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15th Workshop of the International Isotope Society–Central European Division: The synthesis and applications of isotopes and isotopically labelled compounds

Bad Soden, Germany, June 12–13, 2008

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ORAL PRESENTATIONS

ORGAN FUNCTION TESTS WITH THE STABLE ISOTOPE ^{15}N

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Introduction: Several medical organ function tests with ^{13}C, known as breath tests, have been used in research and–later on–in clinical routine for about 30 years.

Results and Discussion: For about 25 years our Leipzig group has focused their efforts on an alternative test principle based on ^{15}N to be used especially in newborn children and infants. The diagnostic agent is chemically the same as the one used for the breath test, but it is labelled with ^{15}N instead of ^{13}C. As a consequence, the test result mostly comes from urine instead of breath.

In parallel studies, the new ^{15}N test principle was found to be equally good as the ^{13}C principle, but even more beneficial to the very young patients. The advantages apply to the test procedure as well as to the reliability of test results.

Two ^{15}N urine tests and a ^{15}N breath test, each for a specific diagnostic aim, were developed:

(1) Liver function test with [^{15}N]methacetin[1]
(2) *Helicobacter pylori* test with [^{15}N]urea[2]
(3) Lung function test with [^{15}N]arginine.[3]

The test principles and some application examples are discussed.

Extensive studies in epidemiology and environmental medicine have been carried out.

In follow-up studies of a specific population group over several years we then changed from the urine test to the corresponding breath test which is best suited to over-six-years-olds.

References

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LABORATORY OF RADIOISOTOPES OF IOCB ASCR–RECENT RESULTS OF LABELING BY ^{3}H AND ^{125}I

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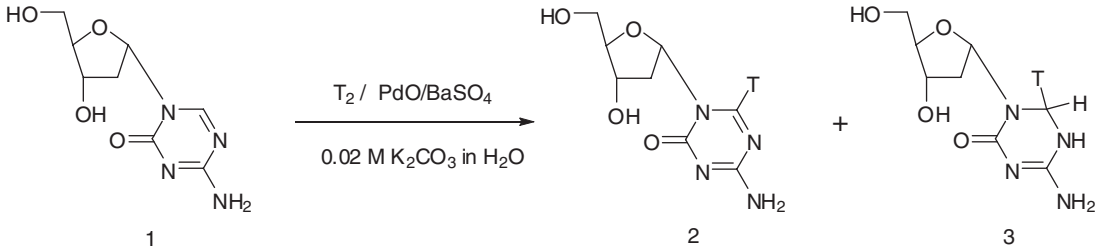
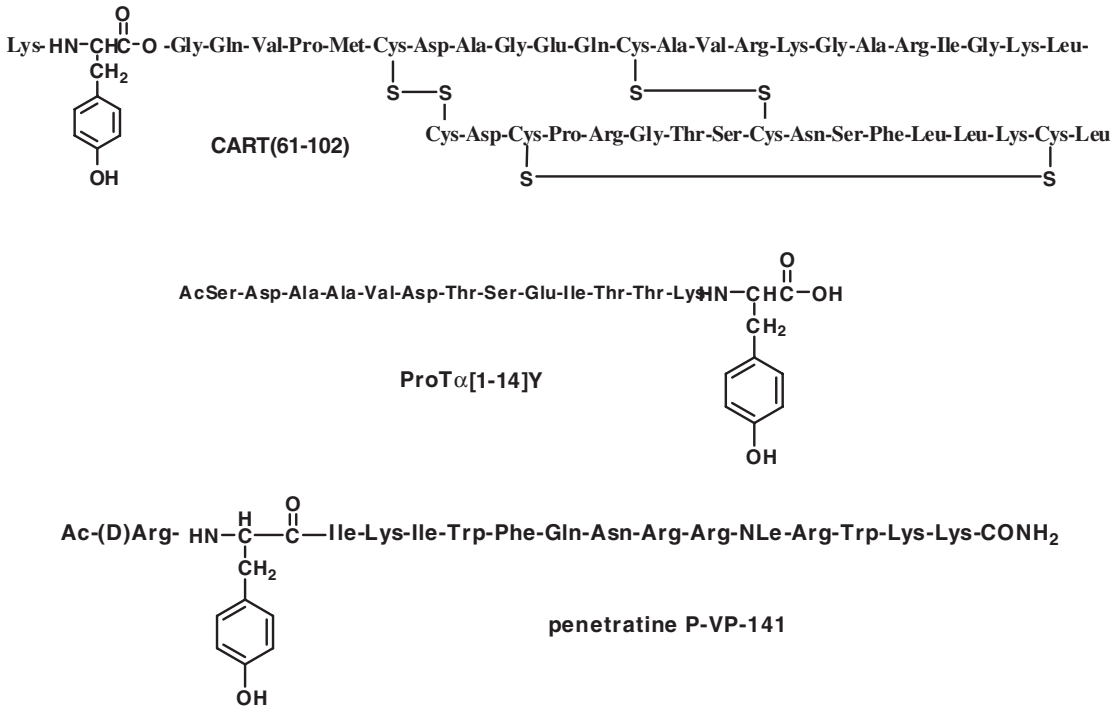
Introduction: Laboratory of Radioisotopes of IOCB ASCR is primarily charged to provide commercially non available labeled compounds for biochemical experiments and experiments in vivo. At present time we are able to prepare tritium labeled compounds and peptides labeled by ^{125}I. We are able to characterize the tritium labeled compounds not only by radio-HPLC but also by ^{3}H NMR.



Scheme 1.



Scheme 2.

Results and Discussion: Recently we prepared alfa-5-aza-2′-deoxy-[6-^{3}H]cytidine (**2**) by CESG method, characterization of the byproducts by ^{3}H NMR confirmed, that in a small extent the double bond in heterocyclic ring is reduced (**3**) during the exchange reaction.[1] The stability of alfa-5-aza-2′-deoxy-[6-^{3}H]cytidine during storage was followed by radio-HPLC (Scheme 1).

In recent 2 years we have labeled 13 peptides by ^{125}I. Three representative peptide sequences are given below (Scheme 2).

For labeling of peptides we used aromatic electrophilic substitution of hydrogen in tyrosine aromatic ring by ^{125}I as a standard method. For conversion of carrier free Na[^{125}I] to [^{125}I]Cl, the water insoluble chloramine IODO-GEN^{TM} coated on the wall of Eppendorf tube was used. After 15 to 20 minutes of shaking the reaction mixture was directly injected on HPLC column. Good resolution of starting peptide and its monoiodo and diiodo derivatives for the peptides with 15 to 30 amino acids was obtained on reverse phase columns in water – acetonitrile gradient.

Using much less excess of IODO-GEN^{TM} to Na[^{125}I] than used in commercially available glass IODO-GEN^{TM} precoated test tubes we were able to label even the peptides otherwise sensitive to oxidative decomposition (containing tryptophane and methionine residues).

Working in PE and PP ware was advantageous also from the point view of tendency of these peptides to stick to the walls of glass vessels. The techniques to further suppress the gluing of the peptides to PE and PP tubes are discussed.

References

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IN VITRO AND IN VIVO EVALUATION OF ^{64}CU-LABELED BISPIDINE LIGANDS

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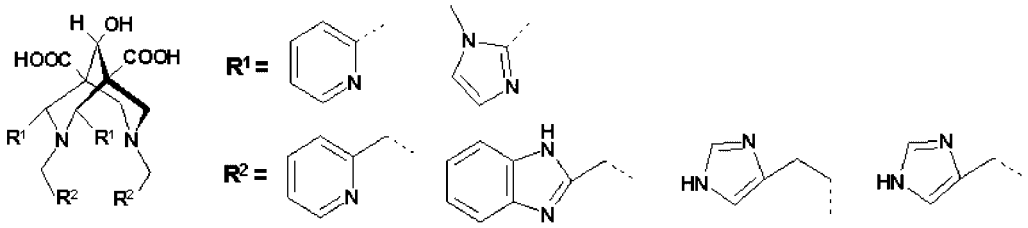
Introduction: The synthesis, characterization and evaluation of novel hexadentate bispidine derivatives containing pyridine and/or imidazole units as donor groups are presented. Bispidine ligands (bispidine = 3,7-diazabicyclo[3.3.1]-nonane) show unique

complexation behaviour towards transition metals.[1,2] The high thermodynamic stability of the complexes of these structurally reinforced ligands with Cu(II) offers the possibility to apply such complexes for diagnostic (^{64}Cu) and therapeutic (^{67}Cu) purposes.[3] Moreover the bispidine structure opens suitable chemical approaches to connect bio-molecules onto the skeleton, an important feature in view of the targeting of such complexes.

Experimental: The ligands were prepared by two consecutive Mannich condensations according to the known procedure.[1] Cyclic voltammetry (CV) measurements were recorded on a BASinstrument with a standard three-electrode cell (glassy carbon electrode, $AgNO_3$/Ag reference electrode, Pt wire with auxiliary electrode) at 25°C in degassed water in an Ar atmosphere. Bispidines were labelled with ^{67}Cu using $^{67}CuCl_2$. To 200 µl of the ligand solution (10^{-4} M ligand in 0.05 M MES/NaOH buffer, pH = 5.4) 250 kBq of $^{67}CuCl_2$ were added. ^{67}Cu-labelling yields were studied by TLC using RP18 TLC plates which were developed in acetonitrile /water (0.1%TFA) = 4/1.

Results and Discussion: CV measurements were performed in order to estimate the stability of the copper(II) bispidine complexes. Strongly negative redox potentials were found for all compounds investigated indicating the high stability of the Cu(II) complexes.[2] Labelling experiments of the new bispidines with ^{67}Cu and ^{64}Cu indicate the rapid formation of radiocopper complexes under mild conditions in almost quantitatively yields (Scheme 1).



Scheme 1.

Conclusion: The radiocopper complexes were found to be stable in the presence of a high excess of competing ligands, and showed a high in vitro stability in rat plasma up to 24 h. Studies on the bioconjugation of the bispidine ^{64}Cu complexes are now in progress.

Acknowledgement: Roger Schibli (Paul Scherrer Institute, Villigen, Switzerland) is gratefully acknowledged for providing copper-67.

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PREPARATION OF RADIO-PHARMACEUTICALS

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The last few years, the number of human studies involving radiolabelled compounds has increased.

Besides Human ADME studies, radiolabelled compounds are more and more used in so called exploratory IND studies. These are clinical trials that are conducted early or even before phase I, and that involve very limited human exposure, and that have no therapeutic or diagnostic intent, based on the microdosing concept. An example of such a clinical trial is the early characterization of a substance's pharmacokinetic-/distribution properties or receptor selectivity profile using Positron Emission Tomography (PET) imaging or accelerator mass spectrometry (AMS).

What all these studies have in common, is the use of a Drug Product containing the radiolabelled Active Pharmaceutical Ingredient. The preparation of such a Drug Product can either be carried out 'in House' or it can be outsourced to specialised CRO's. This presentation will give you an impression of our 'in House' procedures for the preparation of solid as well as non-solid radio-labelled Drug Products.

STABLE AND RADIOLABELLED PHA-690509: A CASE STUDY OF ISOTOPIC LABELLING USE IN THE PHARMACEUTICAL INDUSTRY

ERMINIA FONTANA

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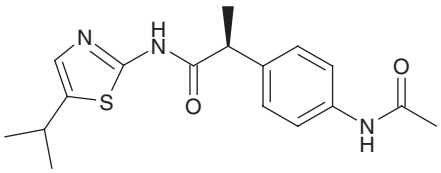
Introduction: The preparation of stable and radiolabelled versions of a new molecular entity and its metabolite(s) are powerful tools in the drug development process.

Results and Discussion: PHA-690509 (namely (*2S*)-2-[4-(acetylamino)phenyl]-N-(5-isopropyl-1,3-thiazol-2-yl)propanamide) is a potent, specific and selective CDK2/Cyclin E and A activity inhibitor and blocks both retinoblastoma protein phosphorylation and DNA synthesis.[1,2]

The compound was selected as drug candidate for its promising *in vitro* and *in vivo* profile.

Therefore more extensive investigations had to be performed that required also suitable isotopically labeled versions of PHA-690509.

The syntheses of [^{14}C], [^{13}C, $^{15}N_2$], [$^{2}H_7$], [$^{2}H_3$, ^{13}C]PHA-690509 and its [^{13}C, $^{15}N_2$]N-deacetylated metabolite as well as selected examples of their use for compound characterization and issue solving will be presented.



PHA-690509

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100 YEARS NOBEL PRICE 1908–ERNEST RUTHERFORD

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1871–1908: Born August, 30th near Nelson, South Island of New Zealand as 1 of 12 children of his parents, Ernest grew up in subtopic climate and pioneer conditions. Having finished classical school and Nelson College he started 1889 with magnetization of iron wires, useful as the best detectors for wireless waves. In 1894 at the age of 23 he applied to a special competition for further studies in England, but he received the second place only. When he was working on the field collecting remaining potatoes, the postman delivered a letter that the winner had resigned: Rutherford cried: 'this was the last potato I have scratched out in my life!'

1895–1898 Cambridge, England: In September 1895 he arrived in the Cavendish Laboratory, Cambridge, early enough to participate in historical scientific discoveries; Rutherford, J.J. Thomson and other scientists too measured the ionization by x-radiation, transferring the results to the ionization by uranium later on.

1898–1907 Mc Gill University, Montreal, Canada: In 1898 he held a professorship at Mc Gill. Now Rutherford had found his final area of research: Radioactivity and Nuclear Physics. Until 1907 early problems of radioactivity have been studied; in most cases the complex and embarrassing results of experiments could be analyzed and successfully solved by Rutherford.

1899–1900: alpha- and ß-radiation, magnetic deflection, speed, charge(+) and (−), active deposit from Th- and later Ra-preparations, identification of emanations as gases, **what had not been solved by Marie Curie; one of the rare errors of Rutherford: erroneous identification of Th- and Ra-emanations as compounds of different boiling points.**

1902–1906: Theory of Atomic Disintegration, historical role of Th-emanation. - Book 'Radioactivity', 1907 in German.-Long lived daughters of Ra: Ra D-Ra E-Ra F (Polonium) and Pb; Otto Hahn as guest from Germany, stories about Radiothorium. - Rutherford President of the Royal Society of Canada.

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1907–1919 Manchester: Now the time of Rutherford's historical results was beginning.

1908: Journey from Manchester via Hamburg and Copenhagen to Stockholm, later back via Berlin (visit Hahn and Meitner) and Leiden (Kamerlingh-Onnes).

Nobel Price in Chemistry 1908: 'We have just returned from our Journey to Stockholm, where we had a great time – in fact, the time of our lives'. Speech at the evening banquet by Rutherford, 'I have dealt with many different transformations with various periods of time, but the quickest was my own transformation in one moment from a physicist to a chemist'.

His Nobel lecture was entitled: 'The Chemical Nature of the Alpha Particles from Radioactive Substances'.

LABELLING METHODS IN (PET-)RADIOPHARMACY: DO WE NEED ALTERNATIVES?

JÖRG STEINBACH

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Introduction: Labelling of molecules was introduced by Georg von Hevesy already decades ago utilizing radiophosphorus. With the availability of C-14 and H-3 a long period of isotopic labelling started in the fifties of the last century following Rudolf Schönheimer's idea to label biomolecules with deuterium. This was the scientific base of the modern biochemistry enabling detailed study of metabolism and of modern pharmacokinetics. With the use of radioiodine fur human investigations and therapy the era of Nuclear Medicine began during that time.

Results and Discussion: All these applications require different labelling techniques for the radiotracer synthesis. Most of them have been derived from organic microchemistry and have been further developed for the special radiochemical conditions such as radiation protection and the limited amount of substance matter.

The introduction of radiometals to Nuclear Medicine, first of all Tc-99m, opened a new challenge. This was the need to mimic organic molecules by coordination compounds–utilizing knowledge from inorganic, organic and radiochemistry.

At the end of the seventies the Positron Emission Tomography came into the focus of science and later on of medicine. Completely new methods had to be developed due to the application of the no carrier added positron emitting radionuclides in connection with their short half lifes first of all for F-18 and C-11.

Nowadays thousands of compounds have been labelled–organic and inorganic. Hundreds of methods have been developed to introduce e.g. carbon isotopes, radiohalogens and radiometals into the desired positions of molecules comprising a wide variety of substance classes.

Have we reached a saturation of demand for labelling methods?

Of course–for small molecules the requirements for real new developments may be limited. But new demands are at the horizon: This are biomacromolecules such as proteins, peptides, oligo-nucleotides which are sensitive to the 'classic' conditions of chemical reactions. Other problems are connected with the need for regioselective, site specific labelling.

There is and will be done a lot of work–also in the future for applied purposes.

HYDROXY[^{14}C]METHYLPHOSPHONIC ACID DIETHYL ESTER, [2,3-^{14}C]FUMARIC ACID, [U-^{14}C]GLUCOISOSACCHARINIC ACID & 1-[(TRIMETHYLSILYL)OXY]-1,3-[1-^{14}C]BUTADIENE

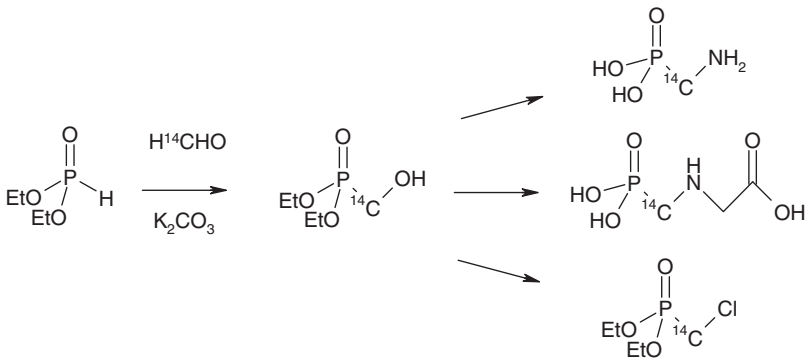
JON BLOOM

GE Healthcare, Cardiff, United Kingdom

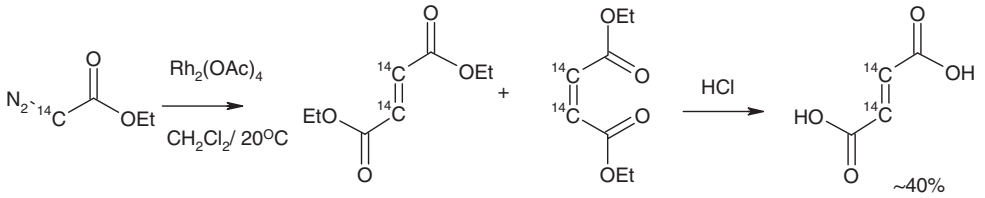
Hydroxy[^{14}C]methylphosphonic acid diethyl ester is prepared from aqueous [^{14}C]formaldehyde, diethyl phosphite and potassium carbonate. This compound has been used a precursor to synthesize amino[^{14}C]methylphosphonic acid, chloro[^{14}C]methylphosphonic acid diethyl ester and [^{14}C]glyphosate (Scheme 1).

[2,3-^{14}C]Fumaric Acid is prepared by the self coupling of ethyl [2-^{14}C]diazoacetate followed by acid hydrolysis (Scheme 2).

[U-^{14}C]Glucoisosaccharinic acid is prepared by alkaline degradation of [^{14}C]cellulose. The compound is purified by conversion into the tribenzoyl lactone, followed by chromatography and hydrolysis (Scheme 3).



Scheme 1.



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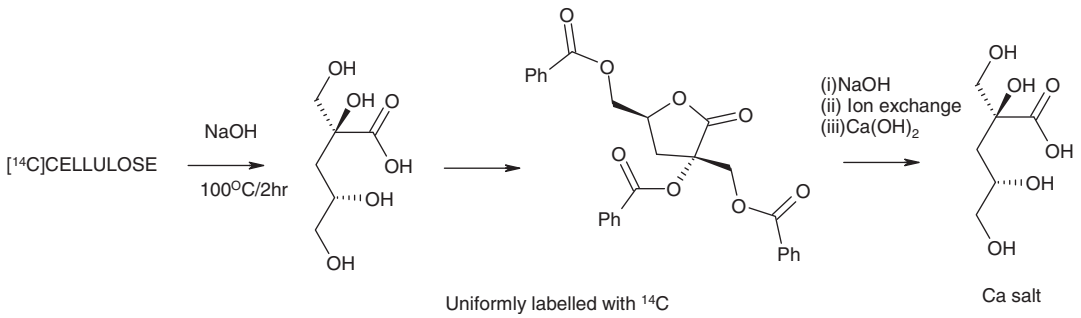
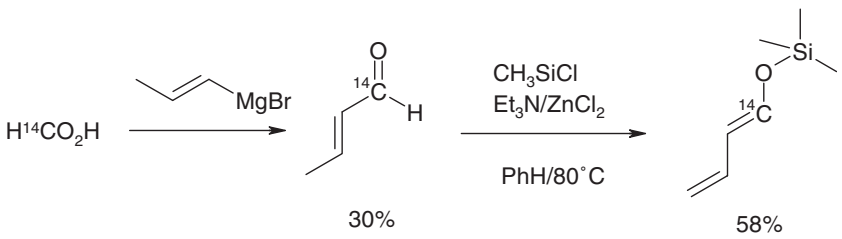


Scheme 3.



Scheme 4.

[^{14}C]Crotonaldehyde is prepared by reaction of [^{14}C]formic acid with 1-propenyl magnesium bromide. This compound is converted into 1-[(Trimethylsilyl)oxy]-1,3-[1-^{14}C]butadiene by reaction with trimethylsilyl chloride, zinc chloride and triethylamine. The product is a diene for Diels-Alder reactions (Scheme 4).

IN VITRO BIOLOGICAL EVALUATION OF ^{188}RE-ANTI-VEGF-MAB AND ^{188}RE-ANTI-MUC1

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Introduction: Radiolabelled antibodies with beta emitters offer a promising alternative for management of different malignancies. For the radioimmunotherapy of cancer, the development of the ^{188}W/^{188}Re generator, gives the possibility of having a radionuclide with therapeutic effect by virtue of its specific nuclear properties as: $E_{\beta} = 2{,}12$ Mev, $E_{\gamma} = 155$ keV, $T_{1/2} = 16.9$ h and easy chemistry which allows to make similar labelling approaches to those used for ^{99m}Tc.

Aim: The aim of the present work was radiolabelling of VEGF and MUC1 monoclonal antibodies with ^{188}Re and the evaluation of the saturation binding of ^{188}Re-VEGF and ^{188}Re-MUC1 to HeLa tumour cells as well as the induced cytotoxicity of radiolabelled antibodies in tumour cells by inhibition of the specific receptors functions and delivery of β-radiation doses.

Methods: Direct labelling method was employed for the synthesis of the ^{188}Re-anti-VEGF-Mab and ^{188}Re-anti-MUC1, using 2-mercaptoethanol (1;10 v/v 2-mercaptoethanol:ethanol) as a reducing agent of –S-S- cysteine bounds. ^{188}Re-labeling yield was proportional to antibody/2-mercaptoethanol molar ratio. Optimization studies of the reaction time and temperature indicate an incubation time of 90 min. at 90°C was required for obtaining maximum complexation yield.

The tumour cell lines (HeLa, MCF-7 and MDA-MB-231) were characterized as heterogenous tumour cells with high expression of the VEGF/MUC1 receptors. 4×10^5 HeLa cell samples were used in evaluation of the saturation binding of ^{188} Re-VEGF respectively ^{188}Re-anti-MUC1. The induced radiotoxicity of ^{188}Re-VEGF and ^{188}Re-anti-MUC1 was evaluated by MTT method using MDA-MB-231 respectively MCF-7 cell lines. The citotoxicity of these two radiolabelled antibodies was also tested by MTT using HeLa cell lines after 24 and 48 h incubation time.

Results and Discussion: Radiochemical purity of ^{188}Re-VEGF and ^{188}Re-anti-MUC1 were higher than 95% while the specific activities of the radiolabelled antibodies were 100 mCi/mg.

The maximum binding of ^{188}Re-MUC1 to expressive tumor cell receptors was reached for 0.3 μg antibody. The induced radiotoxicity of ^{188}Re-MUC1 is high, as the MCF-7 cells viability decreases fast to zero at 100 μCi. The ^{188}Re-MUC1 induces a cytotoxicity which decrease the HeLa cells viability up to 62.34% (at 48 h, 100 μCi).

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The results also show that for 0.3–0.6 μg antibody there is maximum binding of ^{188}Re-VEGF to expressive tumour cell receptors. The graph of the induced radiotoxicity of ^{188}Re-VEGF registers a decreasing of the MDA-MB-231 cells viability in 50–100 μCi radioactivity range up to 90% while the cytotoxic effect of the ^{188}Re-VEGF on HeLa cells was also high.

These results will be used for the in vivo studies regarding radiopharmacokinetics and the therapeutic effect of ^{188}Re-anti-VEGF-Mab and ^{188}Re-anti-MUC1.

RADIOCHROMATOGRAPHY: ANALOG VERSUS DIGITAL RECORDING

GÜNTER DIETZEL

Raytest GmbH, Straubenhardt, Germany

Introduction: The analog output of a radioactivity flow detector 0–1V was developed in the last century, when pen-recorders were used for recording the radioactivity countrate over eluate flow (time) in LC and distance in TLC and gas flow (time) in GC.

For peak integration the paper record was used to be cut out the peak-area using a scissor and weighing the paper-peak on a laboratory balance. Such archaic peak-integration methods were replaced by electronic peak integrators, which were often derived from pen-recorders.

From 1980 on raytest used personal computers to record, integrate and later control chromatogramic systems. Although personal computers can only record and integrate digital information, it was fashionable to connect chromatography detectors by the auxilliary analog input of 0–1V.

Radioactivity detectors in all chromatography applications are 'counting' single radiation events' over flow, distance and time and the recorded information of the 'counting speed' could only be entered in computerized chromatographic recorders and integrators by the auxilliary anlog input 0–1V.

Results and Discussions: All radioactivity detector manufacturers were using digital ratemeter units, in order to convert the recorded number of radioactive counts 'single events' into an anlog information of 0–1V for recording. The result was the conversion from the original digital (counting information) into a suitable analog input information 0–1V and since computers can only handle digital information, an suitable ADC (Analog-to-Digital Converter) converted the information back to digital.

Unfortunately on this way from digital to analog to digital the property radioactive radiation, the statistical variation, was lost for ever. Therefore raytest recorded since 1980 the original information of radioactivity measurement over flow, distance and time directly, digitally in the connected personal computer.

Conventional chromatography instrument manufacturer did never follow that idea, because radioactivity detection in chromatographic application was compared to the huge number of non-radioactive application rather small and there was simply not enough market inquiries in order to follow this trend. The frequency of radioactivity detection in chromatography has not increased but decreased in recent years and there is simply not enough market volume for the curious requirement of radioactive 'single event counting' in radiochromatogarphy.

Meanwhile the industry of chromatographic instruments nevertheless is following the trend to digital data transfer, processing, control and storage by introducing the advantages of LAN-local area network-technology in their system control and recording. But the chromatographic instruments industry did not pay any attention to direct single event counting and digital radioactivity recording of radioactivity.

Even with 'modern' digital LAN control and recording, the properties of the statistics of the radioactivity detection are not regarded.

Raytest is demonstrating in this presentation, that 'SEC' with statistical analysis is much more (about 100 times more) sensitive than conventional analog recording or even modern LAN based systems. This digital recording method and statistical analysis can be integrated in any chromatographic controlling, recording and integrating using PC. Sensitivity of radioactivity detection in chromatographic applications is needed very much for better analysis of metabolism, pharamacokinetic, excretion and quality control of radioactive labelled compounds.

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

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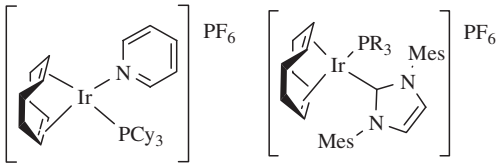
[a]Dept. of Pure and Applied Chemistry, University of Strathclyde, Glasgow, United Kingdom
[b]Dept. of Medicinal Chemistry, AstraZeneca R&D Mölndal, Mölndal, Sweden

***Hydrogen Isotope Exchange*:** Hydrogen isotope exchange (HIE) represents an effective way of following the metabolic pathways of potential drug candidates and, therefore, is of increasing importance to the pharmaceutical industry. The ideal situation would be

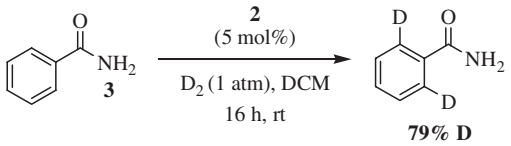


1 2

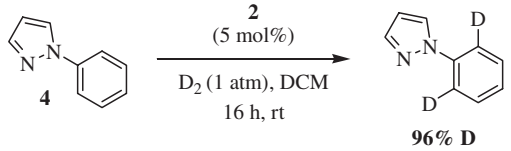
to utilise catalytic quantities of a transition metal species to carry out this transformation on a fully functionalised compound. A limited range of iridium catalysts has been developed and applied to this process with varying success. Traditionally, the most effective catalyst used in this field is Crabtree's catalyst **1**.[1] However, often super-stoichiometric amounts of this species and long reaction times are required, with low levels of labelling being encountered. With this in mind, a series of novel iridium(I) complexes of type **2**, bearing specifically bulky *N*-heterocyclic carbene ligands together with bulky phosphine ligands, have been developed and applied to HIE.[2] At only 5 mol% complex loading, high incorporation is achieved into substrate **3** (Scheme 1).

Furthermore, the site of labelling is highly predictable and reproducible. Substrate **3** is a traditionally difficult substrate to label and, indeed, *110 mol% of Crabtree's catalyst* is required to achieve a mere 65% labelling.[1] Scheme 2 shows another impressive result, with substrate **4** being labelled to an extremely high degree. Again, a *stoichiometric amount of Crabtree's catalyst* was previously required to induce a similar level of labelling.[3] Finally, after conducting loading and time studies on substrate **5**, it was found that only 0.5 mol% of the catalyst was required to deliver an extremely high level of labelling over a remarkably short reaction time (Scheme 3).

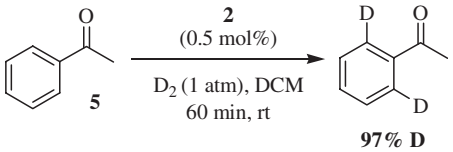
***Selective Hydrogenation*:** Based on the outstanding levels of activity shown in C-H activation, we felt that these same iridium-based species had the potential to act as selective hydrogenation catalysts. To investigate this hypothesis, we chose substrate **6**, containing a benzyl group, which is easily removed under hydrogenation conditions. As shown in Scheme 4, using only 0.5 mol% of the complex, substrate **6** was fully reduced in extremely short order, and we were pleased to observe that the benzyl group stayed intact throughout the reaction. Under the same conditions with *Crabtree's catalyst, only a 28% conversion to product was obtained after 1 hour*. Alongside this, we have investigated whether our complexes could be used for the semi-hydrogenation of alkynes, i.e. reducing an alkyne to an alkene. With only 0.1 mol% of complex **2**, and employing benzamide as a poison, the alkyne, **7**, can be selectively reduced, yielding 85% of the corresponding alkene with a *Z*:*E* ratio of 42:1 (Scheme 5).



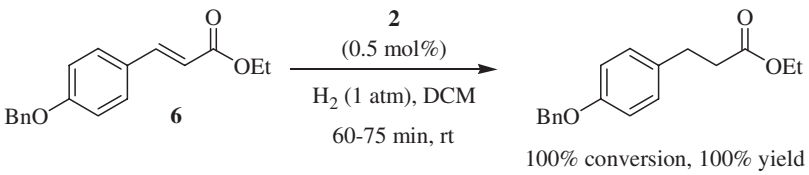
Scheme 1.



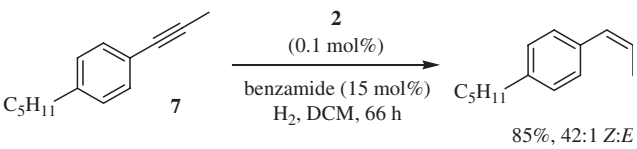
Scheme 2.



Scheme 3.



Scheme 4.



Scheme 5.

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PRODUCTION AND QUALITY CONTROL OF IODINE-123 SOLUTION

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Agriculture and Medicine Institute, Karaj, Iran

Aim: Sodium Iodide-123 is well known as a radioisotope that is used for studies of the thyroid and its metastases. ^{123}I labeled compounds are used for kidney, heart and brain studies. Since demand for ^{123}I is increasing a serious effort for its production has performed.

Material and Methods: For production of ^{123}I, a home made target was designed. The main part of the target contained; conical target vessel that was mounted in an aluminum cylinder of length 30 cm and diameter 8.5 cm for water cooling during bombardment. Front and rear target windows made by titanium foil and cooling system. In addition, some other part such as a cold finger, decay vessel, vacuum pump and four fingers also designed and installed on the system. ^{123}I was produced from the reaction of 28 Mev protons with 99.9% enriched ^{124}Xe with Cyclon-30. Bombarded xenon was transferred to the vessel and stored there up to about 6 hours to allow ^{123}CS decay to ^{123}I with the maximum efficiency. The remaining xenon gas was then transferred into the reservoir capsule using a cryogenic technique. The produced, ^{123}I which is deposited on the wall of the decay vessel, was rinsed out by dissolution in 0.05 μs water at 80°C. Nitrogen gas pressure was used for the transfer the ^{123}I solution to the chemical hot lab. The pH, and adjusted with sodium hydroxide.

Results and Conclusion: A sample of production was send to quality control. Quality control results were in good accordance with the United States Pharmacopoeia. Using this system with the above specification, the production yield could be increased by increasing the current. The results show no chemical or radionuclide impurity, and enable to produce I-123 without any more purification step.

BREATH TESTS IN PERSONALIZED MEDICINE: A COMPREHENSIVE OVERVIEW

A. S. MODAK

Cambridge Isotope Laboratories Inc., Andover, USA

Introduction: Physicians have long recognized that the smell of their patients' breath can provide clues to what is ailing them since the time of Hippocrates. Antoine Lavoisier was the first to analyze breath and demonstrate that it contains carbon dioxide. More than 3000 gases have now been identified in samples of exhaled human breath and several have been specifically associated with a disease process.

Results and Discussion: In this presentation, I would like to discuss novel innovative non invasive phenotype breath tests currently being evaluated at academic and pharmaceutical organizations and discuss the trends and future directions of breath tests in the field of personalized medicine.

Stable isotope ^{13}C labeled compounds have been widely used as diagnostic probes in research laboratories for over 30 years.[1] ^{13}C stable isotope diagnostic probes are now being expanded in their scope, to provide precise evaluations of the presence or absence of etiologically significant changes in metabolism due to a specific disease or the lack of a specific enzyme. The concept exploits the use of the ^{13}C-label that is incorporated at the appropriate site into a selected substrate specifically designed for the targeted enzyme in a discrete metabolic pathway. The enzyme-substrate interaction, results in the release of $^{13}CO_2$ in the expired breath. The subsequent quantification of $^{13}CO_2$ allows for the indirect determination of pharmacokinetics and the evaluation of enzyme activity.

Conclusion: The diagnostic breath tests will enable physicians and patients to benefit from rapid, novel and noninvasive ways to detect enzyme deficiencies, to monitor the progress of disease severity or medication efficacy, to trace acquired and/or congenital metabolic defects, to study *in vivo* the pharmacokinetics of xenobiotics, and to optimize individually tailored treatment therapies.

Reference

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POSTER PRESENTATIONS

^{14}C-LABELLED DITHIOCARBAMATE FUNGICIDES

TAMÁS VARGADI, ALIZ FUCHS, ERNŐKOLTAI, ANDRÁS ALEXIN, and ELISABETH B. FAIGL

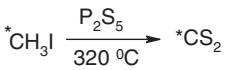
Institute of Isotopes Co., Ltd., Budapest, Hungary

Indroduction: Nowdays, the efficient production in the agriculture requires the application of various types of pesticides. Isotopically labelled compounds are essential requisites in the research and development of environmentally benign agricultural technologies.

Results and Discussion: SDDC (sodium dimethyldithiocarbamate), Ziram (zinc bis(dimethyldithiocarbamate)) and Thiram (bis(dimethylthiocarbamoyl)disulfide) are the important members of the dithiocarmate fungicides. All these fungicides are produced at industrial scale.

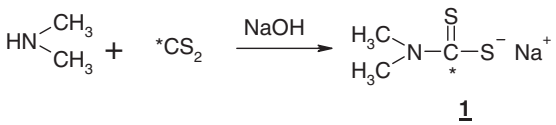
However, we could not find any data on the synthesis of C-14 labelled molecules in the literature. Therefore, as a part of our ongoing program, we aimed at the syntheses of the C-14 dithiocarbamates labelled in different positions.

SYNTHESIS OF [CARBOXYL-^{14}C]SDDC: The key intermediate of the syntheses of the carboxyllabelled dithiocarbamates is the C-14 labelled carbon disulfide which was synthesized by a method known from the literature (Scheme 1):[1]



Scheme 1.

Carbon disulfide was reacted with dimethylamine in an aqueous solution in the presence of an equivalent base to give SDDC (1): (Scheme 2):



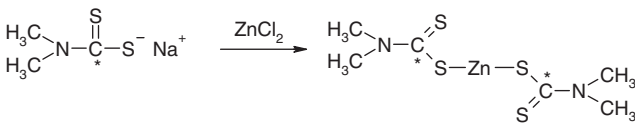
Scheme 2.

SDDC is sensitive against the oxidation, thus it was isolated by lyophilyzation.

The yield was 80% based on [^{14}C]methyl iodide. Molar activity: 1300 MBq/mmol.

SYNTHESIS OF [CARBOXYL-^{14}C]ZIRAM and [CARBOXYL-^{14}C]THIRAM: Ziram (2) was prepared from SDDC (1) with zinc chloride in aqueous medium.

It was purified by crystallization from chloroform/hexane (Scheme 3).

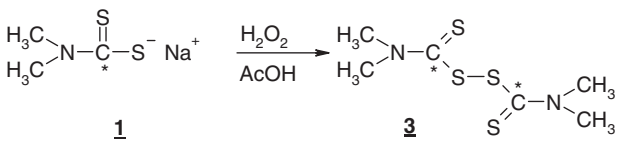


Scheme 3.

The yield was 70% based on [^{14}C]methyl iodide. Molar activity: 666 MBq/mmol.

Thiram (3) was prepared from SDDC (1) oxidizing it with peracetic acid.[2]

The crude product was washed with ethanol resulting in the purity higher than 95% (Scheme 4).



Scheme 4.

The yield was 60% based on [^{14}C]methyl iodide. Molar activity: 2035 MBq/mmol).

SYNTHESIS OF [METHYL-^{14}C]ZIRAM: The key intermediate in the synthesis of [methyl-^{14}C]Ziram is the [^{14}C]dimethylamine which was synthesized from [^{14}C]methyl iodide.

4-Toluenesulfonic acid amide was alkylated in protic (water/NaOH) or in aprotic (DMF/NaH) solvent and the formed dialkylamide was hydrolyzed with 48% HBr aqueous solution (Scheme 5).

The [^{14}C]dimethylamine was liberated with aqueous NaOH and it was removed from the reaction mixture with a carrirer gas. SDDC (1) and then Ziram (2) was prepared as it was described above (Scheme 6).

The yield was 61% based on methyl iodide. Molar activity: 3850 MBq/mmol).

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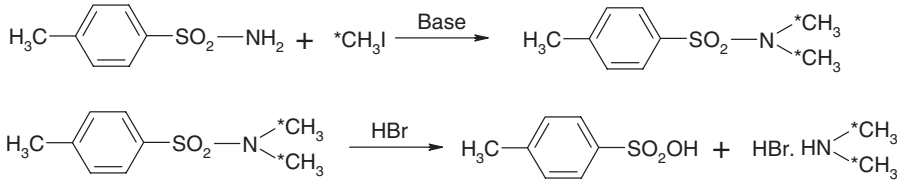
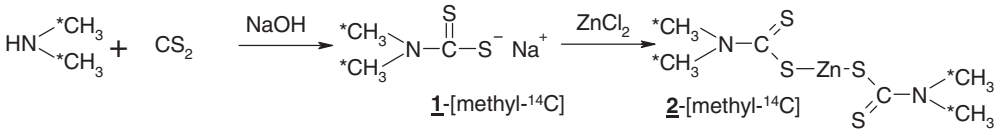


Scheme 5.



Scheme 6.

References

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[2] DE 19930625 German patent (**2001**).

HUMAN ALBUMIN NANOSPHERES LABELLED WITH ^{99M}TC

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[a]National Institute of R&D for Physics and Nuclear Engineering 'Horia Hulubei', Bucharest, Romania
[b]National Research & Development Institute for Chemistry & Petrochemistry, Bucharest, Romania
[c]National Institute of Materials Physics, Bucharest, Romania

The aim of this paper is to present a method for the labelling of human albumin nanospheres with ^{99m}Tc. Nanospheres of 200–500 nm were prepared by a desolvation technique. The influence of different parameters like pH, the quantity of stannous chloride, the time on the reaction yield was studied. Under optimal conditions yields more than 97% were obtained. The ^{99m}Tc binding to the nanospheres was found to be stable in vitro, 6 hours after labelling. The labelled albumin nanospheres are intended to be used in the imaging studies of the inflammation.

PREPARATION AND IMAGING STUDIES OF ^{67}GA-DTPA IN RAT

A. SATTARI, M. POLADI, S. MORADKHANI, F. SADDADI, A. KARIMIAN, and S. DANESHVARI

Nuclear Medicine Group, Agriculture, Medical and Industrial Research School, Karaj, Iran

Introduction: Indication of ^{67}Ga-DTPA in biokinetic studies has been reported recently. In this article preparation and imaging of ^{67}Ga-DTPA (diethylene triamine pentaacetic acid) comparing distribution of ^{67}Ga-citrate in normal mice is presented.

Methods: Zinc-68 target was irradiated by 22 MeV protons in cyclotron, Carrier free ^{67}Ga was obtained after separation and purification by ion exchange chromatography using AG 50 W, 200–400 mesh, H^+ form (high:7 cm, :1 cm) and liquid-liquid extraction which had performed by Di-isopropyl ether. 0.2 ml sodium acetate buffer (0.5 M, pH = 4.5) and 0.5 ml DTPA solution containing 4 mg DTPA in 0.4 ml, normal sodium hydroxide were added to 0.5 ml ^{67}Ga-chloride solution. 200°μCi, ^{67}Ga-DTPA was injected in the tail vein of a normal mice aged 40 days, Images after 2 and 24 hours were investigated.

Results: Quality control results showed less than 0.1 ppm zinc and copper in the final solution beside, 96% radiochemical purity which was performed by TLC method, ammonium acetate10% and methanol (1:1), Rf = 0.7.

In first imaging, 2 h after administration ^{67}Ga-DTPA in head, chest, heart, kidneys, liver and bladder was accumulated while within 24 h, in second imaging, kidneys and bladder have been detected and there was no detection on head, chest and heart. ^{67}Ga-citrate has no detection in blood and kidneys while was detected in liver and spleen after 24 h. Regarding to our results ^{67}Ga-DTPA could indicate in medical imaging of kidney and its inflammatory disease study.

NEW TOTAL SYNTHESIS OF [$^2H_5$]-OCHRATOXIN A

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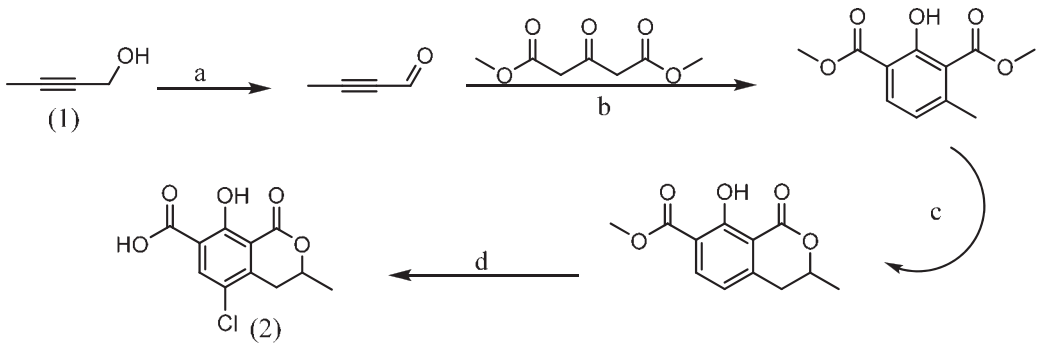
[b]Dep. of Chemistry, University of Calabria, Calabria, Italy

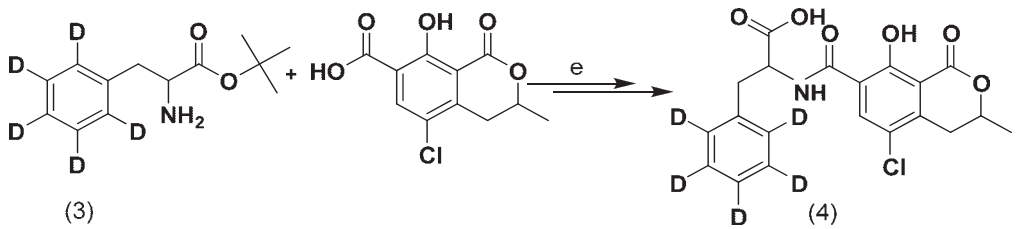
Introduction: Stable isotope dilution assay (SIDA) has recently attracted considerable attention for the quantification of micro-components in food due to its r excellent accuracy and sensitivity. In particular the quantification of the mycotoxin Ochratoxin A in wine and beer samples can be performed using [$^2H_5$]-OTA as internal standard.[1]

Results and Discussion: Recently we have developed a new facile procedure for the total synthesis of [$^2H_5$]-OTA (4) based on the use of low cost and readily available starting materials. In this procedure we have firstly synthesized ochratoxin α (2) starting from 2-butynol in four steps (a, b, c, and d) with an overall yield of 23%, and then we have coupled ochratoxin α with [$^2H_5$]-L-Phenylalanine (3). Eventually deprotection of the carboxylic group gave [$^2H_5$]-OTA as a mixture of diastereomers with a yield of mixture of diastereomers with a yield of 86% (Scheme 1).

The quantification of Ochratoxin A in beer and wine samples will be done by the development of George J. Soleas's method using [$^2H_5$]-OTA as internal standard by GC-MSD (Scheme 2).[2]



Scheme 1.



Scheme 2.

References

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SYNTHESIS AND H/D ISOMERIZATION OCCURRED DURING THE GAS-PHASE FRAGMENTATION(MS/MS MODE) OF RING-LABELED D_3 AND D_4- TRANS-RESVERATROL

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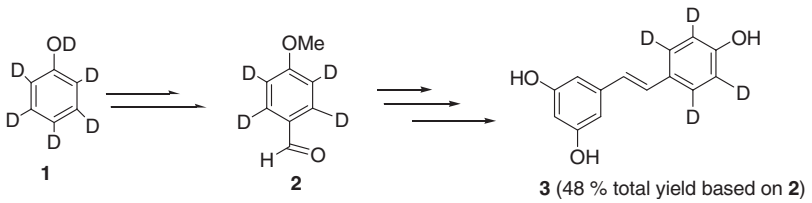
Introduction: Resveratrol (3,4′,5-trihydroxystilbene) is a dietary antioxidant polyphenol occurring in high to moderate quantities in various foods including grapes, peanuts and wine. Resveratrol as a constituent of red wine[1] has been implicated in the French paradox:[2] the incidence of coronary heart disease is relatively low in southern France despite high dietary intake of saturated fats and suggested to mediate the cancer chemoprevention proprieties of red wine.



Scheme A.

Results and Discussion: Resveratrol has also been reported to possess a variety of anti-inflammatory, and anti-estrogenic effects, so its presence in wine is an important qualitative parameter because of the several beneficial effects on human health. In the

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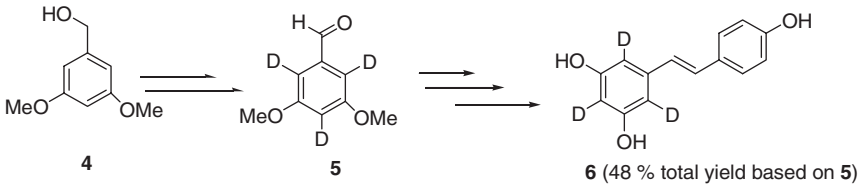
present work we describe two alternative strategies for the preparation of ring-labeled-d_3 and d_4- *trans*-resveratrol. We have synthesized the ring-labeled-d_4-*trans*-resveratrol **3** starting from d_6-phenol **1**,[3] according to scheme A with an isotopic purity exceeding 96% of d_4. The latter is stable under Electrospray Ionisation MS conditions, but it undergoes deuterium exchange[4] in MS/MS mode. We therefore suggest that this derivative can be used as a standard for metabolic and analytical studies based on ESI/MS. The ring-labelled-d_3-*trans*-resveratrol **6** was synthesized from commercially available 3,5-dimethoxybenzylalcohol **4** (scheme B). In this case the **H/D** exchange and redistribution occurs during in the last step of the synthesis to give instead a mixture of isotopologues, d_0, d_1, d_2, d_3 with a distribution of 18%, 12%, 19.5% and 50.5% ^{2}H yields, respectively. A similar behavior occurred in the gas-phase fragmentation in MS/MS experiments on ring-labeled-d_3- *trans*-resveratrol **6** (Scheme B).



Scheme B.

References

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[2] N. Labinskyy, A. Csiszar *et al. Curr. Med. Chem.* **2006**, *13*, 989–996.
[3] B. Gabriele, H. Benabdelkamel, P. Plastina, G. Sindona, A. Tagarelli, *7th Sigma Aldrich Young Chemists Symposium,* Riccione (RN), October 22–24, pp. P10.
[4] D. Kuck, *Int. J. Mass Spectrom.* **2002**, *213*, 101–144.

DETERMINATION OF TRACE ANIONS IN WATER FROM THE SPENT NUCLEAR FUEL WET STORAGE

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[a]National Institute of R&D for Physics and Nuclear Engineering 'Horia Hulubei', Bucharest, Romania
[b]Faculty of Chemistry, University of Bucharest, Bucharest, Romania

Introduction: This paper presents the results of trace anions determination in high purity water from spent nuclear fuel pools at IFIN-HH storage facility. There has been considerable interest in the determination of anions at trace levels by ion chromatography: chloride, bromide, fluoride, nitrite, nitrate, sulphate and phosphate.

Results and Discussion: Equipment used was Dionex ICS-3000 Ion Chromatography System consisting of:

- Isocratic pump SP;
- Chromatography module with single temperature zone;
- CD20 Conductivity detector;
- IonPac-AS4A (4 mm) Anion Exchange Column;
- IonPac-AG4A (4 mm) Guard Column;
- AAES Anion Atlas Suppressor;
- TAC-2 Trace Anion concentrator;
- Auxiliary AXP pump;
- Software Chromeleon version 6.8 CHM-1.

A preconcentration or trace enrichment technique has been utilized. With this method, the analytes of interest are preconcentrated on a small pre-column in order to ≪ strip ≫ ions from a measured sample volume.

Reagents and standards were deionized water, Type I reagent grade, 18MΩ-cm resistance, 1,8 mM Na_2CO_3/1,7 mM $NaHCO_3$ as eluent solution and stock seven anions combination standard solutions 1000 mg/l.

Method detection limits were established the standard deviation of nine replicates multiplied by the Student's *t* value for the 99% confidence level. Calibration curves were obtained with standards prepared in deionized water. Results for the 7 anions of interest yielded a linear response with coefficients of determination (R^2) greater than 0.99.

ENHANCED ON-LINE DETECTION FOR RADIOCHEMICAL MEASUREMENT

K. A. HALL, and H. LOARING

LabLogic Systems Limited, Sheffield, United Kingdom

Recent moves towards the use of increasingly lower levels of radioactivity in samples for metabolite profiling whilst still favouring the speed and convenience of on-line detection have led to the development of SoFie. On-line radio detection of compounds

separated by both HPLC and the newer fast LC/Rapid resolution techniques remains an important tool for researchers. The challenge is to detect ever decreasing levels of activity whilst remaining in the highly controlled and regulated environment of the trusted software systems. Here we show that the new SoFie system, controlled by the Laura radio chromatography system offers up to 8 times the level of sensitivity of standard on-line detection. This eliminates the need for fraction collecting which is always time consuming and potentially destructive to the delicate sample as further processing is required for counting on plate readers. The SoFie system also offers enhanced resolution as well as improved limits of detection.

AUTOMATED PREPARATION FOR LIQUID SCINTILLATION COUNTING OF C-14 AND H-3 LABELLED SAMPLES

W. ZINSSER

Zinsser Analytic GmbH, Frankfurt, Germany

Introduction: 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.

Results and Discussion: 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 oxidiser 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.

With its enclosure, Robox is a remote enclosed workstation providing a safe environment for the user.

SYNTHESIS OF TRITIATED BIOTIN BY ISOTOPE EXCHANGE CATALYST METHOD

C. POSTOLACHE, L. MATEI, and G. BUBUEANU

National Institute of Research and Development for Physics and Nuclear Engineering 'Horia Hulubei', Bucharest, Romania

The labelled biotin was obtained by isotopic exchange reaction in heterogeneous catalysis, using biotin sodium salt as substrate and T_2 as labelling agent. Pd/C and Pd/$BaSO_4$ were used as isotope exchange catalyst and dimethylformamide-phosphate buffer as solvent. Reaction time was 8 hours at room temperature. The raw labelled compound was purified by preparative TLC using silica gel 60F and butanol: acetic acid: water (4:1:1 v/v/v) as mobile phase. Distribution of the radioactivity on the plate was identified using LB 2723 Berthold scanner with proportional counting detector.

The labelled biotin was conditioned as aqueous solution at 37 MBq/ml radioactive concentration. Characterization of labelled compound was accomplished by determination of chemical concentration, radioactive concentrations and radiochemical purities. Chemical concentration was determined using SPECORD 210 UV VIS spectrometer at 490 nm. Radioactive concentration was determined using TRICARB 2800 TR LSC spectrometer and ULTIMAGOLD as liquid scintillation cocktail. Radiochemical purity was determined using the previous presented TLC system.

The physical and chemical characterization of labelled biotin reveals a radiochemical purity higher than 95%, and a specific activity of 314 GBq/mmol.

The labelled compound was used as radioimunodiagnostic in virology.

SYNTHESIS OF TRITIATED METHADONE AND NALOXONE BY ISOTOPE EXCHANGE CATALYST METHOD

L. MATEI, C. POSTOLACHE, C. TUTA, and G. BUBUEANU

National Institute of Research and Development for Physics and Nuclear Engineering 'Horia Hulubei', Bucharest, Romania

The labelled methadone was obtained by isotopic exchange reaction in heterogonous catalysis, using T_2 as labelling agent. Pd/C was used as isotope exchange catalyst and dioxane: acetic acid mixture as solvent. Reaction time was 14 hours at room temperature (Scheme 1).

The raw labelled compound was purified by preparative TLC using silica gel 60F and water: acetic acid: ethanol 10:30:60 (v/v/v) as mobile phase.

Naloxone was labelled by isotopic exchange reaction in heterogonous catalysis, using lyophilised NARCANTI (naloxone 0.02 mg/ml water solution) as substrate, T_2 as labelling agent.

Pd/$BaSO_4$/piridine was used as catalyst and dioxane as solvent. Reaction time was 4 hours at room temperature (Scheme 2).

The raw labelled compound was purified by preparative TLC using silica gel 60F and methanol 5 ml: 95 ml (60 ml ammonia 25% water solution and 100 ml N-butyl alcohol mixture) as mobile phase.

After purification, labelled methadone and naloxone were obtaining with physical-chemical characteristics show in Table 1.

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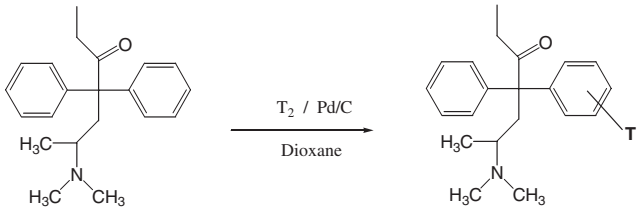
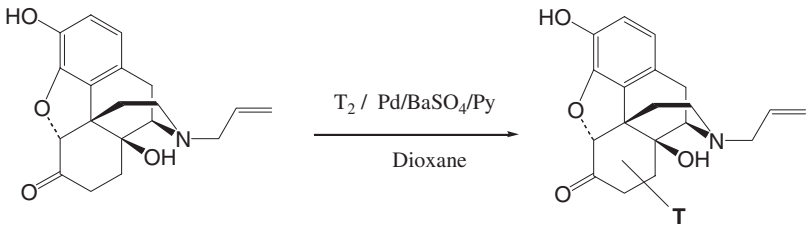


Scheme 1.



Scheme 2.

Table 1. Labelled methadone and naloxone characteristics

	Methadone-T	Naloxone-T
Raw product activity [MBq]	853	245
Purified product activity [MBq]	597	159
Purified product radiochemical purity [%]	>95	>95
Radioactive concentration [MBq/mL]	35	18
Chemical conc. [mg/mL]	0.020	0.027
Specific activity [GBq/mmol]	548	218

IN VITRO AND IN VIVO RADIOMETRICAL STUDIES FOR EVALUATION OF NEW NUCLEOSIDE ANALOGUE BEHAVIOR

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Introduction: Nucleosides and their analogues are for a long time a recognized class of clinically useful drugs possessing antiviral and anticancer activity. Intensive research is developed worldwide for new more efficient and more selective antitumor and antiviral nucleoside analogues, with limited side-effects.

We investigated the behavior of a novel pyrimidine nucleoside analogue-U-34 (Figure 1), in which the sugar moiety was modified by replacing the furan ring with a functionalized oxabicyclo [3.3.0]octane fragment and using the naturally occurring pyrimidine uracil, as base.

Results and Discussion: In this study has been observed the in vivo and in vitro action of the U-34 compound.

The in vitro action of U-34 was studied on human T lymphoblasts (Jurkat cell line). U-34 (30 μM) exerts cytotoxic effects on Jurkat cells (decrease of tetrazolium salts reduction, accompanied by enhancement of LDH release) and hinders uridine or thymidine uptake (evaluated using tritium-labeled nucleosides).

The limited tritium-labeled uridine and thymidine incorporation, suggests that this inhibition is not sufficient for altering cell viability and proliferation capacity. We point out that U-34 uptake by Jurkat lymphoblasts was highly dependent on the compound concentration per cell.

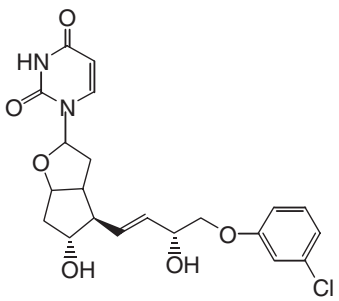

Figure 1. U-34 Nucleosidic Analogue.

Using Wistar rats and a tritium-labeled variant of U-34, we showed that the nucleoside analog accumulated rapidly and persistently in various organs and blood. Accumulation of U-34 in the bowel, shortly after drug administration, may be ascribed to the route of drug inoculation. Protection mechanisms are seemingly acting in liver, at least by limiting drug concentration after an initial phase of accumulation. The small quantity of U-34 detected in secondary lymphoid organs and in blood suggests that U-34 may have in vivo only minimal effects on the immune response.

SYNTHESIS OF THYMIDINE-METHYL-T AND URIDINE-5-T

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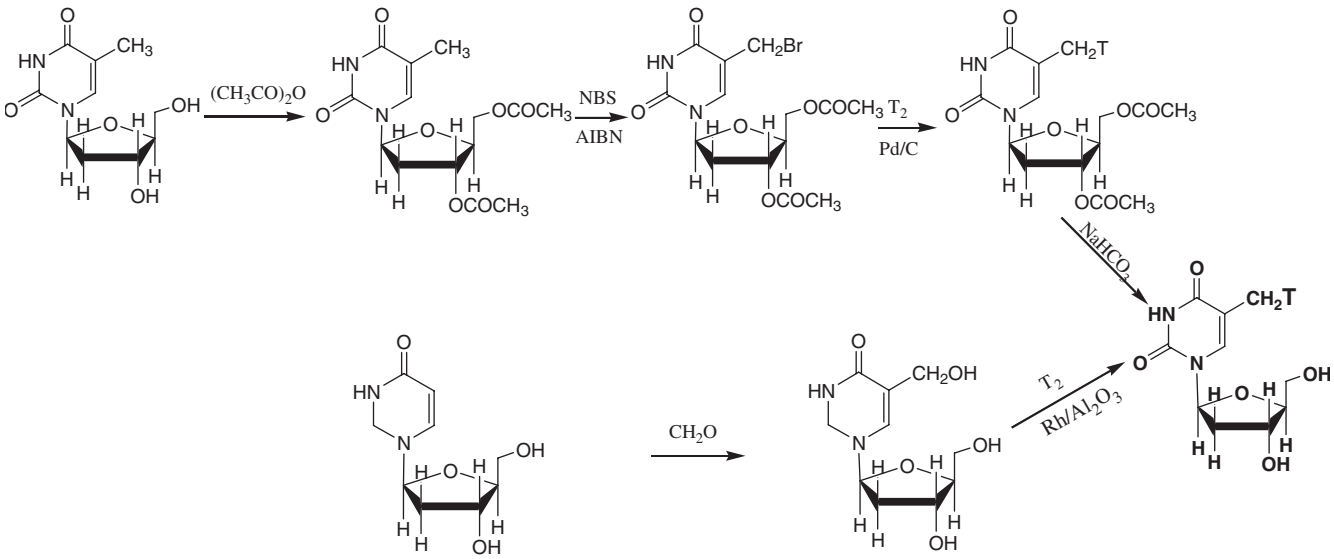
In this study, the labelling of thymidine in the methyl position is described. The synthesis has been performed using two different methods: hydrogenation of a brominated derivative and of hydroxymethylthymidine, respectively (Scheme 1).

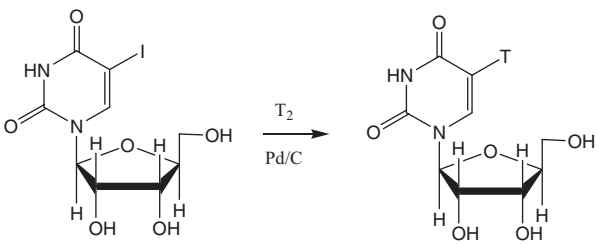
The uridine has been synthesized by hydrogenation of 5-I-uridine following the reaction (Scheme 2):

After purification, the labeled compounds with characteristics presented in Table 1 have been obtained.

The obtained tritiated uridine and thymidine have been used for in vitro radiometrical studies for evaluation of new nucleoside analogue behaviour.



Scheme 1.



Scheme 2.

Table 1. Characteristics of obtained tritiated thymidine and uridine

	Thymidine-Me-T from Br derivative	Thymidine-Me-T from hydroxyl methyl derivative	Uridine-5-T
Raw product activity [MBq]	25086	10767	9805
Purified product activity [MBq]	13986	9472	7955
Raw product radiochemical purity [%]	59	92	85
Purified product radiochemical purity [%]	>95	>95	>95
Radioactive concentration [MBq/mL]	35	37	34
Chemical conc. [mg/mL]	0.008	0.011	0.010
Specific activity [GBq/mmol]	999	814	851