Journal of Labelled Compounds and Radiopharmaceuticals
J. Labelled Cpd. Radiopharm. **44**, 305-322 (2001)
DOI: 10.1002/jlcr.459

International Isotope Society

Abstracts of the

XTH I.I.S. (U.K. GROUP) SYMPOSIUM.

Astra-Zeneca R&D, Alderley Park, UK

Current Themes in Isotopic Chemistry

10th October 2000

Meeting Summary

The tenth annual symposium of the International Isotope Society's United Kingdom Group took place at the Alderley Park site of AstraZeneca plc. The symposium was attended by over 80 delegates from academia, the pharmaceutical and agrochemical industries, life science and fine chemical companies. Delegates were welcomed to the site by Dr Peter Warner of the Medicinal Chemistry Department.

The scientific programme consisted of presentations on isotopic chemistry and applications of labelled compounds, or of chemistry with significant implications for isotopic synthesis. Both short-lived and long-lived isotopes were represented, as were stable isotopes. The programme was split into a morning and afternoon session chaired by Dr John Harding (AstraZeneca, who also organised the excellent domestic programme) and Dr Karl Cable (Glaxo Wellcome). The meeting ended with concluding remarks from Dr Ken Lawrie (SmithKline Beecham), chairman of the UK Group. This years symposium had a larger attendance from other European countries, an excellent level of sponsorship was achieved, and the symposium proved self-financing.

The next UK group symposium is planned for early October 2001.

Received 23 December 2000
Accepted 3 January 2001

Meeting Programme

Morning Session

Paul Pringle [University of Bristol, UK] *To what extent can Homogeneous Catalysts be Designed?*

Henrick Olsen [Novo Nordisk, Denmark] *Mapping of Pharmaceutical Degradation Products in the Solid Dosage Form using Radiotracers.*

Marie-Claire Lasne [University of Caen, France] *Synthesis of New Ligands of Cholinergic Receptors. Strategies for their Radiolabelling with Positron Emitters, Carbon-11 or Fluorine-18.*

Christopher Rayner [University of Leeds, UK] *Catalysis in Supercritical Carbon Dioxide – What advantages does it have to Offer?*

Afternoon Session

Gunnar Antoni, [University of Uppsala, Sweden] *Position Specific Labelling of Biomedically Interesting Compounds using Short-Lived Radionuclides.*

Hugh Wiltshire [Hoffmann-La Roche, UK] *The Use of Stable-Labelled Isotopes in Bioequivalence Studies with the HIV-Protease inhibitor Saquinavir .*

Alison Hill [Kings College, UK] *Elucidation of Soraphen A Biosynthesis using ^{2}H, ^{13}C and ^{18}O-Labelled Precursors.*

Nick Shipley [Glaxo Wellcome, UK] *Preparation of P450 Substrates for use in Drug-drug Interactions*

Andrew Mather [AstraZeneca , UK] *Aspects of Isotopic Labelling by Parallel Chemistry.*

Helen Booth [Astra Zeneca, UK] *Synthesis of ^{14}C-labelled forms of a series of Human Leukocyte Elastase Inhibitors.*

Meeting Abstracts

TO WHAT EXTENT CAN HOMOGENEOUS CATALYSTS BE DESIGNED?

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Ever since the invention of Wilkinson's Catalyst over 30 years ago, chemists have been attracted by the prospect of tailoring phosphorus(III) ligands to increase the catalytic activity and selectivity of synthetic metal complex catalysts. The fruit of this research endeavour (which has been carried out as much by industrial chemists as by academics) is the emergence of some principles that enable the design of better catalysts.

In this paper, it is shown how the application of some simple ligand design led to the invention of an asymmetric hydrogenation catalyst which is superior in terms of ee's to the outstandingly successful bis(phospholano)ethane-rhodium catalysts (one of the DuPhos family).

The limitations of very well established ideas on hydrogenation catalyst design will be illustrated with a class of chiral monodentate phosphorus(III) ligands that outperform their bidentate analogues in terms of the enantioselectivity of their rhodium complex catalysts.

Finally the rôle of serendipity in catalyst discovery is discussed with reference to the application of some unusual phospha-adamantane cage ligands in olefin carbonylation catalysis.

Reference:

Fernandez, E.,Gillon, A., Heslop, K., Horwood, E., Hyett, D. J.; Orpen, A. G., Pringle, P. G. *Chem. Commun. (Cambridge)*, (17), 1663-1664 (2000)

MAPPING OF PHARMACEUTICAL DEGRADATION PRODUCTS IN THE SOLID DOSAGE FORM USING RADIOTRACERS

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The determination and identification of the actual impurities present in stability indicating samples is an important aspect of analysis of pharmaceutical products. An accurate analytical profile of a drug and its formulation fulfils the requirements of regulatory agencies, and is essential since the formation of degradation products can affect the safety and efficacy of the pharmaceutical product. According to the ICH guideline covering impurities in new drug products [1], those impurities in drug products classified as degradation products of the active ingredient or reaction products of the active ingredient with an excipient should be identified to a certain level dependent of the maximum daily dose. Attention should also be paid to reviewing the adequacy of the mass balance, e.g. the process of adding together the assay value and levels of degradation products to see how closely these add up to 100 per cent of the initial value [2].

We have recently demonstrated that radiolabelled material of the new drug product can be used for alleviating the degradation pathways and mass balance of drug products in the solid dosage form [3,4]. The technique can be used to test whether the extraction procedure developed for the analysis is sufficiently efficient, whether any volatile products produced by the degradation processes escape detection, whether all major degradation products are detected by the analytical method, if any reaction products with an excipient are produced after storage, and also to determine the assay values by an independent method. A good estimate of the response factors of the degradation and reaction products - not easy or even impossible to synthesize - can also be obtained by comparing the UV/MS response with the molar content obtained by scintillation

counting. The methodology will be illustrated by our work on the degradation of Gabtril®, an antiepileptic drug, Levormeloxifene. a partial oestrogen receptor agonist, and the well known steroid Norethisterone Acetate used for HRT treatment of menopausal. The studies clearly demonstrate that radiolabelled tracers provide a valuable tool to determine the fate of the degradation products in solid pharmaceutical products.

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SYNTHESIS OF NEW LIGANDS OF CHOLINERGIC RECEPTORS. STRATEGIES FOR THEIR RADIOLABELLING WITH POSITRON EMITTERS, CARBON-11 OR FLUORINE-18.

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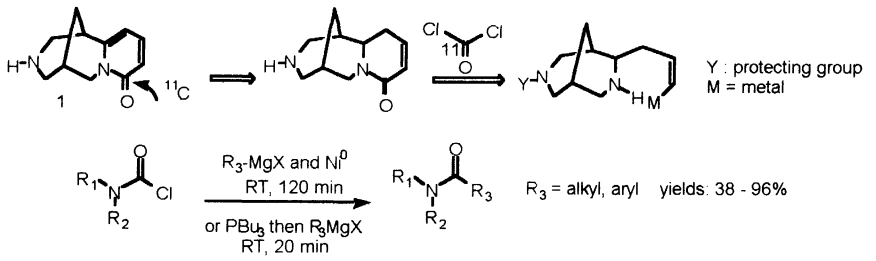
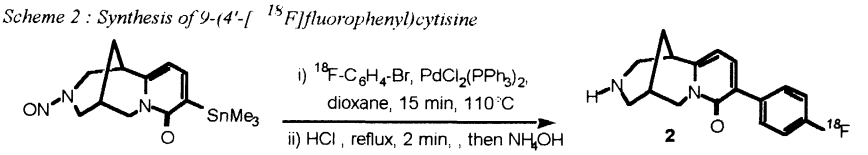
Nicotinic acetylcholine receptors (nAChRs) are involved in various physiological effects observed with nicotine. The nAChR densities are altered in various pathologies such as Alzheimer and Parkinson's diseases.[1] As a result, the *in vivo* quantitation of receptors using positron emission tomography (PET)[2] has attracted tremendous interest as a tool to investigate the role of the nicotinic system in neurodegenerative diseases.[3] Recently, several radioligands have been prepared to visualize nAChRs *in vivo* by PET,[4] or Single Photon Emission tomography (SPET).[5] However, due to their toxicity or their high non specific binding, none of these ligands is ideally suited for PET imaging studies of the human brain. It is therefore crucial to develop a radiotracer to be able to map these receptors *in vivo*. Owing to its nanomolar affinity[6] and its high selectivity towards the $\alpha_4\beta_2$ receptor subtypes,[7] (-)-cytisine **1** is often used as a reference ligand in the studies of the nicotinic neurotransmission. Its long half life *in vivo* compared to nicotine,[8] and its ability to cross the blood brain barrier[9] make cytisine a good candidate for PET studies. The synthetic approaches developed in order to access [8-^{11}C]-(-)-cytisine and the preparation of 9-(4'-[^{18}F]-fluorophenyl)cytisine **2**, a fluoro analogue of (-)-cytisine are summarized in schemes 1 and 2.

Scheme 1 : Synthetic approaches towards [8- ^{11}C*]cytisine*



Scheme 2 : Synthesis of 9-(4'-[^{18}F*]fluorophenyl)cytisine*



10% radiochemical yield from [^{18}F*]KF, 150 min total synthesis time*

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CATALYSIS IN SUPERCRITICAL CARBON DIOXIDE – WHAT ADVANTAGES DOES IT HAVE TO OFFER?

Christopher M. Rayner
University of Leeds.

The development of new methods for addressing environmental problems associated with organic synthetic procedures continues to be one of the major challenges facing synthetic organic chemists, and will remain so for the foreseeable future. Relatively few synthetic organic reactions can be carried out in the absence of solvent, particularly on a large scale. Recently, supercritical carbon dioxide ($scCO_2$) has been demonstrated as being an effective medium for a limited number of organic transformations (*vide infra*), and this, coupled with its environmental advantages has led to it being considered as a solvent of the future.

Supercritical fluids are substances above their critical temperatures and pressures, whose properties are intermediate between those of gases and liquids, which can be controlled by variation of both temperature and pressure. Pure carbon dioxide has a critical temperature of 31°C and critical pressure of 74 atmospheres, both of which are readily achievable using commercially available equipment. Advantages include: low toxicity, ready availability, ease of removal and disposal and/or recycling. Other advantages which are particularly relevant for carrying out reactions in $scCO_2$ are: fine control of solvent properties by changes in temperature and pressure, the ability to homogenise reaction substrates, electrically neutral metal complexes and gases like oxygen and hydrogen; enhanced diffusion rates; and potential for product processing which is particularly useful in the pharmaceutical area, which is where much of our synthetic work is targeted.

The presentation will concentrate on 3 main topics. An introduction into the area of green chemistry and the use of supercritical CO_2 as a solvent will be given. This will include aspects such as phase behaviour and the unique properties of supercritical fluids, and reactor design and construction.

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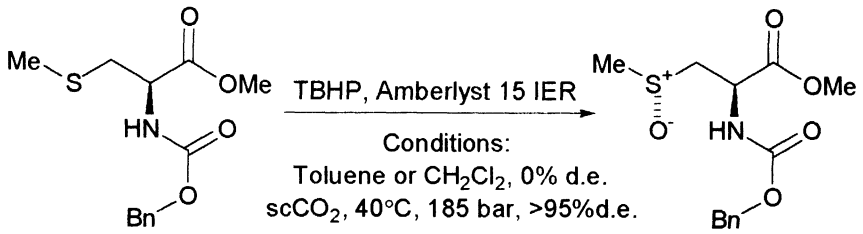
The remainder of the presentation will describe two areas of chemistry developed in Leeds, namely Pd-mediated C-C bond formation in $scCO_2$;[1] and enhanced stereocontrol by solvent tuning.[2,3] The former will be presented as an example to demonstrate the approaches often used to enable reactions to be transferred from conventional solvents to $scCO_2$, and also demonstrates some of the advantages (some unexpected) of using $scCO_2$ such as enhanced reaction rates, reduced side reactions, and unusual reactivity.

The final part of the talk will describe reactions where dramatic enhancements of stereocontrol can be achieved in $scCO_2$ compared to those possible in conventional solvents, for example achieving almost total stereocontrol in $scCO_2$ where no selectivity is observed in conventional media. Examples presented will include the Diels-Alder reaction,[2] and diastereoselective sulfur oxidation (Scheme 1).[3] A brief explanation will be provided which suggests that the density of the reaction medium plays a crucial role in determining the outcome of the reaction.

Scheme 1.



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POSITION SPECIFIC LABELLING OF BIOMEDICALLY INTERESTING COMPOUNDS USING SHORT-LIVED RADIONUCLIDES

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Labelling synthesis using short-lived positron emitting radionuclides e.g. ^{11}C ($t_{1/2}$=20.4 min) requires fast and efficient reactions due to the need for a short reaction time. The uses of catalysts such as enzymes or transition metal complexes are thus of interest in rapid labelling synthesis. Enzymes are particularly useful for the ^{11}C-labelling of endogenous compounds such as amino acids where enzymatic catalysis can be advantageous in terms of speed of reaction, stereochemical requirements as well as the possibility to perform the reaction at physiological conditions with respect to pH and temperature. The possibility to use multi-enzymatic catalysis and combined chemo/enzymatic syntheses will be presented.

Palladium and other transition metal catalysts can also be used for labelling of both exogenous and endogenous compounds. The introductions of a methyl or a cyano group on an aromatic ring are well-known examples. Other important applications where $Pd^{(0)}$ complexes are used is in the synthesis of amides, lactones and ketones using [^{11}C]carbon monoxide as the labelled precursor. Compounds where the carbonyl group is bound to two heteroatoms (e.g. ureas) can be obtained using selenium as catalyst. The use of [^{11}C]carbon monoxide is technically difficult due to the problems of trapping carbon monoxide in a suitable solvent. However, by applying a high-pressure approach in a micro-autoclave this obstacle is by-passed.[1] Other metal catalysed reactions will also be presented as well as the synthesis of precursors for labelling synthesis such as phosgene and ethyl iodide from [^{11}C]carbon monoxide.

In this presentation enzyme catalysed syntheses of ^{11}C-labelled aromatic[2] and aliphatic amino[3] acids as well as other endogenous compounds such as sugars[4] and acetyl-L-carnitine[5], will be presented. The emphasis will be on labelled compounds that have

been used as tracers in animal and/or human positron emission tomography (PET) investigations. The importance of labelling in different positions and how this approach can be used to unravel *in vivo* biochemistry in *non-invasive* PET investigations are also discussed.

The study of *in vivo* synthesis of neurotransmitters such as serotonin and dopamine from ^{11}C-labelled 5-hydroxy-L-tryptophane and 3,4-dihydro-L-phenylalanine (DOPA), respectively, will be presented and the implications of this in clinical tumour PET investigations pointed out. The use of acetyl-L-carnitine, labelled in three different positions, for the study of the coupling between this compound and the tricarboxylic acid cycle and the use of LC-MS for evaluation of the metabolites formed will be discussed.[6] Other examples of the biological significance of labelling in different positions will be presented.

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THE USE OF STABLE-LABELLED ISOTOPES IN BIOEQUIVALENCE STUDIES WITH THE HIV-PROTEASE INHIBITOR SAQUINAVIR.

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Hoffmann-La Roche.

Saquinavir was the first HIV protease inhibitor to be licensed for the treatment of AIDS in 1995. In common with other members of its class of drug, saquinavir exhibits rather wide variations, both inter- and intra-subject, in exposure when administered orally. This property makes the conduct of classic bioequivalence studies difficult. When first marketed, saquinavir was formulated as its solid mesylate salt in a hard gelatine capsule under the name of Invirase™. Approximately three-fold higher exposure was obtained when the drug, Fortovase™, was formulated as its free base in solution in Capmul™ (a mixture of mono- and di-glycerides of medium length fatty acids) in soft gelatine capsules.

Many different formulations were examined during the development of Fortovase™ and both relative bioavailability and formal bioequivalence studies were carried out. The latter required some 45 subjects for statistically valid results. Since saquinavir is administered as several capsules per dose, the possibility of the use of a capsule containing a stable-labelled "internal standard" was explored. Tetra- or pentadeuterated saquinavir were formulated as their mesylate salts, packed into hard gelatine capsules and co-administered with non-labelled saquinavir made in in the formulations of interest. The relative plasma concentrations of deuterated and non-deuterated saquinavir were then determined mass spectrometrically using a second stable-labelled form of saquinavir ($^{2}H_{5}$, $^{15}N_{1}$, $^{13}C_{6}$) as the analytical internal standard.

This talk will discuss the merits of this approach to bioequivalence studies with drugs which exhibit high coefficients of variation in terms of systemic exposure.

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Wiltshire, H. R., Prior, K. J., Dhesi, J., Trach, F., Schlageter, M., Schonenberger, H. *J. Labelled Compd. Radiopharm.* **41**(12), 1103-1126 (1998).

ELUCIDATION OF SORAPHEN A BIOSYNTHESIS USING ^{2}H, ^{13}C AND ^{18}O LABELLED PRECURSORS

Alison M. Hill
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Soraphen A **1** is a polyketide metabolite produced by the myxobacterium *Sorangium cellulosum* and exhibits a spectrum of activity that is particularly suited for the control of fungal plant pathogens. Soraphen A is derived from a benzoate starter unit, three acetate, three propionate extender units and two glycerol units which are incorporated into the vicinal hydroxy groups at C-3,4 and C-11,12 by an unknown mechanism. Experiments with ^{13}C and ^{2}H labelled glycerol samples have shown that the *pro*-(***R***)-hydroxymethyl group is retained and incorporated into **1** at C-3 and 11 (consequently the *pro*-(***S***)-hydroxymethyl group is lost) and C-2 of glycerol is incorporated into C-4 and 12 of **1** (figure 1). Oxidation of C-2 takes place prior to incorporation into C-4 and C-12.

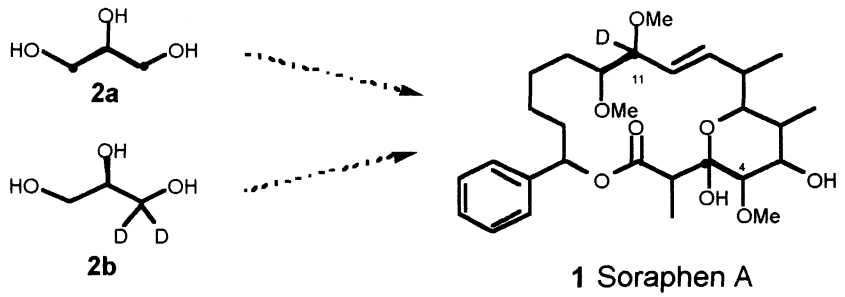
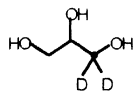


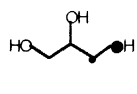
Figure 1. Incorporation of [1,3-$^{13}C_2$]-and (***R***)-[1-$^{2}H_2$]-glycerol (**2a** and **2b**) into soraphen A **1**.

There are two possible explanations for the incorporation of deuterium at H-11: Firstly, intact incorporation of the C-D bond from the *pro*-(***R***)-hydroxymethyl group of

glycerol has taken place (and consequently hydroxymalonate cannot be the immediate precursor in soraphen A biosynthesis). Alternatively, oxidation of the hydroxymethyl group does take place and subsequent reduction, with redelivery of a deuterium from a co-factor results in deuterium incorporation at H-11. To ascertain which pathway was operating, [1-^{13}C, $^{2}H_2$]-glycerol **2c** was synthesised and fed to the organism. Biosynthetic results from this experiment and from the incorporation of other labelled glycerols, glycolates **3** and methoxyacetates **4** are described.



2c



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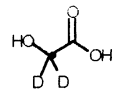
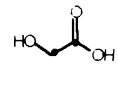
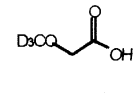


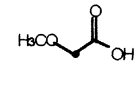
3a



3b



4a



4b

Reference:

Hill, A. M., Harris, J. P., Siskos, A. P. *Chem. Commun. (Cambridge)* (21), 2361-2362 (1998).

PREPARATION OF P450 SUBSTRATES FOR USE IN DRUG-DRUG INVESTIGATIONS

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The importance of predicting possible deleterious effects of drug-drug interactions is increasing within the pharmaceutical industry. Most work in this area has been carried out with cytochrome P450 enzymes (CYP) which are a group of mixed function monooxidases, accounting for over 90% of human metabolism. Probe substrates have now been identified which are predominantly metabolised by each of the major isoforms of CYP. These can be used to determine the effects of drug candidates on CYP mediated metabolism and hence identify potentially harmful drug-drug interactions. Stable isotopically labelled versions of probe drugs and their metabolites were required for use as internal standards in co-incubation studies with GlaxoWellcome development compounds. The synthesis of these will be discussed.

ASPECTS OF ISOTOPIC LABELLING BY PARALLEL CHEMISTRY

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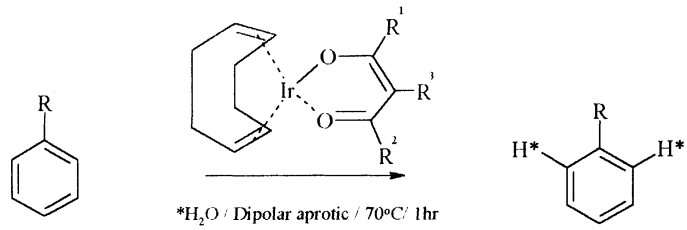
The introduction of parallel chemistry techniques is one of the most far-reaching innovations of the last decade. This paper describes the use of such techniques for the rapid discovery and/or optimisation of catalytic systems for labelling organic compounds with hydrogen isotopes.

Until recently, rapid parallel screening was limited by the absence of appropriate apparatus for parallel isotopic work and by the difficulty of analysing the large quantity of labelling-data generated. Apparatus is now easily constructed to enable parallel screening of hydrogen isotope exchange reactions, hydrogenation reactions, etc, whilst programs for the automated analysis of labelled products by MS and, more recently, by ^{1}H- and ^{2}H-NMR are also available.

Examples are given of the use of the above approaches to investigate two series of very efficient new catalysts (below) for labelling organic compounds with deuterium and tritium via exchange with either deuterium or tritium gas or isotopic water.



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Kingston, L.P., Lockley, W.J.S., Mather, A.N., Spink, E., Thompson, S.P., Wilkinson, D. J. *Tetrahedron Letters.* **41**(15), 2705-2708 (2000).

SYNTHESIS OF CARBON-14 LABELLED FORMS OF A SERIES OF HUMAN LEUKOCYTE ELASTASE INHIBITORS

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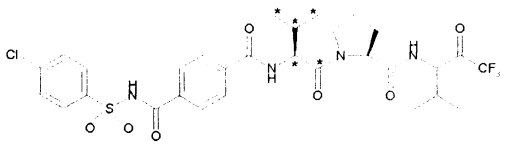
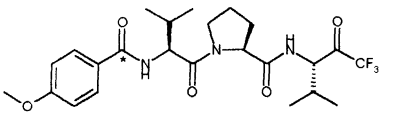
A series of potent and selective inhibitors of human leukocyte elastase have been developed to aid in the treatment of number of respiratory diseases including emphysema, chronic bronchitis and adult respiratory distress syndrome. Carbon-14 labelled forms of inhibitors ZM200880 **(1)**, ZD0892 **(2)** and ZD8321 **(3a), (3b)** were required for metabolism and distribution studies in animals and man.

[^{14}C]-ZM200880 **(1)** was prepared in a seven stage synthesis from L-[U-^{14}C]valine in 28% overall radiochemical yield, at a specific activity of 32.9 mCi/mmol. In view of the limited metabolism seen with this compound, a simpler strategy was used to label ZD0892. [^{14}C]-ZD0892 **(2)** was prepared in a two stage synthesis from 4-methoxy[carbonyl-^{14}C]benzoic acid in 44% overall radiochemical yield, at a specific activity of 28.2 mCi/mmol.



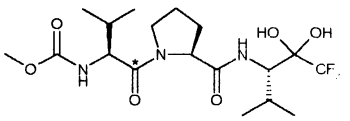
(1)



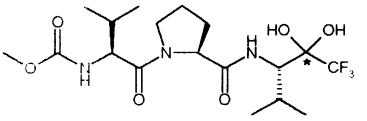
(2)

With ZD8321, however, two carbon-14 labelled forms were required to fully define the metabolism. The first, **(3a),** was prepared in a five stage synthesis from L-[1-^{14}C]valine in 17% overall radiochemical yield, at a specific activity of 25.1 mCi/mmol. The second, **(3b),** was prepared in two stages from (2*S*,3*S*)-3-amino-1,1,1-trifluoro-4-

methyl-[2-^{14}C]pentan-2-ol in 51% overall radiochemical yield, at a specific activity of 8.8 mCi/mmol.



(3a)



(3b)