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Abstracts

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Selected Abstracts

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PREPARATION OF CARRIER-FREE |METHYL-^{3}H|METHYL NOSYLATE AND ITS USE AS A RADIOCHEMICALLY STABLE METHYLATION REAGENT

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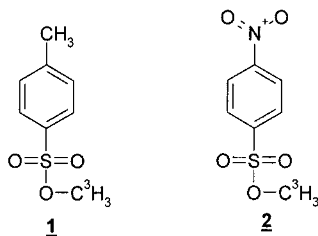
Carrier-free [methyl-^{3}H]methyl iodide is a powerful methylating reagent; however, it must be used soon after it is synthesized, as it degrades rapidly. We wished to extend the useful lifetime of the labelled methyl group by increasing its chemical stability. Transfer of the methyl group from iodide to benzenesulfonate derivatives gave methylating reagents which had a much longer useful lifetime than the parent [methyl-^{3}H]methyl iodide with no loss of specific activity. Described are the preparation and reactions of [methyl-^{3}H]-methyl *para*-toluenesulfonate 1 and [methyl-^{3}H]methyl *para*-nitro-benzene-sulfonate 2.



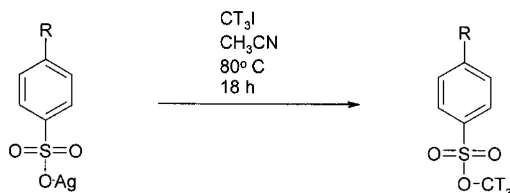
Tritiated methyl iodide is a useful radiochemical reagent. It readily reacts with amines, alcohols, thiols, and enolates to give specifically-labelled radiochemicals with specific activities that are routinely greater than 80 Ci/mmol.

Tritiated methyl iodide has been prepared in many ways. The simplest methods involve reduction of carbon monoxide with tritium gas over a catalyst or lithium aluminum tritide to give tritiated methanol which is reacted with HI to give the iodide. These two methods can be run on a large scale and can give high specific activity. Other methods that have been used involve generation of a tritiated methyl ether which is cleaved with HI. The advantages of tritiated methyl iodide as a radiochemical reagent are that is it reactive, it is readily prepared, and it can be made in large quantities. The disadvantages are that it is not stable, it is volatile, and – to accurately meter out high specific activity material – it must be made in large quantities.

We thought it would be convenient to have a methylating agent that retained the high specific activity and reactivity of tritiated methyl iodide but

was more stable and less volatile. It would be useful to have on hand a methylating agent of high specific activity which could be dispensed by the millicurie. The methyl benzenesulfonates methyl tosylate and methyl nosylate seemed like good candidates. These cold methylating reagents are reactive and chemically stable.

Using conditions from the literature,[1] the silver benzenesulfonates reacted with carrier-free tritiated methyl iodide in acetonitrile at 80°C to give a quantitative yield of material which was radiochemically very pure by TLC. The benzenesulfonates are well-behaved on silica gel and can be purified by flash chromatography.



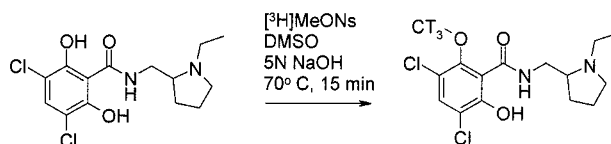
Initially, the tosylate was prepared and used to methylate alcohols and amines in good yields. The radiochemical purity of the reagent was unchanged after storage at 600 mCi/ml for 20 days at 4°C in hexane-ethyl acetate. The tosylate was so stable that we decided to investigate the utility of the more reactive nosylate. Similar reaction conditions provided a good yield of the nosylate.

Tritiated methyl nosylate was reacted with a weak and hindered nucleophile, the carboxylate group of carfentanil carboxylate. The nosylate was used in only 30% excess, but the reaction gave a 56% yield of purified material at 82 Ci/mmol.

The nosylate also proved to be quite stable. It showed no appreciable decomposition after storage at 39 mCi/ml for 14 weeks at 4°C in hexane-ethyl acetate.

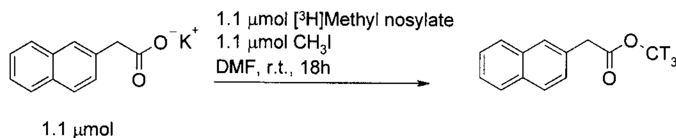
[methyl-^{3}H]Methyl nosylate was used to produce some catalog products which had before been made with methyl iodide. One advantage of the low volatility of the nosylate was seen in methods development. The reaction is initiated and monitored in THF, for example, and if methylation is not proceeding as fast as desired, the solvent may be stripped off and replaced with a different one. The reaction may also be heated without loss of the

methylating reagent.



Raclopride was prepared at 80.5 Ci/mmo1 by heating the reaction to 70°C in DMSO. The nosylate is able to be dispensed by volume, and the solvent removed to leave the reagent ready for use in the reaction vessel. In the methylation of the raclopride precursor, the stoichiometry of the reaction was able to be carefully controlled to minimize demethylation.

The methylating ability of methyl iodide versus methyl nosylate was compared in a competition experiment. The potassium salt of 2-naphthylacetic acid was stirred in DMF with one equivalent of cold methyl iodide and one equivalent of tritiated methyl nosylate. The purified material was determined to be 86 Ci/mmol. In this experiment, the nucleophile had been preferentially methylated bv the tritiated methyl nosylate with only a small fraction reacting instead with the unlabelled methyl iodide.



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TRITIO-DEHALOGENATION: NEW VARIANTS ON AN OLD THEME

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The use of iodoarenes as precursors for radiolabelling allows tritium to be introduced selectively and at high isotopic incorporation. Iodoarenes act as a poison to the catalyst during these reactions and such poisoning is responsible for the selectivity observed when reducing these iodoarenes in the presence of other easily reduced functionality.

Homogeneous exchange using Crabtree's catalyst has provided the radiochemist with a very powerful tool for the one-step labelling of a wide variety of compounds, allowing the introduction of tritium both rapidly and regiospecifically. There are, however, numerous limitations to this approach as the molecule of interest has to possess a suitable directing group to coordinate with the iridium metal center, as well as being soluble in dichloromethane. Frequently the presence of other functionality capable of binding to the iridium without delivering any useful isotopic incorporation makes it necessary for the exchange reactions to be run with a stoichiometric excess of the catalyst. This often leads to the isolation of complex mixtures from the exchange reaction, in which the compound of interest may represent only a small percentage of the total tritium activity. This can make purification of the desired compound problematic.

Tritio-dehalogenation has long been used as a method for the incorporation of tritium into organic compounds. The methodologies employed have not changed significantly over the years and have relied upon the reduction of a suitable halogenated precursor using tritium gas and a heterogeneous catalyst, typically palladium on carbon. The halogenated precursor may have been prepared either via a multi-step synthesis or in a single step via a suitable electrophilic halogenation of the molecule of interest. Choices as to which halogen should be employed, and why, have always been somewhat arbitrary and have never been properly investigated. This paper describes some initial studies undertaken to investigate what factors are important for the tritio-dehalogenation reaction, and how these might be exploited in future to achieve more effective labelling protocols.

Our desire has been to introduce tritium into target compounds regardless of their functionality and solubility, and yet to achieve material with high radiochemical purity in a single step. For this tritio-dehalogenation is a promising approach but presents two challenges (a) selective introduction of an appropriate halogen in the presence of various substituents and (b) selective

removal of the halogen without compromising these substituents. It was felt that through the use of appropriate electrophilic reagents, effective chromatographic separation and the judicious choice of halogen we might be able to routinely accomplish this objective.

The tritio-dehalogenation of iodoarenes normally gives rise to the highest isotopic incorporations, although the reasons for this are not well understood. In our own laboratories, the typical ranges for the molar specific activities of products obtained by tritio-dehalogenation are outlined in the table below:

Halogen	Specific activity range (Ci/mmol)
Chlorine	8–16
Bromine	14–25
Iodine	20–28

Recently there has been an increased interest in accessing iodoarenes due to their utility in syntheses involving metal-catalyzed coupling reactions. This, in turn, has led to the development of new reagents, some of which are capable of iodinating even very deactivated aromatics. This ability to introduce a halogen directly into an aromatic system independent of the existing substitution pattern could provide a very versatile approach to the tritium labelling of compounds. Moreover, in many cases the radiochemist need not rely upon quantitative yields of the correct regioisomer, since techniques such as mass-directed purification permit the isolation of the appropriate halogenated species even though it may be only a minor product of the reaction.

Of particular interest to this approach is the removal of one halogen, usually bromine or iodine, in the presence of another, often fluorine or chlorine, or indeed in the presence of other reducible groups. Rousseau demonstrated that only iodoarenes could be selectively dehalogenated in the presence of functional groups such as azido, nitro, benzophenone, etc.[1,2] This selectivity was attributed to a marked adsorption of the iodoarene by the catalyst. However, in many of the examples quoted, even after complete reduction of the iodoarene, the vulnerable functional groups still remained intact. This led us to consider an alternative explanation for the unique selectivity offered by iodoarenes.

A series of experiments were defined to determine the rate of dehalogenation of a number of different haloarene substrates, both individually and as a mixture of chloro-, bromo- and iodoarenes in the same reaction. For the individual substrates, the rate of dehalogenation was always bromo $>$ chloro $>$ iodo, whilst for the mixture, the order was iodo $>$ bromo $>$ chloro (see Figures 1 and 2). The main difference observed was the effect of the substituent

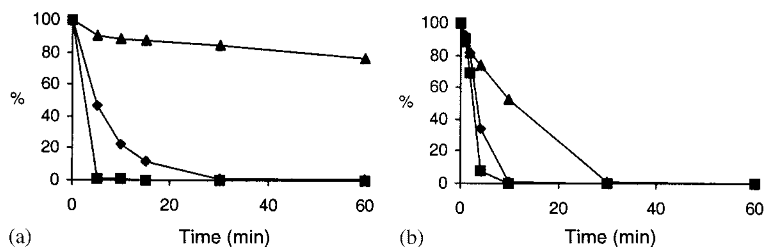

Figure 1. Rates of reduction of (a) 4-halobenzoic acids, (b) 4-halobenzylamines. ◆ Chloro, ■ Bromo, ▲ Iodo

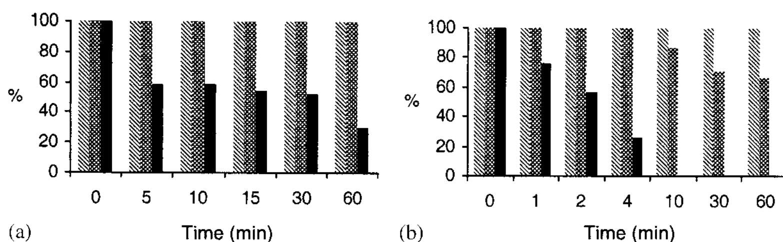

Figure 2. Reduction of an equimolar mixture of (a) 4-halobenzoic acids and (b) 4-halo-benzylamines. Chloro(slash), bromo(chequered), iodo (block)

on the overall rate of dehalogenation, as expected from electronic and regiochemical factors.

For the mixtures, it was only the more reactive substrates that showed any further reduction of the bromo- and chloroarenes once the iodoarene had been consumed. Even then, the rate was much slower than had been observed for the same substrate when examined individually. We postulated that this was due to the activity of the catalyst having been modified during the reduction of the iodoarene and that this was responsible for the selectivity observed when reducing iodoarenes in the presence of other vulnerable functionality. The effect of the release of hydriodic acid on the rate of reduction was examined using 4-bromobenzylamine. Adding increasing quantities of hydriodic acid to samples of 4-bromobenzylamine showed that it was possible to slow down the rate until at one equivalent, the reaction was effectively halted (Figure 3). Of course the effective catalyst poison during the deiodination reaction may not be hydriodic acid as such, though the data above suggests this possibility. Further support for this comes from gas-phase thermal desorbtion studies[3] where it was demonstrated that the normally dissociative attachment of phenyl iodide to the catalyst surface, giving rise to iodide, was inhibited in the presence of excess iodine.

The tritio-dehalogenation of iodoarenes is a preferred method for the introduction of tritium into compounds. There are many new iodination

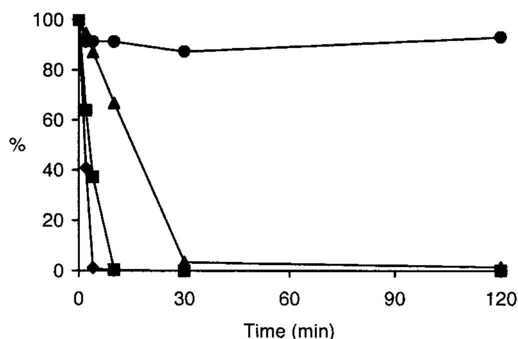

Figure 3. Effect of hydriodic concentration on the rate of reduction of 4-bromobenzyl-amine. ● 1 eq. HI, ▲0.1 eq. HI, ■ 0.01 eq. HI, ◆ 0.001 eq. HI

reagents that may be exploited to introduce iodine into the compound of interest, even those containing deactivated aromatics systems. Preparative HPLC allows the most appropriate derivative to be selected allowing one to isolate multiply iodinated species if specific activity is the main driver or a particular regio-isomer if position of incorporation is important. Typically the products from tritio-dehalogenation are already of good purity thereby making further repurification trivial and minimizing the amount of radioactive waste generated.

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THE SYNTHESES OF LABELLED 3′-AZIDO-3′-DEOXYTHYMIDINE DERIVATIVES, DISPLAYING ANTI-HIV ACTIVITY

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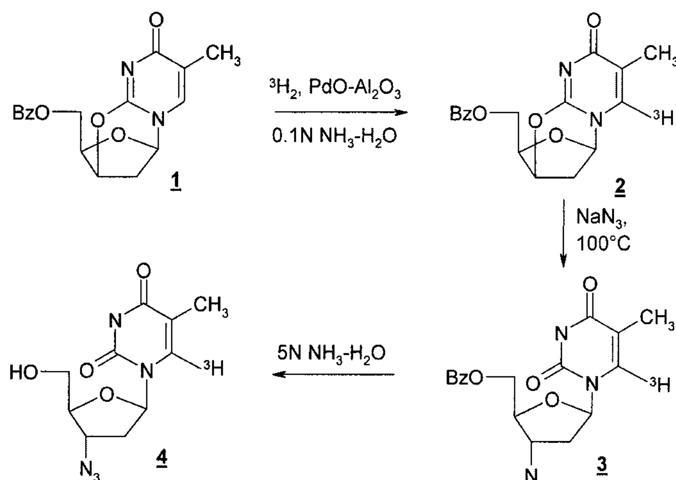

Tritium-labelled 3′-azido-3′-deoxythymidine (AZT) 4 was obtained via a three-step synthesis as shown below. 5′-O-benzoyl-2,3′-anhydrothymidine 1 was first subjected to a catalytic isotope exchange in solution in the presence of gaseous tritium followed by azidation and hydrolytic debenzoylation to give 3′-azido-3′-deoxy[6-^{3}H]thymidine 4. The specific activity of the resulting compound exceeded 18 Ci/mmol. According to ^{3}H-NMR spectroscopy, more than 98% of tritium was localized at the thymine C-6 atom.

Labelling of 3′-azido-3′-deoxythymidine-5′-H-phosphonate (Phosphazide) with tritium were accomplished by using isotope exchange as well as chemical methods. The direct homogeneous liquid isotope exchange procedure allowed the introduction of tritium into the H-phosphonate fragment. In order to introduce the label into the 5′-position, we used the known method of oxidation and NaB[^{3}H]$_4$ reduction of AZT followed by chemical phosphitylation. Finally, Phosphazide labelled at the 6-position of the thymine residue was

prepared by reacting compound **4** with phosphorous acid in the presence of DCC. The specific activities and stability of the tritium labels for each compound were determined. The methods discussed above were also applied to the tritium and phosphorus-32 labelling of 3′-azido-3′-deoxythymidine-5′-cholinephosphate.

SYNTHESES OF RADIOLABELLED ABT-578 FOR ADME STUDIES

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A. B. Susan[b] and G. Rotert[c]

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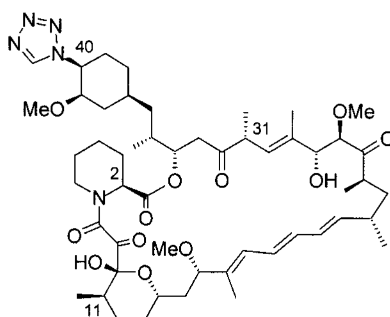
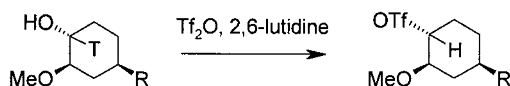
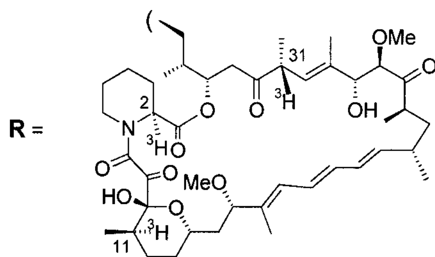
ABT-578 (structure below) is a patented analog of rapamycin and the active ingredient in Abbott's drug-coated stent device.



Since it is a new chemical entity, radiolabelled ABT-578 was needed for animal ADME and human AME studies. It was first labelled in the tetrazole moiety with tritium, then ^{14}C at Amersham for this work. Unfortunately, in both animal studies the majority of the activity proved to be highly polar degradation product(s) that meant that many relevant metabolites were not being observed. Hence, alternative radiolabelling methods had to be pursued. Labelling of the rapamycin ring with ^{14}C by a combined chemical and microbiological synthesis using the method of Moenius *et al.*[1] was not chosen because of the high cost and low specific activity product associated with it. An attempt to make [40-^{3}H]ABT-578 using the method of Moenius, Andres *et al.*,[2] to synthesize the [40-^{3}H]rapamycin precursor was unsuccessful due to apparent loss of the tritium label from the 40-triflate intermediate required to make [40-^{3}H]ABT-578:

This loss of tritium at the 40 position might be due to the increase in its acidity/exchangeability caused by the inductive effect of the strongly electron withdrawing triflate group.





The [2,11,31-^{3}H]ABT-578 was synthesized in three steps from rapamycin (Figure 1). The rapamycin was labelled by exchange with tritiated water in the presence of a noble metal catalyst and found by tritium NMR analysis to be labelled mostly in the two position with lesser amounts in the 11 and 31 positions. To our knowledge, this is the first reported successful tritium exchange labelling of the very delicate rapamycin molecule. This was done by

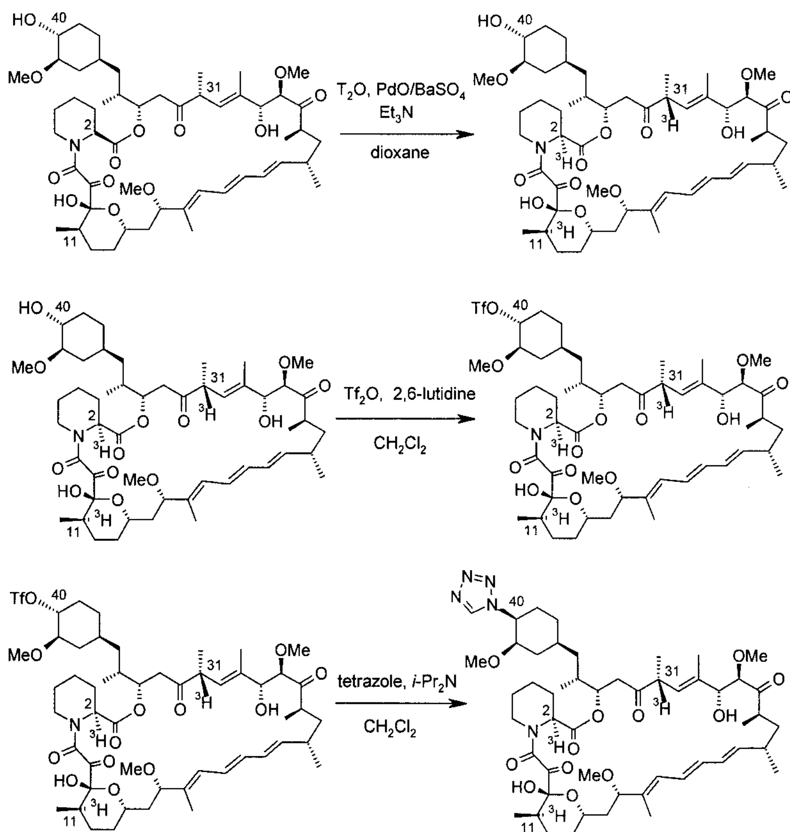


Figure 1. Synthesis of [2,11,31-3 H]ABT-578

International Isotopes Clearing House (IICH) at the Institute of Molecular Genetics, Russian Academy of Sciences, Moscow, Russia. Conversion to the title product in two steps via a 40-triflate intermediate was quite difficult due to the instability of the both the intermediates and products.

Use of the [2,11,31-^{3}H]ABT-578 gave good results in the animal ADME and human AME studies. The relevant metabolites could be observed, recoveries of total radioactivity were excellent and the amount of labile (volatile) tritium found was acceptable.

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(-)-[^{3}H)GALANTHAMINE – APPLICATION AS A PHOTOAFFINITY PROBE

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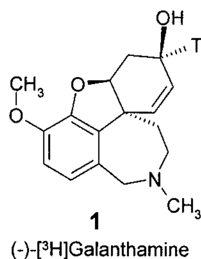
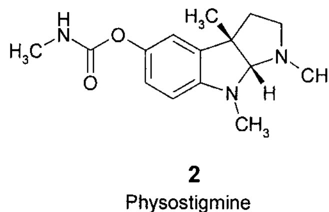
Galanthamine (**1**) is a tertiary alkaloid that has been first isolated from the bulbs of the caucasian snowdrop (*GALANTHUS woronowi*)[1]. It shows reversible, competitive acetyl-cholinesterase inhibition and acts as noncompetitive nicotinic receptor agonist, and allosterically potentiating ligand (APL) for the electric response induced by acetylcholine at the nicotinic acetylcholine receptor (nAChR).[2] Based on these functions, galanthamine (**1**) has been approved for symptomatic treatment of Alzheimer's disease.[3] Here we describe photoaffinity labelling studies using tritium labelled galanthamine (**1**) with the aim of localizing the allosteric binding side of **1** at the nAChR. The receptor protein consists of five transmembrane subunits, two α- and one each of the β-, γ- and δ-subunit, arranged as a rosette around a central hole which is believed to represent the receptor ion channel.[4] Allosteric potentiation of nAChR requires the corresponding binding site to reside on the extracellular surface of the receptor, and to be interlinked with the neurotransmitter binding site. As a first indication for the allosteric binding site, physostigmine (**2**) was shown to covalently attach to amino acid Lys 125 on the receptor α-subunit by photoactivation.[5] When compared with the acetylcholine binding protein (AChBP, 1I9B),[6] which is accepted as a template for the extracellular part of nAChR, Lys 125 is located on the α-subunit at the lower part of the central pore close to the contact surface of the γ- and δ-subunit, respectively.

In order to locate the binding site of [^{3}H]-(-)-galanthamine (**1**) on nAChR, a series of photoaffinity labelling studies was performed using membrane bound receptor from *Torpedo marmorata* (TMF).[7] The synthesis of tritiated galanthamine (**1**) has been described in detail elsewhere.[8]



1
(-)-[^{3}H]Galanthamine



2
Physostigmine

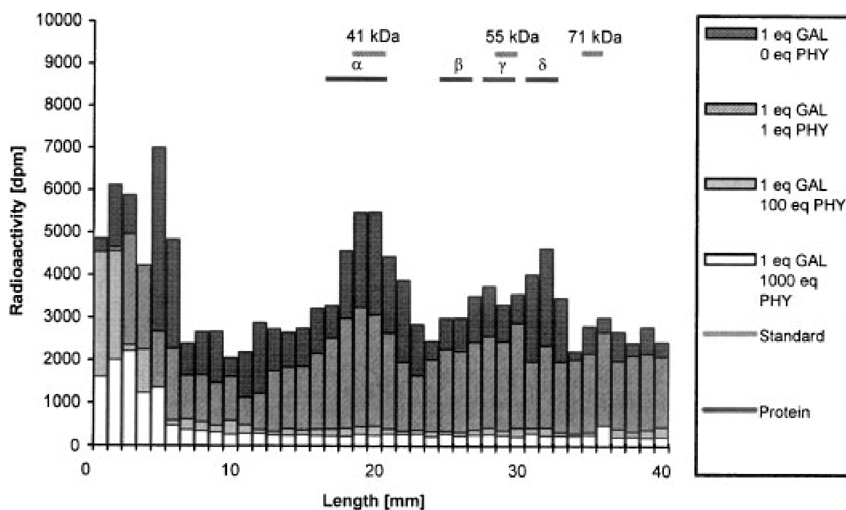


Figure 2. Binding of [^{3}H]-(-)-galanthamine to the membrane bound nAChR and competition with physostigmine

Micromolar concentrations of TMF were incubated with 25 fold excess of radioactively labelled galanthamine (**1**) for 30 min. Irradiation with UV-light for 2.5 min produces galanthamine radicals that react with surrounding amino acids to form a covalent bond and thereby attaching the radiolabel to the protein. Irradiation times have to be minimized to avoid too much destruction or interprotein reactions. The reaction mixture is after workup by electrophoresis and blotting of the separated subunits onto nitrocellulose membrane, which finally is cut in small 5 mm wide and 1 mm high slices and analyzed for radioactivity by liquid scintillation counting. The results are shown in Figure 2. The dark gray curve clearly demonstrates binding of **1** to the α-subunit of nAChR. In addition, the δ- and, to even smaller extend, also the γ-subunit is labelled, which can be rationalized by the close distance between the assumed APL-binding site around Lys 125 on the (two) α-subunits and the contact surface of the neighboring δ- and γ-subunit, respectively. No binding occurs to the β-subunit. Galanthamine (**1**) binding can be totally inhibited by preincubation of the membrane bound nAChR with physostigmine (**2**), suggesting that both ligands are competing for the same binding site.

With the assumption of the APL-binding site being located around Lys 125 on the α-subunit, APL-ligands would be able to enhance the observed twisting of nAChR-subunits during neurotransmitter binding,[5] which in turn is postulated to trigger gating of the ion channel.[9] In order to further narrow down the location of the APL-site, we are currently modifying galanthamine (**1**) by introducing 2-diazocyclopentadien-carboxylic acid as photosensitive group, to improve the peak to noise ratio during photoaffinity labelling, and to more precisely trace the radiolabel on the α-subunit.

We thank Dr H. Ardres, NOVARTIS Pharma AG Basel, Switzerland for the donation of a TriCarb Liquid Szintillation Counter.

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CARBON-11 LABELLED COMPOUNDS IN THE DEVELOPMENT OF PHARMACEUTICALS

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Positron emission tomography (PET) is a powerful noninvasive clinical and research imaging technique which allows *in vivo* measurements and quantification of biochemical processes at the molecular level.[1–3] Besides the success story of PET as an indispensable diagnostic tool in oncology, neurology and cardiology, PET technology has also emerged the process of drug research and development.[4–6]

The combination of recent developments on novel high-resolution PET cameras including micro-PET scanners with the advances in organic synthesis of PET radiotracers opens an intriguing molecular window on the distribution, metabolism, bioavailability and modes of action of pharmaceuticals in humans and laboratory animals. Mathematical methods for the evaluation of PET measurements within the framework of compartment models are well established.[7] All these facts place PET in a unique position to provide quantitative information on the molecular mechanisms of interactions and pathways of novel and established drugs.

To fully exploit PET technology in drug development, appropriately labelled compounds are needed. The drug usually must be labelled with ^{11}C ($t_{1/2}$ = 20.4 min) to avoid alterations of the properties of the parent compound. Alternatively, ^{18}F ($t_{1/2}$ = 109.6 min) can be used if the drug contains a fluorine atom. The short half-lives of ^{11}C and ^{18}F require rapid and selective labelling techniques, and time dominates all aspects of PET.[8,9] In this connection, the extensive performance of organic radiochemistry using ^{11}C and ^{18}F is fundamental but also a special challenge. The presentation addresses the recent developments of organic PET radiochemistry in the synthesis of PET radiotracers with special focus on the potential role of ^{11}C-labelled compounds in the design, development and evaluation of pharmaceuticals.

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SYNTHESIS OF A ^{11}C-LABELLED TAXANE DERIVATIVE BY [1-^{11}C]ACETYLATION

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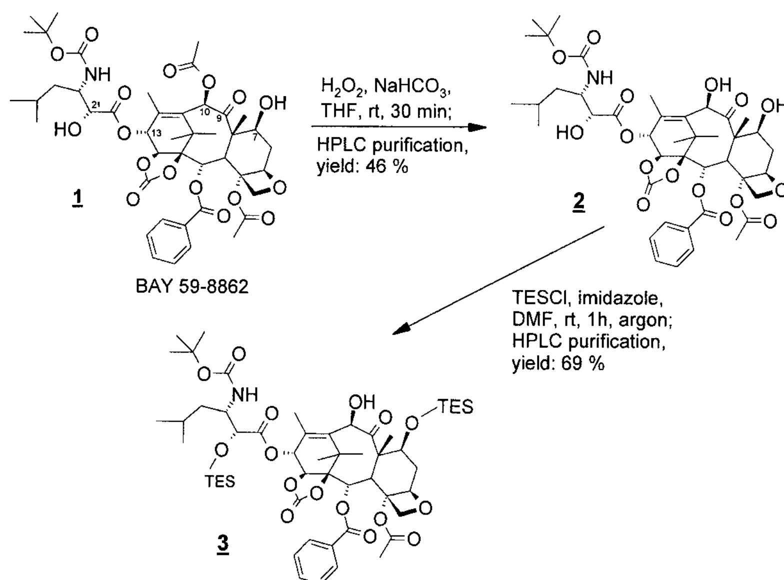
Taxanes are an important class of antitumor agents. These compounds bind to the microtubuli and inhibit their depolymerization into tubulin. Subsequently, the mitosis is disrupted and the cells are not able to divide into daughter cells.[1] Investigations with positron emission tomography (PET) are an important tool to determine the *in vivo* distribution and the pharmacokinetics of such drugs when they are labelled with positron-emitting radionuclides. An example for such a labelling is the synthesis of [^{11}C]paclitaxel by reacting [carbonyl-^{11}C]benzoyl chloride with the corresponding primary amine precursor.[2] Paclitaxel (Taxol) is an effective anticancer drug against solid tumors.

This report describes the ^{11}C-labelling of the taxan derivative BAY 59-8862 1. Compound 1 was shown to be a new highly potent anticancer drug which is intended for the therapy of breast cancer metastases in the brain. A PET study of female patients with this clinical syndrome is supposed to be carried using **[^{11}C]1**.



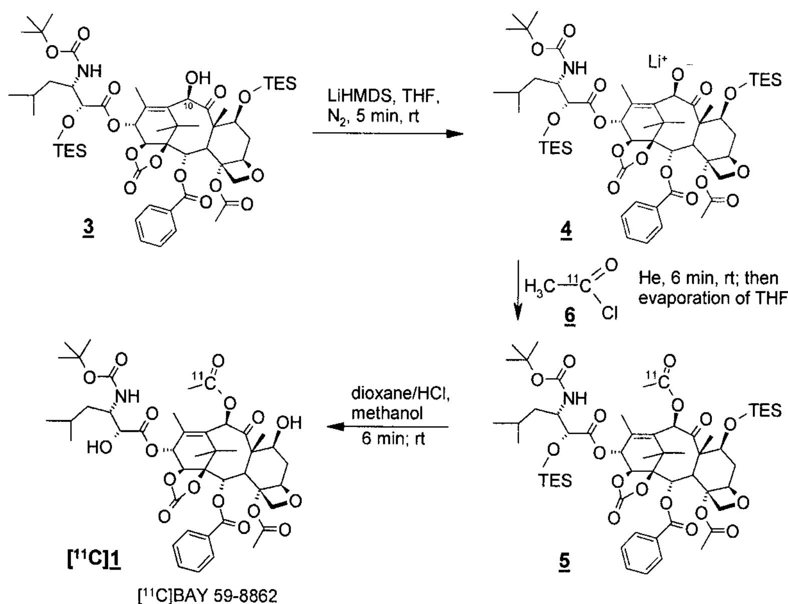
Scheme 1. Synthesis of the precursor 3

The acetyl moiety of compound 1 at position 10 was chosen to introduce carbon-11 by reaction of a suitable 10-deacetyl precursor compound 3 with [1-^{11}C]acetyl chloride with respect to drug metabolism, experiments in human hepatocytes showed that this acetyl position is metabolically stable.

For synthesis of the required precursor 3 according to Scheme 1 the taxane 1 was selectively deacetylated at position 10 by treatment with sodium hydrogencarbonate and hydrogen peroxide yielding compound 2. To avoid the known epimerization of the hydroxy group in position 7 and to stabilize the ester function in position 13 the 2′ and 7 hydroxy groups were selectively protected with triethylsilyl moieties yielding the precursor 3.

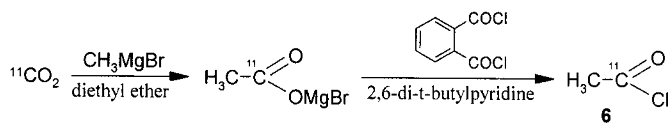
[^{11}C]1 was synthesized in a multi-step procedure according to Scheme 2 using an automated synthesis module. The remotely controlled synthesis includes: radiosynthesis of [1-^{11}C]acetyl chloride 6 and its conversion with 4 into the intermediate 5, hydrolysis of 5 to **[^{11}C]1**, HPLC purification of the final product, and its solid phase extraction and formulation.

The used module was commercially available as ^{11}C-methylation module from GE Medical Systems (former Nuclear Interface), Münster, Germany. It was modified in terms of program and hardware.



Scheme 2. Synthesis of [^{11}C]BAY 59-8862 ([^{11}C]1

[1-^{11}C]Acetyl chloride 6 was synthesized as previously described[3] with some modifications[4] (Scheme 3).



Scheme 3. Synthesis of [1-^{11}C]acetyl chloride 6

Briefly, [^{11}C]carbon dioxide was bubbled slowly into a solution of methylmagnesium bromide (0.15 M in diethyl ether). The resulting [^{11}C]acetate solution was quenched by adding phthaloyl dichloride and 2,6-*di*-*t*-butylpyridine. Diethyl ether containing phthaloyl dichloride was evaporated by means of a helium gas stream. Then, gaseous 6 was transferred at 85–100°C into a cooled THF solution of the lithium salt 4 of precursor 3. Impurities of phthaloyl dichloride can prevent the [^{11}C]acetylation reaction. To avoid contamination of the reaction mixture with phthaloyl dichloride the helium gas stream was carefully flushed over the quenched Grignard reaction mixture for the most time. It was bubbled through the quenched Grignard reaction mixture in very short time intervals.

The [^{11}C]acetylation reaction to form 5 was carried out at room temperature. After evaporation of the solvent a solution of hydrochloric acid in MeOH/dioxane was added for cleavage of the triethyl-silyl protecting groups at room temperature yielding [^{11}C]1. After neutralization of the reaction mixture with NaOH, the crude product [^{11}C]1 was purified by semi-preparative RP-HPLC and solid phase extraction at an RP-18-cartridge. The resulting ethanolic solution of [^{11}C]1 and a saline solution was transferred successively through a sterile filter to obtain the final formulation of [^{11}C]1 in pharmaceutical quality.

The decay-corrected radiochemical yields were in the range of 6–19% (related to [^{11}C]CO_2; $n = 11$) within a synthesis time of 45–50 min. The radiochemical purity exceeded 96%. The specific radioactivity of the final product reached 18 GBq/μmol starting from 26 GBq of [^{11}C]CO_2.

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THE PREPARATION OF 2-METHYL[RING-2-^{14}C]BENZYL CHLORIDE

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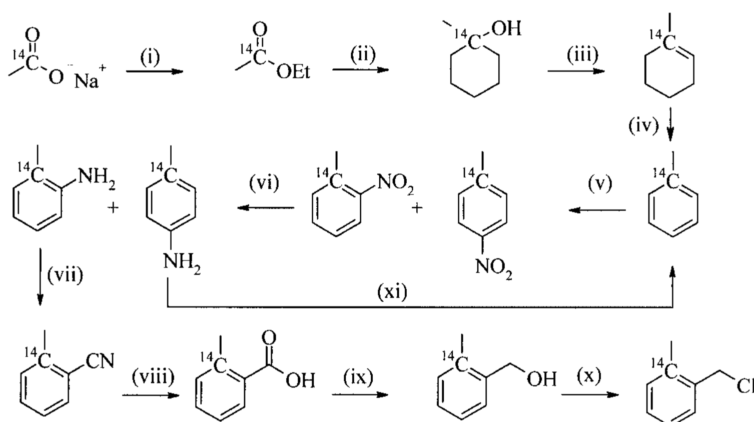

Reagents: (i) $(EtO)_3PO$, (ii) $C_5H_{10}Br_2Mg_2$, (iii) H_3PO_4, (iv) Pd/C, (v) TFAA/ NH_4NO_3, (vi) H_2-Pd/C, (vii) HONO, CuCN, (viii) NaOH, (ix) B_2H_6, (x) $C_3Cl_6O_3$

The title compound was prepared in 10 steps and 5% yield from [1-^{14}C]acetic acid sodium salt [1-^{14}C]Acetic acid sodium salt was converted into ethyl[1-^{14}C]acetate using triethyl phosphate.[1] This compound was reacted with the commercially available pentamethylene *bis*(magnesium bromide) to give l-hydroxy-l-methyl[1-^{14}C]cyclo-hexane[2] which was dehydrated to 1-methyl[1-^{14}C]cyclohexene with phosphoric acid.[3] In the key reaction, 1-methyl[1-^{14}C]cyclohexene was converted into [1-^{14}C]toluene by vapor phase dehydrogenation over a palladium on charcoal catalyst at high temperature.[4] This gave [1-^{14}C]toluene contaminated with a small amount of [^{14}C]methylcy-clohexane. The [1-^{14}C]toluene was nitrated using ammonium nitrate/trifluor-oacetic anhydride in dichloromethane.[5] The isomeric [^{14}C]nitrotoluenes produced were difficult to separate so they were reduced to a mixture of [^{14}C]toluidines using standard hydrogenation conditions. The *o*-[2-^{14}C]tolui-dine was separated by HPLC, converted into the nitrile and hydrolyzed to *o*-[2-^{14}C]toluic acid using sodium hydroxide. The *o*-[2-^{14}C]toluic acid was reduced by diborane to the benzyl alcohol which was then converted into the product using triphosgene.[6] The *p*-[4-^{14}C] isomer was recycled by conversion back to [1-^{14}C]toluene by diazotization and reduction with hypophosphorus acid.

[1-^{14}C]toluene: a flask containing 1-methyl[1-^{14}C]cyclohexene (1.5 g, 810 mCi, 15.6 mmol) under nitrogen was attached to a column ($\sim$45 $\times$ 2.5 cm) of 30% Pd-C suspended on glass wool. Around the column, heating tape was wound and a dry-ice/2-propanol trap was connected to the top. The column was evacuated using a water pump and heated. The flask containing the 1-methyl[1-^{14}C]cyclohexene was evacuated so that the contents slowly distilled through the column. The product was collected in the cooled trap. When all further trapping of product ceased, the apparatus was allowed to cool and filled with nitrogen to 1 atm. The contents of the trap were distilled out under vacuum, analyzed by GLC and found to be 80% pure [1-^{14}C]toluene (1 g, 450 mCi, 8.7 mmol, 55%).

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SYNTHESIS OF ^{14}C-LABELLED ANGIOTENSIN-(1-7)-RECEPTOR AGONISTS AVE 0991

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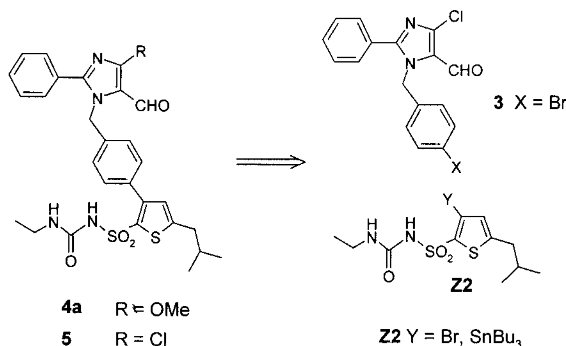
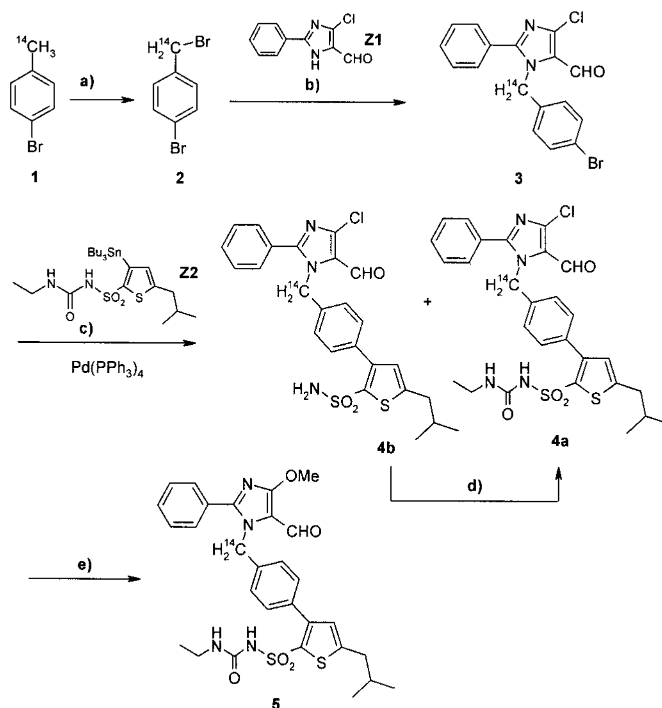
Substituted imidazole derivatives have proven to be potent ligands for angiotensin receptors, and a number of them are used as valuable cardiovascular drugs. The stimulation of the endothelial cell connected angiotensin-(1-7)-receptor initiates the release of vasodilating and cardioprotective messengers such as cyclic 3′ ,5′-guanosine monophosphate (cGMP) and nitrogen monoxide (NO). As a result, imidazole derived angiotensin-(1-7)-agonists may serve as valuable agents in the treatment or prophylaxis of hypertension, heart hypertrophy, heart failure, and coronary heart diseases such as angina pectoris, myocardial infarct, and endothelial dysfunction, (e.g. as a result of diabetes mellitus and arterial or venous thrombosis).[1] For pharmacokinetic and metabolism studies on AVE 0991, an interesting, pharmacologically active representative of this structural class, the synthesis of carbon-14 labelled material (5-[^{14}C]) became necessary.[2]



The retrosynthetic analysis pointed to the Stille cross-coupling of carbon-14 labelled imidazole (**3**) and thiophene (**Z2**) as the crucial step for the synthesis of **5**-[^{14}C].

Imidazole (3-[^{14}C]) was prepared in 50% overall yield by AIBN-mediated bromination of 4-bromo-toluene (1-[^{14}C]) with *N*-bromosuccinimide followed by alkylation of imidazole (Zl) with the resulted *p*-bromo-[α-^{14}C]benzyl bromide (2). Stannane (Z2), needed as a coupling partner in the Stille reaction, was readily available through metal/halogen exchange on the respective brominated precursor with excess *n*-BuLi followed by quenching of the *in situ* generated lithiated derivative with tributyltin chloride. The subsequent coupling of one equivalent imidazole (3) and two equivalents stannane (Z2)

succeeded in refluxing THF in the presence of 10 mol% $Pd(PPh_3)_4$. Work up of the reaction mixture after 3 days afforded a mixture of the requested cross-coupling product (4a-[^{14}C]) and unsubstituted sulfonamide (4b-[^{14}C]) in a ratio of 1.6:1. Treatment of this mixture with ethyl isocyanate converted (4b-[^{14}C]) into additional product (4a-[^{14}C]). In the last reaction step, the cross-coupling product (4a-[^{14}C]) was methoxylated under basic conditions in methanol to yield the final product (5-[^{14}C]) in 45% yield. The crude product was purified by HPLC and furnished angiotensin-(1-7)-receptor agonist (5-[^{14}C]) in an overall radiochemical yield of 15% and a radiochemical purity of >98%.



Reaction conditions: (a) NBS, AIBN, cyclohexane, reflux, 4h, 75%; (b) K_2CO_3, DMF, reflux, 48h, 73%; (c) $Pd(PPh_3)_4$, THF, reflux, 72h; (d) EtNCO, K_2CO_3, DME, reflux, 2h, 62 (2 steps); (e) NaOH, MeOH, 70°C, 9h, 45%

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C-14 LABELING OF THE *Β*-AMINO CARBOXYL-MOIETY: ALTERNATIVES TO THE ACRYLATE-APPROACH

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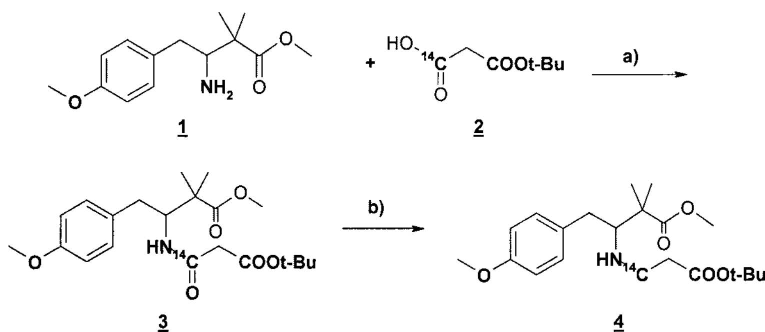
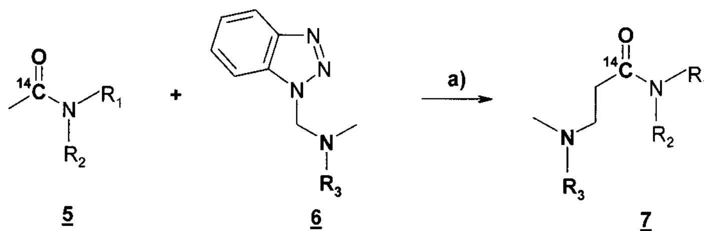
An established procedure for the synthesis of (*β*-amino carboxylates is the d0-a3 combination of acrylates (a3) with amines (d0). In the radioactive case, however, due to their tendency to polymerize — especially when unsubstituted and of high specific activities — this type of reaction is of limited value for the synthesis of C-14 labelled material. In order to avoid the preparation of [^{14}C]acrylates we used the following two strategies:

(a) d0-a3 combination of *mono*-alkyl [^{14}C]malonates 2 (see also Mc Carthy *et al.*)[1] with amines, selective thionation of the amide 3 with Lawesson's reagent and subsequent reduction of the resulting thioamide gave the required *β*-amino carboxyl-moiety.



Reaction conditions: a. DCC, THF, r.t., 12 h, 85%, b) 2,4-bis (4-methoxyphenyl)-1,3-dithia-2,4-diphosphetane-2,4-disulfide (Lawesson's reagent), toluene, 100°C, 4 h, 85%, Ra-Ni, EtOH/H_2O 8/2, 100%;

(b) Alternatively, d2-al combination of lithiated dialkyl[carbonyl-^{14}C]acetamides 5 with the benzotriazolylmethyl amino derivatives 6 [2], as illustrated by the synthesis of compound 7.



Reaction conditions: a) diisopropylamine, THF, n-BuLi, 0.5h, −78°C, 5, 20 min., 6, n-BuLi, 16h, r.t., 30%;

Both strategies were successfully applied to the radiosynthesis of isotopically highly enriched compounds.

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EFFECTIVE CARBON LABELLING UNDER MICROWAVE IRRADIATION

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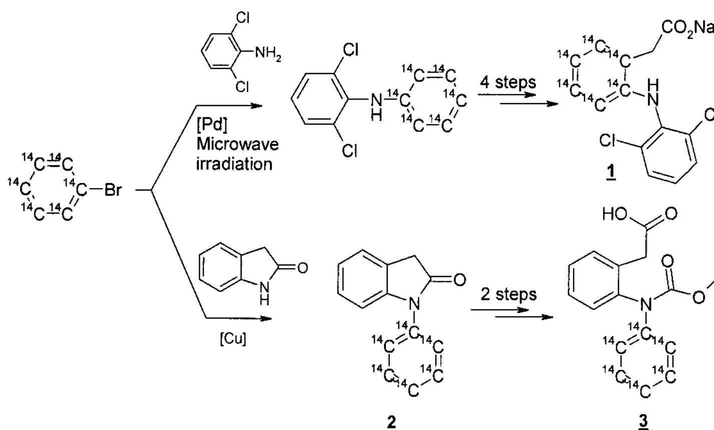
Microwave-assisted organic synthesis has gained considerable attention over the last decade and has become commonplace in modern research laboratories. The advantages of using microwave energy include dramatic decrease of reaction times, improved reaction yields, cleaner product formation, and access to new reaction conditions that are not available through traditional means.[1–3] The benefit of microwave with particular reference to the synthesis of ^{3}H, ^{18}F and ^{11}C has been the subject of recent reviews.[4,5]

For different environmental compatibility studies C-14 labelled Diclofenac **1** (active ingredient of VOLTAREN), the intermediate phenyloxindole **2** and the carbamic acid derivative **3** were needed. Since a label in the ring position was requested, the metal catalyzed C–N bond-formation between the appropriate nitrogen nucleophile and labelled bromobenzene was the most attractive process to prepare all the required compounds. Only a few reports of microwave assisted Pd-catalyzed aryl amination (*Buchwald reaction*) have been reported in the literature.[6,7] An efficient synthesis of C-14 labelled Diclofenac **1** via microwave-assisted Pd-catalyzed aryl amination of labelled bromobenzene will be described (Scheme 1).

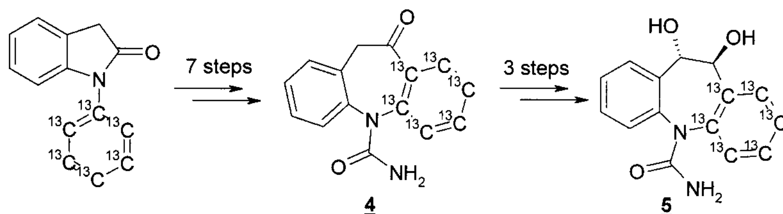


Scheme 1.

Furthermore, the extension of the Pd-catalyzed reaction of amides and aryle bromides[8] to the lactame 2-indolinone was unsuccessful. However, we report

the application of a mild and suitable copper-catalyzed amide arylation procedure (*Goldberg reaction*) under both, conventional[9] and microwave conditions to the preparation of phenyloxindole **2** and the carbamic acid derivative **3**.

As internal standard for mass spectrometry the stable label of Oxacarbazepine **4** (antiepileptic drug) and one of its metabolites **5** was prepared as well (Scheme 2).



Scheme 2.

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STABLE-ISOTOPE LABELLED COMPOUNDS (STILS) AS INTERNAL STANDARDS FOR LC–MS/MS. REQUIREMENTS AND SOME SYNTHETIC APPROACHES

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Recent evolutions in the development of LC–MS/MS make it the present day's most important analytical technique in the bioanalytical laboratory, at the cost of HPLC-UV, Radio Immuno Assay and GC–MS. The unprecedented selectivity of tandem mass spectrometry allows minimal sample preparation and fast LC-separation, resulting in high throughput. The major drawback of LC–MS/MS is ion-suppression, caused by insufficient ionization capacity and by difference in ionization preference of the different constituents eluting from the LC-column.

The availability of Stable Isotope Labelled (STIL) analogues as internal standards allows the bioanalytical scientist to compensate for ion suppression and to maximize on the advantages of LC–MS/MS. The incorporation of (a number of) deuterium, ^{13}C and/or ^{15}N atoms in a molecule, creating an internal standard which has virtually identical physicochemical properties with respect to extraction, chromatography and ionization as compared to the drug of interest, cured all unfavorable properties of an LC–MS/MS assay observed with non-STIL internal standards.

Initially both the requestor of STILs as well as the chemists who prepared them went through a learning process, which after some failures brought us to a mutual understanding. Minimum requirements to be fulfilled resulted in highly efficient, and hence, less time consuming approaches towards the synthesis of a useful internal standard.

Since no metabolism is involved, the positions of the labels are of minor importance.

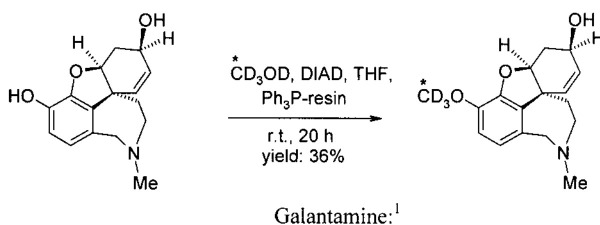
Most assays are run on RP columns. Hence, even if a compound contains an optical center, the synthesis of a racemic mixture is sufficient.

Multi-labelling is required. As a rule of thumb, compounds which do not contain chlorine need an STIL with $M+3$ or higher. Compounds with one chlorine prefer an internal standard with $M+4$ or higher.

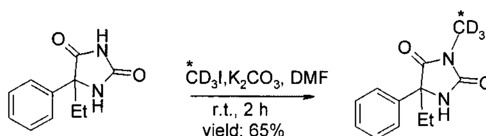
Quality standards to be met are higher than for unlabelled compounds. The internal standard's chemical purity must be $>98\%$ with a critical demand for isotopic purity: $M+0<0.1\%$ and the isotopic ratio should be much in favor of the highest label number.

With these requirements in mind, it is the chemists' job to find the most efficient way towards the synthesis of such molecules. The strategy, of course, hinges on the desired structure and the availability of adequate starting materials, building blocks and labelled molecules. This presentation illustrates our laboratories' present-day situation in which STILs are developed containing preferentially a combination of different isotopes (e.g.: ^{13}C-D, multi-^{13}C, ^{13}C-^{15}N, etc.). In general the strategy falls apart into three approaches, each of which will be illustrated with one or more examples.

1. For *N*- or *O*-methylated compounds, a demethylation from the unlabelled molecule in question, or the availability of the desmethyl compounds allows easy access to the STIL, using $^{*}CD_3I$ or $^{*}CD_3OD$.



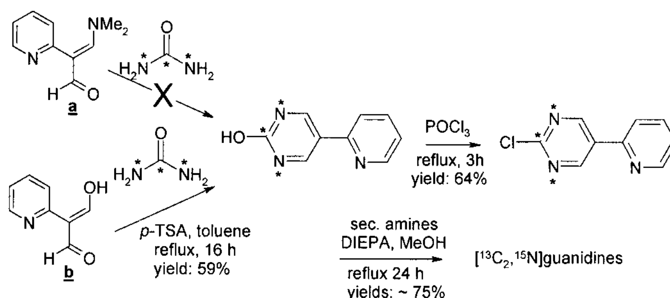
Galantamine:[1]



- Mephenytoin:

-$^{*}CD_3$-Dextromethorphan, numerous J&J compounds, secondary methylamines via benzyl protection (reductive amination), methylation and deprotection.

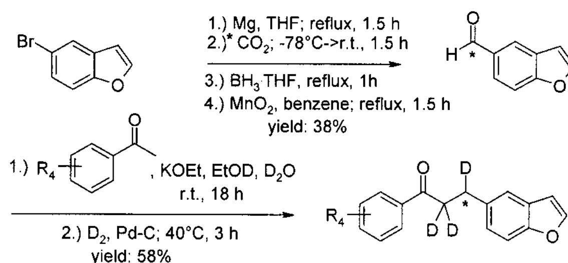
2. A large or an elaborate portion of the molecule is available as building block. The labels can be positioned into the smaller and easier attainable part, with an ensuing coupling to the final compound.



2-pyrids pyrimidine as intermediate towards guanidine compounds:

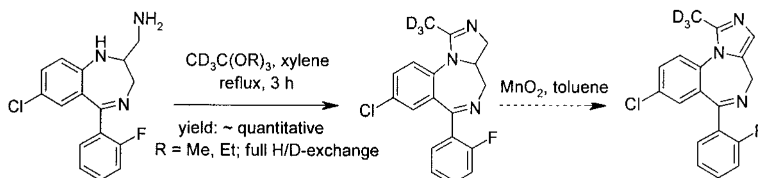
Vilsmeier approach towards a in analogy to literature[2] gave unsavory black tars. A direct approach from commercial dialdehyde b provided easy access to the desired structures.

- Aldol condensation of an aromatic aldehyde with a tetra substituted acetophenone,



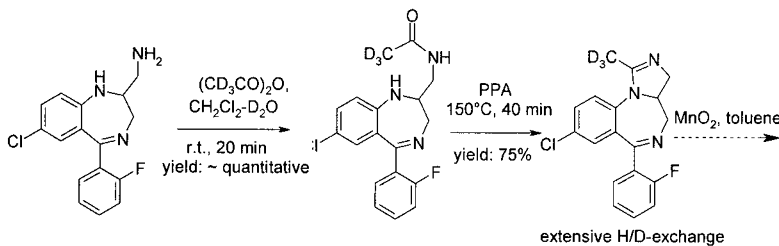
- followed by one-pot deuterium saturation of the double bond:

3. Most unfavorable and quite time consuming is the full construction of the labelled compound, as exemplified by the synthesis of a 3-^{13}C-midazolam STIL.

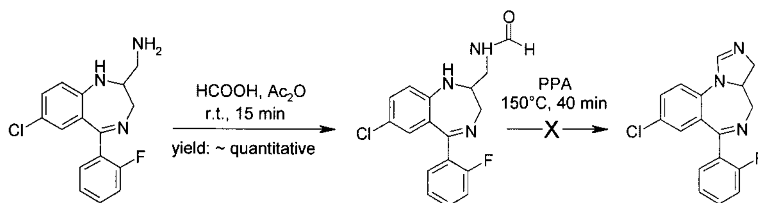


The easiest approach seemed the reaction of a suitable precursor with fully deuterated triethyl or trimethyl ortho acetate. Full exchange during the ringclosure led to unlabelled midazolam:

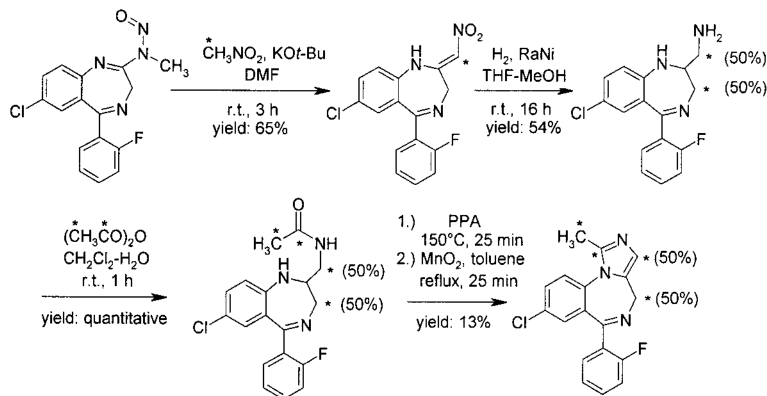
Formation of the N-CD_3-acetyl compound, and cyclization as for the unlabelled compound likewise gave unlabelled midazolam:



Creation of desmethyl midazolam to finally methylate the imidazole-2 position failed in the synthesis of the precursor:



No other choice was left but to go for [$^{13}C_3$]midazolam via the shortest possible construction:[3]



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MODELS FOR CALCULATING THE RADIATION DOSE AFTER INCORPORATION OF ^{3}H AND ^{14}C

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Most countries have radiation protection legislation mandating both the monitoring of radionuclide incorporation and the limitation of the effective committed dose (E_{50}) to which higher-risk workers are exposed. Guidelines for calculating these doses vary from country to country, but all are based on the models and dose limits of the International Commission for Radiation Protection (ICRP [1]). Unfortunately, these models do not correspond to the biokinetic pattern of the ^{3}H and ^{14}C labelled compounds used in chemical and pharmaceutical laboratories, which normally have a very high excretion rate. The aim of this paper is:

1. to discuss a biokinetic and dosimetric model of inhalation of an ^{3}H labelled compound with a high excretion rate,
2. to compare this model with the ICRP model for tritiated water (HTO),
3. to show how a compound-specific model for ^{3}H and ^{14}C can be constructed, and specify what data must be known,
4. to present the urine data and dose calculation of an actual incorporation incident.

To demonstrate the calculation of the effective committed dose, a model based on the ICRP reference man is used and the following hypothesis is made:

(a) Inhalation of 80 MBq ^{3}H,

(b) the labelled compound is immediately resorbed into the blood and evenly distributed throughout the body,

(c) the labelled compound is not metabolized and is excreted only by the kidneys,

(d) the activity curve of an organ, e.g., the liver, and the excretion rate in the urine, A_u, are known (Figures 1 and 2).

The effective dose is the sum of the weighted organ doses H_T (T stands for both tissue and organ). The weighting factors w_T reflect the radiosensitivity, i.e., the risk of cancer and genetic effects. The organ dose H_T is the absorbed radiation energy per mass m_T.

The absorbed energy is the number of β-disintegrations multiplied by the mean β-energy. The number of disintegrations is called the cumulated activity $\tilde{A}$ and equals the area under the activity curve AUC. AUC is calculated by multiplying the mean activity in Bq by the time in seconds. The mean energy $\bar{e}$ for ^{3}H is $5.6\,keV/dis = 9 \times 10^{-16}\,J/dis$. If the labelled compound is homo-

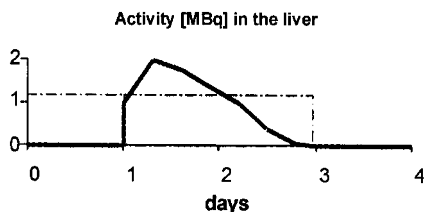

Figure 1. Activity curve in the liver after inhalation of 80 MBq ^3H of a rapidly excreted compound

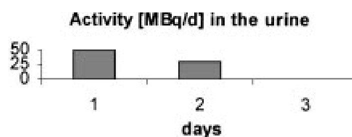

Figure 2. Excretion in the urine (day 1: 50 MBq, day 2: 30 MBq). Same chemical compound as in Figure 2

geneously distributed in the body, then the effective dose is equal to any organ dose. Thus [**1**]:

$$[\mathbf{1}] \quad E_{50} = \sum_T w_\mathrm{T} \cdot H_\mathrm{T} = \sum_T w_\mathrm{T} \cdot \frac{\tilde{A}_\mathrm{T} \cdot \bar{e}}{m_\mathrm{t}} = H_\mathrm{Liver} = \frac{\tilde{A}_\mathrm{Liver} \cdot \bar{e}}{m_\mathrm{Liver}} = \frac{\mathrm{AUC}_\mathrm{Liver} \cdot \bar{e}}{m_\mathrm{Liver}}$$

For the example in question, $\mathrm{AUC}_\mathrm{Liver}$ is approximately 1.1 MBq · 2 days = 1′100′000 dis/s · 172′800 s = 1.9×10^{11} disintegrations. m_Liver is 1.8 kg. Thus [**2**]:

$$[\mathbf{2}] \quad E_{50} = \frac{\mathrm{AUC}_\mathrm{Liver} \cdot \bar{e}}{m_\mathrm{Liver}} = \frac{1.9 \times 10^{11}\ \mathrm{dis} \cdot 9 \times 10^{-16}\ \mathrm{J/dis}}{1.8\ \mathrm{kg}}$$
$$= 9.5 \times 10^{-5}\ \mathrm{J/kg} = 9.5 \times 10^{-5}\ \mathrm{Sv} = 95\ \mu\mathrm{Sv}$$

The dose coefficient $e(50)_\mathrm{inh}$ [**3**] as well as the excretion ratio $m(t)$ for day 1 [**4**] and 2 [**5**] can now be calculated:

$$[\mathbf{3}] \quad e(50)_\mathrm{inh} = \frac{E_{50}}{A_\mathrm{inh}} = \frac{9.5 \times 10^{-5}\ \mathrm{Sv}}{80 \times 10^6\ \mathrm{Bq}} = 1.2 \times 10^{-12}\ \mathrm{Sv/Bq}$$

$$[\mathbf{4}] \quad m(\mathrm{day}\ 1) = \frac{A_\mathrm{u}(\mathrm{day}\ 1)}{A_\mathrm{inh}} = \frac{50\ \mathrm{MBq}}{80\ \mathrm{MBq}} = 0.625$$

$$[\mathbf{5}] \quad m(\mathrm{day}\ 2) = \frac{A_\mathrm{u}(\mathrm{day}\ 2)}{A_\mathrm{inh}} = \frac{30\ \mathrm{MBq}}{80\ \mathrm{MBq}} = 0.375$$

The committed effective dose E_{50} in a radiation worker who has had an inhalation incident and whose urine activity A_u is known can now be calculated as follows [**6**]:

$$[\mathbf{6}] \quad E_{50} = A_\mathrm{inh} \cdot e(50)_\mathrm{inh} = \frac{A_\mathrm{u}(t)}{m(t)} \cdot e(50)_\mathrm{inh} = A_\mathrm{u}(t) \cdot \frac{e(50)_\mathrm{inh}}{m(t)}$$

with $e(50)_\mathrm{inh}/m(\mathrm{day}\ 1) = 1.92 \times 10^{-12}$ Sv/Bq and $e(50)_\mathrm{inh}/m(\mathrm{day}\ 2) = 3.2 \times 10^{-12}$ Sv/Bq.

The ICRP model for HTO is based on the following hypothesis: HTO is immediately reabsorbed, homogeneously distributed in the body and rather

slowly excreted by urine with a biological half-life of 10 days. Figures 3 and 4 show the activity curve of an organ, e.g., the liver, and the excretion rate in the urine after inhalation of 80 MBq HTO.

Based on this model, the dose coefficient and $e(50)_{inh}/m(t)$ are as follows:[2,3]

$e(50)_{inh} = 1.8 \times 10^{-11}$ Sv/Bq

t(day)	$\frac{e(50)_{inh}}{m(t)}$ (Sv/Bq)
1	5.6×10^{-10}
2	6.1×10^{-10}
3	6.4×10^{-10}
4	6.8×10^{-10}
5	7.8×10^{-10}
6	7.8×10^{-10}
7	8.6×10^{-10}
15	14×10^{-10}
30	37×10^{-10}
45	92×10^{-10}

Based on these values for $e(50)_{inh}/m(t)$, the guideline limit for a screening program can be calculated. The aim of screening is to use monthly urine checks to find radiation workers whose annual effective dose due to ^{3}H inhalation may be more than 1 mSv. Let us assume that an incident occurs in the middle of the month. The probe is taken on day 15, and urine production is 1.4 l. Thus [7]:

$$[7] \quad C_{u,15th\ day} = \frac{A_{u,day\ 15}}{1.4\ l} = \frac{E_{50}}{e(50)_{inh}/m(\text{day } 15)} \cdot \frac{1}{1.4\ l} = \frac{1/12\ \text{mSv}}{1.4 \times 10^{-10}\ \text{Sv/Bq} \cdot 1.4\ l} = 42'000\ \text{Bq/l}$$

As the guideline limit depends on the biokinetic model, the use of monthly screening for urine concentrations >42 kBq/l is debatable when applied to workers at laboratories where rapidly excreted compounds are handled.

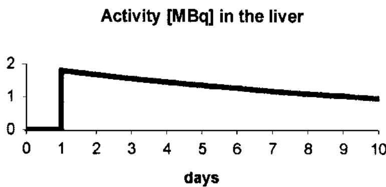

Figure 3. Activity curve in the liver after inhalation of 80 MBq HTO

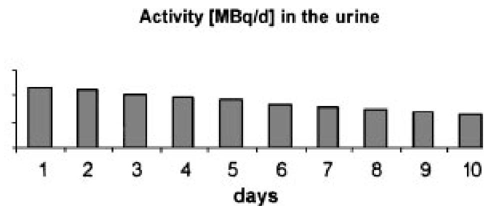

Figure 4. Excretion of HTO in the urine

A comparison of the values of $e(50)_{inh}/m(t)$ for HTO (Chapter 2) with those for rapidly excreted ^{3}H labelled compounds (Chapter 1) shows that dose calculation depends to a large extent on the posited model. Therefore, the values of $e(50)_{inh}/m(t)$ must be determined if the biokinetic pattern of the inhaled labelled substance differs considerably from the ICRP model. In order to do so, experiments with at least five animals should be performed according to the following protocol:

- administer known activity through the lungs (A_{inh}),
- sample urine and blood,
- sacrifice an animal shortly after administration, after completion of one-third and two-thirds of the excretion period, close to the end of the excretion period, and after the excretion period has ended,
- measure the activity A_T and mass m_T of each relevant organ as well as the urine ($A_u(t)$) and blood samples,
- calculate the AUC and H_T for each organ,
- calculate $e(50)_{inh}/m(t)$ and correct these results by the factor f = animal weight/73 kg.

In many countries, however, animal protection legislation does not permit such experiments solely for the purpose of retrospective dose calculation.

Finally, a ^{14}C incident is presented and discussed. The urine curve (Figure 5) clearly shows that the ICRP ^{14}C-model (Figure 6) cannot be applied, because the ICRP model is based on the assumption that the biological half-life of the labelled compound is 40 days,[2,3] whereas in this case it is about 12 h. Unfortunately no blood samples were taken and no ADME studies were available for this compound. Animal experiments were considered unjustifiable (as discussed in Chapter 3).

The effective dose for technician B was estimated based on the following assumptions:

(a) the labelled compound is immediately resorbed into the blood and distributed evenly throughout the body,

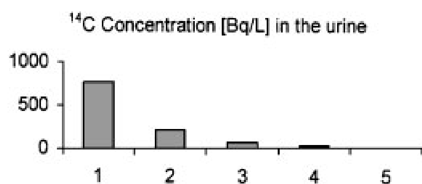

Figure 5. ^{14}C concentrations in urine samples taken from technician B, April 2000

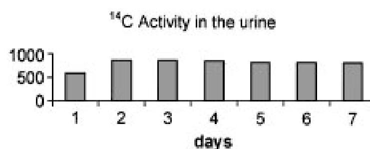

Figure 6. Relative urine activity according to the ICRP ^{14}C model

(b) at any given time, the ^{14}C concentrations in the urine, blood and organs are equal,

(c) the ^{14}C is excreted only by the kidneys. Thus, the following simplified formula **[8]** was applied:

$$[8] \quad E_{50} = H_{\text{whole body}} = \frac{\tilde{A}_{\text{body}} \cdot \bar{e}}{m_{\text{body}}} = \tilde{C}_{\text{urine}} \cdot \bar{e}$$

with $\tilde{C}_{\text{urine}} = (754\,\text{Bq/l} + 215\,\text{Bq/l} + 72\,\text{Bq/l} + 25\,\text{Bq/l})86400\,\text{s} = 9.2 \times 10^{7}$ disintegrations/kg and $\bar{e}_{^{14}C} = 49.5\ \text{kV} = 8 \times 10^{-15}$ J. Therefore:

$$[9] \quad E_{50} = \frac{9.2 \times 10^{7}\ \text{disintegrations} \cdot 8 \times 10^{-15}\ \text{J/disintegrations}}{\text{kg}}$$

$$= 7.4 \times 10^{-7}\ \text{Sv} = 74\ \mu\text{Sv}.$$

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   - #89 (volume 32, 2002): Basic anatomical and physiological data in radiological protection.
   - #80 (volume 28, 1999): Radiation dose to patients from radiopharmaceuticals.
   - #78 (volume 27, 1998): Individual monitoring for internal exposure of workers.
   - #72 (volume 26, 1996): Age-dependent doses to the members of the public from intake of radionuclides. Part 5. Compilation of ingestion and inhalation coefficients.
   - #68 (volume 24, 1995): Dose coefficients for intakes of radionuclides by workers.
2. $e(50)_{\text{inh}}$ values for ^{3}H, ^{14}C and most other radionuclides are listed at: www.bfs.de/bfs/recht/teil2_1.pdf and www/admin.ch/ch/d/sr/8/814.501.de.pdf
3. $e(50)_{\text{inh}}/m(t)$ values for ^{3}H, ^{14}C, ^{32}P, ^{33}P, ^{35}S, ^{45}Ca, ^{51}Cr, ^{59}Fe, ^{58}Co, ^{60}Co, ^{85}Sr, ^{89}Sr, ^{90}Sr, ^{99m}Tc, ^{123}I, ^{125}I, ^{131}I, ^{134}Cs, ^{137}Cs, ^{232}Th, ^{235}U, ^{238}U, ^{237}Np, ^{239}Pu and ^{241}Am can be found at: www/admin.ch/ch/d/sr/8/814.43.de.pdf

DOSIMETRY OF C-14 LABELLED ORGANIC COMPOUNDS

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While dealing with C-14 labelled organic compounds activity may unintentionally be incorporated into the human body. Further, in drug research C-14 labelled development candidates are administered to test persons in the course of ADME studies. In both cases, the radiation dose resulting from the activity intake has to be determined. Internal dosimetry is usually based on biokinetic models recommended by the International Commission on Radiological Protection (ICRP). Special biokinetic models are published for some C-14 labelled radiopharmaceuticals. But for C-14 labelled starting materials for the synthesis of higher compounds as well as for C-14 labelled development candidates in drug research the situation is much less satisfactory. Mostly, the general biokinetic model of the ICRP for C-14 in organic compounds is used. Its major characteristics are a homogeneous activity distribution within the body and a biological half-life of the activity of 40 days. Both assumptions do not apply for many substances used in pharmaceutical industry. Such compounds may accumulate in certain organs or tissues and frequently experimental results indicate a very rapid elimination of the substances or their metabolites from the body. Thus, the use of the general biokinetic model for C-14 in organic compounds may lead to a significant overestimation of the dose in this case.

To overcome this limitation, two different approaches in dose determination are proposed. One possibility is to adopt a biokinetic model, which was developed for toxicological purposes. The prerequisites are that the metabolism of the compound and the resulting activity distribution within the *human* body are already established. This holds for a large number of substances that are used as starting materials for the synthesis of higher organic compounds. Benzene is one prominent example. Dose coefficients derived from a benzene-specific biokinetic model turned out to be below 20% of those expected from the general model.[1]

A first-order compartment model is proposed for calculating dose coefficients from activity excretion data in drug research. The model includes main physiological compartments and depends on only six parameters, which are adjusted with the help of the measurement data. The model covers different activity distribution patterns within the body like the accumulation in one target tissue or a uniform activity distribution. The results from animal experiments could indicate the suitable distribution pattern. A comparison of

measured and simulated activities in excreta based on four examples revealed considerable agreement, though some limitations have to be accepted.[2]

References

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2. Krins, Fidorra J, Pleiss U, Sahre P, Schönmuth T. Biokinetic model for the calculation of dose coefficients for oral and intravenous administration of 14C labelled drugs. *Kerntechnik* 2003; accepted.

A NEW OPTION OF ^{14}C-DETECTION IN COMBINATION WITH MICRO-HPLC

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For studies of a drug's fate in intact organisms (ADME studies) or *in vitro* systems, compounds labelled with radioactive isotopes are a prerequisite. The benefits of ^{14}C-detection go along with a lack in sensitivity which is until now the main reason why ^{14}C-HPLC is mostly employed with columns of larger inner diameters (up to 8 mm). For structure elucidation of biotransformations, HPLC-^{14}C-MS/MS is today widely used since it was for the first time reported in 1993[1] and for the coupling to mass spectrometry, capillary-HPLC is excellently suited[2,3] because of its low flow rates. In the present contribution, we will show and discuss the data recorded with a recently developed radio detector, being to our knowledge the first one to be compatible to capillary HPLC.

For a long time the only way to adapt radio detection to capillary HPLC was the use of miniaturized detection cells, while the rest of the detector remained the same. The starting point for the construction of a new radio detector was the availability of smaller photo-multipliers with a significantly lower dark current which results in a reduced background. This new

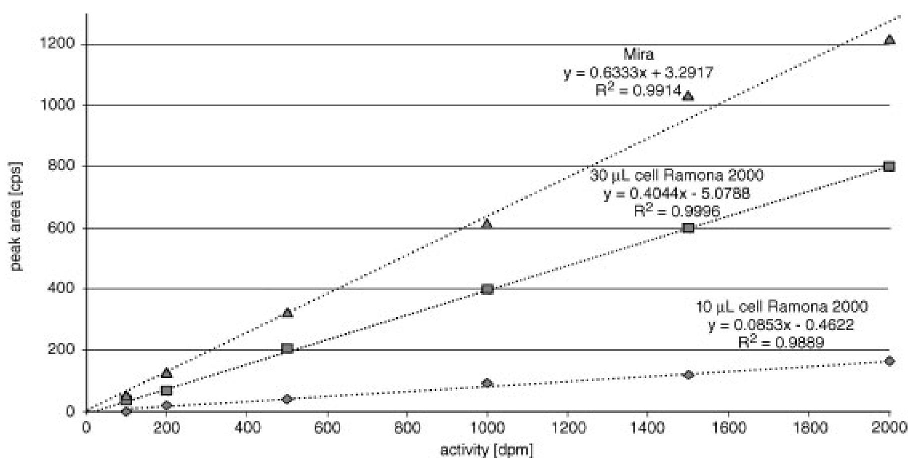

Figure 1. Comparison of peak areas against different amounts of radioactivity on column from three different detection cells. A conventional 10 µl cell (blue), a conventional 30 µl cell (red) and MIRA cell (green)

instrument allowed the realization of the idea to combine up to four pairs of photo-multipliers – each pair with separate coincidence-circurt – in order to achieve an improved S/N ratio while keeping chromatographic parameters in an acceptable range. The new MIRA cell (detector prototype name) proved to be superior to the conventional 10 μl cell with regard to various criteria (s. Figure 1): (i) Already peaks of 100 dpm can be detected while the conventional 10 μl cell required at least 500 dpm for reliable peak recognition. (ii) The MIRA cell exhibited a better correlation coefficient as its competing 10 μl cell (0.9914 versus 0.9889) which is an important issue for quantification purposes. If (iii) the signal-to-noise ratios of the different cells are compared, the advantages of MIRA appear more clearly: MIRA gives an S/N ratio of $33 \pm 11.3\%$ versus $7.1 \pm 12.7\%$ of the 10 μl cell (and $6.5 \pm 7.7\%$ of the 30 μl cell).

The data from Figure 1 might suggest that the conventional 30 μl cell is almost as good as MIRA, but one has to keep in mind that the diagram does not completely reflect the chromatographic reality as is shown in Figure 2. There, it can be seen that the peak broadening resulting from the diffusion into the large detection cell leads to unsatisfactory peak shapes. The data were included rather to show the effect of a high cell volume than to make a chromatographic comparison. As can also be seen in Figure 2, the MIRA chromatogram has a by far smoother base line with peak areas comparable to the ones from the 30 μl cell, which is due to the fourfold higher amount of data points that allow a much better description of the baseline. It is self-evident that such a smoother baseline improves peak recognition and facilitates the integration of smaller peaks. So the MIRA combines in an excellent way the advantages of both other cells: it keeps on one hand the cell volume small in order to keep chromatographic resolution acceptable but reveals on the other hand high peaks with great areas with an excellent S/N ratio. We therefore conclude that MIRA provides a very promising improvement in sensitive ^{14}C-online detection.

Ammonium acetate R.G. No. 32301, Water Chromasolv® No. 34877, acetonitrile Chromasolv® No. 34851, were purchased from Riedel de Haen (Seelze, FRG). The radioactive standard, BAY 59-7939, 2.694 MBq/mg, was dissolved in H_2O/acetonitrile 70:30. The stock solutions were adjusted to 10,000 dpm/μl.

Chromatography. Column: Nucleosil 100 C18 HD, 3 μm, 75 × 0.8 mm with guard column, (Grom Analytik GmbH, FRG). Flow rate: 64 μl/min with 60% 10 mM NH_4Ac/acetonitrile (60/40, v/v). Apparatus: Cap LC-System HP1100 with DAD, A/D-converter HP 35900 C (Agilent, Waldbronn, FRG) and Mistral Slave® (Spark, Emmen, NL) and a Ramona® 2000 on-line radioactivity flow detector as well as an MIRA (Prototype) ^{14}C-online detector (raytest GmbH, Straubenhardt, FRG) both connected with Gina-box

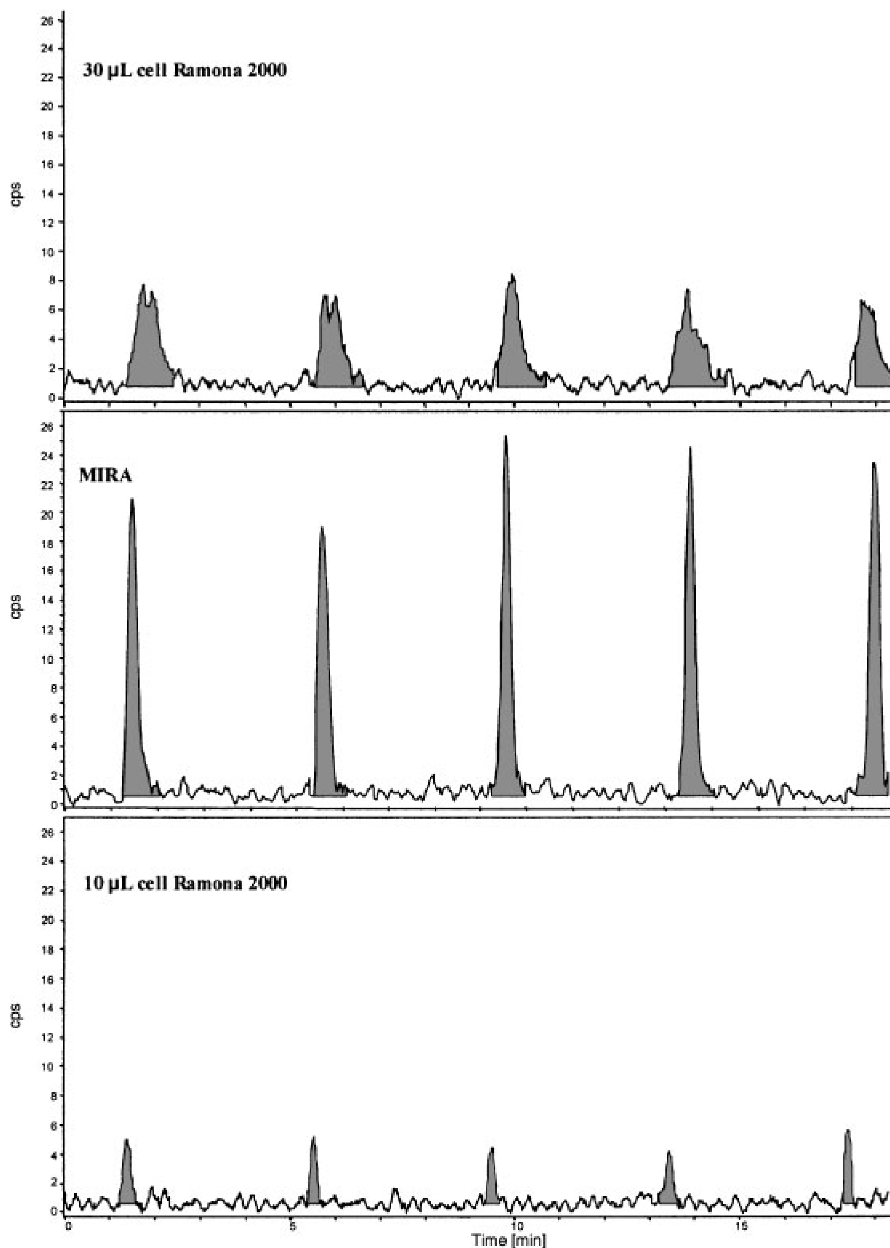

Figure 2. Comparison of chromatograms (500 dpm on column, five injections, all chromatograms normalized to the same y-scale) recorded with the three different detection cells

and Ginasoftware. For testing the sensitivity, a stock solution of BAY 59-7939 in H_2O/acetonitrile (70:30, v/v) was diluted to receive six solutions with 100, 200, 500, 1000, 1500, 2000 dpm/µl. The injection volume was 1 µl. Each

concentration was injected five times and the procedure applied to all three cells.

The authors would like to thank Mrs Andrea Kohn, raytest, for her reliability and her continued engagement and support. We also thank the members of the Isotope Chemistry Group of Bayer Health Care AG, Dr U. Pleiss, Dr D. Seidel and Dr M. Gerisch for having provided us with material.

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