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Metal-catalysed isotopic exchange labelling: 30 years of experience in pharmaceutical R&D

Paul H. Allen, Michael J. Hickey, Lee P. Kingston, and David J. Wilkinson*

Metal-catalysed exchange has been used extensively in the laboratories of AstraZeneca plc at R&D Charnwood to label a variety of molecules of pharmaceutical interest with the isotopes of deuterium and tritium. Despite early prejudices against the use of tritiated compounds, particularly in ADME studies, the development of directed isotopic exchange techniques has enabled timely and economic support for many pharmaceutical projects.

Keywords: tritium; deuterium; metal-catalysed exchange; rhodium; ruthenium; iridium

Introduction

The synthesis of radiolabelled compounds has played a pivotal role in pharmaceutical R&D since the isotopes of tritium and carbon-14 became readily available commercially. Despite many predictions that their use would be superseded by alternative technologies and methodologies, radiolabelled compounds remain an essential part of drug discovery and development. A major driver for this is the requirement of regulatory bodies for specific information on the *in vivo* biological fate of drugs and other small molecule entities. How a compound is absorbed, distributed, metabolized and excreted from the body (ADME) are still questions which can only be satisfactorily addressed through the use of radioactively labelled compounds. As a consequence, no novel small molecule entity has received regulatory approval and successfully proceeded to market without first having been radiolabelled.

Early approaches to the incorporation of radioactive isotopes into molecules involved a rational synthesis from a suitable carbon-14 precursor. Alternatively, tritium might be incorporated via a reductive procedure involving a suitable precursor or via the isotopic exchange of protium in a molecule for tritium. As the original source for all carbon-14 labelled compounds is barium [^{14}C]carbonate, accessing even simple molecular building blocks, such as cyanide, ethyl acetoacetate, diethyl malonate and simple substituted aromatics, can be time consuming and synthetically challenging for anyone other than a commercial supplier of such products. As such, the choice of what chemical starting point to use for a carbon-14 synthesis can be a significant decision which is influenced by such considerations as budget, resource availability as well as the competing internal demand for other radiolabels. In some pharma organizations, this situation has led to restrictions on the availability of radiolabels as they are perceived as being too expensive and requiring long lead times, a view which restricts their supply to solely supporting development candidates.

Tritium has always offered a cheaper and more readily accessible alternative to carbon-14 for the radiolabelling of compounds. However, the early methods of isotopic exchange were often non-specific and led to generally labelled compounds. While these radiolabels were perfectly acceptable when used in some applications, the lack of regioselectivity was often a disadvantage if the compound was employed for *in vivo* studies, since all or part of the label could be lost as a result of metabolism occurring at one or other of the labelled positions. This complication could often compromise the interpretation of results arising from these studies and was one of the prime reasons for the early mistrust of tritium-labelled compounds. In contrast, site-specific labelling procedures such as double and triple bond reduction, reductive dehalogenation and certain exchange reactions taking advantage of particular functionality e.g. benzylic exchange have always been possibilities. However, the opportunities to take advantage of these approaches required either significant chemistry effort to synthesize the required precursors or that the molecule of interest contained a specific functionality.

An early discovery (1980–1990)

It has always presented as an attractive prospect to have available versatile methodologies through which tritium could be directly introduced into a drug molecule with high efficiency and regioselectivity in a one-step process. The ability to synthesize quality radiolabels both rapidly and cheaply would allow them to be used in early investigational studies, thus opening new or more efficient research options. Using the data generated from these studies provides an opportunity for higher quality and better decision making which is often not possible using data generated using other methodologies.

In our laboratories we had been involved in a long running discovery programme looking for a successor to sodium

AstraZeneca plc, R&D Charnwood, Bakewell Rd, Loughborough, Leicestershire LE11 5RH, UK

**Correspondence to: David J. Wilkinson AstraZeneca plc, R&D Charnwood, Bakewell Rd, Loughborough, Leicestershire LE11 5RH, UK.*
E-mail: david.j.wilkinson@astrazeneca.com

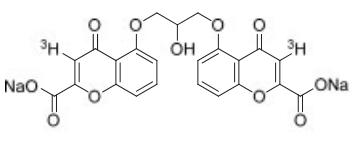
cromoglycate (Intal), a prophylatic anti-allergy agent used in the treatment of asthma and other diseases with an allergy basis such as seasonal rhinitis. The drug was delivered via inhalation and had a novel mechanism of action which was not fully understood. The molecule itself was derived from a chemical class known as the chromone-2-carboxylic acids and this chemical motif was a common feature of potential successors arising from the ongoing discovery programme. Traditionally, the compounds were radiolabelled with carbon-14 introduced using diethyl [^{14}C]oxalate via a Claisen condensation reaction. However, the specific activity achievable with carbon-14 was inadequate for the type of investigations that were being contemplated. These included explorations of metabolism, kinetics, mechanism of action and there was also a requirement for the development of a radioimmunoassay for the drug. Therefore, a suitable route to the tritiated radiolabelled drug was sought.

Reports[1,2] had been published highlighting the use of rhodium chloride trihydrate as a homogeneous catalyst capable of promoting the general exchange of deuterium in a number of simple aromatic systems. Using this as a starting point, conditions were subsequently identified under which isotopic exchange of a range of aromatic carboxylic acids was observed. However, NMR studies with the deuterium isotope showed that, in contrast to the early work, the isotope was introduced with a high degree of regioselectivity (>95%) into the position *ortho* to the carboxylate function. Typical conditions used to effect isotopic exchange were:

The substrate (100 mg) together with rhodium(III) chloride trihydrate (20 mg) were dissolved in a mixture of DMF/deuterium oxide, 2:1 by volume (3 ml) and heated at 107°C for 18 h. The labelled substrate was isolated using solvent extraction and purified by crystallization from methanol or water, if necessary.

Deuterium incorporations of between 30 and 98% were typically obtained from this procedure. Further elaborations of the conditions were necessary to make the approach feasible for the incorporation of tritium.

Tritium oxide can be accessed at near carrier-free specific activity; however, to do so using manageable volumes (up to 10 μl) necessitates employing significant amounts of radioactivity (>1 TBq). This introduces particular challenges associated with the handling of this volatile material and having to deal with the large quantities of radioactive waste that are invariably generated. Consequently, this work is more often carried out in the laboratories of commercial contract suppliers using specialized equipment. Despite this limitation, it is possible to purchase tritium oxide at lower specific activities in the range of 3–30 GBq mmol^{-1} and this is more than adequate for the purposes of producing radiolabelled material suitable for ADME studies.



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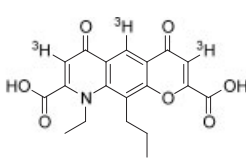


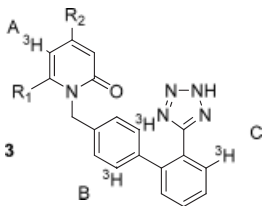
2

Typical of the experimental procedure that was developed to synthesize high specific activity radioligands is the one described below for [^{3}H]nedocromil sodium (**2**):

Tritium oxide (>90% atom abundance, 9 μl, 925 GBq) was added to a solution of nedocromil diacid (2 mg) and rhodium(III) trichloride trihydrate (1 mg) in DMF (0.4 ml). The solution was heated at 90°C for 24 h and, after cooling and removal of any labile tritium by lyophilization, a portion of the crude product was purified by reversed-phase HPLC using a methanol-aqueous ammonium acetate gradient. A batch of [^{3}H]nedocromil sodium was obtained with a specific activity of 796 GBq mmol^{-1} and a radiochemical purity of > 95%[3]. Interestingly, isotope was found not only in the expected 3- and 7-positions but there was also a small amount of isotopic incorporation at the 5-position. Similarly, [^{3}H]sodium cromoglycate (**1**) *was synthesized via a comparable procedure to afford material with a specific activity of 610 GBq mmol^{-1} and with a radiochemical purity of > 97%[4].*

The versatility of this approach was subsequently demonstrated via the tritiation of a wide range of drug molecules, including phenacetin, paracetamol, probenecid, procainamide[5] and pentamidine[6]. In all cases, high regioselectivity was observed (>98%) and acceptable specific activities (1–4 GBq mmol^{-1}) were obtained using tritium oxide with a specific activity of 3.33 GBq mmol^{-1}.

The versatility of the rhodium chloride trihydrate-directed exchange is not confined to carboxylic acids[7]. Amides, anilides and benzylamines are also capable of realizing useful levels of isotopic incorporation[8,9]. An interesting and challenging variant presented when we tried to make use of the tetrazole functionality, often introduced as an isostere for carboxylic acids, as a directing group to promote *ortho*- isotopic exchange. We were working with a group of biaryl tetrazoles compounds (**3**) and there was interest in having a number of these radiolabelled to support some initial *in vitro* and *in vivo* metabolism studies.



3

A trial exchange reaction using deuterium oxide revealed significant deuterium incorporation into several positions. Deuterium NMR confirmed that labelling was not only occurring at the position *ortho*- to the tetrazole, as anticipated, but that isotope was also being incorporated into the 1,4-disubstituted aryl ring as well as into the 5-position of the pyridinone ring. However, we obtained an unexpectedly low recovery of the desired labelled product, in addition to noting the presence of considerable quantities of more polar material.

The tritiated compound was subsequently prepared by exchange reaction with tritium oxide using the following conditions:

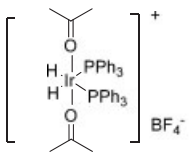
The substrate (80 mg), rhodium(III) chloride trihydrate (27 mg), tritium oxide (80 μl, 15 GBq) and DMF (1 ml) were heated at 105°C for 18 h in a sealed vial. Under these conditions recoveries are generally expected to be good; however, in this case only 20% of the total radioactivity was found to be associated with the required product. The remainder was present as an uncharacterized polar compound, probably a rhodium complex involving the labelled substrate.

Fortunately, the complex could be broken down by heating the crude radiolabelled product in methanol with 5 M aqueous sodium hydroxide at 85°C. The labelled substrate was isolated by preparative HPLC, which yielded 60 mg of the tritiated product with a radiochemical purity of 99% and a specific activity of 860 MBq mmol^{-1}. Tritium NMR confirmed the positions of incorporation to be as indicated in the structure above in the ratio of 42% (A), 41% (B) and 17% (C).

This approach was followed up to radiolabel a series of compounds from the same project which allowed the selection of the candidate possessing the best DMPK properties to go forward for further development.

A decade of innovation and development (1990–2000)

Subsequently, an additional fruitful avenue of isotope exchange was provided by the development of another class of *ortho*-exchange catalysts, exemplified in the first instance by the Heys catalyst[10] (**4**). This new approach complemented and, in some areas, replaced the use of the rhodium(III) chloride trihydrate-based exchange systems. It had some clear attractions, comprising mild reaction conditions, the use of a tritium gas donor and the provision of a new range of directing groups. Hence, the group at Charnwood began investigating the use of this catalyst in 1994. A further spur to our Ir(I) investigations was provided by a publication by Hesk who showed that the commercially available Crabtree's catalyst[11] (**5**) also exhibited *ortho*-exchange ability with a range of substrates[12]. Subsequently, the use of Crabtree's catalyst became an established labelling approach within the group.



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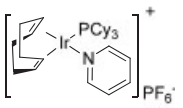
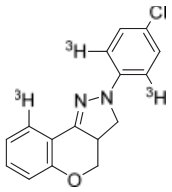


5

One of the first projects to benefit from this new approach was aimed at developing novel Th2 selective immunosuppressive agents[13]. In the mid-90's, the lead compound (**6**) for the project was required in radiolabelled form to support *in vivo* ADME studies. It was recognized that the pyrazole portion of the molecule might be utilized to effect *ortho*-exchange tritiation via the new Ir(I) catalysts. A series of deuterium model studies with the compound provided the group with experience of the use of Crabtree's catalyst and subsequently led to a successful tritium labelling reaction:

The substrate (3.5 mg, 12.3 μmol) in dichloromethane (0.75 ml) and Crabtree's catalyst (0.5 mg, 12.3 μmol) were stirred with tritium gas (37 GBq, 17.4 μmol) for 18 h at room temperature. Labile tritium was removed by lyophilization with ethanol and the crude residue dissolved in ethanol to afford a stock solution (13.3 GBq). HPLC purification of a portion of the crude stock afforded the product (888 MBq) with a radiochemical purity of 99.9% and a specific activity of 740 GBq mmol^{-1}. Tritium NMR: δ(533 MHz, DMSO-d_6) 7.14 (d, 8.5 Hz), 7.81 (d, 8.5 Hz).

The tritium NMR of the above product showed that as well as exchange occurring in the chlorophenyl ring there was also a small amount of exchange in the phenyl ring of the tricyclic system. The corresponding labelling reaction employing rhodium(III) chloride trihydrate and tritiated water (9.25 GBq, approx. 3.3 GBq mmol^{-1}) of tritiated water afforded 37.5 MBq of product with a radiochemical purity of 74%. Hence, it was apparent that the new technique would be a useful addition to our in-house labelling methods.

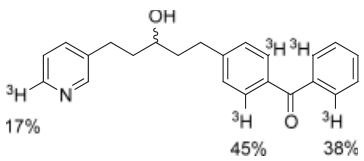


6

The successful labelling of the above target led to similar successes with other compounds from the same project. Moreover, we found that by selecting compounds with different chromatographic retention times it was possible to pool these and perform the exchange reaction in the same tritiation pot; this both increased productivity and reduced the quantities of radioactive waste generated. It should, however, be noted that there can also be drawbacks to this approach first due to the differential competition with the substrates for the catalyst and second, due to the need for extensive chromatographic purification of the product mixture to achieve satisfactory radiochemical purities. Nevertheless, the benefits far outweigh the additional effort required and we often use this approach to radiolabel multiple candidates in a single exchange reaction.

Another interesting aspect of iridium-catalysed labelling was provided by the exchange reaction of a benzophenone-containing photoaffinity probe (**7**) required for target identification studies. Deuterium trials with Crabtree's catalyst revealed that the experimental atom % abundance of the compound was higher than anticipated for the four available positions suggesting that label had been introduced elsewhere in the molecule. The experiment was repeated using tritium as the isotope source via the following procedure:

The substrate (1.2 mg) and Crabtree's catalyst (3.6 mg) were dissolved in DCM (1 ml) and tritium gas (115 GBq, 1.2 ml) introduced and the contents left to stir at ambient temperature for 18 h. Following removal of labile tritium via lyophilization and subsequent purification by HPLC, the tritiated product was obtained with a specific activity of 4525 GBq mmol^{-1} and a radiochemical purity of 95.4%.



7

Tritium NMR analysis of the pure product revealed that tritium had been introduced into the anticipated positions *ortho* to the keto functionality but that there was also 17% isotope present *ortho* to nitrogen in the pyridine ring. This phenomenon was subsequently exploited in labelling other compounds from this project and is now well known and understood and is widely applicable across a range of heterocyclic-containing compounds. Further examples of this approach are presented later in this paper.

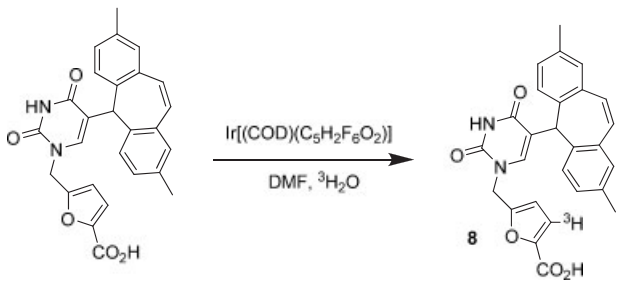
Although the use of Crabtree's catalyst opened up new labelling options, it was still far from being the ideal labelling option. Exchange efficiency tended to be substrate specific and

non-productive complexation reactions by heteroatoms in the substrate led to the need to employ super-stoichiometric amounts of catalyst. This, in turn, produced complex reaction mixtures which were sometimes difficult to purify. It was these challenges that encouraged researchers to investigate other iridium catalysts, not necessarily as a replacement but to complement the existing methodology.

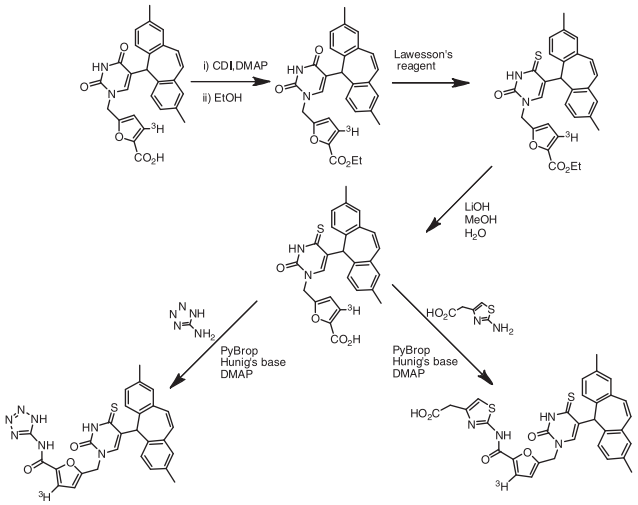
Lockley[14] produced a series of iridium-based catalysts used for homogeneous labelling. Using the newly available parallel techniques for reaction optimization and analysis, a wide selection of transition metal complexes were screened for catalytic activity against substrates with a range of functionality. From this work, cycloocta-1,5-diene.iridium(I)acetylacetonate (COD.Ir.acac) was shown to exhibit activity similar to that of rhodium(III) chloride. Optimization of the reaction conditions using high throughput NMR and further ligand fine-tuning delivered a second catalyst, cycloocta-1,5-diene.iridium(I)-1,1,1,5,5,5-hexafluoropentane-2,4-dionate (COD.Ir.F_6acac)[15]. This catalyst also had further advantages over its predecessor in that it had been shown to exhibit catalytic activity using deuterium gas in both DMA and DMF.

This was first put to good use in a project where we needed to demonstrate acceptable levels of skin penetration for a possible topical drug candidate intended for the treatment of psoriasis. With a total of seven drug candidates to evaluate in these skin absorption studies using a human *in-vitro* model and a relatively short timeframe to deliver within, there was a requirement for a rapid and efficient approach to radiolabelling the compounds. While not all the compounds were amenable to labelling via this approach, it was possible to access these through other means including acid-catalysed exchange and keto reduction using LiB^3H_4. However, several of the compounds contained suitable directing groups which made them amenable to homogeneous exchange catalysis using the new (COD.Ir.F_6acac) catalyst. Moreover, the potential existed to access them all from a common intermediate (**8**) as shown below. The conditions for the exchange of this compound were investigated and optimized resulting in the following procedure which gave the best compromise between tritium incorporation and overall yield:

The substrate (5 mg), COD.Ir.F_6acac (2 mg) and tritiated water (740 GBq) in DMF (0.09 ml) were heated at 70°C for 20 h in a sealed vial. The contents were allowed to cool and labile tritium removed by lyophilization. Following HPLC purification, a total of 483 MBq of **8** *was obtained with a specific activity of 70 GBq mmol^{-1} and a radiochemical purity of > 96%.*



The intermediate (**8**) was further elaborated using mainline synthetic chemistry techniques to provide the required final products that were required for evaluation in the *in vitro* model.



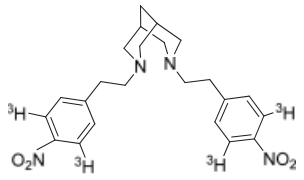
Further advances in homogeneous metal-catalysed exchange (2000–2010)

Developments continued in the area of homogenous iridium(I) catalysis, specifically around the incorporation of different phosphine ligands in an attempt to modify the activity and selectivity of the catalytic species created. These catalysts further extended the range of compounds that could be accessed and radiolabelled via this approach and include such species as: [COD.Ir.$(PPh_2Me)_2$]PF_6, [COD.Ir.$(PPh_3)_2$]BF_4 and the bidentate phosphine complex [COD.Ir.(dppe)]BF_4 (dppe = 1,2-*bis*(diphenylphosphinyl)ethane). This increase in choice provides the opportunity for introducing tritium in a regioselective fashion via either a 5- or a 6-membered metallocyclic intermediate. For more detailed information on the role of phosphine and arsine ligands in the determination of labelling regioselectivity with COD.Ir(I)-based catalysts, see the contributions by R Salter and J Herbert in this Special Issue of the Journal. In addition to catalyst selection, the use of parallel screening technologies within the group has also enabled the optimization of reaction conditions with respect to time, concentration, solvent and mole ratio of catalyst to substrate.

An example of how this approach was employed involved an exercise to maximize the specific activity of a radioligand (**9**) which was required for use in a high-throughput binding assay. It was anticipated that any radiolabelling procedure requiring reductive conditions and tritium gas would likely be limiting due to the vulnerability of the nitro groups. However, the nitro group is recognized as a weak director for *ortho*-exchange and it was felt that by screening a number of iridium phosphine complexes, it might be possible to identify one which would prove suitable for this substrate. In fact, [COD.Ir.$(PPh_2Me)_2$]PF_6 was found to be very efficient in promoting labelling of this compound as indicated by the levels of isotope incorporation with over three deuterons per molecule being achieved when using deuterium gas. The corresponding tritium reaction was performed as below:

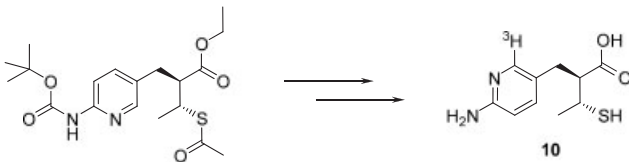
[COD.Ir.$(PPh_2Me)_2$]PF_6 (8.6 mg) and substrate (1.6 mg) were dissolved in dichloromethane (1 ml) and stirred under an atmosphere of tritium gas (130.6 GBq) at a partial pressure of approximately 300 mbar for 18 h. Evaporation of the solvent,

removal of labile tritium by two cycles of evaporation from ethanol provided a stock material. HPLC purification of a portion of the stock afforded a total of 880 MBq of labelled product with a molar specific activity of 2.94 TBq mmol^{-1} and a radiochemical purity of 97.5%.



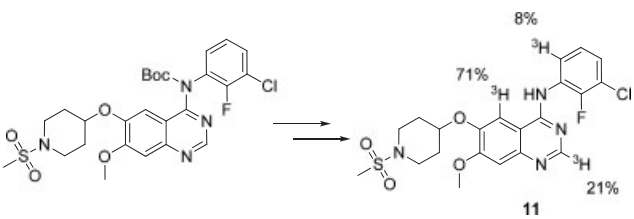
9

Parallel optimization was also used to identify experimental exchange conditions for radiolabelling compound (**10**). Initial attempts to radiolabel the BOC-protected precursor using Crabtree's catalyst and tritium gas were hampered by low isotopic incorporation (approx. 5%) and poor recoveries (<25%). Using a parallel screening approach with deuterium gas, 16 homogeneous catalysts were assessed in either dichloromethane (DCM) or *N,N*-dimethylacetamide (DMA) and using varying substrate/catalyst ratios[16]. Using this approach allowed an initial set of conditions with DMA and Crabtree's catalyst to be identified which gave rise to significant isotopic incorporation. This then served as the starting point for a further round of optimization of the exchange reaction using DMA and varying the stoichiometry of the Crabtree's catalyst. The tritium-labelling reaction was finally run with Crabtree's catalyst (100 mol%), substrate (6 mg) in DMA (3 ml) and tritium gas (1480 GBq) and a much improved recovery of 98% was obtained together with a greatly increased isotopic incorporation of 62%.



10

The use of the BOC-group as a suitable directing group to facilitate *ortho*-exchange was demonstrated in the labelling of compound **11**. The compound was labelled efficiently and exclusively at the indicated positions using BOC-protection of the aniline nitrogen of the parent molecule. Isotopic exchange with Crabtree's catalyst and tritium gas in dichloromethane and subsequent deprotection afforded the desired product with a specific activity of 400 GBq mmol^{-1}; the positions of tritium incorporation were confirmed by tritium NMR and are indicated below.



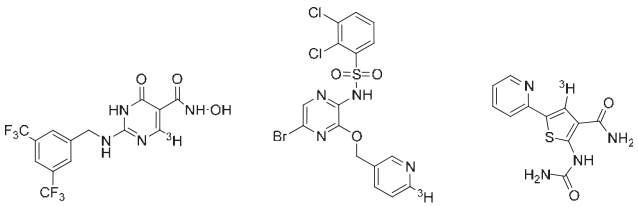
11

The utility of Crabtree's catalyst to label complex molecules is illustrated by the examples given with compounds **12–14**. We were able to utilize the hydroxamate group to exchange deuterium into compound **12**, albeit with low efficiency (approx. 5%), using Crabtree's catalyst and while this was inadequate in terms of the required specific activity for a possible tritium radiolabel, it does provide an example of a novel directing group which is capable of promoting isotope exchange.

The introduction of tritium into **13** using reductive conditions would prove difficult due to the presence of the chlorine and bromine moieties. However, this is another example of the potential to use a pyridyl nitrogen and Crabtree's catalyst to direct exchange *ortho* to the heteroatom. As such, we were able to successfully introduce tritium into the pyridine derivative **13** using Crabtree's catalyst and tritium gas. Unfortunately, the radiochemical purity of the initial crude product was disappointing (<10%); however, it proved possible after extensive HPLC purification to achieve material with a radiochemical purity of > 99%.

Another possible example of the pyridyl function being used to radiolabel a molecule is compound **14**. However, since the carboxamide is also an effective *ortho*-director, it is possible that either or both of these functions could be responsible for the observed labelling. Owing to the limited solubility of **14** in DCM, the tritiation was performed using a slightly modified procedure:

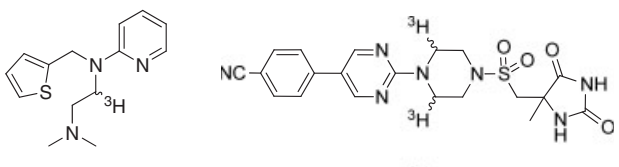
Compound **14** *(0.4 mg) and Crabtree's catalyst (1.2 mg) were dissolved in 10% v/v acetone/DCM (1 ml) and tritium gas (74 GBq) was introduced into the reaction flask and the contents left to stir at room temperature for 18 h. Following lyophilization to remove any labile tritium, the crude product was found to have a radiochemical purity of 25% and a specific activity of 814 GBq mmol^{-1}.*



12 13 14

In addition to the labelling of suitable *ortho*-positions, the Ir(I) catalysts are known to effect exchange of protons on suitable sp^3 carbons, such as *N*-alkyl groups. Co-workers at AstraZeneca R&D Alderley Park have used this approach to successfully label methapyrilene using the heteroaryl nitrogen function to direct isotopic exchange[17]. During their investigations, a set of model heteroaryl compounds were reacted with Crabtree's catalyst in the presence of deuterium gas and in all examples, a high degree of isotopic incorporation was achieved at the adjacent alkyl positions. We have successfully used this methodology to label compound **15** via the following procedure:

Compound **15** *(0.5 mg) and Crabtree's catalyst (3.1 mg) were dissolved in DCM (1 ml) and tritium gas (81.4 GBq) introduced into the reaction flask which was stirred at room temperature for 18 h. Following lyophilization to remove any labile tritium, crude product was obtained with a specific activity of 181.3 GBq mmol^{-1}, albeit with a low radiochemical purity of 12%. Tritium NMR: δ(426 MHz, DMSO-d_6) 3.85 ppm.*



methapyrilene 15

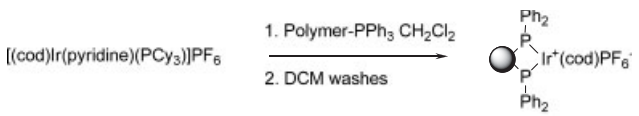
From the examples given above, it becomes clear that one of the major limitations to using such common catalysts as

Crabtree's catalyst and variants of the type $[COD.Ir.(L)_2]PF_6$ is the challenging isolations of the labelled product that has to be performed. Labelling of compounds containing additional functional groups often requires the exchange reactions to be carried out with a super-stoichiometric excess of the catalyst as a result of non-productive ligation by other functional groups to the iridium metal centre. Frequently this leads to complex reaction mixtures which make purification of the labelled compound problematic. In 2005, we identified a novel solution to this problem and set about developing a new catalytic system to promote the isotopic exchange of *ortho*-directing substrates[18]. An iridium-based solid-phase catalyst (**16**) was conveniently synthesized in a single step from commercially available starting materials which, after a simple filtration step, afforded labelled products of good radiochemical purities, thereby simplifying the purification effort. The catalyst possessed similar activity to $[COD.Ir.(PPh_3)_2]PF_6$ and Crabtree's catalyst and labelling was found to occur both efficiently and regioselectively with simple and complex molecules. The polymer-supported catalyst was readily amenable to including in the labelling screen alongside conventional homogeneous complexes and is now routinely used within our laboratories for radiolabelling drug candidates.

The polymer-supported catalyst was prepared as described below:

(1,5-Cyclooctadiene)(pyridine)(tricyclohexylphosphine) iridium(I) hexafluorophosphate (Crabtree's catalyst, 144 mg) was dissolved in dichloromethane (9 ml) and the resulting solution added to the polymer-bound triphenylphosphine (Aldrich item 3645, nominal 3 mmol of P per gram of resin, 60 mg). The reaction flask was capped, flushed thoroughly with nitrogen and stirred for 2 h at ambient temperature. The orange supernatant was decanted from the deep-red polymer and the polymer washed five times by resuspension and stirring in dichloromethane (4 ml each time). The supernatant was clear and colourless after the second wash. After drying to constant weight under vacuum, a blood-red solid was obtained (89.3 mg).

The polymer-supported catalyst showed no loss of activity when stored for 10 weeks under nitrogen at −20°C. However, when stored in air at room temperature only around 30% of the activity remained after the same period.

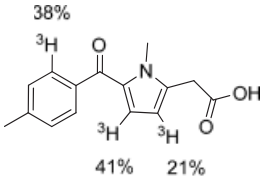


16

A typical labelling reaction is described below to demonstrate the utility of the catalyst:

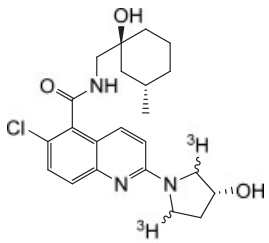
*A mixture of the non-steroidal anti-inflammatory drug, tolmetin (***17,** *1 mg) and the solid phase catalyst (**16,** 7 mg, 1.1 equiv.) in dichloromethane (1 ml) were stirred in the presence of tritium gas (66.6 GBq) at a partial pressure of approximately 52 mbar for 3 h. The catalyst was removed by filtration and the solvent evaporated, subjected to two cycles of lyophilization with ethanol to afford [^{3}H]tolmetin (4.81 GBq, 1517 GBq mmol^{-1}) with a radiochemical purity of 98.5%. Tritium NMR analysis confirmed the distribution of tritium at the indicated positions.*

The radiochemical purity of 98.5% for the tritiated tolmetin was achieved directly without the need for any further purification. When the exchange reaction was conducted using a traditional homogeneous catalyst, the product had a radiochemical purity of only 55% and a specific activity of 1228 GBq mmol^{-1}.



17

A similar experience was had when we repeated the exchange labelling of the aryl nitro compound (**9**) described earlier using 2 equiv. of polymer-supported catalyst (**16**). This afforded crude material with a radiochemical purity of 92% which compared with a radiochemical purity of only 35% using the homogeneous catalytic approach. Interestingly, the specific activity achieved using the polymer-supported catalyst (1.22 TBq mmol^{-1}) was lower than that achieved using $[COD.Ir.(PPh_2Me)_2]PF_6$ (2.84 TBq mmol^{-1}). Consequently, driven by the requirement for higher specific activity in this particular application, we continued to use the homogeneous catalytic approach to label this compound.



18

Another example of the use of the polymer-supported catalyst was in the case of the pyrrolidino-quinoline compound (**18**) which was required for *in vivo* ADME studies. This was radiolabelled using the following procedure:

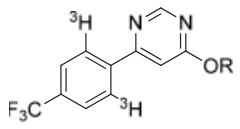
*Substrate (***18,** *3 mg) and polymer-supported catalyst (**16,** 12 mg, 1 mol equiv.) were suspended in DCM (1 ml) and stirred under tritium gas (80 GBq) at a partial pressure of 64 mbar at room temperature for 20 h. Following two cycles of lyophilization using ethanol, a crude tritiated product was obtained with a specific activity of 1500 GBq mmol^{-1} and a radiochemical purity of 80%.*

Although the compound (**18**) had only low solubility in DCM, the preferred solvent for use in these exchange procedures, it was possible to identify conditions using deuterium gas that achieved both good isotopic incorporation and relied upon a simple isolation procedure through the use of a fine suspension of the substrate together with the polymer-supported catalyst in DCM.

During the investigations into the labelling of this substrate (**18**), alternative solvents such as DMF were tried; however, while these overcame the issue of poor solubility of the substrate, as might be expected, no isotopic incorporation was observed due to inactivation of the catalyst through solvent coordination. Catalyst stoichiometry was also found to have a critical role and when used sub-stoichiometrically (0.2 mol equiv.), no incorporation of isotope was observed. When this was increased to 4 mol equiv. of catalyst, substantial isotopic incorporation was achieved; however, it proved impossible to isolate the labelled product from the polymer-supported catalyst by filtration as the substrate appeared to be irreversibly bound to the polymer support and could not be liberated. Consequently, a compromise was reached by using 1 mol equiv. of the polymer-supported catalyst which gave acceptable levels of isotopic

incorporation and a good recovery of product. This just serves to highlight that while there are significant advantages to be gained with respect to the isolation and purification of the tritiated products using the polymer-supported catalyst, it does still suffer from some of the drawbacks associated with the homogeneous version.

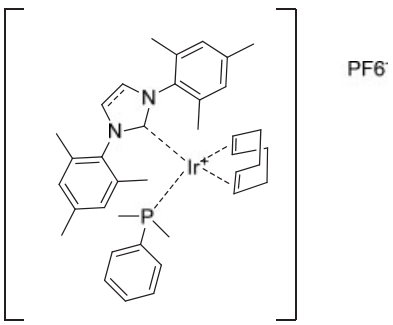
One final example from our laboratories of the use of polymer-supported catalyst highlights the more typical situation of a nitrogen heteroatom in the pyrimidine directing into an adjacent aromatic ring (**19**).



19

The substrate (1.84 mg) together with polymer-supported catalyst (6 mg, 1.5 equiv.) were stirred in dichloromethane (1 ml) under an atmosphere of tritium gas (92.5 GBq) at a partial pressure of 74 mbar for 23 h at room temperature. Following lyophilization, a crude stock solution of product was obtained which had a specific activity of 374 GBq mmol^{-1} and a radiochemical purity of 55%. Following HPLC repurification the radiochemical purity was increased to > 98%.

The publication of the work by Kerr[19] in which they describe the development of a series of novel iridium carbene-based catalysts with very different activity and selectivity profiles to those of the phosphine-based catalysts has now further extended the options available for radiolabelling compounds of pharmaceutical interest. Examples of the utility of these catalysts in labelling simple and complex molecules with deuterium and tritium are given in the paper by Kerr and Nilsson in this Special Edition of the Journal. Previously, it has only been possible to overcome the limitations imposed by non-productive complexation by using super-stochiometric quantities of the catalyst. However, the new carbene-based catalysts may provide an advantage over the conventional catalysts in this regard by offering a different kind or degree of discrimination between productive and non-productive complexation. The catalyst we routinely use is based upon the 1,3-bis(2,4,6-trimethylphenyl)imidazol-2-ylidene (IMes) and dimethyl phenylphosphine ligands, i.e. [COD.Ir.PMe$_2$Ph.(IMes)]PF$_6$ (**20**).



20

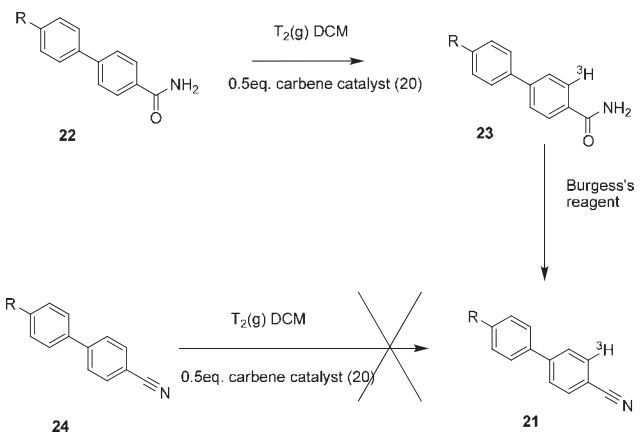
We routinely synthesize this catalyst using the procedure described below:

To a suspension of chloro(1,5-cyclooctadiene)iridium(I) dimer (200 mg) in dry benzene under nitrogen, was added sodium ethoxide (2 equiv., freshly prepared) and the mixture allowed to stir for 10 min at room temperature. 1,3-Dimesityl-1H-imidazol-3-ium chloride (2 equiv.) was added and the resulting orange suspension stirred at room temperature for 4 h. The solvent was removed under high vacuum and the residue triturated with a small amount of dry ether. The ether was passed through a pad of Celite under nitrogen and the solvent removed under high vacuum and reconstituted into dry THF. Silver(I) hexafluorophosphate (2 equiv.) was added and the slurry left to stir overnight. The resulting dark brown slurry was passed through a pad of Celite under nitrogen to give a dark orange solution. Dimethyl(phenyl)phosphine (2 equiv.) was added and the dark red solution left to stir under nitrogen for 5 h at room temperature. The organics were concentrated under vacuum (water-bath at room temp.) to give 458 mg of a red glassy solid. NMR confirmed the presence of the desired product but that the purity was low. The solid was dissolved in DCM (2 ml) and dry ether (5 ml) added slowly with stirring causing the product to crystallize. The red solid was filtered and dried under vacuum at room temperature to afford 115 mg of product which was pure by NMR (yield 22%). It should be noted that fresh phosphine, silver hexafluorophosphate, 1,3-dimesityl-1H-imidazol-3-ium chloride should be used throughout as well as anhydrous solvents.

The carbene catalyst (**20**) has been successfully used to label a range of drug molecules which might otherwise have proved difficult to radiolabel using the conventional Ir(I)-type catalysts, or indeed, by any other method. One such compound, the 4-cyanobiphenyl (**21**) was found to be unstable to standard hydrogenation conditions, suggesting that reductive dehalogenation, as an approach, would not be applicable. When an initial attempt was made at labelling the parent (**21**) directly using 0.5 equiv. of the carbene catalyst (**20**) and a partial pressure of deuterium gas, no incorporation of isotope was observed. However, by using a masked analogue of the cyano function, for example the primary carboxamide (**22**), to initially direct the isotopic labelling it was felt that this could then be converted to the required cyano group by reaction with Burgess reagent ((methoxycarbonylsulfamoyl)triethylammonium hydroxide inner salt). The tritiation was performed as described below:

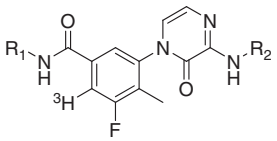
*The carboxamide (**22**, 2.5 mg) with 0.5 equiv. of carbene catalyst (**20**, 2.2 mg) in DCM (1 ml) was stirred under a partial pressure of tritium gas (65 mbar, 81 GBq) for 19 h. Following two cycles of lyophilization with ethanol, the crude tritiated carboxamide (**23**) was obtained with a specific activity of 494 GBq mmol^{-1} and a crude radiochemical purity of 40%.*

The labelled carboxamide was then dehydrated using Burgess's reagent to afford the desired nitrile (**21**) as shown below. Following HPLC purification, material was obtained with a specific activity of 456 GBq mmol^{-1} and a radiochemical purity of 97.5%.



The different selectivity and activity exhibited by the carbene catalyst (**20**) has now led to it being included as part of our routine exchange labelling screen. The benefits that can be obtained are illustrated using the following example of an amide precursor (**25**). Using a partial pressure of deuterium gas to reflect the experimental conditions that would be required when using tritium gas, a selection of catalysts were screened to assess which would give the highest incorporation of isotope; the three catalysts used were Crabtree's catalyst (0.6 equiv.), the polymer-supported catalyst (0.05 equiv.) and the carbene catalyst (0.2 equiv.). Very different results were obtained with Crabtree's catalyst giving 21% incorporation of deuterium and the polymer-supported catalyst giving 63% incorporation. However, when the results for the carbene catalyst were examined it was found that there was an additional peak at M +3 in the mass spectrum. This suggested that in addition to the expected exchange of isotope into the molecule, the catalyst was sufficiently active to reduce one of the double bonds present in the molecule. By reducing the carbene catalyst loading to just 0.06 equiv. it was possible to eliminate the reduction of the double bond and achieve a very useful 70% incorporation of deuterium. Subsequently the tritiation reaction was performed using the following procedure:

*Substrate (**25**, 2 mg) and the carbene catalyst (0.25 mg, 0.07 equiv.) were dissolved in dichloromethane (1 ml) and stirred under tritium gas (92.5 GBq) at a partial pressure of 75 mbar for 3 h at room temperature. Following two cycles of lyophilization with ethanol, a crude stock of tritiated product was obtained with a specific activity of 370 GBq mmol^{-1} and a radiochemical purity of 80%.*



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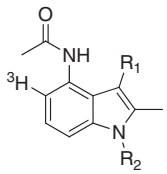

26

Using a similar approach, the exchange labelling of the indole acetamide (**26**) was also investigated. At a partial pressure of deuterium (50 mbar), very different results were obtained with each catalyst employed. The polymer-supported catalyst (2.7 equiv.) afforded 27% isotopic incorporation while the carbene catalyst (**20**, 0.1 equiv.) gave no isotopic incorporation. However, when the reaction was repeated with the commercial catalyst, $[(COD)_2.Ir.(PMePh_2)_2]PF_6$ (0.5 equiv.), a 66% incorporation of deuterium isotope was obtained. The tritiation was performed as described below:

*The indole acetamide (**26**, 1.3 mg) together with $[(COD)_2.Ir.(PMePh_2)_2]PF_6$ (1.8 mg, 0.6 equiv.) in dichloromethane (1 ml) were stirred at room temperature under tritium gas (72 GBq) at a partial pressure of 57 mbar for 16 h. Following lyophilization to remove any labile tritium, a stock solution of the tritiated indole acetamide (**26**) was obtained with a specific activity of 74 GBq mmol^{-1} and a crude radiochemical purity of 52%.*

Conclusions

The last 30 years have seen a huge expansion in the application of metal-catalysed isotopic exchange procedures employed in the synthesis of tritiated compounds. This growth has been driven by the recognition that having early and rapid access to good quality radiolabels can add significant value to the decision-making processes involved in drug discovery. It may, therefore, be surprising to find that these advances and the expanding range of catalytic tools that are now available to the isotopic chemist have been achieved by the efforts of a relatively small number of groups and individuals based predominantly in the pharmaceutical industry. However, despite all these efforts, there is still no single universal catalyst that can be called upon for all our isotopic labelling needs. Using the wealth of knowledge that we now have, it is possible to predict the type of catalyst and the experimental conditions that will give rise to useful isotopic incorporation in any particular substrate. These parameters can then be further optimized using a simple screening procedure to ensure maximum specific activity is achieved.

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