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Abstracts of the 20th International Isotope Society (UK group) Symposium: Synthesis & Applications of Labelled Compounds 2011

F. Aigbirhio, M. V. C. L. Appleyard, R. L. Arrowsmith, S. A. Baldwin, M. Bayrakdarian, N. P. Botting, L.-D. Cantin, D. R. Carbery, M. A. Carroll, L. I. Dixon, P. N. Dorff, G. Ellames, C. S. Elmore, C. W. G. Fishwick, O. Foot, N. J. Geach, J. Gowdy, R. S. Grainger, T. Gregson, W. R. R. Harker, P. J. F. Henderson, J. R. Heys, S. W. Homans, Z. Hu, S. Jackson, J. Johnston, P. Johnson, A. Kalverda, C. Kay, S. L. Kitson, B. Lanoue, M. H. Levitt, Y. Li, W. J. S. Lockley,* X Luo, P. Ma, D. A. Middleton, J. Newsome, B. Pandya, S. I. Pascu, S. G. Patching, M. K. Phillips-Jones, M. E. Powell, P. Riss, J. Simmons, T. M. Simpson, A. D. Smith, A. M. Thompson, L. Trembleau, R. Turtle, K. W. Watters, and Q. Zhang

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Meeting Summary

The 20th annual symposium of the International Isotope Society's United Kingdom Group took place at the Wellcome Genome Campus, Hinxton, Cambridge, UK on Tuesday 18 October 2011. The meeting was attended by around 70 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 session chaired by Prof. Chris Willis (University of Bristol, UK) and afternoon sessions chaired by Mr Mike Chappelle (Quotient Biosciences, UK) and by Dr Nick Bushby (AstraZeneca, UK). The UK meeting concluded with remarks from Dr Ken Lawrie (GlaxoSmithKline, Stevenage, UK).

Synthesis & Applications of Labelled Compounds 2011

20th International Isotope Society (UK Group) Symposium.
Tuesday 18 October 2011 Hinxton Hall, Wellcome Trust Genome Campus, UK.

9:00 am	**Registration/Morning Coffee/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	**Dr Karl Cable [GlaxoSmithKline, Stevenage, UK]**
	"*Nigel Botting. In memoriam*"
10:00 am	**Dr Richard Grainger [University of Birmingham, UK]**
	"*Dithiocarbamate-mediated synthesis of nitrogen heterocycles and beyond*"
10:30 am	**Dr Oliver Foot [Quotient, UK]**.
	"*Two examples of the Diels-Alder reaction in ^{14}C-radiosynthesis*"
11:00 am	**Manufacturers Exhibition/Coffee & Tea/Poster viewing**.
11:30 am	**Dr Sofia Pascu [University of Bath, UK]**

(*Continues*)

	"*Studies towards the ^{68}Ga, ^{64}Cu, ^{89}Zr and ^{111}In radiolabelling of small molecules and nanoparticles – multimodality PET or SPECT and optical imaging devices*"
12:00 pm	**Dr Bhaumik Pandya [CoNCERT Pharmaceuticals, Lexington, USA]**
	"*Old drugs yield new discoveries: case studies of site-selective deuterium incorporation*"
12:30 pm	**Manufacturers Exhibition/Buffet Lunch/Poster viewing**
Afternoon Session I:	Chair:- Mr Mike Chappelle, [Quotient, UK]
1:30 pm	**Dr Ken Lawrie [GlaxoSmithKline, Stevenage, UK]**
	"*Presentation on the forthcoming Heidelberg IIS Symposium 2012*"
1:40 pm	**Dr Dave Carbery [University of Bath, UK]**
	"*Carbon labelling strategies to unravel sigmatropy in β amino acid synthesis*"
2:10 pm	**Dr Jeff Newsome [Selcia, UK]**
	"*A review of the synthesis of fluorine substituted [phenyl-U-^{14}C]aromatic compounds*"
2:40 pm	**Dr Mike Carroll [University of Newcastle upon Tyne, UK]**
	"*Introduction of carbon isotopes into organic frameworks using alkynyliodonium salts*"
3:10 pm	**Manufacturers Exhibition/Coffee & Tea/Poster viewing**
Afternoon Session 2:	Chair:- Dr Nick Bushby [AstraZeneca, UK]
3:30 pm	**Dr Qingzhi Zhang [University of St Andrews, UK]**
	"*Synthesis of [6,8,10,3′,5′-^{13} C_5]cyanidin-3-glucoside, for human in vivo metabolism studies*"
4:00 pm	**Dr Tim Smith [University of Aberdeen, UK]**
	"*Biotin labelling with 1$^{[18}$F]FDG, [^{18}F]fluoride and dendrimers for pretargeted imaging*"
4:30 pm	**Dr Chad Elmore [AstraZeneca]**
	"*Small C-14 building blocks for use in Pd-catalysed coupling reactions*"
5:00 pm	**Concluding Remarks**. Dr Ken Lawrie [GlaxoSmithKline, Stevenage, UK]

Oral Presentation Abstracts

Dithiocarbamate-mediated synthesis of nitrogen heterocycles and beyond

Richard S. Grainger

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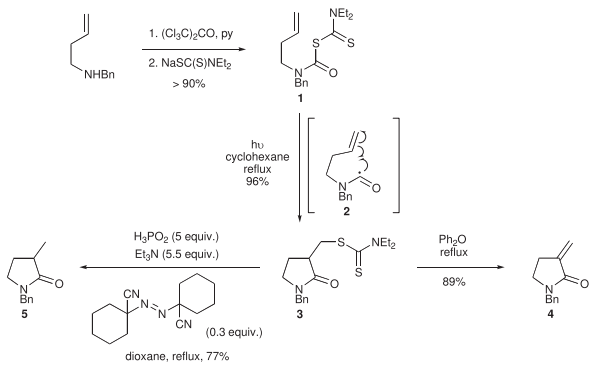
We have reported a methodology for generating carbamoyl (aminoacyl) radicals **2**, based on the use of readily prepared carbamoyl dithiocarbamates such as **1**.[1] Initiation of a radical chain process by the action of a simple halogen lamp or convenient and safe peroxide (lauroyl peroxide) gives lactam **3** in high yield in an operationally simple procedure, which requires neither high dilution nor syringe-pump techniques. The overall sequence is related to studies by Zard using xanthate precursors to generate acyl and carbonyl radicals,[2] but differs in the radical source. Crucial to success in the case of carbamoyl radicals is the use of a dithiocarbamate moiety instead of a xanthate. Although we have found both xanthates and dithiocarbamates undergo the group transfer radical process, a xanthate radical precursor corresponding to **1** can only be obtained in low yield.



A range of lactams of various ring sizes (4–8) can be prepared using this chemistry. Further synthetic utility is gained through manipulation of the dithiocarbamate group in the product. Thermal elimination of the dithiocarbamate generates alkenes such as **4**,[3] whereas desulfurization to **5**. can be achieved through a radical-mediated reduction under tin-free conditions.[4] Application of this methodology in target synthesis,[3,5] and opportunities for isotope incorporation, will also be presented.

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Two examples of the Diels-Alder reaction in ^{14}C radiosynthesis

Oliver Foot[a], Robert Turtle[b] and James Johnston[a]

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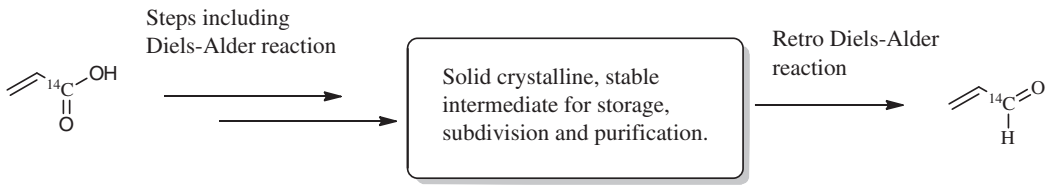
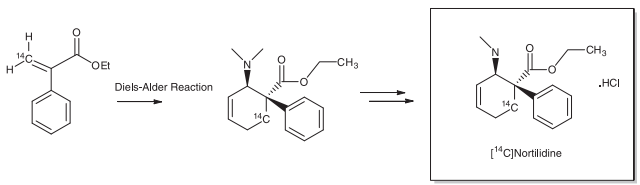
Here, we demonstrate how [^{14}C]acrolein can be prepared in small samples for synthesis or other studies on demand from a stable intermediate that can be readily stored, subdivided and purified.



[^{14}C]Nortilidine was required by our client and is made inactively in industry by use of the Diels-Alder reaction to form the cyclohexene ring. The radiosynthesis by the same route proved challenging. Our chemists modified the Diels-Alder step to deliver the target, [^{14}C]nortilidine.



Studies towards the ^{68}Ga, ^{64}Cu, ^{89}Zr and ^{111}In radiolabelling of small molecules and nanoparticles—multimodality PET or SPECT and optical imaging devices

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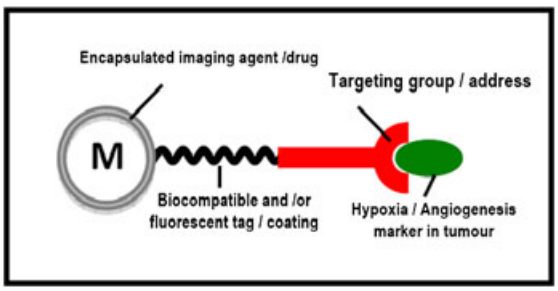
Our recent research interests are towards the development of new multimodal hypoxia imaging agents equipped with fluorescent tags (Figure 1). For this, we have been using flat aromatic tags, which may act as fluorophors (naphthyldiimides), and as near IR fluorescent emitters (naphthyldiimides and perylenes) to act as synthetic scaffolds, which will eventually enable whole body *in vivo* imaging or blood stability measurements. We have been seeking to develop entirely new dual mode ^{68}Ga-based, ^{64}Cu-based, ^{89}Zr-based and ^{111}In-based hypoxia imaging agents with extremely high kinetic stability in healthy and cancerous cells (MCF-7, HeLa, PC3). With the availability of Ge/Ga generators and rapid labelling kinetics, such Ga-labelled agents would be viable replacements for F-MISO for PET imaging. A further new concept is the development of potentially trimodal imaging agents (PET/MRI/optical) using filled carbon nanotubes and silica-encased iron particles functionalised with fluorophores including near IR emitters, $CdZnSe_2$ quantum dots and hypoxia-targeting vectors. An efficient route towards the radiolabelling of single-walled carbon nanotubes with ^{64}Cu and ^{89}Zr in aqueous conditions is also described. This route builds on our strategy developed previously, with high yield encapsulation of metallic species in the hydrophobic cavity of the nanotube being discussed.

Studies into the biological effects of all metalloprobes using MTT assays and confocal fluorescence imaging will aid our understanding of their properties and thus help the achievement of added bioimaging benefits. Standard radioanalytical techniques have been used to detect

the radiolabelling yield. New synthetic routes to radiolabelled *bis*(thiosemicarbazones) and nanoparticle-anchored *bis*(thiosemicarbazones) are therefore required, and our recent progress in their synthetic methodology involved is described.



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Old drugs yield new discoveries: case studies of site-selective deuterium incorporation

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Site-selective deuterium incorporation of physiologically active compounds has the unique effect of preserving the biochemical receptor potency and selectivity while, in select cases, modifying the metabolic fate to substantially alter the therapeutic profile of the agent. Deuterium incorporation has previously been utilized as a tool to probe metabolic or pharmacokinetic hypotheses in both pre-clinical and clinical settings. Although the impact of deuterium substitution to alter metabolic rates and pathways has been well documented, its application in drug discovery has only recently been implemented. The observation of a deuterium isotope effect in complex biological systems has been unpredictable and lends to non-obvious nature of the discoveries made during lead optimization. This presentation will highlight several examples in which site selective deuteration has been applied to clinically validated compounds, potentially providing a streamlined approach to important new drugs.

Carbon labelling strategies to unravel sigmatropy in β-amino acid synthesis

Wesley R. R. Harker and David R. Carbery[a]
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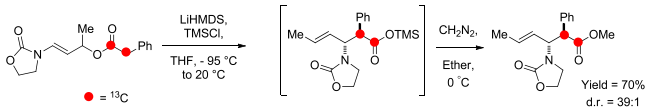
β-Amino acids represent an important family of compounds, naturally embedded within molecules of higher complexity within all five kingdoms of living organisms (animals, plants, fungi, bacteria and protista). In the field of proteomics, the inclusion of β-amino acids can have profound effects from modifying hydrolytic stability of peptides to the control of peptide conformation. Accordingly, novel stereocontrolled approaches to β-amino acid synthesis are of continued pertinence.

We have recently reported an Ireland-Claisen rearrangement approach to $\beta^{2,3}$-amino acid derivatives.[1] This entailed the rearrangement of novel enamide substrates, with particularly high levels of stereocontrol seen with a phenylacetate. This talk will discuss the work completed to expand our initial observations and, in particular, the ^{13}C labelling of rearrangement substrates in order to explore intriguing remote electronic stereocontrolling effects.



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A review of the synthesis of fluorine substituted [phenyl-U-^{14}C]aromatic compounds

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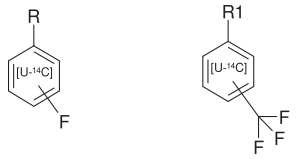
The use of fluorine substituted aromatic compounds in both pharmaceuticals and agrochemicals is widespread with at least 220 fluorinated drugs on the market by 1990.[1] Over the years, Selcia has been asked to prepare ^{14}C-labelled fluorinated compounds for DMPK and registration studies. These have included compounds containing a uniformly labelled benzene ring substituted with one or more fluorine atoms (F) or a trifluoromethyl group (CF_3). There are a number of techniques available to the organic chemist to introduce F or CF_3. These include halogen or nitro group exchange,[2] palladium catalysed coupling,[3] diazotisation[4] and electrophilic substitution.



The purpose of our presentation is to review the synthesis of fluorine substituted [phenyl-U-^{14}C]aromatic compounds. We will include examples from the literature and also a number of compounds that Selcia has prepared.

Fluorine is also useful as a functional group in aromatic chemistry being both an ortho-lithiation directing group[5] and a good leaving group for a number of aromatic nucleophilic substitution reactions.

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Introduction of carbon isotopes into organic frameworks using alkynyliodonium salts

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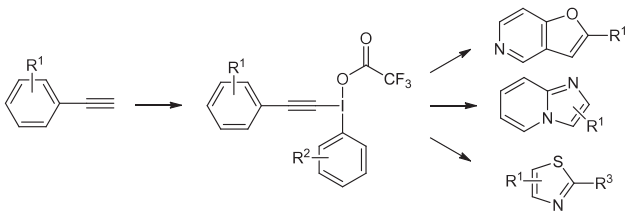
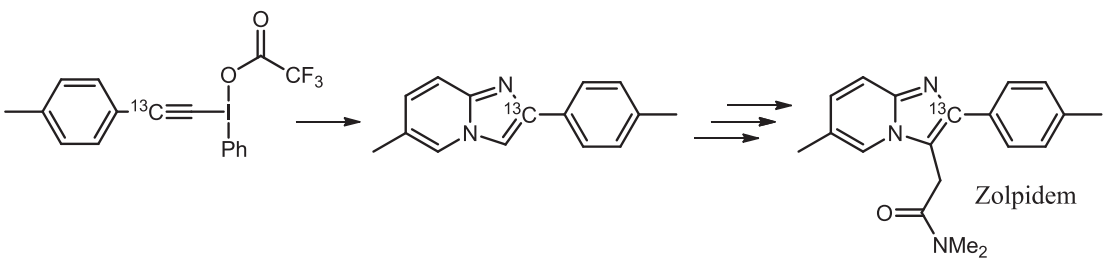
Iodine, like the other halogens, is found typically in its monovalent form (oxidation state: −1). However, because of its large size and polarisability, it is able to form stable polycoordinate, multivalent compounds. Compounds of this type, containing hypervalent iodine, have been known for over a century and continue to receive considerable attention because of their ability to act as both selective reagents and useful intermediates.[1]



Alkynyliodonium salts are highly versatile reactive species, which can be used to create a vast array of aromatic targets; however, their syntheses often require hard to prepare or unstable starting materials and produce products, which are difficult to purify and often unstable.[2] To address this limitation, we have developed a fast and efficient method for the synthesis of a range alkynyliodonium trifluoroacetate salts from commercially available and inexpensive starting materials and demonstrated their application in the preparation of a range of heteroaromatic systems[3] (Scheme 1).



The first generic route to alkynyliodonium trifluoroacetates and their application to the synthesis of a range of functionalised heteroaromatic systems are presented. We have also demonstrated the application of this novel methodology through the synthesis of the carbon-13 labelled imidazo[1,2-*a*]pyridine based drug, Zolpidem (Scheme 2).

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Small C-14 building blocks for use in Pd catalysed coupling reactions

Charles S. Elmore[1], Peter N. Dorff[2], Mark E. Powell[2], J. Richard Heys[2], Yan Li[2], Bernard Lanoue[2], Xuehong Luo[3], Malken Bayrakdarian[3] and Louis-David Cantin[3]

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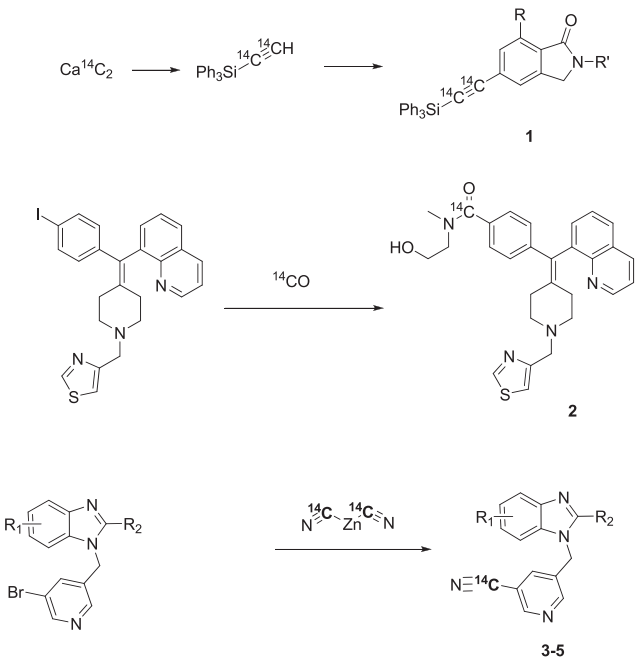
The use of Pd-catalyzed reactions in drug discovery especially Suzuki and Sonogashira Coupling reactions is expansive. A recent investigation[1] found that almost 60% of C–C bond forming reactions used by medicinal chemists were one of these two reactions. This can be a challenge for the radiochemist as the incorporation of C-14 into an arylboronic acid or alkyne is often not trivial.

Recently, significant progress in the development of reagents and reactions has made the Sonogashira reaction, carbonylation, and aryl cyanation more accessible for C-14 reactions. Compound **1** was required by DMPK labeled with C-14. The isoindolinone portion of the molecule was a long, low-yielding synthesis. After forming the bromoisoindolone, Medicinal Chemistry performed a Sonogashira reaction to give the arylalkyne. However, there were few reports of using labeled compounds in the Sonogashira reaction and even fewer reporting the preparation of a C-14 labeled alkyne substrate. A route to triphenylsilyl[^{14}C]acetylene was developed, and it was used to prepare an intermediate for a drug discovery project.[2]

Compound **2** was required labeled with C-14 for metabolism studies by DMPK. Using now standard C-14 carbonylation methodology, we prepared the compound in one radioactive step. The synthesis of several other compounds, which was prepared by a similar synthetic route, will also be presented.[3]

Compounds **3**, **4**, and **5** were required labeled with C-14 to determine their propensity to form reactive metabolites. This was accomplished in a single radioactive step; Pd-catalyzed zinc cyanation of an aryl bromide gave compounds **3**, **4**, and **5** high yield and purity in less than 3 weeks.



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Biotin labelling with [^{18}F]FDG, [^{18}F]fluoride and dendrons for pre-targeted imaging

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[^{18}F]Fluorine is an ideal nuclide for positron emission tomography (PET) imaging, as 97% of its radioactive emission is by β+ decay; its β+ energy is only 0.63 MeV so the positron range in tissue is short. High tumour-to-background ratios are essential for good image quality and are achieved by allowing blood clearance of non-target bound tracer from the blood prior to imaging. PET imaging using tracers directly labelled with short-lived tracers including [^{18}F]-fluorine ($t_{1/2}$ = 110 min) is restricted to molecules of <40,000 g molecular weight (renal filtration limit) so it excludes whole antibodies.

Pre-targeted imaging involves the administration of avidin–antibody conjugate, followed some hours or days later by radio-labelled biotin. PAMAM dendrons (half-dendrimers), tree-like structures with NH_2-terminal branches, have lipophilic cores that can carry hydrophobic drugs such as doxorubicin. Attachment of a radiolabel facilitates *in vivo* detection of their biodistribution.

We have developed three biotin precursors for [^{18}F] labelling: oxy-amine-biotin conjugate. which was labelled using [^{18}F]FDG as a prosthetic group;[1] a conjugate of biotin and boroaryl, which was [^{18}F]labelled in aqueous solution;[2] a conjugate of biotin with 4, 8 and 16 branched dendrons functionalised with trifluoroboroaryl,[3] which were labelled using isotopic exchange.

Each [^{18}F]-labelled biotin interacted with avidin. [^{18}F] binding to HER2-expressing tumour cells pre-incubated with the anti-HER2 antibody, trastuzumab, conjugated to avidin, followed by incubation with [^{18}F]-biotin, was demonstrated.

Acknowledgements: This work was funded by BBSRC, Friends of Anchor and The Breast Cancer Campaign.

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Synthesis of [6,8,10,3′,5′-$^{13}C_5$]-cyanidin-3-glucoside, for human *in vivo* metabolism studies

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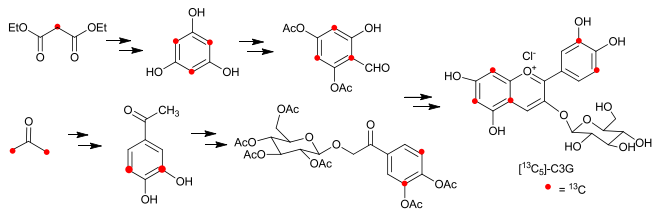
[†] Nigel P. Botting Died 4th June 2011

Anthocyanins are naturally occurring polyphenols belonging to the flavonoid family of natural products. These pigments are responsible for the rich colours from red, purple to blue displayed in various flowers, seeds, leaves, fruits and vegetables. Epidemiological evidence suggests that those who consume the highest proportions of anthocyanins in the diet are at the lowest risk of cardiovascular diseases such as hypertension and stroke.[1] However, because of their low stability at physiological pH and high propensity to bind to other molecules, their absorption, distribution, metabolism and elimination in humans has yet to be fully established. This calls for gram scale synthesis of multi-isotopically labelled anthocyanins for human feeding studies to identify the metabolites of anthocyanins' degradation products *in vivo*.

Herein, we synthesised 4.3 g of [6,8,10,3′,5′-$^{13}C_5$]cyanidin-3-glucoside (C3G), the multi-^{13}C-labelled counterpart of the most commonly occurring anthocyanin C3G in fruits, by adopting Robinson's method,[2] which allows us to incorporate several ^{13}C atoms in both building blocks, phenolic aldehyde[3] and glycosylated aryl ketone,[4] from commercially available simple molecules [2-^{13}C] diethyl malonate and [1,3-$^{13}C_2$]acetone, respectively. The distribution of ^{13}C-atoms in both A- and B-rings means that both moieties can be easily tracked during the analysis of metabolites; 4 g of [$^{13}C_5$]C3G has been capsuled and used in a human trial, and the remaining product can be used as an internal standard for LCMS analysis.



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Poster Abstracts

Isotope chemistry for investigating ligand binding and transport by Mhp1 and its homologues

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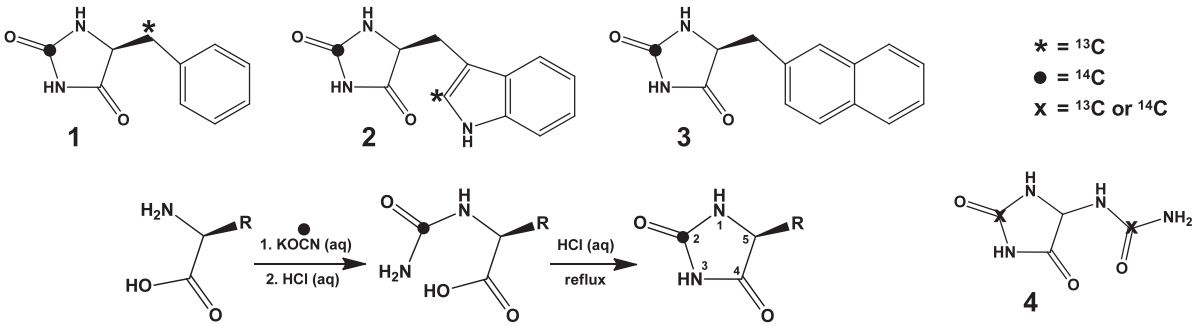
We demonstrate the importance of isotope chemistry for assisting our structure-function investigation of hydantoin transporters from pathogenic bacteria that required a number of ^{13}C-labelled and ^{14}C-labelled 5-substituted hydantoins for solid-state NMR experiments and for transport assays, respectively.

The Mhp1 protein from *Microbacterium liquefaciens*,[1] for which we have a number of X-ray crystal structures,[2,3] had been shown to transport L-5-benzylhydantoin (L-BH) **1** and L-5-indolylmethylhydantoin (L-IMH) **2**.[1] Efficient methods were developed for synthesising separate ^{13}C-labelled and ^{14}C-labelled versions of the Mhp1 substrates L-BH and L-IMH by cyclising the amino acids L-phenylalanine and L-tryptophan, respectively, with potassium cyanate.[4] A transport assay for Mhp1 developed with the [^{14}C]-L-IMH was used to screen 49 unlabelled competing hydantoin analogues and other compounds for their ability to bind to Mhp1. The highest affinity compound tested was L-5-(2-naphthylmethyl)hydantoin (L-NMH) **3**, which was then synthesised ^{14}C labelled, and its transport properties were investigated. This information is being combined with new crystal structures of Mhp1 with bound ligands and with docking models and kinetics experiments to understand the substrate specificity and mechanism of ligand binding and transport by the protein.

A homologous protein, PucI from *Bacillus subtilis*, that we have also cloned and expressed in *Escherichia coli*, was predicted to transport 5-ureidohydantoin (also known as allantoin) **4** based on phylogenetic analysis. A ^{14}C-labelled version of allantoin was synthesised from parabanic acid by using a route that introduced the ^{14}C label at the final step using [^{14}C]urea.[5] The synthesis was also performed using [^{13}C]urea to confirm the purity of the product and integrity of the label; ^{13}C NMR revealed a partial scrambling of the label from the ureido carbonyl group to C-2 by a re-arrangement of allantoin that goes via a symmetrical hydroxyglycouril intermediate. Additionally, this work made the first full characterisation of 5-hydroxy-5-methoxyhydantoin, obtained as a side product through reaction of 5-hydroxyhydantoin with the methanol solvent. The ^{14}C-labelled allantoin was used in an assay with PucI that confirmed the protein as an allantoin transporter and allowed an investigation of its ligand binding specificity.



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NMR methods for structure-function investigation of membrane proteins and their ligands

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Membrane proteins represent up to 30% of the proteins in all organisms; they are involved in many biological processes and are the molecular targets for around 50% of validated drugs. Despite this, membrane proteins represent less than 1% of all high-resolution protein structures because of various challenges associated with applying the main biophysical techniques used for protein structure determination.[1]

We are developing and applying novel solution-state and solid-state NMR experiments, labelling and sample preparation strategies for structure-function investigation of membrane proteins and their ligands, all of which require stable isotope-labelled compounds (labelled ligands, labelled proteins and other labelled sample components).[2] Our aim was to contribute new knowledge about the molecular mechanisms of membrane proteins and their roles in human disease and to provide information that will assist drug design.[3] We have principally been using bacterial transport and receptor proteins, which are targets for developing new antibacterials and/or are homologous with human membrane proteins.

Some of our recent and current work presented here includes the following:

(i) Achievement of high-resolution ^{1}H-^{15}N-TROSY and methyl-TROSY solution-state NMR spectra of the 12-helix (51 kDa) sugar transporter GalP selectively labelled at Trp residues and at methyl groups in Ile/Leu/Val residues, respectively, and reconstituted in deuterated detergent micelles.

(ii) Solid-state NMR methods for measuring torsion angles[4] and multiple distances to elucidate the structures/conformations of uniformly-labelled ligands developed with the nucleoside transporter substrate [U-^{13}C, U-^{15}N]uridine.

(iii) Selective ^{13}C,^{15}N-labelling of Trp residues in the sugar transporter GalP and use of solid-state NMR to detect a selective interaction between C-1 in the α-anomer of the bound substrate D-[U-^{13}C]glucose and C-1 in Trp residues in the binding site of the protein in native membranes.[5]

(iv) Development of sample conditions using ^{2}H,^{13}C-labelled ligands and with the sample in D_2O for structural measurements on ligands and their binding sites with membrane proteins that have weakly binding ligands.

(v) The first NMR measurements on membrane proteins with the sample at cryogenic temperatures (down to 5 K = −268 °C) performed on amino acid-selective labelled samples of GalP purified and reconstituted in lipids. This includes measurement of ^{1}H T_1 relaxation times.

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Carbon-14-labelled peptides: Solid phase peptide synthesis, biotinylation and PEGylation

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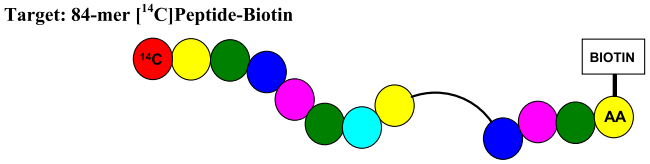
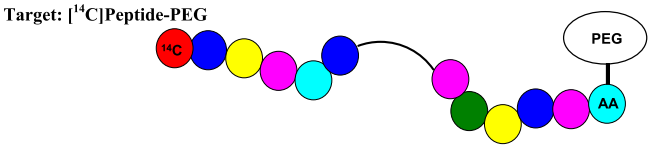
The radiolabelling group at Almac have synthesised a number of peptide APIs containing carbon-14 amino acid residues by using the solid-phase peptide synthesis (SPPS) approach. A number of these carbon-14-labelled peptides were modified by the addition of polyethylene glycols (PEGs) to produce a new chemical entity with a different pharmacological profile. In some cases, carbon-14-labelled peptides can undergo biotinylation to provide targeted drug substances. Two examples are given to provide an overview of SPPS, PEGylation and biotinylation towards the synthesis of carbon-14-labelled peptides.

Strategy. The simplest way to introduce carbon-14 into a peptide is via a glycine residue. The chemical synthesis of the achiral carbon-14-labelled glycine is straightforward and high yielding as no resolution of unwanted enantiomers is required. Glycine can be labelled on one or both carbon atoms to yield specific activities up to 120 mCi/mmol.



In this target, the unlabelled 83-mer resin bound peptide was first synthesised using the SPPS approach. The terminal Fmoc amino acid protecting group was cleaved and the carbon-14 label was introduced via *N*-Boc-*L*-[U-^{14}C]isoleucine. Cleavage of the protecting group followed by biotinylation then *N*-Boc cleavage produced the 84-mer carbon-14-labelled peptide. Resin cleavage released the

[^{14}C]peptide-biotin, which was purified and lyophilised giving product with a radiochemical purity (HPLC) $>$ 98 area%, chemical purity (HPLC) $>$ 98 area% and specific activity $>$300 mCi/mmol.



For the PEGylated target, the unlabelled resin bound peptide was synthesised by the SPPS approach, and the terminal Fmoc amino acid was cleaved to enable the coupling of *N*-Boc-[^{14}C]glycine. The carbon-14-labelled peptide was cleaved from the resin and purified followed by lyophilisation to give pure [^{14}C]peptide. PEGylation of the peptide followed by deprotection and purification gave [^{14}C]peptide-PEG with a radiochemical purity (HPLC) $>$ 98 area%, chemical purity (HPLC) $>$ 98.0 area% and specific activity $>$20 mCi/mmol.

Both [^{14}C]peptide-biotin and [^{14}C]peptide-PEG were used in ADME studies.