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Abstracts

14th International Isotope Society (UK group) Symposium

Synthesis & Applications of Labelled Compounds 2004

P. S. Aburel, F. Aigbirhio, E. Alexakis, H. Audrain, C. A. Austin, C. Barry, D. Bender, N. Bushby, K. Cable, M. A. Carroll, H. Deng, G. Ellames, I. Fellows, J. M. Gardiner, N. J. Geach, A. D. Gee, M. Gerhard, E. J. Guthrie, D. W. Hamprecht, J. R. Harding, R. C. Hartley, S. J. Harwood, J. M. Herbert, M. J. Hickey, J. R. Jones, L. M. Kamara, L. P. Kingston, K. W. M. Lawrie, R. J. Lewis, A. Lockhart, W. J. S. Lockley*, J. Macritchie, R. MacGlinchey, C. Macleod, L. Martarello, A. N. Mather, J. C. Matthews, B. M. McAuley, G. J. McKiernan, A. McNeill, V. Murrell, D. O'Hagan, M. F. Oldfield, N. Panchal, J. Passchier, V. W. Pike, C. F. Roberts, D. C. Rustidge, T. Smith, W. Stimpson, K. Taylor, D. A. Widdowson, C. L. Willis, D. J. Wilkinson, I. Wilson, W. Zinsser

Meeting Summary

The 14th annual symposium of the International Isotope Society's United Kingdom Group took place at the Wellcome Genome Campus, Hinxton, Cambridge, UK on Thursday 4th November 2004. The meeting was attended by around 100 delegates from academia, life sciences and fine chemical companies.

Delegates were welcomed by Professor John Jones (University of Surrey, UK). 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- and long-lived isotopes were represented, as were stable isotopes. The symposium programme was divided into a morning and afternoon session

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chaired by Professor Christine Willis (University of Bristol, UK) and Dr Franklin Aigbhirio (Wolfson Brain Imaging Centre, University of Cambridge, UK), respectively. The meeting was concluded with remarks from Dr Karl Cable [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 and will remain unchanged for the next IIS UK group symposium which is planned for 3rd November 2005.

Meeting Programme

Prof David O'Hagan [University of St Andrews, UK]—*Enzymatic C–^{18}F Bond Synthesis: A New Strategy for PET Synthesis.*

Dr Michael Carroll [University of Newcastle, UK]—*Studies Towards 6-[^{18}F]Fluoro-m-tyramine using Iodonium Salts.*

Dr John Gardiner [University of Manchester, UK]—*Methods Towards Isotopomer-Versatile Synthesis of ^{13}C-Labelled Carbohydrates.*

Prof William Lockley [University of Surrey, UK]—*Some New Catalytic Systems for Isotope-Exchange Labelling.*

Dr David Rustidge [Scynexis Europe, UK]—*^{14}C-Synthesis—Not Always as Easy as it Looks.*

Dr Richard Hartley [University of Glasgow, UK]—*The Potential for using Titanium Alkylidene Chemistry in Solid-Phase Radiochemical Synthesis.*

Dr Ian Wilson [Turku Imanet, Finland]—*Transition of PET Tracers from Clinical Research Tools to Commercial Diagnostics.*

Dr Simon Harwood [GlaxoSmithKline, UK]—*The Synthesis of Stable Labelled Ketamine.*

Dr John Herbert [Sanofi-Aventis, UK]—*Towards Robust Conditions for Iridium-mediated Exchange.*

Dr Conor Barry [University of Bristol, UK]—*Total Synthesis of Clavosolide A.*

ABSTRACTS: ORAL PRESENTATIONS

ENZYMATIC C–^{18}F BOND SYNTHESIS: A NEW STRATEGY FOR PET SYNTHESIS

David O'Hagan[a], Hai Deng[a], Laurent Martarello[b], Anthony D. Gee[b], Andrew Lockhart[b] and Ryan MacGlinchey[a]

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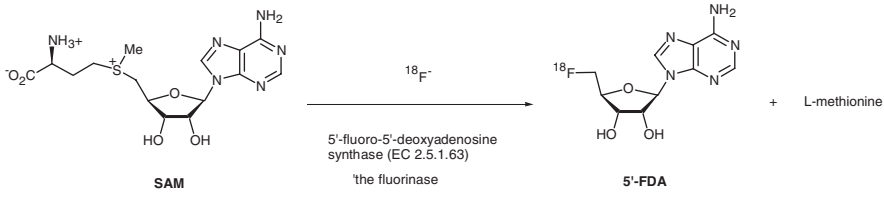
New and efficient methods for carbon–fluorine bond synthesis are much in demand to facilitate the growing interest in positron emission tomography (PET). The recent isolation[1] of a fluorination enzyme from *Streptomyces cattleya* has opened up prospects, for the first time, for a biocatalytic approach to the synthesis of carbon fluorine bonds from inorganic fluoride. The enzyme converts fluoride ion and *S*-adenosyl-*L*-methionine (SAM) into 5′-fluoro-5′-deoxyadenosine (5′-FDA) plus *L*-methionine. Our first radiolabelling investigations[2] gave rise [^{18}F]-5′-FDA with a radiochemical yield of ~1%.



The fluorinase enzyme has now been purified, cloned and crystallized[3] and this has further improved its prospects as a catalyst for radiochemical labelling with fluorine-18. Optimization of this reaction has now allowed [^{18}F]-5′-FDA to be prepared in radiochemical yields above 50%, very competitive with synthetic methods. Our latest results on this method will be reported.

References

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STUDIES TOWARDS 6-[^{18}F]FLUORODOPA USING IODONIUM SALTS: PREPARATION OF 6-FLUORO-*m*-TYRAMINE

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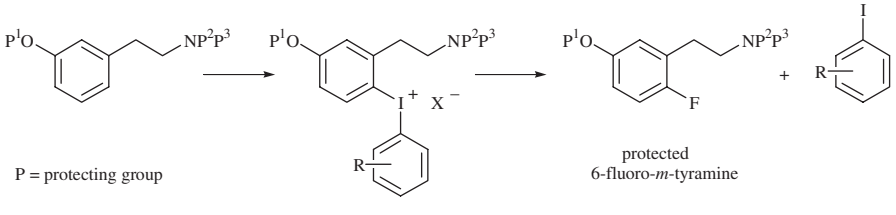
Diaryliodonium salts have been shown to be suitable precursors for the preparation of fluorine-18 labelled aromatics[1] which are employed extensively in clinical research using the medical imaging technique—Positron Emission Tomography. This new approach has several distinct advantages over conventional procedures to this important class of materials.

- The use of [^{18}F]fluoride, which may be produced in much higher amounts and higher specific radioactivity than [^{18}F]F_2 and derived reagents (cf. fluorodestannylation).
- Iodonium salts place little or no restriction on the nature and pattern of aromatic substituents of the target (cf. S_NAr processes).

The efficient production, in high specific radioactivity, of 6-*L*-[^{18}F]fluoro-DOPA which is used for the study of brain dopaminergic neuron density in movement disorders such as Parkinson's disease, remains a significant challenge. We recently established mild conditions for the formation of a range of diaryl- and aryl(heteroaryl)iodonium salts[2,3] and gained valuable insight into the chemistry of these hypervalent iodine systems.[4,5] Initial studies, towards an 'iodonium salt' approach to 6-*L*-[^{18}F]fluoroDOPA and analogous compounds, detailing the first highly functionalized diaryliodonium salts and their subsequent fluoridation chemistry will be presented.



Thus the preparation and fluoridation of a range of *m*-tyramine derived diaryliodonium salts will be described. The influence of nitrogen protecting groups and aromatic substituents (R in the non-target aromatic ring) on yields and selectivity in the nucleophilic substitution process will be discussed. Mechanistic rationale for the observed trends in selectivity will be proposed.

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METHODS TOWARDS ISOTOPOMER-VERSATILE SYNTHESIS OF ^{13}C-LABELLED CARBOHYDRATES

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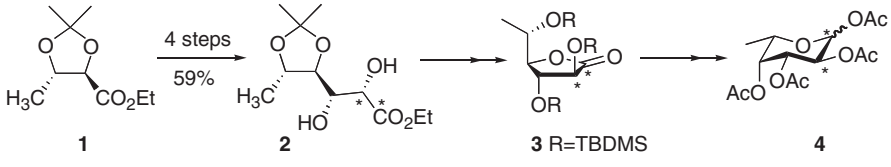
Synthetic routes towards enabling stereoselective synthesis of fucose and galactose with labelling versatility will be described, where a common synthetic route can be applied to the synthesis of different ^{13}C-labelled targets (e.g. regioisomerically labelled, multi-labelled targets). In this way, a catalogue of ^{13}C isotopomers is viable without a variety of different synthetic routes. The heterogeneity of potential sugar linkage isomers makes availability of a diversity of partly labelled monosaccharides important. Alternatives to the known iterative homologations of lower sugars[1] (which are rather label-target specific) are needed.

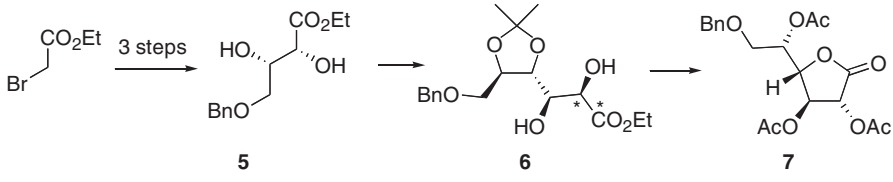
We undertook a synthesis towards L-fucose labelled at C1 and C2, starting from **1**. Wittig reaction enabled introduction of labels, and asymmetric dihydroxylation introduces the remaining L-fucose stereocentres with very high diasterecontrol (matching) giving **2**. Removal of the acetal protecting group with concurrent lactonization could be directed exclusively to the γ-lactone furanone (**3**, after silylation). Reduction to the lactol then leads towards the [1,2-$^{13}C_2$]L-fucopyranose **4** (Scheme 1).



Scheme 1.

The stereochemical analogy between D-Gal and L-Fuc (L-Fuc being 6-deoxy-L-Gal) allows for a similar tactic to be employed from the oxygenated variant of **1**, prepared in a labelling-versatile manner via **5**, using ethyl bromoacetate as the ultimate source of all 4 carbons. Since this is available as either mono-^{13}C-labelled isotopomer, or dilabelled, the single route *de facto* provides access to any possible number and location of ^{13}C labels in these 4

carbons (Scheme 2). The intermediate **5** (after protection as its isopropylidine acetal) can then be elaborated towards Gal using chemistry directly analogous to that employed towards L-fucose from **1**.



Scheme 2.

Reference

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SOME NEW CATALYTIC SYSTEMS FOR ISOTOPIC EXCHANGE LABELLING

Efstathios Alexakis[a], Michael J. Hickey[b], Lee P. Kingston[b], John R. Jones[a], William J. S. Lockley[a], Andrew N. Mather[b], Barry M. McAuley[b], Traci Smith[a] and David J. Wilkinson[b]

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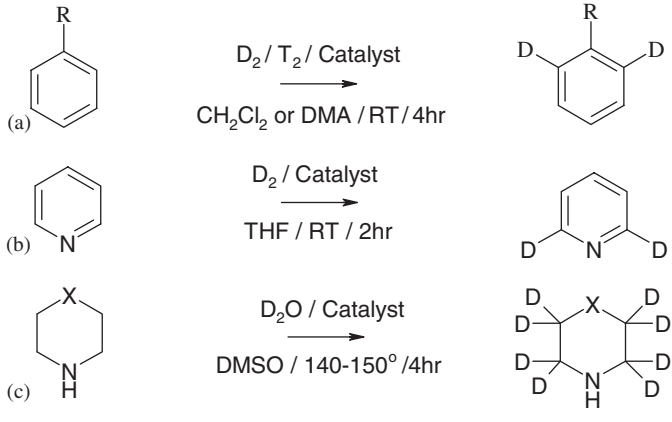
The labelling of compounds via isotopic exchange procedures continues to provide one of the simplest and most convenient techniques for introducing tritium or deuterium into organic molecules. This presentation together with the accompanying posters and leading references summarizes work in this area carried out at the University of Surrey in the course of collaborations with AstraZeneca Charnwood and with the ATHENA catalysis consortium (a collaboration between several universities and Synetix to develop newer and more efficient catalytic systems for hydrogenation, dehydrogenation and oxidation).[1] During this work new catalytic systems have been developed and older systems improved so as to provide methods for the efficient one-step labelling of several target compound classes with hydrogen isotopes. The classes comprise (a) substrates amenable to directed *ortho*-exchange including benzylamines and anilines in addition to the standard *ortho*-directors,[2] (b) pyridines and other nitrogen heterocyclics with available α-positions[3] and (c) piperazines/piperidines and other secondary amines with a free NH group.[4]



All the catalysts identified are either commercially available or prepared in single-step procedures requiring a minimum of handling precautions. A summary of the advantages and drawbacks of these new catalytic systems will be briefly presented.

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CARBON-14 SYNTHESIS—NOT ALWAYS AS EASY AS IT LOOKS

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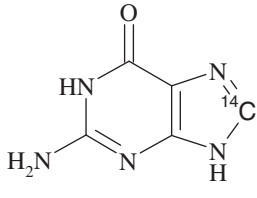
Often, the carbon-14-labelled synthesis of a molecule for use in, for example, DMPK studies will be based on a route developed by Process Chemistry. Although this has advantages in terms of the process being optimized, albeit in relation to a much larger scale than a radiochemist is ever likely to contemplate, there are other issues that generally arise. One of these is that the 'starting material' used for the process route is invariably a number of synthetic steps removed from a cheap and readily available carbon-14 source, such as barium carbonate, potassium cyanide, acetanilide, etc. Unless you are very lucky and the radiolabelled 'starting material' that you require has been investigated previously **and** reported, then the synthesis will have to be based on the non-labelled literature for, possibly, the same or a similar compound; occasionally, a relevant stable or short-lived isomer synthesis may have been reported, which can be advantageous. This can give rise to various problems, some of which, will be discussed using the carbon-14 synthesis of [8-^{14}C]guanine, **1**, from sodium [^{14}C]formate as an example.



1

THE POTENTIAL FOR USING TITANIUM ALKYLIDENE CHEMISTRY IN SOLID-PHASE RADIOCHEMICAL SYNTHESIS

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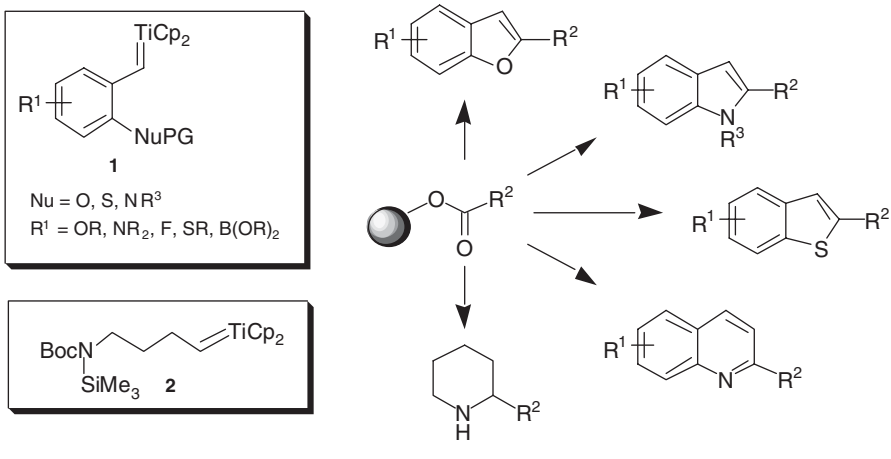
Recently, we have developed a method by which simple resin-bound esters can be converted into a range of bioactive heterocycles (benzofurans,[1–3] indoles,[3,4] benzothiophenes,[5] quinolines and racemic piperidines[6]) using titanium alkylidene reagents (for review see Reference)[7] **1** and **2**. The heterocycles are produced in very high purity and require no chromatography. Our method exploits the key advantages of solid-phase synthesis, i.e. compounds not attached to the resin can be washed away following each reaction and the resin is easily handled. However, our chemistry also overcomes some major difficulties in solid-phase synthesis: (i) we employ the cheapest resin and simplest linker; (ii) switching the nature of the linker to the resin from acid-stable to acid-sensitive ensures the high purity of products; (iii) cleavage from resin is often not an extra synthetic step analogous to deprotection, but is intrinsic to heterocycle formation; and finally, (iv) many methods distort pharmaceutical structure–activity relationships by leaving polar functionality in the products at the site where the resin was attached, but ours is a 'traceless' method leaving no such functionality. The method was originally developed



for drug discovery as using directed sorting techniques that combine parallel and split-and-pool synthesis, large numbers of discrete compounds could be synthesized. However, it should also have advantages for radiochemical synthesis and the lecture will provide an overview of our work, highlighting this potential.

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TRANSITION OF PET TRACERS FROM CLINICAL RESEARCH TOOLS TO COMMERCIAL DIAGNOSTICS

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The clinical use and application of PET tracers is becoming more and more widespread. The multi-disciplined technique of PET imaging is in an exciting phase of development where tracers are moving from use as research tools to established tracers with clinical applications in the diagnosis of neurodegenerative diseases, cardiovascular disease and malignancy.

Innovations within academic PET centres and industry have led to advancement that will lead to PET becoming increasingly more accessible and available. With a focus on the commercialization of PET imaging, a status report on new radiolabelling methods, synthesis box fabrication, PET tomography and PET tracer development will be discussed.

Radiosynthesis of FDG using a resin-linked precursor to enable fast and simple manufacture of PET radopharmaceuticals has been established and may provide a platform for establishing other PET tracers in clinical centres. The design of new markers to measure angiogenesis in oncology using ^{18}F synthon chemistry, will be used to explore how new label methods will provide a method to screen new molecular targets and help design the next generation of PET tracers.

There is potential that PET tracers that visual amyloid with Alzheimer's disease patients will help with patient diagnosis and selection. The clinical experience of ^{11}C 6-OH BTA-1(PIB) and new fluorine analogues development will be reviewed.

THE SYNTHESIS OF STABLE LABELLED KETAMINE HYDROCHLORIDE

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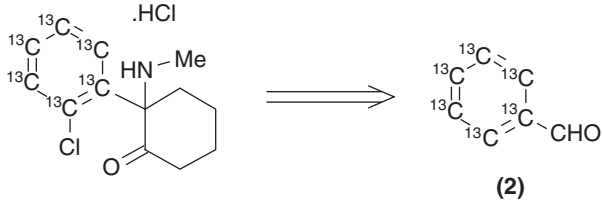
Ketamine hydrochloride is a non-barbiturate, rapid-acting disassociative anesthetic used on both animals and humans; it has also been used in experimental psychotherapy. Stable labelled ketamine hydrochloride was required as an internal standard for use in LCMS assays. The preparation of [$^{13}C_6$]ketamine hydrochloride (**1**) from commercially available [$^{13}C_6$]benzaldehyde (**2**) will be presented, and the synthetic challenges encountered in this work will be discussed.



(1) **(2)**

TOWARDS ROBUST CONDITIONS FOR IRIDIUM-MEDIATED EXCHANGE

John M. Herbert

Isotope Chemistry and Metabolite Synthesis Department, Sanofi-Synthélabo Willowburn Ave., Alnwick, Northumberland NE66 2JH, UK

As a method for isotopic labelling, iridium-mediated hydrogen exchange suffers from problems with transferability between laboratories. In addition, there is still no good general catalyst for exchange in homologous substrates such as benzyl ketones and phenylacetamides. This presentation describes some of our recent efforts to understand and overcome the former, and to develop better catalysts for the latter.

Many of the problems encountered with unexpectedly low levels of exchange prove, in effect, to be a consequence of the slowing of exchange as a result of a low partial pressure of tritium in the system, a low concentration of the exchange solution, or partial poisoning of the catalyst by a coordinating solvent such as water. This effect in turn appears to be related to degradation of the active catalyst within a relatively short time. This is illustrated (Figure 1) by the kinetics of deuteration of acetophenone in the presence of $Ir(cod)(PPh_3)_2^+$ and $Ir(cod)(PPh_3)_3^+$: whereas exchange with the former stops abruptly within four hours, the latter remains active until exchange is complete. Separate NMR studies have shown that the hydrogenated form of $Ir(cod)(PPh_3)_2^+$ is stable in solution for an extended period.

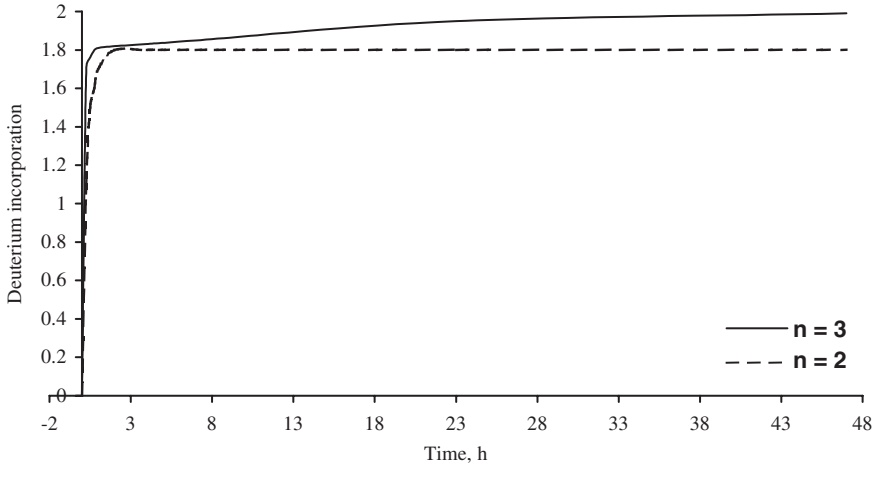


Figure 1. Kinetics of deuteration of acetophenone with deuterium in the presence of $Ir(cod)(PPh_3)_n^+.BF_4^-$

The effects described above are exacerbated in the case of exchange into the *N*-methyl groups of a dimethylamide, where exchange is extremely sensitive to conditions. This can be rationalised in terms of the lesser stability of agostic bonds from a metal centre to an aliphatic, rather than to an aromatic hydrogen.

In the course of efforts to improve exchange into benzyl ketones and phenylacetamides, we have found that the bidentate ligand-containing complexes that are most promising as exchange mediators for these substrates act more slowly than simple phosphine complexes do, so that extended reaction times are required. The observation of similar activity on the part of $Ir(cod)(AsPh_3)_2^+$ has led to the development of the bidentate catalyst, Ir(cod)(dpae)+, which is the most efficient exchange mediator yet described for phenylacetone, in particular, and is proposed as the prototype for a new series of exchange catalysts.

STUDIES TOWARDS THE TOTAL SYNTHESIS OF CLAVOSOLIDE A

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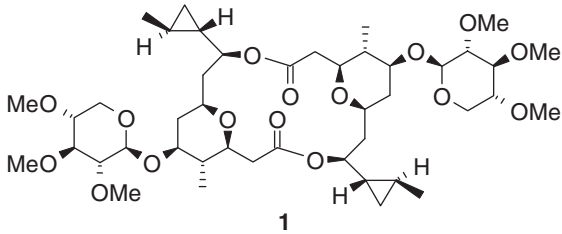
[b]*AstraZeneca UK Ltd, Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG, UK*

The clavosolides are a novel class of dimeric macrolactones isolated from the marine sponge *Myriastra clavosa*.[1] They represent a new family of marine natural products incorporating several unusual structural features. Crude extracts of *Myriastra clavosa* have displayed promising cytotoxic and anti-proliferative effects in antitumor screens however thorough evaluation of the cytotoxic properties of the clavosolides has not been possible due to the limited natural abundance of these compounds. The simplest member of the family, clavosolide A **1**, was chosen as a synthetic target.



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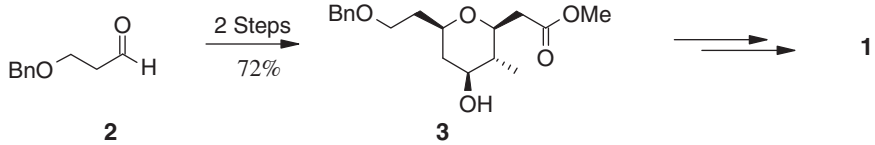
We have shown that highly substituted tetrahydropyrans can be rapidly accessed via an acid-promoted Prins cyclization between an aldehyde and a homoallylic alcohol.[2] The tetrahydropyran core of clavosolide A **1** has been synthesized using this methodology and further side-chain manipulations followed by macrolactonization would give the target dilactone **1**.

We have prepared the tetrahydropyran core **3** of clavosolide A from 3-benzyloxypropanal **2** in just two steps and in 72% yield via an enantioselective crotyl transfer reaction[3] to the required homoallylic alcohol, followed by a Prins cyclization.



2 **3**

Elaboration of the core tetrahydropyran towards the natural product will be described. A discussion of the mechanistic aspects of the Prins cyclization relevant to the planning of syntheses will also be presented.

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ABSTRACTS: POSTER PRESENTATIONS

EFFICIENT DEUTERIUM EXCHANGE-LABELLING OF PYRIDINES AND OTHER AROMATIC *N*-HETEROCYCLICS USING A DEUTERIUM GAS DONOR AT ROOM TEMPERATURE AND PRESSURE

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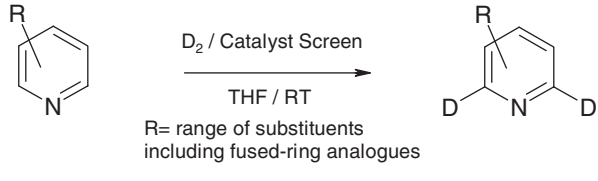
Pyridine and other *N*-heteroaromatic sub-units occur in many agrochemical and pharmaceutical agents. Methods for labelling these units with isotopes of hydrogen are therefore of interest since they provide routes to the tritium-labelled compounds for use in environmental, disposition and other ADMET radiotracer studies. They also make available the deuterium-labelled compounds for use as GC-MS and LC-MS internal standards or stable-isotope tracer studies.

Isotopic exchange methods for labelling such *N*-heterocyclics are quite limited. Most involve the use of isotopic water with the consequent problems of potential radiotoxicity and of product radiolysis if high specific activity tritium-labelling is required. However, one report of the use of a deuterium gas donor in conjunction with a 5% ruthenium on carbon catalyst appears in the literature.[1] We have now shown that in most cases the isotope incorporation observed results largely from the deuterated solvent (CD_3OD) employed in conjunction with the D_2 gas. An additional drawback is that the activity of the catalyst is low, necessitating the use of D_2 under pressure, an undesirable operation with the tritium isotope. We have therefore carried out a screen of potential catalysts for the deuterium gas exchange process (Scheme) to identify systems with greater potential for use with both the deuterium and tritium isotopes.



A wide range of supported and unsupported noble metal catalysts were screened and three catalysts were identified which transferred the isotope efficiently from gas to substrate. Moreover, these catalysts were far more active than the literature catalyst, allowing their use at ambient temperature and pressure. The catalysts identified were rhodium black, ruthenium black and

5% rhodium on alumina. Using these systems a range of pyridines and other *N*-heterocyclics could be efficiently labelled using deuterium by simply stirring with the catalyst under a deuterium atmosphere in THF for 1–2 h. The procedure is usually highly regioselective for labelling α to nitrogen, product isolation is simple and recoveries are often excellent. In some cases a degree of reduction accompanies the isotope exchange, though for most substrates examined this behaviour is absent or of marginal significance.

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ORTHO-^{2}H-LABELLED ANILIDES: AN UNUSUALLY LARGE DEUTERIUM ISOTOPE EFFECT

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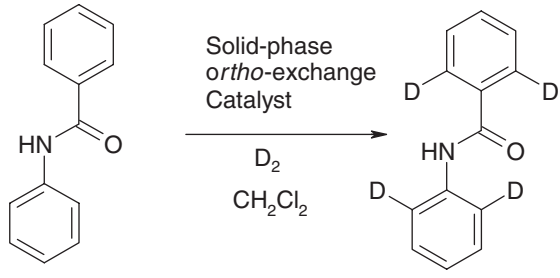
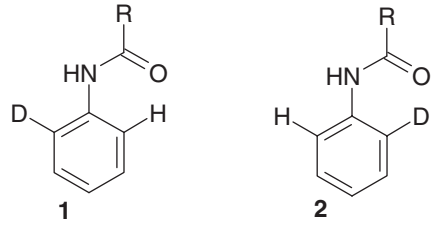
Isotope effects associated with the introduction of deuterium into a molecule are well recognized for both ^{1}H- and ^{13}C-NMR. Generally these effects are both small and additive.[1]



Scheme



Figure

Investigations of the deuteriation of benzanilide (Scheme) over novel solid-phase *ortho*-exchange catalysts[2] has revealed an unusually large deuterium isotope effect (a 0.016 ppm upfield shift) upon the remaining anilide *ortho*-proton in those isotopomers which are mono-deuterated in the anilide ring. The isotope shift is many fold larger than is normal. It is also observed with many other anilides, but not with other *ortho*-labelling directors. The size of this isotope effect may be best rationalized by a deuterium-induced disturbance of the conformation of the monodeuterated anilide molecules. It

has long been recognized that the deshielding effect of the carbonyl group on the *ortho*-proton affords a sensitive probe into the conformation of anilides about the N–Ar bond, and changes in chemical shifts of the *ortho*-proton have been used to investigate the conformations of both mono-*ortho*[3a–c]- and mono-*meta*[3d]-substituted anilides. We presume that the large isotope effect we observe arises from a slight preference for conformer **2** over conformer **1**, occasioned by the shorter, more polar C–D bond (Figure) and possibly mediated by differential dipole–dipole interactions, steric effects or even by hydrogen bonding. The large isotope effect now provides a useful probe with which to investigate other isotopic replacement reactions at the *ortho*-position by ^{1}H-NMR. We are currently utilizing the effect to study isotopic scrambling and poisoning[4] during deuterodehalogenation.

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SYNTHESIS OF [$^{13}C_7$]3,5-DICHLOROBENZYLAMINE HYDROCHLORIDE

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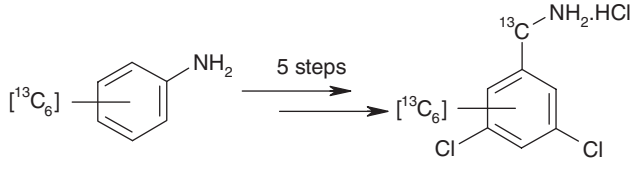
Commercially available [$^{13}C_6$]aniline, **1**, was readily converted into [$^{13}C_6$]1-bromo-3,5-dichlorobenzene. A further stable label was introduced by cyanation using K^{13}CN and cuprous iodide. Borane reduction of the subsequent benzonitrile, followed by an acid work up, furnished [$^{13}C_7$]3,5-dichlorobenzylamine hydrochloride, **6**, which was further elaborated to provide an internal standard for use in LC-MS assays.



1 **6**

AUTOMATED SAMPLE OXIDATION & SCINTILLATION COUNTING

Manfred Gerhard and Werner Zinsser

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Combustion of C-14- or H-3-labelled biological samples, e.g. whole blood, tissue and plants or inorganic materials such as soil and paint has proven to be superior for efficient and reproducible scintillation counting. However, as each sample needs to be handled individually this technology is slow and time consuming. For high throughput, automation is a must.

A new automatic system has been designed by Zinsser Analytic, where up to 200 samples are automatically processed on a unique robotic system. The sample material arrives pre-weighed on barcode-labelled sample boats and are delivered by the robot to the combustion furnace. The C-14 or H-3 samples are collected together with scintillation cocktail in scintillation vials, capped and then placed in racks from the scintillation counter. Barcode labelling of samples and the receiving scintillation vials allows complete tracking of the sample material. The samples are processed in a catalytic oxidizer at a high temperature with a controlled oxygen flow. The efficiency of the combustion process is constantly monitored by an integrated scintillation counter. Hardware and software are GMP compliant and meet the 21CFR Chapter 11 requirements of the FDA. The powerful software controls the workflow and operations as well as providing a complete log-file of all combustion and operational parameters.

THE SYNTHESIS OF [$^{13}C_6$] *N*-(3,4-DICHLOROPHENYL)-2,2-DIMETHYLPROPANAMIDE

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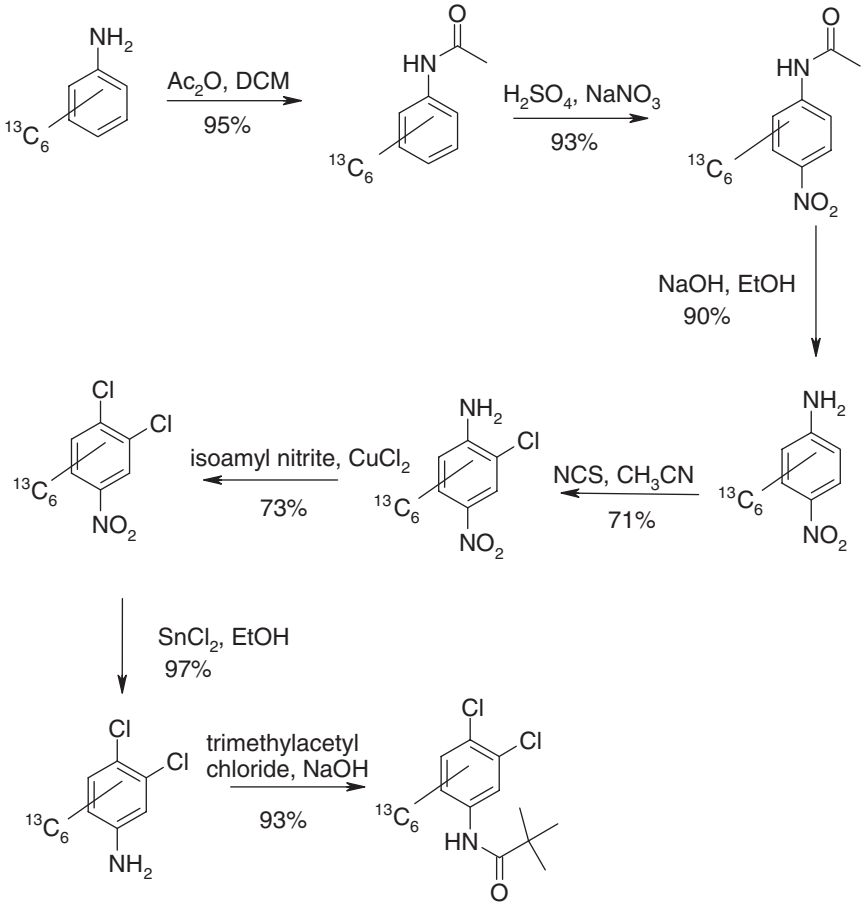
A high yielding regiospecific synthesis of the title compound is described.



SYNTHESIS OF ^{11}C-AMIDES USING [^{11}C]BORANE CARBONYL ([^{11}C]BH_3.CO) AS A CO SOURCE

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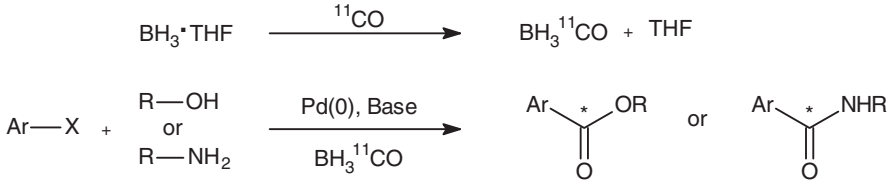
Recent literature reports have described [^{11}C]carbon monoxide as a practical building block for the synthesis of radiolabelled compounds for positron emission tomography (PET). A wide range of compounds have been prepared by palladium-/selenium-promoted carbonylation reactions using dedicated automated robotic systems. Difficulties encountered with the trapping [^{11}C]CO gas in a small solvent volume were overcome using high-pressure or recirculation systems. As an alternative we have developed a new method to convert [^{11}C]CO to [^{11}C]BH_3.CO and to react this boron-based carbonylating complex in palladium-mediated reactions at atmospheric pressure (Scheme 1).



Scheme 1.

The conversion of [^{11}C]CO to [^{11}C]BH_3.CO was easily achieved by passing [^{11}C]CO in a stream of nitrogen through a 1 M solution of BH_3.THF at room temperature. Subsequently [^{11}C]BH_3.CO was carried with a flow of nitrogen into a reaction vial loaded with reactants in solution cooled at -78°C. Following the trapping step, thermal heating was applied for the reaction vessel for ca. 10 min. Using standardized reaction conditions described above, model compounds such as [^{11}C]phthalide and [^{11}C]*N*-benzylbenzamide were successfully labelled from 2-bromobenzyl alcohol and, iodobenzene and benzylamine, respectively. A wide variety of bases, solvents and reaction conditions were investigated. In summary, [^{11}C]BH_3.CO can provide a good alternative to autoclaving methods with [^{11}C]CO for palladium-catalyzed carbonylation reactions.

SYNTHESIS OF THE ANTI-MALARIAL [*QUINOLINE*-3-^{14}C]-FERROQUINE FROM [2-^{14}C]-MALONIC ACID

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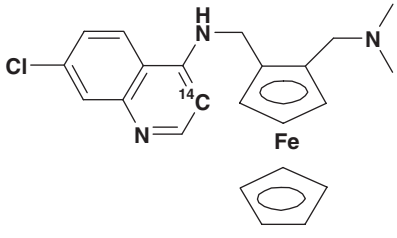
The antimalarial [*quinoline*-3-^{14}C]-ferroquine, **1**, an analogue of chloroquine, **2**, was synthesized from [2-^{14}C]-malonic acid with an overall radiochemical yield of 15%. The synthetic route via [^{14}C]-Meldrum's acid was designed to minimize the intermediacy of radiolabelled volatiles. This synthesis involves a four-step route to labelled 4,7-dichloroquinoline, which is the key intermediate for the synthesis of many analogues of chloroquine.



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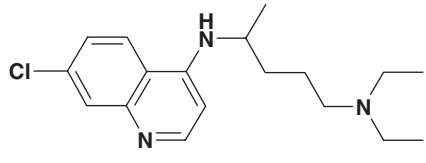

2

SYNTHESIS OF ISOTOPICALLY LABELLED CARBOHYDRATES

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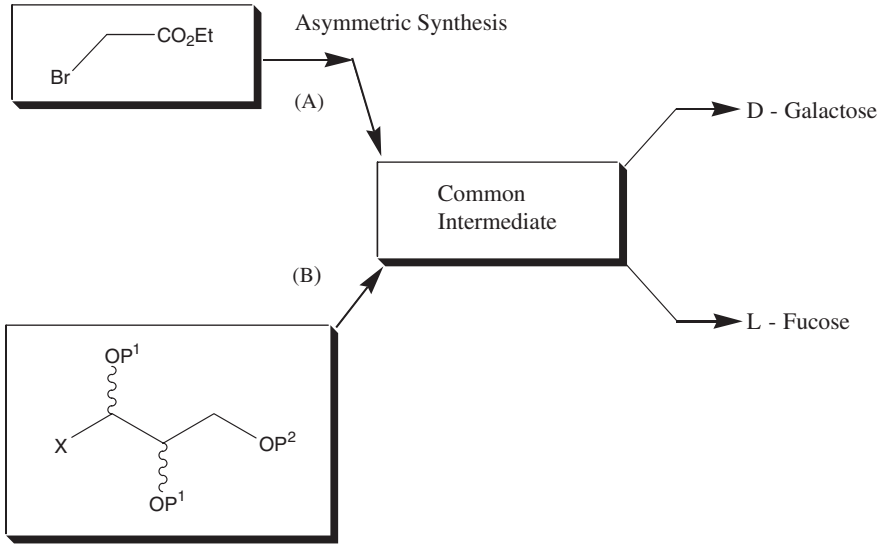
Oligosaccharide structures and their conjugates play key roles in a variety of biological phenomena. To fully understand the nature of these interactions, the conformation of the ligand bound to the receptor needs to be determined. However, due to the inherent flexibility of carbohydrates (about glycosidic linkages) this is a very demanding task to achieve practically. NMR is the method of choice to study carbohydrate structure and conformation in solution, and can be applied to conformational studies of both the bound and free carbohydrate. Due to severe resonance overlap in carbohydrate NMR spectra, multidimensional techniques are invariably applied for these studies. The use of isotopically labelled oligosaccharides can greatly facilitate NMR analysis of sugar conformations.

However, though at present stable isotopic enrichment of proteins is widely employed, this is not yet true for isotopically labelled saccharide. While certain labelled carbohydrates are available commercially at high cost, this is limited to mono-labelled glucose isomers and per-labelled glucose, [1-^{13}C]-galactose and [1-^{13}C]-mannose (with some other isotope patterns available by further synthesis). For extending the utility of NMR for carbohydrate conformational analysis, availability of different multiple labelled monosaccharides is essential to allow versatility in the preparation of differing labelling patterns of ^{13}C-labelled oligosaccharides.

A large number of strategies have been employed in the stereoselective syntheses of monosaccharides. However, few of these reported approaches are readily applicable to the preparation of isotopically labelled analogues. Most involve diastereoselective homologation steps, which are generally unsuitable for isotope incorporation. In this work two strategies are proposed: one based on chiral building blocks to allow rapid access to singly or doubly labelled monosaccharides and a completely *de novo* synthesis from achiral starting materials where any pattern of labelling is accessible as all carbons are designed to be derived from 1- or 2-^{13}C materials which can be obtained labelled.

NB: (A) for more labelling versatility, (B) for 1,2 labelling or patterns thereof.



[^{11}C]LOPERAMIDE AS A HIGHLY SENSITIVE PET PROBE TO ASSESS CHANGES IN CEREBERAL *P*-GLYCOPROTEIN FUNCTIONALITY

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Introduction: The ATP-driven efflux pump *P*-glycoprotein (*P*-gp) is known to play a major role in multidrug resistance, leading to therapy failure in e.g. oncology and antiretroviral treatment. In addition, it is also evident that *P*-gp has a major impact on the development of novel drugs targeting e.g. the brain. A substantial amount of work has been performed to characterize the functionality of *P*-gp in the blood–brain barrier (BBB) *in vivo* but unfortunately, most of the PET and SPECT probes developed lack sufficient sensitivity to accurately measure the difference between baseline extraction fraction, the extraction fraction under conditions where *P*-gp is not fully inhibited, and the extraction fraction under conditions where *P*-gp is assumed to be fully inhibited. Loperamide, an opioid receptor agonist, has been shown to be a good substrate for *P*-gp, with 13.5-fold higher concentration in the brain of mdr1(-/-) knock-out versus wild-type mice. The aim of the current work is to (a) develop a novel way of preparing the precursor to [^{11}C]loperamide, (b) demonstrate the feasibility of labelling loperamide and, (c) determine if it has potential as a highly sensitive PET probe to measure changes in *P*-gp functionality *in vivo*.

Methods: Desmethyl loperamide was prepared in two steps by a fusion reaction between 3,3-diphenyl-dihydrofuran-2-one and 4-(4-chlorophenyl)-piperidin-4-ol and reacting the resulting butyric acid with trifluoroacetic anhydride and methylamine. Subsequently, [^{11}C]loperamide was prepared through *N*-methylation by reacting 1 mg desmethyl loperamide in 300 µl DMSO with [^{11}C]MeI using 5 mg freshly ground KOH as base, for 5 min at 80°C (1.04 $\pm$ /0.9 GBq, $n = 8$, unoptimized). Ketamine-induced isoflurane-aneasthetized pigs ($n = 3$, Yorkshire landrace, 37.5 kg) were scanned under baseline conditions and either following consecutive doses of cyclosporin A (CsA: pig1: 1, 10, 30 mg/kg CsA, pig2: 5, 15, 30 mg/kg CsA), 20–30 min prior to administration of [^{11}C]loperamide or under baseline conditions and

following consecutive pre-treatment with 10 mg/kg indomethacin and 10 mg/kg quinidine.

Results: The synthesis of desmethyl loperamide using the method described above proceeded in good yield (56% overall yield). Subsequent reaction with [^{11}C]MeI yielded sufficient [^{11}C]loperamide to perform the *in vivo* evaluation (0.5 ± 0.3 GBq, $n = 14$). Upon intravenous administration, little uptake of ^{11}C-derived radioactivity was observed in the CNS (<1% ID/l). The apparent extraction fraction compared to [^{15}O]H_2O was approx. 10%. Pretreatment with CsA led to a dose-dependent increase in ^{11}C-derived radioactivity signal in the CNS. Whole brain CNS levels increased by a maximum of seven-fold compared to baseline conditions. The rate of metabolism appeared slightly decreased at doses of CsA >10 mg/kg (from 60% parent to ~80% parent at 60 min post-admin). Clearance of [^{11}C]loperamide from plasma also appeared slightly reduced at doses of CsA >10 mg/kg, leading to increased plasma concentrations at later time points. No change in CNS uptake was observed following pre-treatment with 10 mg/kg of the non-subtype selective MRP inhibitor indomethacin, in contrast to subsequent pre-treatment with 10 mg/kg of the competitive *P*-gp substrate quinidine which led to a slight increase of CNS penetration, similar to what could be expected of a ~3 mg/kg administration of CsA.

Conclusion/Discussion: The novel methodology for the synthesis of desmethyl loperamide has greatly facilitated our ability to produce cGMP grade starting material for human studies. In addition, this method has opened the way to the manufacture of close analogues, e.g. for the introduction of longer lived isotopes to enable a broader clinical application. Overall yields for the synthesis of [^{11}C]loperamide are low and the production method requires further optimization. As evidenced from the results in pig, [^{11}C]loperamide appears to be a very promising PET probe for measuring changes in *P*-gp functionality *in vivo*. The large difference between baseline and ~100% *P*-gp inhibition, together with its apparent selectivity for *P*-gp over MRP, indicates that [^{11}C]loperamide has potential as a research tool to investigate the effect of small (15%) changes in *P*-gp functionality on pharmacology (e.g. drug–drug interactions, disease therapy) and in disease. Efforts are currently ongoing to further investigate the kinetics of [^{11}C]loperamide in pig and to assess and validate the utility of this novel PET probe in man.

