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Abstracts

13th IIS (UK Group) Symposium*

Synthesis & Applications of Labelled Compounds 2003

Dedicated to Dr E. A. (Tony) Evans (1931–2003) for his contributions to the field of radiochemistry and in particular for the significant advances made in the chemistry and applications of the tritium isotope

Meeting Summary

The 13th annual symposium of the International Isotope Society's United Kingdom Group took place at the Wellcome Genome Campus, Hinxton, Cambridge, UK on Thursday 16th October 2003. The meeting was attended by over 100 delegates from academia, life science and fine chemical companies. Delegates were welcomed by Dr Ken Lawrie, of GlaxoSmithKline, Chairman of the IIS UK group.

The 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 programme was divided into a morning and afternoon session chaired by Dr Karl Cable (GlaxoSmithKline, Stevenage, UK) and Dr Franklin Aigbhirio (Wolfson Brain Imaging Centre, University of Cambridge, UK) respectively.

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 proved very popular and will remain unchanged for the next IIS UK group symposium which is planned for 4th November 2004.

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Meeting Programme

Prof Stephen Caddick [University College London, UK]—*Studies on the chromoprotein antibiotic neocarzinostatin.*

Prof Sharon Stone-Elander [Karolinska Institute, Sweden]—The 'special effects' of microwaving in radiochemistry.

Dr Simon Patching [University of Leeds, UK]—Stable isotopes and membrane transport proteins, chemical synthesis and biochemical analysis by solid state NMR.

Dr Jason Eames [Queen Mary College, London, UK]—Synthetic aspects of the C-deuteration of enolates.

Dr Alan Spivey [Imperial College, London, UK]—Opportunities for isotope labelling via solid phase synthesis with organogermanium linkers.

Dr Ian Blagbrough [University of Bath, UK]—Labelled methyllycaconitine (MLA)—a key nicotinic acetylcholine receptor ligand.

Dr Jim Ballinger [Guy's and St Thomas' Hospital, London, UK]—Challenges in preparation of radiolabelled drugs for nuclear medicine.

Dr Gary Shemilt [Amersham, Cardiff, UK]—The synthesis of carbon-14 labelled PEGs.

Dr Julian Knight [University of Newcastle, UK]—Development of palladium catalysed carbonylations for asymmetric synthesis.

Dr Neil Geach [Scynexis, Ongar, UK]—The versatility of labelled cyanide.

STUDIES ON THE CHROMOPROTEIN ANTIBIOTIC NEOCARZINOSTATIN

Stephen Caddick

Department of Chemistry, University College London, Christopher Ingold Laboratories, 20 Gordon Street, London WC1 H OAJ, UK

The enediyne chromoproteins are a fascinating class of naturally occurring antibiotic, which exhibit potent anti-cancer activity. These substances are isolated from soil bacteria and comprise a highly reactive enediyne chromophore, a ligand, which is non-covalently bound to an apo-protein. It is believed that the biological activity resides with the chromophore, with the apo-protein responsible for stabilisation of the ligand.

This talk will focus on our studies directed toward Neocarzinostatin (NCS). Recent work has enabled the production of isotopically labelled recombinant apoNCS, which has been used to probe the structural requirements for recruiting small drug-like substances into the protein binding site. The use of high field NMR techniques has enabled the level of promiscuity in small molecule binding to be assessed. Further work has examined the potential for the apo-NCS system to stabilize novel nitrogen mustard derivatives. The ability of apoNCS to enter cells will also be discussed.

THE 'SPECIAL EFFECTS' OF MICROWAVING IN RADIOCHEMISTRY

Sharon Stone-Elander[a,b]

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[b]*Karolinska Institute, Department of Clinical Neurosciences, Clinical Neurophysiology Section, R2:01, Karolinska Hospital, SE-17176 Stockholm, Sweden*

Microwaves are electromagnetic waves in the frequency region 0.3–300 GHz, with most non-radar equipment being required to operate at a frequency of 2.45 or 0.915 GHz. When dipolar or ionic molecules are exposed to microwaves, they attempt to follow the directions of the rapidly oscillating field. However, they cannot move freely in liquid or solid phases and the friction from these hindered rotations induces heat in the sample. Temperatures increase very rapidly (sometimes denoted as 'flash' heating). This essentially instantaneous heating occurs throughout the sample, instead of, as in conventional heating, slowly inward from the walls of the vessel that are in contact with the heating source.

The high temperatures which can be reached with this dielectric heating are increasingly being used to dramatically reduce reaction times in chemical transformations (see e.g. a review[1]). Microwave applications in radiolabelling (see reviews[2–4]) have paralleled and sometimes preceded developments in other areas of microwave-enhanced chemistry. Furthermore, a number of microwaving advantages present very 'special' and/or unique possibilities for our abilities to perform transformations with radionuclides.

This presentation relates experiences from this broadening field, which have been selected to illustrate 'special' opportunities for the development and practice of radiochemistry.

The author would like to acknowledge her present and former collaborators in microwave radiochemistry: Nils Elander, Jan-Olov Thorell, Peter Johnström, Anna Fredriksson, Ester Vázquez and Gareth Getvoldsen and for financial support from the Swedish National Board for Technical Research (210-1997-494).

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STABLE ISOTOPES AND MEMBRANE TRANSPORT PROTEINS: CHEMICAL SYNTHESIS AND BIOCHEMICAL ANALYSIS BY SOLID STATE NMR

Simon G. Patching,[a] Richard B. Herbert,[a] Peter J. F. Henderson,[a] Adrian R. Brough[b] and David A. Middleton[c]

[a]*Astbury Centre for Structural Molecular Biology, University of Leeds, Leeds LS2 9JT, UK*
[b]*Institute of Materials Research, University of Leeds, Leeds LS2 9JT, UK*
[c]*Department of Biomolecular Sciences, UMIST, P. O. Box 88, Manchester M60 1QD, UK*

Membrane proteins perform essential roles in the cells of all living organisms, where they function as cell surface receptors or as transporters that mediate the uptake of nutrients into the cell or the expulsion of waste products and toxins. Consequently, membrane proteins represent over 50% of current drug targets; however, the unique physical properties of membrane proteins and their demand for a lipid environment to retain structure and function, generally makes them very difficult to crystallize and unsuitable for structural or functional analysis using conventional NMR techniques.

Using solid state (SS) NMR we have detected the binding of substrates and inhibitors, labelled with NMR-active stable isotopes, to a range of transport proteins from *Escherichia coli* expressed in their native membranes. These systems were used to develop sample preparation and spectral editing methods that reduce interference from natural abundance background resonances and that eliminate signals that originate through the non-specific binding of hydrophobic ligands. Further, we have developed methods to quantify substrate affinities for membrane proteins using SS NMR.

There are SS NMR techniques available that measure precise through-space distances between close proximity NMR active nuclei. The preparation of ligands with isotopic labels at selected positions enables the measurement of intramolecular distances and these can be used to determine the conformation of the molecule.[1] We are using this approach to attempt the measurement of distances within a ligand that is bound to a nucleoside transporter and to probe distances between the ligand and isotopic labels incorporated into specific amino acids in the binding site of the protein.

Reference

1. Patching SG, Middleton DA, Henderson PJF, Herbert RB. *Org Biomol Chem* 2003; **1**: 2057.

SYNTHETIC ASPECTS OF C-DEUTERIATION OF ENOLATES

Gregory S. Coumbarides, Jason Eames, Stephanos Ghilagaber, Michael J. Suggate and Neluka Weerasooriya

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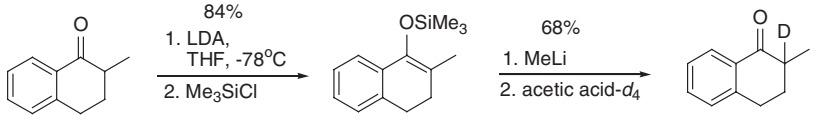
The continuing development of novel synthetic methodology for direct incorporation of non-radioactive isotopic labels within organic molecules for both chemical and biological mechanistic studies is paramount.[1] Some of this attention has been concerned with deuterium incorporation involving simple carbon–hydrogen bond exchange reactions,[2] many of which have relied on a deprotonation–deuteriation strategy involving relatively acidic centres,[3] most noticeably adjacent to a carbonyl group.[4]



([D]:[H]) = >98:<2

For synthetic ease, most H–D exchange reactions are usually performed under thermodynamic control[5] – by using an excess of the deuterium source, which generally is used as the solvent – to drive the reaction to completion.[6] However, there are some problems associated with this protocol, such as overall D-efficiency, cost and the difficulty associated with product separation (due to incomplete substitution or over incorporation). Whereas, deuteriation under kinetic control could potentially solve many of these problems, such as single incorporation of deuterium, but this type of methodology has been studied far less.

This lecture will focus on the deuteriation of a variety of structurally distinct enolates,[7] and will discuss factors[8] (such as the presence of additives and the structural nature of both the enolate and deuterium source) and comment on the role they play within the deuteriation step.[9]

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OPPORTUNITIES FOR ISOTOPE LABELLING VIA SOLID PHASE SYNTHESIS WITH ORGANOGERMANIUM LINKERS

Alan C. Spivey,[a] Chris Diaper,[b] Ratnasothy Srikaran,[b] Teyrnon Jones,[b] Catherine Noban,[a] George J. Ellames,[c] Andrew Kohler [c] and Harry Wadsworth[d]

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[d]*Amersham Health, Amersham, Buckinghamshire HP7 9LL, UK*

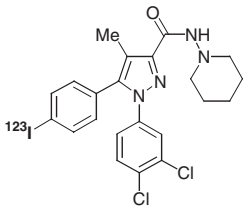
The development of germanium-based linkers for solid-phase synthesis will be outlined and their utility for the preparation of libraries of heterocyclic compounds overviewed.[1–3] Specific applications of this type of linker relevant to isotopic labelling procedures will then be presented. Specifically, three areas of work will be discussed:

Electrophilic ipso-iodo-degermylative linker cleavage for the synthesis of SR141716 analogues: SR141716 (Rimonabant) is a cannabinoid antagonist under development within Sanofi-Synthelabo. Our work towards the development of a solid phase synthesis applicable to the preparation of radio-iodinated analogues of this compound suitable for SPECT imaging will be discussed.

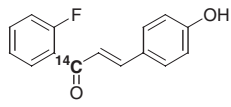
The devolatilisation of intermediates en route to SR47035: SR47035 is an advanced intermediate *en-route* to the Sanofi-Synthelabo 5-HT_2 antagonist, SR46439B. During the development of ^{14}C-labelled SR47035 volatility of a key fluoroacetophenone intermediate was noted. Our work towards the development of a solid phase synthesis of SR47035, circumventing the containment issues associated with the solution phase route, will be discussed.

Electrophilic ipso-fluoro-degermylative linker cleavage for the synthesis of 6-fluoro-L-DOPA: 6-[^{18}F]-L-DOPA is used clinically for the diagnosis of Parkinsons disease by PET. The commercial synthesis of this compound employs electrophilic *ipso*-fluorodestannylation of a 6-trimethylstannyl-L-DOPA derivative in solution followed by rapid HPLC purification to remove toxic tin by-products. Our work towards the development of a method for the synthesis of 6-[^{18}F]-L-DOPA via electrophilic *ipso*-fluorodegermylation of an

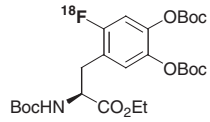
L-DOPA derivative bound to a solid support by a germanium linker will be discussed.



[I^{123}]-SR141716 analogue



[^{14}C]-SR47035



6-[^{18}F]-L-DOPA derivative

References

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LABELLED METHYLLYCACONITINE (MLA) – A KEY NICOTINIC ACETYLCHOLINE RECEPTOR LIGAND

Ian S. Blagbrough

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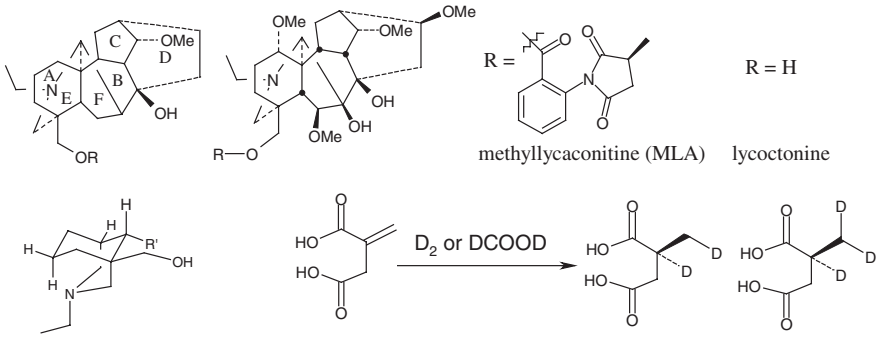
In this presentation, I will cover the design of practical synthetic organic routes to norditerpenoid alkaloids (*N*-ethylpiperidines in the lycoctonine class), regioselective deuteriation, and tritium incorporation, within the area of biochemical probe studies. We have completed such a project by making methyllycaconitine (MLA), the most potent and selective, competitive nicotinic acetylcholine receptor (nAChR) antagonist. As a small molecule, MLA is an important lead compound in current pharmaceutical and agrochemical nAChR studies at neuronal target sites. Many insecticides work at nAChR and much current pharmaceutical research interest is focussed at neurodegeneration and understanding nAChR in neurochemistry. As a labelled probe, MLA is of significance for highlighting and for selectively blocking the $\alpha 7$ nAChR. A tritiated or radio-iodinated MLA probe will be an important biochemical probe. In our design of the synthesis of such probes, we disconnected the key neopentyl ester of the norditerpenoid alkaloid to afford an accessible anthranilate moiety. We also carried out a mild hydrolysis of the succinimide moiety, in the presence of the ester. We had to determine the unknown stereochemistry at the key methine carbon carrying the methyl group in MLA (the methyl substituent on lycaconitine, from where MLA takes its name). We achieved this by double menthyl ester diastereoisomer formation and detailed comparative NMR and HPLC studies. This is an *S*-stereo centre. We then achieved the semi-synthesis of cold and of labelled MLA, introducing this key stereo centre by a chiral hydrogenation with H_2 gas and by transfer hydrogenation from formic acid. We performed the deuteriation as a model reaction for the tritiation. One key feature in the preparation of the labelled probe is that 2.4 deuterons (on average) are incorporated per MLA molecule. However, as well as achieving regioselective deuteriation, here we uncovered a new chemical mechanism. Furthermore, these results were reproducible by Tocris in their synthesis of this important radio-labelled probe containing at least two tritium atoms.



These studies were generously funded by grants from the Wellcome Trust.

CHALLENGES IN THE PREPARATION OF RADIOLABELLED DRUGS FOR NUCLEAR MEDICINE

James R. Ballinger

Department of Nuclear Medicine, Guy's and St Thomas' Hospital, St Thomas Street, London SE1 9RT, UK

Nuclear medicine involves the administration of drugs labelled with a gamma- or positron-emitting radionuclide for diagnosis of disease. Beta-emitting radiopharmaceuticals are used therapeutically.

The most commonly used radionuclide for imaging, technetium-99 m (^{99m}Tc), has a half-life of 6 h which means it has to be prepared on a daily basis. ^{99m}Tc is obtained on-site by decay of the parent radionuclide molybdenum-99 ($t\ \frac{1}{2}$ 3 days). Longer-lived gamma-emitters are supplied ready to use via overnight couriers from commercial manufacturers. Positron-emitters generally have $t\ \frac{1}{2} < 2$ h and are prepared in a cyclotron on-site.

The challenges in preparation of radiopharmaceuticals on a routine basis include: *half-life*: the short half-lives necessitate rapid synthesis, purification, formulation, and quality control; *radiation exposure*: require shielding and remote operation or robotics where possible to minimize radiation dose to the operator; *sterility*: most radiopharmaceuticals are administered by intravenous injection and final preparation must be under EC-GMP Grade A conditions.

^{99m}Tc radiopharmaceuticals are generally prepared in a single step from commercially available 'kits' which contain all the non-radioactive ingredients (ligand, reducing agent, stabiliser) in sterile and apyrogenic form. Solutions of ^{99m}Tc are transferred between lead-shielded septum-sealed vials manually by syringe using a lead or tungsten syringe shield with a leaded-glass window to allow volumes to be read. Most of the labelling reactions take place with $>95\%$ efficiency in a few minutes at room temperature and no further processing is involved. Labelling efficiency can be checked by instant thin-layer chromatography techniques or with solid-phase extraction cartridges.

Preparation of positron-emitting radiopharmaceuticals is much more complex, usually multi-step and sometimes requiring purification of intermediates by low- or high-pressure column chromatography. Often the solvent used for separation must be removed by evaporation prior to reconstitution for injection. The short half-lives necessitate starting with large amounts of radioactivity in order to have an adequate dose for administration at the end of the process. Preparation is usually carried out remotely within a

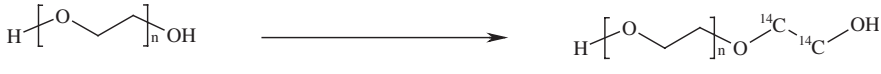
shielded hot cell. For the most commonly required radiopharmaceuticals, 'black boxes' are available with disposable modular components. Some aspects of radiopharmaceutical preparation have not changed in 30 years, while others have continually improved. Both novel chemistry and innovative technology have contributed to the development of the broad range of radiopharmaceuticals now available and the reliability with which they can be produced.

THE SYNTHESIS OF CARBON-14 LABELLED PEGS

Gary Shemilt, R Gordon Reid, Philip D Wilde and Andrew Mangion

Amersham Biosciences, Forest Farm Whitchurch, Cardiff CF14 7YT, UK

Molecules can be modified by the addition of polyethylene glycols (PEGs) to give the new molecule different characteristics. Amersham is often asked to label PEGs with low polydispersity ratios (sometimes approaching 1) without affecting the molecular weight distribution. This generally involves the addition of one carbon-14 labelled monomer unit and sometimes isolation of the $n + 1$ chains. It was necessary to develop a method that did not allow the polymer chains to propagate or the monomer to polymerize to generate its own low molecular weight fragments. Details of the methodology and the advantages this gives to the molecular weight characteristics of the final labelled product will be provided.



DEVELOPMENT OF PALLADIUM CATALYSED CARBONYLATIONS FOR ASYMMETRIC SYNTHESIS

Julian G. Knight

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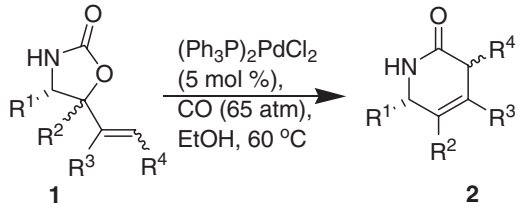
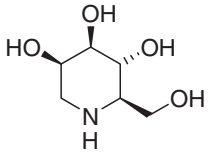
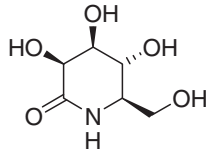
We have found that the carbonylation of 5-vinyloxazolidinones **1** gives δ-lactams **2** rather than the expected β-lactam. The carbonylation allows a simple route from amino acid derivatives to chiral piperidine derivatives and has been used as the key step in the total syntheses of piperidine alkaloids deoxymannojirimycin and mannolactam, and towards the synthesis of the indole alkaloid lysergine. Modification of the vinyloxazolidinone substrate has allowed the development of a low pressure carbonylation and a new route to isoquinolines.



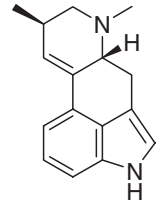
1 **2**



Deoxymannojirimycin **3**



D-Mannolactam **4**



Lysergine **5**

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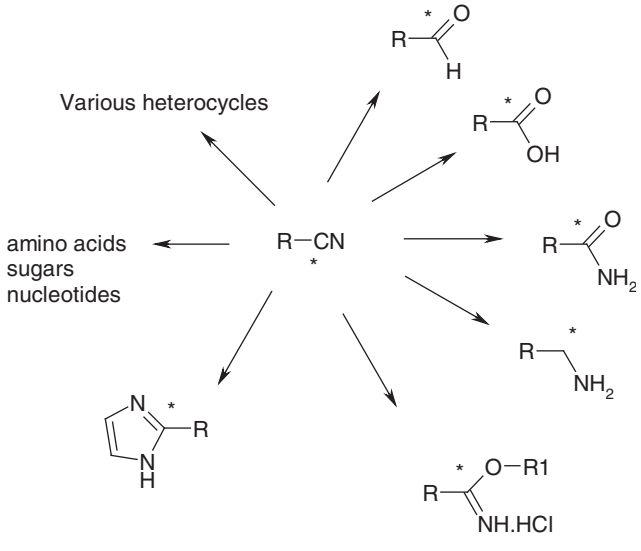
THE VERSATILITY OF LABELLED CYANIDE

Neil J. Geach and Mark F. Oldfield

SCYNEXIS Europe Limited, Fyfield Business and Research Park, Fyfield Road, Ongar, Essex CM5 0GS, UK

For many years radiochemists have made use of readily available ^{13}C and ^{14}C-building blocks to prepare radiolabelled drugs and agrochemicals. We at SCYNEXIS are no different in wanting to make use of ^{13}C or ^{14}C labelled cyanide. There is a vast array of transformations[1] that can be carried out on the cyano group in order to prepare single labelled intermediates and final compounds required by our customers.



The presentation describes some work by SCYNEXIS on the incorporation of the cyano group into a number of compounds and their conversion to some useful labelled intermediates.

Reference

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POSTER ABSTRACTS ON THE STABILITY AND STORAGE OF ^{14}C-LABELLED COMPOUNDS

Jens Atzrodt and Claudia Loewe

Aventis Pharma Deutschland GmbH, DI&A, Chemical Development, Global Radiosynthesis Center, Frankfurt, Germany

Radiolytic decomposition of labelled compounds is a well known though not completely understood phenomenon. Increasing the knowledge about this specific decomposition and identifying suitable storage conditions and precautionary measures is a continuing challenge to all involved in synthesis and application of radiolabelled compounds. We want to contribute by sharing recent experience with ^{14}C-labelled material. We refer to published theory and experimental studies that successfully lead to some rules for stabilization of radiolytically challenged compounds.[1] We describe examples showing the effects of radiolysis,[2] including mechanism,[3] classification[4] and attempts for quantification[5] of decomposition and typical parameters influencing this phenomenon. Examples are also presented for successful modification of storage conditions in order to achieve reasonable stabilization.

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THE PREPARATION OF THE P_2 RECEPTOR AGONIST 2-[METHYL-^{3}H]METHYLTHIOADENOSINE-5′-DIPHOSPHATE

Michael R. Chappelle and Calvin R. Hawes

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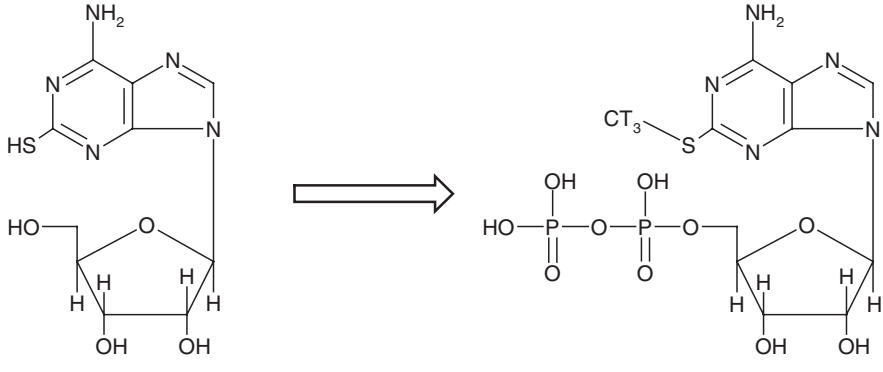
There are two types of purinoceptor, namely P_1 and P_2. The P_1 receptors are nucleoside activated with agonists such as adenosine and have been widely studied with more agonists and antagonists for each receptor sub-type.

The P_2 receptors are activated by nucleotides with agonists such as adenosine triphosphate. There are a large number of P_2 receptor sub-types and the P_2 receptor agonist,[1] 2-[methyl-^{3}H]methylthioadenosine-5′-diphosphate (2), may help in the study and understanding of some of these P_2 receptor sub-types.



(1) (2)

The poster details the multi-stage synthesis of 2-[methyl ^{3}H]methylthioadenosine-5′-diphosphate (2), from 2-thioadenosine (1) and high specific activity [^{3}H]methyl iodide.

Reference

1. Cusack NJ, *et al. Br J Pharmacol* 1982; **77**: 329.

TRITIUM LABELLING OF AROMATIC AND ALIPHATIC ALDEHYDES USING CRABTREE'S CATALYST , $[IR(COD)PY(PCY_3)]PF_6$

Michael R. Chappelle and Alan D. Morgan

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The iridium catalysed regiospecific ring labelling of aromatic compounds with tritium ortho- to certain coordinating groups such as ketones, esters, amides and certain heterocyclic groups has been well documented.[1–5] However, there are few if any literature reports on the iridium catalysed tritium exchange-labelling of aldehydes, useful intermediates in Knoevenagel and similar condensation reactions. This work describes the tritium labelling of both aromatic and aliphatic aldehydes using Crabtree's catalyst, $[Ir(cod)py(PCy_3)]PF_6$.

Aromatic aldehydes were labelled to high specific activities (22–74 Ci/mmol) in both the ring and formyl group except in cases of diortho-substituted rings or sterically hindered rings when exclusive formyl labelling was observed (8–24 Ci/mmol). Aliphatic aldehydes were labelled to high specific activities (>20 Ci/mmol) in the formyl group.

Ring-labelling of aromatic aldehydes can be explained in terms of a 5-membered intermediate complex formed by the iridium, ortho- ring-carbon and hetero atom as postulated by Heys.[3] However, labelling of the formyl group in both aromatic and aliphatic aldehydes must be occurring by a different mechanism since formation of a 5- membered intermediate would not be possible.

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THE SPECIFIC ACTIVITY DETERMINATION AND CONFIRMATION OF IDENTITY OF A RADIOLABELLED PEPTIDE USING TANDEM MASS SPECTROMETRY

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The confirmation of the integrity of any product is an essential element of any manufacturing process. The production of radiochemicals is no exception. Traditionally three fundamental quality criteria, namely radiochemical purity, chemical identity and specific activity are considered important for radiochemicals. The purpose of this poster is to demonstrate that modern instrumentation and techniques are now able to reveal far more information about a molecule. Amersham Biosciences, in support of opiate receptor research, manufactures [leucyl-^{3}H] Nociceptin. It is prepared by the tritiation of [dehydro-Leu] Nociceptin using tritium gas over a palladium catalyst:

Phe-Gly-Gly-Phe-Thr-Gly-Ala-Arg-Lys-Ser-Ala-Arg-Lys-[dehydro Leu]-Ala-Asn-Gln

Tritium gas/Pd ↓

Phe-Gly-Gly-Phe-Thr-Gly-Ala-Arg-Lys-Ser-Ala-Arg-Lys-[^{3}H]Leu-Ala-Asn-Gln

Specific activity[1–3] of a radiolabelled compound and specificity of the labelled position is of considerable importance to the research scientist. Until now technology has only allowed us to establish the amount of radioactivity associated with the molecular ion and provided no real structural confirmation or indication of the site of labelling. Utilising the power of tandem mass spectrometry we can now quantify and demonstrate the position of the radiolabel within the structure.

The chosen example, [leucyl-3H] Nociceptin has sites, certainly within the benzyl group of the phenylalanine and quite possibly the arginine and lysine moieties, for labile exchange to occur. Ideally, to satisfactorily demonstrate that this situation has not arisen, the peptide needs to be sequenced and the isolated residues carefully examined for evidence of any label within its isotope pattern.

Unfortunately not all peptides give satisfactory MS-MS spectra, Nociceptin is one of those. In this instance they require digestion with an enzyme and chromatographic separation of the resulting fragments prior to

MS-MS analysis. This work describes the use of on-line liquid chromatography electrospray tandem mass spectrometry to achieve this (LC-ESI-MS-MS).

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DESIGN AND APPLICATION OF STABLE OR RADIOLABELLED CARBAMATES

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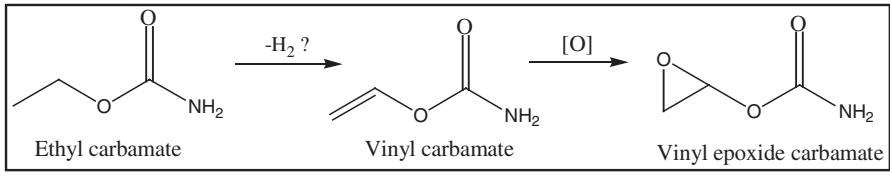
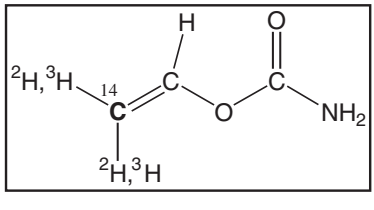
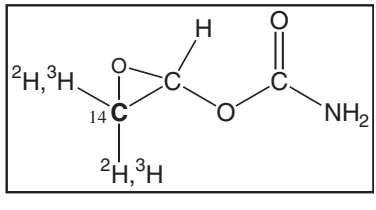
The studies of Scherer[1] and Miller[2] have led to the hypothesis that urethane (ethyl carbamate) is converted *in vivo* to vinyl carbamate, which is then epoxidized by the microsomal P-450 dependent mono-oxygenase system to vinyl epoxide carbamate, which is the *ultimate carcinogen*.



To elucidate the mechanism by which urethane is activated, potential metabolites which include the labelled vinyl and vinyl epoxide carbamates are required to be synthesized. This allows for investigations into the chemical and biological behaviour of each compound. Studies[3] to date have established novel synthetic routes to specifically labelled (^{2}H and ^{13}C) vinyl carbamate and their epoxides. This synthetic methodology can be applied to give the corresponding ^{3}H and ^{14}C vinyl and vinyl epoxide carbamates.

Labelled vinyl and vinyl epoxide carbamates





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SYNTHESIS OF ISOTOPOMERS OF THE MHC II ANTAGONIST AZD2315

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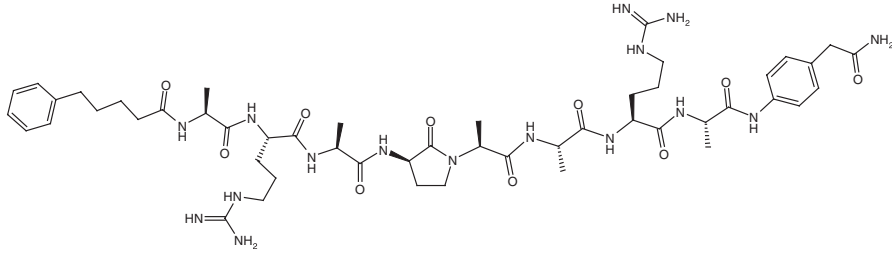
AZD2315 **(1)** is a major histocompatibility class II (MHC II) antagonist being developed primarily for the treatment of rheumatoid arthritis.

This presentation describes the synthesis of the various isotopomers of AZD2315 that were required during the development programme. The drug has been labelled with ^{14}C using both D-[ring carbonyl-^{14}C]gammalactam (derived from D-[1-^{14}C]methionine) and 4-aminophenyl[1-^{14}C]acetic acid and with ^{2}H in each alanine residue from L-[3,3,3-2H]alanine. A high specific activity ^{3}H labelled form was prepared by reduction of the corresponding phenylbutadiene derivative with tritium gas. All labelled and unlabelled peptides were prepared by the Fmoc solid phase synthesis approach.



PHV-Ala-Arg-Ala-[D-Gammalactam]-Ala-Arg-Ala-PAPA-NH_2

(1)

SYNTHESIS OF ISOTOPOMERS OF GEFITINIB

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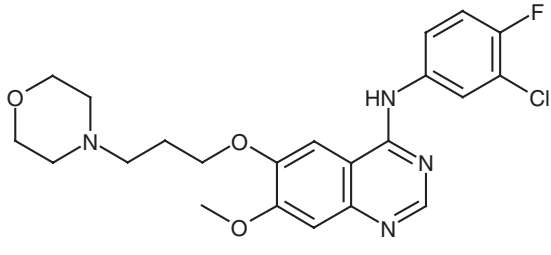
Gefitinib (IRESSA^{TM}, AstraZeneca ZD1839) **(1)** is the first in a new class of anti-cancer drugs known as Epidermal Growth Factor Receptor (EGFR) inhibitors. Gefitinib targets and blocks, within the cell, signalling pathways that are implicated in the growth and survival of cancer cells. Gefitinib was approved in Japan for the treatment of inoperable or recurrent non-small cell lung cancer in July 2002.

This presentation describes the synthesis of the various isotopomers of gefitinib that were required during the development programme. The drug has been labelled with ^{14}C from 3-(4-morpholino)[2,3-^{14}C]propyl chloride and 3-chloro-4-fluoro[U-^{14}C]aniline and with ^{2}H from d_8- morpholine. High specific activity ^{3}H labelled forms were prepared by tritium-iodine exchange of an iodinated precursor and by tritiomethylation of an *O*-desmethyl precursor using [^{3}H]-methyl iodide.



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MERCURATION/IODODEMERCURATION/DEIODINATION: A PRACTICAL FIRST-LINE STRATEGY FOR THE RAPID PREPARATION OF IODINATED LABELLING PRECURSORS

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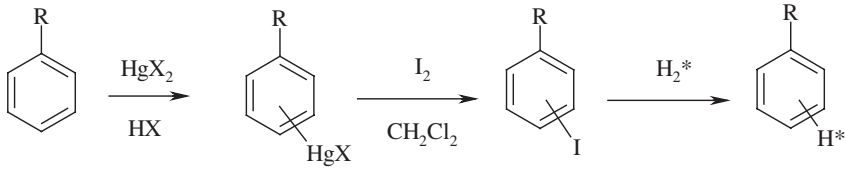
Labelling of molecules by electrophilic halogenation followed by reductive dehalogenation with isotopic hydrogen gas is a well-tried and successful approach to the preparation of hydrogen-isotope labelled organic compounds. However the method often fails with substrates of low nucelophilicity due to the need to use a powerful halogenation medium (e.g. bromine or iodine plus an oxidising agent). We have investigated an alternative approach using an intermediary mercuration[1,2] step and exploiting the high reactivity[3] achievable for the mercury electrophile when the reaction is carried out in perfluorinated organic acids.



HX= CF_3CO_2H or $CF_3CF_2CF_2CO_2H$

Under these conditions the rapidity and ease of electrophilic mercuration combined with the low steric requirements and low oxidising power of the mercurating species make the approach particularly applicable for the small-scale, one-pot, preparation of iodinated precursors from both activated and deactivated aromatics.

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DEUTERIUM LABELLING WITH CYCLOOCTA-1,5-DIENYLIRIDIUM(I)1,1,1,5,5,5-HEXAFLUOROPENTAN-2,4-DIONATE: A COMPARISON OF D_2O AND D_2 ISOTOPE DONORS

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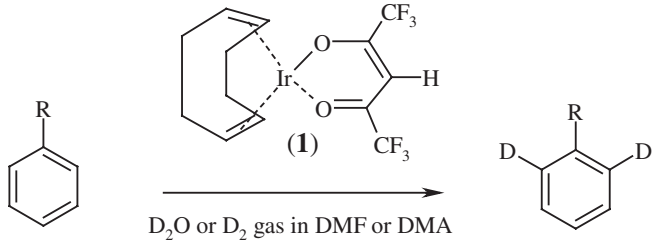
Cycloocta-1,5-dienyliridium(I)1,1,1,5,5,5-hexafluoropentan-2,4-dionate (**1**) is an efficient catalyst[1,2] for the *ortho*-labelling of aromatic substrates which possess suitable directing substituents (Scheme). However the ability of substituents to direct the labelling has been shown to vary radically, depending upon which isotope donor, D_2 or D_2O, is utilized.



R = Directing group for *ortho*-metallation.

The implications of these results for the mechanism of the labelling process and the nature of the active species in the reactions will be discussed. In addition, the resulting opportunities for the preparation of deuterium and tritium labelled compounds via isotope-exchange using this catalyst will be presented.

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MARTIN D. KAMEN (1913–2002): THE CO-DISCOVERER OF CARBON-14

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Martin D. Kamen, Emeritus Professor of Chemistry at both the University of California, San Diego and the University of Southern California died on August 31, 2002 at his Montecito California home.

An accomplished scholar and musician, all of these achievements paled in significance to his momentous discovery of Carbon-14 with Samuel Ruben at the Berkeley Ernest Lawrence Laboratory on February 27, 1940.

Given the extraordinary impact that his discovery catalyzed in so many areas of science and the later Nobel Prize winning work it spawned, it is indeed puzzling that he was not also recognized with this the arguably highest of scientific accolades.

As captured in the title of his autobiography, *Radiant Science, Dark Politics – A Memoir Of The Nuclear Age,* his life was punctuated not only with this most brilliant discovery, but also victimized by the irrational fear and prejudice that touched so many in post World War II America.

This poster will chronicle the story of Martin D. Kamen, his incredibly key discovery and later brave and turbulent life.

AN EFFICIENT SYNTHESIS OF [2-^{14}C] ETHYL NIPECOTATE

Giliyar Ullas and Terry Kelly

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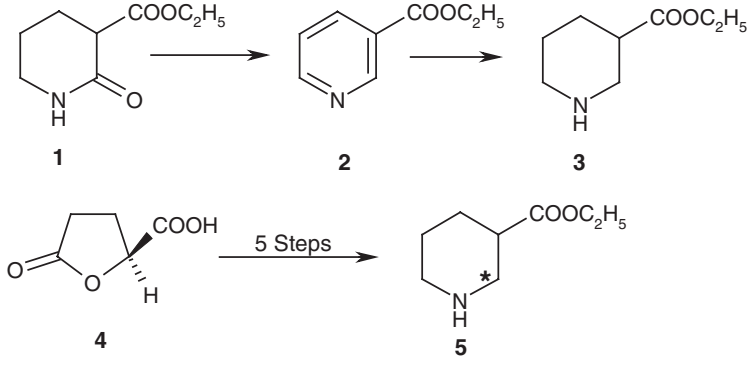
Ethyl 2-oxo-3-piperidinecarboxylate **1** has been reported to be the precursor for nicotinic acid and related compounds **2**. Compound **1** can also be the starting material for ethyl nipecotate **3**. Herein we report a more efficient synthesis of ethyl [2-^{14}C]nipecotate **5** from commercially available 5-oxo-2-tetrahydrofurancarboxylic acid **4** and sodium [^{14}C]cyanide. Details of this five-step synthesis will be presented.



RADIOSYNTHESIS CONDUCTED UNDER CGMP COMPLIANCE

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In the development of drugs for human use, regulatory authorities require that absorption, distribution, metabolism, and excretion (ADME) studies be conducted prior to the initiation of clinical trials. It is common to label drug candidates with radioactivity as a tool to measure candidate performance in these studies.

Clinical trial materials must be manufactured in compliance with current Good Manufacturing Practice (cGMP). Consistent with the stage of clinical development, the intent of these requirements also apply to the synthesis of radioactive drug substances. This has compelled the creative integration of cGMP principles with the many challenges already inherent in radiosynthesis and analysis. Also, because of the time sensitive nature of these expensive human studies, a further demand facing organizations conducting cGMP projects is the adherence to strict and absolute deadlines.

Our laboratories have performed cGMP synthesis and analysis for decades and have participated in the evolution of this important service for the pharmaceutical industry. This presentation will describe the features and scope of our current cGMP radiosynthesis process as well as our perspective in this uniquely important technical area.

COMPUTER-SIMULATIONS FOR THE REACTOR-SYNTHESIS OF HIGH-ACTIVITY, HIGH SPECIFIC ACTIVITY, TELETHERAPY SOURCES OF EUROPIUM-152

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We present results from computer analyses and simulations performed, partly on our behalf, by workers at the US Department of Energy's Pacific Northwest National Laboratory in Hanford, Washington, USA. These confirm reactor parameters needed to produce clinically useful total and specific activities of europium-152. Reactor variables were principally chosen to ensure markedly lower costs for teletherapy sources of europium-152 when compared with cobalt-60.

Earlier papers[1–4] proposed ^{152}Eu to succeed ^{60}Co in external-beam radiotherapy, as cobalt sources have, in recent years, become both more costly and less available. Data in support of that proposal are also displayed including depth dose tables and curves comparing the clinical value of the two isotopes. The parameters have been optimized for specific activities from 3 to 4 terabecquerels per gram (80–110 Ci g^{-1}) of target material in order to allow encapsulated activities between 600 and 800 TBq ($\approx$ 16 000–22 000 Ci) in such form that the source capsules themselves may, *without alteration*, be freely exchanged for exhausted capsules of cobalt-60 in existing teletherapy machines throughout the world. Such capsules containing intrinsic filtration for hardening the γ-spectrum to >0.6 MeV could be relied upon to deliver between 1 and 5 Gy min^{-1} of $\bar{1}$14 MeV gammas at 80 cm to 1 m source-to-skin distance (SSD) over a useful lifetime of 15–20 years. The simulations assumed the use of europium nitride, enriched to $>92\%$ ^{151}EuN by low-cost plasma separation (PSP). The simulations confirm practical reactor irradiation times <45 days for 'ready-to-use' pre-encapsulated ^{151}Eu targets vs 2 to 4 years of irradiation for bulk pellets of ^{60}Co.

Rough figures are presented to show that very high activity sources can be made whose cost in real terms to a purchasing hospital is likely to fall between 4 and 8% that of cobalt-60, considering useful lifetimes and γ-beam collimation; or alternatively, between 0.5 and 1% of a clinical accelerator, when the costs of engineering support for hospital accelerator installations are included. Savings of that magnitude are especially poignant viewed from the standpoint of Third World nations.[5]

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PARALLEL OPTIMISATION OF HYDROGEN ISOTOPE EXCHANGE MEDIATED BY HOMOGENEOUS CATALYSTS: A COLLABORATION BETWEEN THE ISOTOPE GROUPS AT MöLNDAL AND CHARNWOOD R&D

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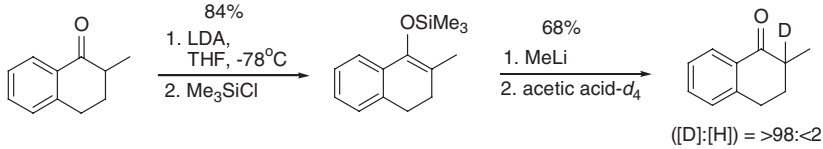
Compounds containing the structural feature depicted in Scheme 1 are of interest to our research at AstraZeneca R&D Mölndal. To support this work, there was a requirement for radiolabelled material, however, our initial efforts using exchange methodology[1,2] were hampered by poor yields and low isotopic incorporation producing insufficient quantities of the tritiated product. Following discussions with colleagues at AstraZeneca R&D Charnwood, an approach involving the parallel evaluation of a range of homogeneous catalysts, using different stoichiometries and solvents was adopted.



Scheme 1.

Over 60 experiments were performed using different catalysts and either DMA (N,N-dimethylacetamide) or DCM (dichloromethane) as solvent together with deuterium gas. From this initial round, the best reaction conditions were taken and used in a further round of optimization involving 11 more parallel experiments before going into the hot lab.

As a prelude to running the hot reaction, an identical experiment was conducted using the Tritec manifold and substituting deuterium for tritium gas. The catalyst/substrate complex (15 μmol substrate, 100 mol% Crabtree's catalyst in 3 ml DMA) was pre-reduced with hydrogen for 2 h before re-evacuating and charging with 1115 mbar of deuterium gas and then left to stir overnight; an isotopic incorporation of 72% was observed by LC/MS. Using the same conditions with tritium gas, afforded an isotopic incorporation of 62% as observed by LC/MS.

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NEW OPPORTUNITIES FOR ISOTOPE LABELLING USING SOLID PHASE ORGANIC SYNTHESIS

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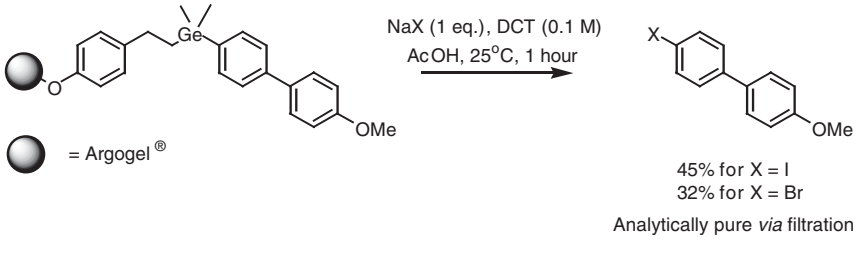
[c]*Isotope Chemistry and Metabolite Synthesis, Sanofi-Synthelabo, Alnwick Research Centre, Alnwick, UK*

Radiolabelled organic compounds have many industrial uses. These include medical applications (as both diagnostic and therapeutic agents) and in the investigation of pharmaceutical drug metabolism. The synthesis of such compounds is generally accomplished using 'traditional' solution syntheses but can be complicated by the need to incorporate a label at a specific molecular location, using a limited range of radioactive reagents that must be carefully handled in order to prevent environmental damage.

This poster describes Ge polymer based solid phase organic synthesis as a convenient route to access potentially radiolabelled compounds. Such an approach can help avoid some of the problems mentioned above, such as the containment of volatile radiolabelled intermediates, as well as providing an operationally simple route to radiohalide labelled compounds.



RAPID SYNTHESIS OF ^{11}C-FLUMAZENIL IN A MICRO-REACTOR PLACED IN THE HPLC LOOP: A FAST CAPTIVE SOLVENT METHOD

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Neurology Unit and Wolfson Brain Imaging Centre, University of Cambridge, Cambridge, UK

Objectives: Two microreactors which can be built in minutes were tested for the preparation of ^{11}C-flumazenil using the capture solvent technique.

Method: Reactor **1** is a commercially available HPLC column (30 mm × 2.1 mm) and reactor **2** is a custom-made column (10 mm × 2 mm). Both are packed with stainless steel powder (< 45 μm). The reactor is placed in the position of the HPLC injector sample loop (using low volume Peek tubing). 100 μg of precursor in 28.5 μl DMF and 1.5 μl 10 M aqueous KOH are injected into the reactors. A stream of helium is briefly passed through the reactor which elutes a proportion of the precursor solution out of the loop. ^{11}C-MeI produced at high specific radioactivity by a MeI MicroLab (GE Medical Systems) is released in a stream of helium through the reactor. After delivery of the ^{11}C-MeI HPLC purification is started.

Results: The trapping efficiency of reactor **1** is excellent (90%), with the smaller reactor 2 somewhat less efficient (70%). On average only 60 and 25 μg are retained by the reactors **1** and **2**, respectively. The yields are between 6–8 GBq which demonstrates a highly efficient use of precursor. The small amount of precursor used allows rapid HPLC purification to be performed on an analytical C18 column using an eluent of ethanol/water (15/85 v/v). The product elutes after 2.5 min in a volume of only 2.75 ml. The combination of using ethanol as co-solvent and the small peak volume means that only saline has to be added to the product to make it ready for injection.

Conclusion: The combination of using ^{11}C-MeI of high specific radioactivity with rapid synthesis and purification (20 min after EOB) produces ^{11}C-flumazenil at very high specific radioactivity; up to 15 Ci/μmol. These are ideal levels for imaging receptor systems in small animals using the MicroPET scanner. In addition the highly efficient use of precursor helps in its conservation. We aim to apply this method for the synthesis of other radioligands to be used in MicroPET studies.

SPATIOTEMPORAL IMAGING OF RECEPTOR SYSTEMS USING PET

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Seven trans-membrane G-protein coupled receptors are targeted by more than 50% of the currently used drugs. We have discovered in the human vasculature a number of these novel peptide receptor systems, originally predicted from the human genome, that may become targets for new therapies. The aim of our work is to understand the role of these vasoactive peptides and their receptor sub-types in the human cardiovascular system and to quantify changes associated with disease. Endothelin-1 (ET-1) is the most potent constrictor of human vessels with a unique long lasting action. We have established that ET-1 causes vasoconstriction in humans via the ET_A receptor but the role of the ET_B sub-type is unclear. The ET system (including the precursor peptide, big ET-1) is upregulated in disease. Two strategies are being pursued to prevent the vasoconstrictor actions of ET-1: selective receptor antagonists or blocking synthesis of ET-1 from big ET-1 by inhibition of endothelin converting enzyme (ECE).

Vascular receptors for peptides are a diverse group of potential drug targets that have not been studied extensively using positron emission tomography (PET) owing to the lack of suitable radioligands. Our objective is to use PET to study the pharmacodynamics and pharmacokinetics of these receptor systems *in vivo* and for this purpose we have established a generic method for ^{18}F-labelling of peptides by conjugation with *N*-succinimidyl 4-[^{18}F]fluorobenzoate. Using this reagent we have successfully labelled ET-1[1] and big ET-1[2] in the Lys^9 group.

Using [^{18}F]-ET-1, we have dynamically imaged ET receptors *in vivo* for the first time and followed modulation of ligand binding by drug antagonists for up to two hours. The combination of the ^{18}F radionuclide with the improved resolution and sensitivity of a small animal tomograph (microPET) has permitted a remarkably detailed anatomical distribution of the receptors to be

visualized in organs such as the kidney. We have discovered that selective blockade of the ET_B receptor prevents ET-1 from binding in the lungs: thus the ET_B sub-type has a beneficial function as a 'clearing receptor' removing the peptide from the plasma, confirming our hypothesis predicted from *in vitro* studies that the optimum pharmacological profile for an ET blocker is ET_A selectivity.

High circulating levels of big ET-1 are present in heart failure and ECE activity is upregulated in atherosclerosis. Big ET-1 is not physiologically active but must be converted to the mature peptide by cleavage of a unique scissile bond catalysed by ECE. Therefore conversion of [^{18}F]-big ET-1 by ECE to [^{18}F]-ET-1 can be visualized as binding to ET receptors. We hypothesized that significant tissue specific conversion may occur within the vasculature, leading to increase vasoconstriction. Using [^{18}F]-big ET-1, we demonstrated conversion and binding to blood vessels which could be blocked by inhibitors of ECE, analogous to the strategy of ACE inhibition.

This work demonstrates for the first time the imaging of a vascular peptide receptor system using a dedicated small animal tomograph and ^{18}F-labelled ligands. Furthermore, it shows the value of an animal model informed by a receptor system that has been well characterized in humans and the potential for studying other emerging orphan receptor systems such as apelin, ghrelin and urotensin II facilitating the rapid translation of information from the human genome into function.

References

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