

## Advancing Isotope Labeling Technologies at AstraZeneca through Academic-Industrial Collaboration

Ryan A. Bragg,<sup>a,\*</sup> Jonas Bergare,<sup>b</sup> Markus Artelsmair,<sup>b</sup> William J. Kerr,<sup>c</sup> Troels Skrydstrup,<sup>d</sup> Charles S. Elmore<sup>b,\*</sup>

<sup>a</sup> Early Chemical Development, Pharmaceutical Science, R&D, AstraZeneca, Cambridge, UK

<sup>b</sup> Early Chemical Development Pharmaceutical Science, R&D, AstraZeneca, Gothenburg, Sweden

<sup>c</sup> Department of Pure and Applied Chemistry, University of Strathclyde, 295 Cathedral Street, Glasgow, Scotland, G1 1XL (UK)

<sup>d</sup> Interdisciplinary Nanoscience Center (iNANO), Department of Chemistry, Aarhus University, Gustav Wieds Vej 14, 8000 Aarhus C, Denmark

[ryan.bragg@astrazeneca.com](mailto:ryan.bragg@astrazeneca.com); [chad.elmore@astrazeneca.com](mailto:chad.elmore@astrazeneca.com)

### Abstract

Academic-Industrial collaborations have played an important role in developing new synthetic methodologies for the isotope chemistry team at AstraZeneca. In this communication, we present the outcome of two collaborations and illustrate how the innovative methodologies developed through these partnerships have been effectively applied within an AstraZeneca project.

**Key Words:** Collaboration Isotopes, Carbonylation, Tritium, Radiolabeling.

### Introduction

Isotopically labeled compounds are used at many points in the drug development process. In drug discovery, they are typically used to characterize the binding of the compound to the target receptor, in binding assays, or in initial DMPK assays.<sup>1</sup> As the compound advances into the development phase, a stable isotope labeled (SIL) version of the molecule will be prepared that can be used in mass spectrometry assays to allow for quantification of materials in biological fluids.<sup>2</sup> A carbon-14 labeled version of the compound is generally prepared between single compound selection and phase II clinical trials. These compounds are initially used for safety and DMPK studies, which include quantitative whole body autoradiography (QWBA) and mass balance studies. They are then later used for human absorption, distribution, metabolism, and excretion (hADME) and environmental fate studies. The synthesis of short lived isotopically labeled compounds and therapeutic compounds represents a further and separate endeavor.

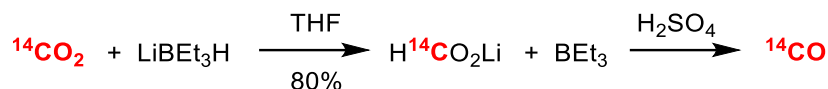
The synthesis of SIL compounds often parallels that of medicinal chemistry compounds as many reagents are available and the site of labeling is typically not critical so long as the label is stable under HPLC conditions.<sup>3</sup> The synthesis of tritium (H-3) and C-14 labeled compounds involve the use of much more simple starting materials since the commercial availability of complex building blocks is quite limited. The location of the radioactive labels in a given molecule needs to be chosen more selectively than is the case for SIL compounds, due to their use in metabolism studies.<sup>4</sup> Most H-3 syntheses preferentially employ the use of tritium gas, although there are many procedures that also use tritiated water or an activated tritium labeled methyl group (such as tritiated methyl iodide or tritiated methyl nosylate). All C-14 labeled compounds are prepared from Ba<sup>14</sup>CO<sub>3</sub>, which is readily converted into useful building blocks including, cyanides, CO<sub>2</sub>, MeI, and similar small molecules.<sup>5</sup>

The need for selective, high-yielding, and simple methodologies for isotope incorporation has driven considerable research into the labeling of molecules with C-14 and H-3. Below we discuss how recent collaborative efforts in this field have directly affected the chemistries run in the AstraZeneca isotope chemistry group.

### Academic-Industrial collaborations applied to carbon-14 labeling.

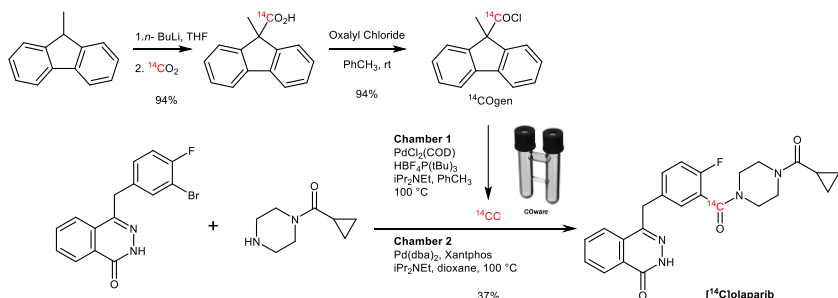
Carbonylation is an attractive target for C-14 labeling as it is, in general, very tolerant of functional groups and is, thus, amenable for late-stage incorporation of the C-14 unit. This is crucial since it minimizes the number of radioactive steps and the amount of radioactive waste generated.  $^{14}\text{C}$ O is also a one-carbon synthon that can be generated from  $\text{Ba}^{14}\text{CO}_3$  (or  $^{14}\text{CO}_2$ ) with only minimal manipulations. Moreover, carbonylation reactions can often be carried out in stoichiometric or near-stoichiometric amounts of  $^{14}\text{CO}$ .

$^{14}\text{CO}$  was first reported in 1947,<sup>6</sup> but only one instance of it actually being used in a carbonylation was reported prior to 2000.<sup>7</sup> Then in 2000, the synthesis of  $^{14}\text{CO}$  by dehydration of  $\text{H}^{14}\text{CO}_2\text{Li}$  and its subsequent application in several carbonylation reactions was reported (Scheme 1).<sup>8</sup> However, this method suffered from a cumbersome reaction apparatus, and generated a considerable amount of sulfuric acid contaminated with radioactive by-products. In addition, no attention had been paid to the  $^{14}\text{CO}$  not consumed in the reaction, and this limitation prevented zero-emission sites from using the methodology.



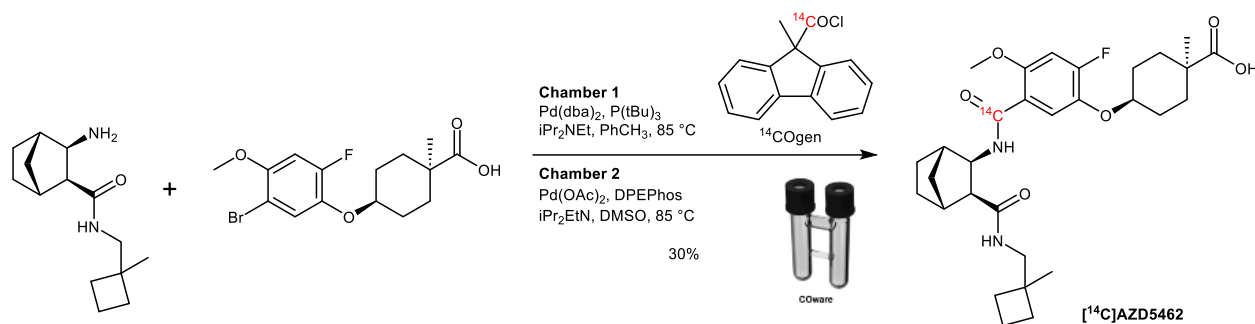
Scheme 1. Dehydration of  $\text{H}^{14}\text{CO}_2\text{H}$  to give  $^{14}\text{CO}$ .<sup>8</sup>

In 2011, the Skrydstrup group reported the use of COgen (Scheme 2) as a CO-releasing molecule and a convenient apparatus for conducting these reactions (COware).<sup>9</sup> The COware consists of two-reaction chambers connected by a glass tube. COgen is placed in the CO releasing chamber and once released the CO diffuses through the tube into the other reaction chamber where it is consumed to afford the carbonylated product. Promisingly, this methodology appeared to alleviate many of the problems encountered with the dehydration of  $\text{H}^{14}\text{CO}_2\text{H}$ . To investigate this technology for its applicability to/feasibility for C-14 chemistry in an industrial setting, a collaboration between the Skrydstrup group at Aarhus University and AstraZeneca-Gothenburg was initiated. AstraZeneca (AZ) provided funding for reagents and lab space while Aarhus placed two synthetic chemists in the AstraZeneca labs.  $^{14}\text{COgen}$  was successfully prepared and the utility of the methodology was demonstrated in the synthesis of several pharmaceutically relevant molecules including [ $^{14}\text{C}$ ]olaparib (Scheme 2) on the 1 mmol scale (2 GBq or 60 mCi).<sup>10</sup> To further improve the methodology for its use with C-14, a method to capture excess CO was also developed using *n*-BuLi.



Scheme 2. Synthesis of  $^{14}\text{C}$  from  $^{14}\text{CO}_2$  *via* the Skrydstrup method and application to the synthesis of  $[^{14}\text{C}]$ olaparib.<sup>10</sup>

In the decade prior to 2012, no C-14 carbonylations were conducted at AstraZeneca in Europe; however, since developing this method, 10% of the C-14 labeled compounds prepared by AstraZeneca and subcontractors have utilized the carbonylation methodology. The utility of this methodology is further exemplified in the synthesis of an AstraZeneca development compound,  $[^{14}\text{C}]$ AZD5462 (Scheme 3).<sup>11</sup> The radiolabeled drug molecule was synthesized in a single step, exemplifying an exceptionally efficient process despite the modest yield obtained. This collaboration has been extended using funding from two EU Horizon programs and by supporting visiting scientists to develop novel carbonylation chemistries.<sup>12, 13</sup>



Scheme 3. The synthesis of  $[^{14}\text{C}]$ AZD5462 *via* a C-14 carbonylation.

### Academic-Industrial collaborations applied to tritium labeling

In the 1990s, a novel way of handling tritium gas was developed, which allowed for safe and precise use of the gas in a stainless steel manifold.<sup>14</sup> The gas could be stored as a solid as uranium tritide ( $\text{UT}_3$ ) and then liberated as tritium gas with heat. Once the reaction was complete, the remaining tritium gas could be recovered onto the uranium bed to again give  $\text{UT}_3$ . This allowed safe and easy handling of tritium gas and a significant reduction in tritium releases to the environment. Accordingly, this technology has caused many isotope chemistry groups in industry to focus on the use of tritium gas instead of alternatives such as  $\text{T}_2\text{O}$ .

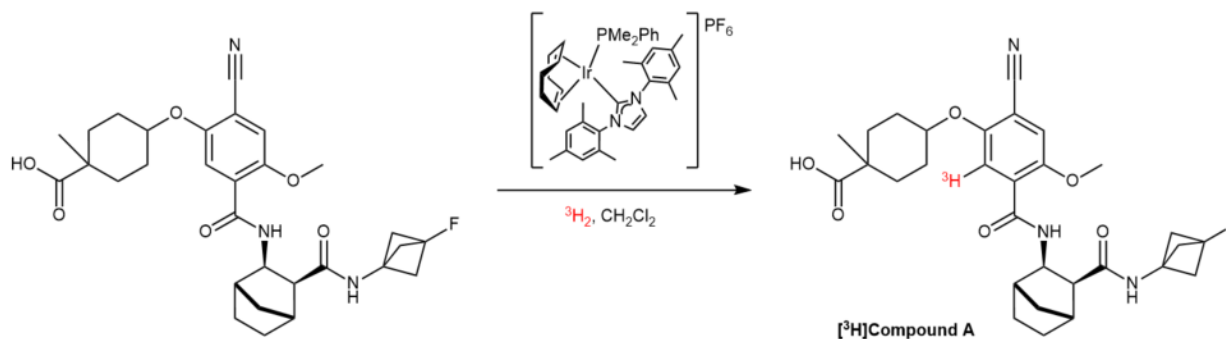
As previously mentioned, for some DMPK assays, the location of the radioactive label is critical and multiple radioactive labels (or non-specific labeling in multiple locations) can affect the ease of data interpretation. Therefore, the chemoselectivity of the labeling methodology is very important. Typical methods for incorporating tritium into drug molecules from tritium gas include reductive dehalogenation, multiple bond reductions, and hydrogen isotope exchange (HIE).<sup>3, 15</sup> However, there are also alternatives that do not rely upon tritium gas and many groups also use methylation and the incorporation of tritiated water to good effect. For HIE with  $\text{T}_2$ , the state of the art method for exchange chemistry used Crabtree's catalyst until approx 2008.<sup>16</sup> One of the main drawbacks of Crabtree's Catalyst is the large amount of radioactive impurities produced by the catalyst<sup>17</sup> and rapid catalyst deactivation.<sup>18</sup> Consequently, purification of crude reaction mixtures often necessitated multiple HPLC purification steps to obtain pure compounds.

Based on relationships developed following a series of lectures delivered by Professor Billy Kerr within AstraZeneca sites, discussions on improved HIE reactions were initiated between AstraZeneca scientists and Prof. Kerr. These talks focused on emerging research directions within the Kerr laboratory, and rapidly led to an industry-funded PhD studentship targeted at further developing a series of *N*-heterocyclic

carbene (NHC) containing catalysts. These NHC catalysts have now become the state of the art for conducting HIE.<sup>16</sup> These catalysts are not only more active at promoting hydrogen isotope exchange reactions at lower tritium pressures but are also typically more selective for certain directing groups, as detailed in a recent review, thereby generating cleaner reaction profiles and more stereospecific labeling.<sup>19, 20</sup> Furthermore, they can often be used with lower catalyst loading although they are frequently still used in stoichiometric amounts within AZ laboratories.

Over the past decade at AZ, 65% of tritium labeled compounds were prepared via tritium reductions of aryl halides or multiple bond reductions. Hydrogen isotope exchange chemistry accounted for 31% of all tritium labeling, of which 56% used Kerr catalysts, 28% Crabtree's catalyst, and the remainder a mixture of other catalysts. Therefore, the Kerr-developed catalysts were used in the synthesis of 17% of all tritium labeled compounds synthesized at AZ over the past decade.

An example showing the application of these newly developed catalysts at AstraZeneca is illustrated in Scheme 4. A tritium-labeled Relaxin Family Peptide Receptor 1 (RXFP1) agonist, [<sup>3</sup>H]Compound A, was directly obtained from its non-labelled precursor in one step as directed by the adjacent amide group. The pure compound was isolated after a single prep HPLC run at a high molar activity (94% labeled with tritium, 990 kBq/nmol), and the <sup>3</sup>H NMR showed the labeling to be completely regioselective to the indicated location (Figure 1).<sup>20</sup>



Scheme 4. The synthesis of [<sup>3</sup>H]Compound A using a Kerr-catalyst to effect hydrogen isotope exchange.

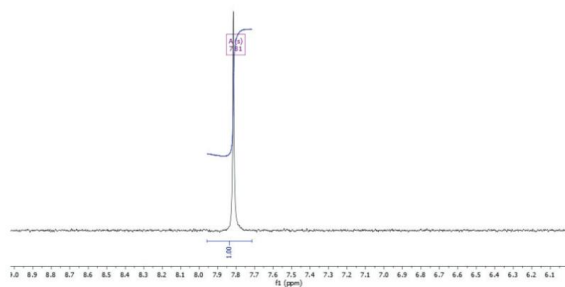


Figure 1. <sup>3</sup>H NMR of [<sup>3</sup>H]Compound A showing the exquisite selectivity of the exchange reaction.

This initial collaboration with the Kerr group has since progressed through a series of additional collaborative industry-funded studentships aimed at exploring further novel catalysts. These efforts have focused on facilitating selective hydrogen isotope exchange with a variety of directing groups and under

different reaction conditions, thereby enhancing our understanding and, indeed, the applicability of this methodology.<sup>21,22</sup>

## Conclusion

Academic-industrial collaborations have played a crucial role in advancing synthetic methodologies for AstraZeneca's isotope chemistry team by integrating academic innovation with industry expertise. These partnerships have enabled the efficient synthesis of radiolabeled drug molecules and has delivered key tools for drug discovery and development projects, thereby streamlining the process of bringing medicines to patients.

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**Abbreviations:** QWBA quantitative whole body autoradiography; hADME human absorption, distribution, metabolism, and excretion; DMPK Drug Metabolism and Pharmacokinetics; SIL Stable Isotope Labeled; COgen - 9-Methyl-9H-fluorene-9-carbonyl chloride; COD 1,5-cycloctadine; DBA Dibenzylideneacetone; DPEPhos Bis[(2-diphenylphosphino)phenyl] ether; HIE hydrogen isotope exchange (HIE); NHC *N*-heterocyclic carbene (NHC); AZ AstraZeneca; RXFP1 Relaxin Family Peptide Receptor 1

**Conflict of Interest:** R.A.B, J.B, M.A., and C.S.E. are current or former employees and shareholders of AstraZeneca. T.S. is co-owner of SyTracks A/S, which commercializes the two-chamber system (COware), SilaCOgen and COgen.

**Safety statement.** No unexpected or unusually high safety hazards were encountered. The handling of radioactive compounds requires specialized training, facilities, and permits.

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