JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
J Label Compd Radiopharm 2002; **45**: 1159-1198
DOI: 10.1002/jlcr. 658

10TH CONFERENCE

of the Central European Division e.V. of the International Isotope Society

Bad Soden, Germany,
6 - 7 June 2002

Selected Abstracts

Edited by

Rolf Voges
Preclinical Safety, Novartis Pharma AG,
CH-4002 Basel, Switzerland

Ulrich Pleiss
Drug Metabolism and Isotope Chemistry, Bayer AG,
D-42096 Wuppertal, Germany

LIST OF ABSTRACTS:

Preparative HPLC: a Useful Approach to Speed-up the Purification of Labelled Compounds.

A Pignatti, E Fontana, Global Drug Metabolism Dept., Pharmacia, Italy

Use of Stable Isotopes for the Biosynthesis and Biodegradation of Fosfomycin

JW McGrath[1], JP Quinn[1], BP Simov[2], W Preusser[2], F Wuggenig[2], S Schmidt[2], A Woschek[2], F Hammerschmidt[2]

[1]School of Biology and Biochemistry, The Queen's University of Belfast, Ireland

[2]Institut für Organische Chemie der Universität Wien, Austria

Metalloradiopharmaceuticals for Nuclear Medicine

H Spies, FZR Rossendorf, Germany

Strategic outlook: Future Methods in DMPK and subsequent requirements for isotopically labelled compounds

J Maas, Aventis Pharma, Germany

Isotopic Imaging of Glass-based cDNA Microarray Hybridization

F Diehl, J Coy, J Hoheisel, M von Knebel Doeberitz, M Nees,

Department of Molecular Pathology, University of Heidelberg, Germany

New Developments in Radiation Measuring Technique

A Klett, Berthold Technologies GmbH & Co. KG, Germany

Use of Microwaves in Organic Synthesis: Application in the Synthesis of Isotopically Labelled Compounds

J R Jones*, S-Y Lu
Chemistry Department, University of Surrey
Guildford GU2 7XH, UK
j.r.jones@surrey.ac.uk

The last 20 years has seen great changes in the way in which we carry out synthetic organic chemistry and many of the improvements are being transferred to the area of labelled compounds, both stable and radioactive. Here we focus on how microwaves can be used to provide faster, more selective, routes to the synthesis of deuterium and tritium labelled compounds, and, in the latter case, to much reduced levels of radioactive waste; additional benefits with potential application to C-14, C-13 and C-11 also emerge. Further details of the work described can be found in a review [1] and a chapter in a forthcoming book [2] as well as in the original literature.

Microwaves lie in the 0.3-300 GHz frequency range, but as most of this is assigned to radar and telecommunications it is necessary for chemists to operate at a fixed frequency - in this case 2450 MHz - corresponding to a wavelength of 12.2 cm. Micro-wave energy is transferred not by convection or conduction but by dielectric interaction and this produces efficient heat transfer, resulting in very even heating throughout the sample. Polar liquids and solids interact best with microwave radiation and this is one of the reasons why ionic liquids may be extremely useful in the labeling area.

Much of the early work was performed in household units which are of the multimode type (radiation not focused), but there is now beginning to emerge a number of monomode instruments specifically designed for work in a chemical laboratory. These are more efficient and allow the temperature, and sometimes the pressure, to be carefully controlled so that more quantitative and reproducible data can be obtained.

There are five major reactions that are used to prepare deuterium and tritium labelled compounds: hydrogen isotope exchange [3, 4], hydrogenation [5], dehalogenation [6], borohydride reduction [7] and methylation [8] and in all cases we have shown that these reactions can benefit from being subjected to microwave irradiation.

In the case of hydrogen isotope exchange reactions we have taken advantage of the fact that many organic compounds can be protonated in acid media, and in the presence of a suitable donor, usually deuteriated or tritiated water, exchange can be dramatically accelerated [3]. Careful choice of solvent can extend the benefits even further [4].

Deuterium or tritium gas (both are non-polar and poorly soluble in many organic solvents) is the customary source of the label in hydrogenation (both homogeneous and heterogeneous) reactions but if a solid polar donor such as formate is employed the reactions are invariably very rapid (5-10 min) and good isotopic incorporation is achieved [5]. Furthermore, there is no

longer a need to use excess donor thereby reducing the amount of waste produced and, in the case of tritium, considerable cost.

Aromatic dehalogenation [6] with deuterium/tritium gas only leads to 50 % isotopic incorporation and is therefore a very wasteful process. Once again, by using labelled formate there is no excess reagent required and 100 % isotopic incorporation is usually achieved when the best solvent is chosen.

The above-mentioned reactions are usually performed separately but there is no reason why, given careful choice of substrate, two or even three reactions can not be carried out concurrently. We have recently shown [9] how combined hydrogenation/aromatic dehalogenation of p-bromocinnamic acid under microwave-enhanced conditions can lead to the rapid (<1 min) incorporation of three deuterium atoms.

Varma [10] was the first to show that borohydride reductions of ketones and aldehydes under microwave-enhanced conditions can be performed extremely rapidly and in the absence of a solvent and when the corresponding borodeuteride was employed [7] good isotopic incorporation was achieved in a matter of a few minutes.

Methyl iodide, despite its many disadvantages (a low boiling liquid of poor stability and accompanying health hazard, especially if it is radioactive), is still the most widely used methylating agent. Our thoughts therefore turned towards a replacement made up of both a primary and secondary donor [8]. The combination of formic acid and formaldehyde methylation of amines - the so-called Eschweiler-Clarke reaction - is a method of wide synthetic utility. In the first application of the microwave-enhanced reaction in the labeling area we have deuteriated a number of amines, including desmethyl tamoxifen.

There are other reactions which through the use of microwaves have now become more attractive for labeling purposes. Decarboxylation reactions [11] fall into this category and others will surely emerge as the potential for creating reactive intermediates on microwave irradiation becomes appreciated by a wider audience.

Of the five main methods mentioned above, only the first - hydrogen isotope exchange - is truly reversible. By reference to some tritiated oils we have been able to successfully perform microwave-enhanced detritiations, some 500 times faster than the corresponding thermal reactions [12]. This procedure does therefore have the potential to treat tritiated waste, so that the tritated solvent, usually water, thus formed can therefore be re-used, although at a lower specific activity.

One can therefore conclude from the examples given above that microwave-enhanced labeling procedures can be of considerable benefit and as well as deuterium and tritium there may well be new opportunities [13] for C-14, C-13 and even C-11 where the short half-life demands very rapid reactions.

Acknowledgements:

The tritium work at Surrey has been generously funded over many years by EPSRC (previously SERC and at the beginning, SRC), the EU, NATO and the chemical industry. The present publication was undertaken as part of the EU sponsored D10 COST Program (Innovative Methods and Techniques for Chemical Transformations).

References:

1. Elander N, Jones JR, Lu SY, Stone-Elander S, *Chem Soc Rev* 2001; **29**:239-249.
2. Jones JR, Lu SY.Microwave-enhanced radiochemistry in Microwaves in Organic Synthesis, Loupy A (ed), John Wiley &Sons Ltd; Chichester, 2002; 435-462.
3. Barthez JM, Filikov AV, Frederiksen LB, Huguet ML,Jones JR, Lu SY, *Can J Chem* 1998; **76**:726-728.
4. Anto S, Getvoldsen GS, Harding JR, Jones JR, Lu SY, Russell JC, *J Chem Soc* Perkin Trans 2000; **2**, 2208-2211.
5. Al-Qahtani MH, Cleator N, Danks TN, Garman RN, Jones JR, Stefaniak S, Morgan AD, Simmonds A. *J Chem Res (S)*1998; 400- 401.
6. Jones JR, Lockley WJS, Lu SY, Thompson SP, *Tetrahedron Lett.* 2001; **42**, 331-332.
7. Erb ThW, Jones JR, Lu SY. *J Chem Res(S)*1999; 728-729.
8. Harding JR, Jones JR, Lu SY, Wood R *to be published.*
9. Chappelle MR, Kent BB, Jones JR, Lu SY, Morgan AD, *Tetrahedron Lett* 2002; **43**:5117-5118.
10. Varma RS, Saini RK, *Tetrahedron Lett* 1997; **33**, 4337-4343.
11. Frederiksen LB, Grobosch TH, Jones JR, Lu SY,Zhao CC. *J Chem Res(S)* 2000; 42-43.
12. Jones JR, Langham PB, Lu SY. *Green Chem* 2002, in press.
13. Hernandez L, Casanova E, Loupy A, Petit A. *J Label Compd Radiopharm* 2002; **45**, *in press.*

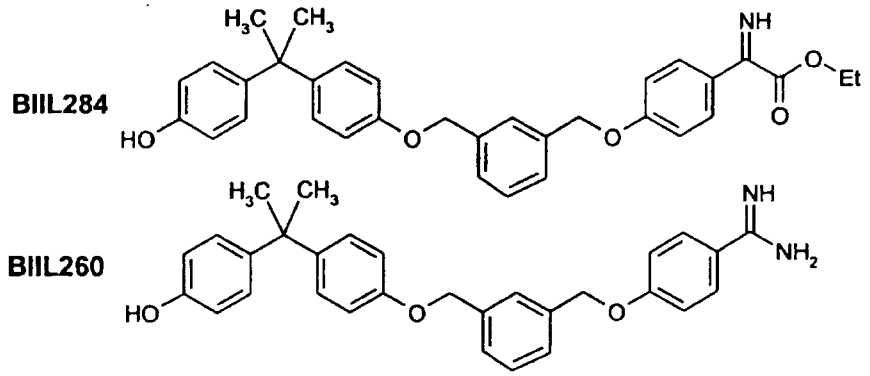
Preparation of Tritium-Labelled BIIL260 with High Specific Radioactivity

V. P. Shevchenko, I. Yu. Nagaev, N. F. Myasoedov
Institute of Molecular Genetics, Russian Academy of Sciences,
123182 Moscow, Kurchatov Sq., RUSSIA
A. B. Susan*
International Isotopes Clearing House, Inc.,
9110 Lee Blvd., Leawood, KS 66206, USA.
asusan@kc.rr.com

R. Anderskerskevitz, F.W. Birke, K-H.Switek
Boehringer Ingelheim Pharma KG
Birkendorfer Str.65, D-88397 Biberach an der Riss, Germany

Introduction:

BIIL 284 BS is a new LTB4 receptor antagonist developed as a novel anti-inflammatory principle for several chronic inflammatory conditions. It is a prodrug, which is metabolised to the active metabolite **BIIL 260**. **BIIL 260** has a high affinity to the LTB4-receptor with a Ki value of 1.7 nM on human neutrophilic granulocytes. For the characterization of **BIIL 260** in its interaction with the LTB4 receptor the preparation of the tritium labelled isotopomer with a high specific radioactivity was required. Methods allowing the direct introduction of tritium into the final product would have been preferable over the more time-consuming and complex synthetic methods which need to be used if the compound is unstable under the given reaction conditions.

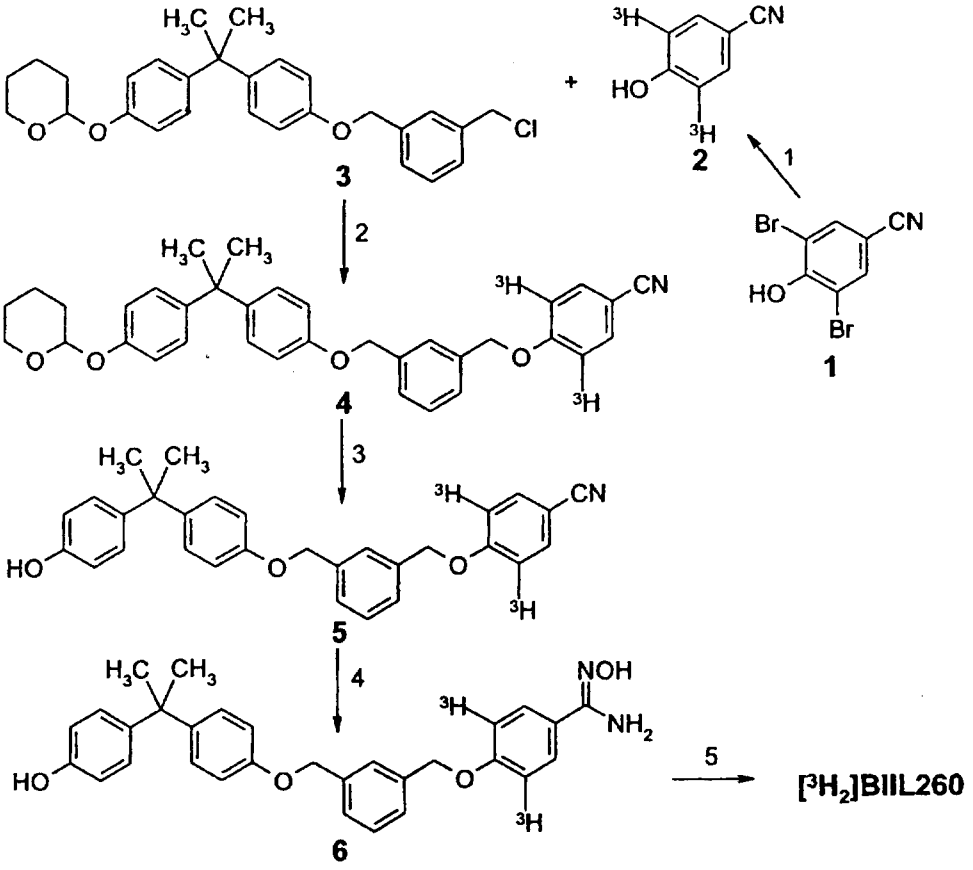


Results and Discussion:

The first attempts to introduce tritium directly into **BIIL260** with tritium gas over various palladium catalysts (Pd-C, Pd-$BaSO_4$ etc.) following solid-phase isotope exchange techniques (reaction times ranging from 15 to 120 min and temperatures from 140°C to 200°C) as well as by Pd-catalyzed exchange labelling with tritium water of high specific radioactivity (reaction times from 0.5 to 16 h, reaction temperatures ranging from 23°C to 160°C) failed. The specific radioactivities which could be achieved under the reaction conditions applied were

only in the range of 2 to 7 Ci/mmol and therefore too low for the planned receptor binding studies. Furthermore, treatment of **BIIL260** for 10 to 30 min with gaseous tritium at 400 hPa and Pd-catalysts along with solvents, such as methanol, ethanol, acetonitrile or their mixtures, led to a complete decomposition of the substrate. From the reaction mixture 2,2-bis-(4-hydroxyphenyl)propane, m-xylene, and HO-C_6H_4-C(NH_2)=NH could be isolated indicating a hydrogenolytic cleavage of the compound into R'-OH and R"-C_6H_4-CH_3 components as known for compounds of the general formula R'O-CH_2-C_6H_4-R. Consequently, in order to achieve our goal to prepare **BIIL260** with a higher specific activity we devised the two labeling pathways described below (scheme 1 and 2).

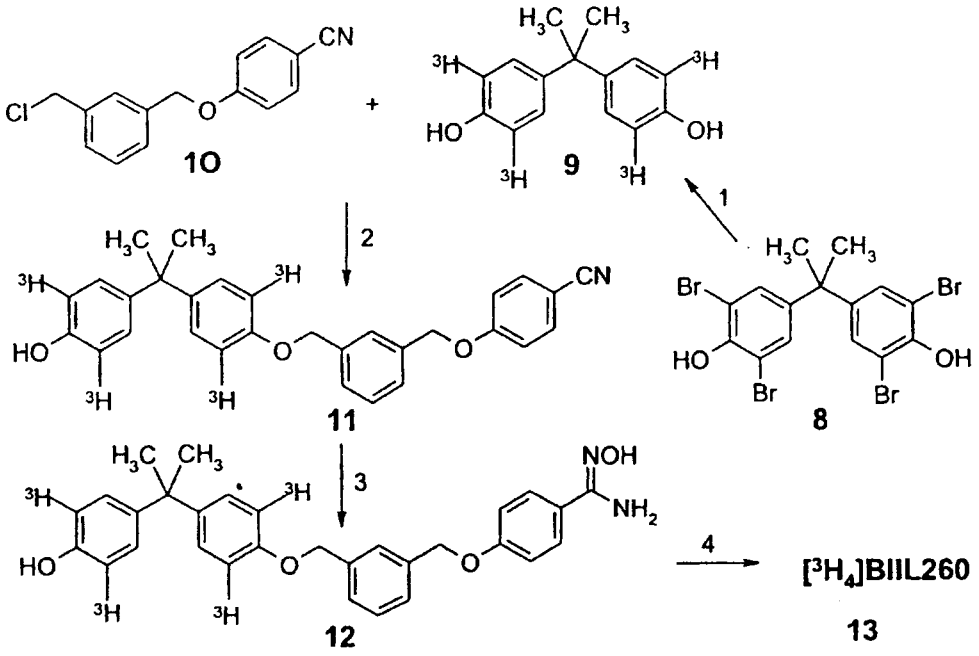
Reaction scheme 1. Synthesis of **[$^3H_2$] BIIL 260 7** *according to procedure 1*



Reaction conditions: 1 (liquid-phase conditions): $^3H_2$, 5% Pd/$BaSO_4$, Et_3N, EtOAc, r.t., 80 min; 2: **3**, K_2CO_3, KI, CH_3CN, 85°C, 2 h; 3: p-TosOH, EtOAc-MeOH 2:1, r.t., 2 h; 4: H_2NOH·HCl, Na_2CO_3, MeOH, 100°C, 2 h; 5: H_2, Raney-Ni, r.t., 80 min

As described in Scheme 1, 4-hydroxy[3,5-$^3H_2$]bezonitrile **2** was prepared by Pd-catalyzed tritio-dehalogenation of 4-hydroxy-3,5-dibromobenzonitrile **1**, followed by coupling of its potassium salt to the protected benzyl chloride **3**, acid-catalyzed cleavage of the protecting group, treatment of the nitrile intermediate **5** with hydroxylamine and concluding Raney-Ni catalyzed reduction of the resulting amidoxime **6** to **[$^3H_2$]BIIL 260 7**.

Reaction scheme 2. Synthesis of **[$^3H_4$]BIIL 260 13** *according to procedure 2.*



Reaction conditions: 1 (solid-phase conditions): $^3H_2$, 5% $Pd/BaSO_4$, 160 °C, 15 min; 2.: **10**, K_2CO_3, KI, CH_3CN, 85°C, 2 h; 3.: $H_2NOH{\cdot}HCl$, Na_2CO_3, MeOH, 100°C, 2 h; 4.: H_2, Raney-Ni, r.t., 80 min

As described in Scheme 2, the preparation of 2,2-bis-(4-hydroxy-[3,5-$^3H_2$]phenyl)propane **9** was achieved by Pd-catalyzed tritio-dehalogenation of the corresponding tetrabromo-precursor **8**., subsequent etherification of its potassium salt with 4-(3-$ClCH_2C_6H_4CH_2O)C_6H_4CN$ **10**, reaction of the nitrile **11** formed with hydroxylamine and finally Raney-Ni mediated reduction of the resulting amidoxime **12** into **[$^3H_4$]BIIL 260 13.**

The first reaction pathway yielded tritium labeled **BIIL 260** with a specific radioactivity of 25 Ci/mmol, while the specific radioactivity of the compound could be increased to 71 Ci/mmol when the second pathway was followed. In both cases the introduction of the label and the Raney-Ni catalyzed reduction of the [^{3}H]amidoximes **6** and **11** proved to be the most critical processes.

Both tritio-dehalogenation reactions leading to **2** and **9** were carried out following solid-phase as well as liquid-phase techniques. In the case of the tritio-dehalogenation of nitrile **1** the best results were achieved by the liquid-phase method. Solid-phase procedures proved to be unsuitable because of considerable losses of substrate by sublimation (at reaction temperatures of above 100°C as needed for an efficient high-temperature solid-phase dehalogenation). In the case of the tritio-dehalogenation of 2,2-bis-(4-hydroxy-3,5-dibromophenyl)propane **7**, however, the solid-phase method gave better results.

The difficulty of completing the remaining reaction steps resided in the fact that only milligram quantities of the labelled key starting materials **2** and **9** for the following multi-stage syntheses could be prepared. Following reaction pathway **1**, high yields (up to 90 % and above) of intermediate **4** were obtained when a large excess of benzyl chloride **3** was

employed in comparison to nitrile **2**. Furthermore, for the etherification of **9** with benzyl chloride **10** an equimolar ratio of both reagents had to be used in order to minimize the formation of the dimeric by-product. Because of these constraints, the yield of the desired labelled final products did not exceed 50% of the theoretical value.

The removal of protecting group and the transformation of the tritium labeled nitriles **5** and **10** to the labelled amidoximes **6** and **11** did not cause any problems since these processes could be carried out using large excesses of unlabeled reagents. For reasons of safety, all reactions involving labeled intermediates were carried out in sealed ampoules. The stirring of the reaction mixtures was achieved by rotation of the ampoule in a rotary evaporator while the reaction temperature was kept by means of a heating bath.

The most critical reaction step was the Raney-Ni catalyzed reduction of the labeled amidoximes **6** and **11**. This reaction had to be performed with microgram amounts of both intermediates. The yield was found to be strongly influenced by the conditions of the preparation of the Raney nickel catalyst. It could be raised up to 40 % when Raney nickel pre-treated with acetic acid and a substance-to-catalyst ratio of 1:40 (by weight) was employed into the reaction.

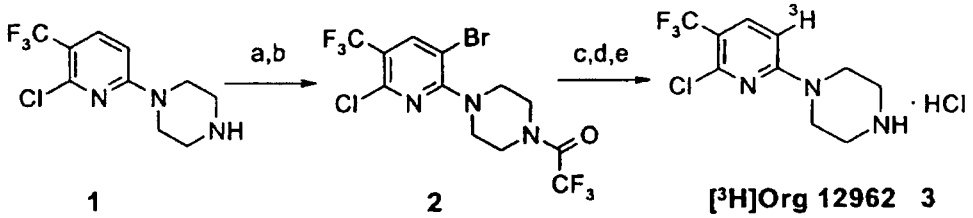
Synthesis of [^{3}H]-, [^{14}C]- and [^{13}C,^{2}H]-labelled Org 12962 Derivatives

Peter Wiegerinck*, Olaf Post, Leontine Hofstede, Marcel van den Heuvel
NV Organon, PO Box 20, 5340 BH Oss, The Netherlands
peter.wiegerinck@organon.com

Major Depressive Disorder is a psychiatric disease which can be treated with compounds acting on different receptor systems. In behavioral pharmacological studies, **Org 12962 1** acts as a full 5-HT_{2C} receptor agonist. It was found to induce changes which are characteristic for antidepressant drugs. The EEG-sleep profile also indicated anxiolytic properties. Due to the agonistic action of **Org 12962** on 5-HT_{2C} receptors it is believed that the drug will stimulate the receptors more directly and more effectively than the selective serotonin re-uptake inhibitors (SSRIs). Accordingly, it is expected to exert an improved therapeutic efficacy with fewer 5-HT related side-effects, such as nausea, vomiting and dizziness, than SSRIs.

For preclinical development studies both [^{3}H]- and [^{14}C]-labelled isotopomers of **Org 12962** were synthesized.

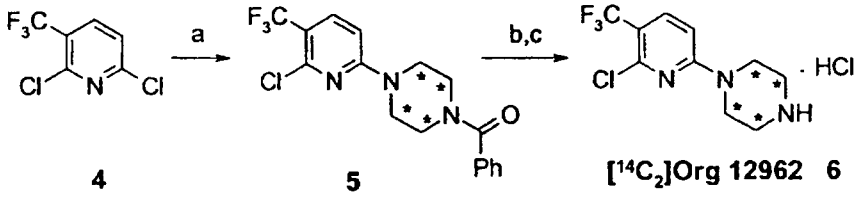
Protection of the free base of **Org 12962 1** upon treatment with trifluoroacetic anhydride in the presence of pyridine followed by halogenation with bromine in the presence of acetic acid yielded **2**. Subsequent catalytic tritio-dehalogenation with $^{3}H_2$-gas and deprotection of the amino-function with a solution of aqueous HCl in ethanol furnished **[^{3}H]Org 12962 3**.



1 **2** **[^{3}H]Org 12962 3**

a) trifluoroacetic anhydride, pyridine, dichloromethane (C.Y. 98 %). b) bromine, acetic acid, 60 °C (C.Y. 60 %). c) $^{3}H_2$, Pd-$CaCO_3$ (10 %) in ethyl acetate. d) Na_2CO_3 (7% m/v), H_2O (20 % v/v), methanol (80 % v/v). e) 36 % HCl (aq) in ethanol (C.Y. 48 % from **2**).

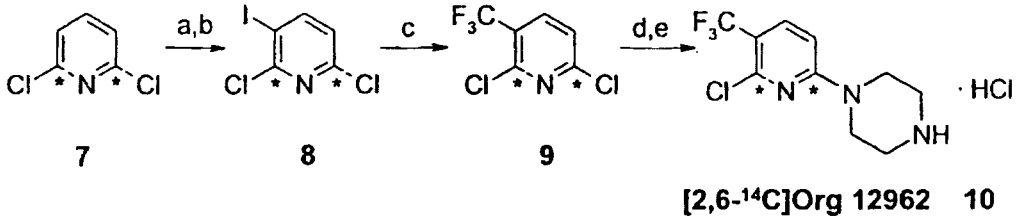
[$^{14}C_2$]Org 12962 6 was synthesized by reaction of 1,6-dichloro-3-trifluoromethyl-pyridine with 1-benzoyl[2,3-$^{14}C_2$]piperazine followed by reductive removal of the benzoyl group and subsequent treatment with a solution of aqueous HCl in ethanol.



4 **5** **[$^{14}C_2$]Org 12962 6**

a) 1-benzoyl[2,3-$^{14}C_2$]piperazine, $NaHCO_3$, KF, DMSO, 120 °C, 20 hours. b) DiBAlH, - 78 °C, 3 hours. c) 36 % HCl (aq) in ethanol (C.Y. 59 % from **4**; 40 % after recrystallization).

[$^{14}C_2$]Org 12962 10 was synthesized starting from 1,2-dichloro[1,2-$^{14}C_2$]pyridine **7**. Treatment of **7** with LDA during three hours followed by quenching with iodine [1] yielded **8**. Regioselective introduction of the 3-trifluoromethyl group was achieved upon reaction of **8** with trifluoromethyltrimethylsilane in the presence of potassium fluoride and copper(I)iodide [2]. Subsequent reaction of **9** with piperazine and treatment with aqueous HCl in ethanol furnished **10**.

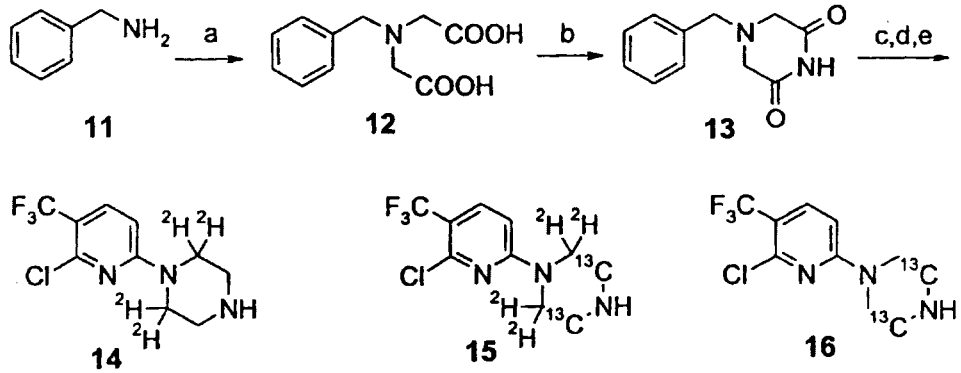


a) LDA, - 78 °C, 3 hours. b) I_2, 15 min. c) $F_3CSi(CH_3)_3$, KF, CuI, 85 °C, 20 hours in DMF/THF (5/1). d) piperazine in ethanol,* . e) 36 % HCl (aq) in ethanol (R.C.Y. 6 % from 7).

Additional data for the compounds **3, 6 and 10**: The amount synthesized, the specific activity, the chemical stability and the relative metabolic stabilty.

	Amount (mCi)	Specific activity (mCi/mmol)	Chemical Stability	Metabolic Stability
[^{3}H]Org 12962 3	150	$14.2 \cdot 10^3$	> 18 months (toluene)	- -
[$^{14}C_2$]Org 12962 6	23	108	> 6 months (methanol)	-
[$^{14}C_2$]Org 12962 10	15	102	> 8 months (ethanol)	+

Some specifically [^{2}H]- and/or [^{13}C]-labelled derivatives of **Org 12962** were synthesized [3] to enable the elucidation of the fragmentation mechanism in EI-MS-MS, as depicted below.



a) bromoacetic acid, NaOH (aq) ($BaCl_2$, H_2SO_4 ; C.Y. 78 %). b) urea, 176 °C, 20 min (C.Y. 31 %). c) $LiAL^2H_4$, THF, 70 °C (C.Y. 77 %). d) 2,6-dichloro-3-trifluoromethylpyridine, DMSO (C.Y. 88 %). e) H_2 , Pd-C (10 %) (C.Y. 47 %).

The free base of **[$^{2}H_4$]Org 12962 14** was synthesized as depicted above. By employing bromo[2-$^{13}C_2$]acetic acid, **[$^{13}C_2$,$^{2}H_4$]Org 12962 15** could be prepared following the same synthetic route. Finally, **[$^{13}C_2$]Org 12962 16** was readily available by reducing the carbon-13 labelled diketopiperazine intermediate with $LiAlH_4$.

References:

1. (a) Radinov R, Chanev, C, Haimova M. *J Org Chem.* 1991; **56**: 4793-4796.
   (b) Rocca P, Marsais F, Godard A, Queguiner, G. *Tetrahedron* 1993; **49**: 49-64.

2. Robic, N.; Noël, J-P. *J Labelled Cpd Radiopharm* 1999; **42**: 109-119.

3. (a) Chase BH, Downes AM. *J Chem Soc* 1953; 3874-3875.
   (b) Berchet GJ. *Org Synth* 1943; **2**: 397.
   (c) Dischino DD, Combs CM, Gammans RE. *Labelled Cpd Radiopharm* 1992; 57: 1179-1190.

Labeling of BAY 58-2667, an Activator of sGC

U. Pleiss*, D. Seidel
Bayer AG, PH-PD P Drug Metabolism and Isotope Chemistry, Aprather Weg 18 a, 42096 Wuppertal, Germany
ulrich.pleiss.up@bayer-ag.de

The soluble guanylate cyclase (sGC) has long been considered as an enzyme that is regulated exclusively by NO. **BAY 58-2667** was identified as a very potent and NO-independent sGC activator and may offer a new approach for the treatment of cardiovascular diseases [1]. For preclinical studies and for the elucidation of the mode of action carbon-14 and tritium labeled substance was needed. As an internal standard for mass spectrometry a stable labeled compound was prepared as well.

Since the starting material **2** (scheme 1) for the carbon-14 labeling was commercially not available bromo ketone **1** had to be reduced. The carbon-14 label was introduced by halogen-metal exchange and carboxylation with $^{14}CO_2$. The carboxylic acid **3** formed was reduced to alcohol **4** which was subsequently transformed into the respective chloro derivative **5** using thionyl chloride [2]. O-alkylation of phenolate **6** and subsequent saponification led to [^{14}C]BAY 58-2667 **8**.

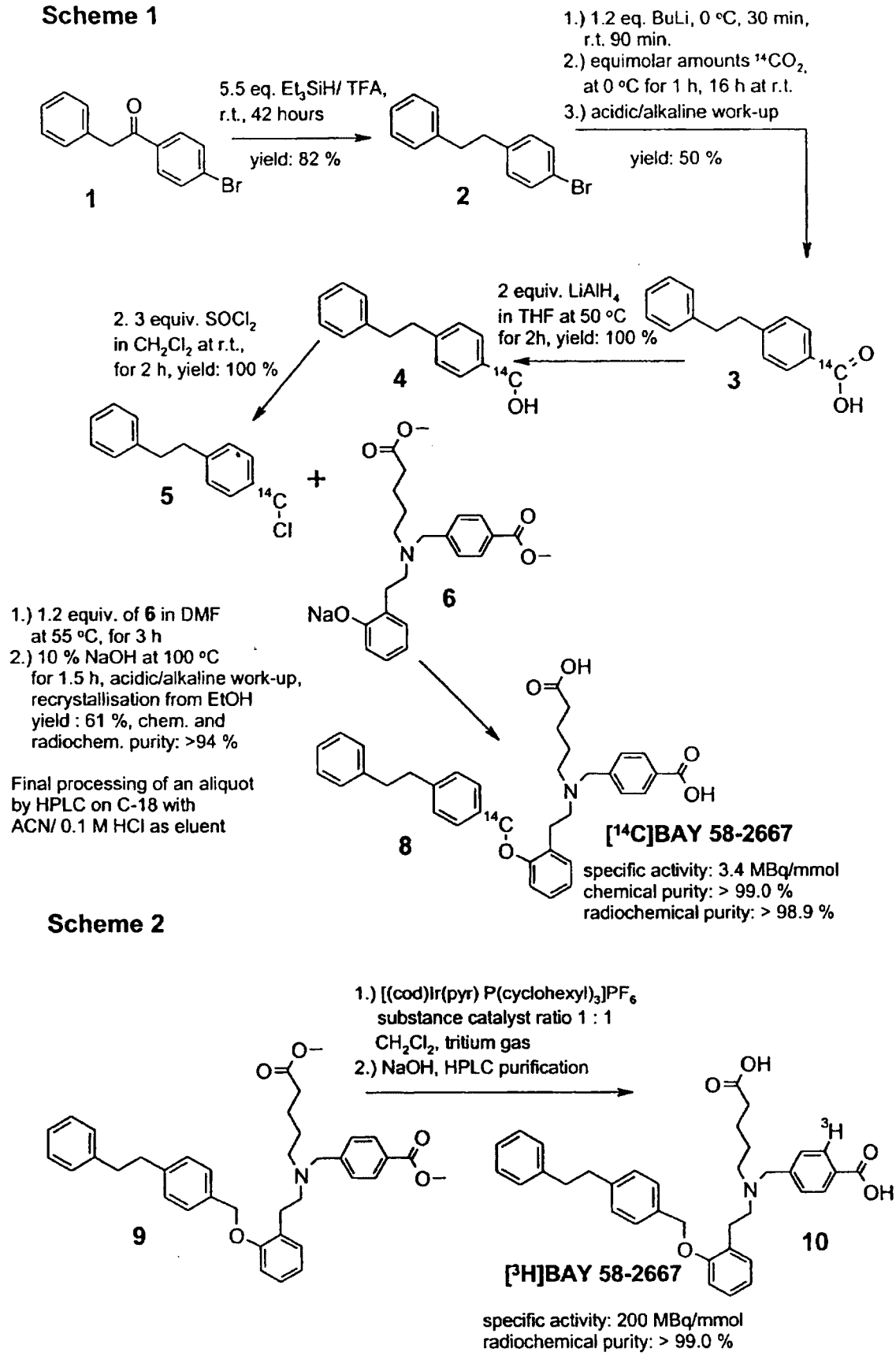
The tritium labeling was realized by an organo-iridium exchange [3] using diester **9** and [(cod)Ir(pyridine)(triscyclohexylphosphine)]PF_6 as a catalyst (scheme 2). The expected introduction of tritium in the ortho positions of the benzoic acid unit was confirmed by ^{3}H-NMR. Other tritium labeled positions were not found.

For the stable labeling (scheme 3) the substituted benzyl chloride **11** was reacted with potassium [^{13}C,^{15}N]cyanide yielding nitrile **12**. Hydrogenation of **12** using Raney-nickel catalyst led to amine **13**, which was reacted with the carbon-13 labeled aldehyde **15** to to give Schiff base **16**. After hydrogenation with platinum on charcoal the secondary amine **17** was alkylated with methyl 5-bromovalerate. The final product **[$^{13}C_2$,^{15}N]BAY 58-2667 19** was obtained by saponification of the corresponding dimethylester **18**. [^{13}C]aldehyde **15** was prepared by reaction of methyl 4-bromobenzoate with potassium [^{13}C]cyanide and direct reduction of the resulting nitrile **14** using Raney-nickel alloy and formic acid.

References:

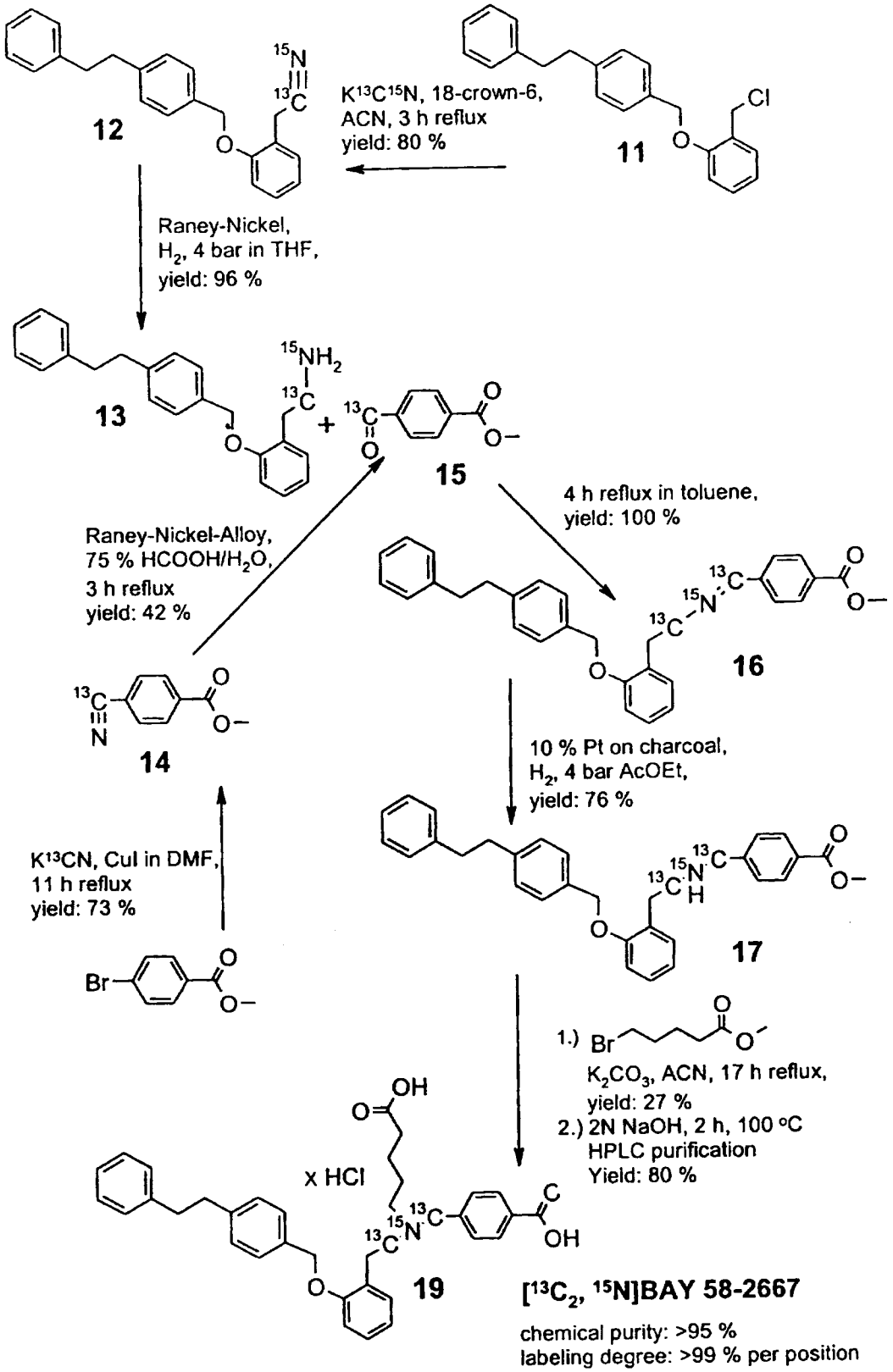
1. J.-P. Stasch et al., *British J. Pharmacol. accepted*
2. M. Carrara et al., *Arzneimittelforschung* 1997; **47**: 803.
3. Shu AYL, Heys JR, *Proceedings of the 7th Intern. Symposium on the Synthesis and Application of Isotopically Labelled Compounds* **2000**, *Dresden, edited by U. Pleiss and R. Voges, John Wiley & Sons, p. 68.*

Scheme 1



Scheme 2

Scheme 3

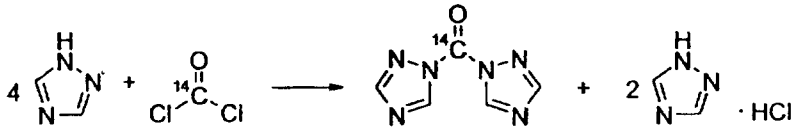


Preparation and Use of [^{14}C]N,N'-Carbonylditriazole in the Labelling of Urea Derivatives

K.-H. Switek*, H. Braunger,
Boehringer Ingelheim Pharma KG, Biberach an der Riß, Germany
karl-heinz.switek@bc.boehringer.ingelheim.com

In order to label the new calcitonin gene-related peptide (CGRP) receptor antagonist BIBN 4096 BS **7a**, with carbon-14 in a quick and economic way, the carbonyl function of the central urea subunit was identified as the most suitable position for the introduction of the label. In the course of preceding experiments we found **N,N'-carbonyl-di-1,2,4-triazole (CDT)** to be the most effective coupling reagents to meet the mentioned requirements.

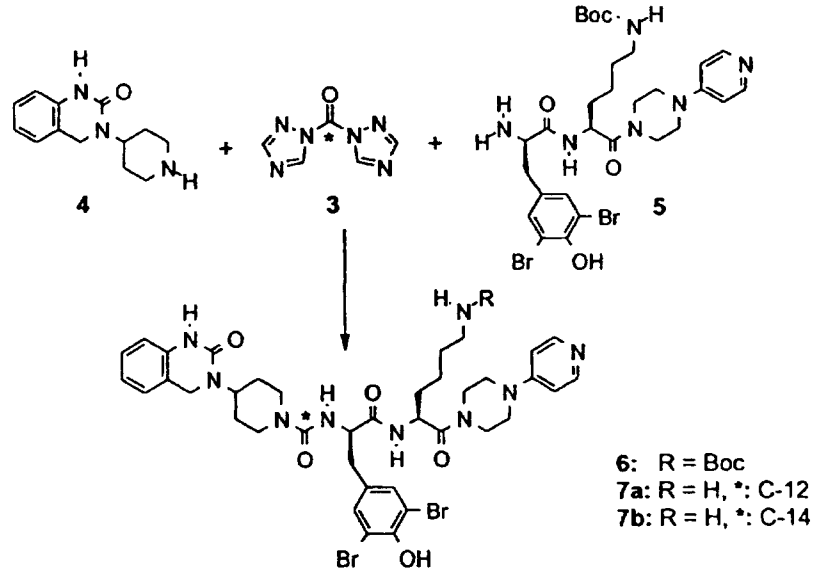
Scheme 1: Synthesis of N,N'-[^{14}C]carbonyl-di-1,2,4-triazole ([^{14}C]CDT) 3



1 2 3

Due to its sensitivity towards hydrolysis, [^{14}C]CDT **3** is commercially not available. For its preparation we followed essentially the method reported by Staab [1]. Under dry N_2 at ambient temperature to a stirred solution of [^{14}C]phosgene **2** in toluene the fourfold molar excess of 1,2,4-triazole **1** in absolute tetrahydrofuran was added within 10 min. Stirring was continued for further 10 min, the formed precipitate (1,2,4-triazole hydrochloride) was removed by filtration and the filtrate evaporated almost to dryness. The crude residue was immediately used in the following coupling reaction without further treatment.

Scheme 2: Synthesis of [^{14}C]BIBN 4096 BS 7b

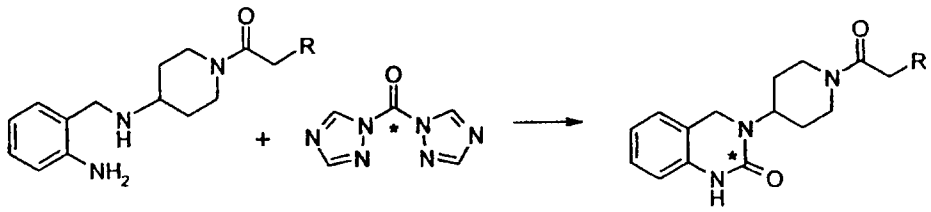


4 3 5

[^{14}C]**BIBN 4096 BS 7b** was obtained by successive reaction of **3** with equimolar amounts of the amines **5** at 0 °C and **4** at 65 °C in dry DMF. The N-BOC protected intermediate **6** formed was isolated by precipitation with water directly from the reaction solution and purified by column chromatography on silica gel. Cleavage of the protection group with TFA and the subsequent precipitation of **7b** led to a white crystalline product. Its chemical and radiochemical purity was > 97 %. The overall yield was 42.5 % relative to [^{14}C]phosgene used as precursor.

Encouraged by this result we applied the [^{14}C]CDT labelling method also to the labelling of compounds showing the same central subunit. Furthermore, we were able to introduce the C-14 label into position 2 of the 3,4-dihydro-1*H*-quinazolin-2-one moiety (scheme 3) of another drug candidate following similar reaction conditions.

Scheme 3: C-14 Labelling of **3,4-dihydro-1*H*-quinazolin-2-one** derivatives



Reference:

1 Staab HA, *Liebigs Ann Chem* 1957; **609**: 75

Efficient Carbon-14 Labeling of 2-(3-Hydroxypropyl)-1*H*-benzimidazole

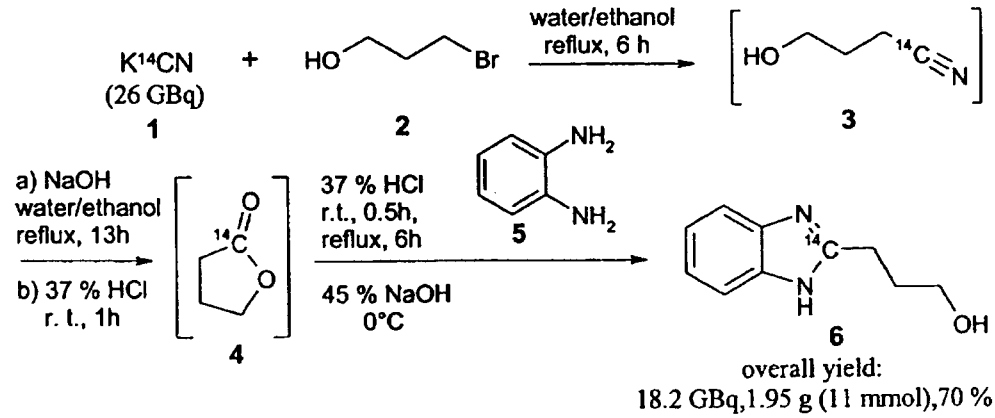
Dietrich Seidel
Bayer AG, PH-PD P Drug Metabolism and Isotope Chemistry, Aprather Weg 18 a, 42096 Wuppertal, Germany
dietrich.seidel.ds@bayer-ag.de

2-(3-Hydroxypropyl)-1*H*-[2-^{14}C]benzimidazole **6** was needed as an intermediate in the carbon-14 labeling synthesis of a drug substance for pharmacokinetics and drug metabolism studies. An efficient one-pot procedure is described.

The synthesis started with the formation of 4-hydroxybutyro[^{14}C]nitrile **3** from 3-bromopropanol **2** and K^{14}CN **1** diluted with non-labeled carrier. Saponification of the resulting labeled nitrile led to 4-hydroxy[1-^{14}C]butyrate. Subsequent treatment with hydrochloric acid furnished the intermediate [1-^{14}C]butyrolactone **4**. The sequence for the respective non-labeled butyrolactone starting from chloropropanol has been described by Boorman and Linstead [1]. All intermediates including the labeled butyrolactone were used without isolation and purification in the present synthesis.

The [1-^{14}C]butyrolactone was then reacted with 1,2-diaminobenzene **5** in half-concentrated hydrochloric acid to form 2-(3-hydroxypropyl)-1*H*-[2-^{14}C]benzimidazole **6** as published by Feedman et al. (non-labelled) [2]. The labeled benzimidazole derivative was isolated as a solid in an amount of 1.95 g and a total radioactivity of 18.2 GBq. The yield corresponds to 70 % of the theory referred to the K^{14}CN employed. A small amount of 2-[3-(3-hydroxypropoxy)-propyl]-1*H*-[2-^{14}C]benzimidazole was determined as a by-product.

Scheme: Synthesis of 2-(3-hydroxypropyl)-1*H*-[2-^{14}C]benzimidazole 6



overall yield:
18.2 GBq,1.95 g (11 mmol),70 %

References:

1. Boorman EJ, Linstead RP. *J Chem Soc* 1933; 577
2. Freedman AR, Payne DS, Day AR. *J Heterocycl Chem* 1966; 3: 257

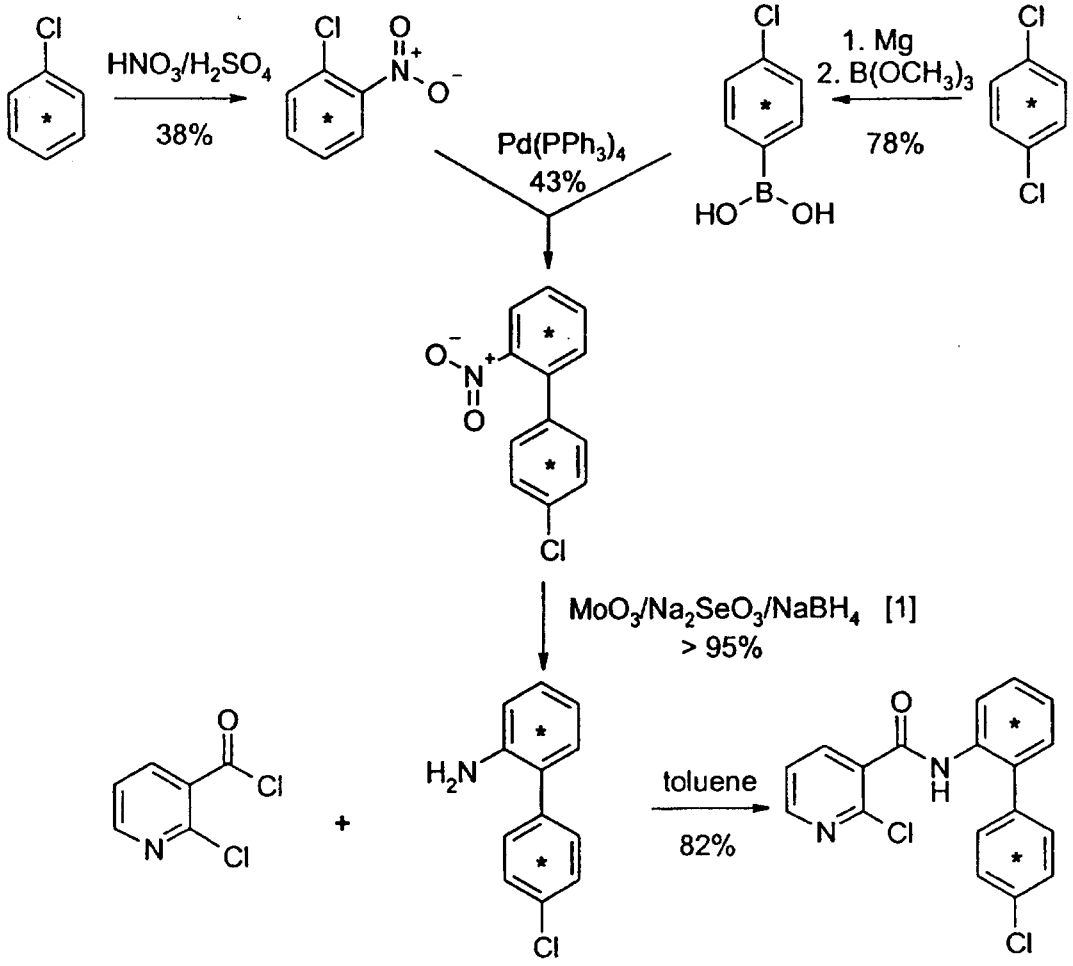
Synthesis of Some ^{14}C-Labelled Fungicides

Rainer Schlecker
BASF AG, Specialty Chemical Research, Isotope Laboratory, Germany
rainer.schlecker@basf-ag.de

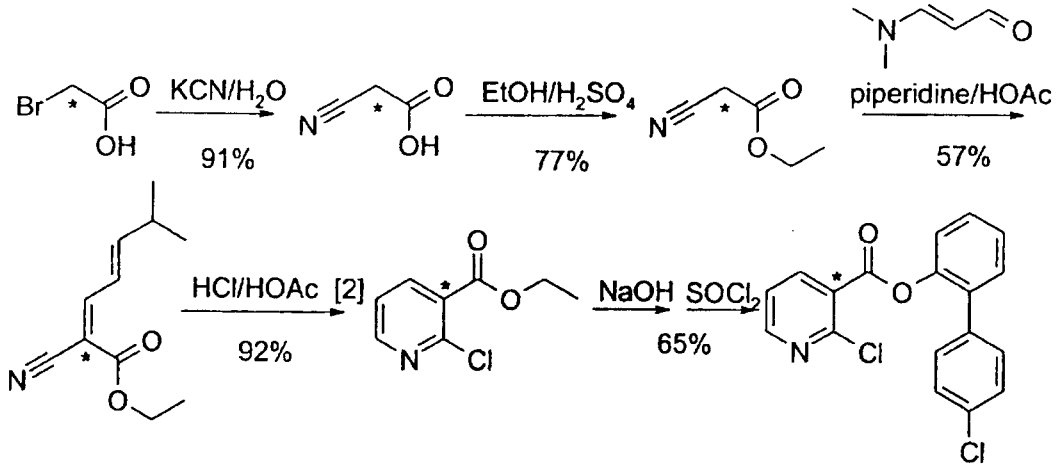
BAS 500 F and **BAS 510 F** are two novel compounds developed by BASF AG for the treatment of fungal diseases in a broad variety of plants. For ADME-studies radiolabelled samples of these fungicides were required.

The synthesis of these ^{14}C-isotopomers started from simple ^{14}C-labelled educts as described in the following.

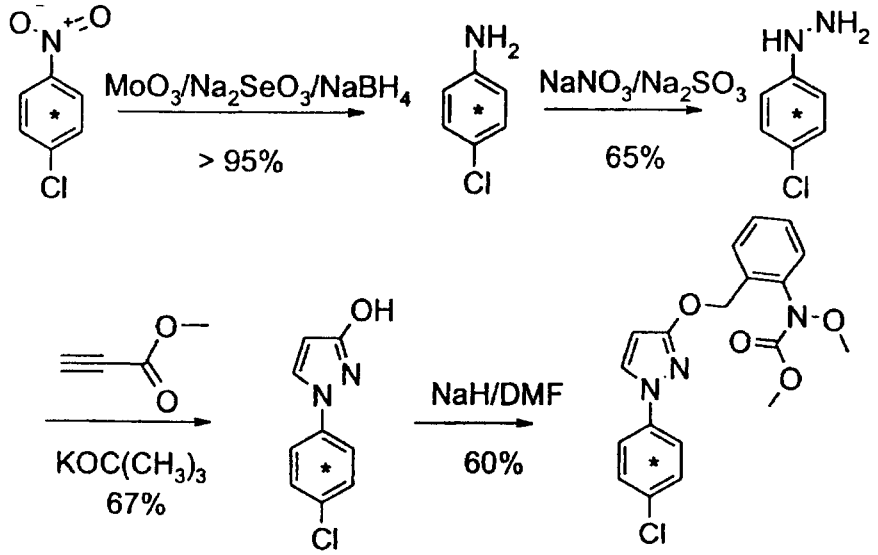
Scheme 1: Synthesis of **[biphenyl-^{14}C]BAS 510F**



Scheme 2: Synthesis of **[pyridine-3-^{14}C]BAS 510F**



Scheme 3: Synthesis of **[phenyl-U-^{14}C]BAS 500F**



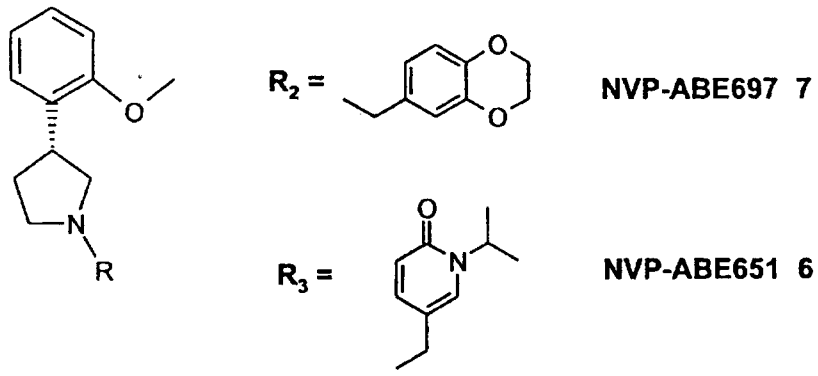
References:

1 Yanada K, Yanada R, Meguri H. *Tetrahedron Lett* 1992; **33**: 1463
2 Schröder L. 1988; *DE 3840954*

C-14 Labelling of NVP-ABE651 and NVP-ABE697

Thomas Moenius*, Max-Peter Seiler, Joachim Nozulak und Paul Burtscher
Novartis Pharma AG, CH-4002 Basel, Switzerland
Thomas.Moenius@Pharma.Novartis.com

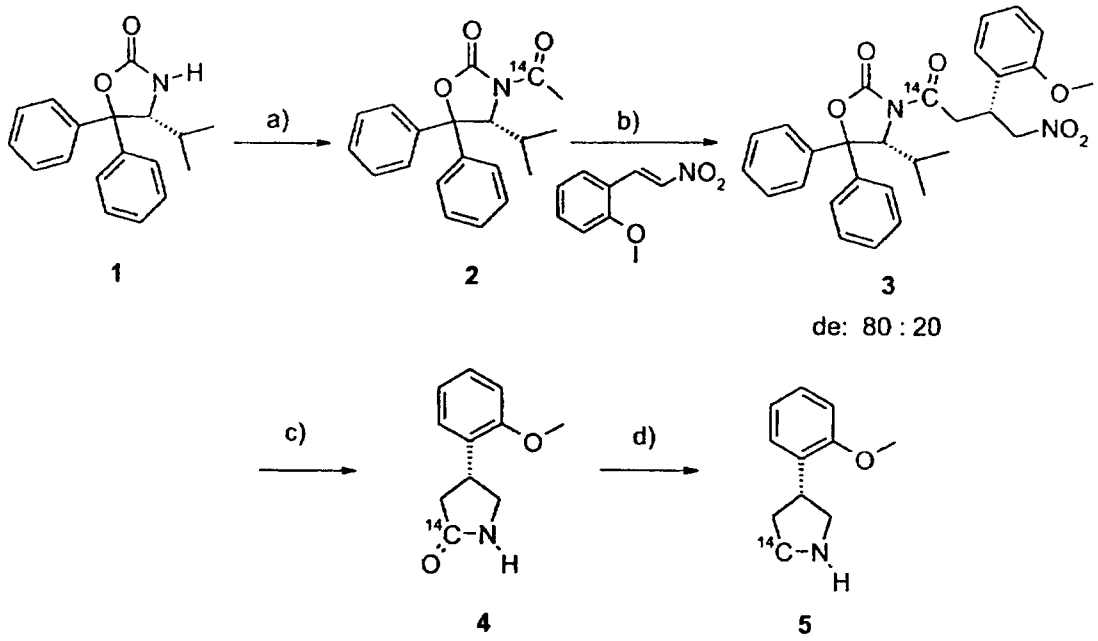
The highly selective α_{2C}-antagonists **NVP-ABE651 6** and **NVP-ABE697 7** are being developed by NOVARTIS PHARMA AG in the indication of schizophrenia. In order to establish a general C-14 labelling strategy we concentrated our efforts on the common structural subunit **NVP-AAL953 5**.



Based on results published by Seebach [1] we started the diastereoselective synthesis of [^{14}C]-**5** from (R)-4-isopropyl-5,5-diphenyl-oxazolidinone **1** as a chiral auxiliary. The key step of this sequence was the $TiCl_4$-catalysed Michael-addition of 1-methoxy-2-(2-nitrovinyl)-benzene to the titanium-enolate of the respective N-[1-^{14}C]-acetyl-oxazolidinone **2**. Since the reaction conditions were not fully optimised reaction the desired diastereomer **3** could only be isolated in a ratio of 80 : 20. Subsequent reduction of the purified Michael-adduct (Ra-Ni, H_2, EtOH-H_2O) resulted in elimination of the auxilary and spontaneous cyclisation to the pyrrolidinone-derivate **4**, which in turn was reduced (THF, $LiAlH_4$, rt, 6 h) to **NVP-AAL953 5**.

Reductive amination of [^{14}C]-**5** ($NaCNBH_3$, MeOH, rt, 2 h) with 1,4-benzo-dioxan-6-carboxaldehyde and condensation with 1-isopropyl-1H-pyridin-2-on-5-methylalcohol under Mukaiyama-conditions (DEAD, TPP, THF, rt, 16 h) resulted in C-14 labelled **NVP-ABE697 6** and **NVP-ABE651 7**, respectively.

Figure 1: C-14 Labelling of **NVP-AAL953 5**



a) n-BuLi, THF, 0°C, [1-^{14}C]acetyl chloride, 3 h, rt, 82%;
b) $TiCl_4$, *N*-ethyldiisopropylamine, 7 h, -75°C,separation of diastereomeres, 42%;
c) Ra-Ni in water, H_2, ethyl acetate - ethanol 1: 1, 17 h, rt, 100%;
d) $LiAlH_4$ (1 M in THF), THF, 2 h, 90°C; 76%;

Reference:

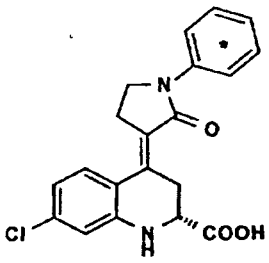
1. Brenner M, Seebach D, *Helv.Chimica Acta* 1999; **82**: 2365

Synthesis of Carbon-13,14 Isotopomers of a Novel 4-Substituted-2-carboxy Tetrahydroquinoline

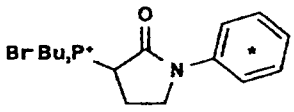
Mario Almi, Simone Guelfi and Maria Luisa Navacchia
Chemical Development Department, GlaxoSmithKline S.p.A., Via Fleming 4, 37135 Verona, Italy

INTRODUCTION

A novel group of 4-substituted-2-carboxy tetrahydroquinolines (THQ) having interesting activity as specific glycine antagonists at the strychnine-insensitive binding site of the N-methyl-D-aspartate (NMDA) receptor has been recently discovered.[1,2]
Among these, compound THQ-1 **(1)** was selected for further drug development studies, therefore carbon-13 and carbon-14 labelled derivatives were prepared respectively as LC-MS standard and for ADME studies.[3]



(1) THQ-1



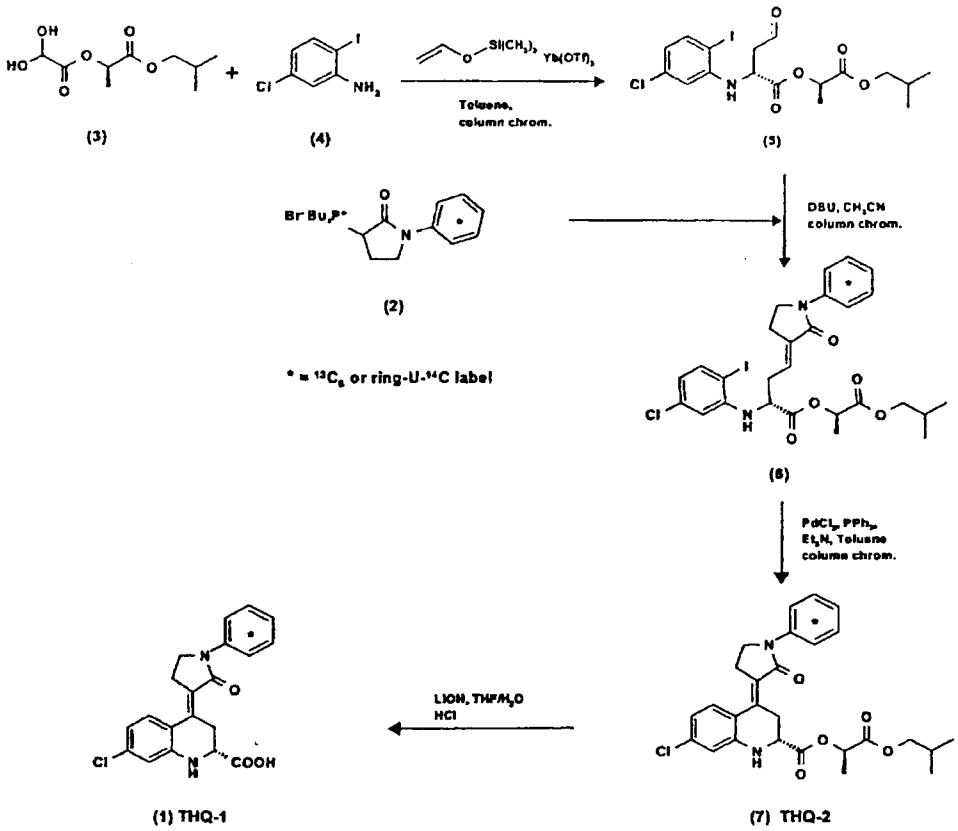
(2)

* = $^{13}C_6$ or ring-U-^{14}C label

RESULTS AND DISCUSSION

The synthetic approach relied on the labelled key intermediate phosphonium salt **(2)** which was prepared in three steps starting from commercially available [$^{13}C_6$] and [U-^{14}C] aniline, according to a described procedure.[4] Labelled aniline, either [$^{13}C_6$] free base or [U-^{14}C] aniline hydrochloride, was easily reacted with 2,4-dibromobutyryl chloride to give the corresponding anilide which underwent cyclization in aq.NaOH/DCM to afford 3-bromo-N-phenyl-pyrrolidin-2-one. This promptly reacted with n-But_3P to provide labelled phosphonium salt **(2)** that was isolated as white solid. Typical overall yield of the reaction sequence was in the range 55-75% *vs.* starting aniline.
For the preparation of THQ derivatives, a non-stereoselective route followed by enzymatic resolution of a racemic advanced intermediate was initially performed to afford carbon-13 labelled THQ-1 **(1)** in 14% overall yield from [$^{13}C_6$] phosphonium salt **(2)** (see Scheme 1 where ethyl glyoxalate was employed in place of **(3)**).
Subsequently, a more efficient, high-yielding stereoselective route was employed to synthesize both a second batch of carbon-13 derivative and the carbon-14 labelled version. Chiral isobutyl-(R)-(+)-lactate glyoxalate **(3)** was reacted with 5-chloro-2-iodo-aniline **(4)** *via* ytterbium catalyzed nucleophilic addition of vinyloxytrimethylsilane on the imine intermediate.[5] Diastereomeric aldehyde **(5)** was purified by column chromatography providing the desired diastereoisomer in > 98:2 ratio (Scheme 1).

Scheme 1



Wittig reaction of chiral aldehyde (5) with labelled phosphonium salt (2) was then performed, followed by Heck cyclization to afford labelled ester THQ-2 (7) in 45-50% overall yield. Hydrolysis of ester (7) provided final labelled THQ-1 (1) in 90-95% yield, enantiomeric ratio 99/1. By employing this route, [$^{13}C_6$]THQ-1 and [^{14}C]THQ-1 were prepared respectively in 37 and 41% overall yield from labelled phosphonium salt (2).

CONCLUSIONS

The novel 4-substituted-2-carboxy tetrahydroquinoline THQ-1 (1) was successfully labelled with carbon-13 and carbon-14 in the phenyl ring linked to the pyrrolidinone moiety.

Initially, a non-stereoselective synthetic approach followed by enzymatic resolution provided carbon-13 labelled material in 8% overall yield from [$^{13}C_6$]aniline.

Subsequently, a more efficient stereoselective route was employed in the syntheses of both C-13 and C-14 labelled versions with respectively 25 and 23% overall yields from corresponding labelled aniline.

REFERENCES

1. Di Fabio, R. PCT Int. Appl. 1999; *WO 99/64411* (Glaxo Wellcome S.p.A.)
2. Di Fabio, R.; Alvaro, G.; Bertani, B.; Giacobbe, S. *Can. J. Chem.* (2000), **78**, 809-815.
3. Orlandi, A. PCT Int. Appl. 2001; *WO 0142238* (Glaxo Wellcome S.p.A.)
4. Almi, M.; Guelfi, S. *Synthesis and Applications of Isotopically Labelled Compounds*, Vol. 7; U. Pleiss, R. Voges Eds; John Wiley & Sons (2001), 197-201
5. Kobayashi, S.; Nagayama, S. *J. Am. Chem. Soc.***1997**, *119*, 10049-10053.

[^{14}C]CH_2N_2: A Suitable Precursor for the Preparation of ^{14}C-Labelled Anthracyclines

C. Felicini*, D. Giribone, A. Pignatti and E. Fontana
Global Drug Metabolism Dept., Pharmacia,
viale Pasteur 10, 20014 Nerviano (MI), Italy
chiara.felicini@pharmacia.com

Introduction:

Since the discovery of the antitumoural activity of daunorubicin and doxorubicin in clinical trials, anthracyclines have been intensively investigated to find new analogs with a broader spectrum of activity and/or reduced toxicity. At present, drugs belonging to this chemical class such as Daunoblastina®, Adriblastina® (EU)/Adriamycin® (US), Farmorubicin® (EU)/Ellence® (US) and Zavedos® (EU)/Idamycin® (US), are on the market and have a key role in the antitumour therapy. In order to perform the distribution, pharmacokinetics and metabolism studies of these compounds, the preparation of ^{14}C-labelled materials was required. A convenient method to prepare [^{14}C]daunorubicin and [^{14}C]doxorubicin patented in 1980 involved the reaction of [^{14}C]diazomethane with 9-deacetyl-9-formyl-N-trifluoroacetyl-daunorubicin [1]. More recently, the same approach was followed to obtain [^{11}C]daunorubicin starting from [^{11}C]diazomethane [2]. To date, several anthracyclines were successfully synthesized in our company using [^{14}C]diazomethane as radiolabelled precursor, and the stability of the C-14 position was found to be quite satisfactory *in vivo* [3]. In this paper, an overview of the strategy used in our labs to successfully introduce ^{14}C in the anthracycline structures is described as shown in Figure 1.

Methods and results:

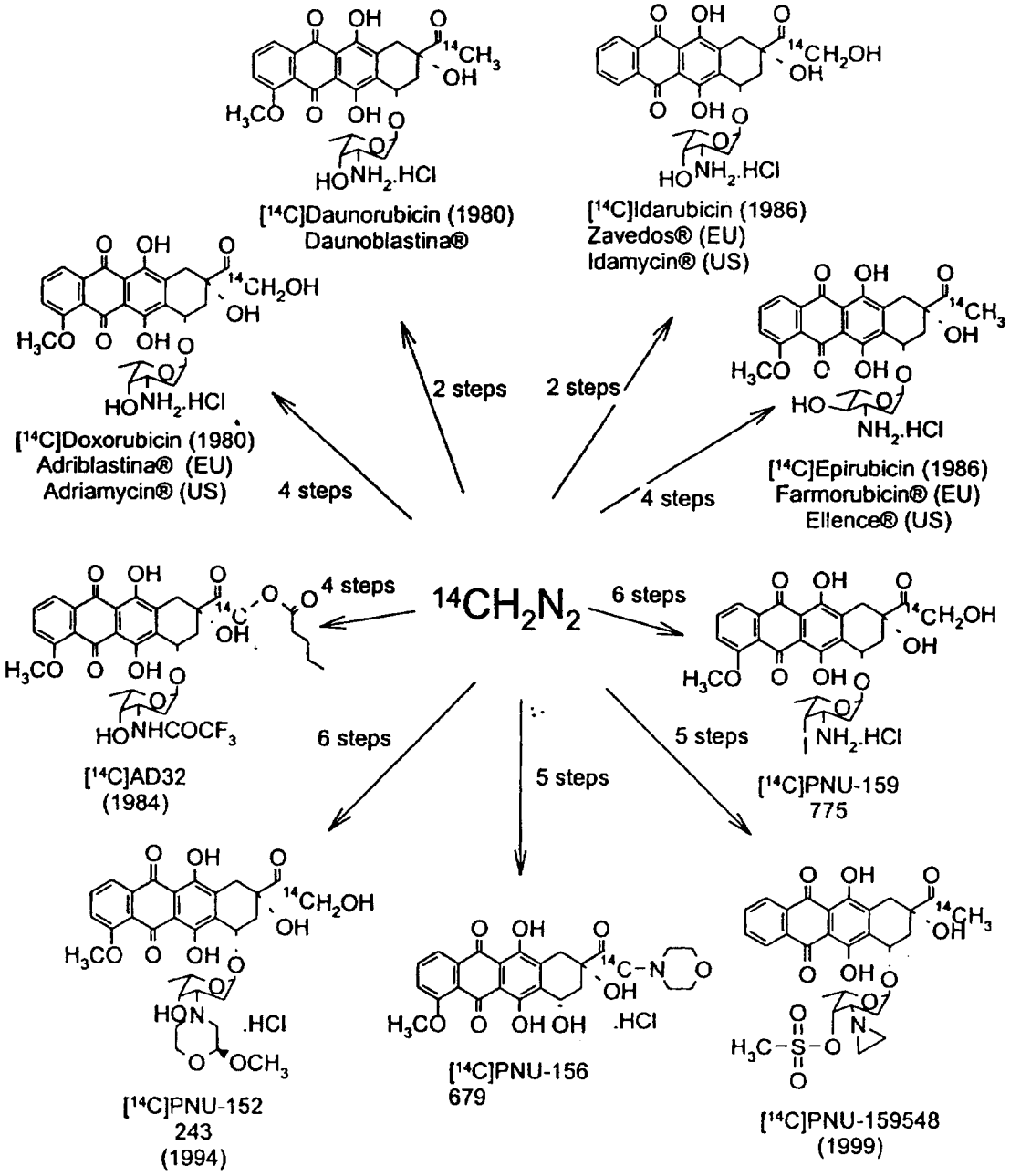
[^{14}C]Diazomethane was generated from N-nitroso-N-[^{14}C]methylurea, obtained from methylamine hydrochloride, in an aprotic organic solvent such as methylene chloride and/or diethyl ether at room temperature for 1 hour. The introduction of a ^{14}C in the C-14 position of the anthracycline skeleton was accomplished by reacting [^{14}C]diazomethane with a suitable N-trifluoroacetyl protected aldehyde precursor in methylene chloride at room temperature for 1 hour [1]. The removal of the protecting group was obtained by mild alkaline treatment (aqueous 0.1 N sodium hydroxide) of the N-trifluoroacetyl derivatives at 0-5 °C for 30-60 minutes. When needed, the dauno-derivatives were converted to the corresponding hydrochloride salts by adding methanolic 0.01N HCl. According to this approach, [^{14}C]daunorubicin 1 [1] and [^{14}C]idarubicin 2 [3] were prepared and used also as precursors for the preparation of compounds of the same chemical class. The conversion of daunorubicin derivatives into doxorubicin derivatives was accomplished by reaction with bromine in mixtures of methanol and dioxane at 8°C followed by treatment with an aqueous solution of sodium formate at room temperature. This synthetic pathway was followed to prepare [^{14}C]doxorubicin and [^{14}C]epirubicin in an overall radiochemical yield of 10-15% from [^{14}C]diazomethane[1]. [^{14}C]AD32 was synthesized starting from the conversion of 1 into the corresponding 14-bromoderivative, which was N-trifluoroacetylated and subsequently submitted to nucleophilic substitution to give the final product in an overall radiochemical

yield of 24% from 1 [4]. The synthesis of [^{14}C]PNU-159775 was carried out starting from 4-epi-N-trifluoroacetyl- [^{14}C]daunorubicin 3 in five steps. The substitution of C-4'-OH with iodine was performed with sodium iodide in acetone. The subsequent hydroxylation of the obtained 4'-iodo-4'-[^{14}C]deoxydaunorubicin side chain was accomplished *via* the 14-bromoderivative. Finally, [^{14}C]PNU-159775 was obtained in an overall radiochemical yield of 20% from 3 [5]. [^{14}C]PNU-152243 was prepared by reacting 1 with an excess of the diiododerivative (2S)-1,5-diiodo-2-methoxy-3-oxapentane in dimethylformamide in the presence of triethylamine. After stirring at room temperature in the dark for 48 hours, the corresponding [^{14}C]daunoderivative was recovered as a free base and then converted to the hydrochloride salt form by adding 0.22 N anhydrous HCl in methanol. The subsequent three-step hydroxylation of the intermediate side chain was carried out according to the already described procedure for the conversion of dauno-derivatives into doxo-derivatives. [^{14}C]PNU-152243 was obtained in an overall radiochemical yield of 24% from 1 [6,7]. The synthesis of [^{14}C]PNU-156679 was performed in five steps starting from 1. The hydrolysis of 1 in 0.2 N HCl at 100°C gave [^{14}C]daunomycinone which, after treatment with bromine in 1,4-dioxane at room temperature, yielded the corresponding [^{14}C]bromoderivative. The final product was then obtained as a free base by reaction with morpholine in methylene chloride at room temperature. The addition of methanolic HCl gave the corresponding hydrochloride. The overall radiochemical yield was 37% from 1. [^{14}C]PNU-159548 was prepared starting from the reaction of 2 with 2-bromoethanol (30 molar excess) in methylene chloride in the presence of N,N-diisopropylethylamine (DIEA). The obtained 4-demethoxy-3'-N-(2-hydroxyehtyl)-[^{14}C]daunorubicin was converted to the corresponding dimethylsulfonyl derivative by reacting with methanesulfonyl chloride in anhydrous pyridine. The aziridinyl ring closure was achieved by stirring in acetone in the presence of DIEA. [^{14}C]PNU-159548 was obtained in an overall radiochemical yield of 27% from 2 [8].

References:

1. Vicario GP, Penco S, Arcamone F. *U.S. Patent* 4, 211, 864, July 8, 1980.
2. Elsinga PH. *The Journal of Nuclear Medicine* 1996; **37** (9): 1571-75.
3. Zini G, Vicario GP, Lazzati M, Arcamone F. *Cancer Chemother Pharmacol* 1986; **16**: 107-115.
4. Zini G, Vicario GP, Lazzati M, Arcamone F. *Cancer Chemother Pharmacol* 1984; **12**: 154-156.
5. Fontana E, Suarato A, Caruso M, Vicario GP. *Journal of Labelled Compounds and Radiopharmaceuticals* 1988; **25** (10); 1129-1134.
6. Arcamone F, Lazzati M, Vicario GP, Zini G. *Cancer Chemother Pharmacol.* 1984; **12**: 157.
7. Fontana E, Giribone D, Bargiotti A, Faiardi D, Suarato A, Dostert P. *Synthesis and Applications of Isotopically Labelled Compounds* 1994; 719-722.
8. Felicini C, Fontana E. *Journal of Labelled Compounds and Radiopharmaceuticals*, 2002; **45** (7): 543-550.

Figure 1



Preparative HPLC: a Useful Approach to Speed-up the Purification of Labelled Compounds.

A. Pignatti* and E. Fontana
Global Drug Metabolism Dept., Pharmacia
viale Pasteur 10, 20014 Nerviano (MI), Italy
alberto.pignatti@pharmacia.com

Introduction:

The purification of reaction products or the separation of mixtures is recognized as a non trivial as well as a time-consuming part of a chemist's everyday work. Due to different factors including the required high purity of the final compounds, low amounts of available material etc., this step is often particularly challenging in radiochemistry. Moreover, as usually radiolabelled compounds are used during periods of months, degradations can occur and a purification could avoid expensive re-synthesis of additional batches starting from the beginning. Therefore it is not unusual that the development of a suitable purification method could require more effort than the chemical reaction set-up. In our experience, preparative HPLC was found to be the most effective and versatile method compared to other purification techniques such as flash-chromatography, crystallization, preparative-TLC etc. Due to time-constraints and the variety of chemical classes to be purified, our goal was the development of a simple reverse phase-HPLC procedure of purification applicable to the widest range of compounds with different chemical and physical-chemical characteristics (e.g. presence of acidic and/or basic functional groups, polarity, solubility etc.). In this paper we report the approach used in our labs, when the purification of a radiolabelled compound by preparative-HPLC is required.

Methods and results:

Preparative HPLC separations were performed using a Varian PrepStar SD-1 binary high-pressure gradient system (flow rate range 0.01-200 ml/min) equipped with a Varian Prostar 320 UV-Vis detector with a dual pathlength flowcell (4 mm/0.15 mm), a Varian Prostar 701 fraction collector and a Rheodyne preparative valve mod. 3725i with a 10 ml stainless steel loop. The HPLC system is controlled through the PrepStar software package.

Raw products were dissolved in the HPLC mobile phase as first choice. In case of solubility problems, dimethyl sulfoxide was a valid alternative, which did usually not affect the chromatographic separation.

Before setting up the preparative HPLC system, the HPLC conditions were optimized by using an analytical column with the same packing of the preparative column to be used to purify the compound. Therefore a direct scale-up could be applied to predict the loading capacity as well as the flow rate of the preparative column using the well-known scale-up conversion factors. A good separation of most of the mixtures was achieved by using Xterra MS18 5μ 30-50mm ID x 100mm from Waters, Symmetryprep C18/C8 5μ 19mm ID x 100-150mm from Waters and Zorbax SB C18 5μ 21.2mm ID x 150mm from Agilent. A scouting gradient run using

acetonitrile:water: trifluoroacetic acid (5:95:0.1 v/v) as mobile phase A, acetonitrile:water: trifluoroacetic acid (95:5:0.1 v/v) as mobile phase B and a linear gradient from 100% A to 0%A over 30 minutes was found to be successful for an easy and quick development of the most convenient elution condition.

The pure compound recovery from the collected HPLC fractions was usually performed by mobile phase evaporation or liquid/liquid extraction. According to the characteristics of the compound, an alternative easy procedure to remove the salts from eluate was applied as following described. After acetonitrile removal from eluate by evaporation, the remaining aqueous solution was loaded on a LC system (e.g. column: Kromasil KR100 C18 10 mm 10x250 mm; flow rate: 5 ml/min), the column was washed with 100% water for about 15 minutes and the eluate discarded. The column was then washed with 100% acetonirile up to compound elution and, after solvent evaporation , the pure compound was recovered.

Conclusions:

The use of preparative HPLC increased considerably the productivity of the purification steps during the preparation of labelled compounds. The standard protocol developed in our labs was successfully applied to more than 90% of the mixtures to purify. Moreover, the yield and purity achieved were usually higher than those ones obtained by using other traditional techniques such as flash-chromatography, crystallization, preparative-TLC etc. The purification average time from sample preparation to final work-up was about one day.

Use of Stable Isotopes for the Biosynthesis and Biodegradation of Fosfomycin

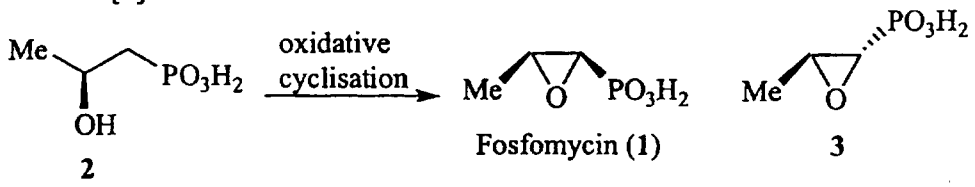
J. W. McGrath[1], B. Peric Simov[2], W. Preusser[2], J. P. Quinn[1], S. Schmidt[2], A. Woschek[2], F. Wuggenig[2], **F. Hammerschmidt** *[2]

[1]*School of Biology and Biochemistry, The Queen's University of Belfast,*
[2]*Institut für Organische Chemie der Universität Wien.*

friedrich.hammerschmidt@univie.ac.at

Fosfomycin **1** is a clinical antibiotic interfering with cell wall biosynthesis. It is produced by various strains of *Streptomyces* and bacteria and is one of the natural products containing a P-C bond [1].

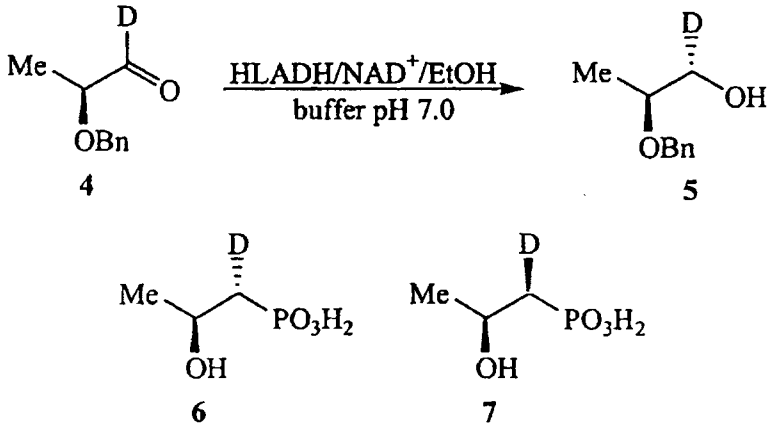
The P-C bond is formed by the phosphomutase-catalysed rearrangement of phosphoenol-pyruvate. The phosphonopyruvic acid formed is decarboxylated and methylated to give (S)-2-hydroxypropylphosphonic acid **2**. Oxidative cyclisation of **2** yields fosfomycin (Scheme 1). The oxygen atom of the oxirane ring is not derived from dioxygen, but the hydroxyl group of **2** as proven by use of [hydroxy-^{18}O]-**2** as precursor [2]. Recently, **3** was isolated a co-metabolite of **1** [3].



2 Fosfomycin (1) **3**

Scheme 1

We have investigated the stereochemistry of the formation of the C-1-O bond using deuterium labelled phosphonic acids **6** and **7**. They were prepared from chirally deuteriated 2-benzyl-oxypropanol **5** obtained by HLADH-catalysed reduction of the corresponding aldehyde **4** (Scheme 2).

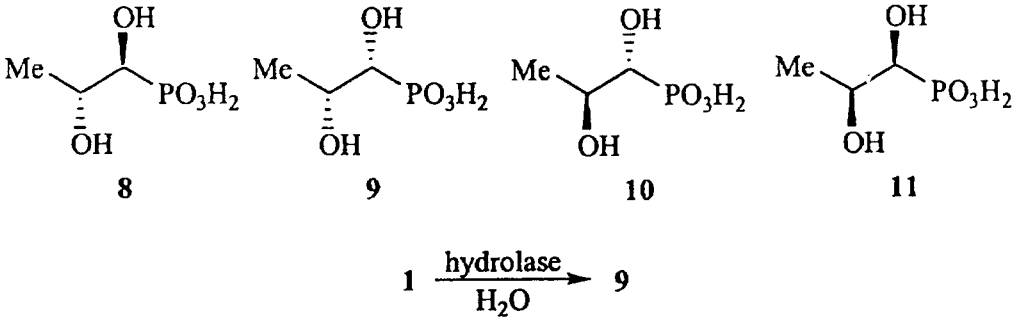


4 **5** **6** **7**

Scheme 2

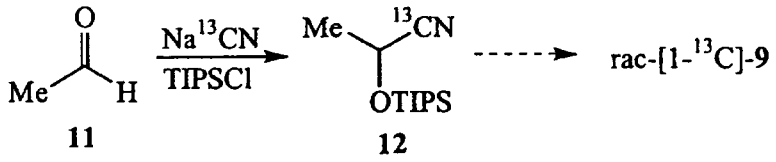
When 2-hydroxypropylphosphonic acids **6** and **7** were incorporated into **1** and **3** in *Streptomyces fradiae*, only the deuterium of **6** was retained. The C-1-O bond of fosfomycin is formed with inversion, that of **3** with retention of configuration. We assume that an intermediate radical is generated which accounts for the formation of fosfomycin (97%) and its *trans*-isomer **3** (3%).

Fosfomycin is degraded by *Rhizobium huakuii* PMY1 [4]. The antibiotic is used as carbon and phosphorus sources with accompanying P_i release. All four 1,2-dihydroxypropylphosphonic acids (**8-11**) were prepared and used as precursors for biodegradation studies. Only **9** was degraded thus demonstrating that the first step of the biodegradation of fosfomycin is the opening of the oxirane ring with inversion of configuration at C-2 (Scheme 3).



Scheme 3

To study the further degradation of **9**, its carbon-13 labelled isotopomer was prepared using commercially available sodium [^{13}C]cyanide as cabon-13 source (Scheme 4).



Scheme 4

Acknowledgments:

We are grateful to the Fonds zur Förderung der wissenschaftlichen Forschung (project no. P13017-CHE and 6537C) for financial support.

References:

1 Seto H, Kuzuyama T. *Nat Prod Rep* 1999; **16**: 589-596.
2 Hammerschmidt F, *J Chem So. Perkin Trans 1* 1991; 1993-1996.
3 Peric Simov B, Wuggenig F, Lämmerhofer M, Lindner W, Zarbl E, Hammerschmidt F. *Eur. J. Org. Chem.* 2002, 1139-1142.
4 McGrath JW, Hammerschmidt F, Quinn JP. *Appl Environ Microbiol* 1998; **64**: 356-358.

Metalloradiopharmaceuticals for Nuclear Medicine

H. Spies
Forschungszentrum Rossendorf, PF 510 119, 01314 Dresden, Germany
H.Spies@fz-rossendorf.de

Radiopharmaceuticals are drugs containing a radionuclide and are used routinely in nuclear medicine for the diagnosis or therapy of various diseases. They are mostly small organic or inorganic compounds, but can also be macromolecules such as monoclonal antibodies or their fragments labeled with a radionuclide. Those agents whose biodistribution is determined by biological interaction, e. g. receptor binding, are termed target-specific radiopharmaceuticals.

For **diagnostic imaging agents**, technetium-99m is frequently the radionuclide of choice because it has optimal nuclide properties (half-life of 6 h and appropriate γ-energy of 140 keV). The inconvenience of purchasing a short-lived radionuclide was overcome by the development of the $^{99}Mo/^{99m}Tc$ generator, which is based on transient equilibrium between the parent radionuclide ^{99}Mo (66 h half-life) and the daughter radionuclide ^{99m}Tc (6 h half-life). The generator makes this radionuclide both routinely available and economical. Finally, the ^{99m}Tc-radiopharmaceutical can easily be prepared in the clinic by means of prefabricated kits. So it is no surprise that more than 90 % of routine *in vivo* imaging is performed with technetium-99m [1, 2].

Table 1. Metal nuclides for diagnostic or therapeutic application

diagnostic nuclides			**therapeutic nuclides**		
Nuclide	**$t_{1/2}$ [h]**	**mode of decay**	**nuclide**	**$t_{1/2}$ [h]**	**Mode of decay**
Tc-99m	6	γ	Sr-89	1212	β
In-111	67.9	γ	Y-90	64.8	β
Cu-67	62	γ	Re-186	91.2	β
			Re-188	16.8	β
Cu-64	12	β^+	At-211	7.2	α
Ga-66	9.5	β^+	Bi-212	1.0	α
Y-86	14.7	β^+	Bi-213	0.76	α

Beside technetium, many other radiometals have been or are being investigated for their uses in nuclear medicine [3]. This comprises not only radiometal-labeled agents used in gamma scintigraphy and positron emission tomography (PET), but also beta and alpha emitters for radionuclide therapy [4] (Table 1).
The development of effective metalloradiopharmaceuticals is a complex task which is not simply accomplished by attaching a radionuclide to a nonradiolabeled targeting vector (Fig. 1).

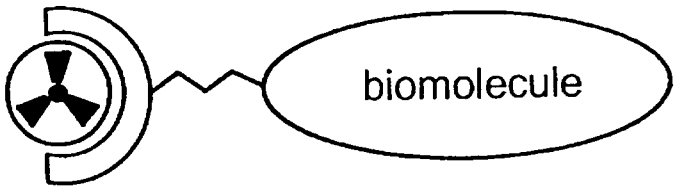


Fig.1. Schematic representation of the bifunctional approach as a strategy of radiopharmaceutical design

Because of the unphysiological nature of the radiometal, the structure of the biomolecule where the chelate is appended, will be altered and the biodistribution will change drastically. The search for metalloradiopharmaceuticals involves therefore chelate systems that stable bind the radiometal, and, since it will be directly involved in optimizing the biochemical properties of the radiopharmaceutical, it has to be compatible in structure and physicochemical properties. In recent years, **new chelate systems** have been developed (Fig. 2) for binding technetium (as well its congener rhenium) at oxidation states V, III, and I involving metallnitrido cores **1** [5], mixed-ligand complexes **2** [6] and metal-tricarbonyl derived complexes **3** [7, 8].

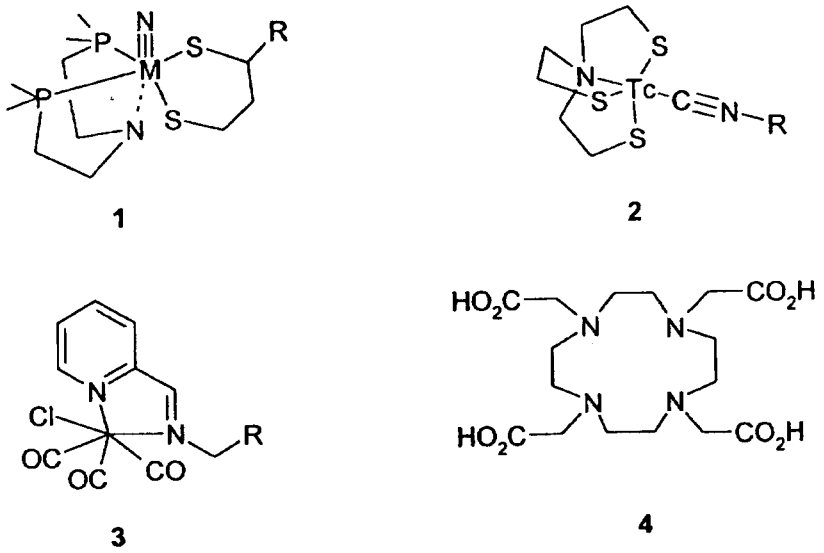
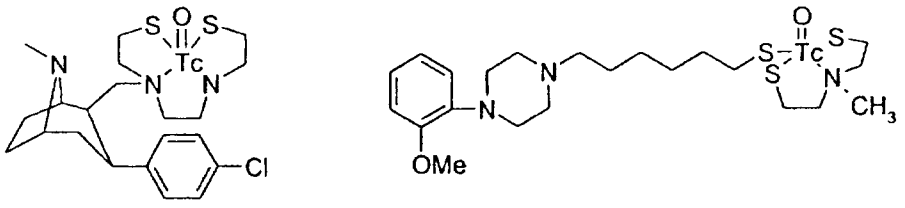


Fig.2. Some chelating systems for stable binding of metal nuclides

A variety of cyclic tetraaza-ligands is used for more "hard" metals, e.g. indium-111, yttrium-90, or lutetium-177. In particular, 1,4,7,10-tetraazacyclododecane-N,N',N",N"'-tetraacetic acid (DOTA, **4**) [9] has been shown to form complexes with high in vivo stability.
Beside metal binding the most important aspect is the transport into and the accumulation in the target. The considerable interest in **imaging CNS receptors** led to [^{99m}Tc]TRODAT-1 for imaging the dopamine transporter in the brain [10]. More recently, a series of Tc-complexes with subnanomolar affinites to the serotonin 5 HT_{1A} receptor [11] have been designed (a representative **6** is shown in Fig. 3).



5 6

Fig. 3: Receptor-affine Tc-tracers [^{99m}Tc]TRODAT-1 **5** and **6**

Challenges are also evident in the field of **peptide labeling** [12 - 14]. Bioactive peptides such as peptide hormones, immunomodulators and growth promotors, have been found to modulate a wide variety of biological functions. The development of the somatostatin analogues ^{111}In-DTPA-Octrotide (Octreoscan®) and ^{99m}Tc-P829 (NeoTect®) for tumor imaging illustrates how basic science advances are translated into health care [12].

Therapeutic radiopharmaceuticals [4] are radiolabeled molecules designed to deliver therapeutic doses of ionizing radiation to specific disease sites (most often cancerous tumors). The design of each radiotherapeutic agent requires optimizing the balance between specific in vivo targeting of the disease site and the clearance of radioactivity from nontarget radiosensitive tissues. Because of the requirements that have to be fulfilled by radiotherapeuticals (high effective dose for the target but practically no interference with healthy tissue, fast excretion of non-target activity) the number of therapeutic radiopharmaceuticals for routine use and available on a commercial base is limited. Present radiopharmaceutical research aims at to overcoming the problems in order to exploit the whole therapeutic potential of available metalloradionuclides.

References:

1. Jurisson SS, Lydon JD. *Chem. Rev.* 1999; **99**: 2205-2218.
2. Johannsen B, Spies H. *Topics. Curr. Chem.* 1996; **176**: 77-121.
3. Anderson CJ, Welch MJ. *Chem. Rev.* 1999; **99**: 2219-2234.
4. Volkert W A, Hoffmann TJ. *Chem. Rev.* 1999; **99**: 2269-2292.
5. Bolzati C, Boschi A, Duatti A, Prakash S, Uccelli L, Refosco F, Tiasto F, Bandoli G.*J. Am. Chem. Soc.* 2000; **122**: 4510-4511.
6. Pietzsch HJ, Gupta A, Syhre S, Leibnitz P, Spies S. *Bioconjugate Chem.* 2001; **12**: 538-544.
7. Alberto R, Schibli R, Angst D, Schubiger PA, Abram U, Abram S, Kaden TA. *Trans. Met. Chem.* 1997; **22**: 597-601.
8. Metzler-Nolte N, *Angew. Chem.* 2001;**113**: 1072-1076.
9. Li M, Meares CF, Zhong GR, Miers L, Xiong CY, DeNardo SJ. *Bioconjugate Chem.* 1994; **5**: 101.
10. Kung HF. *Nucl. Med. Biol.* 2001 **28**: 505-508.
11. Johannsen B, Pietzsch HJ. *Eur. J. Nucl. Med.* **29**: 263-275.
12. Liu S, Edwards DS. *Topics Curr. Chem.* 2002; **222**: 259-285.
13. Langer M, Beck-Sickinger AG. *Curr. Med. Chem.* 2001; **1**: 71-93.
14. Liu S, Edwards DS. *Chem. Rev.* 1999; **99**: 2235-2268.
15. Johannsen B, Spies H. *Transition Met. Chem.* 1997; **22**: 318-320.
16. Reubi JC. *J. Nucl. Med.* 1997; **41**: 63-70.
17 Okarvi SM. *Eur. Nucl. Med.* 2001; **28**: 929-938.

Strategic outlook: Future methods in DMPK and subsequent requirements for isotopically labelled compounds

Jochen Maas*, Volker Krone
Aventis Pharma, Germany
Jochen.Maas@aventis.com

The classical activities of DMPK such as

- Mass balance in animals and humans incl. blood/plasma levels,
- Clarifying the routes of elimination
- Metabolite identification, isolation and structural determination from body fluids like blood, plasma, urine and bile (feces)
- Tissue distribution in animals incl. diaplacental transfer and excretion via milk
- Protein binding, binding to special cells, enterohepatic circulation etc.

are largely based on the synthesis of radiolabelled material. Although the sensitivity of methods using radioactivity is virtually reached also by other approaches (e.g. LC-MS/MS), there seems to be no general alternative in the near future. The essential prerequisites for radiolabelled compounds are the appropriate position of the label, the biological stability, the radiochemical purity and the radiochemical stability (at least for the time interval until the DMPK studies can start).

With increasing molecular weight of future drugs, the solubility will more often reach the limit of the biocompatibility of the solutions to be administered. Thus, the tendency to use suspensions might increase. Already today much more attention has to be paid to the solid phase properties of the radiolabelled material, such as crystal morphology, particle size and specific surface.

The classical advantage of the parallel detection of parent compounds and all (labelled) metabolites cannot be fulfilled by any other approach. Additionally, a tendency to obtain as many results as possible from a small number of radiokinetic studies is clearly visible: We sometimes use one study in parallel for the investigation of the mass balance, the excretion routes, the blood plasma profile (of radioactivity and parent compound), the metabolite profiling, the identification, isolation and structural determination of the metabolites. Such intraindividual approaches make interpretation easier and omit additional studies with scattering results (besides saving of resources and ethical questions of additional animal and human studies).

In summary, studies with specifically and unspecifically labelled radioactive material will be performed also in the future and used for the both *in vitro* as well as *in vivo*-investigations.
For selected purposes like mass balance studies in humans, alternative approaches are available: as an example, A(ccelerated) M(ass) S(pektrometry) could provide data similar to those obtained by a conventional hADME-study but due to the technical device required, costs and some practical details, most companies will prefer to continue with the classical approach for routine studies. Exceptions could be, for example, compounds with a demonstrated

melanin binding, which require a considerably lower radioactivity burden for the volunteers, as well as a possible application in early hADME studies. The last example demonstrates that even potential alternatives may require radiolabelled compounds – but considerably lower amounts.

A relatively new approach in DMPK are *early* ADME-activities: Data regarding absorption, distribution, metabolism and elimination of compounds have often to be present at very early stages of development to be used as selection criteria for the further development of chemical scaffolds. In the past, these activities had to be performed using non-radiolabelled material since at this stage of development radiolabelled compounds were not available. Based on experiences of the last two to three years, many companies started "*early labelling*" initiatives to support the eADME work. For these purposes it has to be discussed whether ^{3}H or ^{14}C is the label of choice, both have their pros and cons. The decision has to be made case-by-case. Thus, some issues, which would have required several "cold" studies for clarification, can now be solved in one study using radiolabelled compounds.

The requirements on radiolabelled compounds differ significantly in early and late (registration) studies: In early studies, the focus is clearly on the rapid availability and low costs (as far as possible) of numerous compounds. Documentation, radiochemical purity, excellent radiochemical stability etc. are of minor interest. In later studies, when timelines are more comfortable, quality requirements are considerably higher.

On the other hand, isotopically labelled compounds does not automatically mean radiolabelled! For several years and due to the increasing requirements of regulatory authorities there is a growing demand for stable isotope labelling for competitor technologies such as LC-MS/MS: Stable isotopes have proved to be absolutely essential for the analysis of biological samples using this technology since it only isotopically labelled compounds are ionised in the same manner as the drug to be detected – independent from the matrix.
Currently, there is no other technology visible yet which may replace the LC-MS/MS technology or will render the isotopically labelled "internal standard"-methodology unnecessary.

Isotopic Imaging of Glass-based cDNA Microarray Hybridization

Frank Diehl[2], Johannes Coy[3], Jörg Hoheisel[2], Magnus von Knebel Doeberitz[1], **Matthias Nees[1*]**

[1]Department of Molecular Pathology, University of Heidelberg, Im Neuenheimer Feld 220/221, 69120 Heidelberg, Germany; [2]Department of Funktional Genome Analysis, German Cancer Research Center; Im Neuenheimer Feld 506, 69120 Heidelberg, Germany, [3]MTM Laboratories, Im Neuenheimer Feld 519, 69120 Heidelberg, Germany.
neesmatthias@yahoo.com

Abstract

Microarrays allow the analysis of expression of thousands of genes in parallel. To generate cDNA microarrays, DNA fragments or oligonucleotides that represent particular genes are immobilized on glass or other support materials. Analysis of gene expression by microarrays is performed by hybridisation of cDNA probes that are generated by reverse transcription of mRNA, which is isolated from the cells or tissues of interest. Frequently, two probes labelled with different fluorescent dyes are hybridised in parallel, allowing direct comparison of differential gene expression between the samples. However, it is also possible to label cDNAs using isotopic methods, which in fact has been used for a number of years for filter array hybridisations on nylon membranes. Isotopic imaging has so far not been used for glass microarrays. Individual genes, that show high ratios of differential expression between two cell populations, can be readily identified and analysed in detail. To identify and confirm differential expression of genes involved in the development and progression of colorectal cancer, an epithelia-specific cDNA microarray was designed that represents 3500 human genes, covered by 5275 individual cDNA fragments. Gene expression profiles of cancers from 22 patients were compared with matching normal colorectal mucosa, using a combination of fluorescent and isotopic labelling methods. Radioactive labelling was used for RNA isolated from microdissected tissue. Microdissection of tumor cells allows the isolation of very small amounts, but highly enriched tumor cell RNA. Marked differences in data generated by isotopic and fluorescent detection methods were observed, based primarily on the higher sensitivity and the increased dynamic range of radioactive microarray hybridisations and detection methods (MicroImager, Biospace Inc., France). A subset of genes expressed at low as well as very high levels could be identified exclusively using isotopic detection, and were elusive using standard fluorescent detection methods. Semiquantitative reverse transcription-PCR (RT-PCR) was used to confirm differential expression for >50 genes. Our set of data provides an additional source of potential candidate genes for early detection of colorectal cancer, disease monitoring, and possible novel targets for cancer immunotherapy.

Technical Aspects

In combination with tissue microdissection, we have used a highly sensitive radioactive detection method (MicroImager; Biospace Inc., France) to increase the frequently restricting sensitivity of cDNA microarray hybridisations, in particular in clinical research. This method

allows the direct use of small amounts of total RNA (0.1 – 1.0 μg) for microarray hybridisation, which is in a range that can be routinely extracted from small clinical tissue samples. Efficient labelling of mRNA/cDNA for hybridisation is the most critical step in the microarray technology. The fluorescent dyes commonly used for labelling are expensive, and dye incorporation rates are exceedingly low. For this reason, the amount of RNA necessary for the generation of strong and reproducible hybridisation signals on microarrays is usually very high. The standard dual labelling methods require 1-2 micrograms of purified mRNA or 30-50 micrograms of total RNA. This usually exceeds the amounts of RNA that can be routinely obtained from patient samples in clinical practice. Therefore, two general possibilities to increase microarray sensitivity are frequently used: amplification of the hybridisation signal or linear RNA amplification to increase the amount of starting material available. Usually, several series of linear amplification have to be performed to obtain sufficient and measurable amounts of RNA that can be used for fluorescent labelling. With each cycle of amplification, the risk to generate artefacts due to non-linear amplification increases. Furthermore, amplification is a tedious and time consuming process.

As a possible solution for these common technical difficulties and limitations, we investigated the possibility of isotopic labelling and detection methods for microarray hybridisations. Radioactive hybridisation on glass cDNA microarrays combines the general advantages of radioactivity (very high sensitivity, broad dynamic range) with the advantages of chemically modified glass surfaces (smooth surface, low background) as the support material of choice. The use of the Biospace MicroImager allows the detection of disintegrations on glass microarrays with very high accuracy and resolution, low non-specific background and excellent sensitivity. The use of the low-energy beta emitters ^{33}P and/or ^{3}H combines good incorporation rates of isotopically labelled nucleotides with moderate exposure times and acceptable scattering of signals on the MicroImager. Competitive parallel co-hybridisations of ^{33}P and ^{3}H -labelled probes on one array can also be measured using the MicroImager, based on the clear distinction of the characteristic emission energy for each isotope. For the sensitive detection of disintegrations, ordinary photographic emulsions, phosphor imager screens or other reader devices proved to be insufficient and did usually not provide the high resolution of 5-10 μm necessary for microarrays with spot sizes ranging between 50 – 200 μM. Using an ultra thin scintillation foil (grain size 3 μm) and a linear amplification device that multiplies the photons generated by scintillation prior to recording on a CCD camera, the measuring of disintegrations in real-time is possible with very high sensitivity on the MicroImager. The minimum amount of starting material necessary to generate good microarray hybridisations and high quality expression data is in the range of 0.1 – 0.2 μg total RNA. This typically requires exposure times between 8 and 16h. Therefore, with the application of isotopic labelling and detection methods, we were able to reduce the minimal starting amount of total RNA that is necessary to generate reproducible microarray hybridisations by a factor of 200, compared to standard fluorescent labelling techniques. By using quantitative realtime RT-PCR, we were also able to successfully validate most of the expression data. This indicates the reliability of the expression patterns generated by radioactive hybridisation on microarrays. This technology represents an attractive alternative to commonly used RNA amplification methods and allows the direct and straightforward use of minute amounts of RNA for routine microarray applications.

New Developments in Radiation Measuring Technique

Alfred Klett
Berthold Technologies GmbH & Co KG,
P.O. Box 100163, D-75312 Bad Wildbad, Germany
Alfred.Klett@BertholdTech.com

Berthold Technologies is a well-known manufacturer of radiation protection instrumentation for many years. The regulations in radiation protection have been widely changed and harmonized in Europe since a couple of years. One consequence was the definition of new operational quantities as for instance in dose rate monitoring the ambient dose equivalent H*(10). There were even more changes which require new probes as well as new instruments for applications in radiation protection. Besides of this the availability of innovative technologies has enforced the development of new instruments for new applications. This is for instance true in the field of handheld gamma spectroscopy with automatic identification of radionuclides. There are also other issues like illicit trafficking radioactive sources or illegal transfer of nuclear materials, which need special detection techniques.

Berthold offers a wide variety of dose rate monitors for both, gamma and neutron radiation fields. Together with the nuclear research center Karlsruhe the neutron monitor LB 6411 has recently been developed for neutron dose rate monitoring. This detector has superior characteristics to measure the Ambient Dose Equivalent H*(10) for neutrons. It's sensitivity is extremely high and the energy dependend response matches the new requirements quite perfectly. Currently there are also new gamma dose rate probes being designed to measure the new quantities. These probes cover a wide range of dose rate levels.

In contamination monitoring Berthold offers the new handheld contamination monitor LB124. This monitor is based on proven detector technology with Xenon-filled proportional counters. The instrument is ideally suited for the measurement of photon emitting nuclides as widely used in nuclear medicine. It has a low weight and the user interface is easy-to-be-used. The software offers many useful functions, tools and access to all parameters for experienced users. For unskilled users the instrument's configuration can be predefined by a supervisor as a simple or even as an extremely simple system depending on the special needs on site. Calibration according to ISO 7503-1 is provided for most common nuclides including the new PET nuclides.

One of the most interesting new products is the hand-held gamma spectrometer LB125. The portable battery operated instrument for the laboratory and for the field is ideal to search, localize, identify and measure radioactive materials. The software provides intelligent analysis and visualization of the measured data and even users which are not nuclear experts can achieve fast and reliable isotope identification. The radiation is detected by NaI scintillator, and pulse height analysis is done with a fast multi-channel analyzer. Applications of this monitor are mainly in nuclear industry, nuclear decommissioning, nuclear waste, steel and scrap, customs, police and military, especially upon the occurrence of unidentified radiation sources. Several examples of measured gamma spectra are shown.

Several cases of illegal plutonium were reported. Most spectacular was the recent case in Karlsruhe, where plutonium was stolen by an employee. Plutonium is extremely dangerous and hard to detect over larger distances. The alpha-particles, gammas and X-rays which are emitted by the plutonium isotopes can easily be shielded by the material itself or by the surroundings. Therefore we designed a new type of instrument to search for plutonium. It is based on the detection of fission neutrons. Neutrons are generated by spontaneous fission of the even numbered plutonium isotopes. These neutrons are very penetrating and the method is therefore very effective. The detection of 10g reactor plutonium at a distance of 1m is feasible within only 10 seconds.

