Iridium(I)-Catalyzed *Ortho*-Directed Hydrogen Isotope Exchange in Continuous-Flow Reactors

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Deuterated compounds are important in drug discovery and development, e.g., to prepare the so-called stable isotopically labeled internal standards. However, their preparation can be challenging, and its scalability could be difficult with regard to safety aspects. Herein, we report the first continuous-flow iridium(I)-catalyzed *ortho*-directed hydrogen–deuterium exchange reaction in microreactors. An immobilized iridium(I) catalyst was loaded in continuous-flow microreactors, such as continuous stirred tank reactors and packed-bed reactors. Best results were obtained in a packed-bed reactor allowing deuterium labeling up to M +7.

Keywords: isotope exchange, deuterium labeling, flow chemistry, C–H activation, microreactor, immobilized catalyst

1. Introduction

Within a drug discovery process, drug metabolism and pharmacokinetic studies play a crucial role in the identification and optimization of new drug candidates [1]. In order to understand the metabolic and kinetic behavior of compounds, appropriate analytical techniques have been developed. One such technique for the quantification of analytes is liquid chromatography coupled with a tandem mass spectroscopic detection (LC–MS/MS). To improve the accuracy of the technique, stable isotopically labeled internal standards (STILs) are typically used [2]. Such compounds are considered to be ideal internal standards since they have nearly identical physicochemical properties as their unlabeled counterpart. Consequently, they are considered to be matrix independent, which results in a very high degree of accuracy.

The preparation of STILs is often achieved via a hydrogen isotope exchange reaction [3]. A variety of different metals, including platinum [4], rhodium [5], ruthenium [6], nickel [7], and iridium [8], has demonstrated to facilitate a controlled and selective C–H functionalization to enable the hydrogen–deuterium (H–D) exchange. Such catalysts offer the advantage of mild reaction conditions while providing a high degree of regio- and chemoselectivity. Recently, an immobilized iridium catalyst (**1**) was developed by Hickey et al. who used deuterium or tritium gas as a source of isotopes [9]. The use of an immobilized catalyst facilitates the purification of the desired deuterium or tritium labeled compounds by simple filtration [10]. This advantage together with the ease of preparation and the good activity makes this immobilized catalyst an interesting catalytic system for the preparation of labeled pharmaceuticals. However, there is a concern regarding safety when this process is scaled to produce larger amounts of the labeled compound due to explosive nature of deuterium and tritium gas. Another point of attention is the lower C–H exchange activity depending on the solid support utilized for immobilization of the catalyst.

Continuous-flow chemistry has emerged as an enabling tool to facilitate gas–liquid reactions [11]. It provides fast mixing between the gas and liquid phase due to the large and well-defined interfacial areas, and it allows to scale the chemistry without reoptimizing the reaction and process parameters [12]. Due to the small dimensions of the gas–liquid reactor, safety risks are minimized substantially. This is of great interest when using flammable gases like deuterium and radioactive tritium [13]. In addition, it is important to note that, while cross-coupling reactions have been carried out extensively in continuous-flow microreactors [14,15], examples of C–H activation in flow are rare [16]. Herein, we report the first iridium(I)-catalyzed C–H deuteration in a continuous-flow microreactor.

2. Results and Discussion

We commenced our investigations by performing the hydrogen–deuterium exchange reaction in batch on *N*-(4-methoxyphenyl)-*N*-methylbenzamide (**2**). This benchmark molecule could provide insight in a wide range of labeling opportunities of both sp^2, sp^3, and 5- and 6-membered metallocyclic *ortho* aromatic exchange reactions [10]. The solid phase immobilized iridium catalyst (**1**) selection was based on its ease of preparation and its reported good degree of exchange activities. Catalyst (**1**) was prepared via a ligand exchange starting from Crabtree's catalyst with polystyrene-based triphenylphosphine as shown in Scheme 1 [9]. Batch exchange reactions were executed as follows: to *N*-(4-methoxyphenyl)-*N*-methylbenzamide (**2**) and iridium catalyst (**1**) dichloromethane was added. The reaction mixture was subsequently exposed to deuterium gas. Results show the incorporation of deuterium, which increases as a function of time (Scheme 2). Both M +1 and M +2 products are obtained, while higher degree of deuterium incorporation remains low (~2% after 30 min).

Next, we evaluated the reaction in various continuous-flow microreactors. In this study, we have evaluated two different designs, i.e., a continuous stirred tank reactor (CSTR) and a packed-bed reactor (PBR). A CSTR is a common reactor in chemical engineering and allows to suspend the catalyst as a fluidized bed which enables an intense contact between the gas, liquid, and solid catalyst phase [17]. The CSTR used in this study consists of a 0.7-mL mixing chamber which is typically used for high-performance liquid chromatography (HPLC) setups (see Scheme 3). The catalyst was loaded in the chamber, and the fluidization was achieved by using a magnetic stir bar. *N*-(4-methoxyphenyl)-*N*-methylbenzamide (**2**), dissolved in dichloromethane, was introduced in the CSTR by syringe pump and was mixed with deuterium gas, which was delivered by means of a mass flow controller. The best results were obtained with lower flow rates and, thus, a higher residence time (see Table 1, Entries 1–3). Within the CSTR, a maximum conversion of 16% could be obtained with a maximum deuterium incorporation of M +2.

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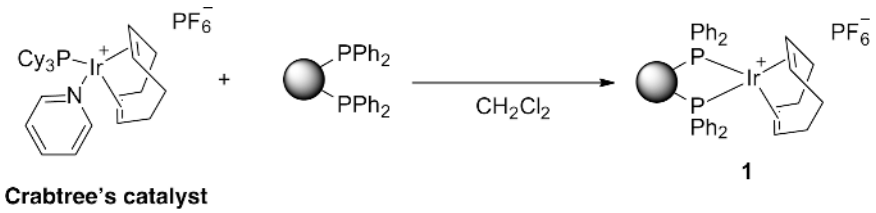
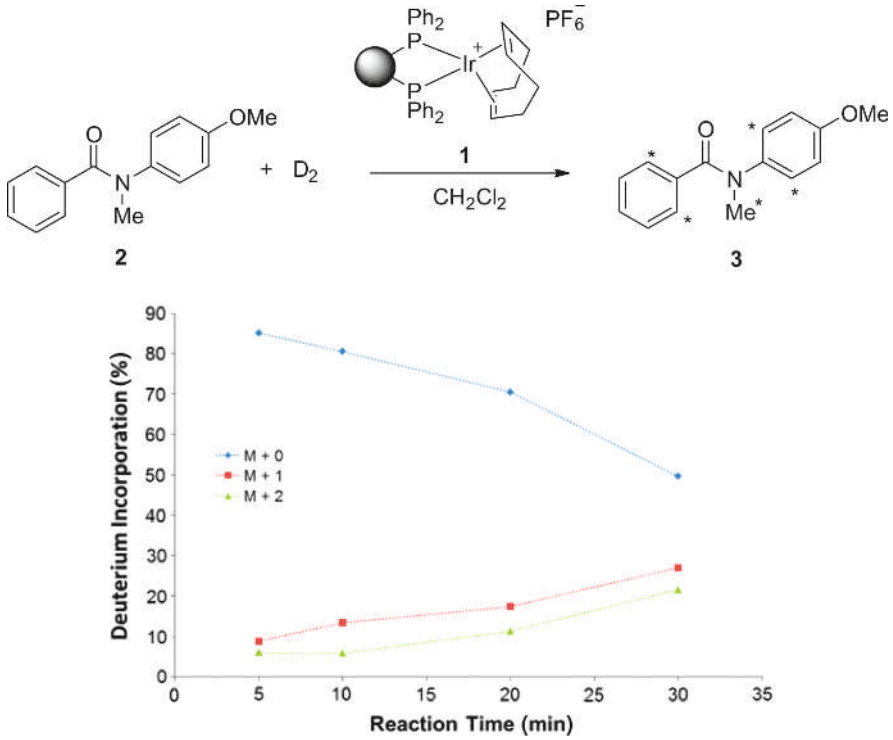
Scheme 1. Synthesis of an immobilized Ir(I)-catalyst via a ligand exchange starting from Crabtree's catalyst



Scheme 2. Ir(I)-catalyzed H–D exchange of *N*-(4-methoxyphenyl)-*N*-methylbenzamide (**2**) in batch. Deuterium incorporation in function of time. Positions that can be deuterated are marked with an asterisk



A micro packed-bed reactor (stainless steel, 20 ×2.1 mm) was next assembled in which 10 mg of immobilized catalyst **1** was loaded (Scheme 4). Such packed-bed reactors demonstrate an excellent mixing efficiency of the biphasic reaction mixtures [18,19]. Difficulties were encountered due to swelling of the polystyrene beads when dichloromethane was directed over the bed. A swelling of 30 to 40% was observed and resulted in a complete blockage of the micro packed-bed reactor. The problem could be overcome by packing the reactor with preswelled catalyst beads. The average results of three independent runs obtained in this reactor are shown in Table 1 (Entry 4). A high conversion of 54% was obtained, and deuterium labeling was observed up to M +7.

Given the good results obtained in a micro packed-bed reactor, we anticipated that higher conversions could be obtained when the deuterium pressure was increased. Hereto, we used a commercially available H-Cube Pro™, which we equipped with the micro packed-bed reactor, and filled the cells with deuterated water (Figure 1) [19]. This deuterated water is used to generate the deuterium gas required for the reaction. As such, a pressure of

Scheme 3. Schematic representation of the micro CSTR setup for the iridium(I)-catalyzed *ortho*-directed hydrogen isotope exchange reaction

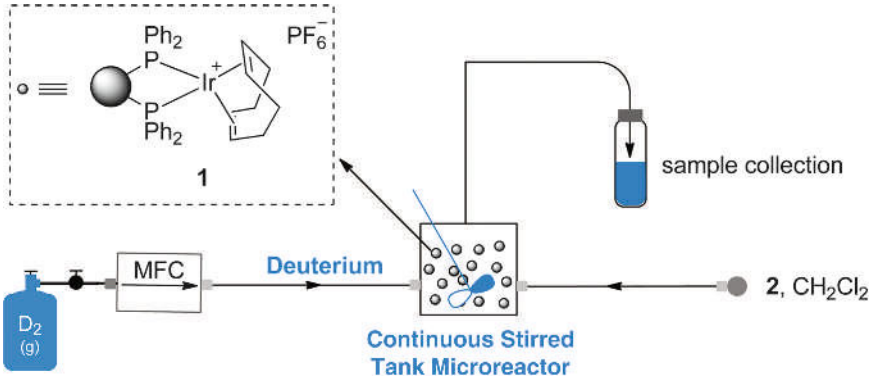
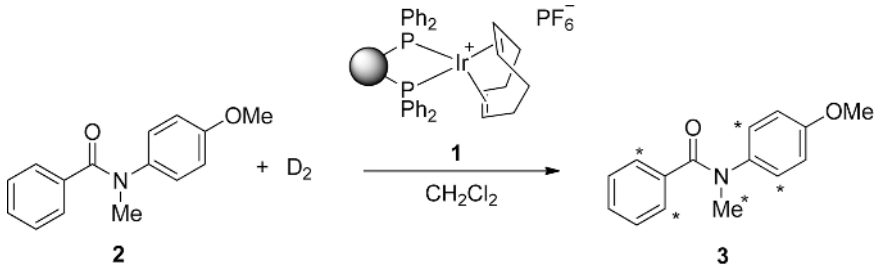


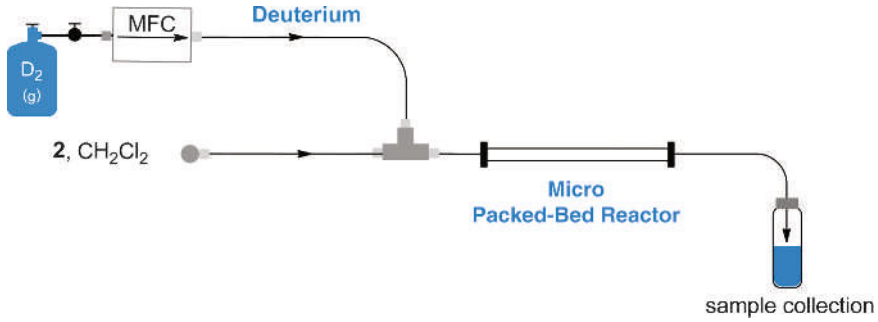
Table 1. Amount of deuterium labeling using different continuous-flow microreactor types



Entry	Reactor type	Gas flow rate (mL/min)	Liquid flow rate (mL/min)	M +0 (%)[5]	M +1 (%)[5]	M +2 (%)[5]	M +3 (%)[5]	M +4 (%)[5]	M +5 (%)[5]	M +6 (%)[5]	M +7 (%)[5]	Conversion (%)[5]
1[1]	CSTR	0.02	0.08	87.8	8.1	3.6						12
2[1]	CSTR	0.02	0.025	84.0	9.8	4.8						16
3[1]	CSTR	0.08	0.085	97.2	2.8							2.8
4[1]	PBR	0.04	0.01	46.0	4.9	16.2	12.1	8.4	5.1	3.9	2.8	54
5[2]	PBR[4]	40 bar	0.3	43.9	14.3	38.4	2.8					56
6[2,3]	PBR[4]	40 bar	0.3	36.8	14.0	43.4	4.5					64

[1] Reaction conditions: **2** (1 mg/mL CH_2Cl_2), catalyst **1** (10 mg), room temperature, yields, and conversion are the average of three independent runs.
[2] Reaction conditions: **2** (1 mg/mL CH_2Cl_2), catalyst **1** (100 mg), room temperature, yields, and conversion are the average of five independent runs.
[3] The liquid stream is the one exiting from entry 5 which is redirected over the catalyst bed.
[4] Micro packed-bed reactor was placed inside H-Cube Pro™.
[5] Yields and conversion are determined by LC–MS.

Scheme 4. Schematic representation of the micro packed-bed reactor setup for the iridium(I)-catalyzed *ortho*-directed hydrogen isotope exchange reaction



40 bar deuterium was obtained. The average results of five independent runs demonstrate that the conversion could be further improved up to 56% (Table 1, Entry 5). A further increase up to 64% labeling was obtained by redirecting the reaction stream for a second time over the catalyst bed (Table 1, Entry 6). Further recycling was impossible since dichloromethane evaporated rapidly, which resulted in precipitation of the reaction product.

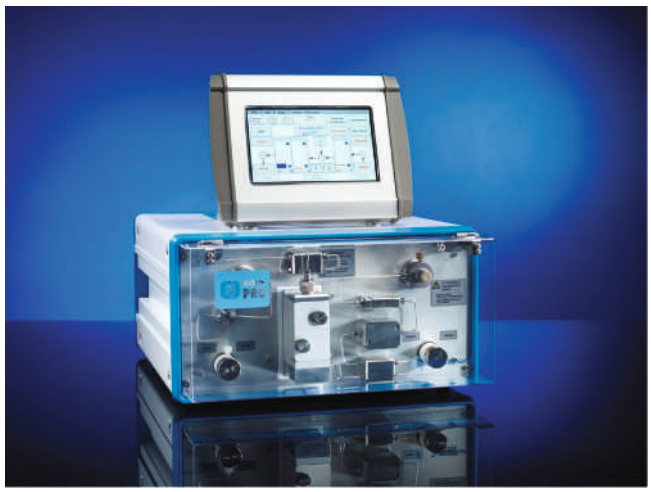


Figure 1. ThalesNano's H-Cube Pro™ for *in situ* generation of deuterium gas starting from deuterated water (reproduced with permission, ThalesNano)

3. Conclusion

We have developed the first continuous-flow procedure to prepare deuterium-labeled organic molecules via an iridium(I)-catalyzed *ortho*-directed hydrogen–deuterium exchange reaction. An immobilized iridium(I)-catalyst was prepared, and two main microreactor types were evaluated during the course of our investigations, including continuous stirred tank reactors and packed-bed reactors. Packed-bed reactors gave the most optimal conditions and deuterium labeling up to M +7 was observed. A further improvement was obtained by using a higher deuterium pressure in an H-Cube Pro™ commercially available reactor system. This system allowed to generate deuterium gas *in situ* from deuterated water and provides opportunities for researchers in academia and pharmaceutical industry. While additional research is required to reach higher degrees of deuterium incorporation, we feel the results reported herein will allow for smooth transfer of future improvements to flow.

4. Experimental Details

4.1. Chemicals. The chemicals and solvents were purchased from Sigma-Aldrich unless otherwise stated and were used without purification unless specified. (Tricyclohexylphosphine)(1,5-cyclooctadiene)(pyridine)-iridium(I)hexafluorophosphate (99% Crabtree's Catalyst) was purchased from Strem Chemicals. The substrate was dissolved in degassed dichloromethane. Deuterium gas with a purity of 99.8% was used.

4.2. Analytical Methods. Liquid chromatography mass spectrometry (LC–MS) analysis was performed using a Waters Separations module, Waters Photodiode array detector, and Waters SQ Detector 2. An aliquot was taken and diluted with methanol in a LC–MS vial and submitted for LC–MS analysis, assuming a concentration of 0.01 mg/mL.

4.3. Preparation of Starting Materials. The preparation of the immobilized iridium-based catalyst **1** was based on a literature procedure [9,20]. *N*-(4-methoxyphenyl)-N-methylbenzamide (**2**) was synthesized according to literature procedures [20].

4.4. Materials and Equipment. In all experiments unless otherwise stated, the flow rate of deuterium gas was controlled with a gas mass flow controller (EL-FLOW Select GAS Mass Flow Controllers F-200CV, Bronckhorst High-Tech). The sample solutions with a concentration of 1 mg/mL were introduced into the microreactor by means of a syringe pump (Pump 11 Elite Infusion Only Dual Syringe, 704501, Harvard Apparatus) equipped with 1000 series syringes (Hamilton). Syringes were connected to PFA tubing (1/16 × 0.020 i.d.) connected by means of Upchurch Flat-Bottom Fittings® (Upchurch Flat-Bottom Fittings, Caps & Plugs, Tools; Upchurch Scientific Inc. USA) assembly parts. For the HPLC setup and H-Cube Pro™ setup, the sample solutions were introduced by means of an HPLC pump.

4.5. Batch Mode. To a 2-mL reaction vessel (Ultra Torr), 5 mg of [(cod)Ir(PS-PPh_3)$_2$]PF_6 (**1**), 2 mg of *N*-(4-methoxyphenyl)-*N*-methylbenzamide (**2**), and 0.2 mL dichloromethane were added. The reaction vessel was connected to a deuterium manifold (RC Tritec). The content of the vessel was cooled down with liquid nitrogen and one time evacuated under vacuum. The flask was allowed to warm to room temperature while stirring. The content was cooled down with liquid nitrogen, evacuated under vacuum, and placed under deuterium gas (200–300 mbar) resulting in an end pressure of approximately 1 bar.

4.6. Continuous Stirred Tank Reactor (CSTR). A mixing chamber, with an internal volume of 0.7 mL, originating from an HPLC setup was filled with 10 mg of the preactivated immobilized catalyst (**1**). The biphasic reactant streams, gas and liquid, were introduced into the system with a mass flow controller or a syringe pump, respectively. The gas phase, which contains only deuterium gas, was introduced at one side of the mixing chamber, whereas the liquid phase, which contains *N*-(4-methoxyphenyl)-*N*-methylbenzamide (**2**) dissolved in dichloromethane, was introduced on the opposite side of the chamber. The mixing chamber was placed horizontally on a stirring plate with maximum stirring rate. After exiting the mixing chamber, samples were collected and analyzed as described in section 4.2.

4.7. Micro Packed-Bed Reactor System. A stainless steel HPLC column threaded SS column (20 ×2.1 mm with 2 μm frit) was loaded with 10 mg of the immobilized catalyst (**1**). The biphasic reactant streams, gas and liquid, were introduced into the system with a mass flow controller or an HPLC pump, respectively. After exiting the HPLC column, samples were collected and analyzed as described in section 4.2.

4.8. Flow Apparatus H-Cube Pro™. An empty cartridge of the H-Cube Pro™ from ThalesNano was filled with 100 mg of immobilized catalyst (**1**). The system was set to 20 °C and 40 bar, with a flow rate of 0.3 mL/min to introduce the liquid phase. Deuterated water, as deuterium gas source, was used. Samples were collected and analyzed as described in section 4.2.

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