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Title: Hydrogen Isotope Exchange Catalyzed by Ru Nanocatalysts: Labelling of Complex Molecules Containing N-Heterocycles and Reaction Mechanism Insights

Authors: Grégory Pieters, Viktor Pfeifer, Marie Certiat, Donia Bouzouita, Alberto Palazzolo, Sébastien Garcia-Argote, Elodie Marcon, David-Alexandre Buisson, Philippe Lesot, Laurent Maron, Bruno Chaudret, Simon Tricard, Iker del Rosal, Romuald Poteau, and Sophie Feuillastre

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Hydrogen Isotope Exchange Catalyzed by Ru Nanocatalysts: Labelling of Complex Molecules Containing N-Heterocycles and Reaction Mechanism Insights

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Abstract: Ruthenium nanocatalysis can provide effective deuteration and tritiation of oxazole, imidazole, triazole and carbazole substructures in complex molecules using D_2 or T_2 gas as isotopic sources. Depending on the substructure considered, this approach does not only represent a significant step forward in practice, but also the unique way to label important heterocycles using hydrogen isotope exchange. In terms of applications, the high incorporation of deuterium atoms allows the synthesis of internal standards for LC-MS quantification. Moreover, the efficacy of the catalyst permits, even under subatmospheric pressure of T_2 gas, the preparation of complex radiolabeled drugs owning high molar activities. From a fundamental point of view, a detailed DFT-based mechanistic study identifying undislosed key intermediates, allowed a deeper understanding of C-H (and N-H) activation processes occurring at the surface of metallic nanoclusters.

Introduction

Nitrogen-based heterocycles like imidazoles, benzimidazoles and triazoles rank on the top of the most frequently approved small molecule drugs by US FDA. For example, 1,2,4-triazoles are prominent structural motifs in many commercial antifungal drugs, anti-tumor, anti-migraine agents and many others. Other heterocycles such as oxazoles and carbazoles are also commonly encountered substructures in drug development, biologically active natural products and in material science as fluorescent molecules where deuterium incorporation can be interesting for the enhancement of fluorescence properties. Due to the occurrence of N-heterocycles in many types of useful molecules, the development of efficient methods for isotopic labelling, particularly with hydrogen isotopes, is of paramount importance. Indeed, titrated analogues of drug candidates are considered as essential tools for studying the in vivo fate of drug candidates during absorption, distribution, metabolism and excretion (ADME) studies. In this context, the development of robust methods allowing the incorporation of at least 0.5 T (corresponding to a molar activity of 15 Ci/mmol) in complex molecules using mild reaction conditions (ideally with a subatmospheric pressure of T_2 gas) is of particular interest. Deuterated compounds are also widely employed in various life science applications, such as metabolomics and proteomics as internal standards for quantitative LC- and GC-MS analyses.

To facilitate the synthetic access to these valuable isotopically labelled compounds, the discovery of new approaches enabling the selective incorporation of at least three deuterium atoms in small complex molecules, is appealing. In the case of molecules containing oxazole and imidazole substructures, high isotopic enrichments can be obtained using Ir(I) catalysts, but only for compounds owning an aromatic ring in position 2 (see Figure 1a). In addition, only C(sp^2)-H bonds in the 2-position relative to the directing atom (DA) can be activated efficiently, and as a consequence only two deuterium atoms can be incorporated (per DA). Concerning the deuterium and tritium labelling of some other N-heterocyclic cores such as 1,2,3-triazole, remarkable results have been described recently by Chirik and coworkers employing an air sensitive bis(arylimidazol-2-yldene)pyridine iron bis(dinitrogen) (see Figure 1b) and a dimeric nickel hydride complex.

![Figure 1: Main previous works on the deuterium/tritium labelling of oxazole, imidazole, triazole and carbazole substructures using HIE procedures.](image-url)
Even if the iron catalyzed hydrogen isotope exchange (HIE) procedure possesses the advantage to work at low pressure of D_2 (1 bar), it afforded moderate deuterium incorporation on the 1,2,3-triazole moiety of suvorexant (0.8 D incorporated). For 1,2,4-triazole- and carbazole substructures (see Figure 1c), no broadly applicable HIE methods have been described so far, to the best of our knowledge. In this paper, we demonstrate that the selective deuterium and tritium labelling of the oxazole, (benz)imidazole, triazole and carbazole pattern in complex molecules can be achieved using Ru nanoparticles (RuNps) as catalyst (see Figure 2). For oxazole and imidazole substructures, this approach possesses a broader scope of applications, a higher solvent applicability, and allows, in most cases, higher isotope incorporations under mild reactions conditions compared to previously described methods.

On top of that, this work describes also the first general method for the selective deuterium and tritium labelling of the 1,2,4-triazole and carbazole scaffold by HIE reactions. From an application point of view, these reactions can be used for the late stage radiolabelling of complex pharmaceuticals (applying a low pressure of tritium gas) and the preparation of internal standards for LC-MS quantification. On a more fundamental aspect, a deeper understanding of the reaction mechanisms involved in C-H and N-H activation processes at the surface of the Ru nanocatalyst was provided by DFT calculations. These studies notably revealed the formation of different types of key intermediates, explaining the isotope incorporation at α, β and γ positions of the directing nitrogen atom.

**Results and Discussion**

**Labelling of oxazole derivatives**

We decided to initiate our studies with the deuteriation of oxazole derivatives because effective methods to label this substructure are still scarce. Based on our previous results, we envisioned that Ru nanoparticles might be used as a catalyst for the deuteration of such nitrogen containing heterocycles. Different oxazole derivatives 1-4 were successfully deuterated at 50°C in THF or DMA as solvent using a catalytic amount of ruthenium nanoparticles (5 mol%) stabilized in a polyvinylpyrrolidone matrix (RuNp@PVP) under 2 bar of D_2 gas (see Figure 3). Under these reaction conditions, diphenyloxazole 1 was labelled with a total uptake of 2.6 deuterium atoms, incorporated at the oxazole core and in the ortho positions of the phenyl group. The possibility to selectively active C-H bonds at α and γ positions to the nitrogen atom of 1 permitted to obtain higher deuterium incorporation than the one achievable over homogeneous Ir(I) catalysis (where only two deuteriums (2 D) can be theoretically introduced). The substrates 2-4 were used to demonstrate the broader substrate scope of RuNp-catalyzed HIE, as they cannot be labeled by other existing approaches such as Ir(I)-catalysis due to their substitution pattern. Indeed, compounds 2 and 3 were successfully deuterated at the C_2 and C_4 position of the oxazole ring (both α-positions relative to the nitrogen atom). Furthermore, regio- and chemoselective labelling at C_2 of carboxylic compound 4 was achieved by using DMA as solvent.

Theoretical calculations at the DFT-PBE level of theory were conducted in order to study the reaction pathway leading to the C(sp^2)-H activation at the α positions of the nitrogen atom. To achieve this study, a 0.5 nm ruthenium cluster with 1.4 H atoms per Ru surface atom (Ru_{1.4}H_{20}) was used as RuNp model. As we can see in Figure 4, the coordination of 3 to the RuNp model through the lone pair of the nitrogen atom is an exothermic process (3\(^\circ\), ca. -19 kcalmol\(^{-1}\); the labelling is explained in the SI). From this intermediate, a stabilizing agostic interaction can be established between the C_2-H (3\(^{agst}\), green pathway) or C_4-H (3\(^{agst}\), blue pathway) group and one of the first-neighbor ruthenium atoms to the one that interacts with the nitrogen atom. The formation of this three-center, two-electron bond between a C-H bonding orbital and an empty metal orbital is evidenced by
the slight carbon pyramidalization (accompanied by the lifting of the hydrogen atom out of the plane of the oxazole ring). From both 4-membered dimetallacycle intermediates $3^{\gamma\text{R}, \gamma\text{H}}$ and $3^{\gamma\text{R}, \gamma\text{D}}$, the C–H bond activation is a kinetically accessible process with an activation barrier of 6.0 kcal.mol$^{-1}$ on C$_2$ position ($3^{\gamma\text{R}, \gamma\text{C}}_{\gamma\text{R}}$) and of 4.2 kcal.mol$^{-1}$ on C$_2$ position ($3^{\gamma\text{R}, \gamma\text{C}}_{\gamma\text{D}}$). However, from a thermodynamic point of view, the C–H bond breaking is an almost athermic process at the C$_2$ position ($3^{\gamma\text{R}, \gamma\text{C}}_{\gamma\text{R}}$; $+1$ kcal.mol$^{-1}$ with respect to $3^{\gamma\text{R}, \gamma\text{C}}_{\gamma\text{D}}$) whereas it is clearly endothermic at the C$_4$ position ($3^{\gamma\text{R}, \gamma\text{C}}_{\gamma\text{R}}$; $+4.3$ kcal.mol$^{-1}$ w.r.t. $3^{\gamma\text{R}, \gamma\text{C}}_{\gamma\text{D}}$). The H$^\text{+}$ atom can then easily exchange its position with one of the numerous deuterides already present at the surface of the RuNps (H$^\text{+}$ $\leftrightarrow$ D$^\text{+}$ step in Figure 4), as previously demonstrated by NMR experiments.$^{34}$ One available deuteride in the vicinity of the active site can then recombine with the C$_2$ atom, provided that kinetics and thermodynamics do not impede it (the full pathway is given in Figure S1). In this mechanism, the first key parameter is the formation of a 4-membered dimetallacycle in both pathways ($3^{\gamma\text{R}, \gamma\text{C}}_{\gamma\text{R}}$, X=2 or 4). But owing to the small barrier heights, a second key parameter is the competition between the (C–H)$^\text{+}$ $\leftrightarrow$ (C)$^\text{+}$([H]) reaction (i.e. $3^{\gamma\text{R}, \gamma\text{C}}_{\gamma\text{R}}$ $\leftrightarrow$ ($3^{\gamma\text{R}, \gamma\text{C}}_{\gamma\text{C}}$)([H])) and the (C)$^\text{+}$([H]) $\leftrightarrow$ (C–H)$^\text{+}$ back reaction. The lower deuterium incorporation at the C$_2$ position (15%) versus the C$_2$ position (98%), experimentally observed for compound 3, can therefore be explained by the small barrier (only 1.7 kcal.mol$^{-1}$) for the back reaction (from $3^{\gamma\text{R}, \gamma\text{C}}_{\gamma\text{R}}$ to $3^{\gamma\text{R}, \gamma\text{C}}_{\gamma\text{D}}$), thereby reducing the efficiency of the global process (see also Figure S2 for a fully detailed pathway, i.e up to the final D incorporation). A similar explanation can probably be invoked for compound 2, where the isotopic labelling at the C$_2$ position (99%) was found to be higher than that measured at the C$_4$ position (26%).

**Labelling of imidazole derivatives**

Further, RuNp@PVP catalyzed deuteration were also conducted on various imidazole derivatives (see Figure 5). 2-Phenylimidazol-5(4H)-one showed high deuterium incorporation in the ortho-positions of the phenyl and at both γ-positions relative to the imidazole nitrogen atom. This result represented another example where the use of RuNps as catalyst yielded a higher deuterium incorporation (3.3 D) than Ir(I)-based catalysts, which would lead to the incorporation of only two deuteriums at a maximum.

<table>
<thead>
<tr>
<th>Imidazole derivatives</th>
<th>RuNp@PVP (5 mol%)</th>
<th>Deuterated Imidazole derivatives</th>
</tr>
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<tbody>
<tr>
<td>D$_2$ (2 bar), 55 °C</td>
<td>THF or DMA, overnight</td>
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Site of the deuterium incorporation [X] isotopic enrichment

<table>
<thead>
<tr>
<th>Site of the deuterium incorporation</th>
<th>Isotopic enrichment obtained after a second run of catalysis</th>
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<tr>
<td>[96]</td>
<td>[62]</td>
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<tr>
<td>[96]</td>
<td>[62]</td>
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<tr>
<td>[12]</td>
<td>[62]</td>
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<tr>
<td>[81]</td>
<td>[98]</td>
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<td>[57]</td>
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* Isotopic enrichment obtained after a second run of catalysis

Figure 5: Examples of deuterated imidazole derivatives

The deuterium incorporation on both sites of 5 has also been considered from a theoretical point of view in order to identify notably, the key intermediate leading to the labelling at the γ-positions of the coordinating nitrogen. In order to answer this question, two competitive pathways were investigated (γ1 in red and γ2 in blue, Figure 6a). Independent of the considered pathway, compound 5 is initially adsorbed at the RuNp surface through the lone pair of the N$_2$ nitrogen atom to give $5^\text{R}$, followed by the formation of a stabilizing C–H agostic interaction. It is noteworthy that two different adducts can be formed, either with the same ruthenium atom ($5^{\gamma\text{R}, \gamma\text{H}}$) or with two neighboring ruthenium atoms ($5^{\gamma\text{R}, \gamma\text{R}}$). They lead respectively to a five-membered metallaacycle (a key intermediate analogue to the one proposed in homogeneous catalysis) or a six-membered dimetallacycle adduct. The pathway that involves a six-membered metallaacycle is both endothermic and kinetically accessible (blue pathway in Figure 6a). This mechanism cannot be excluded for the deuterium incorporation but it is probably inefficient due to the small barrier (1.9 kcal.mol$^{-1}$) and the exothermicity (ca. -4 kcal.mol$^{-1}$) of the (C)$^\text{+}$([H]) $\leftrightarrow$ (C–H)$^\text{+}$ back reaction (i.e. $5^{\gamma\text{R}, \gamma\text{R}}$ $\leftrightarrow$ $5^{\gamma\text{R}, \gamma\text{R}}$). The C–H activation involving a five-membered metallaacycle (red pathway in Figure 6a) is also kinetically accessible, with an activation barrier of 7.4 kcal.mol$^{-1}$, but thermodynamically more favorable (-4.1 kcal.mol$^{-1}$) on the contrary to the γ1 case.

Figure 4: Energy diagram for the first steps of the Langmuir–Hinshelwood-type H/D exchange on the C$_2$ (green pathway) and C$_4$ (blue pathway) position of the oxazole ring of compound 3. Energy values are given in kcal.mol$^{-1}$. The full pathway, i.e. with the D incorporation, is given in Figure S1.

Figure 6: Examples of deuterated imidazole derivatives

The deuterium incorporation on both sites of 5 has also been considered from a theoretical point of view in order to identify notably, the key intermediate leading to the labelling at the γ-positions of the coordinating nitrogen. In order to answer this question, two competitive pathways were investigated (γ1 in red and γ2 in blue, Figure 6a). Independent of the considered pathway, compound 5 is initially adsorbed at the RuNp surface through the lone pair of the N$_2$ nitrogen atom to give $5^\text{R}$, followed by the formation of a stabilizing C–H agostic interaction. It is noteworthy that two different adducts can be formed, either with the same ruthenium atom ($5^{\gamma\text{R}, \gamma\text{H}}$) or with two neighboring ruthenium atoms ($5^{\gamma\text{R}, \gamma\text{R}}$). They lead respectively to a five-membered metallaacycle (a key intermediate analogue to the one proposed in homogeneous catalysis) or a six-membered dimetallacycle adduct. The pathway that involves a six-membered metallaacycle is both endothermic and kinetically accessible (blue pathway in Figure 6a). This mechanism cannot be excluded for the deuterium incorporation but it is probably inefficient due to the small barrier (1.9 kcal.mol$^{-1}$) and the exothermicity (ca. -4 kcal.mol$^{-1}$) of the (C)$^\text{+}$([H]) $\leftrightarrow$ (C–H)$^\text{+}$ back reaction (i.e. $5^{\gamma\text{R}, \gamma\text{R}}$ $\leftrightarrow$ $5^{\gamma\text{R}, \gamma\text{R}}$). The C–H activation involving a five-membered metallaacycle (red pathway in Figure 6a) is also kinetically accessible, with an activation barrier of 7.4 kcal.mol$^{-1}$, but thermodynamically more favorable (-4.1 kcal.mol$^{-1}$) on the contrary to the γ1 case.
Thus, the deuterium incorporation at the γ-position of the nitrogen is most probably due to a process that goes through a five-membered metallacycle intermediate such as in homogeneous catalysis. The very efficient HIE in α to nitrogen atoms (96%) corresponds to a thermodynamically favorable mechanism (-2.1 kcal.mol⁻¹ w.r.t. (C-H)*, green pathway in Figure 6b), involving a 4-membered dimetallacycle as key intermediate and a low activation barrier for the C-H bond breaking step (4.3 kcal.mol⁻¹). Thus, similar to the C-H activation pathway in compound 3 (see Figure 4, green pathway) the competition between the (C)*(H)* ↔ (C-H)* back reaction (i.e. (5N*,α*))(H*) ↔ (5N*,α*H*H*) and the (C)*(D)* ↔ (C-D)* (i.e. (5N*,α*)D* ↔ (5N*,α*D*H*) isotopic exchange is in favor of the latter. An explanation of the difference between the isotopic enrichments of 62% and 96% is somewhat beyond the chemical accuracy of DFT, even though, interestingly, the barrier that leads to the (C-D)* turns out to be higher than its (C-O-D)* counterpart (11.5 vs. 6.5 kcal.mol⁻¹), in agreement with the observed lower isotopic enrichment at this position. Compound 6 was deuterated at the α-position of the unsubstituted nitrogen atom with 99% of isotopic enrichment accompanied by a slight deuterium incorporation on the hydroxymethyl group. The coordination of the nitrogen and the oxygen atom to the surface of the catalyst probably immobilizes the substrate in a certain conformation. This constraint would increase the activation barrier for C-H activation giving rise to a lowered deuterium incorporation at the C(sp²)* center. Very high incorporations of deuterium were obtained for benzimidazoles 7 and 8, which cannot be labelled by other HIE procedures to the best of our knowledge. Indeed, carbon centers in α and β positions of the nitrogen atoms of 7 were deuterated with 98% and respectively 81% of isotopic enrichment. A DFT-based investigation was also achieved for compound 7 in order to identify the key intermediate involved in the labelling of the β-positions of the nitrogens (see Figure 7). Again, the reaction starts with a favorable α-donation of the nitrogen lone pair (7N*) combined with a further stabilization of the adduct as a result of a the C-N-H (7N*,α*) or C-O-H (7N*,β*) agostic interaction on a neighboring Ru atom. The C-N-H HIE reaction involves a 5-membered dimetallacycle and is favored by an exothermic (C-H)* ↔ (C)*(H)* reaction (7N*,α*H*) more stable than 7N*,β*H* by -2.3 kcal.mol⁻¹ leading to the H/D exchange (see the discussion for compound 3) and a relatively low C-H activation barrier (7N*,β*H*; 5.6 kcal.mol⁻¹). As shown in Figure 7, the C-N-H HIE reaction is characterized by a profile (in green), very similar to the profiles calculated for the two other H/D exchanges in α, described above for oxazole and imidazole substructures. It is noteworthy that the most efficient labelling process (98%) goes first through an almost barrierless (C)*(H)* pathway and is then followed by the formation of the new (C-D)* bond which also requires to overcome the lowest barrier (4.5 vs. 7.9 kcal.mol⁻¹, see Figure S.3). Overall, the postulated C-H activation processes excel in three key factors which characterize an efficient HIE at C(sp²)* centers, i.e. the formation of metallacycle intermediates, low barriers and their exothermicities.

Figure 6: a) Energy diagram for the Langmuir–Hinshelwood-type H/D exchange on 5 in the ortho-position of the phenyl (blue and red pathways). b) α-positions relative to the imidazole nitrogen atoms (green pathway); energies are given in kcal.mol⁻¹. See also Figure S2 for a fully detailed pathway, i.e. up to the final D-incorporation.

Figure 7: a) Energy diagram for the Langmuir–Hinshelwood-type H/D exchange on 5 in the ortho-position of the phenyl (blue and red pathways). b) α-positions relative to the imidazole nitrogen atoms (green pathway); energies are given in kcal.mol⁻¹. See also Figure S2 for a fully detailed pathway, i.e. up to the final D-incorporation.
Regarding 8, all C-H bonds situated at the β-positions of nitrogen atoms (C(sp²)-H and C(sp³)-H) were activated, leading to the incorporation of 3.7 deuterium atoms. Interestingly, the reaction conducted in deuterated THF did not lead to an increase of the isotopic enrichment whereas running the reaction twice resulted in a higher isotopic enrichment on the methyl [80].

Labelling of triazole derivatives

To the best of our knowledge, no general approach has been described so far for the labelling of the 1,2,4-triazole scaffold using direct HIE. To our delight, our Ru nanocatalyst also promoted effective HIE on these heterocycles as demonstrated by the labelling of the differently functionalized compounds 9 – 12 (see Figure 8). Both, α and γ-positions of coordinating nitrogen atoms were labelled leading to high deuterium uptakes per substructure unit (up 3.8 D in a single run of catalysis). Similar to previous findings, it appeared very likely, that the underlying C-H activations for the labelling at the α positions of the triazolic scaffold pass through 4-membered dimetallacyclic key intermediates (see Figure 4). In contrast to the targeted C α-H and C β-H of oxazoles, the C α-H and C γ-H of the 1,2,4-triazole unit display almost the same reactivities in most cases, which was reflected in identical isotopic enrichments on C α and C γ of compounds 9 – 12 (see Figure 8). Logically, C-H activations in ortho of adjacent phenyl substituents must also proceed through 5-membered metallacyclic key intermediates as in the case of 2-phenylimidazole (see Figure 6). The efficient deuterium incorporation and the functional group tolerance for the methoxy- in 10, the amino- in 11 and the acetamide unit in 12 supported the potential of this method to prepare stable isotopically labelled internal standards for LC/MS quantification. Indeed, in a later section, the applicability of RuNp@PVP was also confirmed for the deuterium and tritium labelling of more complex triazole based drugs and one agrochemical (see Figures 12 and 13).

Labelling of carbazole derivatives

Up to now, only few methods for the deuteration of carbazoles have been reported using HIE. All of them employed harsh reaction conditions and led to unselective isotope incorporation. Through Ru nanocatalysis, compounds 13-16, were deuterated with high isotopic enrichments at the β-positions relative to the nitrogen using mild reaction conditions (see Figure 9). Interestingly, we found that the addition of a base enhanced the chemoselectivity and efficacy of the deuterium incorporation (see SI for results obtained without base). Indeed, without base, considerable amounts of reduced side-products were formed, reducing the overall yield of isolated products. In contrast, performing the reaction with 1 eq. of Cs₂CO₃ led to the recovery of deuterated compounds 13-16 in nearly quantitative yields. The higher chemoselectivity (i.e. the non-formation of reduced side-products) and efficacy (higher isotopic enrichments), observed for this transformation in the presence of a base, might be explained by the fostering of reaction intermediates leading to the isotope incorporation in β position of the nitrogen. To test this hypothesis, a DFT-based investigation was first performed without base using carbazole as model compound. Different coordination modes of the substrate were considered (see Figure S4). The most favored involved the coordination of N and the simultaneous agostic interaction of C-H to give 13αHβH (see Figure 10). In this case, 13 is weakly coordinated to the surface, just by -9 kcal.mol⁻¹ (compared to the ca. -25 kcal.mol⁻¹ in the previous cases). This is consistent with a α-coordination of the nitrogen atom instead of a β-coordination. Starting from 13αHβH two pathways could be stated: the N-H activation (13αHβH, green line) followed by the C-H activation (13αHβH) and the C-H activation (13αHβH, black line).
followed by the N-H activation (13\textsuperscript{N,\beta}). These both exothermic reactions involve moderate barrier heights, but the C-H activation seems easier, whatever the pathway is. Interestingly, if the N-H activation takes place before (green line), the barrier of the C-H activation is significantly lowered by 5.3 kcal.mol\textsuperscript{-1} (with an activation energy of 9.2 for TS 13\textsuperscript{N,\beta}* vs. 3.9 kcal.mol\textsuperscript{-1} for TS 13\textsuperscript{N,\beta}†). In summary, the optimal reaction consists in the coordination of a 5-membered dimetallacycle that partially breaks the conjugation. This precludes a possible HIE both on N and on C\textsubscript{1} through two pathways that require to overcome a 17.8 kcal.mol\textsuperscript{-1} apparent barrier and going on to 13\textsuperscript{ND,\betaD}. Prompted by this data, we were eager to investigate the role of cesium carbonate (Cs\textsubscript{2}CO\textsubscript{3}) on these activation processes. The exploration of these pathways in the presence of a base is not an easy task for transition-state search algorithms. It is, however, possible to give some energetic and structural clues regarding the role of Cs\textsubscript{2}CO\textsubscript{3} on the C-H and N-H activation in compound 13. As shown in Figure 11(a), Cs\textsubscript{2}CO\textsubscript{3} can favorably interact with both the surface and 13\textsuperscript{N,\beta}. Interestingly, this Cs\textsubscript{2}CO\textsubscript{3} / 13\textsuperscript{N,\beta} complex is more stable by ca. 20 kcal.mol\textsuperscript{-1} than the two species lying far away from each other on the surface. However, this is an energy minimum, i.e. geometry optimizations do not involve a barrierless H transfer from C\textsubscript{1} or N toward the base. Given that the species resulting from this transfer are thermodynamically less stable by \textasciitilde4 kcal.mol\textsuperscript{-1} and \textasciitilde6 kcal.mol\textsuperscript{-1}, such pathways would not facilitate the deuterium incorporation. However, the possible role of the base after a first C-H or N-H activation by the metal surface of the RuNp should be also considered to clarify the situation. As shown in Figure 11(b), a Cs\textsubscript{2}CO\textsubscript{3} molecule in the vicinity of the 13\textsuperscript{N,\beta} or 13\textsuperscript{N,\beta} intermediates spontaneously – and hence efficiently – abstracts the hydrogen of the N-H and C\textsubscript{1}-H bonds, respectively. The reaction is exothermic by \textasciitilde19.5 kcal.mol\textsuperscript{-1}. Given the C and N coordination on the surface, the H/D exchange can then occur from the resulting 13\textsuperscript{N,\beta} compound. To sum up, these mechanistic investigations have shown that after a prior C-H or N-H activation on the surface, a base could potentially facilitate the second rate-determining steps (C-H and N-H activation) of the reactions leading to higher isotope incorporation.\textsuperscript{[19]}
Figure 11: Ability of Cs$_2$CO$_3$ to coordinate to the catalyst surface and to adapt the role of a proton acceptor in the N-H and C-H activation step.

Labelling of pharmaceuticals & other bioactive molecules

a) Deuterium labelling

To illustrate the usefulness and the broad applicability of our RuNps catalyzed HIE, deuterium and tritium labelling of N-heterocycle containing molecules of medical relevance and higher molecular complexity, was then considered (see Figure 12). As a first example, we have chosen the oxazole containing alkaloid, pimprinine 17 possessing anticonvulsant$^{20}$ and antiviral activities$^{21}$. The use of RuNp@PVP (20 mol%) in CD$_3$OD with 1eq of Cs$_2$CO$_3$, allowed a much higher deuteration of this sensitive molecule on its indole moiety than without Cs$_2$CO$_3$, analogous to the findings made with carbazoles$^{13-16}$. Very good isotopic enrichment also occurred on the oxazole core (for the results without Cs$_2$CO$_3$, see SI).

The benzimidazole containing drug astemizole 18 was used as a drug in the treatment of allergies$^{22}$. In the case of this complex molecule, deuteration occurs at the β-position of the unsubstituted nitrogen of the benzimidazole moiety with an isotopic enrichment of 87%. Furthermore, the analysis of $^2$H-NMR data (@14T) also supported slight H/D exchange on one α-methylene of the tertiary amine (22%). Imiquimod 19 has a convincing efficacy against malignant melanoma$^{23}$. This poorly soluble molecule was successfully deuterated using DMA as solvent, highlighting the broad solvent compatibility of our method. The human antifungal drug fluconazole 20, was selectively labelled on the 1,2,4-triazole units leading to a very high isotope incorporation (almost 4 D incorporated). This result repeatedly manifests the potential of our method for the preparation of stable isotopically labelled internal standards of a commercial drug for LC-MS quantifications. This rapid deuteration labelling is insofar beneficiary as we consider that stable isotopically labelled internal standards of fluconazole were originally synthesized from deuterated precursors over four steps$^{24}$. The efficient and selective deuteration of the triazole substructure of the complex agricultural fungicide fluquinconazole 21 is representative for the functional group tolerance of the described catalytic transformation. Despite the presence of different carbon-halogen bonds (1 C-F and 2 C-Cl), the highly deuterated product was obtained in high yield after a simple filtration through a C18 cartridge. The 1,2,3-triazole drug suvorexant 22, administered for insomnia treatment, is known to be labelled by hydrogen isotopes within other methods. Hence, H/D exchange occurs either on the C-H bonds of the 1,2,3-triazole with a homogeneous Fe(0) catalyst (leading to the incorporation of 0.6 D) or in the ortho position of the adjacent phenyl with Crabtree’s catalyst (theoretically limited to the incorporation of 1 D)$^7$. Here, the use of RuNps as catalyst allowed the deuterium labelling of both, the 1,2,3-triazole group (1.6 D) and the adjacent phenyl ring (0.3 D), which gave a considerably higher deuterium incorporation as a whole, compared with the ones obtained using the previously described HIE procedures. Carvedilol 23 is a
nonselective beta/alpha-1 blocker used for treating congestive heart failure, left ventricular dysfunction and high blood pressure. Using carvedilol as a substrate led to the deuteration of the secondary amine with a high isotopic enrichment due to the higher affinity of the alkylamine nitrogen compared to the one embedded in the carbazole moiety. By simply protecting the aliphatic amine with a Boc-group and adding one equivalent of Cs₂CO₃, we were able to exclusively label the carbazole moiety (molecule 24, see Figure 12) with a high isotopic enrichment (99%). The possibility to modify the regioselectivity of the isotopic incorporation on a such complex structure using simple protecting group strategies, highlights the versatility of our RuNps catalyzed HIE reactions for the synthesis of labelled drug compounds for metabolic studies.

b) Tritium labelling of pharmaceuticals

As a next step, we demonstrated that the late-stage tritiation of complex pharmaceuticals can succeed through our catalytic method (see Figure 13). In this context, the use of T₂ gas as isotopic source is a great advantage because it is the easiest raw material to handle for tritium labelling. Typically, reactions involving gaseous tritium were conducted using a subatmospheric pressure of T₂ in order to minimize the risk of leakage and radioactive releases.

Figure 13: Tritium labelling of complex pharmaceuticals.

Up to date, the simplest way to label the N-heterocyclic substructures of astemizole and carvedilol with tritium was the transformation of halogenated precursors with Pd/C and T₂, a procedure that consisted of several other reaction steps. With our method, radioactive analogues of astemizole 18*, fluconazole 20*, and N-Boc-protected carvedilol 24* were obtained with satisfying molar activities and in high yields using subatmospheric tritium gas pressures. It is noteworthy that in every case (18*, 20*, 24*) tritium labelling took place on positions which are not major metabolism sites. The obtained molar activities of 18* and 20* (24 Ci/mmol), under a tritium gas pressure of ~900 mbar, clearly outperformed the prerequisites for ADME studies (10-20 Ci/mmol). Performing the radiolabelling under a lower tritium gas pressure of ~500 mbar, tritiated 24* could be obtained with a tritium incorporation of ~9 Ci/mmol, which was still within an acceptable range.

Conclusion

In this paper, we demonstrated that the application of Ru nanocatalysts can provide efficient deuteration and tritiation of different classes of heterocycles in complex molecules using D₂ or T₂ gas as isotopic sources. For the labelling of oxazole and imidazole substructures, this work represents a significant advance in practicality compared to previously described methods, with notably higher isotope uptakes, a broader substrate scope and a higher solvent compatibility. This method represents also the unique approach allowing the selective deuteration and tritium incorporation on 1,2,4-triazole and carbazole substructures using direct hydrogen isotope exchange. In terms of application, the high incorporation of deuterium atoms allows the synthesis of deuterated internal standards for LC/MS quantification and the efficacy of the catalytic process permits the preparation of complex radiolabelled drugs owning high specific activities by using a subatmospheric pressure of T₂ gas. From a more fundamental point of view, the detailed theoretical calculations have deciphered the processes of C-H bond activation at α, β- and γ-positions of coordinating nitrogen atoms occurring at the surface of Ru clusters. All the key intermediates involved in such reactions were disclosed: 4-membered dimetallacycle for the C-H activation in α, 5-membered dimetallacycle for the C-H activation in β and 5-membered mettallacycle for the C-H activation in γ. Energy profiles qualitatively account for the experimental isotopic enrichment rates. C-H activation equilibria or C-D recombination equilibria exhibit characteristic patterns that drive the overall reaction. In addition to the fact that this study paves the way for an easier access to valuable isotopically labelled complex molecules, the theoretical aspects addressed will be useful for future developments of more efficient and selective nanocatalysts for C-H activations.

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Keywords: Hydrogen Isotopic Exchange • Nanocatalysis • C–H Activation • Deuterium • Tritium

[19] For the moment, no experimental evidence for rate acceleration of the H/D exchange was found in the presence of a base using substrates that do not contain acidic N–H bonds. Therefore, it is likely that the base facilitates the NH activation at the Ru nanocatalyst surface (for carbazole and indole scaffolds) which leads to the decrease of the activation barrier for the C–H bond in β-position of the nitrogen atom.
Ruthenium nanocatalysis can provide effective deuteration and tritiation of oxazole, imidazole, triazole and carbazole substructures in complex molecules using D₂ or T₂ gas as isotopic sources. In terms of application, the high incorporation of deuterium atoms allows the synthesis of deuterated internal standards for LC/MS quantification and the efficacy of the catalytic process permits the preparation of complex radiolabelled drugs owning high specific activities by using a subatmospheric pressure of T₂ gas. From a fundamental point of view, the detailed theoretical calculations have deciphered the processes of C–H bond activation at α, β- and γ-positions of the coordinating nitrogen atoms occurring at the surface of Ru clusters.

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Hydrogen Isotope Exchange catalyzed by Ru nanocatalysts: labelling of complex molecules containing N-Heterocycles and reaction mechanism insights