Isotopic Exchange

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Regioselective and Stereospecific Deuteration of Bioactive Aza Compounds by the Use of Ruthenium Nanoparticles**

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Abstract: *An efficient H/D exchange method allowing the deuteration of pyridines, quinolines, indoles, and alkyl amines with D_2 in the presence of Ru@PVP nanoparticles is described. By a general and simple procedure involving mild reaction conditions and simple filtration to recover the labeled product, the isotopic labeling of 22 compounds proceeded in good yield with high chemo- and regioselectivity. The viability of this procedure was demonstrated by the labeling of eight biologically active compounds. Remarkably, enantiomeric purity was conserved in the labeled compounds, even though labeling took place in the vicinity of the stereogenic center. The level of isotopic enrichment observed is suitable for metabolomic studies in most cases. This approach is also perfectly adapted to tritium labeling because it uses a gas as an isotopic source. Besides these applications to molecules of biological interest, this study reveals a rich and underestimated chemistry on the surface of ruthenium nanoparticles.*

C–H activation has become a powerful tool for the synthesis or functionalization of complex organic compounds through the formation of a large variety of C–C, C–N, C–O, C–B, and C–D bonds.[1] This straightforward method can therefore afford rapid access to deuterated labeled compounds, which are vital tools in many areas of academic and industrial science.[2] Deuterated compounds are essential for mechanistic studies in biology and in organic and organometallic chemistry. Moreover, they are of particular interest for the development of efficient optical devices, such as optical polymer fibers and organic material for organic light-emitting diodes.[3] Recently, the emergence of metabolomics has increased demand for isotopically labeled compounds. In particular, their use as internal standards is essential for the quantitative LC–MS/MS analysis of new drug candidates and of metabolites in biological fluids.[4] For the synthesis of such molecules, isotopic exchange is clearly preferred over a synthetic pathway from labeled building blocks or involving a synthetic precursor. As a consequence, the development of efficient and selective labeling methodologies through catalytic H/D exchange reactions at carbon centers is of great interest.[2a]

Numerous methods based on homogeneous or heterogeneous catalysis for H/D exchange have already been described. Among these methods, the selective deuteration of nitrogen-containing molecules has been investigated with particular intensity because of the presence of this heteroatom in many biologically active compounds. For example, several catalytic systems, such as Rh black, Ru complexes, and PdNp@PVP colloids (Np: nanoparticle, PVP: polyvinylpyrrolidone) have been used to label pyridine-containing compounds with various degrees of deuterium incorporation and substrate tolerance.[5] For indole derivatives, Schnürch and co-workers recently described a ruthenium-catalyzed H/D exchange with a good selectivity and a fair degree of deuterium incorporation.[5b] Concerning alkyl amines, a few methodologies have been devised, but they have essentially been applied to nonfunctionalized substrates.[6] Until recently, labeling procedures for such compounds suffered from major drawbacks, such as harsh reaction conditions, poor selectivity, and low deuterium incorporation. A breakthrough was recently reported by Beller and co-workers, who developed an α,β deuteration of bioactive secondary and tertiary amines.[7] This remarkable method based on the use of a Ru complex as a catalyst enables a high degree of deuterium incorporation. However, it still requires high temperatures (150 °C), which can lead to side reactions and the degradation of fragile substrates. Moreover, it has never been applied to chiral compounds. As a consequence, a regioselective, chemoselective, and stereospecific labeling method that might be applicable to a wide diversity of substrates is urgently required.

Herein, we demonstrate that the use of Ru nanoparticles allows mild (1–2 bar of deuterium gas at room temperature or 55 °C), effective, and selective deuteration of a large diversity of nitrogen-containing compounds, such as pyridines, quinolines, indoles, and primary, secondary, and tertiary alkyl

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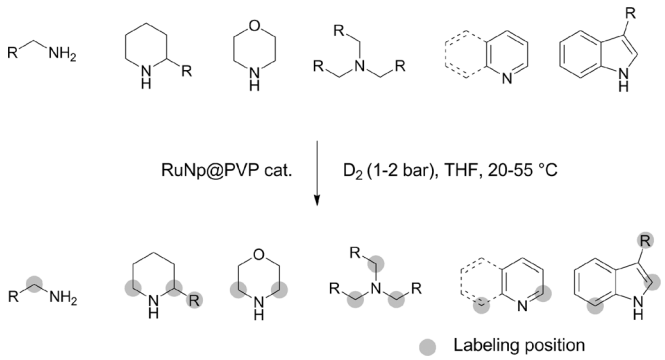


Figure 1. Scope of the deuteration reaction and labeling positions.

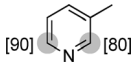
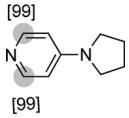
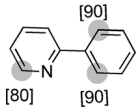
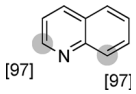
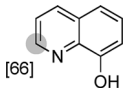
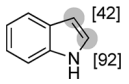
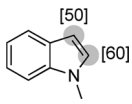
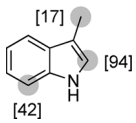
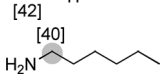
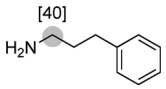
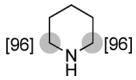
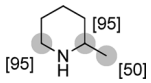
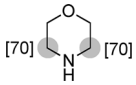
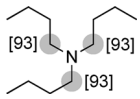
amines (Figure 1). The usefulness of this novel methodology was demonstrated by the labeling of eight bioactive compounds, several of which contain stereogenic centers.

In the course of previous studies, some of us observed that the treatment of Ru nanoparticles with D_2 led to deuterium incorporation into the primary-amine ligand (hexadecylamine) bound to the Ru surface.[8] This incorporation occurred under mild reaction conditions (0.8 bar of deuterium gas, room temperature). This result prompted us to evaluate the catalytic activity of such Ru nanoparticles towards H/D exchange processes on nitrogen-containing substrates through C–H activation. These small nanoparticles (1.1 nm) are readily obtained by decomposition of the organometallic precursor [Ru(cod)(cot)] (cod = 1,5-cyclooctadiene, cot = 1,3,5-cyclooctatriene) under an H_2 atmosphere in the presence of a stabilizer, such as a polymer or a ligand.[9a] These nanoparticles are particularly attractive because their properties can be fine-tuned by surface-ligand modification.[9b] We started our experiments with RuNp@PVP, which can be considered as "naked particles", to facilitate the interaction between the substrate and the reactive Ru surface.[9c]

Deuteration experiments were carried out with 3% RuNp@PVP in THF under a deuterium atmosphere (1 or 2 bar). Pyridine derivatives **1**, **2**, and sterically hindered 2-phenylpyridine (**3**) were regioselectively deuterated with high isotopic enrichment (Table 1). For quinolines **4** and **5**, the C–H activation took place regioselectively with high deuterium incorporation. However, the concomitant formation of labeled tetrahydroquinoline (around 50%) was also observed.

In the indole series (compounds **6**–**8**), RuNp@PVP also catalyzed H/D exchange with high selectivity and no side-product formation. With compounds **6** and **7**, C–H activation took place at the 2- and 3-positions; however, we observed regioselective labeling at three positions of skatol (**8**). The difference in terms of selectivity between indole and skatol might be explained by distinct modes of coordination on the Ru surface and is discussed in more detail below. In the case of hexylamine (**9**), only 20% H/D exchange at the α-position was observed at room temperature, whereas the isotopic enrichment was increased to 40% at 55 °C. Similar results were obtained with 3-phenyl-1-propanamine (**10**). Unidentified side products were formed with primary amines. Nevertheless, the deuterated amino compounds could be isolated readily as hydrochloride salts. Highly efficient labeling of secondary and tertiary amines was observed. Piperidine (**11**) was labeled at both the 2- and the 6-position with almost total replacement of four hydrogen atoms with four deuterium atoms. 2-Methylpiperidine (**12**) was also deuterated at the 2- and 6-positions, along with exchange into the methyl group, thus leading to the incorporation of up to six deuterium atoms. Morpholine (**13**) was deuterated regioselectively with up to four deuterium atoms. Tributylamine (**14**) was deuter-

Table 1: Regioselective catalytic deuteration.[a]

Compound	Structure	Method	Total D[b]
1		**A**	1.7
2		**B**	2.0
3		**B**	2.7
4		**A**	2.0
5		**A**	0.7
6		**B**	1.4
7		**B**	1.1
8		**C**	1.9
9		**B**	0.8
10		**B**	0.8
11		**C**	3.9
12		**C**	4.3
13		**C**	2.8
14		**C**	5.6

[a] Reaction conditions (all experiments were performed for 36 h in THF in the presence of 3% RuNp@PVP): method **A**: D_2 (1 bar), RT; method **B**: D_2 (1 bar), 55 °C; method **C**: D_2 (2 bar), 55 °C. Gray disks show the labeling positions (the number in brackets indicates the isotopic enrichment). [b] Average number of D atoms per molecule.

ated at all C^{α} positions to give a highly deuterated compound (up to six deuterium atoms).

These results prompted us to apply this new method to the labeling of biologically active compounds that contain at least one nitrogen atom. We believed that the mild experimental conditions might be compatible with the labeling of complex chiral compounds. Nicotine (**15**) and anabasine (**16**), two nicotinic acetylcholine receptor agonists that both contain two azacycles (Figure 2), were deuterated regioselectively with a high level of deuterium incorporation. The exclusive exchange with the pyridine ring could be explained by a higher affinity of the pyridine nitrogen atom for the Ru surface than that of the piperidine or pyrrolidine group. In the

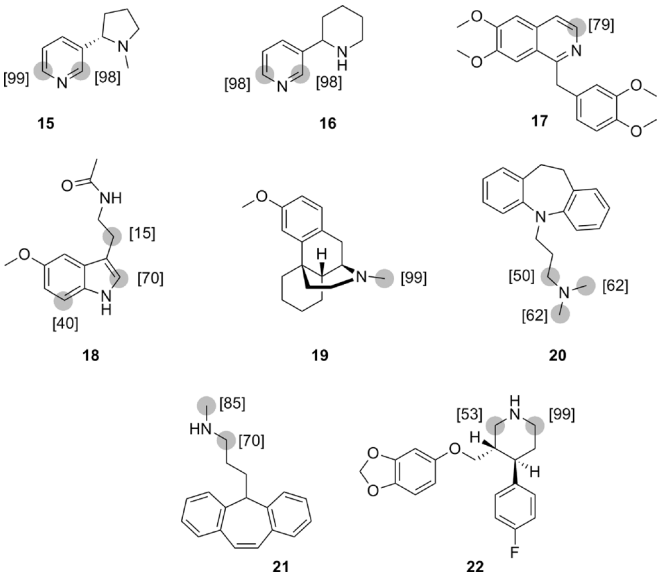


Figure 2. Structure of labeled biologically active compounds (gray disks show the labeling positions; the number in brackets indicates the isotopic enrichment).

case of nicotine (**15**), the enantiomeric purity of the deuterated compound remained unchanged (the measured $[\alpha]_D^{25}$ value was -180.1 before and -176.5 after the labeling experiment ($c = 0.5$, $CHCl_3$)). Furthermore, the mild experimental conditions led to a pure deuterated sample after simple filtration through a pad of alumina, as shown in the case of anabasine (**16**; Figure 3).

Papaverine (**17**), an opium-alkaloid antispasmodic drug, was deuterated at the 2-position in a 79% H/D exchange. Deuteration of melatonin (**18**), a hormone that regulates the circadian rhythms of several biological functions, took place at the equivalent three positions to those at which deuteration occurred in the model compound skatol (**8**). Dextromethorphan (**19**) was selectively deuterated at the *N*-methyl position, with the incorporation of three deuterium atoms. This high regioselectivity could be explained by the low accessibility of other positions α to the nitrogen atom embedded in the six-membered ring. Interestingly, this selectivity suggests that the H/D exchange reaction occurs at the RuNp surface and may not involve the leaching of Ru into solution. Once again, no loss of enantiomeric purity was detected after the selective

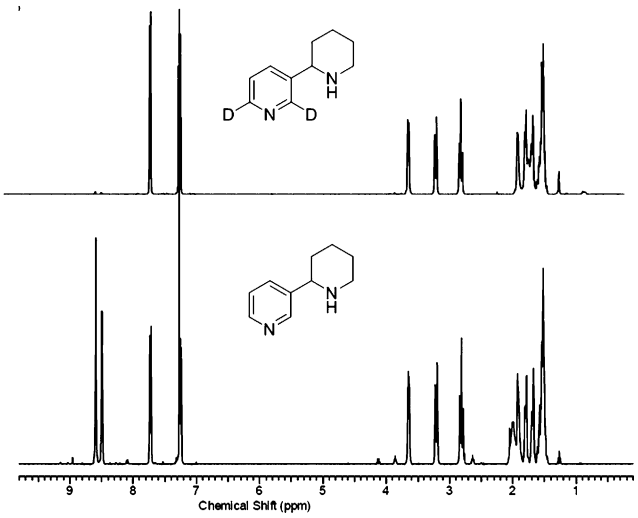


Figure 3. ^{1}H NMR spectra of labeled (top) and unlabeled (bottom) anabasine (**16**) in $CDCl_3$.

deuteration process (the measured $[\alpha]_D^{25}$ value was $+65.1$ before and $+65.4$ after the labeling experiment ($c = 1.0$, $CHCl_3$)). Imipramine (**20**), a tricyclic antidepressant of the dibenzazepine group, incorporated up to eight deuterium atoms into the tertiary alkyl amine. In these six cases, owing to the mild conditions used, no side products were detected; thus, almost quantitative recovery of the product was possible.

Up to five deuterium atoms were regioselectively incorporated in protriptyline (**21**), another tricyclic antidepressant, by our standard exchange procedure. Interestingly, although this compound contains a readily reducible disubstituted double bond, only a limited amount of the reduced compound (around 20%) was observed. The probable anchoring of protriptyline at the surface of RuNps through an amine–ruthenium interaction prevents the reduction of the remote double bond. To the best of our knowledge, no double-bond-containing compound has previously been labeled with up to five deuterium atoms by isotopic exchange with deuterium gas. This result is particularly encouraging because it paves the way to the labeling of complex and fragile biologically active molecules. The power of our C–H activation process for deuterium labeling is highlighted by the case of paroxetine (**22**), an antidepressant drug which belongs to the selective serotonin reuptake inhibitor series. Deuterated **22** was synthesized previously by a pharmaceutical company in a seven-step sequence,[10] whereas our process only required one labeling step. Once again, this complex compound was labeled without significant alteration of chirality ($[\alpha]_D^{25} = -78.5$ ($c = 1.0$, MeOH)).

The use of deuterated molecules as internal standards in metabolomic studies requires very high isotopic enrichment. Such compounds must contain a minimum of two deuterium atoms and usually less than 0.5% nondeuterated material. In most cases, the standard conditions described herein yielded labeled compounds that fulfilled these criteria. In other cases, we investigated the possibility of increasing deuterium incorporation in two different ways. In the case of melatonin (**18**), doubling of the catalytic charge led to higher isotopic

enrichment without a change in selectivity (at the 2-position from 70 to 93%, at the 7-position from 40 to 80%, and at the methylene position from 15 to 25%). Alternatively, in the case of imipramine (**20**), repeating the deuteration process led to a negligible proportion of non- and monodeuterated molecules (see Table S2 in the Supporting Information).

From a mechanistic point of view, the first interesting observation is that under the mild conditions of this system, both $C(sp^2)$–H and $C(sp^3)$–H bonds can be deuterated at comparable rates. The second observation concerns the high regioselectivity of these reactions. No deuteration next to an oxygen donor group was observed, whereas a strong exchange occurred at C–H bonds next to the nitrogen atom. These results clearly show that this H/D exchange process arises from the direct coordination of nitrogen to ruthenium. The affinity of ethers for the Ru surface is presumably too low to initiate the deuteration reaction. These results are similar to those of studies carried out on molecular organometallic catalysts, in which case only the hydrogen atoms α to a nitrogen atom could be activated. They are also comparable to those of older studies on metal complexes, including Ru complexes, in which case only the accessible *ortho* hydrogen atoms of triphenylphosphine or triphenylphosphite could be exchanged as a result of *ortho*-metalation.[11] However, the accessibility of the Ru sites on nanoparticles enables much greater efficiency of these C–H activation processes, which demonstrates the value of such organometallic nanoobjects.

In previous studies, we showed that: 1) the surface of RuNP@PVP was covered by hydrides (1.5 H atoms per surface Ru atom), 2) these hydrides were highly dynamic, and 3) these hydrides could be exchanged with deuterium present in the gas phase.[8,12] The current results therefore suggest that C–H bonds α to a nitrogen atom are readily broken and reformed on a Ru surface. Previous studies have also demonstrated that the fluxional behavior of coordinated nitrogen-donor ligands gives rise to dynamic exchange between free and coordinated ligands.[9] This exchange explains the simple catalytic process observed in this study.

Finally, the case of compounds **6**–**8** provides some information regarding a possible dual mechanism of exchange that implies a competition between nitrogen coordination on the one hand and the formation of a transient carbene on the other hand (see Scheme 1 in the Supporting Information).[13] In compounds **6** and **7**, carbene formation seems to be clearly favored owing to the weakly coordinating nature of the indole nitrogen atom.[14] This behavior may explain the labeling at the 2- and 3-positions and the absence of labeling at the 7-position. In contrast, both pathways coexisted for skatol **8**, which is sterically hindered at the 3-position. This steric hindrance led to the concomitant deuteration of the 7-position through nitrogen coordination, whereas the formation of a carbene enabled the labeling of both the 2-position and the methyl group.

In conclusion, we have developed an efficient H/D exchange method that enables the deuteration of pyridines, quinolines, indoles, and alkyl amines with Ru@PVP nanoparticles as a catalyst and D_2 as the isotopic source. By a general and simple procedure involving mild reaction conditions and simple filtration to recover the labeled product, 22 compounds were isotopically labeled in good yields with high chemo- and regioselectivities. The viability of this procedure was demonstrated by the labeling of eight biologically active compounds. Remarkably, conservation of the enantiomeric purity of the labeled compounds was observed, even though labeling took place in the vicinity of the asymmetric center. The level of isotopic enrichment that can be reached is suitable for metabolomic studies in most cases. Furthermore, this approach is perfectly adapted to tritium labeling because it uses gas as an isotopic source. Extension of the method to other natural compounds, in particular amino acids and peptides, is currently under investigation in our laboratory. Besides these applications to molecules of biological interest, this study reveals a rich and underestimated chemistry on the surface of Ru nanoparticles, which can be further exploited as novel reagents in organic chemistry.

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