Journal of Labelled Compounds and Radiopharmaceuticals

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19th International Isotope Society (UK Group) Symposium: Synthesis & Applications of Labelled Compounds 2010

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The 19th annual symposium of the International Isotope Society's United Kingdom Group took place at the Wellcome Genome Campus, Hinxton, Cambridge, UK on Thursday 14th October 2010. The meeting was attended by around 80 delegates from academia and industry, the life sciences, chemical, radiochemical and scientific instrument suppliers.

Delegates were welcomed by Dr. Ken Lawrie (GlaxoSmithKline, UK, chair of the IIS UK group). The subsequent scientific programme consisted of oral and poster presentations on isotopic chemistry and applications of labelled compounds, or of chemistry with potential implications for isotopic synthesis. Both short-lived and long-lived isotopes were represented, as were stable isotopes. The symposium programme was divided into a morning and an afternoon session chaired by Prof. Chris Willis (University of Bristol, UK) and Dr. Nigel Botting (University of St Andrews, UK) respectively. In addition, a short presentation on the formation of Special Interest Groups within the International Isotope Society was given by Dr. George Ellames (Covance, UK), who asked members with particular isotopic interests to contact him (george.ellames@covance.com) if they would like to be involved in forming or participating in these groups. The UK meeting concluded with remarks from Dr. Ken Lawrie (GlaxoSmithKline, Stevenage, UK).

This year's UK symposium had an excellent level of sponsorship, and the symposium should prove self-financing. The location and facilities of the Hinxton campus again proved very popular and the next IIS UK symposium is provisionally planned for 18th October 2011 at the same venue.

SYNTHESIS & APPLICATIONS OF LABELLED COMPOUNDS 2010

19th International Isotope Society (UK Group) Symposium.
Thursday 14th October 2010, Wellcome Trust Genome Campus, Hinxton, UK.

9:00 am Registration / Coffee / Poster Viewing / Manufacturers Exhibition

9:45 am Welcome : Dr Ken Lawrie [GlaxoSmithKline, Stevenage, UK]

Morning Session: Chair:- Prof. Chris Willis [University of Bristol, UK]

9:50 am Prof. David Harrowven [University of Southampton, UK]
'How rare are boat configured arenes in nature?'

10:20 am Dr John Herbert [sanofi-aventis, UK]
'The synthesis of labelled 4-amino-4-arylpiperidines'

10:50 am Dr Matthew Tredwell [University of Oxford]
'PET ^{18}F-chemistry'

11:20 am Posters Viewing / Manufacturers Exhibition / Coffee & Tea

11:45 am Dr Matt Clarke [University of St Andrews. UK]
'Enantioselective carbonylation and other adventures in atom-efficient asymmetric catalysis.'

12:15 pm Dr Jon Bloom [Quotient-Amersham, Cardiff, UK]
'The labelling of some small molecules with carbon-14'

12:45 pm Posters Viewing / Manufacturers Exhibition / Buffet Lunch

Afternoon Session: Chair:- Dr Nigel Botting [University of St Andrews, UK]

2:00 pm Dr George Ellames
'International Isotope Society: Special interest groups'

2:05 pm Mr David Wilkinson [AstraZeneca Charnwood, UK]
'Isotopic reflections'

2:35 pm Dr Soren Schou [Leo Pharma, Denmark]
'Tuning up rhodium black'

3:00 pm Poster Viewing / Manufacturers Exhibits / Coffee & Tea

3:20 pm Prof. Nicholas Long [Imperial, UK]
'Copper(I) tris(pyrazolyl)borate complexes for use in positron emission tomography'

3:50 pm Dr Nick Bushby [AstraZeneca Alderley, UK]
'Efficient tritium labelling for discovery applications'

4:15 pm Mr Paul Allen [AstraZeneca, Charnwood, UK]
'Synthesis of deuterated isotopic labels using the H-Cube'

4:40 pm Concluding Remarks. Dr Ken Lawrie [GlaxoSmithKline, Stevenage, UK]

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ORAL PRESENTATION ABSTRACTS

HOW RARE ARE BOAT CONFIGURED ARENES IN NATURE?

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Since Kekulé first proposed his ouroboros-inspired structure for benzene in the mid-nineteenth century, it has been regarded as the archetypal aromatic compound – flat, with carbon-carbon bonds of equal length and bond angle.[1] The viability of non-planar benzene rings has been known for many decades, with the first reported example of a boat-configured arene dating to the synthesis of [2.2]paracyclophane in 1949.[2] However, it was arguably the isolation of (+)-cavicularin in 1996, and latterly of the haouamines and hirsutellones, that alerted the wider scientific community to the existence of biosynthetic pathways for the generation of such motifs in Nature (Figure 1).

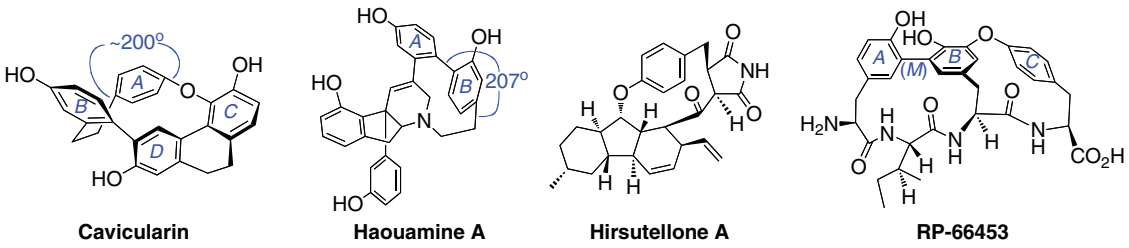

Figure 1. Notable examples of boat-configured arenes in Nature.

Through a combination of chemical synthesis (cavicularin,[3,4] riccardin C[3,4] and RP-66453[4]), molecular modelling and data mining, we have strong evidence to suggest that boat-configured arenes are far more common in natural products than has been traditionally thought, spanning many classes including macrocyclic alkaloids, guaianolides, bisbibenzyls, peptides and biarylheptanoids. In addition, our work provides guidance for addressing the chemical synthesis of such targets.

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THE SYNTHESIS OF LABELLED 4-AMINO-4-PHENYLPIPERIDINES

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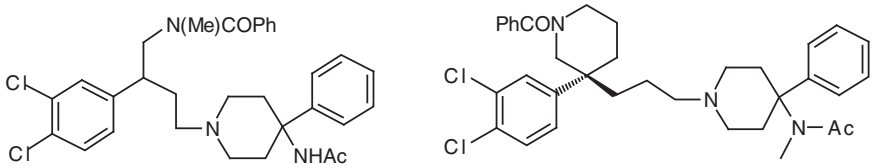
The 4-amino-4-arylpiperidine unit is a common motif in many CNS-active agents including the NK ligands saredutant and osanetant.[1] Over the years, we investigated a number of methods for introducing a single label into these and related development candidates, some of which are summarised in this presentation.



Methods for the introduction of a label into the piperidine ring include the Dieckmann condensation of a labelled precursor (Scheme 1), and the dialkylation of an arylacetic acid ester with a nitrogen mustard, as exemplified in Scheme 2. Both of these methods give intermediates that should be suitable for further functionalisation at C4.

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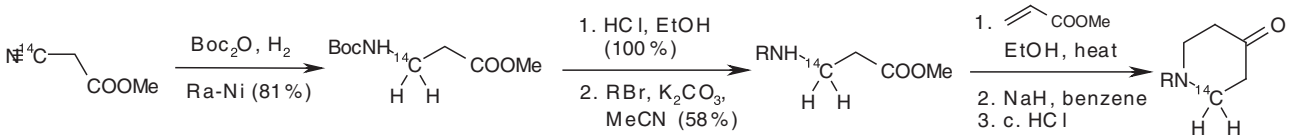
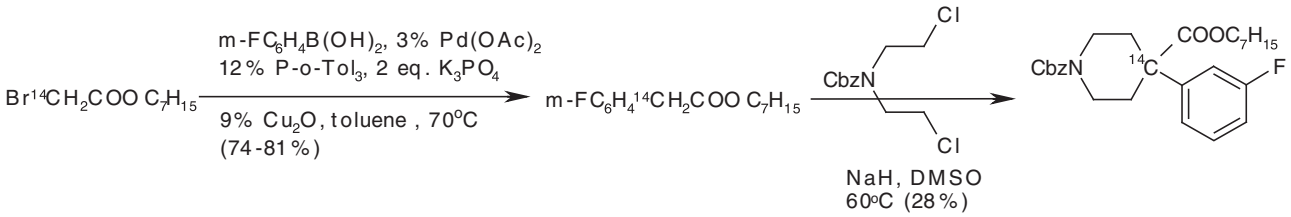
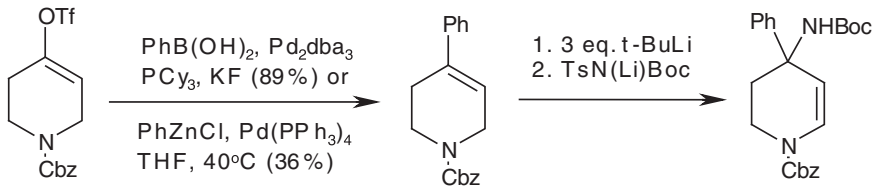


Scheme 1.



Scheme 2.



Scheme 3.

An alternative approach that we have developed involves the generation of a singly-labelled arylboronic acid,[2,3] which is coupled with a tetrahydropyridinyl triflate (Scheme 3).[4] The resulting 4-phenyltetrahydropyridine can be functionalised further at C4 by lithiation and reaction of the resulting ambident anion with an electrophile, as illustrated in Scheme 3.[5]

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PET ^{18}F-CHEMISTRY

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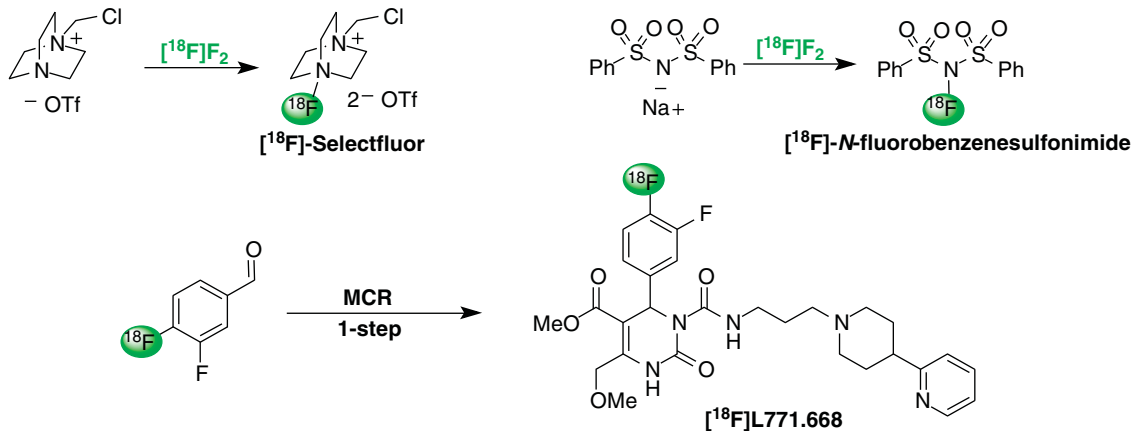
The artificial radioisotope fluorine-18 (^{18}F) is an ideal radionuclide for use in the synthesis of radiotracers due to the relatively long half-life (110 minutes). Introduction of ^{18}F into an organic molecule can be achieved via either electrophilic ($^{18}F^{+}$) or nucleophilic ($^{18}F^{-}$) reagents. Despite the clinical success of fluorinated radiotracers, such as [^{18}F]-FDG, there are relatively few methods for the introduction of ^{18}F in to organic molecules, limiting the scope of accessible ^{18}F-labelled tracers. Our goal is to increase the range of transformations and approaches available to radiochemists, facilitating the synthesis of new molecules.

In the field of electrophilic fluorination we have developed the electrophilic N-F fluorinating agents [^{18}F]-*N*-fluorobenzenesulfonimide and [^{18}F]-Selectfluor, two reagents commonly employed by organic chemists.[1,2] Also presented is the use of multicomponent reactions as a methodology to rapidly access complex [^{18}F]-aromatics.[3]



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ENANTIOSELECTIVE CARBONYLATION AND OTHER ADVENTURES IN ATOM-EFFICIENT ASYMMETRIC CATALYSIS

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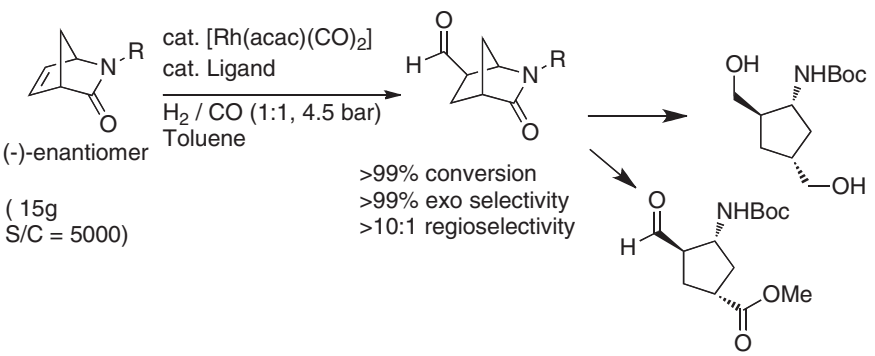
Hydroformylation and alkoxycarbonylation are now quite well established and efficient tools in the synthesis of commodity chemicals. However, these potentially very efficient reactions have been under-exploited in the synthesis of more complex value-added chemicals such as chiral intermediates. Part of the reason for this lies in a lack of catalysts that can simultaneously control chemo-, regio- and enantio-selectivity, although a significant number of improved catalysts have been developed recently. A further complication is the much lower reactivity that more interesting, functionalised substrates have in these reactions, which has led to carbonylations being neglected in organic synthesis until recently. This lecture will present some efficient methods to make chiral building blocks using metal catalysed carbonylations of functionalised alkenes.

Following on from our work building quaternary aldehydes and unusual β-amino acid derivatives using hydroformylation,[1] we have developed enantioselective reactions to convert various α, β-unsaturated amides[2] and allylic amines into useful chiral building blocks including the bicyclic lactam shown in the scheme below.[3] The products from these reactions represent building blocks for drugs and drug candidates.

Pd catalysed carbonylation of alkenes has been even less studied from the perspective of the organic synthetic chemist. Following on from work that produced some highly regioselective catalysts, and used deuterium labelling to rule out a recent mechanistic proposal,[4] we turned our attention to the elusive but highly desired asymmetric hydroxycarbonylation. A radically different catalyst design enables up to 95% e.e. to be realised in these reactions that will hopefully impact significantly on the production of chiral acids and esters in the future.[5]



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THE LABELLING OF SOME SMALL MOLECULES WITH CARBON-14

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The labelling of small molecules with carbon-14 is often very tricky due to the small mass of compound, difficult analysis and high reactivity. Considerable ingenuity is needed to efficiently prepare pure material. The preparation and use of the following small molecules will be discussed:

[^{14}C]Glyphosate, prepared on a large radiochemical scale *via* hydroxy[^{14}C]methylphosphonic acid diethyl ester.
[2,3-^{14}C]Fumaric acid, prepared *via* the dimerisation of ethyl diazo[2-^{14}C]acetate
[U-^{14}C]Glucoisosaccharinic acid, prepared *via* the alkaline degradation of radiolabelled leaf residues ([^{14}C]cellulose).
The use and removal of formic acid as a solvent modifier in the purification of dichloro[U-14C]acetic acid
The preparation of [carbonyl-^{14}C]crotonaldehyde, the conversion into 1-[(trimethylsilyl)oxy]-1,3-[1-^{14}C]butadiene and the preparation of a natural product.
The synthesis of [3-^{14}C]n-hexane
(E)-2-methyl[1-^{14}C]pent-2-enal, and its use in making of the insecticide [^{14}C]vaporthrin

'ISOTOPIC REFLECTIONS'

DAVID J WILKINSON

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The pharmaceutical industry has undergone significant change over the last 30 years and what survives today bears little resemblance to that which was around in the late 1970's. Similarly, the way we do drug discovery has also changed significantly moving from the classical biological testing of single compounds that have been designed and synthesised with a single purpose in mind to the use of parallel approaches such as combinatorial chemistry, high throughput screening and most recently fragment-based lead discovery to provide the leads that can then be refined to deliver new drugs. So it is perhaps surprising that despite the claims of many scientists within the industry that the use of radiolabelled compounds in drug discovery and development would one day be superseded by alternative technologies, they are still as important and indispensable a tool today as they were all those years ago.

This talk will reflect upon the advances in synthetic methodologies that have impacted upon isotopic synthesis, developments in technology and the introduction of new isotope-based applications that have all combined to ensure that isotopic synthesis has remained a critical component in the process that leads to the discovery of new chemical entities and their subsequent development to provide new drugs to market.

TUNING UP RHODIUM BLACK

SØREN SCHOU

Leo Pharma, Denmark

A new catalytic system based on rhodium black using Crabtree's catalyst as an additive for direct hydrogen isotope exchange in aromatic compounds has been investigated. The level of deuterium incorporation can be improved from 16% to 93%. The new catalyst mixture tolerates a variety of solvents. Other rhodium sources can be used, but the degree of crystallinity of the rhodium (metal, black or on support) plays an important role. Rhodium sources with a low degree of crystallinity had the highest catalytic activity.

COPPER(I) *TRIS*(PYRAZOLYL)BORATE COMPLEXES FOR USE IN POSITRON EMISSION TOMOGRAPHY

STEVEN KEALEY,[a,b] LUCY E. JENNINGS,[c] PHILIP W. MILLER,[c] ANTONY. D. GEE,[a,d] CHRISTOPHE PLISSON,[a] ANDREW J. P. WHITE,[c] STEPHEN HUSBANDS,[b] AND NICHOLAS J. LONG[c]

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[b]Department of Pharmacy and Pharmacology, University of Bath, UK
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[^{11}C]Carbon monoxide is a versatile radiolabelling reagent for the synthesis of ^{11}C-labelled tracer molecules for use in positron emission tomography (PET) medical imaging. However, the use of this reagent is hampered by its poor solubility in organic solvents and its delivery at high dilution in an inert gas stream, thus lowering its reactivity, as well as the practical difficulties encountered when working with ionising radiation. Copper(I) complexes supported by tris(pyrazolyl)borate ligands (also known as 'scorpionate' ligands, Figure 1) have proved to be an effective means of solubilising [^{11}C]CO gas through the formation of the corresponding copper(I)[^{11}C]carbonyl complexes, allowing [^{11}C]CO to be effectively 'trapped' in solution (Figure 2).[1,2] These solutions have been used as the [^{11}C]CO source for subsequent palladium-mediated carbonylation reactions to form ^{11}C-labelled amides and ureas. The coordination chemistry of a series of tris(pyrazolyl)borate ligands bearing appended donor groups (phosphine, phosphine oxide, pyridyl, thioether) tethered to the pyrazolyl rings has been explored and their reactivity with copper(I) and [^{12}C]CO and [^{11}C]CO examined.

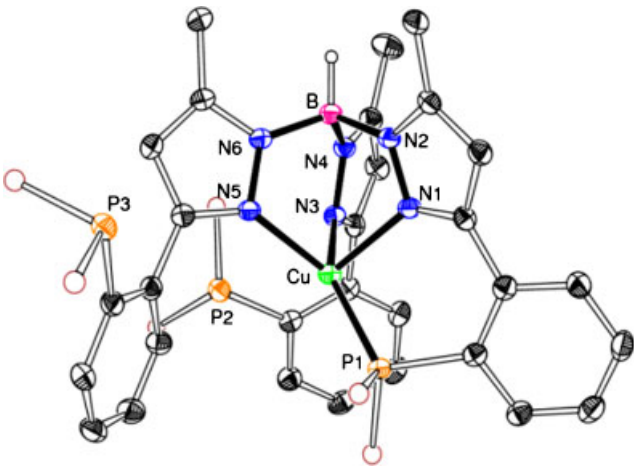

Figure 1. A copper(I) complex containing a new tris(pyrazolyl)borate with appended phosphine donor groups.

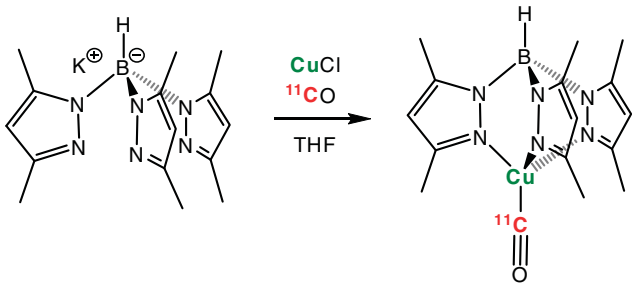

Figure 2. [^{11}C]CO trapping through formation of Cu[Tp*][^{11}C]CO complex.

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EFFICIENT TRITIUM LABELLING FOR DISCOVERY APPLICATIONS

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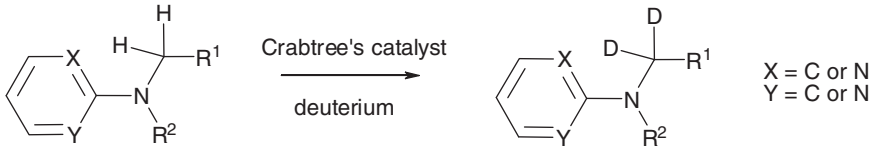
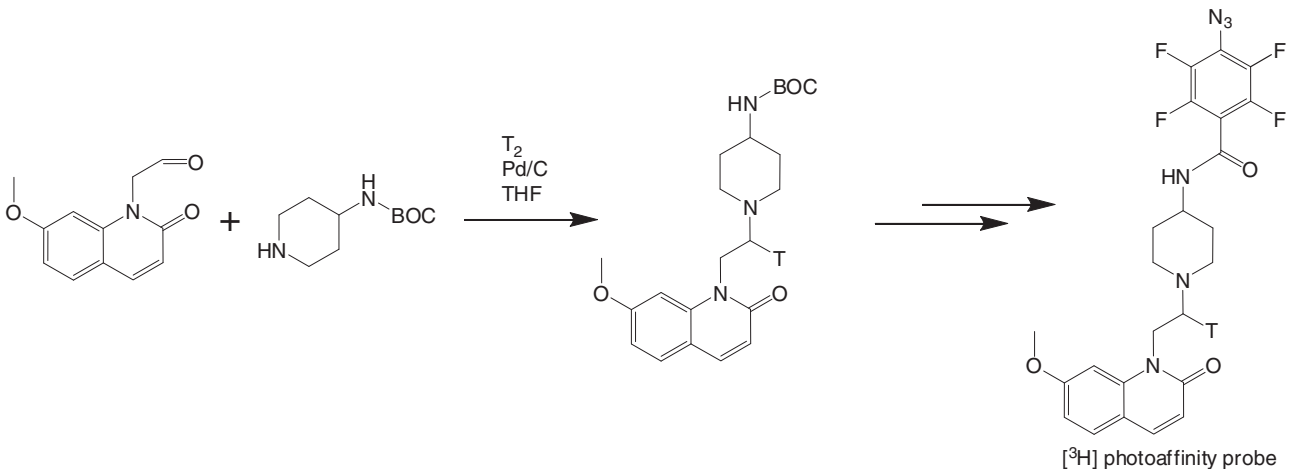
In order for tritium labelling to provide a quick and inexpensive method of radiolabelling for discovery and early development applications, tritium syntheses need to be short and use tritium gas as the tritium source.

Tritio-dehalogenation of brominated or iodinated precursors is often the preferred method for incorporation of tritium into target compounds due to the quick, clean reactions, high incorporation and good specificity of labelling. However, if a halogenated precursor is not available, considerable time and effort may be required to prepare it. Direct hydrogen isotope exchange using iridium(I) catalysts such as Crabtree's catalyst is an established method of introducing tritium labels in one step, however only a limited number of examples were found of direct hydrogen isotope exchange into aliphatic positions rather than aromatic.[1] Investigations into hydrogen isotope exchange at alkyl positions using both Crabtree's catalyst and a more recently developed iridium(I) catalyst[2] with model substrates are presented and its application to the labelling of some drug molecules.



The synthesis of tritiated photoaffinity probes present a particular challenge because they often contain easily reducible and highly sensitive functional groups. The synthesis of a tritiated photoaffinity probe is presented in which the tritium was introduced by reductive amination using tritium gas as the tritium source and reduction of the azide was avoided by altering the synthetic route.



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SYNTHESIS OF DEUTERATED ISOTOPIC LABELS USING THE H-CUBE

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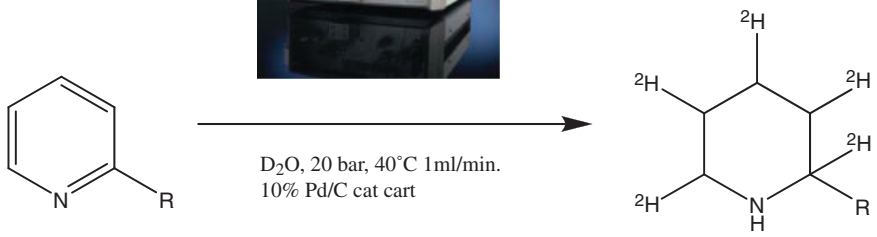
[a]AstraZeneca R&D Charnwood, Bakewell Rd, Loughborough, Leics. LE11 5RH, UK
[b]University of Bristol, School of Chemistry, Bristol BS8 1TH, UK

A series of investigational studies has been performed to assess the potential of the H-Cube for the synthesis of deuterated compounds for use as internal standards in the development of mass spectrometry based bioanalytical methods. The H-Cube is a

compact instrument capable of carrying out hydrogenations of suitable substrates at elevated temperatures and pressures without the need to have access to a gaseous source of hydrogen/deuterium. The hydrogen/deuterium gas required is generated via the electrolysis of water/deuterium oxide *in situ* followed by removal of any water from the gas stream. Final mixing with the substrate in a suitable eluant then occurs prior to pre-heating and passage of the sample through an appropriate catalyst cartridge. Examples will be presented of reductive halogenations as well as the reduction of substituted pyridines and pyrazines to afford the corresponding per-deuterated piperidines and piperazines. These products may then be further elaborated to afford material which is suitable for use as internal MS standards.



POSTER ABSTRACTS: SYNTHESIS OF TRIETHYL[^{2}H]SILANE AND ITS USE IN SELECTIVE DEUTERIODEIODINATION OF A IODINATED PEPTIDE

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There is an enormous and increasing market in the field of therapeutic peptides and proteins. In an era of 'new' synthetic polymeric formulations and bio-conjugated drugs,[1] the demand for tracer studies from the authorities is increasing, thus radiolabelling of these potential drugs are essential. For small peptides (up to approximately 30 AA) tritiodehalogenation is a feasible way of tritium labelling but drastic decreases in yield have been shown to occur when larger substrates are subjected to heterogeneous catalysts and supports.[2] Whether this is due to clotting or sticking of the substrates to the solid catalyst or to the inaccessibility of the labelling site on the peptide or protein by the catalyst is undetermined. There is therefore a need for new and efficient labelling methods for large peptides and proteins - thus we have investigated the use of homogenous catalysis for the tritiodeiodination of peptide substrates.

In this study we have found that synthesised triethyl[^{2}H]silane, as the hydride donor, in combination with a homogeneous Pd(0) catalyst did succeed in selective deuteriodeiodination of a iodinated model peptide (angiotensin-I, Figure 1).

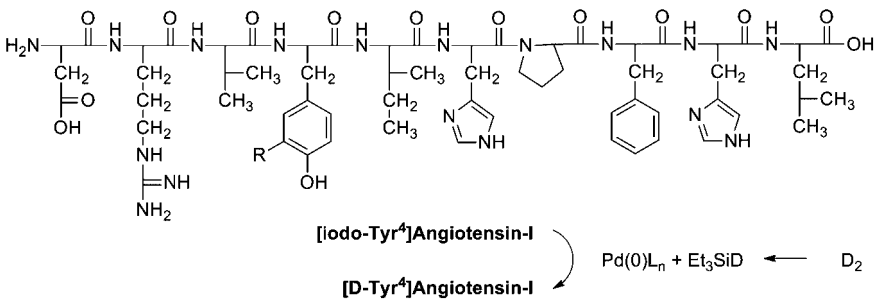

Figure 1. Deuterium labelling of [iodo-Tyr^4]Angiotensin-I with the use of synthesised triethyl[^{2}H]silane and palladium(0).

Synthesis of the triethyl[^{2}H]silane reagent was performed from deuterium gas using *n*BuLi and TMEDA as cosolvent for the formation of lithium deuteride. After thoroughly drying this intermediate, triethyl[^{2}H]silane was then synthesised by the addition of triethylchlorosilane in dry THF. Completion of the reaction was achieved after 30 minutes at room temperature, as monitored by gas chromatography using flame ionisation detection.

Optimisation studies involved screening of the palladium catalyst, phosphine ligands, reaction time and temperature. Eventually it was found that using the bulky tri-*tert.*-butylphosphine as the palladium ligand yielded total conversion of the model peptide after 3 hours of reaction at room temperature in an isolatable yield of 44% of the deuterated product after HPLC purification. The principle of using homogeneous catalysts for hydrogen labelling under these reductive conditions could potentially result in new tritium labelling techniques for peptides or substrates with sensitive functional groups.

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APPLICATION OF STATISTICS TO THE ANALYSIS OF MULTIPLY-LABELLED PRODUCTS FROM ISOTOPIC EXCHANGE REACTIONS

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For multiply-labelled compounds the isotopic cluster pattern of their MS ions holds much information relating to the nature of the molecular labelling sites. Some of this information can be accessed by using the appropriate statistical analysis.

To carry out the title analysis we can define a parameter, the isotope distribution function (IDF), which describes the distribution of the isotope across all the potential isotopomers present in the exchange-labelled compound. For the general case of the IDF we choose a molecule comprising site **1** (with **a** exchangeable positions), site **2** (with **b** exchangeable positions) and so on up to …… site **n** (with **m** exchangeable positions). In which case the IDF is defined by:

$$\mathrm{IDF} = (\mathrm{fH}_1 + \mathrm{fD}_1)^a \times (\mathrm{fH}_2 + \mathrm{fD}_2)^b \ldots \times (\mathrm{fH}_n + \mathrm{fD}_n)^m$$

Where the fractions of H and D at a particular site are denoted as fH_{site} and fD_{site}.

A computer program[1] has been developed to allow the calculation of the IDF for any statistically-labelled molecule, given information about the possible labelling site. This program may be used to compare the theoretical and experimental IDFs for labelled compounds originating from a range of isotopic exchange reactions provided that the appropriate isotope corrected mass spectra[2] are available. This comparison has now been carried out for the following reactions, with good agreement between statistical theory and experimental data in all the following cases: (a) base-catalysed deuteration of 1,3-diacetyl and 1,3,5-triacetyl benzene using deuterium oxide and triethylamine; (b) deuteration of benzophenone catalysed by a heterogenised[3] *ortho*-deuteration catalyst; (c) α-deuteration of 4,4′-bipyridyl[4] using D_2 gas and rhodium black; (d) *ortho*-deuteration of benzanilide using a range of homogeneous and heterogeneous catalysts[3,5,6] with D_2 or D_2O isotope donors; (e) deuterations of isophthalic acid and of benzamide using $CODIrF_6acac$ and a D_2O donor.[5]

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THE SYNTHESIS OF 2-(4-CHLOROPHENYL)[2-^{14}C]THIAZOL-4-YLACETIC ACID (^{14}C FENCLOZIC ACID) AND ITS ACYL GLUCURONIDE METABOLITE

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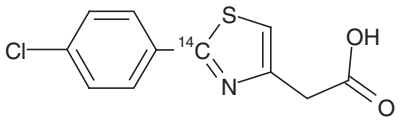
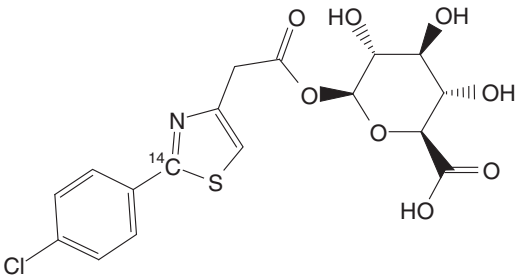
Fenclozic acid (ICI 54,450) was one of a series of substituted phenylthiazolyl acetic acids synthesised at Alderley Park in the 1960's for evaluation in the non-steroidal treatment of rheumatoid arthritis.



Fenclozic acid



Fenclozic acid glucuronide

Early preclinical work showed the compound to be clean in animal toxicology studies but after unexpected adverse effects were recorded in man, development was stopped.[1]

As part of AstraZeneca's strategy to address reactive metabolites, recent academic collaborations have aimed to examine whether the risks associated with fenclozic acid and its acyl glucuronide metabolite would have been highlighted with current screening procedures.

To support this work, high purity ^{14}C material was required, which afforded the opportunity to review the original labelled synthesis of fenclozic acid[2] (from the 1960's) and apply modern synthetic methodology including a palladium coupling approach and microwave assisted heating, to successfully deliver both the radiolabelled parent compound and its glucuronide metabolite.

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SYNTHESIS-TO-CLINIC: CLINICAL APPLICATIONS OF ^{14}C-API WITHIN QUOTIENT BIORESEARCH

IAIN SHAW

Director ^{14}C Enabled Drug Development, Quotient Bioresearch Clinical Sciences

The recent FDA MIST and ICH M3 R2 guidelines have given a new focus to the role of ^{14}C investigations in the overall drug development process. This paper will describe the clinical applications available within ^{14}C-enabled drug development and explain the quality standard required to enable use of the API material in GMP IMP manufacture within Quotient Bioresearch. Finally, by describing how to integrate the complementary services involved in delivering ^{14}C clinical studies from synthesis to clinic we describe how to maximise the value obtained from each ^{14}C-radiosynthesis as opposed to performing multiple synthesis campaigns at different stages of the drug development process.

HYDROLYSIS OF [^{14}C]NITRILE USING NITRILASE (NIT) BIOCATALYSTS

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Biocatalysis[1] in the pharmaceutical and fine chemical industries continues to grow as it moves from the domain of specialised technology to mainstream methodology, a move that has been facilitated by the increasing commercial 'off-the-shelf' availability of biocatalysts at large scale. For example, nitrilase (NIT) biocatalysts have found numerous applications in the transformation of nitrile groups into carboxylic acids. These nitrile manipulating biocatalysts have been applied to the hydrolysis of [^{14}C]-nitrile moieties to preserve sensitive groups within the target compound.[2]

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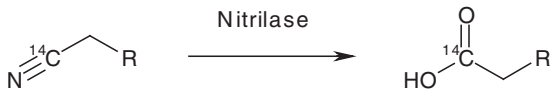
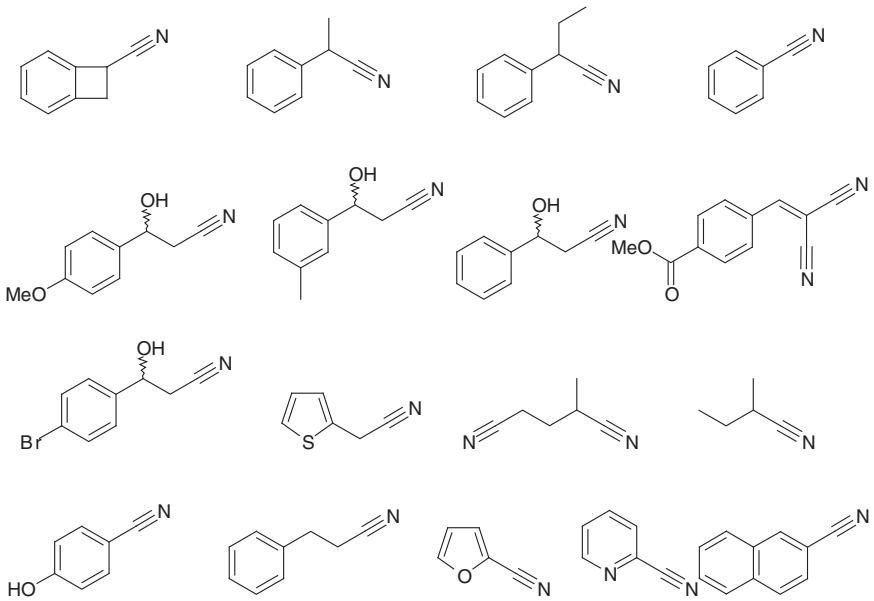


Chirality: If the substrate is a racemic compound, then like many other enzymes, NITs can offer resolution conditions.
Mild: Given the harsh conditions of acid, base or high temperatures that are often required to perform nitrile hydrolysis, a major benefit of using NITs is their ability to perform nitrile hydrolysis under neutral conditions and ambient temperatures.
Selectivity: NITs are often stereo- and regioselective.
Purification: NITs can be used to selectively modify impurities, thereby changing physical properties, and hence aiding in their removal.[3]



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SYNTHESIS OF [METHYL-^{14}C]COMBRESTATIN A1 DIPHOSPHATE

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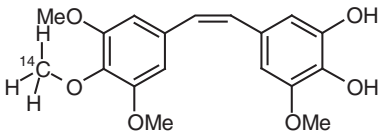
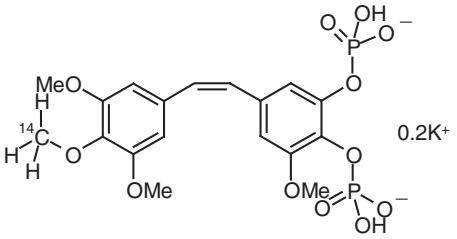
The natural product combretastatin A-1 (CA1) is isolated from the African bush willow tree, a member of the *Combretaceae* family. CA1 has important medicinal value, due, in part, to its ability to inhibit tubulin assembly. The prodrug combretastatin A-1 diphosphate (CA1P; OXi4503) is currently in human Phase I clinical trials as a vascular disrupting agent (VDA). This poster describes the carbon-14 radiosynthesis of [4′-^{14}C]CA1 and the corresponding phosphate prodrug salt [4′-^{14}C]CA1P in high specific activity (55 mCi/mmol).



[methyl-^{14}C]Combretastatin A1



[methyl-^{14}C]Combretastatin A1 diphosphate

The carbon-14 label was introduced by methylation of the C-4′ protected phenolic moiety of the CA1 precursor following removal of the *tert*-butyldimethylsilyl protecting group in the presence of [^{14}C]methyl iodide. This was accomplished in excellent yield without significant *Z* to *E* isomerization. The ^{14}C-precursor ((*Z*)-1-[3′,[4′-^{14}C],5′-trimethoxyphenyl]-2-[2″,3″-di-[(*iso*propyl)oxy]-4″-methoxyphenyl]ethene) was subjected to a de-*iso*propylation reaction with $TiCl_4$. The tetrabenzyl phosphate derivative of the resulting diol was prepared using fresh dibenzyl phosphite. Debenzlyation with trimethylsilylbromide, followed by hydrolysis of the trimethylsilyl ester and adjustment of the pH with dilute aqueous hydrochloric acid yielded [4′-^{14}C]CA1P with an overall radiochemical yield of 8.4%.

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