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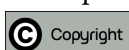
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# Nanocatalyzed Hydrogen Isotope Exchange reactions

*Marco Lepron<sup>†‡</sup>, Marion Daniel-Bertrand<sup>†‡</sup>, Gabriel Mencia<sup>\*</sup>, Bruno Chaudret<sup>\*</sup>, Sophie  
Feuillastre<sup>\*†</sup>, Grégory Pieters<sup>\*†</sup>*

<sup>†</sup>Université Paris-Saclay, CEA, INRAE, Département Médicaments et Technologies pour la  
Santé (DMTS), SCBM, Bat 547, 91191 Gif-sur-Yvette (France).

<sup>\*</sup>LPCNO, Université de Toulouse, UMR 5215 INSA-CNRS-UPS, Institut National des Sciences  
Appliquées, 135, Avenue de Rangueil, F-31077 Toulouse, France.

## **Conspectus.**

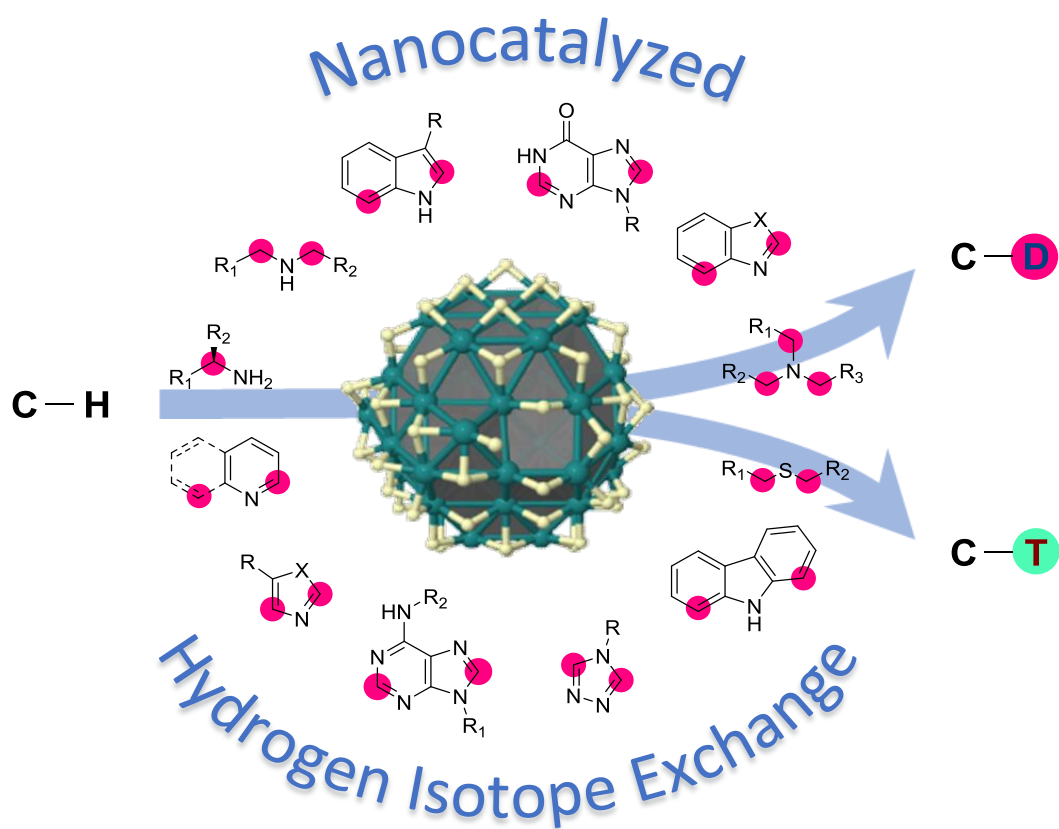
Recently, Hydrogen Isotope Exchange (HIE) reactions have experienced an impressive development due to the growing importance of isotope containing compounds in various fields including materials and life sciences, beside their classical use for mechanistic studies in chemistry and biology. Tritium-labelled compounds are also of crucial interest to study the *in vivo* fate of a bioactive substance or in radioligand binding assays. Over the past few years, deuterium-labelled drugs have been extensively studied for the improvement of ADME (Absorption, Distribution, Metabolism, Excretion) properties of existing bioactive molecules as a

consequence of the primary kinetic isotope effect. Furthermore, in the emergent “omic” fields, the need for new stable isotopically-labelled internal standards (SILS) for quantitative GC- or LC-MS analyses is increasing. Because of their numerous applications, the development of powerful synthetic methods to access deuterated and tritiated molecules with either high isotope incorporation and/or selectivities is of paramount importance.

HIE reactions allow a late-stage incorporation of hydrogen isotopes in a single synthetic step, thus representing an advantageous alternative to conventional multistep synthesis approaches which are time- and resource-consuming. Moreover, HIE reactions can be considered as the most fundamental C–H functionalization processes and are therefore of great interest for the chemists’ community in fundamental research. Depending on the purpose, HIE reactions must either be highly regioselective or allow a maximal incorporation of hydrogen isotopes, sometimes both. In this context, metal-catalyzed HIE reactions are generally performed using either homogeneous or heterogeneous catalysis which may have considerable drawbacks including, respectively, an insufficient isotope incorporation and a lack of chemo- and/or regioselectivity.

Over the past 6 years, our groups have shown that nanocatalysis can be considered has a powerful tool to access complex labelled molecules (e.g. pharmaceuticals, peptides and oligonucleotides) via regio- and chemoselective or even enantiospecific labelling processes occurring at the surface of metallic nanoclusters (Ru or Ir). Numerous heterocyclic (both saturated and unsaturated) and acyclic scaffolds have been labelled with an impressive compatibility regarding sensitive chemical functions and highly deuterated compounds or high molar activity tritiated drugs have been obtained. An insight into mechanisms has also been provided by DFT calculations to explain the regioselectivity obtained throughout the process of labelling. Our studies have suggested that undisclosed key intermediates, including 4- and 5-

membered dimetallacycles, account for the particular regioselectivity observed during the process in contrast with the 5-membered metallacycles key intermediates usually encountered in homogeneous catalysis. These findings together with number of available coordination sites explain the compelling reactivity of metal nanoparticles, between homogeneous and heterogeneous catalysis. They represent innovative tools combining the advantages of both methods for the isotopic labelling and activation of C-H bonds of complex molecules.



#### Key references.

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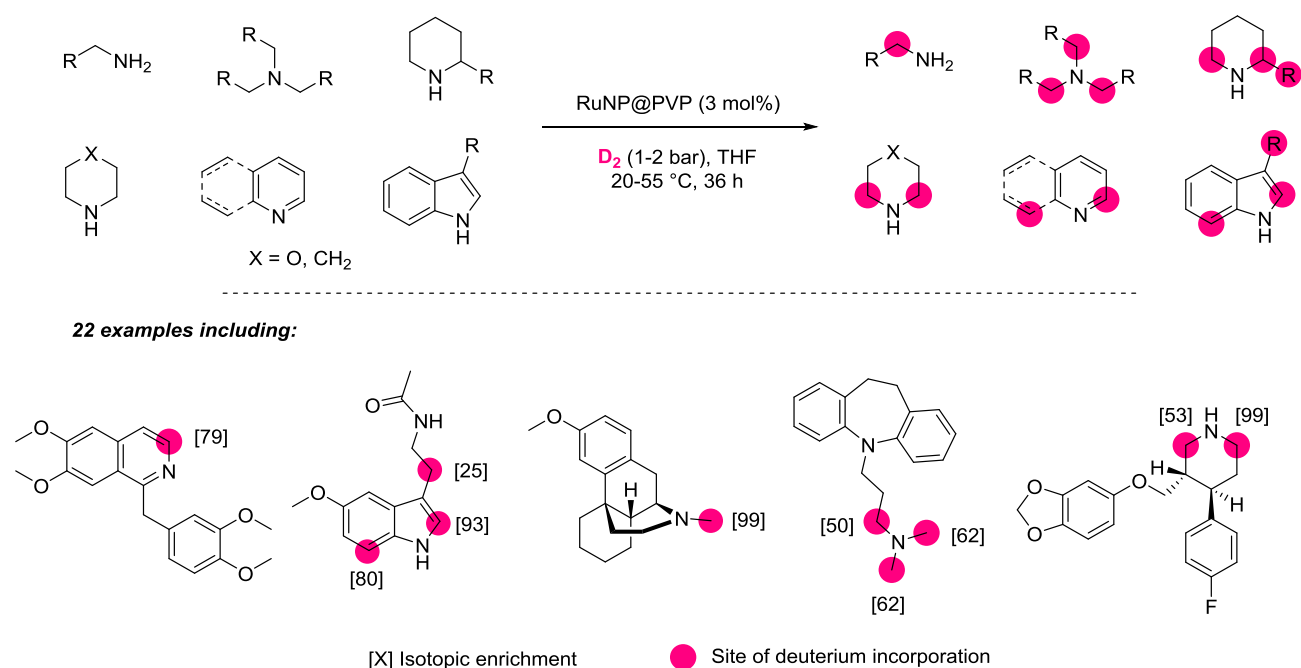
## Introduction.

Hydrogen isotopes play a critical role in a large panel of scientific research areas and technological developments with huge human, economical and societal impacts. In particular, deuterated/tritiated compounds are used: 1) in the health sector as internal mass spectrometry standards or radioactive tracers which are crucial for Drug Discovery and Development,<sup>1,2</sup> 2) in (bio)chemistry for mechanistic studies,<sup>3</sup> 3) in biology for proteomics and metabolomics,<sup>2</sup> 4) for diagnostics as MRI tracers.<sup>4</sup> Moreover, deuterium is the central element of the promising pharmaceutical market of “heavy drugs” with improved properties,<sup>5</sup> and has been recently proposed for improving the metabolic stability of PET (positron emission tomography) diagnostic tracers,<sup>6</sup> as well as for improving the physical properties and lifetime of optoelectronic devices such as OLEDs and optical fibers.<sup>7</sup>

Isotopic labelling had been intensively investigated in the past decades and numerous labelling methods are nowadays available. Among them, metal-mediated hydrogen isotope exchange (HIE) permit the last-stage labelling of compounds, thus reducing wastes, time and costs. Homogeneous catalysis allows the labelling of molecules with high selectivity in mild conditions but generally with moderate isotope incorporation as only specific positions can be activated. On the other hand, heterogeneous catalysis gives access to high isotope incorporation but with a reduced selectivity combined with harsher conditions. To combine advantages and circumvent drawbacks, the use of nanoparticles appeared recently as an attractive solution. Organometallic nanoparticles (NPs) have been developed for the past 25 years.<sup>8</sup> Although they are not defined molecular objects, they can be made monodisperse and their size can be modulated. Regarding our work, we used mainly modified commercial catalysts containing NPs such as Ru/C or Ru

and Ir NPs with controlled sizes between 1 and 2 nm and various surface stabilizers: polymers such as polyvinylpyrrolidone (PVP) or ligands. The most promising ligands are N-heterocyclic carbenes (NHC) which can be either soluble in organic solvents<sup>9</sup> or in water<sup>10</sup> depending on the requirements. These NPs accommodate surface hydrides, which allow facile C-H activation reactions, and their reactivity and selectivity can be modulated by the nature and concentration of surface ligands or by the presence of different metals (bimetallic NPs) and their chemical order. Non noble metal NPs can also be synthesized using the same procedure and in this respect, Ni NPs stabilized by NHCs with a comparable size (2.0 nm) were shown to be catalytically active and to present an interesting reactivity.<sup>11</sup>

Following our pioneer discovery that under a pressure of D<sub>2</sub> gas, primary amine (hexadecylamine) ligands of Ru nanoparticles were labelled and intrigued by this reactivity, we started our journey to the labelling world by focusing on the labelling of nitrogen-containing compounds.<sup>12</sup> Our first results were that either C<sub>sp<sup>2</sup></sub>-H or C<sub>sp<sup>3</sup></sub>-H can be labelled nicely using mild reaction conditions (55°C, 1 to 2 bar of D<sub>2</sub> gas) and a low catalytic amount (3 mol% of RuNP@PVP).



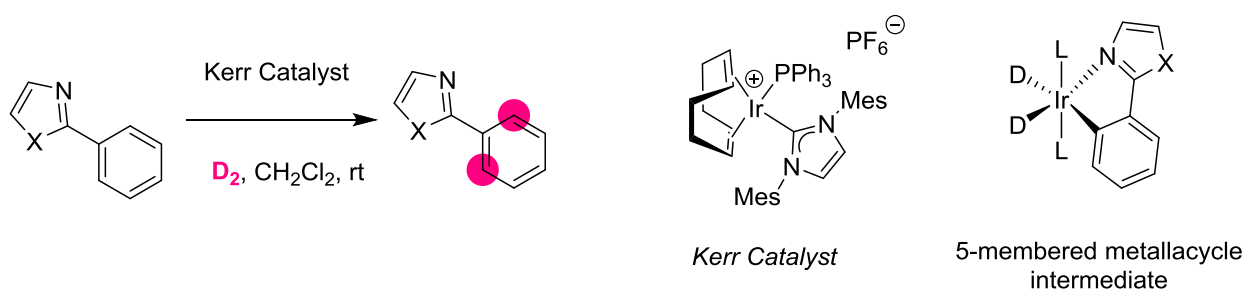
**Figure XX.** Scope and regioselectivity of RuNP@PVP catalyzed HIE reactions on various N-heterocycles.

All compounds tested were obtained in good yields with high chemo- and regioselectivities combined with good to high isotopic enrichments (50 to 99% at the  $\alpha$ -position of the nitrogen atom). At this stage we believed that, for the vast majority of the compounds labelled, the regioselectivity was driven by the *N*-coordination of the substrate at the surface of the catalyst, thus leading to the labelling at the  $\alpha$ -position of the nitrogen atom and at the  $\beta$ - or  $\gamma$ -position for indole and 2-phenylpyridine substructures through a hypothetical 6-membered ring. The mild conditions used permitted the labelling of eight bioactive compounds bearing stereogenic centres without any loss of the enantiomeric purity in all cases, even if the labelling took place in the vicinity of the chiral centre.

## Ruthenium nanoparticles for C(sp<sup>2</sup>)-H activation.

After our first results showing the potency of RuNPs for the H/D exchange reactions on both C(sp<sup>2</sup>)-H and C(sp<sup>3</sup>)-H bonds, we looked forward to expand the scope of this method to relevant *N*-heterocyclic scaffolds and to gain insight on mechanisms. Labelling of nitrogen-containing heterocycles is indeed of substantial interest: triazoles (either 1,2,3- or 1,2,4-), imidazoles and benzimidazoles are all widely encountered moieties in approved US FDA drugs.<sup>13</sup> Oxazoles and carbazoles are also frequent substructures in medicinal chemistry or in material science.<sup>14</sup>

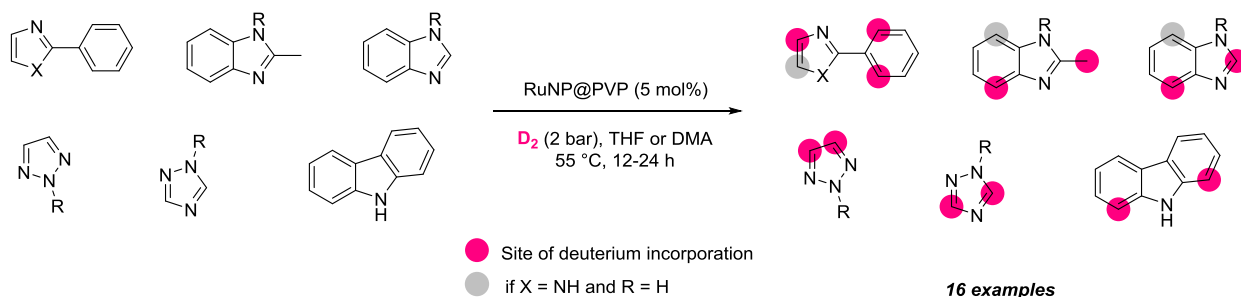
In this context, homogenous Ir(I) catalysts have been used for the selective deuteration of oxazole and imidazole moieties but only if they are substituted by an aromatic group in position 2 and with rather moderate deuterium incorporation (see Figure XX).<sup>15,16</sup>



**Figure XX.** Homogenous Ir(I) catalyst and its corresponding 5-membered metallacycle intermediate

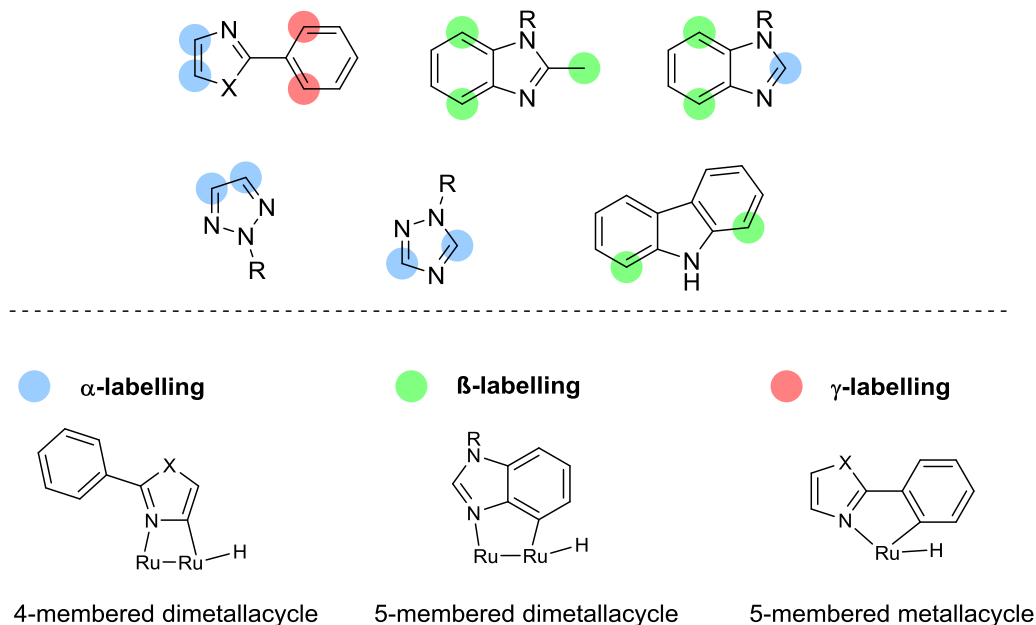
Homogeneous iron and nickel catalysts have demonstrated a moderate activity for the isotopic labelling of 1,2,3-triazoles allied with only low enrichments,<sup>17,18</sup> whereas no broadly applicable HIE method on 1,2,4-triazoles or carbazoles have yet been described.

Using RuNP@PVP, we were able to incorporate up to 5 deuterium atoms at  $\alpha$ ,  $\beta$  and  $\gamma$  positions of a nitrogen atom on a broad scope of the aforementioned N-heterocyclic substrates in a large variety of solvents (see Figure XX).<sup>19</sup>



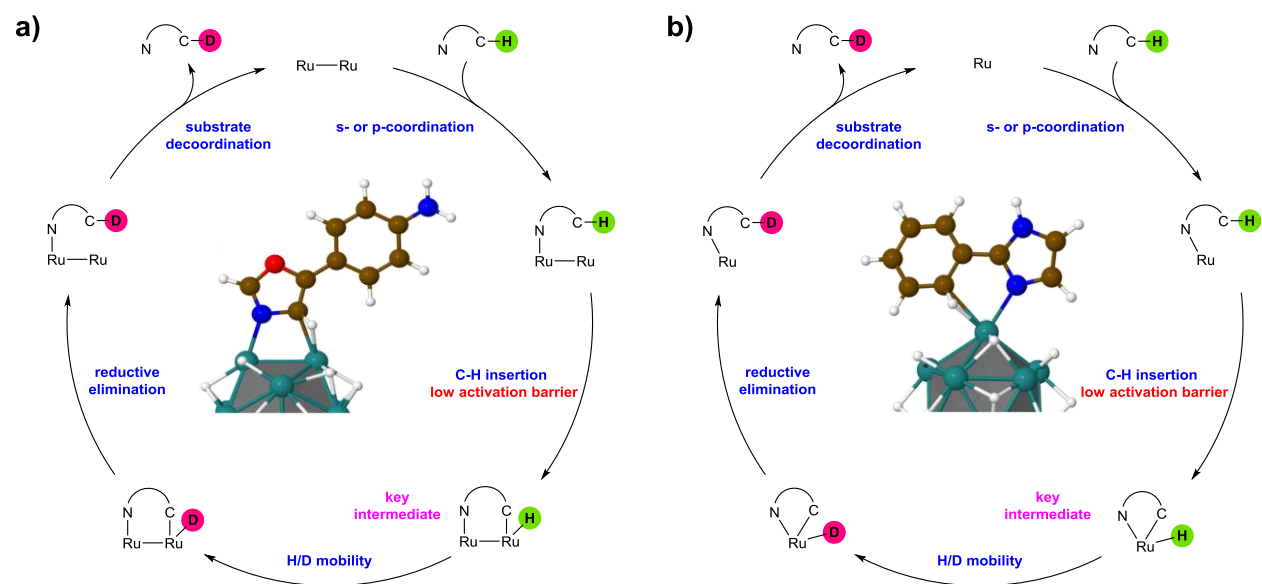
**Figure XX.** Scope and regioselectivity of RuNPs catalyzed HIE reactions on various N-heterocycles.

Beside the demonstration of the very wide scope of application of RuNP catalysis we were interested in the understanding of the reaction mechanism explaining the regioselectivity of the isotope incorporation. Theoretical calculations at the DFT-PBE level of theory carried out by R. Poteau *et al.* succeeded in elucidating the reaction pathways and explaining the different  $\alpha$ ,  $\beta$  or  $\gamma$  selectivities using a 0.5 nm ruthenium cluster model with 1.4 H atoms *per* Ru surface atom (Ru<sub>13</sub>H<sub>17</sub>). It ultimately appeared that, depending on the labelled position, the reaction pathway involves either novel 4- and 5-membered dimetallacycles key intermediates (respectively for  $\alpha$  and  $\beta$  positions) or a 5-membered metallacycle ( $\gamma$  position), explaining these particular regioselectivities (see Figure XX).



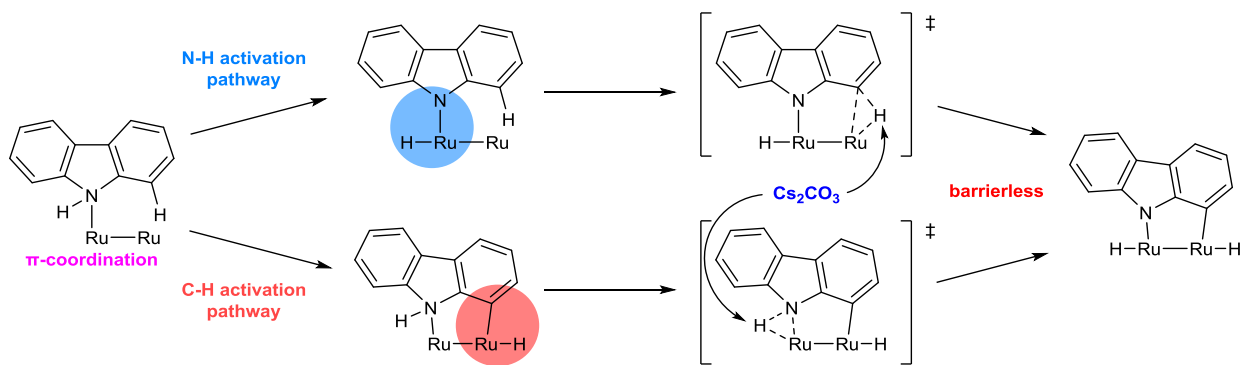
**Figure XX.** Key intermediates involved depending on the regioselectivity of the labelling.

Regardless of the regioselectivity of the labelling, the reaction pathway always involves an initial exothermic adsorption of the substrate through a nitrogen  $\sigma$ - or  $\pi$ -coordination (in the case of carbazoles) on a Ru atom. Then, a stabilizing agostic interaction can be established between the C-H bond and an adjacent Ru atom (in the case of dimetallacycles) or the same Ru atom (in the case of mono-metallacycles) ultimately forming the key intermediate. The consecutive C-H bond breaking step always involves a low to non-existent activation barrier (between 0.0 and 7.4 kcal.mol<sup>-1</sup>, depending on the substrate). The last step revolves around the competition between the (C)<sup>\*</sup>(H)<sup>\*</sup> ↔ (C-H)<sup>\*</sup> and the (C)<sup>\*</sup>(D)<sup>\*</sup> ↔ (C-D)<sup>\*</sup> which is in favour of the latter, leading to the isotope exchange (see Figure XX for the general mechanistic pathway).



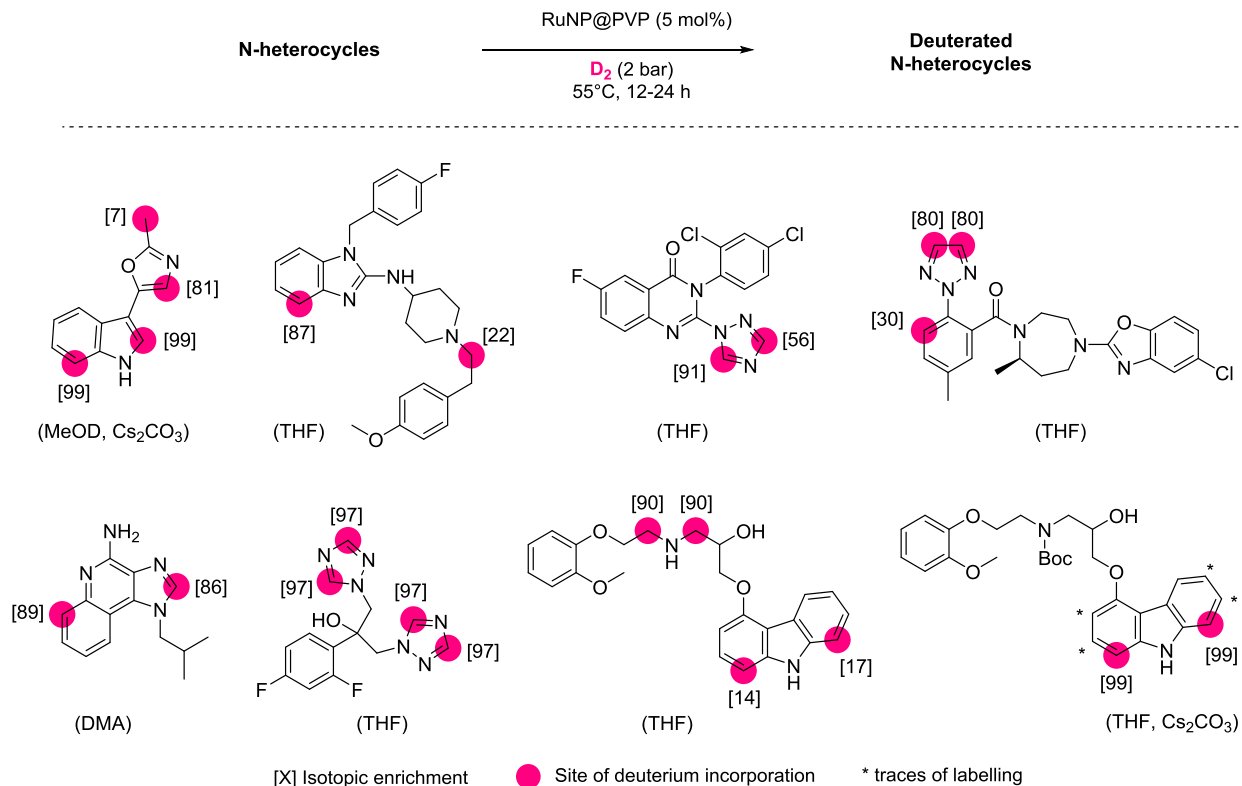
**Figure XX.** General mechanistic pathway for the deuterium labeling of N-heterocyclic compounds using RuNPs (a) with dimetallacyclic intermediates (b) with mono-metallacyclic intermediates.

In the case of carbazole-type derivatives, during the exothermic adsorption on the substrate, the nitrogen atom is only weakly coordinated to the RuNP through a  $\pi$ -coordination instead of a  $\sigma$ -coordination. Afterwards, two pathways were considered, either *via* the N-H activation followed by the C<sub>1</sub>-H activation or the other way around. Calculations showed that both mechanisms were feasible although the barrier for the C-H activation step is significantly lowered if the N-H activation takes place beforehand. We then showed that after a first C-H or N-H activation step, a Cs<sub>2</sub>CO<sub>3</sub> molecule present in the close vicinity of both intermediate could efficiently abstract an hydrogen atom from the N-H or the C<sub>1</sub>-H bond respectively. This leads to an exothermic and barrierless X-H bond (X= N or C) breaking step leading to better isotope incorporations (see Figure XX). Note that Cs<sub>2</sub>CO<sub>3</sub> may be useful for every heterocycle containing an acidic N-H group including indoles (see Figure XX).



**Figure XX.** Two pathways explaining the  $\beta$ -labelling of carbazoles.

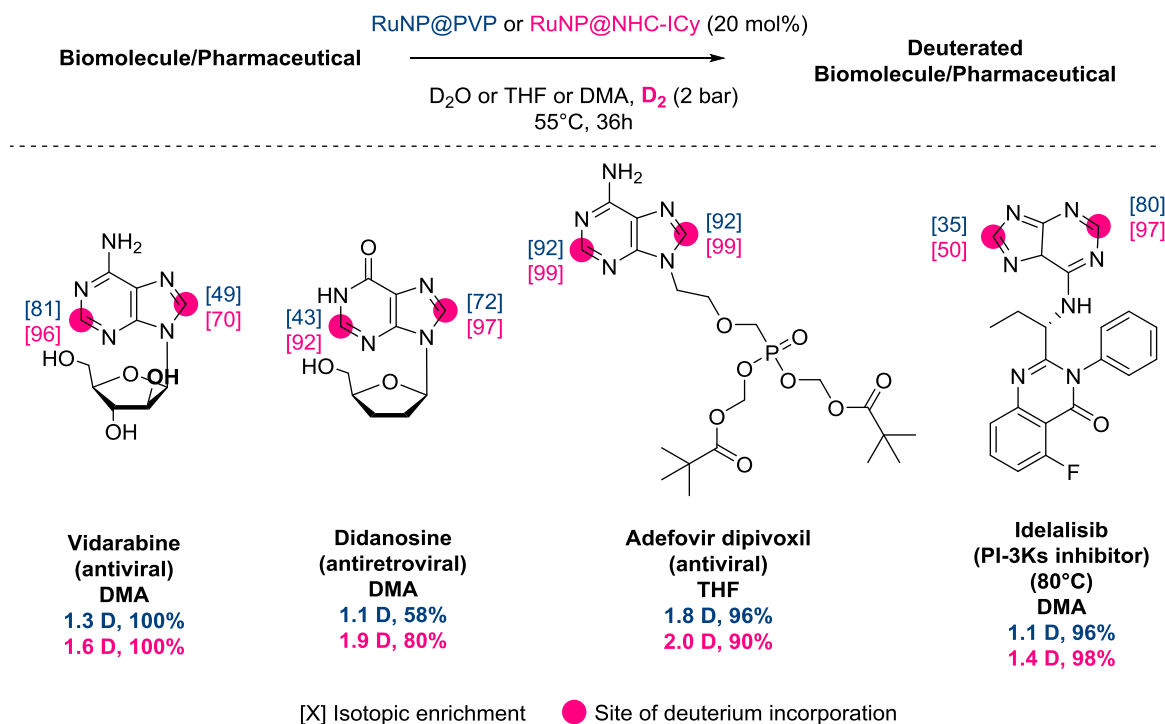
With the mechanistic studies in hand, we turned our attention towards the labelling of complex pharmaceuticals containing the aforementioned N-heterocycles. We obtained good isotopic enrichments over a large variety of scaffolds, thus validating our method for the preparation of stable isotopically labelled commercial drugs for LC-MS quantification (SILS) or for metabolic studies (see Figure XX).



**Figure XX.** Deuterium labelling of complex pharmaceuticals (2 bar of D<sub>2</sub> gas, 55°C, overnight).

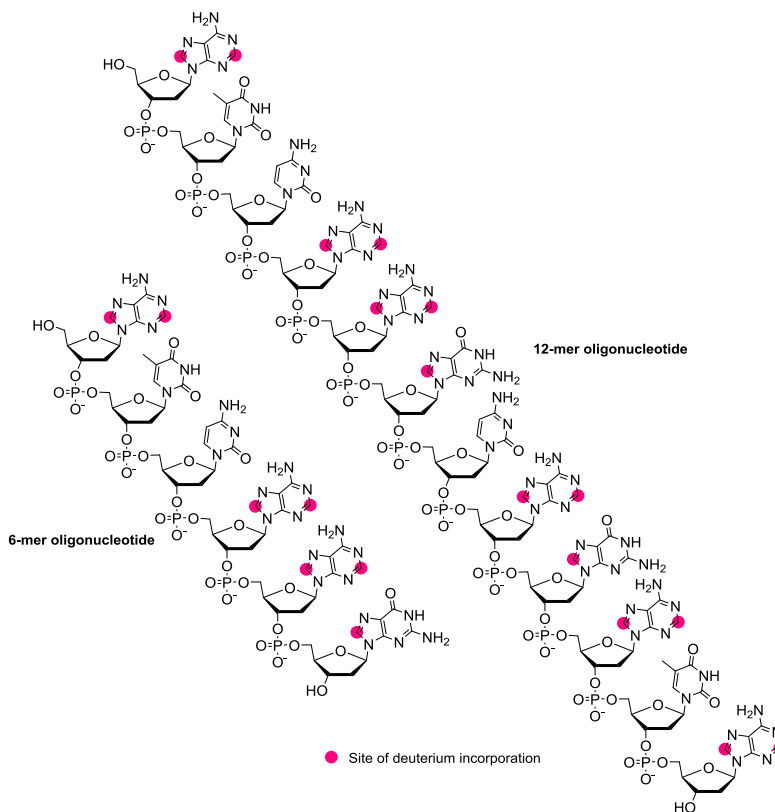
During the mechanistic studies, we discovered that transition states were of low energy, sometimes leading to barrierless steps. This led us to believe that our methodology would be useful for the isotopic labelling of fragile molecules such as purine-containing nucleobase pharmaceuticals and oligonucleotides, which are key substrates for SILS and ADME purposes and for which a reliable labelling method is desperately in need. Generally, deuterated and tritiated nucleobases derivatives are obtained *via* time- and resource-consuming multistep syntheses.<sup>20</sup> Only few examples of direct HIE reactions have been described, either using catalytic amounts of Pd/C in deuterated water<sup>21</sup> or by performing an acido-basic exchange of the 8-position of purine derivatives.<sup>22</sup> However, these methods require harsh conditions and are thereby limited to simple substrates. Using mild reaction conditions, D<sub>2</sub> gas (2 bar) and our RuNP@PVP nanoparticles in deuterated water, we managed to label various purine derivatives with good isotopic enrichments (up to 3.9 D incorporated) over a wide pH range, validating the robustness of our method.<sup>23</sup> We successfully labelled complex purine-based drugs including Doxofylline, Vidarabine, Didanosine, Adefovir and Idelalisib in various solvents depending on the solubility of the compound.

Subsequently, nanoparticle engineering was envisioned in order to improve their solubility in both organic and aqueous media. Using RuNPs stabilized by organosoluble NHCs instead of RuNP@PVP led to faster kinetics for C(sp<sup>2</sup>)-H bonds deuteration (see Figure XX). Furthermore, the higher metal content of the carbene-containing nanoparticles facilitated both work-up and product purification, which is a crucial prerequisite for the method extension to the tritium labelling of oligonucleotides.



**Figure XX.** Deuterium labelling of pharmaceuticals. In blue, isotopic enrichment obtained with RuNP@PVP and in pink with RuNP@NHC.

In order to obtain good deuterium uptakes on hydrosoluble oligonucleotides, we used a new family of RuNP accommodating hydrosoluble NHC carbenes (NHC-PriPr) as ligands and displaying very similar size and shape to the organic soluble ones. We achieved with these NPs the deuteration of 6- and 12-mer oligonucleotides with an average deuterium atom uptake of respectively 5.5 and 7.7 D (see Figure XX) after 3 iterative run of catalysis. The viability of these molecules for SILS purpose in metabolomics and proteomics was confirmed when the relationship between the labelled/unlabelled MS intensity ratio and the oligomer concentration was found linear over at least two orders of magnitude (0.56-56  $\mu\text{M}$  in the case of the 6-mer compound).



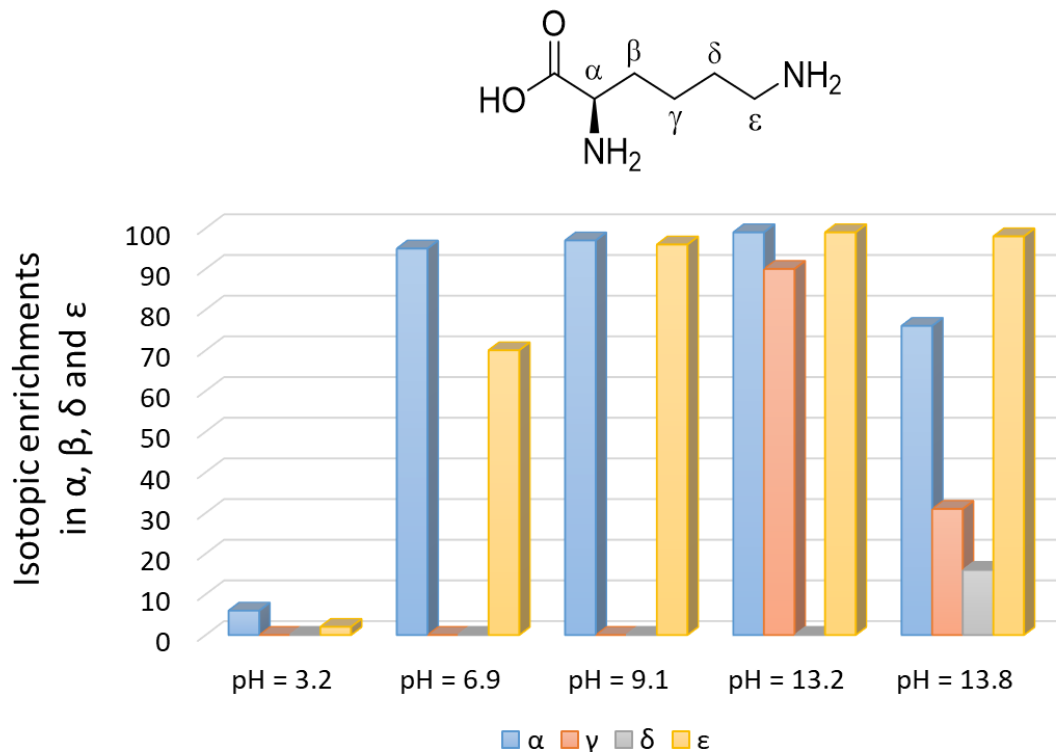
**Figure XX.** Structures of the labelled oligonucleotides and regioselectivity of the labelling.

### Ruthenium nanoparticles for C(sp<sup>3</sup>)-H activation.

As shown in the introduction, C(sp<sup>3</sup>)-H activation is also possible using RuNP but challenges exist as performing the reaction in water with different pH and the labelling on asymmetric centres.

To study the pH dependence of the catalytic process in water, the selectivity of lysine deuteration as a function of the pH was evaluated using either RuNP@PVP or RuNP accommodating hydrosoluble carbenes as catalysts.<sup>10</sup> At acidic pH, the two amino groups are protonated and do not bind to the NPs whereas at basic pH (13), the two amino groups are deprotonated and may be chelating on the NP. This translated into little deuteration up to pH 4, deuteration of the  $\alpha$  and  $\epsilon$

position near pH 7 with a preference for the  $\alpha$  position and deuteration of  $\alpha$ ,  $\gamma$  and  $\epsilon$  positions, with a minor deuteration of the  $\delta$  position at pH 13 (see Figure XX).



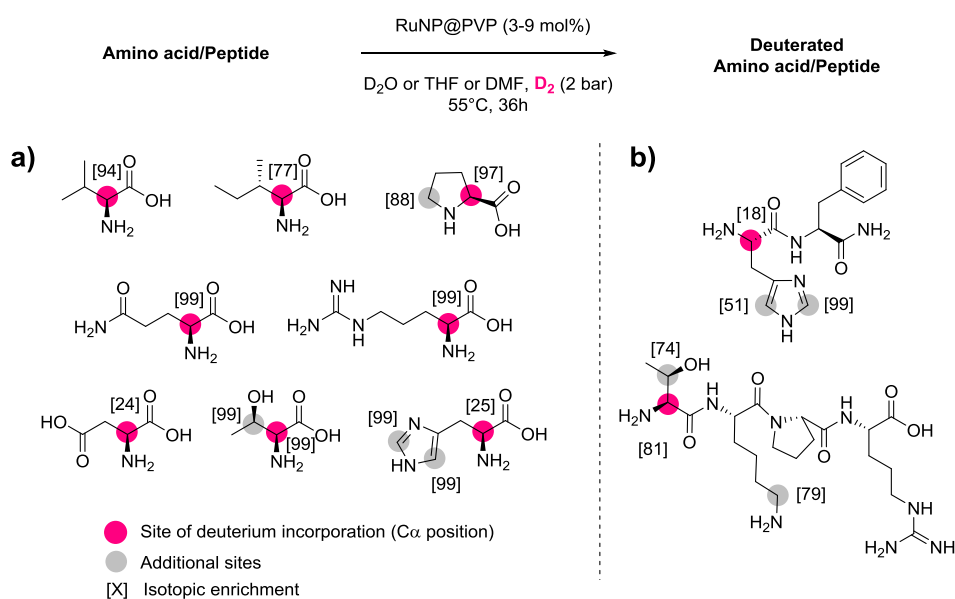
**Figure XX.** Isotopic enrichments in  $\alpha$ ,  $\beta$ ,  $\delta$  and  $\epsilon$  depending on the pH of the solution.

Here, the H/D exchange reaction can be directly monitored by NMR using the Chemical Shift Perturbation technique, which allows the determination of the nature of the chemical groups coordinated on the surface of the NPs. This technique confirmed that the deprotonated groups coordinated at the surface of the NPs and that at pH 7 the amino group of the alkyl chain, although protonated underwent a dynamic exchange with surface deuterides.

Regarding the labelling of C(sp<sup>3</sup>)-H bond on a asymmetric centre, our approach gave very promising results since the chiral amines were isolated in 70-80% yields, with nearly total isotopic enrichments (99%) and full retention of configuration.<sup>24</sup>

As deuterated amino acids are essential substrates, for mechanistic studies of biochemical processes<sup>25</sup> or in metabolomics<sup>26</sup>, methods were developed such as multistep synthesis<sup>27</sup> or through direct isotope incorporation but with either a limited scope<sup>28</sup> or requiring protecting groups<sup>29</sup> or using protic solvents, preventing their use for tritiation experiments.<sup>30</sup>

Using Ru nanocatalysis in water, we were able to perform the efficient deuteration of amino acids bearing aliphatic (Ala, Val, Leu, Ile), amide (Asn, Gln) and nitrogen-containing (Pro, Arg, Lys) side chains along with hydroxyl-containing amino-acids (Ser, Thr) where a labelling at the  $\alpha$ -position relative to the oxygen was also observed.

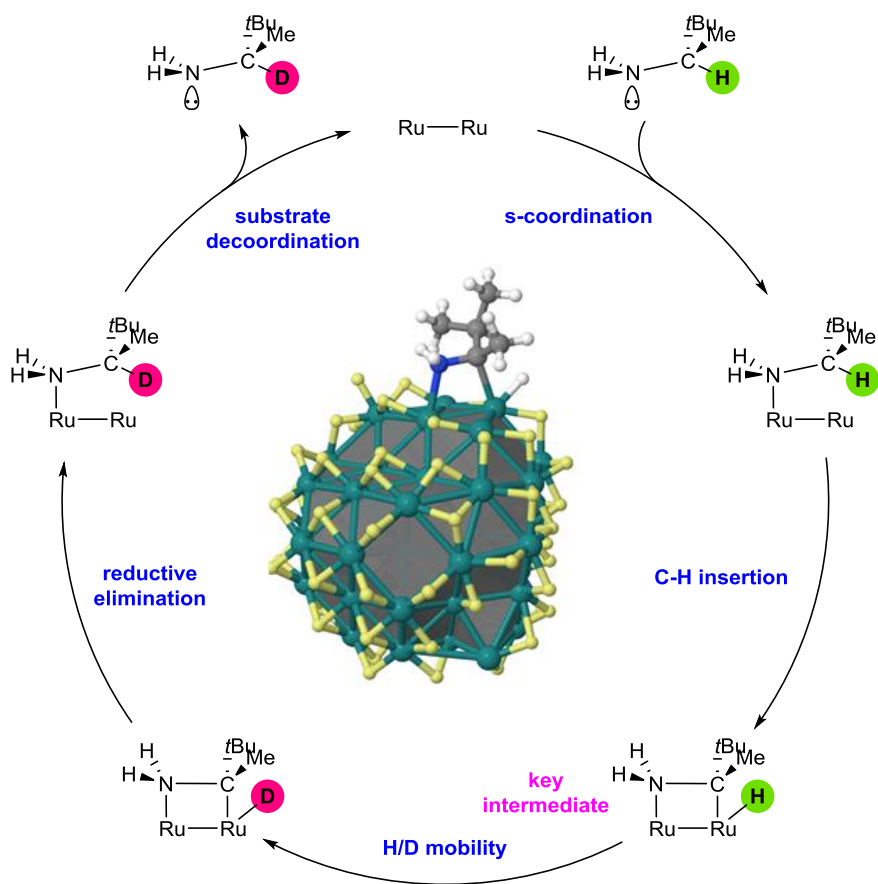


**Figure XX.** Enantiospecific C–H activation/deuteration of (a) amino acids and (b) biologically active peptides.

The labelled products were obtained with full enantiospecificity and good regioselectivity, regardless of the solvent used (THF, DMF or water). Applying this method to more complex and biologically-active peptides we managed to obtain substantial and regioselective deuterium

incorporation without any detectable epimerization, thus demonstrating its usefulness for the late-stage deuteration of complex peptides (see Figure XX).

DFT calculations were also performed in order to investigate the mechanism in order to explain the observed enantiospecificity and regioselectivity. The mechanistic pathway is very similar to the  $\alpha$ -deuteration of N-heterocycles one depicted before, with a four-membered dimetallacycle as key intermediate explaining the selectivities observed. The nitrogen and the vicinal carbon atoms bind to two adjacent Ru atoms with a retention of configuration due to the rigidity of the dimetallacycle and the barrierless H/D exchange process taking advantages of the high mobility and diffusion of the hydrides/deutrides at the surface of the RuNPs (see Figure XX). Interestingly, this theoretical study also suggest that deutrides diffusion may favor the HIE by significantly lowering the activation energy C-H activation step allowing the active metal center to keep its formal oxidation state during oxidative addition.

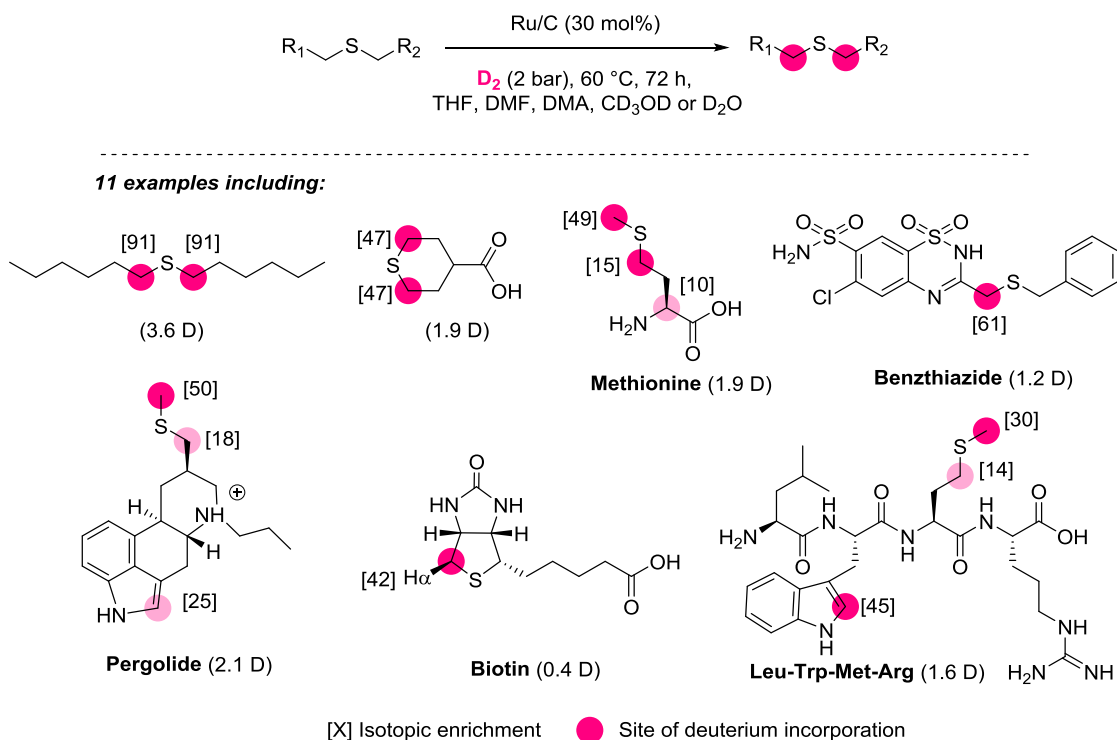


**Figure XX.** General mechanistic pathway for the enantioselective  $\alpha$ -deuteration of chiral amines using RuNP.(adapted from REF XXX)

In the context of the regioselective deuteration of aminoacids, we have also investigated the use of bimetallic RuPt nanoparticles.<sup>31</sup> We have looked for the change of selectivity induced by the change in the chemical order within the particle. For the RuPt system, there is only a slight change in selectivity for lysine deuteration but this opens the door to the use of many different bimetallic systems.

### Ruthenium on carbon.

Sulfur containing compounds, although present in a certain number of pharmaceuticals, are difficult to label by metal-mediated HIE because of the poisoning ability of the sulfur atom.<sup>32</sup> Therefore no HIE methods was described so far for the deuterium labelling of thioether substructures. With our knowledge in metal-catalyzed labelling, we started to address this problem focusing on the deuteration of dihexylsulfide as model substrate.<sup>33</sup> After screening conditions, the optimal heterogeneous catalysts for this transformation were RuNP@PVP and ruthenium on carbon in THF and under a pressure of 2 bar of D<sub>2</sub> gas. However, the large amount of catalyst needed in this case (30 mol%) made Ru/C more advantageous than other RuNP sources. These very simple experimental conditions were then applied to 11 relevant substructures and complex pharmaceuticals (amino acids, peptides and drugs) containing a sulfur atom (Figure x), and the corresponding labelled molecules were obtained with adequate regioselectivities and levels of isotope incorporation. Moreover, no epimerization was observed when the compound contained a stereogenic centre, making this method compatible with sensitive compounds and natural products.



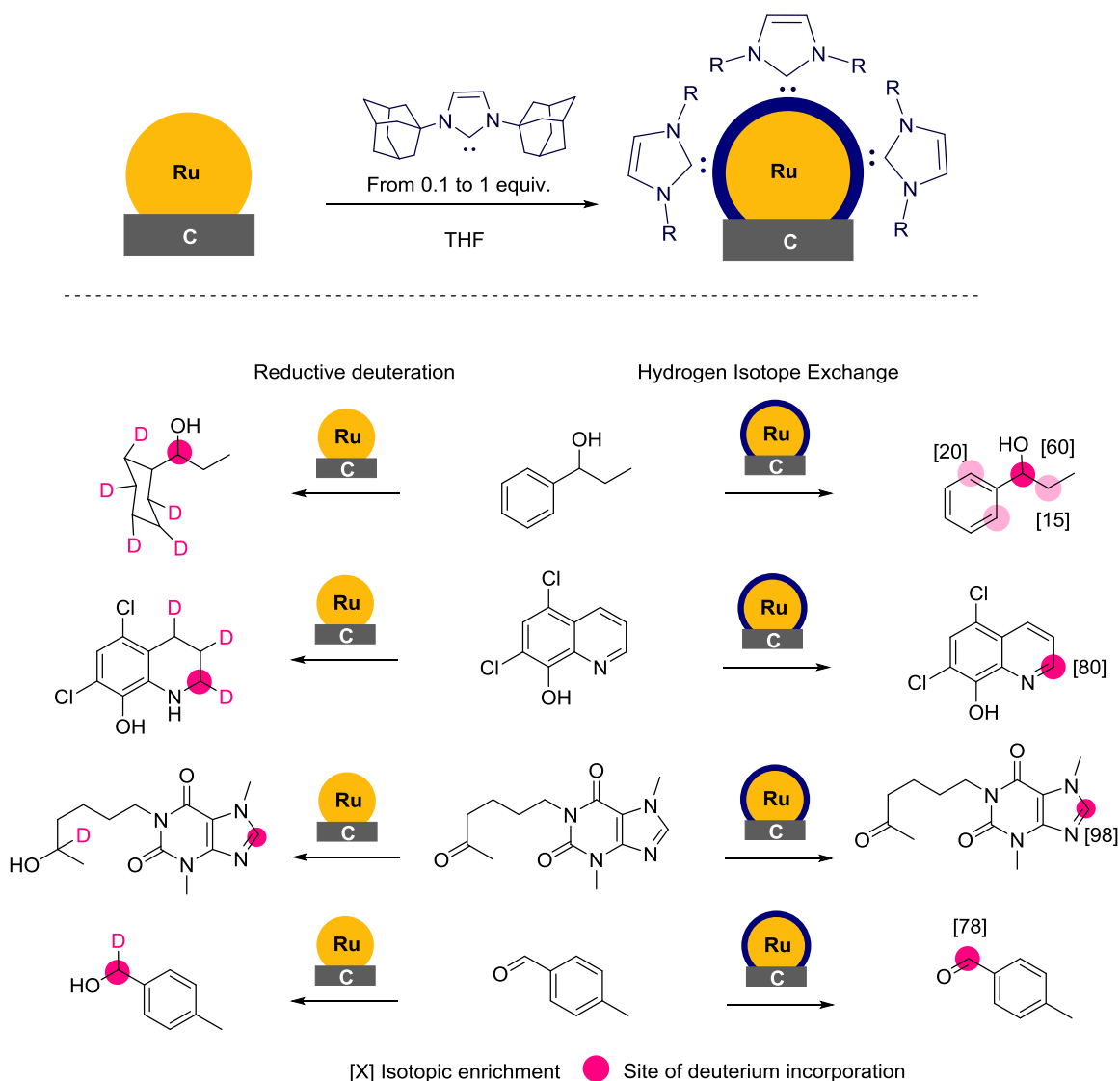
**Figure x.** Deuterium labelling of sulfur-containing compounds using Ru/C (30 mol%) and D<sub>2</sub> gas (2 bar).

The number of deuterium incorporated can be increased by simply reiterating the process: the crude mixture was filtered and the filtrate was put in the same reaction conditions than previously. For instance, for pergolide (a dopamine receptor agonist used for the treatment of Parkinson's disease) three successive runs were performed and a final 4.1 deuterium atoms incorporation was observed.

The proposed mechanism could go through a rigid 4-membered dimetallacycle where the sulfur atom coordinates to the ruthenium surface. The regioselectivity of the reaction is in favour of a C-H activation near the coordination of the ruthenium catalyst. It seemed that the C-S bond could

also be partly activated during the process as some MeSD was formed. This specie can poison the catalyst which could explain the high catalyst loading needed. This work presents therefore an attractive method for the deuterium labelling of sulfur-containing compounds in a context where very few methods already existed, and where it is still one of the only method by HIE so far.

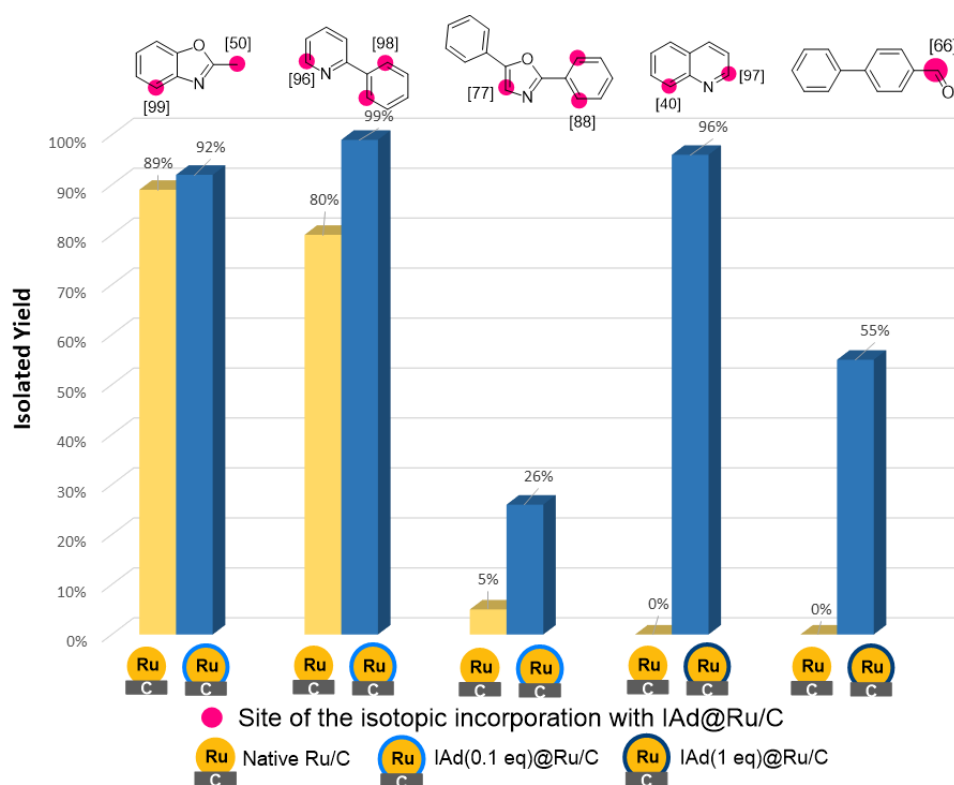
Despite its advantages and the first HIE method for the labelling of sulphur moieties, ruthenium on carbon is also known to be able to reduce moieties such as aromatic rings and unsaturated heterocycles and bonds. The competition between the reductive deuteration and the HIE reaction can be to the disadvantage of the latter. Therefore, efforts have been made by our teams to modulate the reactivity of ruthenium catalysts for HIE reactions by the addition of ligands. NHC ligands were chosen because they are capable of efficiently tuning the reactivity of homogeneous catalysts,<sup>34</sup> but also of heterogeneous catalysts as recently shown by the group of Glorius.<sup>35-37</sup> The addition of various amounts (0.1 to 1 equiv. vs Ru) of NHC ligands on Ru/C permitted the labelling over the reduction for a wide range of sensitive compounds with high regio- and chemo-selectivities (Figure X).<sup>38</sup>



**Figure x.** Synthesis of modified Ru/C catalysts and comparison of the deuterium labelling with Ru/C and IAd@Ru/C.

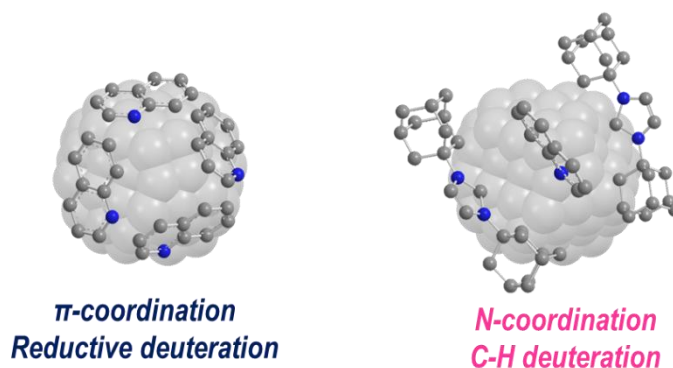
The modified ruthenium catalysts were first applied for the deuteration of alcohols, and the best regioselectivity at the  $\alpha$ -position of the oxygen atom of dodecanol was obtained with IAd(0.1 eq)@Ru/C. In this case, ruthenium nanoparticles gave only poor labelling on this substrate in similar conditions. More interestingly, an impressive switch of reactivity between commercially available Ru/C and modified Ru/C was observed regarding easily reducible phenyl derivatives

such as benzoxazole, phenylpyridine, oxazole and quinoline. High chemoselectivities were observed regarding the labelling versus the reductive deuteration process in all cases (see Figure XX). For quinoline, Ru/C gave only the reductive product whereas a 96% yield of the labelled quinoline was obtained with IAd(1 eq)@Ru/C in THF at 55 °C and under a pressure of 1 bar of D<sub>2</sub> gas hence demonstrating the efficiency of the modified catalyst to favour labelling over reductive reactions. Similarly, applying modified catalysts for the labelling of aldehyde moieties gave access to C-1 deuterated compounds instead of reduced ones (see Figure XX). Indeed, five aldehydes were deuterated using IAd(1 eq)@Ru/C, with labelling up to 78% and yields up to 86%, whereas only alcohols as the reductive products were observed with Ru/C.



**Figure XX.** Deuterium labelling of reducible functions and comparison of reactivity between commercial Ru/C and modified Ru/C.

This switch of reactivity was explained by the incorporation of bulky ligands at the surface of the ruthenium catalyst. Indeed, IAd ligands coordinate strongly at the surface of the catalyst, thus reducing the available surface for the substrate coordination. The  $\pi$ -coordination necessary for the reductive deuteration becomes more difficult than the coordination of a nitrogen or an oxygen atom necessary for the labelling (see Figure X).



**Figure X.** Schematic representation of the coordination of quinoline depending on the nature of the Ru catalyst: Native Ru/C catalyst on the left ( $\pi$ -coordination) and NHC modified catalyst on the right (N-coordination).

Control experiments showed that the rate of the reductive deuteration was therefore reduced compared to the rate of the deuterium labelling. This study represents the first demonstration of the NHC ligands' great potential to tune the reactivity of heterogeneous catalysts in the context of C-H activation reactions, and beyond the domain of hydrogen isotope labelling, offers new avenues in this important field of research.

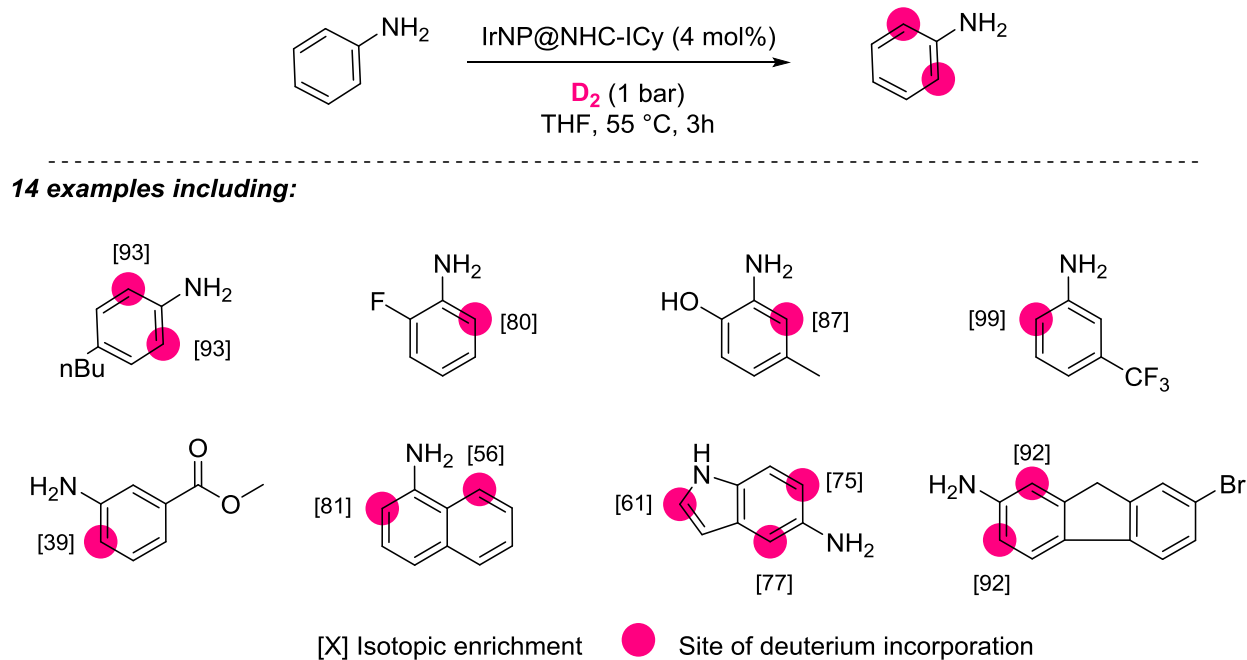
### **Iridium nanoparticles.**

Ruthenium nanoparticles have proved their efficiency and versatility in HIE reactions, but have also demonstrated limitations such as the competition with reductive deuteration on easily reduced substrates. Therefore, some substructures such as anilines were found to be incompatible with HIE Ru nanocatalysis. Other metals were envisioned for the catalytic labelling of such compounds, and iridium seemed a good choice for this investigation as iridium is particularly well-known in homogeneous catalysis for HIE reactions since the discovery of Crabtree catalyst in 1979.<sup>39</sup> Since then, a plethora of iridium catalysts have been described for the homogeneous labelling of heterocycles and pharmaceuticals and some of them are commercially available as Kerr's or Burgess' catalysts.<sup>40-43</sup> These iridium catalysts are known to perform labelling in the *ortho* position of a directing group through 5- or 6-iridacycles and labelled compounds can be obtained with very high efficiency and regioselectivity.<sup>44</sup> However, despite the advantages of heterogeneous catalysis for multiple sites labelling and purification, iridium heterogeneous catalysts and nanoparticles are almost unseen in this field.

Iridium nanoparticles have been synthesized starting from the commercially available  $[\text{Ir}(\text{COD})(\text{OMe})_2]$  (COD = 1,5 cyclooctadiene) precursor and N-heterocyclic carbene ligands under 5 bar of  $\text{H}_2$  gas at room temperature.<sup>45</sup> These very small (1 to 1.5 nm) NPs have been fully characterized and are easy to handle thanks to their air-stability properties.

These new iridium nanoparticles were first tested in HIE reactions on anilines. Anilines are essential substructures in drugs, but no efficient and general methods using  $\text{D}_2$  or  $\text{T}_2$  as isotopic source have been described so far to gain access to deuterated or more complex tritiated anilines with high isotope incorporation along with a high degree of compatibility regarding sensitive functional groups. Using mild conditions, iridium NPs catalyzed the labelling of simple anilines

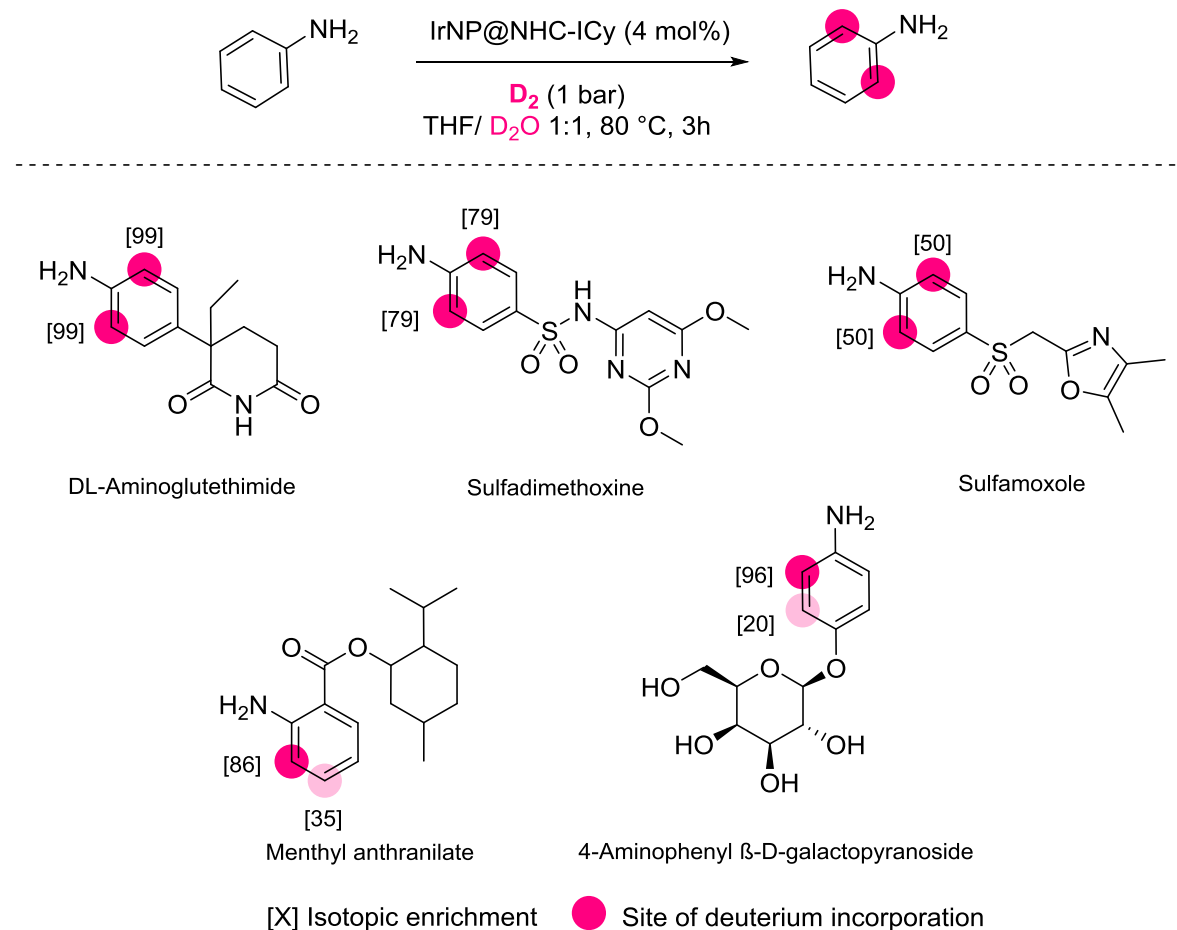
leading to high isotope incorporation in the *ortho* position of the amine group (Figure x), while ruthenium nanoparticles led mainly to the reduction of the substrate.



**Figure x.** Deuteration of anilines with iridium nanoparticles in THF under  $D_2$  gas.

Deuterated anilines were obtained with high regioselectivity but also with good isotopic enrichments. Furthermore, this method is compatible with diverse functional groups such as halogens (fluorine, bromide), alcohols, ketones or esters, thus paving the way to more complex structures. Then, more polar anilines were envisioned, but only moderate deuterium labelling was obtained with the iridium nanoparticles in THF. Heating at 80 °C and adding  $D_2O$  as co-solvent solved the problem and gave nice results for the labelling of complex anilines. These new deuteration conditions were applied to five pharmaceuticals with success: for example DL-Aminoglutethimide was labelled on two positions (Figure x). All these results demonstrate the

importance of iridium nanoparticles for the labelling of large scope of complex anilines with a great tolerance for sensitive functional groups.



**Figure x.** Deuteration of pharmaceuticals with iridium nanoparticles in THF/D<sub>2</sub>O under D<sub>2</sub> gas.

### Tritium labelling using metallic nanoparticles.

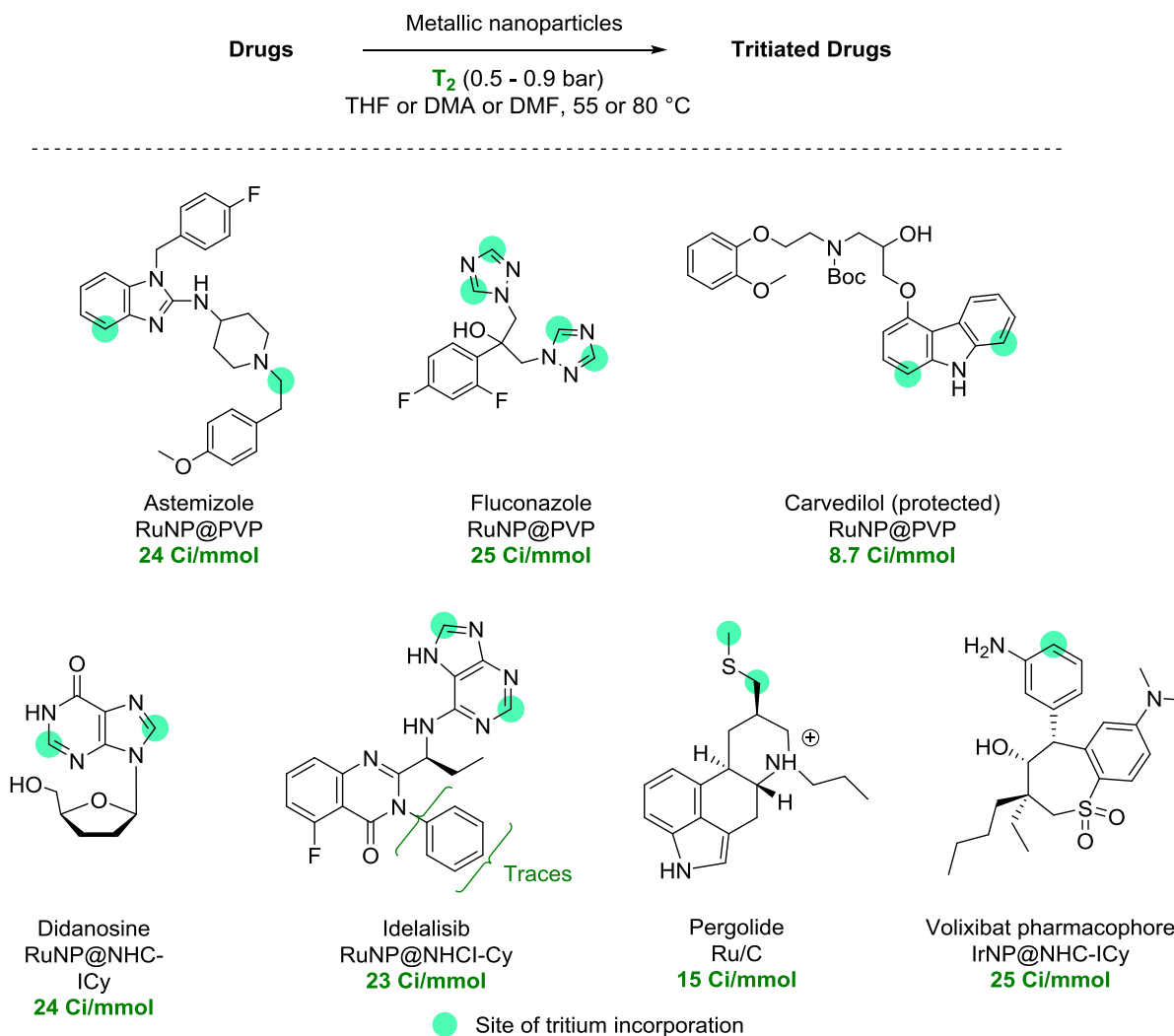
Tritium labelled molecules are of paramount importance in the drug discovery process: they are used for autoradiography or in radioligand binding assays and they can act as radioactive

tracers for *in vivo* fate studies.<sup>1</sup> For these purposes, labelled compounds need to be obtained with a high level of isotope incorporation (around 10-15 Ci.mmol<sup>-1</sup> for most of their applications but up to a minimum of 50 Ci.mmol<sup>-1</sup> for their use in binding assays). Our methods seem suitable for tritium labelling because it may allow the late-stage incorporation of an important number of tritium atoms in only one-step, thus accessing compounds with sufficient molar activity and limiting radioactive waste, time and costs spent for the synthesis. Besides, heterogeneous catalysts and nanoparticles are easy to eliminate by simple filtration, thus facilitating the purification process. Regarding the isotopic source, D<sub>2</sub> gas is easily replaced by T<sub>2</sub> gas, which is the isotopic source of choice since it is by far the easiest accessible raw material.

Methods developed were applied to the tritiation of biologically-relevant compounds under the same reaction conditions except for the use of a subatmospheric pressure of T<sub>2</sub> gas to minimize the risk of leakage and radioactive releases and higher catalytic loadings.

Using RuNP@PVP, we successfully performed the labelling of three main scaffolds: benzimidazole (Astemizole), 1,2,4-triazole (Fluconazole) and carbazole (protected Carvedilol). We obtained molar activities up to 24.7 Ci.mmol<sup>-1</sup>, in accordance with the prerequisites for ADME studies (see Figure XX).<sup>19</sup> Following our NP engineering studies, organo-soluble RuNP@NHC-ICy was used to achieve the tritiation of Didanosine (an antiretroviral agent) and Idelalisib (used for the treatment of blood cancer) which were obtained with good specific activities (> 23 Ci.mmol<sup>-1</sup>).<sup>23</sup> Pergolide, a sulphur-containing compound, was also tritiated with a molar activity of 15 Ci.mmol<sup>-1</sup> in similar conditions using ruthenium on carbon, again making it compatible with ADME requirements.<sup>33</sup> Finally, iridium nanoparticles were used for the

tritiation of the Volixibat pharmacophore, and the drug was nicely labelled with a molar activity of 25 Ci.mmol<sup>-1</sup>, in accordance with the result obtained for deuteration processes.<sup>45</sup>



**Figure XX.** Tritium labelling of complex pharmaceuticals using metallic nanoparticles.

However, despite their numerous advantages, their use for tritiation experiments can be limited by: (i) the need of strict anhydrous conditions, as the nanoparticles can catalyze the  $T_2/H_2O$  exchange, thus diluting the isotopic source; (ii) the formation of by-products coming from a reductive pathway when the substrate is easily reducible (e.g. quinoline); (iii) the need of a  $T_2$  gas pressure above 500 mbar when some homogeneous catalysts are able to perform labelling

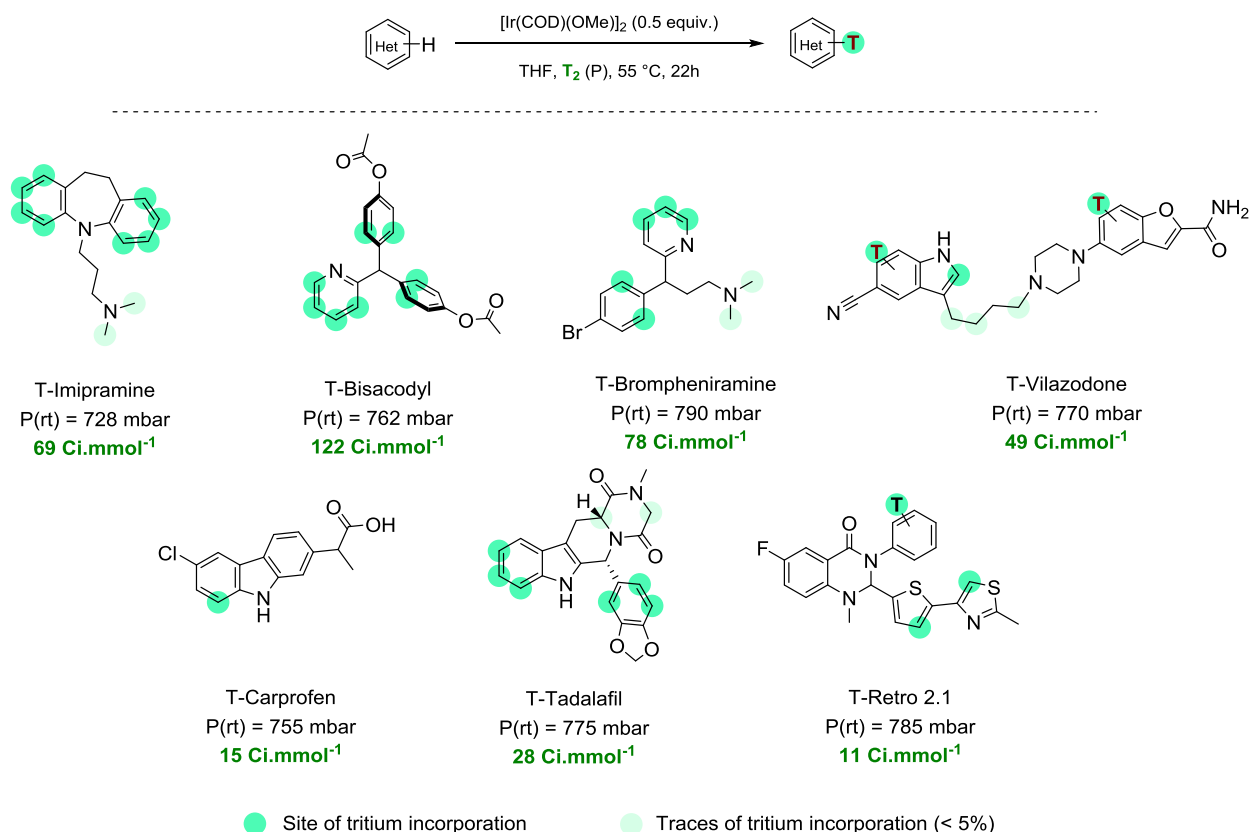
reaction under 200 mbar or even less, thus limiting their application in some radiolabelling laboratories. Nevertheless, we strongly think that the aforementioned advantages of using NP outweigh these weaknesses and we are still working on the development and improvement of new catalysts.

Hence, investigations were carried out to try to overcome some of the mentioned drawbacks. We discovered that the commercially available and air-stable  $[\text{Ir}(\text{COD})(\text{OMe})]_2$  used as precursor for the synthesis of the IrNP particles was a powerful catalyst precursor itself for the labelling of a wide range of compounds.<sup>46</sup> Selected as model substrate, 2-phenylpyridine was labelled not only at the *ortho* position of the phenyl ring as usually obtained using an homogeneous catalyst, or at the pyridinic core usually obtained using nanoparticles, but on both sites as a combination of the two types of catalysis (THF at 55°C and under 1 bar of D<sub>2</sub> gas) under mild conditions leading to a substantial deuteration of almost all positions.

This new and surprising combination of reactivities questioned about the nature of the active species. TEM and HR-TEM experiments, as well as mass spectroscopy measurements, revealed the presence of at least two types of catalytic species formed *in situ*: Ir nanoparticles and homogeneous Ir complexes. Therefore, we concluded that iridium nanoparticles are formed *in situ* during the reaction and are responsible for the labelling on positions not accessible by homogeneous catalysis. Encouraged by this discovery which can lead us to the maximization of the hydrogen isotope incorporation and therefore to applications requiring high molar activities or isotopic enrichments, the scope of the reaction was evaluated and a large number of structures (pyridines, oxa- and thioazoles, carbazole, indole) was deuterated with amazing incorporation rates and a high tolerance towards sensitive functional groups (nitrile and halogen atoms: Br, Cl

and F). We then turned our attention to more complex structures and pharmaceuticals. Thanks to the multiple-sites labelling, high deuterium incorporations were obtained also, often exceeding previous described results.

This method showed promising advantages for the tritiation of pharmaceuticals: the catalyst is easy to handle, the tritium source is T<sub>2</sub> gas and high isotope incorporation is expected. Therefore, we performed the tritiation of seven interesting pharmaceuticals, and the reactions gave, as expected, tritiated molecules with very high molar activities (see Figure x). Bisacodyl was, for instance, obtained with a molar activity of 122 Ci.mmol<sup>-1</sup>, thus overwhelming the activities obtained by HIE previously described so far.



**Figure x.** Tritiation of complex pharmaceuticals with [Ir(COD)(OMe)]<sub>2</sub> in THF under T<sub>2</sub> gas.

Overall, the commercially available  $[\text{Ir}(\text{COD})(\text{OMe})_2]$  is an efficient catalyst precursor of molecular and nanoparticulate species which allow the deuteration and tritiation of a wide range of compounds. The rate of isotope incorporation can be very high, and numerous substrates are compatible with the reaction conditions.

### **Conclusion and perspectives.**

The results summarized in this account illustrate the usefulness of Ru nanoparticles to catalyze Hydrogen Isotope Exchange reactions. Due notably to the polymetallic nature of their surface and the surface hydride mobility, RuNPs are able to activate a broad variety of C-H bonds in complex molecules followed by either deuterium or tritium incorporation in presence of  $\text{D}_2$  or  $\text{T}_2$  gas within low energy barriers. Their great solvent tolerability allied with the possibility to perform multiple isotope incorporation using mild reaction conditions make this approach complementary to the use of homogeneous catalysts to synthesize complex labelled molecules. Recent contributions also highlight the interesting perspectives offered by : 1) the possibility to increase the regio- and chemo-selectivities of such nanocatalysts (or heterogeneous catalysts) by adding organic ligands (e.g. NHC) at their surface; 2) the *in situ* formation of metallic nanoparticles allowing to perform multiple site HIE using easy-to-implement synthetic methods.

On the fundamental viewpoint, using hydrogen isotope incorporation appeared as interesting tools to monitor the impact of nanoparticles engineering on their catalytic activities (regioselectivity, chemoselectivity, kinetics,...) in the context of C-H activation reactions. On a more applicative view point, the discovery of novel well defined nanoparticles or organometallic precursors allowing the *in situ* formation of nanoclusters, involving non-noble metals (Ni,<sup>11</sup> Fe,

...) and owning good performances for HIE would be highly desirable and are currently under investigation.

## AUTHOR INFORMATION

### Corresponding Author

**Sophie Feuillastre and Grégory Pieters**, Université Paris-Saclay, CEA, INRAE, Département Médicaments et Technologies pour la Santé (DMTS), SCBM, F-91191, Gif-sur-Yvette, France ;  
sophie.feuillastre@cea.fr; gregory.pieters@cea.fr

### Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. ‡These authors contributed equally.

### Notes

Any additional relevant notes should be placed here.

### Biographies

**Marco LEPRON** graduated from the Ecole Nationale Supérieure de Chimie de Montpellier in 2016. He then obtained his PhD degree from Paris-Saclay University under the supervision of Prof. Delphine Joseph and Dr. Emmanuelle Drège (2016-2019). He is now working as a postdoctoral fellow in the tritium laboratory at the Molecular Labeling and Bio-organic Chemistry Unit at the CEA.

**Marion DANIEL-BERTRAND** obtained her PhD from the University Pierre et Marie Curie (Paris) in 2013 under the supervision of Prof. Fensterbank. After two postdoctoral experiences (ICSN and ILV, France), she worked in the laboratory of Prof. Cossy at the ESPCI (2016-2018, France). Then she joined the tritium laboratory at the CEA in 2019 where she worked as a postdoctoral fellow.

**Bruno CHAUDRET** graduated from École Nationale Supérieure de Chimie de Paris in 1975. He received his Ph.D. from Imperial College London in 1977 with Sir Geoffrey Wilkinson and the degree of a “Docteur és Sciences” from the University of Toulouse in 1979. He is now “Director of Research CNRS” and Director of the “Laboratoire de Physique et Chimie des Nano-Objets” in Toulouse and is a member of the French Academy of Science and of Academia Europaea. He is a coauthor of more than 480 publications and 17 patents.

**Gabriel MENCIA** obtained his degree in chemistry from the University of Alcalá (Spain) and started his PhD degree in 2015 at the University of Alcalá under the supervision of Prof. Rafael Gomez and Dr. Jesus Cano. Since 2020, he is a postdoctoral research scientist at the LPCNO of Toulouse under the supervision of Dr. Bruno Chaudret.

**Sophie FEUILLASTRE** obtained in 2014 her PhD degree from Lyon 1 University (France) under the supervision of Prof. Piva (2011-2014). She moved next to the CEA as a postdoctoral fellow (2014-2016). After working for a SME supplier in Europe of stable isotopes, as the main leader and senior scientist, she held in 2016 a permanent position at the CEA as a researcher in the tritium laboratory.

**Grégory PIETERS** obtained his PhD from UVSQ (France) in 2010 (Prim group). After a postdoctoral experience at University of Padova (Prins group, until June 2012), he integrated CEA first as a postdoctoral fellow (Rousseau group, until June 2013) then as a permanent researcher. In 2017, he obtained the agreement to supervise research (HDR) and became group leader at the tritium laboratory at the Molecular Labeling and Bio-organic Chemistry Unit. He is a co-author of more than 53 publications and 3 patents.

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## ABBREVIATIONS

HIE, Hydrogen Isotope Exchange; NP, Nanoparticles; PVP, polyvinylpyrrolidone; NHC, N-heterocyclic carbenes.

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