu_{\text{max}}$ (KBr) 3446, 3398, 2963, 2916, 2848, 2567, 2505, 2437, 1599, 1562, 1522, 1422, 1366, and 1261;  $\delta_{\text{H}}$  (400 MHz; CD<sub>3</sub>OD) <sup>a</sup>7.49(0.06H, s), <sup>a</sup>7.44(0.06H, s), <sup>a</sup>7.26 (0.06H, s), 7.01 (0.06H, s);  $\delta_{\text{C}}$ (100 MHz, CD<sub>3</sub>OD) 150.8 (s, C-NO<sub>2</sub>), 150.5(s, C-NH<sub>2</sub>), 130.2 (t, C-D, *J*<sub>C-D</sub> 25),

121.1 (t, C-D,  $J_{C-D}$  24), 112.02 (t, C-D,  $J_{C-D}$  26), 109.1 (t, C-D,  $J_{C-D}$  26);  $m/z$  (EI) 142 ( $M^{+}$ , 100%).

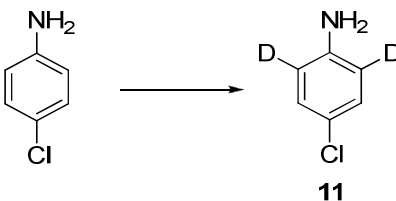
<sup>a</sup>Signals arise from the presence of isotopologues.

### ***N*-[2,4,5,6-Tetradeutero-3-nitrophenyl]acetamide (10a)**



Acetyl chloride (110  $\mu$ L, 1.5 mmol, 1.1 eq.) was added to a stirred solution of *d*<sub>4</sub>-3-nitroaniline (**10**) (200 mg, 1.4 mmol, 1 eq.) in DCM (5 mL) and NEt<sub>3</sub> on ice (462  $\mu$ L, 3.3 mmol, 2.2 eq.) under N<sub>2</sub> atmosphere and reacted according to the general procedure **B**. Purification by chromatography column, eluting with petroleum ether/ ethyl acetate (3/1) gave the *title compound* as a colourless powder (195 mg, 80%): Found  $M^{+}$ , 184.0876. C<sub>8</sub>H<sub>4</sub>D<sub>4</sub>N<sub>2</sub>O<sub>3</sub> [ $M^{+}$ ] requires 184.0873, mp 147-151 °C;  $\nu_{\max}$  (KBr) 3301, 3248, 3174, 3093, 2922, 2852; 2343, 2301, 11674, 1610, 1582 and 1535,  $\delta_{\text{H}}$  (500 MHz; CD<sub>3</sub>OD) 8.49 (0.06H, s), 7.80 (0.06H, s), 7.71 (0.06H, s), 7.41 (0.06H, s), 2.16 (3H, s);  $\delta_{\text{C}}$  (125 MHz, CD<sub>3</sub>OD) 171.1 (s, C=O), 149.8 (s, C-NO<sub>2</sub>), 141.2 (s, C-NH), 130.6 (t, C-D,  $J_{C-D}$  25), 125.6 (t, C-D,  $J_{C-D}$  26), 118.2 (t, C-D,  $J_{C-D}$  26), 115.01 (t, C-D,  $J_{C-D}$  26), 24.0 (s, CH<sub>3</sub>);  $m/z$  (EI) 184 ( $M^{+}$ , 25%).

### **2,6-Dideutero-4-chloroaniline (11)**<sup>208-209</sup>

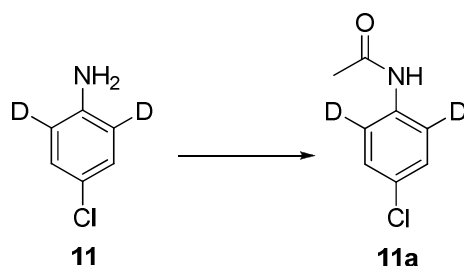


A solution of 4-chloroaniline (300 mg, 2.3 mmol, 1 eq.) was added to a stirred solution of K<sub>2</sub>PtCl<sub>4</sub> (195 mg, 0.47 mmol, 20 mol %) and DCl (35%) (778  $\mu$ L, 9.2 mmol, 4 eq.) in D<sub>2</sub>O (3 mL) and reacted according to the general procedure **A**. To the solution thioglycolic acid (130  $\mu$ L, 1.88 mmol, 4 eq. referred to the catalyst amount) was added and the mixture was stirred

for 30 min, the rest of the the work up followed the general procedure **A** to give the *title compound* as a violet powder (264 mg, 86%): Found  $M^{+}$ , 129.0310.  $C_6H_4D_2ClN$  [ $M^{+}$ ] requires 129.0314; mp 64-68 °C;  $\nu_{max}$  (KBr) 3473, 3427, 3382, 2963, 2533, 1806, 1611, 1583, 1473, 1453, 1261, and 1092;  $\delta_H$  (400 MHz;  $CD_3OD$ ,)  $^a$ 6.95 (1.18H, s),  $^a$ 6.55 (0.10H, m);  $\delta_C$ (100 MHz,  $CD_3OD$ ) 148.12 (s, C-NH<sub>2</sub>), 130.09 (s, C-H), 123.69 (s, C-Cl), 117.22 (t, C-D,  $J_{C-D}$  24);  $m/z$  (EI) 129 ( $M^{+}$ , 100%).

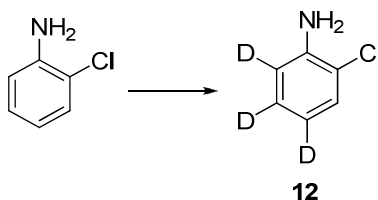
<sup>a</sup>Signals arise from the presence of isotopologues.

### **N-[2,6-dideutero-4-chlorophenyl]acetamide (11a)**



Acetyl chloride (152  $\mu$ L, 2.1 mmol, 1.1 eq.) was added to a stirred solution of *d*<sub>4</sub>-4-chloroaniline (**11**) (255 mg, 1.9 mmol, 1 eq.) in DCM (5 mL) and NEt<sub>3</sub> on ice (586  $\mu$ L, 4.18 mmol, 2.2 eq.) under N<sub>2</sub> atmosphere and reacted according to the general procedure **B** to give the *title compound* as a brown powder (283 mg, 84%): Found  $M^{+}$ , 171.0424.  $C_8H_6D_2ClNO$  [ $M^{+}$ ] requires 171.0420; mp 165-169 °C;  $\nu_{max}$  (KBr) 3272, 3224, 3150, 3062, 2963, 1660, 1571, 1513, 1428, 1396, and 1354,  $\delta_H$  (400 MHz;  $CD_3OD$ ) 7.53 (0.10H, m), 7.27 (1.18H, s), 2.13 (3H, s);  $\delta_C$  (150 MHz,  $CD_3OD$ ) 172.2 (s, C=O), 139.3(s, C-NH), 129.8 (s, C-Cl), 129.6(s, C-H), 122.1 (t, C-D,  $J_{C-D}$  24), 23.8 (s, CH<sub>3</sub>);  $m/z$  (EI) 171 ( $M^{+}$ , 45%).

### **4,5,6-Trideutero-2-chloroaniline (12)**

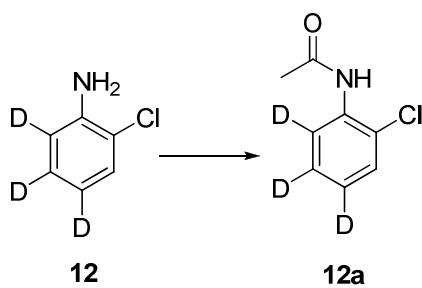


A solution of 2-chloroaniline (300 mg, 247  $\mu$ L, 2.4 mmol, 1 eq.) was added to a stirred solution of K<sub>2</sub>PtCl<sub>4</sub> (199 mg, 0.48 mmol, 20 mol %) and DCl (35%) (778  $\mu$ L, 9.4 mmol, 4 eq.) in D<sub>2</sub>O (3 mL) and reacted according to the general procedure **A**. To the solution

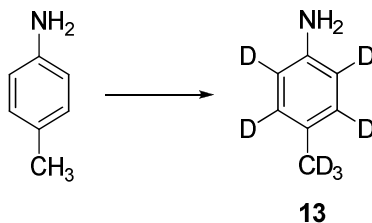
thioglycolic acid (133  $\mu\text{L}$ , 1.92 mmol, 4 eq. referred to the catalyst amount) was added and the mixture was stirred for 30 min, the rest of the the work up followed the general procedure **A** to give the title compound as a brown oil (228 mg, 92%): Found  $M^+$ , 130.0412  $\text{C}_6\text{H}_3\text{D}_4\text{ClN}$  [ $M^+$ ] requires 130.0410;  $\nu_{\text{max}}$  (KBr) 3468, 3383, 3365, 3219, 2534, 1615, 15756, 1464, 1442, 1415, 1372, and 1305;  $\delta_{\text{H}}$  (400 MHz;  $\text{CD}_3\text{OD}$ , 50°C)  $^a$ 7.16 (0.68H, s),  $^a$ 7.00 (0.08H, s),  $^a$ 6.79 (0.08H, m),  $^a$ 6.59 (0.08H, s);  $\delta_{\text{C}}$  (100 MHz,  $\text{CD}_3\text{OD}$ ) 144.01 (s, C-NH<sub>2</sub>), 129.01 (s, C-H), 127.4 (t, C-D,  $J_{\text{C-D}}$  24), 118.9 (s, C-Cl), 118.2 (t, C-D,  $J_{\text{C-D}}$  25), 116.03 (t, C-D,  $J_{\text{C-D}}$  25);  $m/z$  (EI) 130 ( $M^+$ , 50%).

<sup>a</sup>Signals arise from the presence of isotopologues.

### ***N*-[4,5,6-Trideutero-2-chlorophenyl]acetamide (12a)**

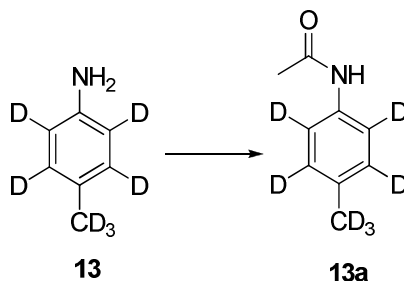


Acetyl chloride (55  $\mu\text{L}$ , 0.78 mmol, 1.1 eq.) was added to a stirred solution of *d*<sub>4</sub>-2-chloroaniline (**12**) (93 mg, 0.71 mmol, 1 eq.) in DCM (5 mL) and  $\text{NEt}_3$  on ice (216  $\mu\text{L}$ , 1.54 mmol, 2.2 eq.), under  $\text{N}_2$  atmosphere and reacted according to the general procedure **B**. Purification by chromatography column, eluting with petroleum ether/ethyl acetate (5/1) gave the *title compound* as a brown powder (50 mg, 41 %): Found  $M^+$ , 172.0545.  $\text{C}_8\text{H}_5\text{D}_3\text{ClNO}$  [ $M^+$ ] requires 172.0549; mp 81-87 °C;  $\nu_{\text{max}}$  (KBr) 3272, 3224, 3150, 3062, 2963, 1660, 1571, 1513, 1428, 1396, and 1354,  $\delta_{\text{H}}$  (400 MHz;  $\text{CD}_3\text{OD}$ ) 7.73 (0.08H, s), 7.43 (0.68H, s), 7.28 (0.08H, s), 7.16 (0.08H, m), 2.18 (3H, s);  $\delta_{\text{C}}$  (100 MHz,  $\text{CD}_3\text{OD}$ ) 170.9 (s, C=O), 134.6 (s, C-NH), 129.9 (s, C-Cl), 129.4 (m, C-D), 127.2 (m, C-D), 127.0 (m, C-D), 126.1 (m, C-D), 22.2 (s, CH<sub>3</sub>);  $m/z$  (EI) 172 ( $M^+$ , 20%).

**2,3,5,6-Tetradeutero-4-(trideuteromethyl)aniline (13)**

A solution of 4-methylaniline (300 mg, 2.8 mmol, 1 eq.) was added to a stirred solution of  $K_2PtCl_4$  (233 mg, 0.56 mmol, 20 mol %) and DCl (35%) (921  $\mu$ L, 11.2 mmol, 4 eq.) in  $D_2O$  (3 mL) and reacted according to the general procedure **A**. To the solution thioglycolic acid (156  $\mu$ L, 2.24 mmol, 4 eq. referred to the catalyst amount) was added and the mixture was stirred for 30 min, the rest of the the work up followed the general procedure **A** give the *title compound* as a brown/yellow powder (271 mg, 85%): Found  $M^{+}$ , 114.1170.  $C_7H_2D_7N$  [ $M^{+}$ ] requires 114.1174; mp 34-40  $^{\circ}C$ ;  $\nu_{max}$  (KBr) 3417, 3343, 3220, 3022, 3006, 2499, 1617, 1597, 1477, 1463, 1442, 1294, 1254 and 1239;  $\delta_H$  (400 MHz;  $CD_3OD$ )  $^a$ 6.91 (0.74H, s),  $^a$ 6.91 (0.12H, s), 2.16(0.23 H, m);  $\delta_C$  (150 MHz,  $CD_3OD$ ) 145.3 (s, C-NH<sub>2</sub>), 130.1 (t, C-D,  $J_{C-D}$  24), 128.5 (s, C-CD<sub>3</sub>), 116.7 (t, C-D,  $J_{C-D}$  24), 19.8 (m, CD<sub>3</sub>);  $m/z$  (EI) 114 ( $M^{+}$ , 100%).

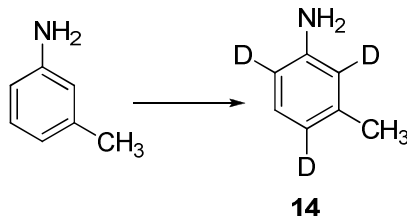
<sup>a</sup>Signals arise from the presence of isotopologues.

**N-[2,3,5,6-Tetradeutero-4-(trideuteromethyl)phenyl]acetamide (13a)**

Acetyl chloride (85 $\mu$ L, 1.21 mmol, 1.1 eq.) was added to a stirred solution of *d*<sub>7</sub>-4-methylaniline (**13**) (125 mg, 1.09 mmol, 1 eq.) in DCM (5 mL) and  $NEt_3$  on ice (340  $\mu$ L, 2.41 mmol, 2.2 eq.) under  $N_2$  atmosphere and reacted according to the general procedure **B** to give the *title compound* as a colourless powder (136 mg, 79%): Found  $M^{+}$ , 156.1281.  $C_9H_4D_7NO$  [ $M^{+}$ ] requires 156.1280; mp 121-125  $^{\circ}C$ ;  $\nu_{max}$  (KBr) 3289, 3245, 3166, 3092, 3031, 2930, 2852, 2782, 1660, 1597, 1533, 1367, 1314, 1263, 1038 and 1013;  $\delta_H$  (400 MHz;  $CD_3OD$ ) 7.38 (0.12H, m), 7.15 (0.74H, s), 2.25 (0.23, s), 2.09 (3H, s);  $\delta_C$  (100 MHz,  $CD_3OD$ ) 171.5 (s,

C=O), 137.2 (s, C-NH), 134.6 (s, C-CD<sub>3</sub>), 129.8 (t, C-D,  $J_{C-D}$  24), 120.9 (t, C-D,  $J_{C-D}$  24), 23.7 (s, CH<sub>3</sub>), 20.0 (m, CD<sub>3</sub>);  $m/z$  (EI) 156 (M<sup>+</sup>, 45%).

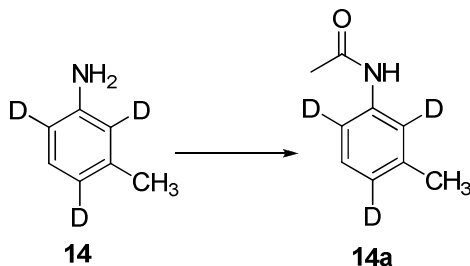
### 2,4,6-Trideutero-3-methylaniline(14)<sup>210</sup>



A solution of 3-methylaniline (300 mg, 2.8 mmol, 1 eq.) was added to a stirred solution of K<sub>2</sub>PtCl<sub>4</sub> (232 mg, 0.56 mmol, 20 mol %) and DCl (35%) (923 μL, 11.2 mmol, 4 eq.) in D<sub>2</sub>O (3 mL) and reacted according to the general procedure A. To the solution thioglycolic acid (155 μL, 2.24 mmol, 4 eq. referred to the catalyst amount) was added and the mixture was stirred for 30 min, the rest of the the work up followed the general procedure A to give the *title compound* as a yellow oil (303 mg, 97%): Found M<sup>+</sup>, 110.1175. C<sub>7</sub>H<sub>6</sub>D<sub>3</sub>N [M<sup>+</sup>] requires 110.1174;  $\nu_{\max}$  (KBr) 3433, 3354, 3219, 3056, 3007, 2921, 1619, 1585, 1445 and 1403;  $\delta_{\text{H}}$  (400 MHz; CD<sub>3</sub>OD) <sup>a</sup>6.82 (0.55H, s), <sup>a</sup>6.38 (0.35H, m), <sup>a</sup>2.05 (1.64H, s),  $\delta_{\text{C}}$  (100 MHz, CD<sub>3</sub>OD) 148.6 (s, C-NH<sub>2</sub>), 140.1 (s, C-CH<sub>3</sub>), 130.3 (s, C-H), 120.7 (m, C-D), 117.7 (t, C-D,  $J_{C-D}$  24), 114.3 (m, C-D), 22.7 (s, CH<sub>3</sub>);  $m/z$  (EI) 110 (M<sup>+</sup>, 80%).

<sup>a</sup>Signals arise from the presence of isotopologues.

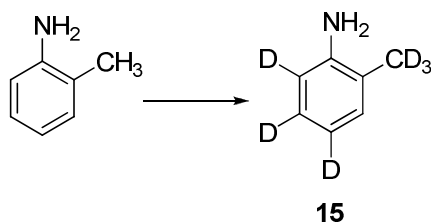
### N-[2,4,6-Trideutero-3-methylphenyl]acetamide (14a)



Acetyl chloride (127 μL, 1.79 mmol, 1.1 eq.) was added to a stirred solution of *d*<sub>7</sub>-3-methylaniline (**14**) (196 mg, 1.63 mmol, 1 eq.) in DCM (5 mL) and NEt<sub>3</sub> on ice (503 μL, 3.57 mmol, 2.2 eq.) under N<sub>2</sub> atmosphere and reacted according to the general procedure B to give the *title compound* as a brown powder (240 mg, 92%): Found M<sup>+</sup>, 152.1284. C<sub>9</sub>H<sub>8</sub>D<sub>3</sub>NO

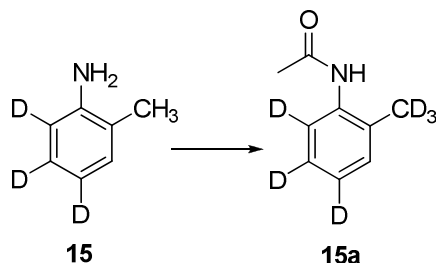
[M<sup>+</sup>] requires 152.1280; mp 48-53 °C;  $\nu_{\max}$  (neat) 3300, 3112, 3059, 2965, 2926, 2393, 2063, 2065 1661, 1652, 1602, 1575, 1538, 1442, 1393, 1310 and 11266;  $\delta_{\text{H}}$  (400 MHz; CD<sub>3</sub>OD) 7.35 (0.11H, s), 7.30 (0.09H, m), 7.15 (0.55H, s), 6.90 (0.11, m), 2.30 (1.65H, m), 2.10(3H,s);  $\delta_{\text{C}}$  (150 MHz, CD<sub>3</sub>OD) 171.8 (s, C=O), 139.7(s, C-NH), 129.5(s, C-CH<sub>3</sub>), 129.4 (s, C-H), 125.6 (m, C-D), 121.5 (t, C-D,  $J_{\text{C-D}}$  25), 118 (m, C-D), 24.05 (s, CH<sub>3</sub>), 21.62 (s, CH<sub>3</sub>);  $m/z$  (EI) 152 (M<sup>+</sup>, 65%).

#### **4,5,6-Trideutero-2-(trideuteromethyl)aniline (15)**

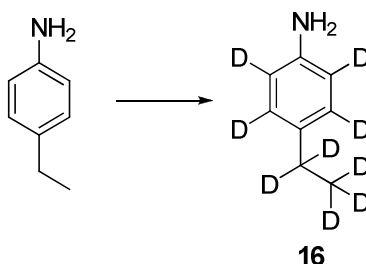


A solution of 2-methylaniline (300 mg, 298  $\mu\text{L}$ , 2.8 mmol, 1 eq.) was added to a stirred solution of K<sub>2</sub>PtCl<sub>4</sub> (232 mg, 0.56 mmol, 20 mol %) and DCl (35%) (923  $\mu\text{L}$ , 11.2 mmol, 4 eq.) in D<sub>2</sub>O (3 mL) and reacted according to the general procedure A. To the solution thioglycolic acid (155  $\mu\text{L}$ , 2.24 mmol, 4 eq. referred to the catalyst amount) was added and the mixture was stirred for 30 min, the rest of the the work up followed the general procedure A to give the title compound as a brown/yellow oil (264 mg, 85%): Found M<sup>+</sup>, 113.1173. C<sub>7</sub>H<sub>3</sub>D<sub>6</sub>N [M<sup>+</sup>] requires 113.1174;  $\nu_{\max}$  (KBr) 3445, 3369, 3228, 3051, 3006, 2957, 2906, 2163, 2517, 1621, 1581, 1448, 1422, 1380 and 1305;  $\delta_{\text{H}}$  (400 MHz; CD<sub>3</sub>OD) <sup>a</sup>6.98 (0.07H, s), <sup>a</sup>6.97 (0.54H, s), <sup>a</sup>6.69 (0.09H, s), <sup>a</sup>6.65 (0.08, m), 2.08 (0.33H, s);  $\delta_{\text{C}}$  (100 MHz, CD<sub>3</sub>OD) 146.3 (s, C-NH<sub>2</sub>), 131.2 (s, C-H), 127.4 (t, C-D,  $J_{\text{C-D}}$  24), 124.1 (s, C-CD<sub>3</sub>), 119.4 (m, C-D), 116.2 (t, C-D,  $J_{\text{C-D}}$  24), 17.5 (m, CD<sub>3</sub>);  $m/z$  (EI) 113 (M<sup>+</sup>, 40%).

<sup>a</sup>Signals arise from the presence of isotopologues.

**N-[4,5,6-Trideutero-2-(trideuteromethyl)phenyl]acetamide (15a)**

Acetyl chloride (109  $\mu\text{L}$ , 1.5 mmol, 1.1 eq.) was added to a stirred solution of *d*<sub>7</sub>-2-methylaniline (**15**) (160 mg, 1.4 mmol, 1 eq.) in DCM (5 mL) and NEt<sub>3</sub> on ice (432  $\mu\text{L}$ , 3.07 mmol, 2.2 eq.) under N<sub>2</sub> atmosphere and reacted according to the general procedure A. Purification by chromatography column, eluting with petroleum ether/ethyl acetate (3/1) gave the title compound as a brown powder (148 mg, 66 %): Found M<sup>+</sup>, 155.1283. C<sub>9</sub>H<sub>5</sub>D<sub>6</sub>NO [M<sup>+</sup>] requires 155.1280; mp 101-110 °C;  $\nu_{\text{max}}$  (KBr) 3292, 3014, 2925, 2851, 2426, 2050, 1837, 1636 and 1515;  $\delta_{\text{H}}$  (400 MHz; CD<sub>3</sub>OD) 7.31 (0.09H, s), 7.20 (0.54H, s), 7.15 (0.08H, m), 7.11 (0.07, s), 2.20 (0.33H, m), 2.13 (3H, s);  $\delta_{\text{C}}$  (150 MHz, CD<sub>3</sub>OD) 172.1 (s, C=O), 136.9 (s, C-NH), 134.1 (s, C-CD<sub>3</sub>), 131.5 (s, C-H), 126.8 (m, C-D), 23.1 (s, CH<sub>3</sub>), 17.6 (m, CD<sub>3</sub>); *m/z* (EI) 155 (M<sup>+</sup>, 45%).

**2,3,5,6-Tetradeutero-4-(1,1,2,2,2-pentadeutero)ethylaniline (16)**

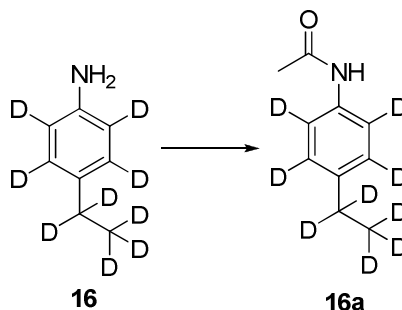
A solution of 4-ethylaniline (300 mg, 309  $\mu\text{L}$ , 2.48 mmol, 1 eq.) was added to a stirred solution of K<sub>2</sub>PtCl<sub>4</sub> (205mg, 0.48 mmol, 20 mol %) and DCl (35%) (816  $\mu\text{L}$ , 9.92 mmol, 4 eq.) in D<sub>2</sub>O (3 mL) and reacted according to the general procedure A. To the solution thioglycolic acid (136  $\mu\text{L}$ , 1.92 mmol, 4 eq. referred to catalyst amount) was added and the mixture was stirred for 30 min, the rest of the the work up followed the general procedure A to give the title compound as a brown oil (180 mg, 56%): Found M<sup>+</sup>, 130.1456. C<sub>8</sub>H<sub>2</sub>D<sub>9</sub>N



[M<sup>+</sup>] requires 130.1456;  $\nu_{\max}$  (KBr) 3349, 2960, 1617, 1477, 1463, 1442, 1301 and 1254;  $\delta_{\text{H}}$  (400 MHz; CD<sub>3</sub>OD) <sup>a</sup>6.93 (0.77H, s), <sup>a</sup>6.67 (0.27H, m), <sup>a</sup>2.46 (0.17 H, m), <sup>a</sup>1.05 (0.24 H, m);  $\delta_{\text{C}}$  (150 MHz, CD<sub>3</sub>OD) 145.7(s, C-NH<sub>2</sub>), 135.5(s, C-C), 128.9 (m, C-D), 116.9 (m, C-D), 28.1 (m, CD<sub>2</sub>), 15.8 (m, CD<sub>3</sub>);  $m/z$  (EI) 130 (M<sup>+</sup>, 50%).

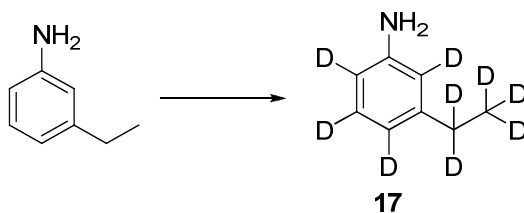
<sup>a</sup>Signals arise from the presence of isotopologues.

**N-[2,3,5,6-tetradeutero-4-(1,1,2,2,2-pentadeuteroethyl)phenyl]acetamide (16a)**



Acetyl chloride (95  $\mu$ L, 1.34 mmol, 1.1 eq.) was added to a stirred solution of *d*<sub>9</sub>-4-ethylaniline (**16**) (150 mg, 1.22 mmol, 1 eq.) in DCM (10 mL) and NEt<sub>3</sub> on ice (270  $\mu$ L, 2.68 mmol, 2.2 eq.) under N<sub>2</sub> atmosphere and reacted according to the general procedure **B** to give the *title compound* as a brown powder (210 mg, 95%): Found M<sup>+</sup>, 172.1560. C<sub>10</sub>H<sub>4</sub>D<sub>9</sub>NO [M<sup>+</sup>] requires 172.1562; mp 45-49 °C;  $\nu_{\max}$  (KBr) 3298, 3094, 3227, 2962, 1664, 1593, 1524, 1372, 1313 and 1264;  $\delta_{\text{H}}$  (400 MHz; DMSO) 7.44 (0.27H, m), 7.15 (0.77H, s), 2.58 (0.17H, m), 2.12 (3H, s), 1.19 (0.24H, m);  $\delta_{\text{C}}$  (150 MHz, CD<sub>3</sub>OD) 171.5 (s, C=O), 140.5 (s, C-NH), 137.3 (s, C-C), 128.6 (t, C-D,  $J_{\text{C-D}}$  24), 121.1 (m, C-D), 28.5 (m, CD<sub>2</sub>), 23.8 (s, CH<sub>3</sub>), 15.3 (m, CD<sub>3</sub>);  $m/z$  (EI) 172 (M<sup>+</sup>, 65%).

**2,4,5,6-Tetradeutero-3-(1,1,2,2,2-pentadeutero)ethylaniline (17)**

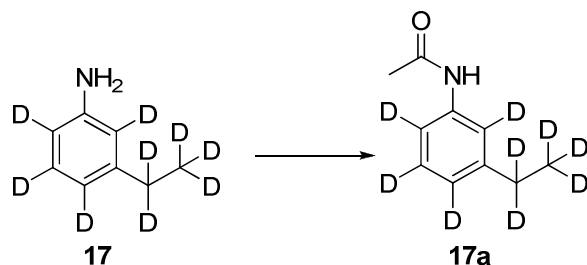


A solution of 3-ethylaniline (300 mg, 307  $\mu$ L, 2.48 mmol, 1 eq.) was added to a stirred solution of K<sub>2</sub>PtCl<sub>4</sub> (205mg, 0.48 mmol, 20 mol %) and DCl (35%)(816  $\mu$ L, 9.92 mmol, 4

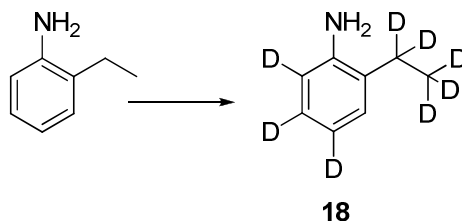
eq.) in  $D_2O$  (3 mL) and reacted according to the general procedure **A**. To the solution thioglycolic acid (141  $\mu$ L, 1.99 mmol, 4 eq. referred to catalyst amount) was added and the mixture was stirred for 30 min, the rest of the the work up followed the general procedure **A** to give the *title compound* as a brown oil (260 mg, 81%): Found  $M^{+}$ , 130.1452.  $C_8H_2D_9N$  [ $M^{+}$ ] requires 130.1456;  $\nu_{max}(KBr)$  3442, 3350, 3220, 2934, 1618, 1518, 1400, 1301, 1258 and 1053;  $\delta_H$  (400 MHz;  $CD_3OD$ )  $^a$ 6.99 (0.08H, s),  $^a$ 6.59 (0.08H, s),  $^a$ 6.54 (0.16H, s),  $^a$ 2.48 (0.21H, s),  $^a$ 1.13 (0.28H, s);  $\delta_C$  (150 MHz,  $CD_3OD$ ) 148.2(s, C-NH<sub>2</sub>), 146.1(s, C-C), 129.4 (t, C-D,  $J_{C-D}$  24), 118.7(t, C-D,  $J_{C-D}$  24), 116.0 (t, C-D,  $J_{C-D}$  23), 113.8(t, C-D,  $J_{C-D}$  23), 29.1 (m,  $CD_2$ ), 15.2 (m,  $CD_3$ );  $m/z$  (EI) 130 ( $M^{+}$ , 80%).

<sup>a</sup>Signals arise from the presence of isotopologues.

**N-[2,4,5,6-tetradeutero-3-(1,1,2,2,2-pentadeuteroethyl)phenyl]acetamide (17a)**

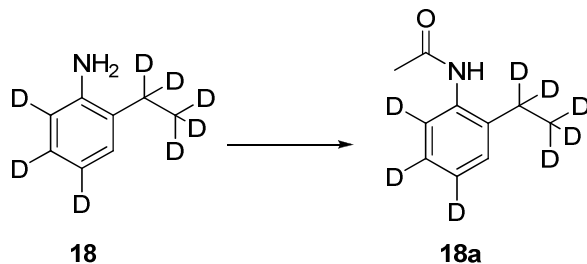


Acetyl chloride (127  $\mu$ L, 1.79 mmol, 1.1 eq.) was added to a stirred solution of *d*<sub>9</sub>-3-ethylaniline (**17**) (200 mg, 1.63 mmol, 1 eq.) in DCM (10 mL) and  $NEt_3$  on ice (503  $\mu$ L, 3.59 mmol, 2.2 eq.) under  $N_2$  atmosphere and reacted according to the general procedure **B** to give the *title compound* as a yellow oil (281mg, 87%): Found  $M^{+}$ , 172.1562.  $C_{10}H_4D_9NO$  [ $M^{+}$ ] requires 172.1562;  $\nu_{max}$  (KBr) 3301, 3152, 3104, 3054, 2931, 2860, 1667, 1537, 1393, 1269, 1127 and 835;  $\delta_H$  (400 MHz; DMSO) 7.37 (0.08H, s), 7.33 (0.08H, s), 7.19 (0.08H, s), 6.93 (0.08H, s), 2.57 (0.21H, s), 2.11 (3H, s), 1.16 (0.28H, m);  $\delta_C$  (150 MHz,  $CD_3OD$ ) 171.4(s, C=O), 145.7 (s, C-NH), 139.5(s, C-C), 129.1 (t, C-D,  $J_{C-D}$  24), 124.2 (t, C-D,  $J_{C-D}$  24), 120.2 (t, C-D,  $J_{C-D}$  24), 118.1(t, C-D,  $J_{C-D}$  25), 28.8 (m,  $CD_2$ ), 23.9 (s,  $CH_3$ ), 15.0 (m,  $CD_3$ );  $m/z$  (EI) 172 ( $M^{+}$ , 20%).

**4,5,6-Trideutero-2-(1,1,2,2,2-pentadeutero)ethylaniline (18)**

A solution of 2-ethylaniline (300 mg, 293  $\mu\text{L}$ , 2.48 mmol, 1 eq.) was added to a stirred solution of  $\text{K}_2\text{PtCl}_4$  (205mg, 0.48 mmol, 20 mol %) and  $\text{DCl}$  (35%) (816  $\mu\text{L}$ , 9.92 mmol, 4 eq.) in  $\text{D}_2\text{O}$  (3 mL) and reacted according to the general procedure A. To the solution thioglycolic acid (136  $\mu\text{L}$ , 1.92 mmol, 4 eq. referred to catalyst amount) was added and the mixture was stirred for 30 min, the rest of the the work up followed the general procedure A to give the *title compound* as a yellow oil (239 mg, 75%): Found  $M^+$ , 128.1395.  $\text{C}_8\text{H}_4\text{D}_7\text{N}$  [ $M^+$ ] requires 128.1394;  $\nu_{\text{max}}$ (KBr) 3227, 3184, 3107, 3046, 3012, 2929, 2784, 1648, 1566, 1524, 1374 and 1272;  $\delta_{\text{H}}$  (500 MHz;  $\text{CD}_3\text{OD}$ )  $^a$ 6.99 (0.77H, s),  $^a$ 6.94 (0.08H, m),  $^a$ 6.71 (0.08H, s),  $^a$ 6.66 (0.08H, d,  $J_{\text{H-H}}$  7.5),  $^a$ 2.49 (0.36H, m),  $^a$ 1.53 (0.24H, m);  $\delta_{\text{C}}$  (150 MHz,  $\text{CD}_3\text{OD}$ ) 145.6 (s, C- $\text{NH}_2$ ), 129.9 (s, C-C), 129.2 (s, C-H), 127.3 (m, C-D), 119.4 (m, C-D), 116.6 (m, C-D), 24.5 (m,  $\text{CD}_2$ ), 13.3 (m,  $\text{CD}_3$ );  $m/z$  (EI) 128 ( $M^+$ , 50%).

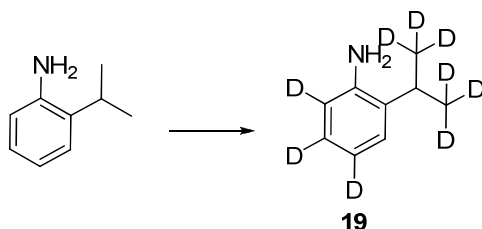
<sup>a</sup>Signals arise from the presence of isotopologues.

**N-[4,5,6-Trideutero-2-(1,1,2,2,2-pentadeuteroethyl)phenyl]acetamide (18a)**

Acetyl chloride (56 $\mu\text{L}$ , 0.81 mmol, 1.1 eq.) was added to a stirred solution of  $d_8$ -2-ethylaniline (**18**) (96 mg, 0.74 mmol, 1 eq.) in  $\text{DCM}$  (10 mL) and  $\text{NEt}_3$  on ice (228.6  $\mu\text{L}$ , 1.63 mmol, 2.2 eq.) under  $\text{N}_2$  atmosphere and reacted according to the general procedure B.

Purification by chromatography column, eluting with petroleum ether/ ethyl acetate (3/1) gave the *title compound* as a yellow powder (39 mg, 31%): Found  $M^+$ , 171.1500.  $C_{10}H_5D_8NO$  [ $M^+$ ] requires 171.1499; mp 106-108 °C;  $\nu_{max}$  (KBr) 3459, 3374, 3227, 3048, 3006, 2936, 1619, 1561 and 1446;  $\delta_H$  (400 MHz; DMSO) 7.32 (0.08H, s), 7.21 (0.77H, s), 7.12 (0.16H, m), 2.55 (0.36H, m), 2.04 (3H, s), 1.05 (0.24H, m);  $\delta_C$  (150 MHz,  $CD_3OD$ ) 172.5 (s, C=O), 140.5 (s, C-NH), 136.3 (s, C-C), 129.8 (s, 1CH), 127.9 (m, C-D), 127.0 (m, C-D), 126.9 (m, C-D), 25.3 (m,  $CD_2$ ), 24.2 (s,  $CH_3$ ), 13.5 (m,  $CD_3$ );  $m/z$  (EI) 171 ( $M^+$ , 60%).

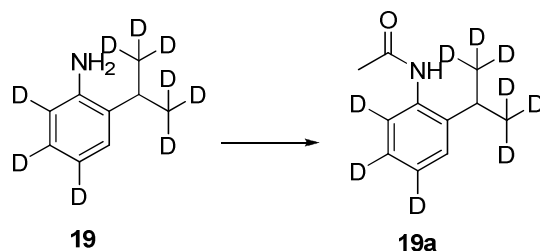
#### **4,5,6-Trideutero-2-(1,1,1,3,3,3-hexadeuteroprop-2-yl)aniline (19)**



A solution of 2-isopropylaniline (300 mg, 314  $\mu$ L, 2.22 mmol, 1 eq.) was added to a stirred solution of  $K_2PtCl_4$  (184 mg, 0.44 mmol, 20 mol %) and DCl (35%) (731  $\mu$ L, 8.88 mmol, 4 eq.) in  $D_2O$  (3 mL) and reacted according to the general procedure A. To the solution thioglycolic acid (136  $\mu$ L, 1.92 mmol, 4 eq. referred to the catalyst amount) was added and the mixture was stirred for 30 min, the rest of the the work up follow the general procedure A to give the *title compound* as a brown oil (229 mg, 72%): Found  $M^+$ , 144.1615.  $C_9H_4D_9N$  [ $M^+$ ] requires 144.1613;  $\nu_{max}$  (KBr) 3464, 3375, 3228, 3060, 2929, 2067, 1619, 1561, 1446, 1383 and 1302;  $\delta_H$  (400 MHz;  $CD_3OD$ ) <sup>a</sup>7.08 (0.94H, s), <sup>a</sup>6.92 (0.09H, s), <sup>a</sup>6.70 (0.18H, m), <sup>a</sup>2.97 (0.71H, m), <sup>a</sup>1.22 (0.98H, d,  $J_{H-H}$  6.98);  $\delta_C$  (150 MHz,  $CD_3OD$ ) 144.9 (s, C-NH<sub>2</sub>), 134.4(s, C-C), 126.8 (t, C-D,  $J_{C-D}$  24), 126.0 (s, C-H), 119.6 (t, C-D,  $J_{C-D}$  24), 117.0 (t, C-D,  $J_{C-D}$  24), 28.0(s, C-H), 22.5 (m,  $CD_3$ );  $m/z$  (EI) 144 ( $M^+$ , 30%).

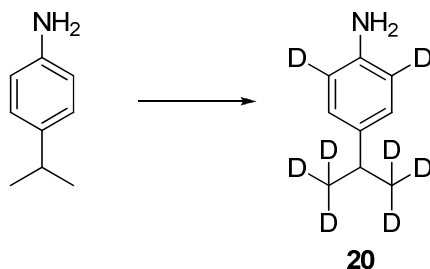
<sup>a</sup>Signals arise from the presence of isotopologues.

**N-[4,5,6-trideutero-2-(1,1,1,3,3,3-hexadeuteroprop-2-yl)phenyl]acetamide (19a)**



Acetyl chloride (92.43  $\mu\text{L}$ , 1.3 mmol, 1.1 eq.) was added to a stirred solution of *d*<sub>10</sub>-2-isopropylaniline (**19**) (180 mg, 1.23 mmol, 1 eq.) in DCM (5 mL) and NEt<sub>3</sub> on ice (379  $\mu\text{L}$ , 2.70 mmol, 2.2 eq.) under N<sub>2</sub> atmosphere and reacted according to the general procedure **B** to give the *title compound* as a yellow powder (162 mg, 72 %): Found M<sup>+</sup>, 186.1787. C<sub>11</sub>H<sub>6</sub>D<sub>9</sub>NO [M<sup>+</sup>] requires 186.1781; mp 43-47 °C;  $\nu_{\text{max}}$  (KBr) 3289, 3013, 2959, 29525, 2780, 2130, 2066, 1653, 1522, 1289, 1011, 973, 694 and 608;  $\delta_{\text{H}}$  (400 MHz; CD<sub>3</sub>OD) 7.34 (0.95H, s), 7.24 (0.09H, s), 7.18 (0.18H), 3.13 (0.71H, s), 2.14 (3H, s), 1.58 (0.98H, d,  $J_{\text{H-H}}$  6.98);  $\delta_{\text{C}}$  (150 MHz, CD<sub>3</sub>OD) 172.7 (s, C=O), 145.6 (s, C-NH), 135.3 (s, C-C), 128.0 (m, C-D), 126.9 (t, C-D,  $J_{\text{C-D}}$  24), 126.8 (s, C-H), 28.8 (m, C-H), 23.5 (m, CD<sub>3</sub>), 22.9 (s, CH<sub>3</sub>); *m/z* (EI) 186 (M<sup>+</sup>, 92%).

**2,6-Dideutero-4-(1,1,1,3,3,3-hexadeuteroprop-2-yl)aniline (20)**



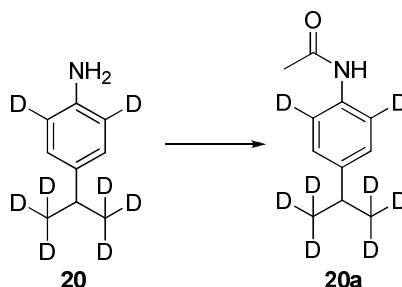
A solution of 4-isopropylaniline (300 mg, 303  $\mu\text{L}$ , 2.22 mmol, 1 eq.) was added to a stirred solution of K<sub>2</sub>PtCl<sub>4</sub> (182 mg, 0.44 mmol, 20 mol %) and DCl (35%) (731  $\mu\text{L}$ , 8.88 mmol, 4 eq.) in D<sub>2</sub>O (3 mL) and reacted according to the general procedure **A**. To the solution thioglycolic acid (122  $\mu\text{L}$ , 1.76 mmol, 4 eq. referred to the catalyst amount) was added and the mixture was stirred for 30 min, the rest of the the work up follow the general procedure **A** to give the *title compound* as a green oil (209 mg, 62%); Found M<sup>+</sup>, 143.1678. C<sub>9</sub>H<sub>5</sub>D<sub>8</sub>N [M<sup>+</sup>] requires 143.1676;  $\nu_{\text{max}}$  (KBr) 3349, 3216, 3022, 2927, 2889, 2065, 1618, 1477, 1460,

1259;  $\delta_{\text{H}}$  (400 MHz; CD<sub>3</sub>OD) <sup>a</sup>6.93 (1.12H, s), <sup>a</sup>6.68 (0.19H, s), <sup>a</sup>2.46 (0.72H, s), <sup>a</sup>1.57(0.53H, s);  $\delta_{\text{C}}$  (150 MHz, CD<sub>3</sub>OD) 145.8 (s, C-NH<sub>2</sub>), 140.2(s, C-C), 127.7 (s, C-H), 116.7 (t, C-D,  $J_{\text{C-D}}$  23), 34.2(s, C-H), 23.9 (m, CD<sub>3</sub>);  $m/z$  (EI) 143 (M<sup>+</sup>, 60%).

<sup>a</sup>Signals arise from the presence of isotopologues.

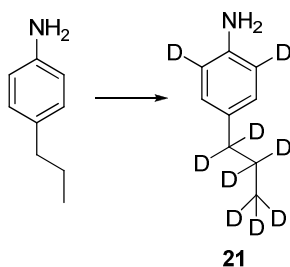
### ***N*-[2,6-Dideutero-4-(1,1,1,3,3,3-hexadeuteroprop-2-yl)phenyl]acetamide**

#### **(20a)**



Acetyl chloride (92.43  $\mu\text{L}$ , 1.3 mmol, 1.1 eq.) was added to a stirred solution of *d*<sub>6</sub>-4-isopropylaniline (**20**) (166 mg, 1.16 mmol, 1 eq.) in DCM (5 mL) and NEt<sub>3</sub> on ice (337  $\mu\text{L}$ , 2.5 mmol, 2.2 eq.) under N<sub>2</sub> atmosphere and reacted according to the general procedure **B** to give the *title compound* as a yellow powder (128 mg, 60 %): Found M<sup>+</sup>, 185.1783. C<sub>11</sub>H<sub>7</sub>D<sub>8</sub>NO[M<sup>+</sup>] requires 185.1781; mp 91-100 °C;  $\nu_{\text{max}}$  (KBr) 3284, 3240, 3167, 3095, 3041, 2963, 2934, 2893, 2065, 1662, 1604, 1540, 1515, 1370 and 1306;  $\delta_{\text{H}}$  (400 MHz; CD<sub>3</sub>OD) 7.42 (0.19H, m), 7.16 (1.12H, s), 2.82 (0.77H, s), 2.10 (3H, s), 1.18 (0.53H, m);  $\delta_{\text{C}}$  (150 MHz, CD<sub>3</sub>OD) 171.6 (s, C=O), 146.1 (s, C-NH), 137.4 (s, C-C), 127.4 (s, C-H), 121.2 (m, C-D), 34.4 (s, C-H), 23.7 (s, CH<sub>3</sub>), 13.5 (m, CD<sub>3</sub>);  $m/z$  (EI) 185 (M<sup>+</sup>, 90%).

### **2,6-Dideutero-4-(1,1,2,2,3,3,3-heptadeuteropropyl)aniline (21)**

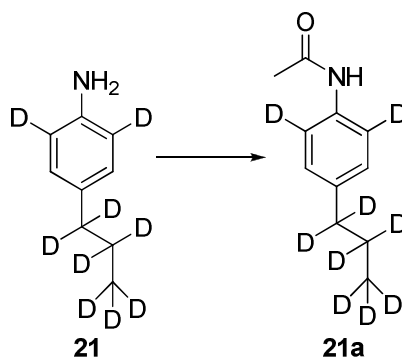


A solution of 4-*n*-propylaniline (300 mg, 326  $\mu\text{L}$ , 2.22 mmol, 1 eq.) was added to a stirred solution of K<sub>2</sub>PtCl<sub>4</sub> (184 mg, 0.44 mmol, 20 mol %) and DCl (35%) (732  $\mu\text{L}$ , 8.88 mmol, 4

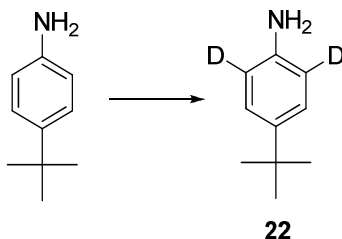
eq.) in D<sub>2</sub>O (3 mL) and reacted according to the general procedure A. To the solution thioglycolic acid (122 μL, 1.76 mmol, 4 eq. referred to the catalyst amount) was added and the mixture was stirred for 30 min, the rest of the work up follow the general procedure A to give the *title compound* as a brown oil (268 mg, 87%): Found M<sup>+</sup>, 144.1734. C<sub>9</sub>H<sub>4</sub>D<sub>9</sub>N [M<sup>+</sup>] requires 144.1738; ν<sub>max</sub>(KBr) 3354, 3240, 3020, , 2907, 2509, 1581, 2102, 1619, 1258, and 1258; δ<sub>H</sub> (400 MHz; CD<sub>3</sub>OD) <sup>a</sup>6.93(0.46H, s), <sup>a</sup>6.68(0.19H, s), <sup>a</sup>2.46(0.40H, s), <sup>a</sup>1.57(0.57H, s), <sup>a</sup>0.91(0.42H, s); δ<sub>c</sub>(100 MHz, CD<sub>3</sub>OD) 144.6 (s, C-NH<sub>2</sub>), 132.7(s, C-C), 128.9 (s, C-H), 115.7 (t, C-D, J<sub>C-D</sub> 24), 36.7(m, CD<sub>2</sub>) , 24.2 (m, CD<sub>2</sub>), 12.4 (m, CD<sub>3</sub>); *m/z* (EI) 144 (M<sup>+</sup>, 70%).

<sup>a</sup>Signals arise from the presence of isotopologues.

**N-[2,6-dideutero-4-(1,1,2,2,3,3,3-heptadeuteropropyl)phenyl]acetamide**  
**(21a)**

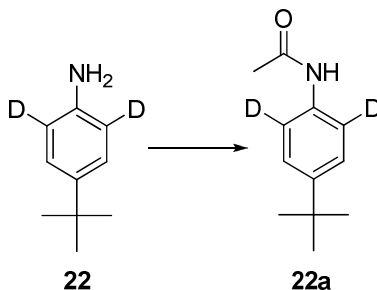


Acetyl chloride (70.3 μL, 0.98 mmol, 1.1 eq.) was added to a stirred solution of *d*<sub>9</sub>-4-*n*-propylaniline (**21**) (125 mg, 0.89 mmol, 1 eq.) in DCM (5 mL) and NEt<sub>3</sub> on ice (274.6 μL, 1.96 mmol, 2.2 eq.) under N<sub>2</sub> atmosphere and reacted according to the general procedure B. Purification by chromatography column, eluting with petroleum ether/ ethyl acetate (3/1) gave the *title compound* as a brown powder (63 mg, 39 %): Found M<sup>+</sup>, 186.1838. C<sub>11</sub>H<sub>6</sub>D<sub>9</sub>NO [M<sup>+</sup>] requires 186.1844, mp 82-86 °C; ν<sub>max</sub> (KBr) 3281, 3168, 3094, 3025, 2962, 2930, 2854, 2386, 2102, 2067, 1637, 1595 and 1479; δ<sub>H</sub> (400 MHz; CD<sub>3</sub>OD) 7.30 (0.19H, m), 6.98 (1.53H, s), 2.39 (0.40H, m), 1.98 (3H,s), 1.45 (0.57H, m), 0.75 (0.42H, m); δ<sub>c</sub> (100 MHz, CD<sub>3</sub>OD) 171.9 (s, C=O), 140.1 (s, C-NH), 137.8 (s, C-C), 130.1(s, C-H), 121.3 (t, C-D, J<sub>C-D</sub> 24), 38.0 (m, CD<sub>2</sub>), 25.3 (m,CD<sub>2</sub>), 24.2 (s, CH<sub>3</sub>), 13.5 (m, CD<sub>3</sub>); *m/z* (EI) 186 (M<sup>+</sup>, 90%).

**2,6-Dideutero-4-tert-butylaniline (22)**

A solution of 4-*tert*-butylaniline (300 mg, 318  $\mu$ L, 2.01 mmol, 1 eq.) was added to a stirred solution of  $K_2PtCl_4$  (166 mg, 0.40 mmol, 20 mol %) and DCl (35%) (662  $\mu$ L, 8.04 mmol, 4 eq.) in  $D_2O$  (3 mL) and reacted according to the general procedure A. To the solution thioglycolic acid (113  $\mu$ L, 1.60 mmol, 4 eq. referred to catalyst amount) was added and the mixture was stirred for 30 min, the rest of the the work up follows the general procedure A was added to give the *title compound* as a brown oil (167 mg, 55%): Found  $M^{+}$ , 151.1334.  $C_{10}H_{13}D_2N$  [ $M^{+}$ ] requires 151.1330;  $\nu_{max}$  (KBr) 3434, 3353, 3215, 3063, 3034, 2927, 2868, 2253, 1619, 1500, 1216, 1043 and 898;  $\delta_H$  (400 MHz;  $CD_3OD$ )  $^a$ 7.13(1.83H, s),  $^a$ 6.67(0.23H, d,  $J_{H-H}$  9.03),  $^a$ 1.25 (7.72H, s);  $\delta_C$  (150 MHz,  $CD_3OD$ ) 145.1 (s, C-NH<sub>2</sub>), 142.1 (s, C-C), 126.5 (s, C-H), 116.2 (t, C-D,  $J_{C-D}$  25), 34.5 (m, C-CD<sub>3</sub>), 31.7 (m, CD<sub>3</sub>);  $m/z$  (EI) 151 ( $M^{+}$ , 40%).

<sup>a</sup>Signals arise from the presence of isotopologues.

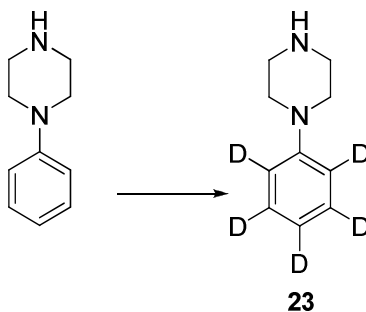
**N-[2,6-dideutero-4-tert-butylphenyl]acetamide (22a)**

Acetyl chloride (65.8  $\mu$ L, 0.88 mmol, 1.1 eq.) was added to a stirred solution of *d*<sub>2</sub>-4-*tert*-butylaniline (**22**) (127 mg, 0.84 mmol, 1 eq.) in DCM (10 mL) and  $NEt_3$  on ice (259.5  $\mu$ L, 1.85 mmol, 2.2 eq.) under  $N_2$  atmosphere and reacted according to the general procedure B to give the *title compound* as a colourless powder (102 mg, 63%): Found  $M^{+}$ , 193.1435.  $C_{12}H_{15}D_2NO$  [ $M^{+}$ ] requires 193.1436; mp 154-159  $^{\circ}C$ ;  $\nu_{max}$  (KBr) 3289, 3248, 3172, 3096, 3034, 2958, 2926, 2865, 1688, 1670, 1604, 1537, 1471, 1382, 1321, 1266, 1043, 1010, 969,



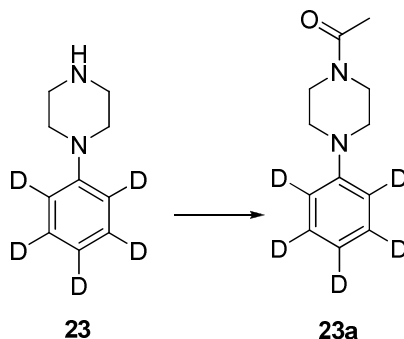
899 and 765;  $\delta_{\text{H}}$  (400 MHz;  $\text{CD}_3\text{OD}$ ) 7.41 (0.23H, d,  $J_{\text{H-H}}$  9.03), 7.34 (1.83H, s), 2.10 (3H, s), 1.30 (7.72H, s);  $\delta_{\text{C}}$  (150 MHz,  $\text{CD}_3\text{OD}$ ) 171.5 (s, C=O), 148.1 (s, C-NH), 137.0(s, C-C), 126.4(s, C-H), 120.7 (t, C-D,  $J_{\text{C-D}}$  24), 35.0 (m, C- $\text{CD}_3$ ), 31.5 (m,  $\text{CD}_3$ ), 23.7 (s,  $\text{CH}_3$ );  $m/z$  (EI) 193 ( $\text{M}^+$ , 30%).

### **1-(2,3,4,5,6-Pentadeuterophenyl)piperazine (23)**

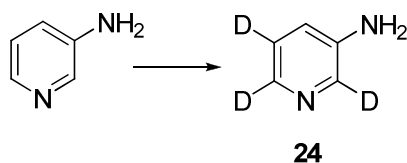


A solution of 1-phenylpiperazine (300 mg, 282  $\mu\text{L}$ , 1.85 mmol, 1 eq.) was added to a stirred solution of  $\text{K}_2\text{PtCl}_4$  (153 mg, 0.37 mmol, 20 mol %) and  $\text{DCl}$  (35%) (609  $\mu\text{L}$ , 7.4 mmol, 4 eq.) in  $\text{D}_2\text{O}$  (3 mL) and reacted according to the general procedures A. To the solution thioglycolic acid (113  $\mu\text{L}$ , 1.61 mmol, 4 eq. referred to catalyst amount) was added and the mixture was stirred for 30 min, the rest of the work up follow the general procedure A to give the *title compound* as a yellow oil (257 mg, 83%): Found  $\text{M}^+$ , 167.1470.  $\text{C}_{10}\text{H}_9\text{D}_5\text{N}_2$  [ $\text{M}^+$ ] requires 167.1471;  $\nu_{\text{max}}$  (neat) 3302, 3047, 2947, 2828, 1575, 1550, 1436, 1381, 1326, 1274 and 1227;  $\delta_{\text{H}}$  (400 MHz;  $\text{CD}_3\text{OD}$ ) <sup>a</sup>7.23 (1.00H, s), <sup>a</sup>7.10 (0.02H, d,  $J_{\text{H-H}}$  3.55), <sup>a</sup>6.96 (0.02H, d,  $J_{\text{H-H}}$  3.55), 3.10 (4H, dt,  $J_{1\text{H-H}}$  16.2,  $J_{2\text{H-H}}$  5.8), 2.95 (4H, dt,  $J_{1\text{H-H}}$  16.2,  $J_{2\text{H-H}}$  5.8);  $\delta_{\text{C}}$  (150 MHz,  $\text{CD}_3\text{OD}$ ) 153.0(s, C- $\text{NH}_2$ ), 129.8 (s, C-H), 120.7 (t, C-D,  $J_{\text{C-D}}$  25), 117.5 (t, C-D,  $J_{\text{C-D}}$  25), 51.1 (s,  $\text{CH}_2$ ), 46.4 (s,  $\text{CH}_2$ );  $m/z$  (EI) 167 ( $\text{M}^+$ , 45%).

<sup>a</sup>Signals arise from the presence of isotopologues.

**1-Acetyl-4-(2,3,4,5,6-pentadeuterophenyl)piperazine (23a)**

Acetyl chloride (94.2  $\mu\text{L}$ , 1.20 mmol, 1.1 eq.) was added to a stirred solution of *d*<sub>5</sub>-1-phenylpiperazine (**23**) (191 mg, 1.09 mmol, 1 eq.) in DCM (10 mL) and NEt<sub>3</sub> on ice (242  $\mu\text{L}$ , 2.40 mmol, 2.2 eq.) under N<sub>2</sub> atmosphere and reacted according to the general procedure **B** to give the *title compound* as a colourless powder (56 mg, 24%): Found M<sup>+</sup>, 209.1572. C<sub>12</sub>H<sub>11</sub>D<sub>5</sub>N<sub>2</sub>O [M<sup>+</sup>] requires 209.1576; mp 43-48 °C;  $\nu_{\text{max}}$  (KBr) 3282, 2916, 2849, 2815, 1627, 1573, 1428, 1277, 1254, 1226, 1043, 1002 and 985;  $\delta_{\text{H}}$  (400 MHz; CD<sub>3</sub>OD) 7.24 (1.07H, s), 7.09 (0.02H, d,  $J_{\text{H-H}}$  3.55), 6.96 (0.02H, d,  $J_{\text{H-H}}$  3.55), 3.73 (4H, dt,  $J_{1\text{ H-H}}$  16.2,  $J_{2\text{ H-H}}$  5.8), 3.17 (4H, dt,  $J_{1\text{ H-H}}$  16.2,  $J_{2\text{ H-H}}$  5.8), 2.14(3H,s);  $\delta_{\text{C}}$  (150 MHz, CD<sub>3</sub>OD) 171.7 (s, C=O), 152.4 (s, C-NH), 129.9 (s, C-H), 121.1 (t, C-D,  $J_{\text{C-D}}$  24), 117.7 (t, C-D,  $J_{\text{C-D}}$  24), 51.0(s, C-NH), 47.4(s, CH<sub>2</sub>), 42.7 (s, CH<sub>2</sub>), 21.1 (s, CH<sub>3</sub>); *m/z* (EI) 167 (M<sup>+</sup> - COCH<sub>3</sub>, 40%).

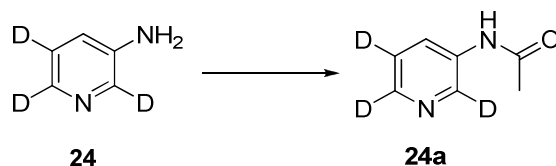
**2,5,6-Trideutero-3-aminopyridine (24)**

A solution of 3-aminopyridine (300 mg, 3.19 mmol, 1 eq) was added to a stirred solution of K<sub>2</sub>PtCl<sub>4</sub> (261 mg, 0.63 mmol, 20 mol %) and DCl (35%) (1 mL, 12.7 mmol, 4 eq) in D<sub>2</sub>O (3 mL) and reacted according to the general procedure **A**. To the solution thioglycolic acid (232 mg, 178  $\mu\text{L}$ , 2.52 mmol, 4 eq referred to the catalyst amount) was added and the mixture was stirred for 30 min, the rest of the the work up follow the general procedure **A** to give the *title compound* as a colourless powder (181 mg, 58 %): Found M<sup>+</sup>, 97.0783. C<sub>5</sub>H<sub>3</sub>D<sub>3</sub>N<sub>2</sub> [M<sup>+</sup>] requires 97.0783; mp 48-51 °C;  $\nu_{\text{max}}$  (KBr) 3338, 3210, 3210, 2522, 1631, 1579, 1441, 1414 and 1260;  $\delta_{\text{H}}$  (400 MHz; CD<sub>3</sub>OD) <sup>a</sup>7.97 (0.04H, s), <sup>a</sup>7.76 (0.17H, s), <sup>a</sup>7.06 (0.26H, s), <sup>a</sup>6.92

(0.60H, s);  $\delta_c$ (100 MHz, CD<sub>3</sub>OD) 146.8 (s, C-NH<sub>2</sub>), 138.5 (t, C-D,  $J_{C-D}$  29), 137.3 (t, C-D,  $J_{C-D}$  29), 125.8 (t, C-D,  $J_{C-D}$  27), 123.4 (s, C-H);  $m/z$  (EI) 97 (M<sup>+</sup>, 100).

<sup>a</sup>Signals arise from the presence of isotopologues.

### **N-[2,5,6-Trideuteropyridin-3-yl]acetamide (24a)**



*d*<sub>3</sub>-3-Aminopyridine (**24**) (89 mg, 0.91 mmol, 1 eq.) was dissolved in acetone (15 mL), and K<sub>2</sub>CO<sub>3</sub> (626.6 mg, 4.54 mmol, 5 eq.) was added, followed by the dropwise addition of acetyl chloride (214 mg, 194  $\mu$ L, 2.73 mmol, 3 eq.) in acetone (5 mL) and reacted according to the general procedure C to give the title compound as a brown powder (82 mg, 64%): Found M<sup>+</sup>, 139.0890. C<sub>7</sub>H<sub>4</sub>D<sub>3</sub>N<sub>2</sub>O [M<sup>+</sup>] requires 139.0888; mp 128-131 °C;  $\nu_{\max}$  (KBr) 3223, 3143, 2963, 2921, 1842, 1685 and 1673;  $\delta_H$  (400 MHz; CD<sub>3</sub>OD): 8.73 (0.04H, s), 8.26 (0.17H, s), 8.12 (0.60H, s), 7.41 (0.26, s), 2.18 (3H, s);  $\delta_c$  (150 MHz, CD<sub>3</sub>OD) 17202 (s, C=O), 144.6 (t, C-D,  $J_{C-D}$  29), 144.2 (t, C-D,  $J_{C-D}$  28), 137.4 (s, C-NH), 128.7 (s, C-H), 125.1 (t, C-D,  $J_{C-D}$  28), 24.4 (s, CH<sub>3</sub>);  $m/z$  (EI) 139 (M<sup>+</sup>, 20%).

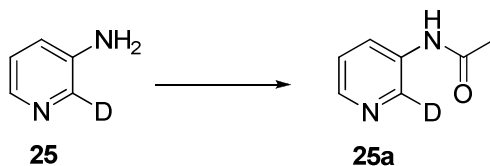
### **2-Deutero-3-aminopyridine (25)**



A solution of 3-aminopyridine (300 mg, 3.19 mmol, 1 eq.) was added to a stirred solution of DCl (35%) (1 mL, 12.7 mmol, 4 eq.) in D<sub>2</sub>O (3 mL) and reacted according to the general procedure A to give the *title compound* as a colourless powder (129 mg, 42%): Found M<sup>+</sup>, 95.0595. C<sub>5</sub>H<sub>5</sub>DN<sub>2</sub> [M<sup>+</sup>] requires 95.0594; mp 48-51 °C;  $\nu_{\max}$  (KBr) 3351, 3175, 3074, 1635, 1582, 1463 and 1435;  $\delta_H$  (400 MHz; CD<sub>3</sub>OD) <sup>a</sup>7.97 (0.05H, s), <sup>a</sup>7.66 (0.79H, dd,  $J_{1-H-H}$  4.45,  $J_{2-H-H}$  1.67), <sup>a</sup>7.00 (1.60H, m); <sup>b</sup> $\delta_c$  (100 MHz, CD<sub>3</sub>OD) 146.8 (s, C-NH<sub>2</sub>), 138.5 (s, C-H), 125.8 (s, C-H), 123.4 (s, C-H);  $m/z$  (EI) 95 (M<sup>+</sup>, 100%).

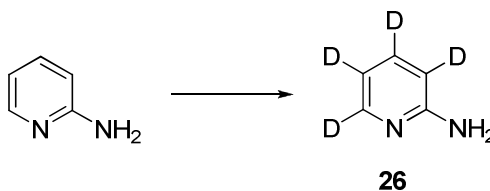
<sup>a</sup>Signals arise from the presence of isotopologues.

<sup>b</sup>Signal presents at too high deuteration level for recording of <sup>13</sup>C.

**N-[2-Deuteropyridin-3-yl]acetamide (25a)**

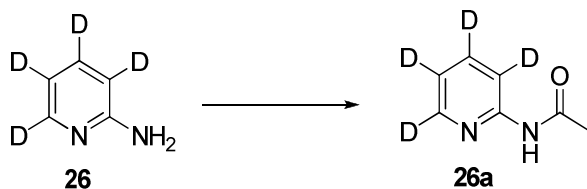
*d*<sub>1</sub>-3-Aminopyridine (**25**) (113 mg, 1.15 mmol, 1 eq.) was dissolved in acetone (15 mL), and K<sub>2</sub>CO<sub>3</sub> (729.3 mg, 5.78 mmol, 5 eq.) was added, followed by the dropwise addition of acetyl chloride (270 mg, 245 μL, 3.45 mmol, 3 eq.) in acetone (5 mL) and reacted according to the general procedure C to give the *title compound* as a colourless powder (40 mg, 24%): Found M<sup>+</sup>, 137.0700. C<sub>7</sub>H<sub>7</sub>DN<sub>2</sub>O [M<sup>+</sup>] requires 137.0699; mp 127-131 °C; ν<sub>max</sub> (KBr) 3294, 3237, 3167, 3102, 2887, 2826, 1686 and 1607; δ<sub>H</sub> (400 MHz; CD<sub>3</sub>OD): 8.73 (0.05H, s), 8.26 (0.79H, dd, *J*<sub>1 H-H</sub> 4.45, *J*<sub>2 H-H</sub> 1.67), 8.12 (0.80H, dd, *J*<sub>1 H-H</sub> 8.37, *J*<sub>2 H-H</sub> 1.42), 7.41 (0.80, dd, *J*<sub>1 H-H</sub> 8.19, *J*<sub>2 H-H</sub> 4.80), 2.18 (3H, s); <sup>b</sup>δ<sub>C</sub> (100 MHz, CD<sub>3</sub>OD) 172.5 (s, C = O), 145.5 (s, C-H), 137.9 (s, C-NH), 129.3 (s, C-H), 125.8 (s, C-H), 24.2 (s, CH<sub>3</sub>); *m/z* (EI) 137 (M<sup>+</sup>, 95%).

<sup>b</sup>Signal presents a too high deuteration level for recording on <sup>13</sup>C.

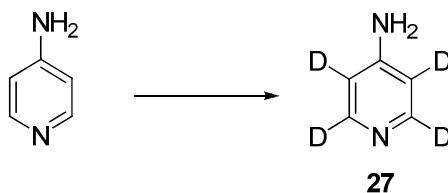
**3,4,5,6-Tetradeutero-2-aminopyridine (26)**

A solution of 2-aminopyridine (300 mg, 3.19 mmol, 1 eq.) was added to a stirred solution of K<sub>2</sub>PtCl<sub>4</sub> (264 mg, 0.64 mmol, 20 mol %) and DCL (35%) (1 mL, 12.7 mmol, 4 eq.) in D<sub>2</sub>O (3 mL) and reacted according to the general procedure A. To the solution thioglycolic acid (181 μL, 2.52 mmol, 4 eq. referred to catalyst amount) was added and the mixture was stirred for 30 min, the rest of the work up follows the general procedure A to give the *title compound* as a colourless powder (247 mg, 79%): Found M<sup>+</sup>, 98.0782. C<sub>5</sub>H<sub>2</sub>D<sub>4</sub>N<sub>2</sub> [M<sup>+</sup>] requires 98.0784; mp 43-49 °C; ν<sub>max</sub> (KBr) 3445, 3304, 3305, 3166, 2532, 1629, 1569, 1535 and 1428; δ<sub>H</sub> (400 MHz; CD<sub>3</sub>OD,) <sup>a</sup>7.87 (0.13H, s), <sup>a</sup>7.44 (0.17H, s), <sup>a</sup>7.44 (0.09H, s); δ<sub>C</sub> (150 MHz, CD<sub>3</sub>OD) 160.8 (s, C-NH<sub>2</sub>), 147.4 (t, C-D, *J*<sub>C-D</sub> 26), 138.8 (t, C-D, *J*<sub>C-D</sub> 24), 113.5 (t, C-D, *J*<sub>C-D</sub> 26), 110.0 (t, C-D, *J*<sub>C-D</sub> 25); *m/z* (EI) 98 (M<sup>+</sup>, 100).

<sup>a</sup>Signals arise from the presence of isotopologues.

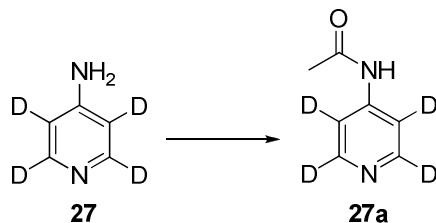
**N-[3,4,5,6-Tetradeuteropyridin-2-yl]acetamide (26a)**

*d*<sub>4</sub>-2-Aminopyridine (**26**) (190 mg, 1.9 mmol, 1 eq.) was dissolved in acetone (50 mL), and K<sub>2</sub>CO<sub>3</sub> (1.34 g, 9.6 mmol, 5 eq.) was added, followed by the dropwise addition of acetyl chloride (447.4 mg, 405 μL, 5.7 mmol, 3 eq.) in acetone (5 mL) and reacted according to the general procedure C. Purification by chromatography column, eluting with DCM/MeOH (100/1) gave the *title compound* as a brown powder (160 mg, 60 %): Found M<sup>+</sup>, 140.0891. C<sub>7</sub>H<sub>4</sub>D<sub>4</sub>N<sub>2</sub>O[M<sup>+</sup>] requires 140.0888; mp 56-63 °C; ν<sub>max</sub> (KBr) 3217, 3139, 3046, 3002, 2321, 1695, 1549 and 1510; δ<sub>H</sub> (400 MHz; CD<sub>3</sub>OD): 8.27 (0.13H, s), 8.07 (0.09H, s), 7.75 (0.09H, s), 7.09 (0.07H, s); 2.08 (3H, s); δ<sub>c</sub> (100 MHz, CD<sub>3</sub>OD) 170.7 (s, C=O), 151.6(s, C-NH), 147.5 (m, C-D), 137.8(m, C-D), 119.2 (m, C-D), 114.1 (m, C-D), 22.7 (s, CH<sub>3</sub>); *m/z* (EI) 140 (M<sup>+</sup>, 45%).

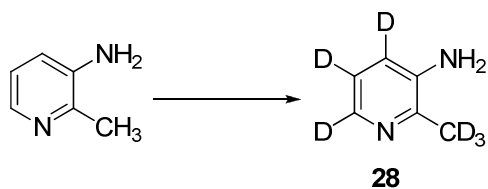
**2,3,5,6-Tetradeutero-4-aminopyridine (27)**

A solution of 4-aminopyridine (300 mg, 3.19 mmol, 1 eq) was added to a stirred solution of K<sub>2</sub>PtCl<sub>4</sub> (264 mg, 0.64 mmol, 20 mol %) and DCl (35%) (1mL, 12.7 mmol, 4 eq) in D<sub>2</sub>O (3 mL) and reacted according to the general procedure A. To the solution thioglycolic acid (181 μL, 2.52 mmol, 4 eq referred to catalyst amount) was added and the mixture was stirred for 30 min, the rest of the work up follows the general procedure A to give the *title compound* as a colourless powder (73 mg, 23%): Found M<sup>+</sup>, 98.0782. C<sub>5</sub>H<sub>2</sub>D<sub>4</sub>N<sub>2</sub> [M<sup>+</sup>] requires 98.0782; mp 143-147 °C; ν<sub>max</sub> (KBr) 3436, 3304, 3074, 3081, 2531, 1647 and 1576; δ<sub>H</sub> (400 MHz; CD<sub>3</sub>OD) <sup>a</sup>7.97 (0.53H, s), <sup>a</sup>6.57 (0.16H, s); δ<sub>c</sub> (150 MHz, CD<sub>3</sub>OD) 156.8 (s, C-NH<sub>2</sub>), 149.5 (t, C-D, *J*<sub>C-D</sub> 26), 110.2 (m, C-D); *m/z* (EI) 98 (M<sup>+</sup>, 100%).

<sup>a</sup>Signals arise from the presence of isotopologues.

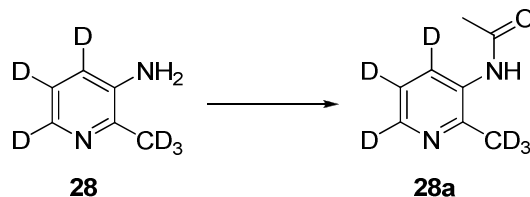
**N-[2,3,5,6-Tetradeuteropyridin-4-yl]acetamide (27a)**

*d*<sub>4</sub>-4-Aminopyridine (**27**) (73 mg, 0.74 mmol, 1 eq.) was dissolved in acetone (15 mL), and K<sub>2</sub>CO<sub>3</sub> (516mg, 3.74 mmol, 5 eq.) was added, followed by dropwise addition of acetyl chloride (174 mg, 157μL, 2.22 mmol, 3 eq.) in acetone (5 ml) and reacted according to the general procedure C to give the *title compound* as a colourless powder (50 mg, 48%): Found M<sup>+</sup>, 140.0892. C<sub>7</sub>H<sub>4</sub>D<sub>4</sub>N<sub>2</sub>O [M<sup>+</sup>] requires 140.0888; mp 138-141 °C; ν<sub>max</sub> (KBr) 3444, 3311, 3146, 3046, 1688, 1615, 1565 and 1508; δ<sub>H</sub> (400 MHz; CD<sub>3</sub>OD): 8.39(0.16H, s), 7.63(0.53H, s), 2.18 (3H, s); δ<sub>c</sub> (150 MHz, CD<sub>3</sub>OD) 172.4 (s, C=O), 150.7(t, C-D, J<sub>C-D</sub> 29), 148.0 (s, C-NH), 114.7 (m, C-D), 24.2 (s, CH<sub>3</sub>); *m/z* (EI) 140 (M<sup>+</sup>, 98%).

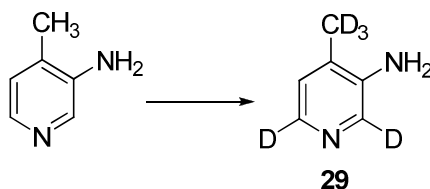
**5,6-Dideutero-3-amino-2-(trideuteromethyl)pyridine (28)**

A solution of 3-amino-2-methylpyridine (150 mg, 1.3 mmol, 1 eq.) was added to a stirred solution of K<sub>2</sub>PtCl<sub>4</sub> (113 mg, 0.27 mmol, 20 mol %.) and DCl (35%) (454 μL, 5.52 mmol, 4 eq.) in D<sub>2</sub>O (3 mL) and reacted according to the general procedure A. To the solution thioglycolic acid (76 μL, 1.08 mmol, 4 eq. referred to catalyst amount) was added and the mixture was stirred for 30 min, the rest of the work up follow the general procedure A to give the *title compound* as a colourless powder (95 mg, 64%): Found M<sup>+</sup>, 114.1061. C<sub>6</sub>H<sub>2</sub>D<sub>6</sub>N<sub>2</sub> [M<sup>+</sup>] requires 114.1064; mp 91-97 °C; ν<sub>max</sub> (KBr) 3345, 3311, 3164, 2536, 2404, 2367, 2194, 1631, 1553, 1436, 1264, 1197, 1042 and 877; δ<sub>H</sub> (400 MHz; CD<sub>3</sub>OD) <sup>a</sup>7.70 (0.04H, d, J<sub>H-H</sub> 5.13), <sup>a</sup>7.06 (0.33H, s), <sup>a</sup>6.99 (0.06H d, J<sub>H-H</sub> 8.05), <sup>a</sup>2.32 (0.44 H, m); δ<sub>c</sub> (150 MHz, CD<sub>3</sub>OD) 144.5 (s, C-NH<sub>2</sub>), 144.4 (s, C-CD<sub>3</sub>), 137.9 (t, C-D, J<sub>C-D</sub> 28), 123.5 (t, C-D, J<sub>C-D</sub> 25), 123.9 (s, C-D, J<sub>C-D</sub> 27), 19.5 (m, CD<sub>3</sub>); *m/z* (EI) 114 (M<sup>+</sup>, 75%).

<sup>a</sup>Signals arise from the presence of isotopologues.

**N-[5,6-Dideutero-2-(trideuteromethyl)pyridin-3-yl]acetamide (28a)**

The solution of *d*<sub>6</sub>-3-amino-2-methylpyridine (**28**) (88 mg, 0.77 mmol, 1 eq.) in Ac<sub>2</sub>O (10 mL, 10.5 mmol, 6 eq.), was refluxed for 2h at 120°C. After cooling to room temperature the reaction mixture was treated by brine and diluted with NaOH 1M. The aqueous phase has been extracted with ethyl acetate (x3). The organics extracts were combined, dried over MgSO<sub>4</sub>, filtered and evaporated under vacuum to obtain colourless powder (50 mg, 41%): Found M<sup>+</sup>, 156.1169. C<sub>8</sub>H<sub>4</sub>D<sub>6</sub>N<sub>2</sub>O [M<sup>+</sup>] requires 156.1170; mp 46-52 °C; ν<sub>max</sub> (KBr) 3841, 3296, 3077, 3006, 2926, 2850, 2785, 1660, 1567, 1511, 1377, 1174, 1013, 954 and 705; δ<sub>H</sub> (400 MHz; CD<sub>3</sub>OD): 8.25 (0.04H, s), 7.88 (0.33H, s), 7.28 (0.04H, s), 2.46 (0.44 H,m), 2.18 (3H, s); δ<sub>c</sub> (150 MHz, CD<sub>3</sub>OD): 172.3 (s, C=O), 153.8 (s, C-NH), 146.5 (t, C-D, J<sub>C-D</sub> 27), 134.7 (t, C-D, J<sub>C-D</sub> 24), 134.1 (s, C-CD<sub>3</sub>), 122.7 (s, C-D, J<sub>C-D</sub> 27), 23.1 (s, CH<sub>3</sub>), 20.1 (m, CD<sub>3</sub>); *m/z* (EI) 156 (M<sup>+</sup>, 80%).

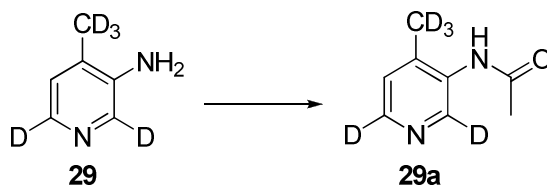
**2,6-Dideutero-3-amino-4-(trideuteromethyl)pyridine (29)**

A solution of 3-amino-4-methylpyridine (300 mg, 2.7 mmol, 1 eq.) was added to a stirred solution of K<sub>2</sub>PtCl<sub>4</sub> (224 mg, 0.54 mmol, 20 mol %) and DCl (35%) (889 μL, 10.8 mmol, 4 eq.) in D<sub>2</sub>O (3 mL) and reacted according to the general procedure A. To the solution thioglycolic acid (153 μL, 2.16 mmol, 4 eq. referred to catalyst amount) was added and left under stirring for half an hour. The rest of the work up follows the general procedure A to give the *title compound* as colourless powder (221 mg, 73%): Found M<sup>+</sup>, 113.1001. C<sub>6</sub>H<sub>3</sub>D<sub>5</sub>N<sub>2</sub> [M<sup>+</sup>] requires 113.1001; mp 100-104°C; ν<sub>max</sub> (KBr) 3441, 3325, 3201, 2573, 2523, 2194, 1643, 1587 and 1550; δ<sub>H</sub> (400 MHz; CD<sub>3</sub>OD,) <sup>a</sup>7.90 (0.05H, s), <sup>a</sup>7.70 (0.05H, d, J<sub>H-H</sub> 5.03), <sup>a</sup>7.03 (0.81H, s), <sup>a</sup>2.14 (0.18H, m); δ<sub>c</sub> (150 MHz, CD<sub>3</sub>OD) 144.7 (s, C-NH<sub>2</sub>), 138.9 (t, C-

D,  $J_{C-D}$  27), 138.6 (t, C-D,  $J_{C-D}$  27), 132.8 (s, C-C), 126.4 (s, C-H), 16.2 (m, CD<sub>3</sub>);  $m/z$  (EI) 113 (M<sup>+</sup>, 100%).

<sup>a</sup>Signals arise from the presence of isotopologues.

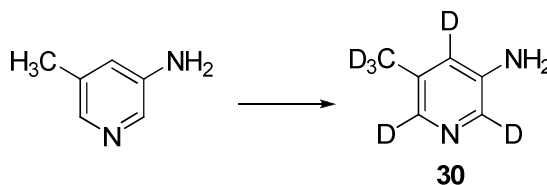
### **N-[2,6-Dideutero-4-(trideuteromethyl)pyridin-3-yl]acetamide (29a)**



*d*<sub>5</sub>-3-Amino-4-methylpyridine (**29**) (175 mg, 1.5 mmol, 1 eq.) was dissolved in acetone (20 mL), and K<sub>2</sub>CO<sub>3</sub> (1.05 g, 7.7 mmol, 5 eq.) was added, followed by the dropwise addition of acetyl chloride (353 mg, 319 μL, 4.6 mmol, 3 eq.) in acetone (5 ml) and reacted according to the general procedure C to give the *title compound* as a brown powder (113 mg, 48%): Found M<sup>+</sup>, 155.1107. C<sub>8</sub>H<sub>5</sub>D<sub>5</sub>N<sub>2</sub>O [M<sup>+</sup>] requires 155.1107; mp 98-103 °C;  $\nu_{\max}$  (KBr) 3950, 3158, 2996, 2806, 1680, 1522, 1445, 1376, 1337, 1292 and 1113;  $\delta_{\text{H}}$  (400 MHz; CD<sub>3</sub>OD): 8.51 (0.05H, s), 8.23 (0.05H, d,  $J_{H-H}$  5.03), 7.33 (0.81H, s), 2.27 (0.18 H, m), 2.19 (3H, s);  $^b\delta_{\text{C}}$  (150 MHz, CD<sub>3</sub>OD) 172.4 (s, C=O), 146.8 (m, C-D), 144.6 (s, C-NH), 134.9 (s, C-C), 126.9 (s, C-H), 23.0 (s, CH<sub>3</sub>), 16.8 (m, CD<sub>3</sub>);  $m/z$  (EI) 155 (M<sup>+</sup>, 85%).

<sup>b</sup>Signals present a too high deuteration level for recording of <sup>13</sup>C.

### **2,4,6-Trideutero-3-amino-5-(trideuteromethyl)pyridine (30)**



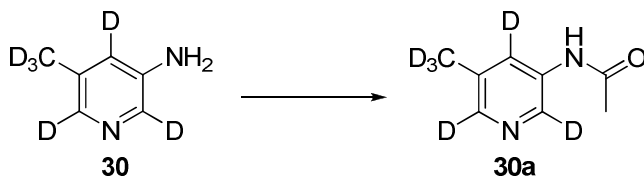
A solution of 3-amino-5-methylpyridine (300 mg, 2.7 mmol, 1 eq.) was added to a stirred solution of K<sub>2</sub>PtCl<sub>4</sub> (224 mg, 0.54 mmol, 20 mol %) and DCl (35%) (889 μL, 10.8 mmol, 4 eq.) in D<sub>2</sub>O (3 mL) and reacted according to the general procedure A. To the solution thioglycolic acid (153 μL, 2.16 mmol, 4 eq. referred to catalyst amount) was added and the mixture was stirred for 30 min, the rest of the work up follows the general procedure A to give the *title compound* as a yellow powder (153 mg, 50%); Found M<sup>+</sup>, 114.1064. C<sub>6</sub>H<sub>2</sub>D<sub>6</sub>N<sub>2</sub> [M<sup>+</sup>] requires 114.1064; mp 53-57 °C;  $\nu_{\max}$  (KBr) 3334, 3210, 1627, 1589, 1575, 1410, 1389,



1259 and 884;  $\delta_{\text{H}}$  (400 MHz;  $\text{CD}_3\text{OD}$ )  $^a7.76$  (0.03H, s),  $^a7.61$  (0.03H, s),  $^a6.92$  (0.35H, s),  $^a2.20$  (0.61H, m);  $\delta_{\text{C}}$  (150 MHz,  $\text{CD}_3\text{OD}$ ) 145.9 (s, C-NH<sub>2</sub>), 138.7 (t, 1CD,  $J_{\text{C-D}}$  26.5), 138.6 (m, C-CD<sub>3</sub>), 134.3 (t, C-D,  $J_{\text{C-D}}$  27), 123.7 (t, C-D,  $J_{\text{C-D}}$  29), 17.6 (m, CD<sub>3</sub>);  $m/z$  (EI) 114 ( $\text{M}^+$ , 60%).

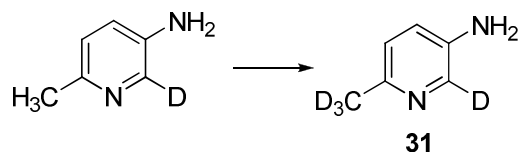
<sup>a</sup>Signals arise from the presence of isotopologues.

### **N-[2,4,6-Trideutero-5-(trideuteromethyl)pyridin-3-yl]acetamide (30a)**



*d*<sub>6</sub>-3-Amino-5-methylpyridine (**30**) (100 mg, 0.88 mmol, 1 eq.) was dissolved in acetone (20 mL), and K<sub>2</sub>CO<sub>3</sub> (607 mg, 4.4 mmol, 5 eq.) was added, followed by the dropwise addition of acetyl chloride (207 mg, 187  $\mu\text{L}$ , 2.6 mmol, 3 eq.) in acetone (5 ml) and reacted according to the general procedure C to give the title compound as a yellow powder (100 mg, 95%): Found  $\text{M}^+$ , 156.1175. C<sub>8</sub>H<sub>4</sub>D<sub>6</sub>N<sub>2</sub>O [ $\text{M}^+$ ] requires 156.1171; mp 125-131 °C;  $\nu_{\text{max}}$  (KBr) 3224, 2922, 1691, 1602, 1531, 1409, 1387, 1289, 1018, 881 and 769;  $\delta_{\text{H}}$  (400 MHz;  $\text{CD}_3\text{OD}$ ):8.51 (0.03H, s), 8.09 (0.03H, s), 7.93 (0.35H, s), 2.31 (0.61H,m), 2.15 (3H, s);  $\delta_{\text{C}}$  (150 MHz,  $\text{CD}_3\text{OD}$ ) 171.8 (s, C=O), 145.0 (t, C-D,  $J_{\text{C-D}}$  26), 138.6 (t, C-D,  $J_{\text{C-D}}$  29), 137.0 (s, C-NH), 135.2 (m, C-CD<sub>3</sub>), 128.9 (t, C-D,  $J_{\text{C-D}}$  28), 23.8 (s, CH<sub>3</sub>), 17.7 (m, CD<sub>3</sub>);  $m/z$  (EI) 156 ( $\text{M}^+$ , 40%).

### **2-Deutero-3-amino-6-(trideuteromethyl)pyridine (31)**

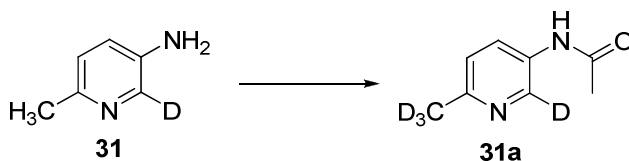


A solution of 5-amino-2-methylpyridine (300 mg, 2.7 mmol, 1 eq.) was added to a stirred solution of K<sub>2</sub>PtCl<sub>4</sub> (224 mg, 0.54 mmol, 20 mol %) and DCl (35%) (889  $\mu\text{L}$ , 10.8 mmol, 4 eq.) in D<sub>2</sub>O (3 mL) and reacted according to the general procedure A. To the solution thioglycolic acid (153  $\mu\text{L}$ , 2.16 mmol, 4 eq referred to catalyst amount) was added and the mixture was stirred for 30 min, the rest of the work up follow the general procedure A to give the *title compound* as a colourless powder (132 mg, 44%); Found  $\text{M}^+$ , 112.0936. C<sub>6</sub>H<sub>4</sub>D<sub>4</sub>N<sub>2</sub>

[M<sup>+</sup>] requires 112.0939; mp 78-81 °C;  $\nu_{\max}$  (KBr) 3396, 3323, 3269, 3030, 2928, 2497, 2402, 1909, 1635, 1597, 1564, 1460, 1371, 1301, 1250, 1153, 1086, 1042, and 914;  $\delta_{\text{H}}$  (400 MHz; CD<sub>3</sub>OD) <sup>a</sup>7.85 (0.09H, s), <sup>a</sup>7.04 (0.67H, d, *J* 8.48), <sup>a</sup>6.92 (0.92H d, *J* 8.48), <sup>a</sup>2.32 (0.50H, m);  $\delta_{\text{C}}$  (150 MHz, CD<sub>3</sub>OD) 147.3(s, C-NH<sub>2</sub>), 143.5(s, C-CD<sub>3</sub>), 148.7(t, C-D, *J*<sub>C-D</sub> 27), 124.8 (s, C-H), 124.5 (s, C-H), 22.1 (m, CD<sub>3</sub>); *m/z* (EI) 112 (M<sup>+</sup>, 80%).

<sup>a</sup>Signals arise from the presence of isotopologues.

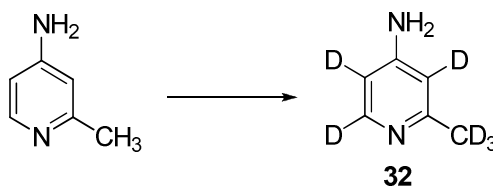
### N-[2-Deutero-6-(tetra-deuteromethyl)pyridin-5-yl]acetamide (31a)



*d*<sub>4</sub>-6-Methyl-3-aminopyridine (**31**) (100 mg, 0.88 mmol, 1 eq.) was dissolved in acetone (20 mL), and K<sub>2</sub>CO<sub>3</sub> (607 mg, 4.4 mmol, 5 eq.) was added, followed by the dropwise addition of acetyl chloride (207 mg, 187  $\mu$ L, 2.6 mmol, 3 eq.) in acetone (5 ml) and reacted according to the general procedure C to give the *title compound* as a colourless powder (67 mg, 50%): Found M<sup>+</sup>, 154.1046. C<sub>8</sub>H<sub>4</sub>D<sub>6</sub>N<sub>2</sub>O [M<sup>+</sup>] requires 154.1044; mp 110-116 °C;  $\nu_{\max}$  (KBr) 3395, 3296, 3232, 3163, 3090, 3009, 2871, 2236, 2151, 2054, 1851, 1685, 1610, 1582, 1463, 1452, 1295, 1139, 1006 and 826; (400 MHz; CD<sub>3</sub>OD): 8.58 (0.09H, s), 7.95 (0.67H, d, *J*<sub>H-H</sub> 8.48), 7.26 (0.92H, d, *J*<sub>H-H</sub> 8.48), 2.45 (0.50H, m), 2.14 (3H, s);  $\delta_{\text{C}}$  (150 MHz, CD<sub>3</sub>OD) 171.9 (s, C=O), 154.2 (s, C-NH), 140.8 (m, C-D), 134.8 (s, C

-CD<sub>3</sub>), 129.7 (s, C-H), 124.7 (s, C-H), 23.6 (s, CH<sub>3</sub>), 22.4 (m, CD<sub>3</sub>); *m/z* (EI) 154 (M<sup>+</sup>, 70%).

### 3,5,6-Trideutero-4-amino-2-(trideuteromethyl)pyridine (32)

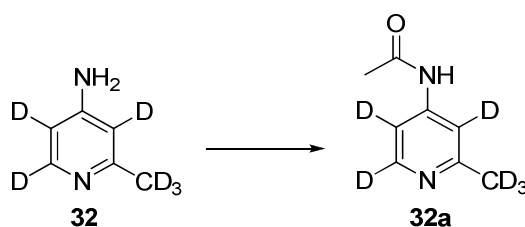


A solution of 4-amino-2-methylpyridine (250 mg, 2.3 mmol, 1 eq.) was added to a stirred solution of K<sub>2</sub>PtCl<sub>4</sub> (190 mg, 0.4 mmol, 20 mol %) and DCl (35%) (754  $\mu$ L, 9.2 mmol, 4 eq.) in D<sub>2</sub>O (3 mL) and reacted according to the general procedure A. To the solution thio glycolic acid (113  $\mu$ L, 1.6 mmol, 4 eq. referred to catalyst amount) was added and the

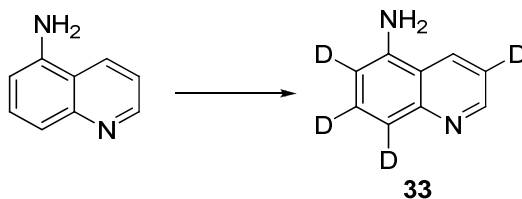
mixutre was stirred for 30 min, the rest of the work up follows the general procedure A to give the *title compound* as a yellow powder (170 mg, 65%): Found  $M^{+}$ , 114.1064.  $C_6H_2D_6N_2$  [ $M^{+}$ ] requires 114.1064; mp 93-97 °C;  $\nu_{max}$  (KBr) 3424, 3393, 3327, 3197, 2924, 2860, 1640, 1583, 1546, 1433, 1408, 1261 and 963;  $\delta_H$  (400 MHz;  $CD_3OD$ )  $^a$ 7.83(0.08H, s),  $^a$ 6.42 (0.01H, s),  $^a$ 6.38 (0.01H, s),  $^a$ 2.28 (0.24H, s);  $\delta_c$  (150 MHz,  $CD_3OD$ ) 158.6 (s, C-NH<sub>2</sub>), 157.1 (s, C-CD<sub>3</sub>), 148.7 (t, C-D,  $J_{C-D}$  26), 109.0 (t, C-D,  $J_{C-D}$  24), 107.6 (t, C-D,  $J_{C-D}$  26), 23.2 (m, CD<sub>3</sub>);  $m/z$  (EI) 114 ( $M^{+}$ , 95%).

<sup>a</sup>Signals arise from the presence of isotopologues.

### **N-[3,5,6-Trideutero-2-(trideuteromethyl)pyridin-4-yl]acetamide (32a)**



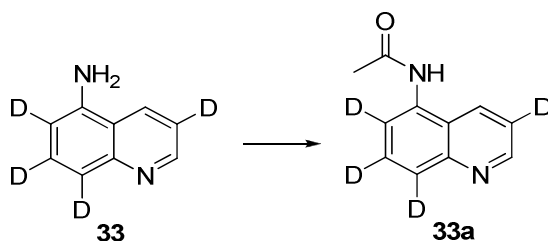
The solution of  $d_6$  4-amino-2-methylpyridine (**32**) (137 mg, 1.2 mmol, 1 eq.) in  $Ac_2O$  (119  $\mu$ L, 12.6 mmol, 10.5 eq.), was refluxed for 2h at 120°C. Another 10.5 equivalent was added and the mixture was left on stirring for 30'. After cooling at room temperature the reaction mixture was treated by brine to precipitate the product, which was filtrated off and re-dissolved in water. The aqueous phase was neutralized with sodium hydroxide and extracted with ethyl acetate (3 x 10 mL). The organic phase was dried by  $Na_2SO_4$  and filtrated. Then the solvent was eliminated and the product dried on the high vacuum, giving a yellow/colourless powder (156 mg, 83%): Found  $M^{+}$ , 156.1173.  $C_8H_4D_6N_2O$  [ $M^{+}$ ] requires 156.1170; mp 104-111 °C;  $\nu_{max}$  (KBr); 3294,3216, 3137, 3050, 2960, 2325,1681, 1577, 1516, 1453, 1408, 1372, 1291, 1233, 1078, 1035, 996 and 973;  $\delta_H$  (400 MHz;  $CD_3OD$ ): 8.24 (0.08H,s), 7.49 (0.01H, s), 7.41 (0.01H, s), 2.45 (0.24H, m), 2.15 (3H, s);  $\delta_c$  (150 MHz,  $CD_3OD$ ) 172.2 (s, C=O), 159.8 (s, C-NH), 149.6 (t, C-D,  $J_{C-D}$  27), 148.1 (s, C-CD<sub>3</sub>), 113.7 (t, C-D,  $J_{C-D}$  26), 111.8 (t, C-D,  $J_{C-D}$  26), 24.3 (s, CH<sub>3</sub>), 23.5 (m, CD<sub>3</sub>);  $m/z$  (EI) 156 ( $M^{+}$ , 45%).

**3,6,7,8-Tetradeutero-5-aminoquinoline (33)**

A solution of 5-aminoquinoline (300 mg, 2.08 mmol, 1 eq.) was added to a stirred solution of  $K_2PtCl_4$  (172 mg, 0.42 mmol, 20 mol %) and DCl (35%) (686  $\mu$ L, 8.33 mmol, 4 eq.) in  $D_2O$  (3 mL) and reacted according to the general procedure A. To the solution thioglycolic acid (117.9  $\mu$ L, 1.66 mmol, 4 eq. referred to catalyst amount) was added and the mixture was stirred for 30 min, the rest of the work up follows the general procedure A to give the *title compound* as a yellow powder (234 mg, 76%): Found  $M^{++}$ , 148.0939.  $C_9H_4D_4N_2$  [ $M^{++}$ ] requires 148.0939; mp 195-202  $^{\circ}C$ ;  $\nu_{max}$  (KBr) 3283, 3194, 1575, 1417, 1374 and 1356;  $\delta_H$  (400 MHz;  $CD_3OD$ )  $^a$ 8.74 (0.74H, s),  $^a$ 8.53 (0.87H, s),  $^a$ 7.51 (0.03H, s),  $^a$ 7.42 (0.03H, s);  $^a$ 7.34 (0.03H, s),  $^a$ 6.86 (0.03H, s);  $^b\delta_C$  (100 MHz,  $CD_3OD$ ) 150.7 (s, C-H), 149.6 (s, C-NH<sub>2</sub>), 146.0 (s, C-C), 133.8 (s, C-H), 121.1 (s, C-C);  $m/z$  (EI) 148 ( $M^{++}$ , 55%).

<sup>a</sup>Signals arise from the presence of isotopologues.

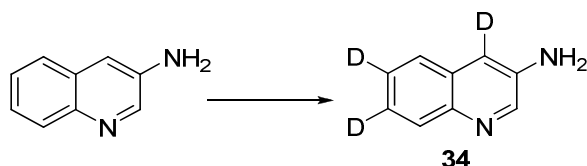
<sup>b</sup>Signals present a too high deuteration level for recording of  $^{13}C$ .

**N-(3,6,7,8-Tetradeuteroquinolin-5-yl)acetamide (33a)**

*d*<sub>6</sub>-5-Aminoquinoline (**33**) (244 mg, 1.6 mmol, 1 eq.) was dissolved in acetone (50 mL), and  $K_2CO_3$  (1.1 g, 8 mmol, 5 eq.) was added, followed by the dropwise addition of acetyl chloride (376 mg, 314  $\mu$ L, 4.6 mmol, 3 eq.) in acetone (5 mL) and reacted according to the general procedure C. Purification by chromatography column, eluting with DCM/MeOH (100/1) gave the *title compound* as a yellow powder (113 mg, 32 %): Found  $M^{++}$ , 190.1037.  $C_{11}H_6D_4N_2O$  [ $M^{++}$ ] requires 190.1040; mp 178-183  $^{\circ}C$ ;  $\nu_{max}$  (KBr) 3269, 3029, 3003, 2417, 1645, 1532, 1426, 1402, 1381, 1288 and 1263;  $\delta_H$  (400 MHz;  $CD_3OD$ ): 8.87 (0.74H, s), 8.49

(0.87H, s), 7.92 (0.03H, s), 7.76 (0.03H, s), 7.71 (0.03H, s), 7.56 (0.03H, s), 2.29 (3H, s),  $\delta_c$  (150 MHz, CD<sub>3</sub>OD) 173.0 (s, C=O), 151.2 (s, C-H), 149.1 (s, C-NH), 134.8 (s, C-C), 133.5 (s, C-H), 130.3 (t, C-D,  $J_{C-D}$  24), 127.0 (m, C-D), 125.0 (s, C-C), 124.1 (m, C-D), 122.1 (t, C-D,  $J_{C-D}$  24), 22.7 (s, CH<sub>3</sub>);  $m/z$  (EI) 190 ( $M^{+}$ , 35%).

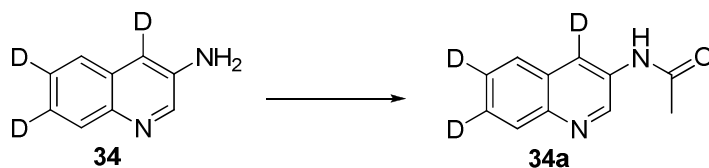
#### **4,6,7-Trideutero-3-aminoquinoline (34)**



A solution of 3-aminoquinoline (300 mg, 2.08 mmol, 1 eq.) was added to a stirred solution of K<sub>2</sub>PtCl<sub>4</sub> (172 mg, 0.42 mmol, 20 mol %) and DCl (35%) (686  $\mu$ L, 8.33 mmol, 4 eq.) in D<sub>2</sub>O (3 mL) and reacted according to the general procedure A. To the solution thioglycolic acid (117.9  $\mu$ L, 1.66 mmol, 4 eq. referred to catalyst amount) was added and the mixture was stirred for 30 min, the rest of the work up follows the general procedure A to give the title compound as an orange powder (267 mg, 87%): Found  $M^{+}$ , 147.0872. C<sub>9</sub>H<sub>5</sub>D<sub>3</sub>N<sub>2</sub> [ $M^{+}$ ] requires 147.0876; mp 78-83 °C;  $\nu_{max}$  (KBr) 3272, 3168, 3077, 2973, 2479, 2401, 2338, 1809, 1648, 1592, 1560, 1498, 1463 and 1401;  $\delta_H$  (400 MHz; CD<sub>3</sub>OD) <sup>a</sup>8.43 (0.87H, s), <sup>a</sup>7.81 (0.65H, s), <sup>a</sup>7.62 (0.65 H, s), <sup>a</sup>7.41 (0.04H, m), <sup>a</sup>7.33 (0.02H, d,  $J_{H-H}$  2.56);  $\delta_c$  (150 MHz, CD<sub>3</sub>OD) 144.0 (s, CH), 143.4 (s, C-NH<sub>2</sub>), 142.6 (s, C-C), 131.2 (s, C-C), 128.5 (s, C-H), 127.7 (t, C-D,  $J_{C-D}$  25), 126.9 (s, C-H), 125.9 (t, C-D,  $J_{C-D}$  24), 115.5 (t, C-D,  $J_{C-D}$  25);  $m/z$  (EI) 147 ( $M^{+}$ , 100%).

<sup>a</sup>Signals arise from the presence of isotopologues.

#### **N-[4,6,7-Trideuteroquinolin-3-yl]acetamide (34a)**

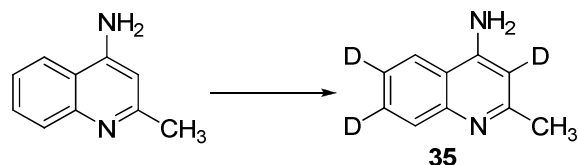


*d*<sub>6</sub>-3-Aminoquinoline (**34**) (180 mg, 1.2 mmol, 1 eq.) was dissolved in acetone (50 mL), and K<sub>2</sub>CO<sub>3</sub> (828 mg, 6 mmol, 5 eq.) was added, followed by the dropwise addition of acetyl chloride (282 mg, 255  $\mu$ L, 3.6 mmol, 3 eq.) in acetone (5 mL) and reacted according to the general procedure C to give the *title compound* as a colourless powder (207 mg, 90%): Found

$M^{+}$ , 189.0983.  $C_{11}H_7D_3N_2O$  [ $M^{+}$ ] requires 189.0981; mp 178-184 °C;  $\nu_{max}$  (KBr) 3496, 3252, 3063, 2320, 1670, 1577, 1552, 1435, 1379 and 1276;  $\delta_H$  (400 MHz;  $CD_3OD$ ): 8.87 (0.87H, s), 8.07 (0.03H, s), 7.96 (0.65H, s), 7.86 (0.65H, s), 7.67 (0.03H, s), 7.57 (0.03H, s), 2.23 (3H, s);  $^b\delta_c$  (150 MHz,  $CD_3OD$ ) 172.4 (s, C=O), 145.6 (s, C-H), 145.5 (s, C-C), 134.81 (s, C-NH), 129.7 (s, C-C), 128.7 (s, C-H), 23.8(s,  $CH_3$ );  $m/z$  (EI) 189 ( $M^{+}$ , 100%).

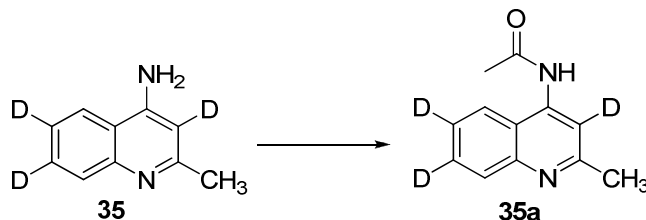
<sup>b</sup>Signals present a too high deuteration level for recording of  $^{13}C$ .

### **(3,7,8-Trideutero-2-methylquinolin-4-yl)amine (35)**



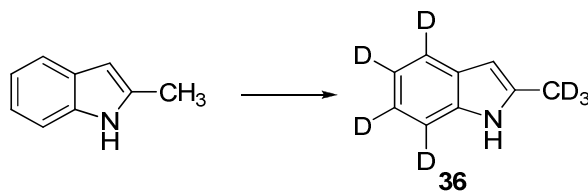
A solution of 2-methylquinolin-4-amine (300 mg, 1.8 mmol, 1 eq.) was added to a stirred solution of  $K_2PtCl_4$  (149 mg, 0.36 mmol, 20 mol %) and DCl (35%) (771  $\mu$ L, 8.33 mmol, 4 eq.) in  $D_2O$  (3 mL) and reacted according to the general procedure A. To the solution thioglycolic acid (102  $\mu$ L, 1.4 mmol, 4 eq. referred to catalyst amount) was added and the mixture was stirred for 30 min, the rest of the work up follows the general procedure A to give the *title compound* as a yellow powder (294 mg, 90%): Found  $M^{+}$ , 161.1034.  $C_{10}H_7D_3N_2$  [ $M^{+}$ ] requires 161.1032; mp 164-168°C;  $\nu_{max}$  (KBr) 3398, 3202, 2466, 2414, 1792, 1658, 1609, 1576, 1485, 1396, 1304, 1212 and 1094;  $\delta_H$  (400 MHz;  $CD_3OD$ ) <sup>a</sup>8.02 (0.98H, s), <sup>a</sup>7.74 (0.60 H, s), <sup>a</sup>7.73 (0.07H, s), <sup>a</sup>7.38 (0.07H, s), <sup>a</sup>6.52 (0.07H, s), <sup>a</sup>2.49 (2.89H, s);  $\delta_c$  (150 MHz,  $CD_3OD$ ) 160.0 (s, C- $CH_3$ ), 154.0 (s, C- $NH_2$ ), 149.3 (s, C-C), 130.2 (t, C-D,  $J_{C-D}$  24.5), 127.9 (s, C-H), 124.3 (t, C-D,  $J_{C-D}$  24), 122.5 (s, C-H), 116.7 (s, C-C), 103.4 (t, C-D,  $J_{C-D}$  23), 24.4 (m,  $CH_3$ );  $m/z$  (EI) 161 ( $M^{+}$ , 100%).

<sup>a</sup>Signals arise from the presence of isotopologues.

**N-[3,7,8-Trideutero-2-methylquinolin-4-yl]acetamide (35a)**

The solution of *d*<sub>6</sub>-2-methylquinolin-4-amine (**35**) (189 mg, 1.2 mmol, 1 eq.) in Ac<sub>2</sub>O (10 mL, 10.5 mmol, 6 eq.), was refluxed for 2h at 120°C. After cooling at room temperature the reaction mixture was treated by brine to precipitate the product, which was filtrated off and re-dissolved in water. The aqueous phase was neutralized with sodium hydroxide and extracted with ethyl acetate (x3). The organic phase was dried by Na<sub>2</sub>SO<sub>4</sub> and filtrated. Then the solvent was eliminated and the product dried on the high vacuum, giving a yellow powder (86 mg, 37%): Found M<sup>+</sup>, 203.1141. C<sub>12</sub>H<sub>9</sub>D<sub>3</sub>N<sub>2</sub>O [M<sup>+</sup>] requires 203.1138; mp 155-159 °C;  $\nu_{\max}$  (KBr) 3244, 3100, 3018, 2921, 2853, 1811, 1666, 1611, 1555, 1520, 1363, 1347 and 1271;  $\delta_{\text{H}}$  (400 MHz; CD<sub>3</sub>OD): 8.19 (0.98H, s), 8.06 (0.07H, s), 7.94 (0.60 H, s), 7.74 (0.07H, m), 7.58 (0.07H, m), 2.68 (2.89H, s), 2.32 (3H, s); <sup>b</sup> $\delta_{\text{C}}$  (150 MHz, CD<sub>3</sub>OD) 172.6 (s, C=O), 161.0 (s, C-CH<sub>3</sub>), 149.4 (s, C-C), 143.5 (s, C-NH), 128.7 (s, C-H), 123.7 (s, C-H), 122.4 (s, C-C), 106 (s, C-H), 24.9 (s, CH<sub>3</sub>), 24.1 (s, CH<sub>3</sub>); *m/z* (EI) 203 (M<sup>+</sup>, 50%).

<sup>b</sup>Signals present a too high deuteration level for recording of <sup>13</sup>C.

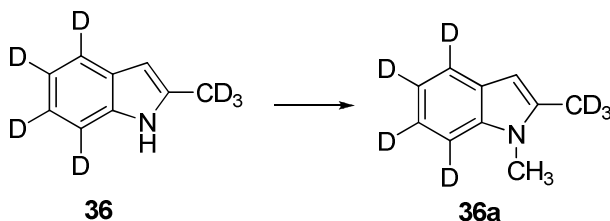
**4,5,6,7-Tetradeutero-2-(trideuteromethyl)-1-H-indole (36)**

A solution of 2-methylindole (300 mg, 2.3mmol, 1 eq.) was added to a stirred solution of K<sub>2</sub>PtCl<sub>4</sub> (190 mg, 0.46 mmol, 20%mmol.) and DCI (35%) (754  $\mu$ L, 9.2 mmol, 4 eq.) in D<sub>2</sub>O (3 mL) and reacted according to the general procedure A. To the solution thioglycolic acid (130  $\mu$ L, 1.84 mmol, 4 eq. referred to catalyst amount) was added and the mixutre was stirred for 30 min, the rest of the work up follows the general procedure A to give the *title compound* as a colourless powder (207 mg, 65%): Found M<sup>+</sup>, 138.1169. C<sub>9</sub>H<sub>2</sub>D<sub>7</sub>N [M<sup>+</sup>] requires 138.1170; mp 44-49 °C;  $\nu_{\max}$  (KBr) 3386, 2883, 1567, 1530, 1381 and 1328;  $\delta_{\text{H}}$  (400 MHz;

CD<sub>3</sub>OD) <sup>a</sup>7.39 (0.07H, s), <sup>a</sup>7.25 (0.07H, s), <sup>a</sup>6.99 (0.07H, s), <sup>a</sup>6.92 (0.07H, s), <sup>a</sup>6.08 (1H, s), <sup>a</sup>2.39 (0.21H, m), δ<sub>c</sub> (100 MHz, CD<sub>3</sub>OD) 138.3(s, C-NH<sub>2</sub>), 136.9 (s, C-C), 130.5 (s, C-C), 121.3 (t, C-D, *J*<sub>C-D</sub> 25), 120.1 (m, C-D), 111.4 (t, C-D, *J*<sub>C-D</sub> 25), 100.8 (s, C-H), 13.3 (m, CD<sub>3</sub>); *m/z* (EI) 138 (M<sup>+</sup>, 40%).

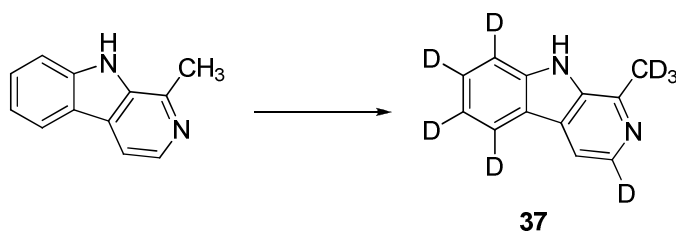
<sup>a</sup>Signals arise from the presence of isotopologues.

#### **4,5,6,7-Tetradeutero-1-methyl-2-(trideuteromethyl)-1-*H*-indole (36a)**



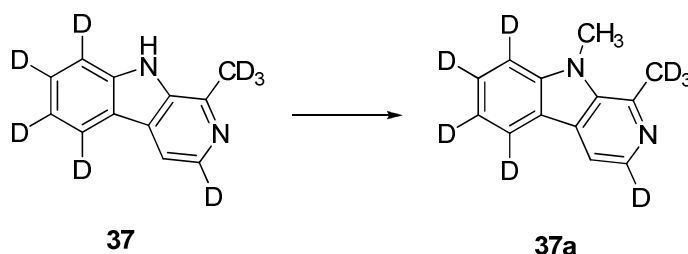
To a solution of *d*<sub>7</sub>-2-methylindole (**36**) (95 mg, 0.7 mmol, 1 eq.) in dry DMF (20 mL), NaH (60% dispersion in oil 55.2 mg, 2.3 mmol, 1 eq.) was added in portions. The mixture was stirred for 30 min, then methyl iodide (MeI) (156 μL, 2.5 mmol, 1.1 eq.) was added in one portion. The reaction became exothermic so it was cooled in an ice bath. After 16 h at RT, the reaction was concentrated under vacuum and the residue was taken up in ethyl acetate. The solution was concentrated under vacuum and the residue taken up in ethyl acetate. Then the mixture was extracted in H<sub>2</sub>O and in brine, dried on Mg<sub>2</sub>SO<sub>4</sub> and concentrated to dryness giving a title compound (34 mg, 32%) as a white solid: Found M<sup>+</sup>, 152.1330. C<sub>10</sub>H<sub>4</sub>D<sub>7</sub>N [M<sup>+</sup>] requires 152.1331; mp 40- 44 °C; ν<sub>max</sub> (KBr) 2925, 2198, 2119, 2055, 1589, 1558, 1529, 1451, 1390, 1329 and 1224; δ<sub>H</sub> (400 MHz; CD<sub>3</sub>OD,) <sup>a</sup>7.39 (0.07H, s), <sup>a</sup>7.25(0.07H, s), <sup>a</sup>7.05 (0.07H, s), <sup>a</sup>6.95 (0.07H, s), <sup>a</sup>6.16 (1H, s), <sup>a</sup>3.61 (3H, s), <sup>a</sup>2.375 (0.21H, m); δ<sub>c</sub> (150 MHz, CD<sub>3</sub>OD) 138.9 (s, C-N), 137.9 (s, C-C), 129.5 (s, C-C), 120.7 (t, C-D, *J*<sub>C-D</sub> 24), 119.9 (m, C-D), 109.3 (t, C-D, *J*<sub>C-D</sub> 24), 100.3 (s, C-H), 29.9 (s, CH<sub>3</sub>), 12.0 (m, CD<sub>3</sub>); *m/z* (EI) 152 (M<sup>+</sup>, 100%).



**3,5,6,7,8-Pentadeutero-1-(trideuteromethyl)-9-*H*-pyrido[3,4-*b*]indole (37)**

A solution of 1-methyl-9*H*-pyrido[3,4-*b*]indole (200 mg, 1.08 mmol, 1 eq.) was added to a stirred solution of  $K_2PtCl_4$  (89.64 mg, 0.22 mmol, 20 mol %), DCI (35%) (356  $\mu$ L, 4.33 mmol, 4 eq.) in  $D_2O$  (3 mL) and reacted according to the general procedure A. To the solution thioglycolic acid (56.6  $\mu$ L, 0.8 mmol, 4 eq. referred to the catalyst amount) was added and the mixture was stirred for 30 min, the rest of the work up follows the general procedure A to give the title compound as yellow powder (125 mg, 60%); Found  $M^{+}$  190.1349.  $C_{12}H_2D_8N_2$  [ $M^{+}$ ] requires 190.1346; mp 195  $^{\circ}C$ -200  $^{\circ}C$ ;  $\nu_{max}(KBr)$  3283, 3194, 1575, 1417, 1374, and 1356;  $\delta_H$  (400 MHz;  $CD_3OD$ )  $^a$ 8.19 (0.06H, d,  $J_{H-H}$  5.56),  $^a$ 8.18 (0.06H, s),  $^a$ 7.97 (0.50H, s),  $^a$ 7.34 (0.06H, s),  $^a$ 6.86 (0.12H, s), 2.80 (1.14H, m);  $\delta_c$  (100 MHz,  $CD_3OD$ ) 150.9(s, C-C), 150.9 (s, C-C), 130.5 (s, C-C), 121.3 (t, C-D,  $J_{C-D}$  25), 120.1 (m, C-D), 111.4 (t, C-D,  $J_{C-D}$  25), 100.8 (m, C-D), 13.3 (m,  $CD_3$ );  $m/z$  (EI) 190 ( $M^{+}$ , 100%).

<sup>a</sup>Signals arise from the presence of isotopologues.

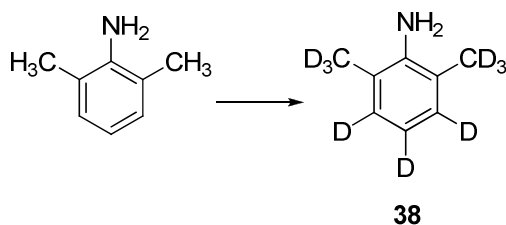
**3,5,6,7,8-Pentadeutero-9-methyl-1-(trideuteromethyl)-9-*H*-pyrido[3,4-*b*]indole (37a)**

To a stirred solution of  $d_8$ -1-methyl-9*H*-pyrido[3,4-*b*]indole (**37**) (120 mg, 0.6 mmol, 1 eq.) in dry DMF (10 mL) and under argon atmosphere, NaH was added in portions. The mixture was stirred for 30', then MeI (93.06, 40.8  $\mu$ L, 0.66, 1.1 eq.) was added in one portion. The reaction became exothermic and was cooled in an ice bath. After 16h, the reaction was concentrated under vacuum and the residue was taken up in ethyl acetate. Then the mixture was extracted in  $H_2O$  and brine. The organic phase was dried by  $Na_2SO_4$  and filtrated. Then the solvent was eliminated and the product dried on the high vacuum, giving a brown powder

(288mg, 40%), the product was purified by chromatography column (DCM 100/ MeOH 1) giving a yellow powder (113 mg, 32 %): Found  $M^{+}$ , 204.1056.  $C_{13}H_4D_8N_2$  [ $M^{+}$ ] requires 204.1053; mp 178 °C-183 °C;  $\nu_{max}$  (KBr) 3269, 3029, 3003, 2417, 1645, 1532, 1426, 1402, 1381, 1288 and 1263;  $\delta_H$  (400 MHz;  $CD_3OD$ ): 8.19 (0.06H,d,  $J_{H-H}$ 5.56), 8.18 (0.06H, s), 7.91 (0.50H, s), 7.61 (0.12H, s), 7.52 (0.06H, s), 7.27 (0.06H, s), 4.04 (3H, s), 3.00 (1.14H, s);  $^b\delta_C$  (150 MHz,  $CD_3OD$ ) 151.2 (s, C-C), 149.1 (s, C- $CD_3$ ), 138.8 (s, C-C), 133.5 (m, C-D), 130.3 (t, C-D,  $J_{C-D}$  24.5), 127.0 (m, C-D), 125.0 (s, C-C), 124.1 (m, C-D), 122.1 (t, C-D,  $J_{C-D}$  24), 110.0 (m, C-D), 109.9 (s, C-C), 32.6 (s,  $CH_3$ ), 22.7 (m,  $CD_3$ );  $m/z$  (EI) 204 ( $M^{+}$ , 100%).

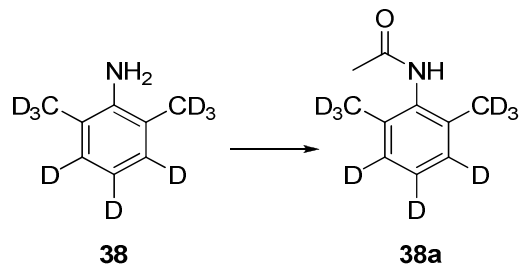
<sup>b</sup>Signals present a too high deuteration level for recording on <sup>13</sup>C.

### **3,4,5-Trideutero-2,6-bis(trideuteromethyl)aniline (38)**

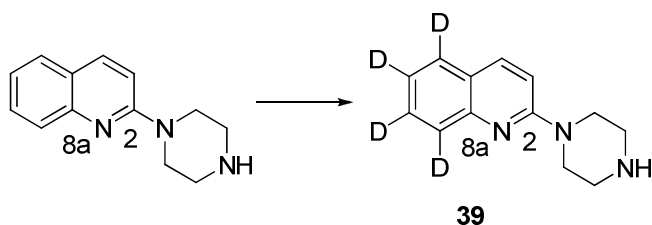


A solution of 2,6 dimethylaniline (300 mg, 306  $\mu$ L, 2.47 mmol, 1 eq.) was added to a stirred solution of  $K_2PtCl_4$  (203 mg, 0.49 mmol, 20 mol %.) and DCl (35%) (813  $\mu$ L, 9.88 mmol, 4 eq.) and reacted according to the general procedure A. To the solution thioglycolic acid (138  $\mu$ L, 1.96 mmol, 4 eq referred to catalyst amount) was added and the mixture was stirred for 30 min, the rest of the work up follow the general procedure A to give the title compound as a violet oil (300 mg, 97%): Found  $M^{+}$ , 130.1452.  $C_9H_3D_4N_2$  [ $M^{+}$ ] requires 130.1456;  $\nu_{max}$  (KBr) 3473, 3390, 3235, 3045, 3002, 2955, 2904, 2618, 1621, 1568, 1441, 1422, 1308, 1276, 1243, 1196, and 1048;  $\delta_H$  (400 MHz;  $CD_3OD$ ) <sup>a</sup>6.85 (0.30H, s), <sup>a</sup>6.54 (0.06H, s), <sup>a</sup>2.12 (0.26H,m);  $\delta_C$  (150 MHz,  $CD_3OD$ ) 144.1 (s, C- $NH_2$ ), 128.9 (s, C-D,  $J_{C-D}$  24), 123.5 (s, C-C), 118.6 (t, C-D,  $J_{C-D}$  24), 17.0 (sept,  $CD_3$ ,  $J_{C-D}$  19);  $m/z$  (EI) 130 ( $M^{+}$ , 30%).

<sup>a</sup>Signals arise from the presence of isotopologues.

**N-[3,4,5-trideutero-2,6-bis(trideuteromethyl)phenyl]acetamide (38a)**

Acetyl chloride (122  $\mu\text{L}$ , 2.02 mmol, 1.1 eq.) was added to a stirred solution of  $d_9$ -2,6-dimethylaniline (**38**) (236 mg, 1.8 mmol, 1 eq.) in DCM (5 mL) and  $\text{NEt}_3$  on ice (523  $\mu\text{L}$ , 4.05 mmol, 2.2 eq.) and reacted according the general procedure **B** to give the title compound as a colourless powder (233 mg, 75 %): Found  $\text{M}^+$ , 172.1560.  $\text{C}_{10}\text{H}_4\text{D}_9\text{NO}$  [ $\text{M}^+$ ] requires 172.1562; mp 168-173  $^\circ\text{C}$ ;  $\nu_{\text{max}}$  (KBr) 3231, 3245, 3130, 3031, 2813, 1713, 1647, 1536, 1428, 1410, 1369, 1291, 1240, 1041, 1012, 975, 735, 615 and 579;  $\delta_{\text{H}}$  (400 MHz;  $\text{CD}_3\text{OD}$ ) 7.19 (0.06H, s), 7.07 (0.30H, s), 2.21 (0.26H, s), 2.12 (3H, s);  $\delta_{\text{C}}$  (100 MHz,  $\text{CD}_3\text{OD}$ ) 172.2 (s, C=O), 136.6(s, C-NH), 135.6 (s, C- $\text{CD}_3$ ), 129.6 (m, C-D), 128.6 (t, C-D,  $J_{\text{C-D}}$  24), 22.4 (s,  $\text{CH}_3$ ), 17.8 (sept,  $\text{CD}_3$ ,  $J_{\text{C-D}}$  19);  $m/z$  (EI) 172 ( $\text{M}^+$ , 75%).

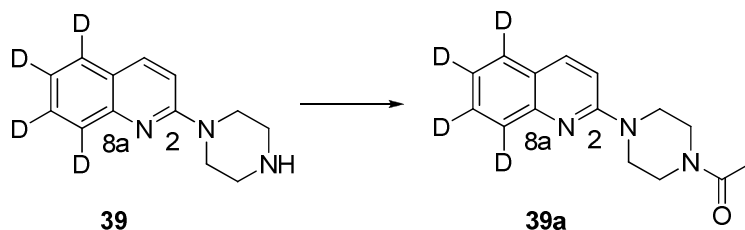
**5,6,7,8-Tetradeutero-2-(piperazin-1-yl)quinoline (39)**

A solution of 2-(piperazin-1-yl)quinoline (200 mg, 0.94 mmol, 1 eq.) was added to a stirred solution of  $\text{K}_2\text{PtCl}_4$  (77.18 mg, 0.19 mmol, 20 mol %) and DCl (35%) (309  $\mu\text{L}$ , 3.76 mmol, 4 eq.) in  $\text{D}_2\text{O}$  (3 mL) and reacted according to the general procedure **A**. To the solution thioglycolic acid (53  $\mu\text{L}$ , 0.76 mmol, 4 eq. referred to catalyst amount) was added and the mixture was stirred for 30 min, the rest of the work up follows the general procedure **A** to give the title compound as a yellow powder (137 mg, 67%): Found  $\text{M}^+$ , 217.1517.  $\text{C}_{13}\text{H}_{11}\text{D}_4\text{N}_3$  [ $\text{M}^+$ ] requires 217.1517; mp 85-89  $^\circ\text{C}$ ;  $\nu_{\text{max}}$  (KBr) 3288, 2986, 2922, 2847, 1609, 1537, 1488, 1437, 1377, 1280, 1236, 1138, 1116 and 807;  $\delta_{\text{H}}$  (400 MHz;  $\text{CD}_3\text{OD}$ )  $^a$ 7.99 (0.80H, d,  $J_{\text{H-H}}$  9.20),  $^a$ 7.65 (0.32 H, s),  $^a$ 7.64 (0.32 H, s),  $^a$ 7.15 (0.80H, d,  $J_{\text{H-H}}$  9.20),  $^a$ 3.72

(4H, m),  $^a$ 2.97(3.93H, m);  $\delta_c$ (100 MHz, CD<sub>3</sub>OD) 159.2(s, C-8a), 148.8 (s, C-2), 138.9 (s, C-H), 130.1 (m, C-D), 128 (m, C-D), 127.0 (m, C-D), 124.5 (s, C-C), 122.9 (m, C-D), 111.1 (s, C-H), 46.9 (s, CH<sub>2</sub>), 49.4 (s, CH<sub>2</sub>);  $m/z$  (EI) 217 (M<sup>+</sup>, 40%).

<sup>a</sup>Signals arise from the presence of isotopologues.

### **1-[4-(5,6,7,8-Tetradeuteroquinolin-2-yl)piperazin-1-yl]ethanone (39a)**



*d*<sub>4</sub>-2-(Piperazin-1-yl)quinoline(**39**) (97 mg, 0.44 mmol, 1 eq.) was solved in DCM (10 ml) at 0°C, followed by the addition of NEt<sub>3</sub> (56.97 μL, 0.44 mmol, 1 eq.). A solution of Ac<sub>2</sub>O (41.79 μL, 0.44 mmol, 1 eq.) in DCM (5 ml) was added dropwise and the mixture has been left on stirring for 3h at RT. The resulting solution has been treated with 10% NaHCO<sub>3</sub> and it has been left on stirring for further 30 min. The mixture was extracted with DCM (x3) and the organic phase washed with brine<sup>c</sup>, dried over NaSO<sub>4</sub> and finally the solvent evaporated under vacuum to obtain a yellow powder (63 mg, 54%); Found M<sup>+</sup>, 259.1623. C<sub>15</sub>H<sub>13</sub>D<sub>4</sub>N<sub>3</sub>O[M<sup>+</sup>] requires 259.1623; mp 176-180 °C;  $\nu_{\max}$  (KBr) 3276, 2993, 2897, 2848, 1659, 1609, 1536, 1493, 1427, 1380, 1349, 1281, 1257, 1235, 1169, 985 and 806;  $\delta_H$  (400 MHz; CD<sub>3</sub>OD)  $^a$ 8.03 (0.80H, d,  $J_{H-H}$  9.20),  $^a$ 7.67 (0.32H, s),  $^a$ 7.66 (0.32H, s),  $^a$ 7.19 (0.80H, d,  $J_{H-H}$  9.20),  $^a$ 3.83 (2H, m),  $^a$ 3.74 (4H, m),  $^a$ 3.70 (2H, m), 2.17 (3H,s);  $\delta_c$ (150 MHz, CD<sub>3</sub>OD) 171.9 (s, C=O),158.3 (s, C-8a),148.3 (s, C-2), 139.3 (s, C-H), 128.4 (m, C-D), 126.4 (m, C-D);<sup>b</sup>120.7 (s, C-C), 111.1 (s,C-H), 47.20 (s, CH<sub>2</sub>), 46.4 (s, CH<sub>2</sub>), 46.2 (s, CH<sub>2</sub>), 42.5 (s, CH<sub>2</sub>), 21.3 (s, CH<sub>3</sub>);  $m/z$  (EI) 259 (M<sup>+</sup>, 60%).

<sup>a</sup>Signals arise from the presence of isotopologues.

<sup>b</sup>Two signals present a too high deuteration level for recording of <sup>13</sup>C.

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