New Opportunities for Advanced Organic Synthesis - Flow-Based Chemical Processing

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16TH INTERNATIONAL ISOTOPE SOCIETY (UK GROUP) SYMPOSIUM

SYNTHESIS AND APPLICATIONS OF LABELLED COMPOUNDS 2007

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Meeting summary

The 16th annual symposium of the International Isotope Society's United Kingdom Group took place at the Wellcome Genome Campus, Hinxton, Cambridge, UK on 1st November 2007. The meeting was attended by around 100 delegates from academia, the life sciences and fine chemical companies.

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 afternoon session chaired by Dr Franklin Aigbirhio (WBIC, University of Cambridge, UK) and Prof. Chris Willis (University of Bristol, UK), respectively. The meeting concluded with remarks from Dr Ken Lawrie (GlaxoSmithKline, Stevenage, UK).

This year's symposium had a large attendance from students. Moreover, an excellent level of sponsorship was achieved, and the symposium proved self-financing. The meeting venue again proved very popular. The next UK symposium is provisionally planned at the same venue for 9th October 2008.

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Meeting programme

Prof. Bill Lockley [University of Surrey, UK]—*In memoriam, John R Jones 1937–2007*.

Dr Simon Patching [University of Leeds, UK]—*Exploiting isotope labelling for solid-state NMR studies of drugs and their targets*.

Dr Martin Christlieb [University of Oxford, UK]—*Imaging metals and imaging with metals: developing metal-containing PET tracers*.

Prof. S David Jackson [University of Glasgow, UK]—*The use of isotopic labels in understanding CO/H_2 reactions*.

Mr Graeme Young [GlaxoSmithKline, Ware, UK]—*Accelerator mass spectrometry (AMS) in drug development at GSK*.

Dr Ryan Bragg [AstraZeneca Alderley Park, UK]—*Stable isotopic labelling strategies for nitrogen heterocycles*.

Prof. Peter Horn [Bavarian State Coll. Paleontol. & Geol., Germany]—*Use of multielement (H, C, N, O, S, Sr, Pb) isotope signatures in forensics and criminology*.

Dr Ian Baxendale [University of Cambridge, UK]—*New opportunities for advanced organic synthesis—flow based chemical processing*.

Ms Tenzeela Ilyas [sanofi-aventis, Alnwick, UK]—*Microwave assisted synthesis of multiply labelled SR244870, a compound related to Ferroquine*.

Dr David Procter [University of Manchester, UK]—*A fluorous synthesis of N-heterocycles using a Pummerer cyclative–capture strategy*.

Dr Neil Geach [Selcia, Ongar, UK]—*Carbon-14 synthesis using microwaves*.

Dr Glynn Williams [GlaxoSmithKline, Stevenage, UK]—*The synthesis of isotopically labelled intermediates from [$^{13}C_6$]aniline and [$^{13}C_6$]phenol*.

Journal of Labelled Compounds and Radiopharmaceuticals

EXPLOITING ISOTOPE LABELLING FOR SOLID-STATE NMR STUDIES OF DRUGS AND THEIR TARGETS

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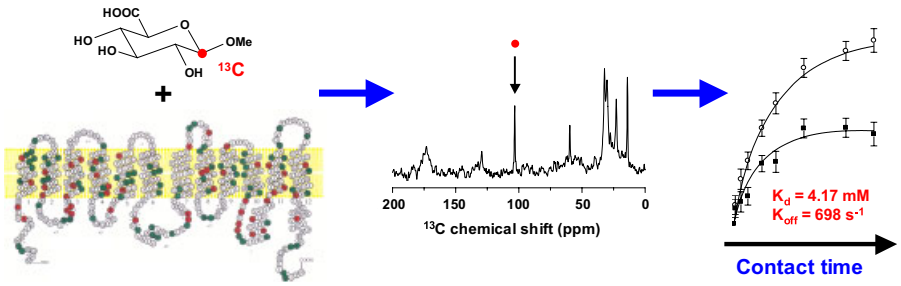
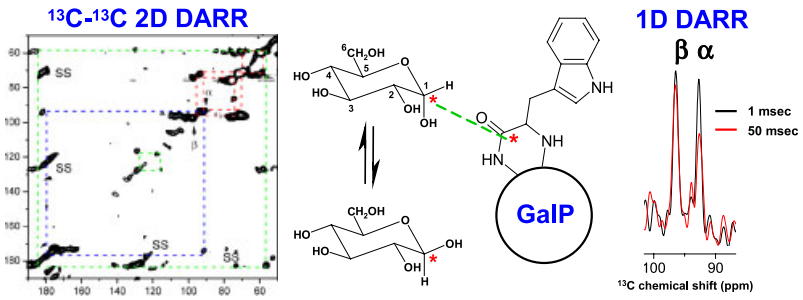
Nuclear magnetic resonance (NMR) spectroscopy is a valuable tool in drug discovery, which contributes to activities ranging from ligand screening through to the structural analysis of ligand–target complexes. Many drug targets, such as the G-protein coupled receptors (GPCRs), are embedded in cellular membranes and not readily amenable to detailed analysis by conventional NMR and crystallographic techniques. Solid-state (SS) NMR spectroscopy is in these cases a valuable technique for characterizing the interactions between small molecules and proteins in their native membranes. Stable isotope labelling (with, e.g. ^{13}C and ^{15}N) of ligands is critical for SSNMR analysis and this presentation will describe how isotope labelling has been exploited in three areas.

Screening small molecule interactions with transmembrane receptors. Using cross-polarization magic-angle spinning (CP-MAS) SSNMR, specific interactions between ^{13}C-labelled ligands and proteins within their native membranes can be detected even when the target of interest represents less than 30% of the total membrane protein.[1] It will be shown how CP-MAS methods can also eliminate interference from non-specific binding of hydrophobic ligands and determine binding constants and dissociation rates (k_{off}).



Characterizing ligand binding sites. SSNMR measurements of dissociation constants (K_D) for different mutants of a target can provide initial indications of the location and topology of the binding site of a ligand. More detailed information can then be obtained by observing dipolar couplings between a bound isotope-labelled ligand and a uniformly or selectively $^{15}N/^{13}C$-labelled protein target, which places constraints on the distance between the ligand and specific residues in the target.



Structures of bound ligands. Ligands must be prepared with isotope labels incorporated at structurally informative sites to provide sufficient interatomic distance and angle constraints. It will be discussed how SSNMR procedures have been developed to maximize the structural information available from sparsely labelled ligands when labelling opportunities are restricted by synthetic feasibility.[2,3]

Examples will be presented of developmental work on transport proteins from *E. coli* and of recent applications of these methods to pharmaceutical targets including GPCRs.

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EXPLOITING TRANSITION METALS TO DIAGNOSE HYPOXIA AND IN TARGETED RADIOTHERAPY

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Radio-medicine uses unstable isotopes to emit radiation either for therapy or imaging.[1,2] The most common elements for imaging come from the p-block of the periodic table, e.g. C, N, O, F, I. Transition metals are very promising but not widely used with the notable exception of ^{99m}Tc.[3] Each transition metal typically has a choice of half-lives available; easy, fast and aqueous labelling chemistry. We are interested in both developing new metal-based radiopharmaceuticals and developing new ways to analyse their behaviour.

PET/cell studies with novel copper complexes. The copper *bis*(thiosemicarbazone) complex, Cu[ATSM] has received a lot of attention as a marker of tumour hypoxia. It has been subjected to study *in vitro*, *in vivo* and is the subject of previous and current clinical trials.[4] However, until recently, the chemistry involved has not been amenable to the ready synthesis of structural analogues.[5] Such analogues would be interesting since Cu[ATSM] still has some problems, which include low solubility and slow clearance rates from oxic tissues. Recent work with Jon Dilworth (Chemistry, Oxford) and Franklin Aigbirhio (WBIC, Cambridge) has not only allowed new compounds to be produced, but has permitted them to be radiolabelled[6] and the first microPET images to be acquired[7] shows not only the beginning of an SAR but also shows that these compounds retain the required electrochemistry and are stable under physiological conditions on the timescale of PET experiments.

Understanding and characterizing. PET is an excellent approach to characterizing the behaviour of compounds *in vivo*, but it cannot provide the resolution needed to look at the behaviour inside a single cell. We are employing fluorescence microscopy in collaboration with Grant Churchill (Pharmacology, Oxford) to study fluorescent analogues of our new compounds.[6]

Looking for metal atoms inside cells. We have been fortunate that some of our compounds could be rendered fluorescent with a rather minor modification of the structure bringing almost no change in molecular weight and a small change in molecular shape. It cannot be hoped that this will always be the case. Most small molecules cannot be 'tagged' without doubling the molecular weight and hopelessly altering the behaviour. In collaboration with Chris Grovenor (Materials, Oxford), we are developing the new technique, based on mass spectrometry, to explore the subcellular behaviour of new metal containing compounds. Since we are looking for either unnatural isotopes or non-biogenic elements, the signal from our desired atom should be easy to spot.

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THE USE OF ISOTOPIC LABELS IN UNDERSTANDING CO/H_2 REACTIONS

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The reaction between carbon monoxide and hydrogen can be catalysed to give a variety of products including methane, methanol, acetic acid and waxes. The mechanisms involved are rarely simple and using ^{13}C, ^{18}O and D_2 as tracers has helped to determine reaction paths. Often a combination of isotopes is needed to fully specify the mechanism. In this talk we will look at complex systems where there is potential for multiple products and where understanding the mechanism is critical to catalyst optimization.

The synthesis of methanol from carbon monoxide–hydrogen and carbon monoxide carbon dioxide–hydrogen over a copper/zinc oxide/alumina catalyst was studied using transient isotope tracing techniques. The non-steady-state period immediately after start-up has been studied and the product distribution and time delay could be explained with reference to the amount of residual oxygen on the catalyst after reduction. Using [^{l8}O]carbon monoxide and [^{18}O]carbon dioxide, it was shown that the label is not detected in the methanol product for 0.3 h. From the steady-state activities and a residence time of 0.3 h, the size of the surface reservoir of methanol precursor was calculated for both carbon monoxide–hydrogen and carbon monoxide–carbon dioxide/hydrogen feedstreams. This figure was in good agreement with the amounts of methanol removed from the catalyst when the feedstream was switched from carbon monoxide–hydrogen or carbon monoxide–carbon dioxide-hydrogen to hydrogen alone.

The production of oxygenate species over rhodium catalysts can under the correct conditions produce a range of oxygenate species including methanol, ethanol, acetaldehyde and acetic acid. Using [^{13}C]CO, [^{18}O]CO and D_2, the mechanism of formation of each species was determined. When labelled carbon monoxide was introduced, neither methane nor ethanol nor methanol showed

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any incorporation; however, the labels were rapidly incorporated into the aldehydic function of ethanal. Labelled water, produced from the hydrogenation of [^{18}O]CO, took more than 0.3 h to desorb. The results suggest that (i) ethanol and ethanal are produced independently with no common intermediate, (ii) the formation of alcohols is slow (taking over 0.5 h), and (iii) carbon monoxide is not hydrogenated directly to methane but goes through the hydrocarbonaceous residue present on the surface. The carbonaceous residue was found to play a central role in the mechanism, supplying in effect both hydrogen and CH_2 units to other reactive surface species.

The details of these and other studies will be reported showing the unrivalled access to mechanistic information that isotopes can give in the field of catalysis.

ACCELERATOR MASS SPECTROMETRY (AMS) IN DRUG DEVELOPMENT AT GSK

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A brief history of the use of accelerator mass spectrometry in GSK studies will be presented, including its use in pilot studies through to clinical, animal and *in vitro* studies. A summary of the status of GSK's in-house AMS capability will be outlined and views on the future of AMS in GSK will be presented, including potential impact on drug development and potential advances in the technology.

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STABLE ISOTOPIC LABELLING STRATEGIES FOR NITROGEN HETEROCYCLES

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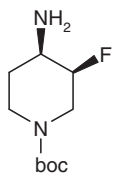
Synthetic approaches to stable isotopically labelled (SIL) heterocyclic fragments of active drug molecules will be presented. Specifically, three areas of work will be discussed:

Piperidine The synthesis of a stable labelled isotopomer of (3*S*,4*R*)-4-amino-3-fluoropiperidine-1-carboxylic acid *tert*-butyl ester **1**, via labelled 1-benzylpiperidin-4-one, will be presented.[1]

Indole Three approaches to stable labelled 5-hydroxy-1*H*-indole-2-carboxylic acid ethyl ester **2** will be discussed, highlighting issues of deuterium lability and ring syntheses.[2]

Pyrazole Synthetic approaches to 5-methyl-2*H*-pyrazol-3-ylamine **3** will be discussed followed by a concise synthesis of a stable labelled version.[3]



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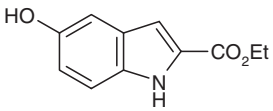

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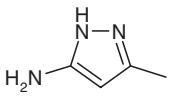

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USE OF MULTIELEMENT (H, C, N, O, S, SR, PB) ISOTOPE SIGNATURES IN FORENSICS AND CRIMINOLOGY

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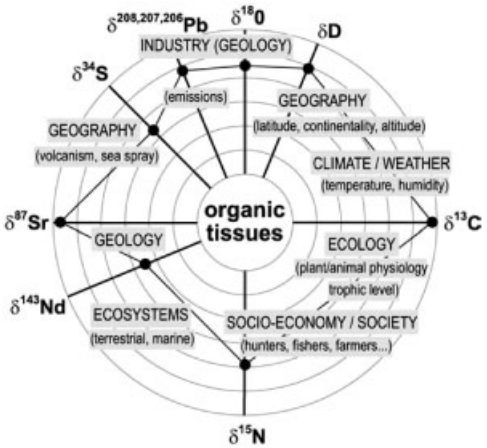
The increasing worldwide mobility of individuals has led to steadily rising numbers of unidentified corpses, which cannot be assigned to any particular region. Often these corpses remain unidentified by the conventional forensic means applied by criminal investigators, anthropologists, forensic doctors, etc. Fortunately isotopic analysis techniques are now providing new approaches to the identification of the origin and places of residence of such deceased persons. The fundamentals of this approach will be described and exemplified.

Important and necessary first steps in the course of an identification of a dead person is to search for leads to former residences of the individual. This may be obtained through mass spectrometrically determinable [1,2] isotope abundance ratios of bioelements, H–S, and geoelements, Sr–Pb. These contain inherent isotopic information about their ultimate provenance, their history and other physicochemical processes occurring at all trophic levels within the food chains. Chemical elements, and with them their history (provenance and specific isotope ratios) enter bodies via foodstuff, drinks and environmental aerosols, and are ultimately found in body tissues[3] where they are in homeostatically controlled elemental concentrations.

Similarly, natural and industrial objects or items that are found with corpses, such as clothing, dental work, bullets/shot, jewellery, etc., also bear isotopic information on the way of processing, and on the provenance of the raw materials.

The prerequisite for successful and convenient use of isotope methods in 'provenancing' is that at different places on the globe chemical elements show different isotope ratios, which depend on geographical, geological/lithological, hydrological, and socio-economical circumstances or settings (Figure).[4] The isotopic ratios of elements very often show not only site-specific regional, but also 'national signatures'.

When the isotopic ratios of an element are determined on tissues with different formation and/or turnover times in a humaṅs life span, one then has the means at hand to deduce isotope changes in foodstuff consumed and, implicitly, in changes of place of residence.[5]



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NEW OPPORTUNITIES FOR ADVANCED ORGANIC SYNTHESIS—FLOW-BASED CHEMICAL PROCESSING

MARCUS BAUMANN, IAN R. BAXENDALE, JOHN J. HAYWARD, MARK D. HOPKIN, JANE JIN, MATTHEW O. KITCHING, STEVE LANNERS, STEVEN V. LEY, NIKZAD NIKBIN-ROUDSARI, CHRISTOPHER D. SMITH, CATHERINE J. SMITH, and LUCIA TAMBORINI

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One of the most important productivity-limiting issues faced by synthetic chemists today is that of product purification and isolation. This problem is even more significant when one considers the increasing availability of automated high-throughput

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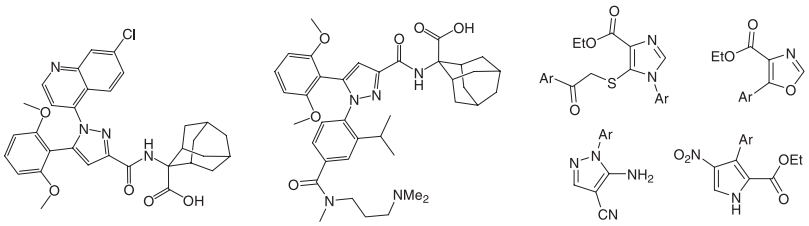
synthetic techniques that can create a large number of diverse products and the inability of modern chromatographic separation techniques to manage this output. We believe that flow chemistry is set to have a major impact on how we conduct modern synthetic chemistry by bypassing the need for purification steps and creating a route to go directly from starting material to the pure isolated product; thus avoiding a costly chromatographic bottleneck. The development of new methods and integrated procedures for the preparation of pharmaceutically interesting chemical entities using multi-step flow methods will be discussed.[1–4] Aspects of scale-up and scale-out as well as in-line compound purification using scavenger agents will be addressed in relation to novel intermediates and known APIs.

Synthesis of the potent neurotensin receptor (NTR) antagonists SR48692 and SR142948A NTR antagonists SR48692 and SR142948A are powerful tools for probing neurotensin (NT)-mediated signalling pathways associated with important physiological and pathological processes in the central nervous system and cancers. Studies towards their large-scale synthesis will be highlighted in terms of a flow chemistry platform.

The flow synthesis of novel heterocyclic cores. The ability to rapidly and on demand furnish gram quantities of key intermediates for further derivatization is an important concern for pharmaceutical companies. Currently the synthesis of such compounds is often outsourced to third party vendors—taking time and significant legal negotiation. Having a small footprint chemical synthesizer capable of delivering such material in-house would be a valuable scientific and commercial asset. Our research towards such a device and its application to the synthesis of small libraries of heterocyclic compounds will be examined



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MICROWAVE-ASSISTED SYNTHESIS OF MULTIPLE-LABELLED SR244870, A COMPOUND RELATED TO FERROQUINE (SSR97193)

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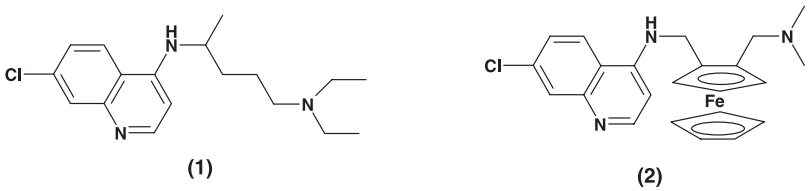
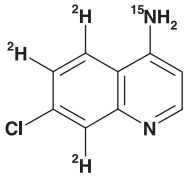
Malaria is caused by protozoan parasites of the genus *Plasmodium* infecting human blood cells. Increased parasite resistance to anti-malarial drugs such as chloroquine, **1**, has led to gradual growth in deaths caused by the disease. This has prompted the development of more potent and effective drugs. One such compound is SSR97193 (Ferroquine, 2)[1], which is presently in phase I of clinical trials.



In order to support our mass spectral studies in this area, we required a multiply stable-labelled derivative of 4-amino-7-chloroquinoline (SR244870). We eventually developed a route to a compound containing an additional four mass units, [$^{2}H_{3}$,^{15}N]SR244870,**3**.



(3)

The overall synthesis is based on our previous synthesis of [*quinoline*-3-^{14}C]-SSR97193, starting from 3-chloroaniline and proceeding via [^{14}C]4,7-dichloroquinoline, recently reported by McNeill *et al.*[2] It was initially necessary to prepare perdeuterated 3-chloroaniline, which we achieved via the homogeneous H/D exchange using platinum tetrachloroplatinate/D_2O described by Garnett,[3] accelerated by microwave radiation using a CEM 'Discover' microwave generation system. The product, [$^2H_4$]3-chloroaniline, was taken on to [5,6,8-$^2H_3$]4,7-dichloroquinoline in three steps.

4-Amino-7-chloroquinoline is normally made by passing ammonia gas into a solution of 4,7-dichloroquinoline in phenol held at 170°C. As this reaction is clearly inappropriate for the insertion of a nitrogen-15 atom, a new amination reaction had to be devised. Unfortunately attempted reaction of 4,7-dichloroquinoline with [^{15}N]ammonium chloride in pyridine gave only ^{14}N-product, presumably by a Chichibabin-style reaction. However, amination could be achieved to afford compound 3 by direct reaction with an excess of aqueous [^{15}N]ammonia, albeit with concomitant hydrolysis. Interestingly, the latter step did not proceed at all under conventional reflux.

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A FLUOROUS SYNTHESIS OF *N*-HETEROCYCLES USING A PUMMERER CYCLATIVE–CAPTURE STRATEGY

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The Pummerer reaction has evolved into a useful tool for the synthesis of heterocyclic compounds.[1] We have developed a new, general strategy for triggering Pummerer cyclizations that involves the addition of thiols to reactive carbonyl compounds such as glyoxamides 1.[2] The resultant hemi-thioacetals can be activated, leading to thionium ion generation and to Pummerer cyclization.

The use of a thiol containing a phase tag results in cyclative-capture of the glyoxamide substrate. The choice of a fluorous thiol[3] allows reactions to be monitored conveniently while also allowing rapid, phase-tag-assisted purification using fluorous solid-phase extraction (FSPE).[4] Our approach utilizes a fluorous phase scavenging reagent in a novel manner. Convenient modification of the fluorous, heterocyclic scaffolds **2** can then be carried out and the products again purified rapidly using FSPE. We have shown the approach to be compatible with a variety of modification strategies including palladium-catalysed cross-coupling technology. Upon completion of the desired sequence, the fluorous tag can be removed in a traceless manner, allowing access to a diverse collection of heterocyclic products (Scheme 1).

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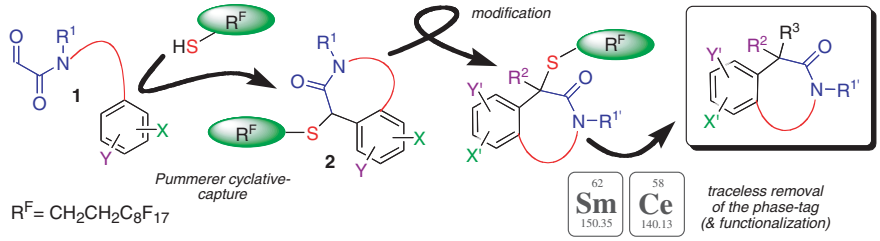
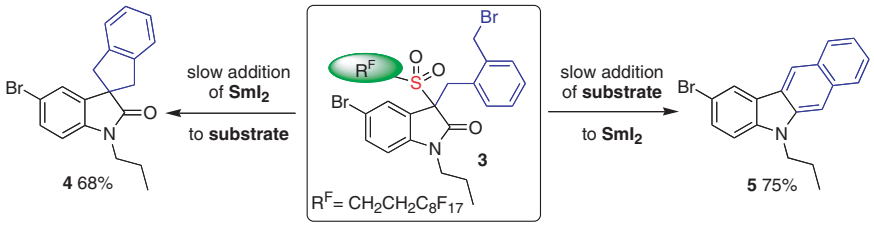


Scheme 1

Our interest in the application of lanthanide reagents in phase tag-assisted synthesis has led us to develop complimentary, reductive (Sm^{II}) and oxidative (Ce^{IV}) strategies for the traceless removal of the fluorous tag at the end of the synthesis.

Finally, we have developed a portfolio of sequential tag-cleavage/cyclization processes using SmI_2.[5] For example, on cleavage of the tag from oxindole **3** using SmI_2, the intermediate Sm(III)-enolate undergoes intramolecular alkylation to give the spirocycle 4 (Scheme 2). Changing the order of addition of SmI_2, gives indolocarbazole 5 as the major product. Thus, the 'order of addition' of a reagent can be used to introduce diversity in the synthesis.



Scheme 2

Further work on the development of sequential fluorous tag-cleavage/cyclization processes and their application in library synthesis will also be described.

References

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CARBON-14 SYNTHESIS USING MICROWAVES

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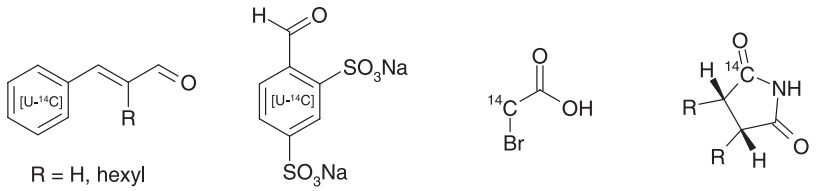
The use of microwaves in organic synthesis was first reported 21 years ago and is now regarded as an essential tool in an organic synthesis laboratory.[1] Microwaves are extremely useful in speeding up reaction times and often fewer by-products are formed thereby resulting in increased yields. More recently this technique has increasingly been applied in the synthesis of radiolabelled compounds.[2]

At Selcia our radiochemists regularly use an industrial microwave reactor to optimize routes and prepare carbon-14-labelled intermediates and final products. In the past these would have needed elevated temperatures, pressure and extended reaction times. The modern industrial microwave reactors are now suitable for performing reactions under pressure and at high temperatures that can be generated by the microwave radiation, often reducing reaction times significantly. A number of examples of where Selcia have used a microwave reactor to prepare carbon-14 material compounds will be presented including the following examples.



Compared with conventional optimization techniques, where it may be necessary to wait for 24 h or more before knowing whether a reaction has gone to completion, a series of microwave reactions can be set up and run in minutes and the crude reaction mixtures analysed for the desired product. The talk will address some of the advantages and disadvantages of using carbon-14 in microwave reactors.

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THE SYNTHESIS OF ISOTOPICALLY LABELLED INTERMEDIATES FROM [$^{13}C_6$] ANILINE AND [$^{13}C_6$] PHENOL

GLYNN WILLIAMS, SIMON HARWOOD, KENNETH LAWRIE, GEOFF BADMAN, IAN WATERHOUSE, RICHARD CARR, NICK SHIPLEY, KARL CABLE, CALVIN MANNING, STEPHEN MONTGOMERY, RON LAWRENCE, MARIO ALMI, YANN AMBACHER, JOHN NEWMAN, AUDREY ATHLAN, PETER WOODWARD, ANDREW BURTON, and GORDON CAMPBELL

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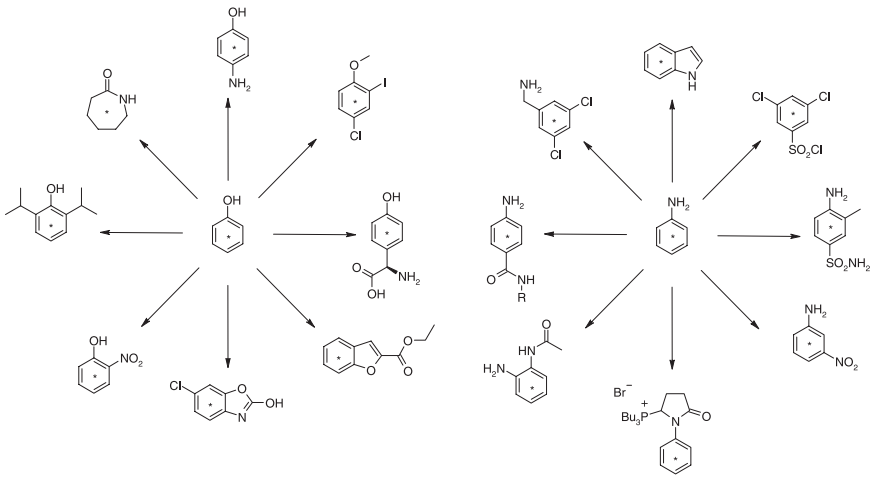
Stable-labelled isotopomers of compounds in drug development are routinely used as internal standards in LC/MS/MS assays.

The prevalence of the phenol and aniline motifs and the importance of discrete skeletal labelling of molecules make [$^{13}C_6$] phenol and [$^{13}C_6$] aniline ideal starting materials.

[$^{13}C_6$] Phenol and [$^{13}C_6$] aniline are both commercially available reagents that can be readily converted to a wide range of intermediates using classical chemistry.

The synthesis of polysubstituted phenols and anilines will be discussed along with the synthesis of aromatic and nonaromatic heterocycles.



* denotes [$^{13}C_6$]

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HIGHLY ACTIVE IRIDIUM(I) COMPLEXES FOR CATALYTIC HYDROGEN ISOTOPE EXCHANGE

JACK A. BROWN,[a] STEPHANIE IRVINE,[a] ALAN R. KENNEDY,[a] WILLIAM J. KERR,[a] SHALINI ANDERSSON,[b] and GÖRAN N. NILSSON[b]

[a]Department of Pure and Applied Chemistry, WestCHEM, University of Strathclyde, 295 Cathedral Street, Glasgow G1 1XL, UK
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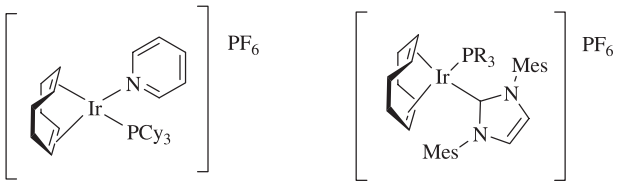
Hydrogen isotope exchange represents an effective way of following the metabolic pathways of potential drug candidates and, therefore, is of increasing importance to the pharmaceutical industry. With this in mind, the ideal situation would be to utilize catalytic quantities of a transition metal species to carry out this transformation on a functionalized compound. To this end, a number of iridium catalysts have been developed and applied to this process with varying success. Traditionally, the most effective catalyst used in this field is Crabtree's catalyst, **1**. However, often super-stoichiometric amounts of this species are required together with long reaction times. This poster reveals a series of novel iridium(I) complexes of type **2**, and highlights their considerable efficiency and general applicability in the field of hydrogen isotope exchange, with levels as low as only 0.5 mol% catalyst loading over short reaction times being required to deliver extremely high levels of labelling.



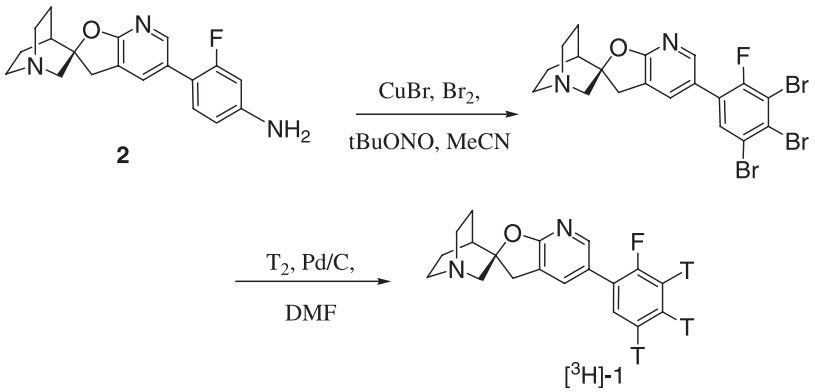
1 **2**

IN VIVO RECEPTOR OCCUPANCY OF ALPHA 7 NEURONAL NICOTINIC RECEPTOR AGONISTS IN RAT BRAIN USING A HIGH-AFFINITY TRITIUM-LABELED RADIOLIGAND

CHARLES ELMORE, JENNIFER L. WERKHEISER, J. RICHARD HEYS, LADISLAV MRZLJAK, and DONNA MAIER

CNS Discovery, AstraZeneca Pharmaceuticals LP, 1800 Concord Pike, Wilmington, De 19850-5437, USA

Neuronal nicotinic acetylcholine receptors (nAChR) have been shown to play an important role in the central nervous system. Recent studies have focused on the alpha-7 nAChR as a potential pharmacologic target for psychiatric diseases. Compound **1** is a high-affinity alpha-7 nAChR ligand with physical–chemical and pharmacokinetic properties preferable for use as an *in vivo* radioligand tool. Labeling of the compound with tritium was accomplished by halogenation of aniline 2 using the Doyle reaction and then tritiodehalogenation to afford [^{3}H]-**1** with a specific activity of 66 Ci/mmol. When the radioligand was administered to rats, it showed rapid uptake in the brain and accumulation in alpha-7 rich regions (frontal cortex, hippocampus, hypothalamus, midbrain) with low uptake in non-target regions (striatum, cerebellum) (Scheme 1).



Scheme 1

A STATISTICAL APPROACH TO THE ANALYSIS OF MS IONS OF COMPOUNDS LABELLED WITH ^{2}H, ^{3}H, ^{13}C AND ^{14}C

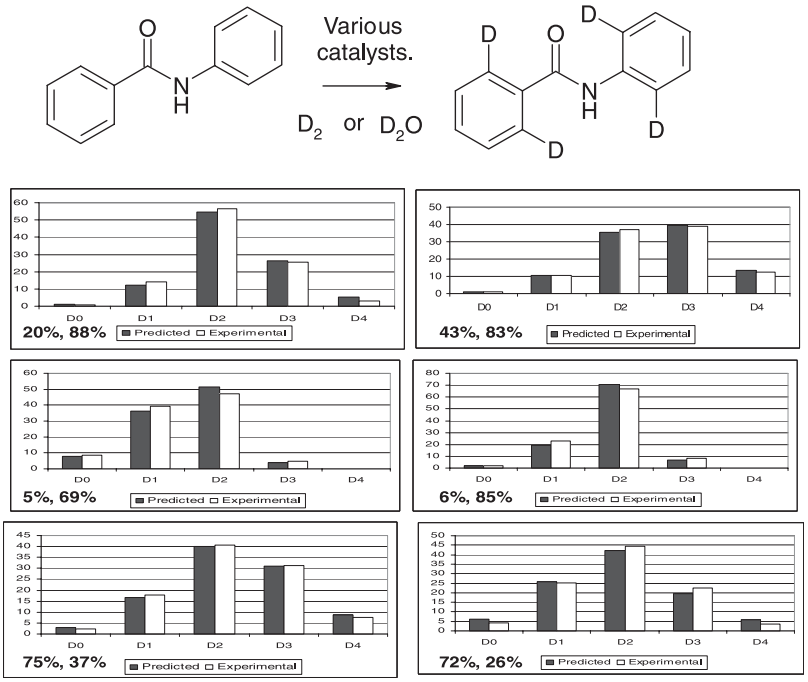
WILLIAM J. S. LOCKLEY, and RICHARD SHERHOD

Division of Chemical Sciences, Faculty of Health and Medical Sciences, University of Surrey, Guildford GU2 7XH, UK

This presentation outlines a specialist data-processing application designed to facilitate the analysis of labelled compounds by modelling the isotopic distributions observed in mass spectrometric ions. The program (Labelled Mass Spec Predictor, LMSP) enables statistical predictions of the MS isotopomer distribution function of a labelled compound, given a model of the number of labelling sites and their degeneracy. It is a Visual Basic development of the Excel six-site MS program[1] with the ability to model up to 15 independent labelling sites.

Use of the LMSP program enables: (a) insight into the mechanism of labelling processes, (b) prediction of the number of labelling sites from best fit data and (c) harmonization of the MS data for labelled compounds with their ^{1}H-, ^{2}H- or ^{3}H-NMR data.

A typical example of the application of the program in harmonizing MS and NMR data is given below:



Experimental and predicted MS isotopic distributions of [^{2}H]benzanilide produced using catalysts with differing specificities for labelling *ortho* to the anilide or amide functions.

The MS data were corrected for natural abundance heavy isotope contributions using NAIC[1] or IsoPat2.[2] The predicted MS isotopic distribution was obtained from LMSP using data from ^{1}H- and ^{2}H-NMR. Percentages refer to the %D *ortho* to the anilide and amide. The catalytic processes were modelled as two independent two-site exchanges and hence had an isotopic distribution function (IDF) given by $IDF = (FH_{amide}+FD_{amide})^2 \times (FH_{anilide}+FD_{anilide})^2$, where FH_{amide} and FD_{amide}, etc. refer to the isotopic fractions of H and D at the subscripted site.

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EXCHANGE LABELLING OF TERMINAL ALKENES OVER GP VIII TRANSITION METALS: A D_2 GAS SCREENING STUDY

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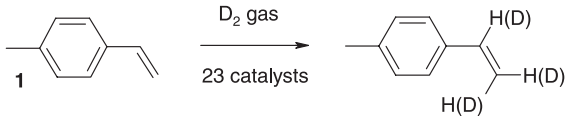
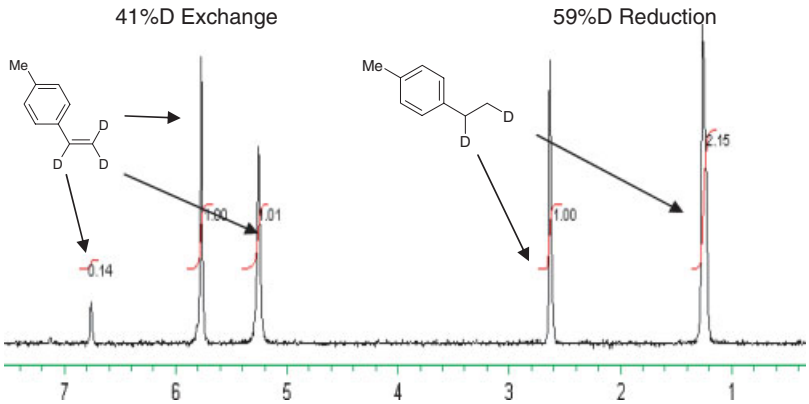
Division of Chemical Sciences, Faculty of Health and Medical Sciences, University of Surrey, Guildford GU2 7XH, UK

The isotopic exchange labelling of the terminal methylene group has been documented previously.[1] As part of a more general series of investigations of the exchange processes accompanying alkene hydrogenations,[2] we have recently investigated this terminal exchange process in some detail by screening a range of transition metal catalysts for such activity using a deficiency of D_2 gas and a model substrate, 4-methylstyrene, **1**. Monitoring was carried out via ^{1}H-NMR, ^{2}H-NMR and GC/MS (Scheme 1).



A number of catalysts were identified where the rate of alkene exchange vs the rate of alkene reduction was such that a useful incorporation of isotope into the alkene could be achieved. A typical ^{2}H-NMR screening result is shown below for a ruthenium black catalyst.



Scheme 1

There was a commonality of behaviour across a wide range of catalysts, with terminal methylene exchange more facile than the methine exchange and with *cis* and *trans* terminal deuterium in a 1:1 ratio. Exceptions were observed with Pt-based catalysts and with two homogeneous catalysts.

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THE FIRST PREPARATION OF ALL REGIOISOMERS OF ETHYL [^{18}F]FLUOROBENZOATES

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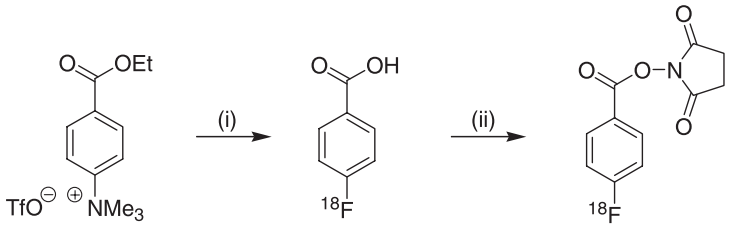
N-Succinimidyl-4-[^{18}F]fluorobenzoate **3** is a widely used prosthetic group for labelling of short peptides, proteins and antibodies in positron emission tomography (PET).[1] Its standard synthesis involves a nucleophilic aromatic substitution of ethyl 4-

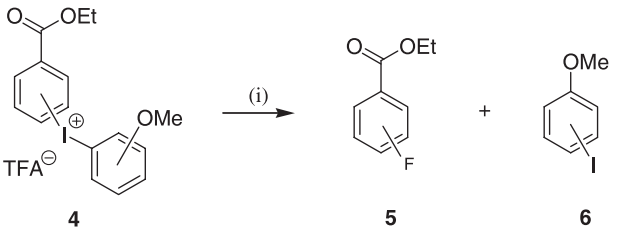
(trimethylammonium triflate) benzoate **1** with $K_{2.2.2}$ complexed [^{18}F]KF to generate the fluorine-18-labelled ester. After hydrolysis, the corresponding acid **2** may be coupled with the succinimidyl group using various coupling reagents (Scheme 1)



(i) a) K^{18}F/$K_{2.2.2}$; b) NaOH/H_2O; (ii) coupling reagents (DCC, DSC or TSTU).

Scheme 1

However, the 2- and 3- fluorine-18-labelled benzoates have never been prepared due to the limitations of the nucleophilic aromatic substitution chemistry. In this presentation, we demonstrate the synthesis of all three regioisomers; ethyl 2-, 3- and 4-[^{18}F]fluorobenzoates using iodonium salts.[2,3] Good to excellent yields and high selectivity were observed in the fluoridation process (Scheme 2)



(i) CsF, TEMPO, CH_3CN, DMF, 130 °C, 1.5 hours.

Scheme 2.

In summary, an efficient method for the preparation of ethyl 2-, 3- and 4-[^{18}F]fluorobenzoates has been developed using iodonium salt precursors. Initial studies, for the *direct* preparation of 3 from [^{18}F]fluoride using an iodonium salt intermediate and the first use of fluorous iodonium salts to expedite isolation/purification of the desired product will also be presented.

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THE SYNTHESIS OF ISOTOPICALLY LABELLED PHYTOCHEMICALS FOR ANALYSIS AND METABOLIC STUDIES

NAWAF AL-MAHARIK, LIRONG CHEN, TARA FRYATT, JOHN J. MORRISON, MARK F. OLDFIELD, AVRIL. A. ROBERTSON, QINGZHI ZHANG, and NIGEL P. BOTTING

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The Food Standards Agency (FSA) funds a large number of research programmes on the biological effects of dietary chemicals. These studies range from surveys of levels in the UK diet to full epidemiological studies and the majority of them rely heavily on accurate analysis of compounds, whether it be in food samples or biological fluids (plasma, urine, etc.). In such work, with large numbers of biological samples, accuracy and reproducibility are vital. Over the past few years we have provided synthetic chemistry support for many of these FSA-funded projects, in particular through the synthesis of isotopically labelled compounds as internal standards for LC-MS and GC-MS analysis.

Initial synthesis was concentrated on the isoflavone phytoestrogens, which are constituents of soya and soya-related products and have a number of biological effects, in particular cancer preventative activity against hormone-related cancers (breast, prostate, etc.). A series of multiply ^{13}C-labelled phytoestrogens were prepared including [3,4,8-$^{13}C_3$]daidzein **1** and [2,3,4-$^{13}C_3$]glycitein **2**. Another group of targets were the lignans, such as [7,8,9-$^{13}C_3$]enterolactone **3**, which also have anti-cancer activity and are present in wholegrains, fruits and vegetables. More recent work involved the synthesis of ^{13}C-labelled isoflavone conjugates, including the glycosides found in plants and glucuronides, such as [2,3,4-$^{13}C_3$]genistein 7-glucuronide **4**, formed via human metabolism.

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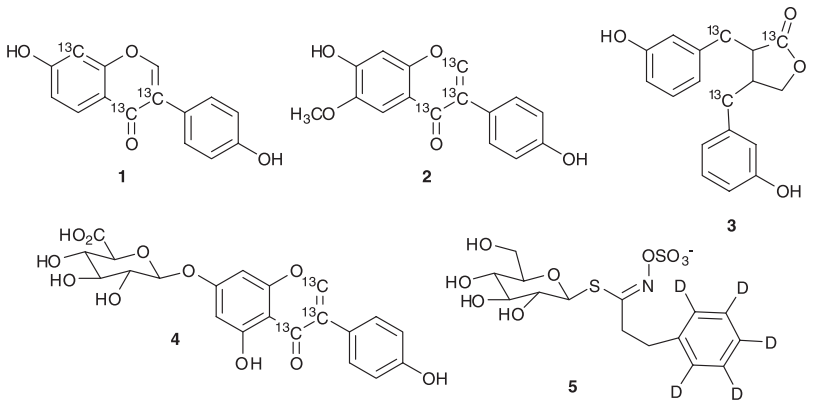

Glucosinolates are another group of dietary compounds with anti-cancer activity. Isotopically labelled glucosinolates, including [*phenyl*-$^{2}H_5$]gluconasturtiin **5**, and their metabolites were synthesized for use both as analytical standards and for metabolic studies.

SYNTHESIS OF [1, 3-$^{13}C_2$]L-PROLINE

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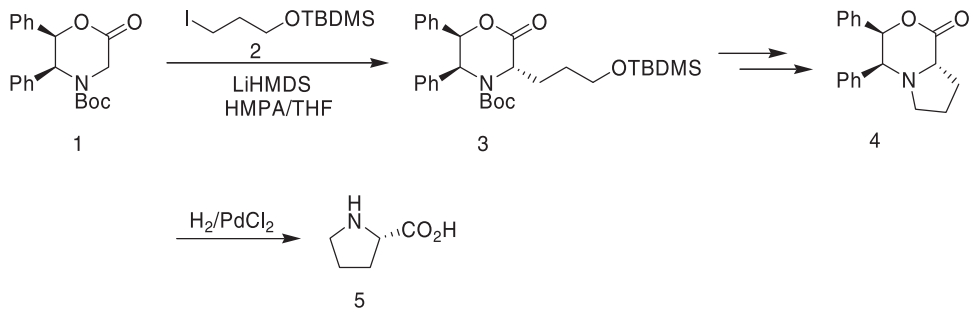
[b]Biomembrane Structure Unit, Department of Biochemistry, University of Oxford, Oxford OX1 3QU, UK

An important application of isotopically labelled amino acids is their incorporation into peptides and proteins for determining the 3D structures of biomolecules at their site of action in receptors by solid-state NMR spectroscopy.

Neurotensin (NT) is a 13-mer peptide that is implicated in the regulation of luteinizing hormone and prolactin release and has significant interaction with the dopaminergic system. The sequence of neurotensin is Glu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu-OH.[1] In order to deduce the $NT_{(8\text{-}13)}$ conformation at its site in the neurotensin-1 brain receptor, in particular whether the proline is *cis* or *trans*, [2-^{13}C]L-proline and [1, 3-$^{13}C_2$]L-proline were required.

One approach involves the use of a camphor sultam to establish the stereogenic centre at C-2.[2] An alternative strategy is the reaction of Williams' oxazinone **1** with iodide **2**, which gave **3** in 42% yield. Further deprotection and cyclization gave the bicyclic product **4** in good overall yield, hydrogenolysis of which then gave L-proline **5** (Scheme 1)



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This chemistry is readily adapted for the synthesis of both [2-^{13}C]L-proline and [1, 3-$^{13}C_2$]L-proline. For example, using [1-^{13}C]bromoacetic acid as starting material, the labelled oxazinone **1** could be synthesized in 59% overall yield following a literature procedure.[3] Reaction of 2-chloroethanol with potassium [^{13}C]cyanide gave [1-^{13}C]3-hydroxypropionitrile in good yield, which could be further transformed into [1-^{13}C]1-iodo-3-(*tert*-butyldimethylsilyloxy)propane.

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