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10th International Symposium on the Synthesis and Applications of Isotopes and Isotopically Labelled Compounds—Synthesis of Compounds Labelled with Long-lived Isotopes

Session 1: Monday, June 15, 2009

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Abstract: This session outlined methods useful for the synthesis of intermediates labeled with carbon-13, carbon-14 or tritium. Additional papers described the utilization of carbon-14 and tritium intermediates in the synthesis of labelled compounds for use in the development of pharmaceutical and agricultural agents.

Keywords: ^{13}C; ^{14}C; Homogeneous metal-catalyzed exchange; tritium; vicarious nucleophilic substitution; electrophilic cyanating reagent; labelling methods

HOMOGENEOUS METAL CATALYSED EXCHANGE LABELLING OF PHARMACEUTICALS WITH TRITIATED WATER

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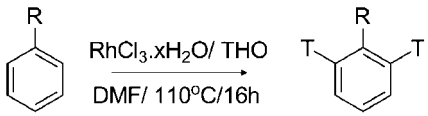
Abstract: Low specific activity tritiated water in conjunction with homogeneous catalysts of rhodium, ruthenium and iridium has been extensively used by the Radiochemistry group at Schering-Plough to rapidly prepare ^{3}H labelled compounds for preliminary drug metabolism work. Such studies are used to aid in the selection of drug candidates for further evaluation and development. Through several examples, the paper will discuss the use of homogeneous metal catalysed exchange with tritiated water to label a wide range of pharmaceutical structures.

Keywords: tritium; rhodium trichloride; tris-triphenylphosphine ruthenium (II) Chloride

Introduction: Tritium labelled compounds are extensively prepared by the Radiochemistry group at Schering-Plough for exploratory metabolism work. A wide range of labelling strategies with both tritium gas and tritiated water are used by the group. Of these methods, homogeneous metal catalysed exchange with tritiated water has proven to be one of the more widely applied techniques, with two catalyst groups in particular used the most frequently. Rhodium trichloride trihydrate in conjunction with tritiated water is well known to catalyze ortho hydrogen isotope exchange in aromatic acids, amides, aralkylamines and acetanilides (Figure 1).[1]



R = CO_2H, $CONH_2$, CH_2NH_2, $NHCOCH_3$

Figure 1. $RhCl_3$ H_2O catalyzed exchange with tritiated water.

Over more recent years, $RuAcAc_3$, IrCOD(AcAc) and Ir $COD(F_6AcAc)$ have been developed as an improvement to the rhodium trichloride system.[2–4] Tris-triphenylphosphine ruthenium (II) chloride, while able to promote ortho exchange in aromatic acids, has found an application as a promoter of hydrogen isotope exchange in alcohols and is particularly useful in amine systems (Figure 2).[5,6]

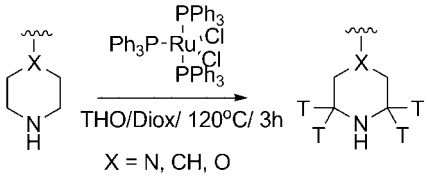


Figure 2. $Ru(Ph_3P)_3Cl_2$ catalyzed exchange with tritiated water.

The late Professor J.R. Jones, to whom this paper is dedicated, spent many years researching this field. As a tribute to John, we would like to illustrate though a series of examples how this chemistry has proved to be an invaluable tool in the preparation of ^3H-labelled pharmaceuticals.

Results and Discussion: The first two examples SCH A and SCH B (Figure 3) involved the use of rhodium trichloride hydrate to label α-ethylbenzylamine and p-chlorobenzylamine as precursors.

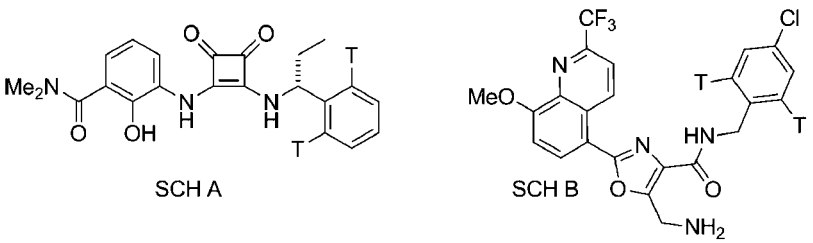


Figure 3. [^{3}H]SCH A and [^{3}H]SCH B.

The resulting ^3H labelled amines, which were purified by a simple extractive work-up and required no additional purification, were then converted to the target compounds using the established medicinal chemistry routes. ^3H NMR analysis showed the expected sites of tritium incorporation.

SCH C (Figure 4) was labelled by rhodium trichloride hydrate with tritiated water on the free pyridine, followed by a rather laborious oxidation process to generate the pyridine N-Oxide.

In the case of SCH D (Figure 4), after a pilot experiment with $Ir(COD)dppePF_6^7$ and deuterium gas failed, presumably due to cyanide poisoning of the catalyst, the acid intermediate was labelled using $RuAcAc_3$ and tritiated water, which had been previously shown to be a more effective promoter of exchange in aromatic acids than the rhodium trichloride system.[2] The isolated acid was converted to the desired product in two additional steps.

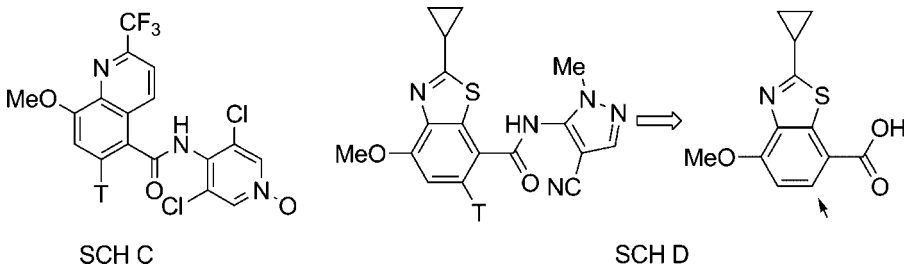


Figure 4. [^{3}H]SCH C and [^{3}H]SCH D.

Initial attempts to label SCH E involved Pt catalysed exchange with tritiated water, which gave a very poor yield. Treatment with nBuLi followed by quenching with tritiated water while very effective in other structurally related analogs, resulted only in decomposition products.[8] Hence SCH E (Figure 5) was prepared by rhodium trichloride hydrate catalysed exchange of the α-methylbenzylamine precursor. The resulting hplc purified amine was treated with triflic anhydride to generate the target compound in respectable yield.

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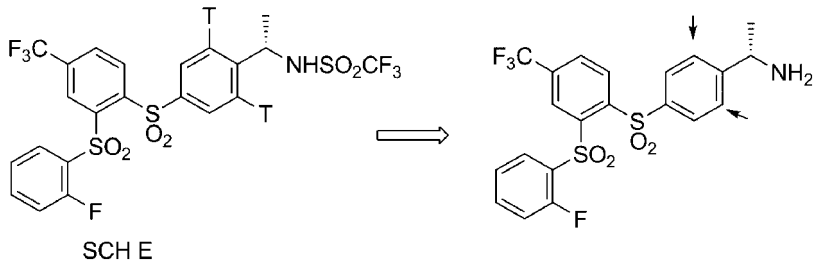

Figure 5. [^{3}H]SCH E.

SCH F (Figure 6) was prepared via reaction with IrCODAcAc and tritiated water with the intention of utilizing the urea and amide functionalities to label the respective phenyl and pyridine rings in the ortho positions.

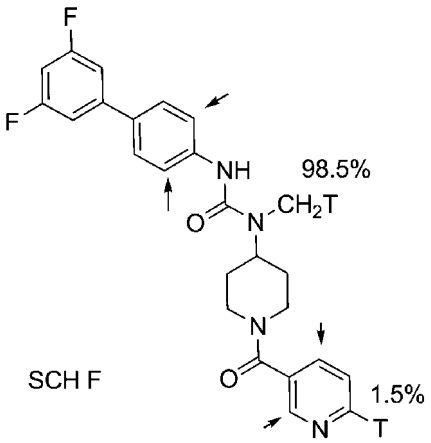

Figure 6. [^{3}H]SCH F.

While a ^{3}H labelled batch was isolated, ^{3}H NMR analysis showed that virtually all of the tritium was incorporated in the urea N-methyl group with a small amount ortho to the pyridine nitrogen. Such incorporation has been previously seen in Ir(I) catalysis with deuterium gas.[9] In the case of the structurally similar SCH G (Figure 7) a request was made for the label to be incorporated on both sides of the urea.

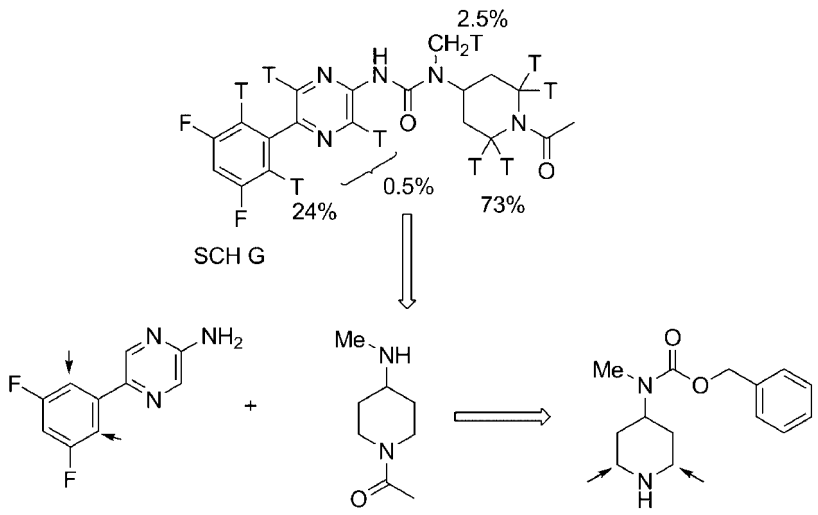

Figure 7. [^{3}H]SCH G.

Hence the aminopyrazine was labelled via rhodium trichloride hydrate in the 3,5-difluorophenyl ring and subsequently converted to the phenylcarbamate. The piperidine moiety was labelled by reacting benzoyl-N-methylpiperidine with $Ru(Ph_3P)_3Cl_2$ and tritiated water. After de-protection and acetylation it was coupled with the phenylcarbamate to generate the target compound. ^{3}H NMR showed tritium was largely incorporated in the expected sites with a small amount in the urea methyl group, which may be due to a Ru complex formed between the carbamate carbonyl and the N-methyl group during the labelling step.

SCH H (Figure 8) was prepared via $Ru(Ph_3P)_3Cl_2$ catalysed exchange of the piperidine precursor, followed by a standard coupling of the pyrimidine acid. ^3H NMR showed the expected labelling pattern. The same methodology was used for examples I to M.

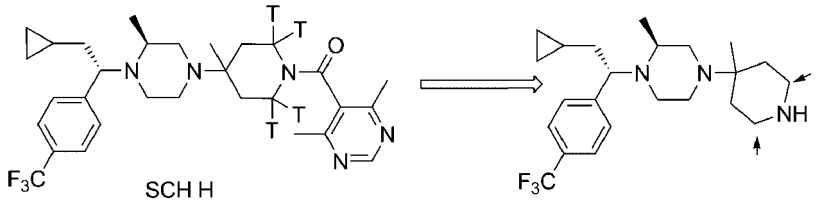

Figure 8. [^{3}H]SCH H.

In the case of SCH I (Figure 9), which was prepared via coupling of the ^3H labelled piperazine with the chloro biaryl under Finkelstein conditions, the ^3H NMR showed that the presence of an axial t-butyl group, hindered the incorporation of the adjacent equitorial position.

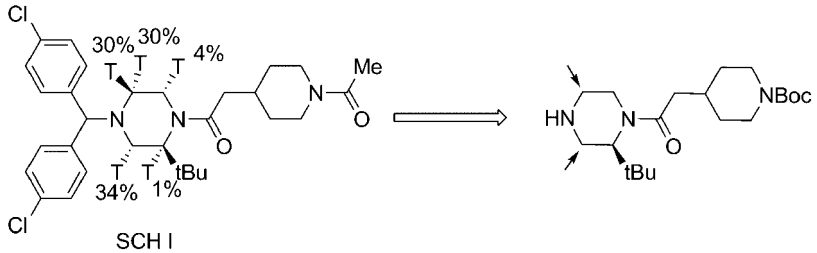

Figure 9. [^{3}H]SCH I.

In the related SCH J (Figure 10), which was labelled via the piperazine containing an isopropyl group adjacent to the active NH, the ^3H NMR analysis showed that in addition to the expected hindrance of the opposite axial site, a substantial amount of label was incorporated in the isopropyl methyl groups.

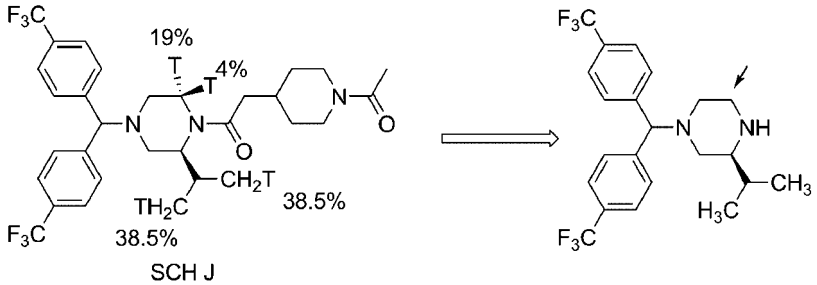

Figure 10. [^{3}H]SCH J.

It is possible that a competing cyclometallation between the methyl group and the piperazine NH is responsible and is worthy of further investigation. Interestingly for SCH K (Figure 11), and in contrast to SCH I, the axial methyl group had no effect on the axial/equitorial distribution in the alpha positions.

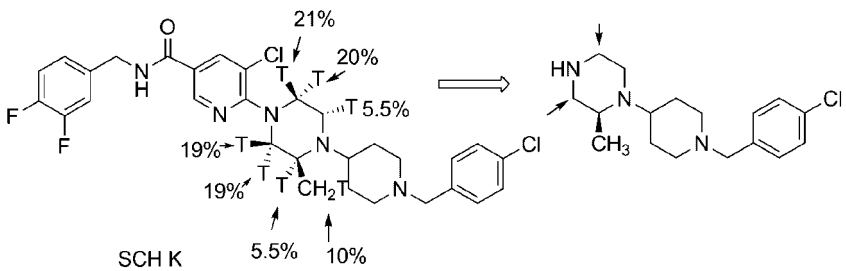

Figure 11. [^{3}H]SCH K.

Additional small amounts of tritium were also incorporated in the equitorial beta positions and the methyl group. SCH L (Figure 12) and SCH M (Figure 12) were labelled via the respective piperidine and piperazine intermediates and converted to the target compounds in two steps and one step respectively.

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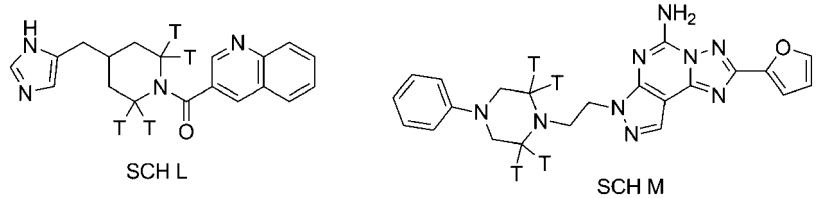

Figure 12. [^{3}H]SCH L and [^{3}H]SCH M.

In the case of SCH L, the imidazole was protected with a trityl group during the labelling step and removed in the final step after the coupling. ^{3}H NMR analysis showed the expected distribution of tritium.

Acknowledgments: The authors would like to thank Dr. T.M. Chan and Dr. M. Senior from the Schering-Plough Research Institute molecular spectroscopy group for the ^{3}H NMR analysis. Thanks are also due to Dr. S. Saluja and Miss S. Hendershot from the Radiochemistry Analytical group and also to SPRI medicinal chemistry for starting materials and procedures.

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SYNTHESIS OF LABELLED TERMINAL [^{14}C]-ACETYLENES

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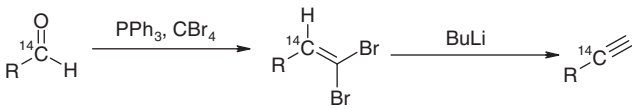
Abstract: There are several literature methods for conversion of ^{14}C precursors into [^{14}C]-acetylenes, and a number of these involve elaboration of [^{14}C]-aldehydes into a terminal acetylenes. One method that involves mild conditions, and in our experience is high yielding and tolerant of a range of functional groups uses the Ohira-Bestmann reagent to effect this transformation. This document briefly describes some of the different methods that have been used for the preparation of [^{14}C]-acetylenes and discusses two worked examples, the synthesis of [*5*-^{14}C]-hex-5-ynoic acid and [*benzene*-(U)-^{14}C]-7-methoxybenzofuran, both of which involve Ohira-Bestmann chemistry being used for the preparation of terminal [^{14}C]-acetylenes.

Keywords: terminal acetylene; Corey-Fuchs; Ohira-Bestmann; 7-methoxybenzofuran; hex-5-ynoic acid

Introduction: Acetylenes are versatile synthetic intermediates that allow access to a wide range of linear, cyclic and heterocyclic molecules. It would be very useful, therefore, to be able to prepare radiolabelled acetylenes conveniently from readily available ^{14}C precursors, and there are various approaches to doing this that can be applied to carbon-14 radiosynthesis. One approach is to use [(U)-^{14}C]-metal acetylides, and while these are accessible, useful and permit higher specific activities in products than singly labelled analogues, [(U)-^{14}C]-metal acetylides are intolerant of many functional groups and can be difficult to handle. One alternative that has been applied to preparation of labelled acetylenes is based upon Corey-Fuchs methodology[1] (Scheme 1). [^{14}C]-Aldehydes can be readily prepared in a number of ways and are commonly made by reduction of [^{14}C]-nitriles or by addition of an organometalic to [^{14}C]-DMF.

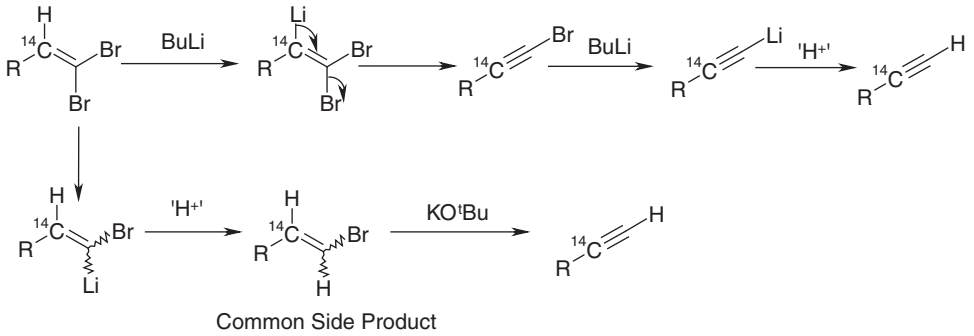


Scheme 1.

[^{14}C]-Aldehydes readily undergo conversion to corresponding vinyl dibromide derivatives and subsequent reaction with butyl lithium gives the terminal lithium acetylide which is then protonated to give the terminal acetylene.

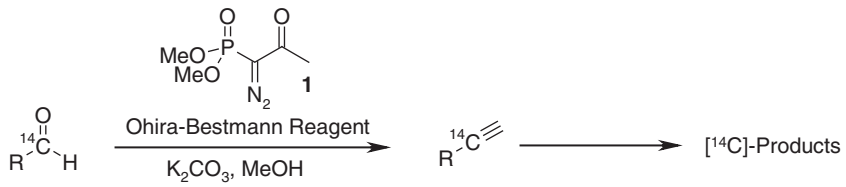
One disadvantage associated with this approach is that yields for the elimination reaction can be low due to side reactions. One side-product often encountered is the corresponding vinyl bromide, which is presumably formed when the vinyl dibromide undergoes lithium halogen exchange as the first step as opposed to elimination (Scheme 2). If isolated, this vinyl bromide can be converted to the desired terminal acetylene by treatment with potassium *tert*-butoxide. Another issue associated with this approach is that it is not tolerant of functional groups that are reactive with butyl lithium.

While this approach is synthetically useful, both of these issues coupled with the fact that there are up to three radiochemical steps required to convert aldehydes to acetylenes (depending upon whether conversion of the vinyl bromide to the terminal acetylene is required to maximise radiochemical yields), have encouraged us to look for alternative literature methods that could be used in radiosynthesis for performing this conversion.



Scheme 2.

Several alternatives to Corey Fuchs methodology have been reported, including development of the Ohira-Bestmann reagent (**1**)[2] (Scheme 3) which allows for a single step conversion of aldehydes to a terminal acetylenes under mild conditions and is tolerant of a number of different functional groups.



Scheme 3.

This communication discusses the synthesis of [*5*-^{14}C]-hex-5-ynoic acid (**2**) and [*benzene*-(U)-^{14}C]-7-methoxybenzofuran (**3**) (Figure 1) which are two examples of carbon-14 labelled intermediates prepared in our laboratories that have involved elaboration of an aldehyde into a terminal acetylene.

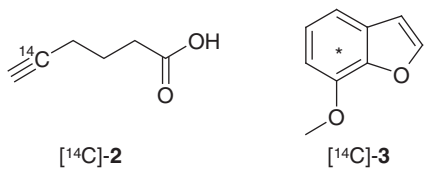


Figure 1. Structures of C-14 labelled **2** and **3**.

Results and Discussion: [*5*-^{14}C]-Hex-5-ynoic acid (2)

Initially it was envisaged that the synthesis would proceed as described in Scheme 4. Nitrile [^{14}C]-**5** would be prepared from commercially available bromoorthoester (**4**) and [^{14}C]-potassium cyanide. This would then undergo reduction to give aldehyde [^{14}C]-**6** which could then be elaborate into the desired [^{14}C]-hex-5-ynoic acid (**2**). As a back-up plan, it was envisaged that nitrile [^{14}C]-**5** could be converted to the methyl ketone [^{14}C]-**7** by reaction with methyl lithium which could be elaborated to the desired product. Both of these routes were initially investigated using unlabelled material or low specific activity carbon-14 labelled materials (Scheme 5).

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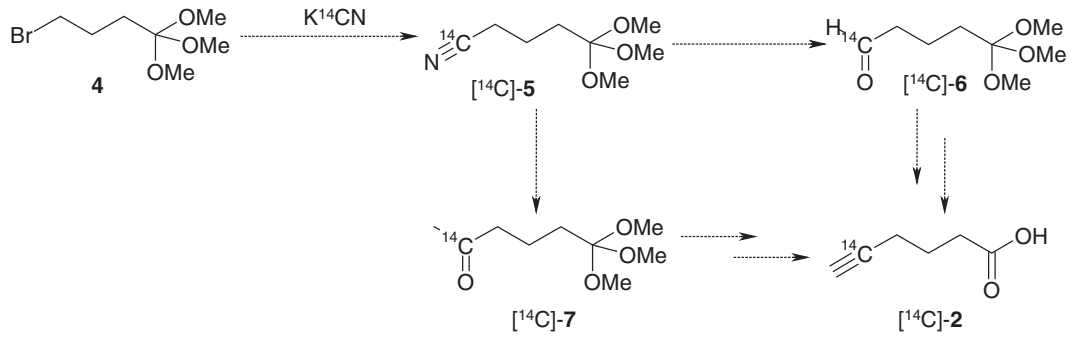
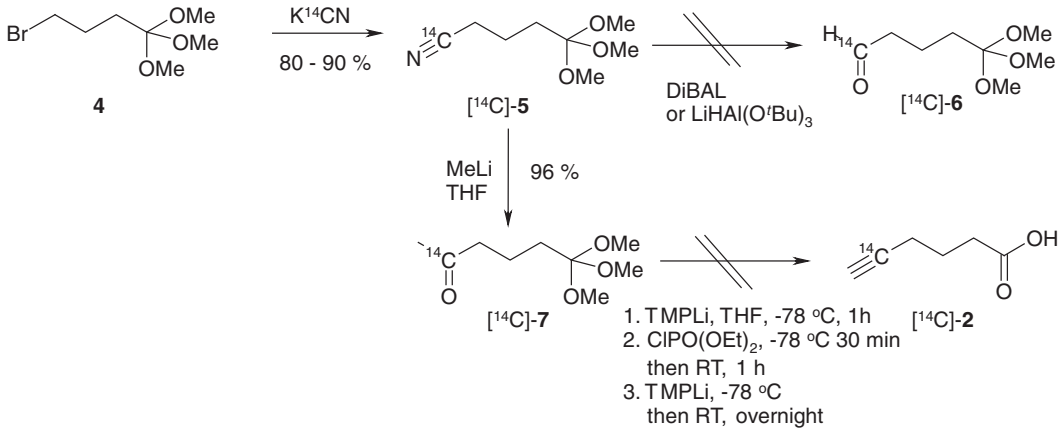


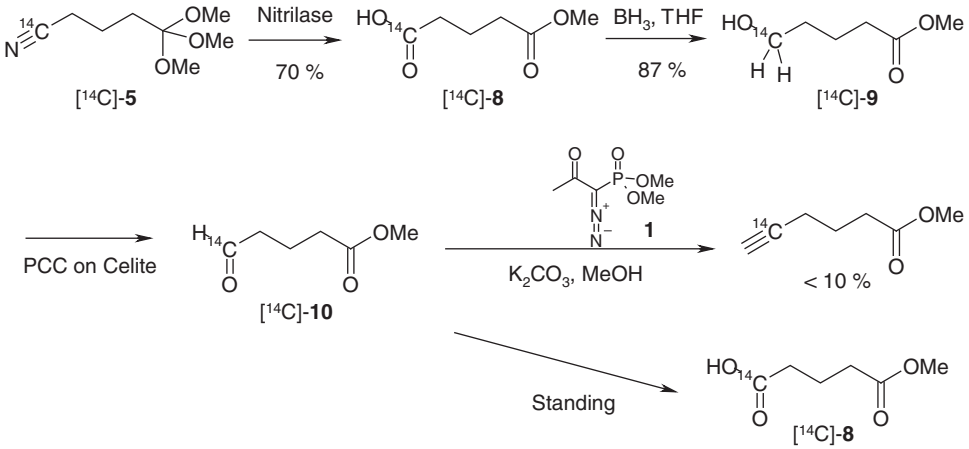
Scheme 4.

Initial formation of nitrile [^{14}C]-**5** worked well in 80–90 % yield. Subsequent reduction of the nitrile, however, failed to yield any useful amounts of product, and it is suspected that the reason for this is that this particular aldehyde is unstable, therefore synthesis *via* the methyl ketone was attempted. Preparation of methyl ketone [^{14}C]-**7** proceeded in good yield. Subsequent de-hydration of the ketone, using a modified procedure[3] involving conversion to the enol phosphate followed by elimination, failed to yield any of the desired product at all, and all attempts to form the desired product from [^{14}C]-**7** afforded complex mixtures.



Scheme 5.

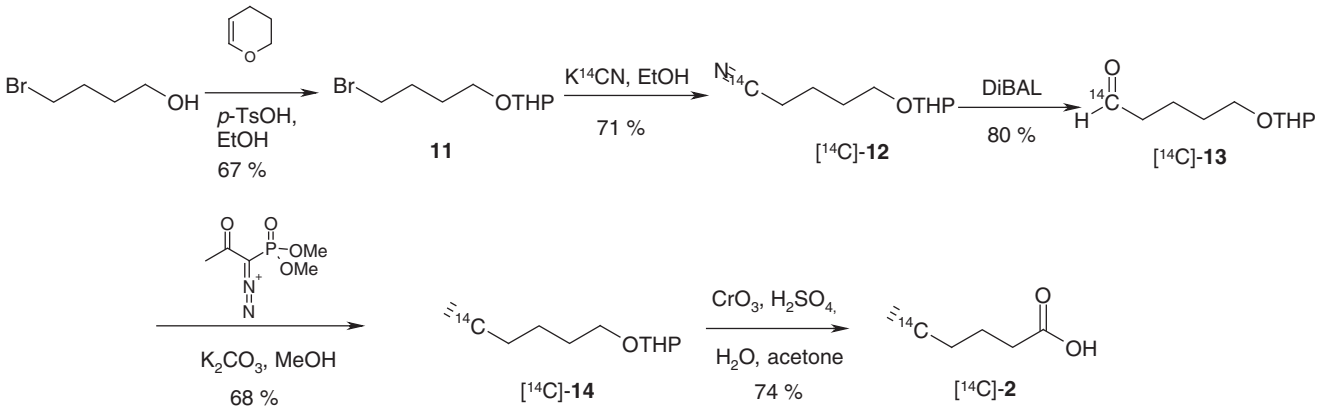
A further approach was attempted (Scheme 6). Nitrile [^{14}C]-**5** was converted to acid [^{14}C]-**8** using a nitrilase enzyme in 70 % yield and the acid was selectively reduced to the alcohol [^{14}C]-**9** in 87 % yield using borane-THF. Subsequent oxidation using PCC on celite to give [^{14}C]-**10** appeared to work by TLC and NMR, however, reaction of the crude product solution failed to give useful yields of the terminal acetylene when reacted with Ohira-Bestmann reagent **1** and on standing re-formed the acid [^{14}C]-**8**, thereby suggesting that this intermediate aldehyde [^{14}C]-**10** was also unstable.



Scheme 6.

[^{14}C]-Hex-5-enoic acid was finally prepared as illustrated in Scheme 7. THP protected bromobutanol **11** was converted to nitrile [^{14}C]-**12** using [^{14}C]-potassium cyanide in 71 % radiochemical yield. Reduction with DiBAL gave aldehyde [^{14}C]-**13** in 80 % radiochemical yield and conversion to acetylenic ether [^{14}C]-**14** using Ohira-Bestmann reagent (**1**) was performed in 68 %

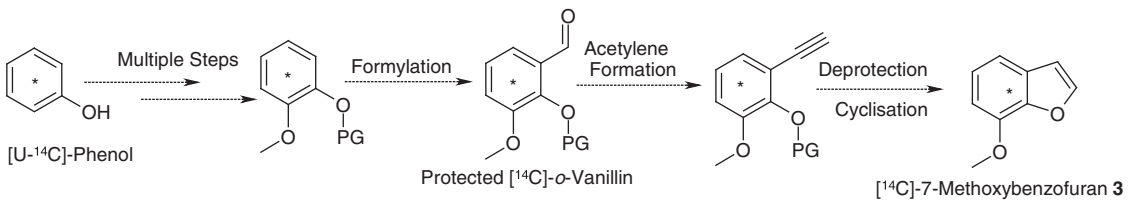
radiochemical yield. Finally, de-protection of the THP protecting group and oxidation of the alcohol to the acid in a single step gave the desired product [^{14}C]-**2** in 74 % radiochemical yield.



Scheme 7.

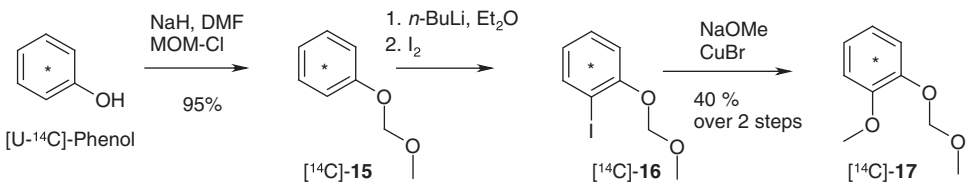
[*Benzene*-(U)-^{14}C]-7-Methoxybenzofuran (3)

It was envisaged that the chemistry would proceed as illustrated in Scheme 8. After a number of exploratory experiments we selected the MOM group as a protecting group and prepared the *O*-MOM, *O*-methyl catechol derivative from [(U)-^{14}C]-phenol as shown in Scheme 9.



Scheme 8.

[(U)-^{14}C]-Phenol was MOM protected in 95 % radiochemical yield to give [^{14}C]-**15**. This was then *ortho*-lithiated with butyl lithium and the anion reacted with iodine to give the iodophenol derivative [^{14}C]-**16**. The iodine was then displaced with sodium methoxide, mediated with copper (I) bromide to give catechol derivative [^{14}C]-**17** in 40 % combined yield for the iodination and displacement. This yield was lower than would have been anticipated from the trial chemistry that we performed, and it was noted that 31% radiochemical yield of an 86:13 mixture of [^{14}C]-**15** and [^{14}C]-**16** was isolated after the methanol displacement. This could have been re-cycled to furnish more product if desired, but this was not done as it was felt that there was more than enough of the catechol derivative [^{14}C]-**17** to complete the synthesis successfully.



Scheme 9.

Conversion of catechol derivative [^{14}C]-**17** to terminal acetylene [^{14}C]-**20** was performed as illustrated in Scheme 10. [^{14}C]-**17** was *ortho*-lithiated with butyl lithium and the resulting anion quenched with DMF. This gave exclusively one regioisomer with the aldehyde being formed *ortho* to the MOM group and this reaction proceeded in 56% yield, however, unfortunately, the reaction or work-up procedures also caused the MOM de-protection to occur to give [^{14}C]-**18** in 56% radiochemical yield. [^{14}C]-**18** was re-protected in quantitative yield to give [^{14}C]-**19** using MOM chloride in acetone in the presence of potassium carbonate. Formation of the terminal acetylene [^{14}C]-**20** was performed using Ohira-Bestmann reagent (**1**) to give [^{14}C]-**20** in 75% yield.

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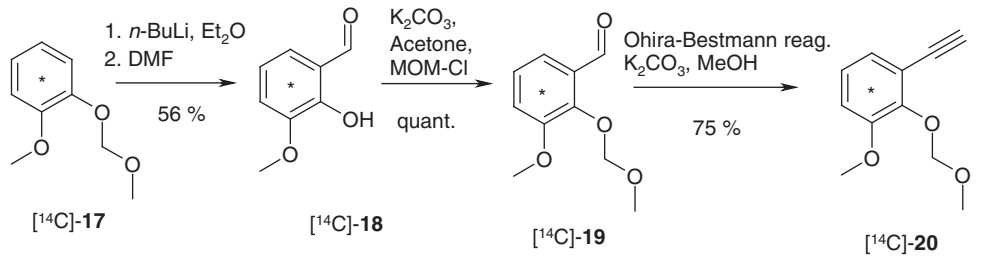
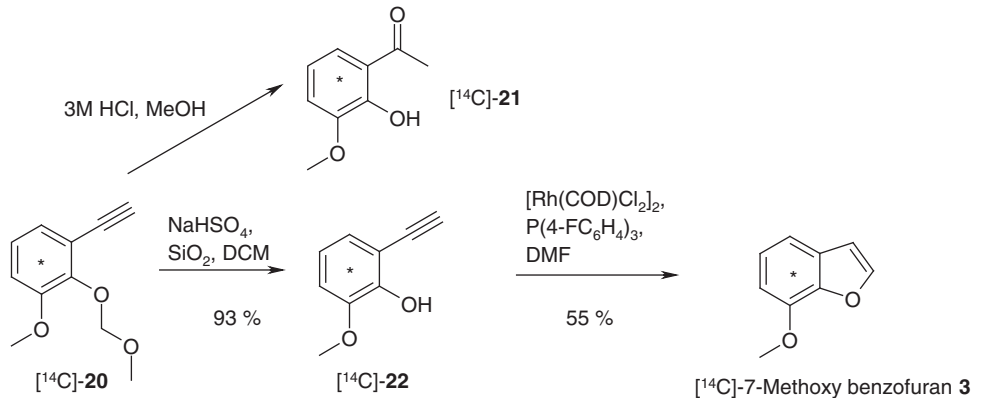


Scheme 10.

Finally, conversion of the terminal acetylene [^{14}C]-**20** to the final product [^{14}C]-7-methoxybenzofuran (**3**) was performed as shown in Scheme 11.



Scheme 11.

Acetylene [^{14}C]-**20** was de-protected using a mixture of sodium hydrogen sulphate and silica gel in DCM to give the phenol [^{14}C]-**22**. Initially, this conversion was attempted using 3*M* HCl in methanol, but this caused the acetylene to hydrate to give acetophenone derivative [^{14}C]-**21**. Formation of benzofurans from acetylenes is often accomplished by Sonogashira reaction of an *o*-iodophenol with an acetylene, and under Sonogashira coupling conditions, cyclisation often occurs spontaneously. Cyclisation of [^{14}C]-**22** was initially attempted by replicating Sonogashira type conditions[4] (heating with a mixture of $PdCl_2$, $CuCl_2$, DCE, and also heating with a mixture of $PdCl_2(PPh_3)_2$, CuI, Et_3N, THF) but this resulted in complex mixtures of products. The final product was ultimately prepared from [^{14}C]-**22** by reaction with a rhodium catalyst in the presence of a phosphine ligand using a literature procedure[5], and this gave the final [^{14}C]-7-methoxybenzofuran (**3**) in 55 % yield from acetylene [^{14}C]-**22**.

Conclusion: [*5*-^{14}C]-Hex-5-ynoic acid (2) was prepared in 4 radiochemcial steps and 29 % radiochemical yield from [^{14}C]-potassium cyanide. [*Benzene*-(U)-^{14}C]-7-Methoxybenzofuran (3) was prepared in 8 radiochemical steps and 8 % radiochemical yield from [(U)-^{14}C]-phenol.

Preparation of a labelled [^{14}C]-aldehyde followed by conversion to the [^{14}C]-terminal acetylene using the Ohira-Bestmann reagent is a convenient and useful approach to a range of labelled compounds provided the corresponding aldehyde is accessible.

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DESIGN AND SYNTHESIS OF A CARBON-14 ELECTROPHILIC CYANATING REAGENT: 1-[^{14}C]CYANO-BENZOTRIAZOLE AND ITS APPLICATION IN ORGANIC SYNTHESIS

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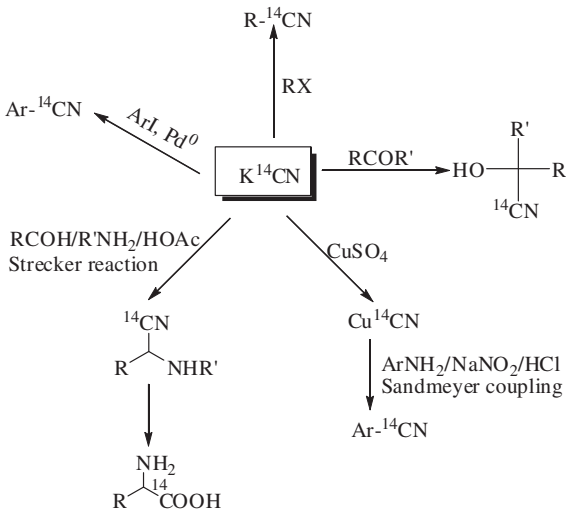
Abstract: 1-[^{14}C]Cyanobenzotriazole (Bt*CN), a Carbon-14 electrophilic cyanating reagent, was synthesized from K^{14}CN in 86% yield in one step. The crude 1-[^{14}C]cyanobenzotriazole was used in the synthesis of two Carbon-14 labeled

compounds: 3-(2-[^{14}C]cyanoacetyl)-1,1-dimethylethyl ester (**1**) and a substituted pyrazolo-triazolo-pyrimidine (**2**). The application of 1-[^{14}C]Cyanobenzotriazole in these syntheses enabled us to prepare the targeted Carbon-14 compounds in two steps from K^{14}CN with high overall yield.

Keywords: Carbon-14; 1-[^{14}C]Cyanobenzotriazole; Electrophilic cyanating reagent

Introduction: Nitriles are versatile intermediates and have broad use in organic synthesis. K^{14}CN is a useful reagent to synthesize ^{14}C nitriles. K^{14}CN is widely used in labeled compound synthesis due to its ready availability, low price and high stability. It is used to introduce a cyano group via carbon-carbon bond formation by CN^- nucleophilic attack at an electrophilic carbon. Some typical transformations are shown in Scheme 1.



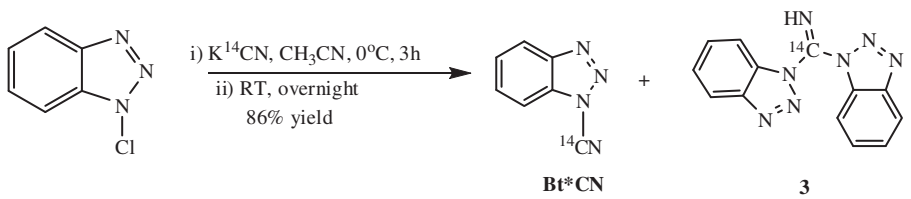
Scheme 1. Synthesis of [^{14}C]Nitriles from K^{14}CN.

An electrophilic cyanating reagent, CN^+, not only broadens the chemistry of C-CN bond formation, but can also be used to form S-CN and N-CN bonds. There are a limited number of reagents that can serve as a cyano cation (CN^+) equivalent, such as cyanogen bromide, cyanogen chloride, phenyl cyanate, tosyl cyanide, 1-cyanobenzotriazole, 1-cyanoimidazole, and 2-cyanopridazin-3(*2H*)-ones.[1,2] Carbon-14 cyanogen bromide was the only reagent reported as a $^{14}CN^+$ reagent.[3,4] However, ^{14}CNBr is difficult to handle due to its low boiling point (61.5°C) and toxicity. A more user friendly $^{14}CN^+$ reagent was needed.

Results and Discussion: Synthesis of 1-[^{14}C]Cyanobenzotriazole (Bt*CN)

1-[^{14}C]Cyanobenzotriazole was chosen as the target electrophilic cyanating reagent based on its physical properties, reactivity and easy preparation from K^{14}CN. Katritzky *et al*. reported synthesis of 1-cyanobenzotriazole from CNBr and benzotriazole in high yield.[1]

Hughes *et al*. synthesized 1-cyanobenzotriazole from 1-chlorobenzotriazole and NaCN in 70% yield after purification by sublimation.[5] We adopted and modified the Hughes' method to prepare 1-[^{14}C]Cyanobenzotriazole from K^{14}CN and 1-chlorobenzotriazole in high yield as shown in Scheme 2. The crude product was used directly without sublimation. Attempts to purify the compound by silica gel column chromatography were unsuccessful; ~10% impurity (imine **3)** was generated.



Scheme 2. Synthesis of 1-[^{14}C]Cyanobenzotriazole.

A typical synthetic procedure follows:

1-Chlorobenzotriazole (290 mg, 1.89 mmol, synthesized according the literature procedure[5]) was added to a suspension of K^{14}CN (100 mCi, 1.72 mmol) in CH_3CN (anhydrous, 8.0 ml) at 0°C. The reaction was stirred at 0°C under N_2, for 3 h and then warmed to RT and stirred for 24 h. The solvent was removed under reduced pressure. The residue was dissolved in EtOAc (50 ml), washed with H_2O, brine, dried over Na_2SO_4, filtered and concentrated to give 85.6 mCi of 1-[^{14}C]Cyanobenzotriazole as a light yellow solid. The RCP was 82% by radio-TLC (silica gel, 2:1 hexanes: EtOAc, Rf = 0.49). The crude product was used in the next step without further purification. The reagent could be stored at −20°C for two weeks. Some decomposition was observed during storage for a longer time. Pretreating the EtOAc extraction solvent with base ($NaHCO_3$) increased the stability of 1-[^{14}C]cyanobenzotriazole.

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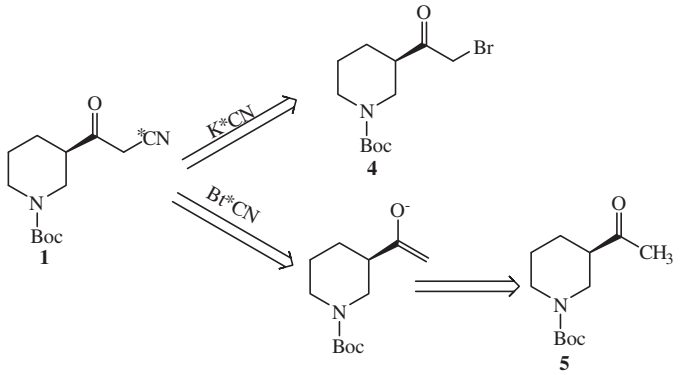
Synthetic applications of 1-[^{14}C]Cyanobenzotriazole

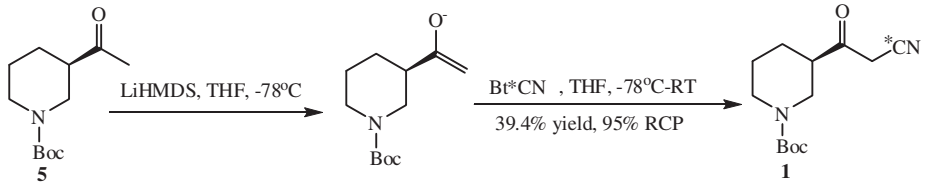
Synthesis of (R)-*tert*-butyl 3-(2-[^{14}C]cyanoacetyl)piperidine-1-carboxylate (1) through electrophilic C-Cyanation

Compound **1** was required with a labeled cyano group. The retrosynthesis is as shown in Scheme 3:



Scheme 3. Retrosynthesis of [^{14}C]compound **1**.

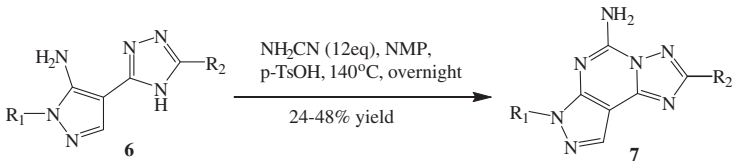
Synthesis of Compond **1** by nucleophilic substitution of **4** with K^{14}CN failed. An alternative approach, eletrophilic cyanation of enolized ketone **5** with 1-[^{14}C]cyanobenzotriazole, was successful. The synthesis is shown in Scheme 4. Ketone **5**, was synthesized according to a literature procedure,[6] enolized with LiHMDS and then reacted with Bt*CN to give compound **1** in 39.4% yield.



Scheme 4. Synthesis of [^{14}C]Nitrile **1**.

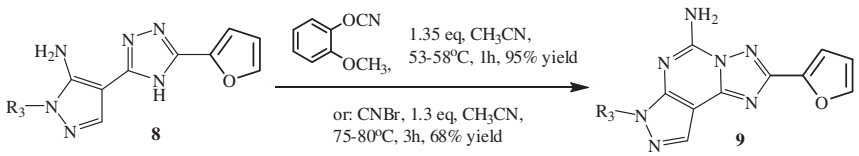
Synthesis of Pyrazolo-Triazolo-[^{14}C]Pyrimidines by eletrophilic N-cyanation and cyclization

Gata and Baraldi have reported the synthesis of compounds containing the Pyrazolo-Triazolo-Pyrimidine core **7** by treating compound **6** with large excess of cyanamide (12 eq) as shown in Scheme 5.[7,8]



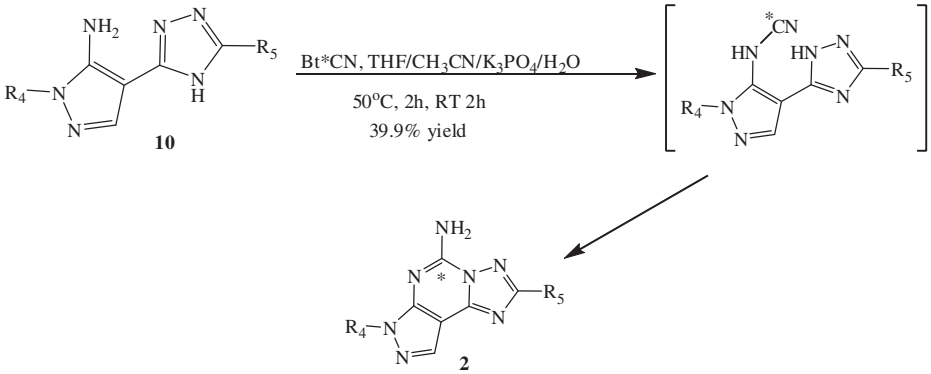
Scheme 5. Synthesis of compound **7**.

Kuo *et al.* reported the similar transformation, with much higher yields, by using eletrophilic cyanating reagents 2-methoxyphenyl cyanate or CNBr as shown in Scheme 6.[9]



Scheme 6. Synthesis of compound **9**.

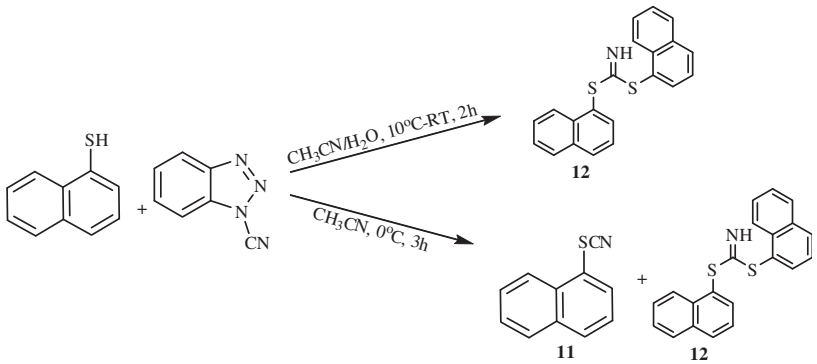
Labeled compound **2** was synthesized using Bt*CN as the eletrophilic cyanating reagent similar to Kuo's approach. Compound **10** was treated with Bt*CN (1.0 eq) in a mildly basic two phase mixture to give labeled compound **2** in 39.9% yield with 97.6% RCP after purification. The synthesis is shown in Scheme 7.



Scheme 7. Synthesis of Carbon-14 labeled compound **2**.

Synthesis of 1-Naphthyl thiocyanate through *S*-Cyanation with Bt*CN

Preliminary *S*-Cyanation chemistry was explored. Naphthalene-1-thiol was reacted with BtCN. The desired 1-Naphthyl thiocyanate **11** and impurity **12** were detected by MS when the reaction was conducted in CH_3CN solvent. Only impurity **12** was detected with CH_3CN/H_2O as solvent as shown in Scheme 8. Further work is planned.



Scheme 8. Synthesis of 1-Naphthyl thiocyanate **11.**

Conclusion: An efficient carbon-14 labeled cyanating reagent, 1-[^{14}C]cyanobenzotriazole, was designed and synthesized in 86% yield from K^{14}CN in one step. It was used in the synthesis of two compounds by electrophilic C-cyanation and N-cyanation. The use of 1-[^{14}C]cyanobenzotriazole enabled us to introduce the carbon-14 at a late step in the synthesis.

Acknowledgements: Special thanks to Dr. Alexei Buevich and Dr. T. M. Chan from Structural Chemistry Group for NMR analysis.

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SYNTHESIS OF [^{14}C] ACIDS FROM THEIR UNLABELED COUNTERPARTS AND APPLICATION TOWARD A NOVEL [^{14}C] LABELED HEPATITIS C VIRUS POLYMERASE INHIBITOR

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Abstract: A strategy has been developed for the rapid construction of a [^{14}C] acid from either an aromatic or an alkyl acid via a decarboxylation-carboxylation sequence. A [^{14}C] labeled heptatitis C virus polymerase inhibitor was prepared. Other examples and the limitations of this methodology are discussed.

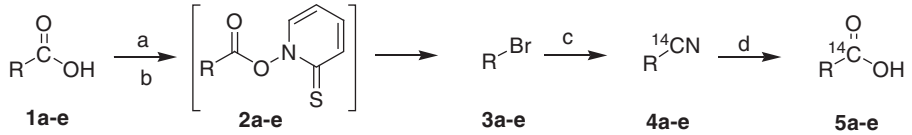
Keywords: ^{14}C-labeled acid; aromatic acid; heptatitis C virus polymerase inhibitor

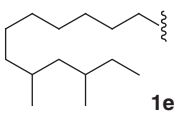
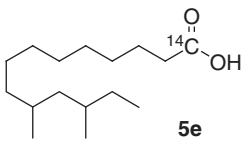
Introduction: The carboxylic acid group plays an important role in organic, medicinal, supramolecular, and material chemistry.[1] For example, recent reports have cited examples of acids as potent HIV-1 protease inhibitors,[2] p38 MAP kinase inhibitors for the treatment of inflammatory diseases,[3a] and peptidemimetics with increased metabolic stability.[3b] Although there are a vast number of methodologies to make an acid, the standard preparation of [^{14}C] acids generally involves use of [^{14}C]CO_2 with Grignard or organic lithium reagents, or hydrolysis of [^{14}C]cyanides.[4] This can be attributed to the fact that halide precursors are readily available commercially or synthetically. When those precursors are difficult to prepare, a method of synthesis of [^{14}C] acids from their unlabeled counterparts becomes very attractive. In this paper a method with mild reaction conditions to convert an unlabeled acid to a [^{14}C] labeled acid is reported.

Results and Discussion: It is known that decarboxylative halogenation of carboxylic acids (the Hunsdiecker-Borodin reaction) leads to a bromide via radical mechanism,[5] This method works well with saturated aliphatic, especially primary, carboxylic acids. The *O*-acyl derivatives of *N*-hydroxy-2-thiopyridone (Barton esters) are commonly used as precursors of radicals,[6] and either primary, secondary, or tertiary carboxylic acids can be converted to the corresponding bromides.[7]

In model studies, decarboxylative halogenations with Barton esters were conducted to obtain bromides, which were then treated with commercially available [^{14}C]KCN to form cyanides. Upon hydrolysis, the desired [^{14}C] acids were realized. The results are listed in Table 1. Thus, unlabeled acids have been transformed to [^{14}C] labeled products. In each case the conversion was monitored by HPLC and LCMS, whereas the yield was based on the isolated product.

Table 1. Synthesis of [^{14}C] aliphatic acids

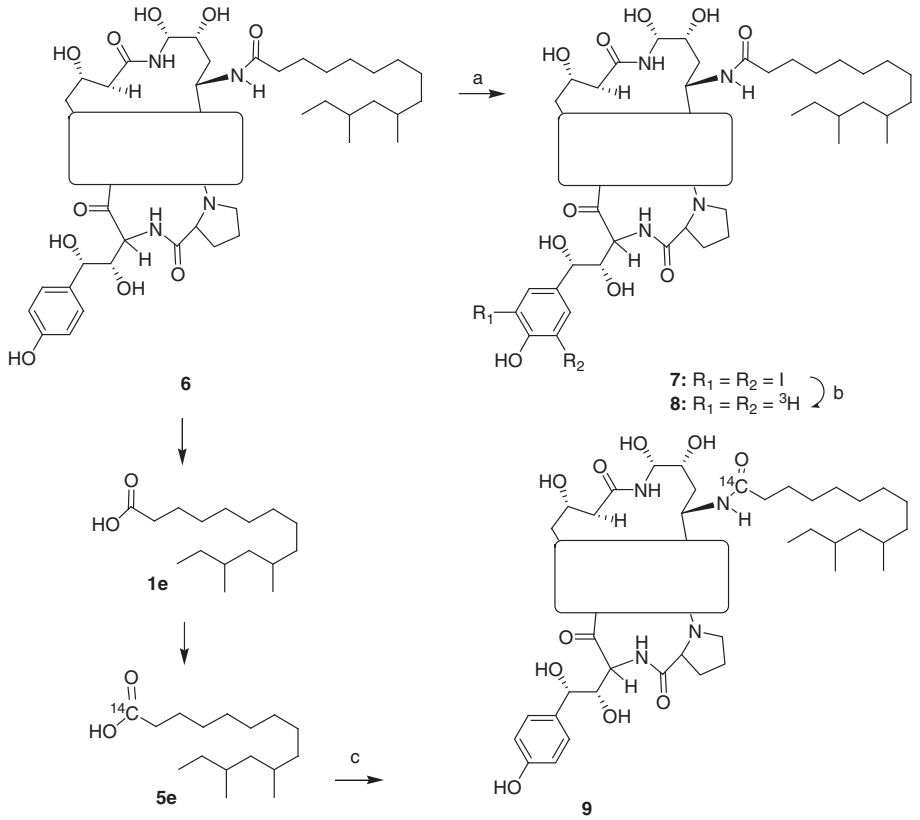


entry	R	Product	% yield
1	n-$C_{11}H_{23}$ (**1a**)	[1-^{14}C]Lauric acid (**5a**)	68
2	n-$C_{13}H_{27}$ (**1b**)	[1-^{14}C]Myristic acid (**5b**)	67
3	n-$C_{15}H_{31}$ (**1c**)	[1-^{14}C]Palmitic acid (**5c**)	62
4	n-$C_{17}H_{35}$ (**1d**)	[1-^{14}C]Stearic acid (**5d**)	60
5	 **1e**	 **5e**	63

Conditions: (a) Oxalyl chloride (1 eq), DMF. (b) 2-Mercaptopyridine *N*-oxide sodium salt, DMAP (0.1 eq), CCl_3Br, reflux, 4 h. (c) [^{14}C]KCN, DMF, reflux, 2 h. (d) 6M HCl, water, reflux, 12 h.

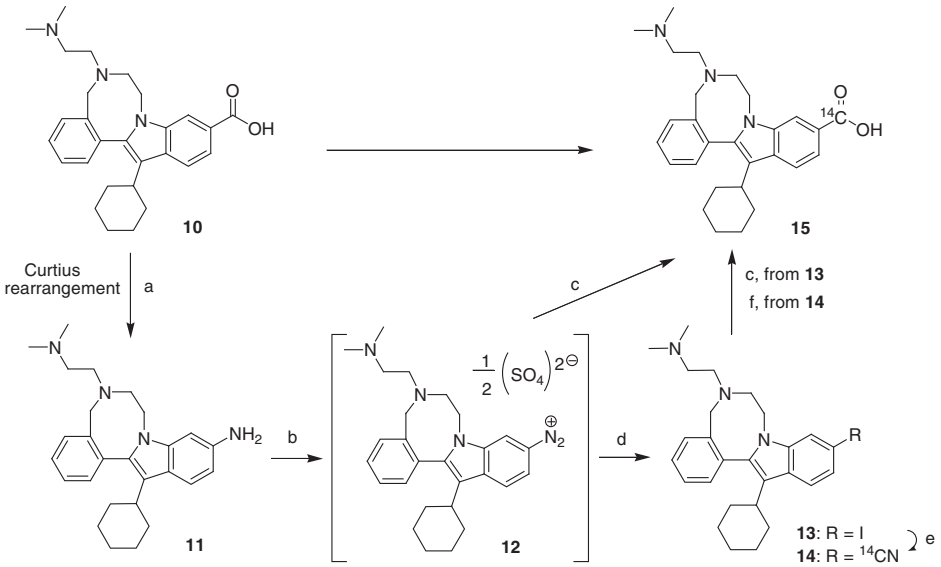
Compound **6** is a fermentation product identified with anti-fungal activities.[8] In order to study its metabolic profile, a radio isotopically labeled tracer was needed. Initially, a [^{3}H] tracer **8** was made via an iodination-tritiation process (Scheme 1).

To make a [^{14}C] labeled tracer, **6** was hydrolyzed to give **1e**, which was then converted to [^{14}C] labeled **5e**. Standard EDC-coupling conditions were used to give final [^{14}C] labeled tracer (**9**) in a short period of time. This [^{14}C] labeled **9** superseded the tritium labeled **8** to became the tracer of choice adopted in metabolic studies.



Scheme 1. Synthesis of [^{3}H] labeled **8** and [^{14}C] labeled **9**. (a) Chloroiodide (ICl), DCM; (b) $^{3}H_2$, 5% $PdCO_3$, DMF; (c) EDC, HOBt, NMM, MeCN, rt, 1 h, 59%.

The limitation of the preparation of [^{14}C] labeled acids *via* the Hunsdiecker-Borodin reaction using Barton esters was that the yields were good for aliphatic substrates but often poor for aromatic ones. As a complement to this labeling method, it was found that [^{14}C] labeled aromatic acids could be synthesized *via* Curtius rearrangement as a key step. Since a great deal of aromatic acids could undergo Curtius rearrangement,[9] this route could expand the availability of [^{14}C] aromatic acids. One example of this effort is shown in Scheme 2. Compound **10** was a lead compound for treatment of hepatitis C virus (HCV) infection.[10] For an absorption, distribution, metabolism, and excretion (ADME) study, a [^{14}C] labeled tracer was needed. However, it was not acceptable to place a label at the *N*-methyl position due to its metabolic instability. It was necessary to put a [^{14}C] label either in the main frame work or at the carboxylic acid. Putting a [^{14}C] label in the skeleton of **10** would involve a 9-step linear sequence[10] based on the medicinal chemistry route to **10**, whereas labeling of the carboxylic acid appeared simpler.



Scheme 2. Synthesis of [^{14}C] acid **15** from its unlabeled counterpart **10.** (a) DPPA, TEA, DMF, 80°C, 4 h, 65%; (b) $NaNO_2$, H_2SO_4, DME, −15°C, 1 h; (c) $Pd(OAc)_2$, AcONa, ^{14}CO, 1%; (d) NaI, water, 91% from **11**; (e) $Zn(^{14}CN)_2$, $Pd(PPh_3)_4$, DMF, 80°C, 12 h, 88%; (f) 6M HCl, 110°C, 57%.

Curtius rearrangement of **10** with diphenylphosphoryl azide (DPPA) gave aniline **11** in 65% yield after flash chromatography. Diazonium salt **12** was obtained by treatment of **11** with $NaNO_2$. Without separation, iodide **13** was prepared, in a one-pot fashion, by addition of NaI to the reaction mixture. Although Pd-catalyzed carbonylation reaction of both **12** and **13** led to the final acid **15**, the yields for these reactions were low (below 1%). Conversion of iodide **13** to cyanide **14** with $Zn(^{14}CN)_2$ was found to be a robust reaction with much higher yield (88%). Hydrolysis of **14** gave the acid **15**. Preparative HPLC purification of **15** was used to achieve an excellent radiochemical purity of 99.4%.

In summary, either an unlabeled alkyl acid or an aromatic acid can efficiently be converted to its [^{14}C] labeled counterpart with only the last two steps involving handling of radio-chemicals.

Acknowledgements: The authors wish to thank Dr. David J. Schenk for analytical support. J.Z.H. gratefully acknowledges Dr. Lee J. Silverberg for help in the preparation of this manuscript.

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SURVEY OF DIFFERENT TRITIUM-LABELLING METHODS USED AT RC TRITEC

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Abstract: The radiosynthesis group of RC TRITEC is specialised in custom tritium synthesis and therefore has gained a lot of experience in a large variety of different tritium-labelling methods. We present a survey of the projects carried out in the past years focusing on the diverse characteristics and popularities of the different methods. All applied reaction types have been classified into different groups of tritium-labelling methods. These groups are illustrated and discussed with regard to specific activities, regioselective labelling, tritium efficiency, possible labelling candidates and precursors. The varying popularities of the different methods used at RC TRITEC in the past years are statistically analyzed.

Keywords: tritium chemistry; tritiation; tritide; tritium-labelling, tritiated

Introduction: Radioisotopically labelled organic compounds are irreplaceable tools for research and development especially in the pharmaceutical and agrochemical industry as well as in academic research. Among the most widely used radioisotopes C-14 and H-3, the latter, tritium, is very much favoured for early applications in the R&D process. From a synthetic viewpoint it is relatively easy to introduce tritium into organic compounds, this allows fast and cost efficient labelling which is of great importance in the fast-paced early development. Beside tritium chemistry's nature of a low-threshold labelling approach the high specific activity of tritium at 29 Ci/milliatom allows the use of such labelled compounds for binding studies, imaging techniques, and many other applications that require highest sensitivities. In contrast, C-14-labelling usually requires multi step syntheses, therefore this time and cost consuming labelling approach is used predominantly for later phases in development.

In this article we present a survey over different methods that are used in tritium chemistry and in special at our facility at RC TRITEC. We would like to offer the researcher the ability to pick out the most suitable tritiation method for his labelling candidate.

Classification of tritium chemistry

There are numerous ways to bring some systematic order to the world of tritium chemistry. Evans[1] has categorised the different preparations of tritium-labelled compounds into isotope exchange reactions, direct chemical synthesis, biochemical methods and recoil labelling (hot-atom reactions). After three decades of advances in tritium chemistry with the introduction of numerous new reagents and methods, Saljoughian and Williams[2] ordered the methods in two groups: 'Hydrogen Isotope Exchange Labeling' and

'Synthetic Labeling'. The recently published new standard work for tritium chemists by Voges, Heys and Moenius[3] again differentiates between isotope exchange methods and chemical synthesis.

When we started to systematically classify all the different chemical methods that have been used at our facility, we found that virtually all methods could be categorised according to the following questions: Does the method involve a direct isotope exchange or a chemical synthesis? Does it use gaseous tritium, a triton containing solvent, tritiated methyl iodide or tritiated intermediates as the tritium source? In cases where a catalyst is used, is it a heterogeneous or a homogeneous one? Are special reagents like tritides utilized? According to this approach, the methods could be divided into five classes distinguished as follows:

1. Direct isotope exchange on the target compound.
2. Catalytic tritium gas reductions of precursors.
3. Tritide reagents are used for selective reductions of suitable precursors.
4. Methylation reactions using tritiated methyl iodide or methyl sulfonates.
5. Coupling of tritiated intermediates.

Tritium-labelling methods

Class 1: Hydrogen isotope exchange

In the past 20 years direct tritium-labelling has gone through ground-breaking developments. The thorough investigation of heterogeneous (Pt, Pd, Rh) and homogeneous catalysts (Rh, Ru, Ir)[4–13] for hydrogen isotope exchange paved the way for new methods affording products with high specific activities. The most obvious advantage of direct isotope exchange labelling is its speed as no precursor is required. Nowadays, a whole bunch of different catalysts, especially Ir(I) based homogeneous ones[11, 13] offer a versatile toolbox for hydrogen exchange on various substrates. Nevertheless, the major drawback is the poor predictability of both extent and position of tritium incorporation resulting in a need for preliminary studies. The problematic generation of high activity tritium waste by Crabtree's catalyst has been relieved by the use of an immobilised version of this versatile Ir(I) catalyst.[14] Alternatively, carrier free tritium oxide or other triton sources like alcohols and carboxylic acids can be used for acid- or base-catalysed exchange reactions or to quench deprotonated C-H acids, e.g. lithiated aromates, see Figure 1.

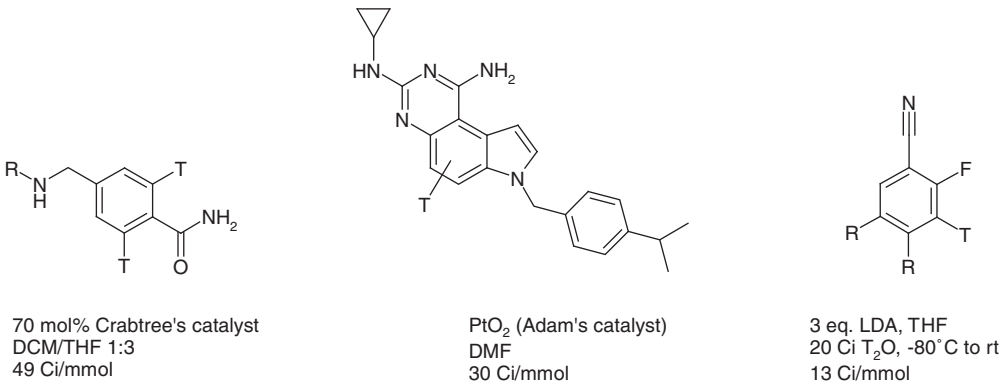

Figure 1. Hydrogen isotope exchange products.

Class 2: Tritium gas reductions

The catalytic reduction of unsaturated or halogenated precursor compounds with carrier free tritium gas is probably the most popular method to introduce tritium into organic molecules. As soon as a precursor is available, heterogeneous catalysis allows rapid tritiation yielding products with high to very high specific activities. A single tritiodebromination typically affords 15–20 Ci/mmol, whereas saturation of an olefinic precursor can give products well above 100 Ci/mmol, especially due to isotope exchange of allylic protons, as can be seen from the second example in Figure 2.

Class 3: Tritide reductions

The development and application of tritide reagents in the last 20 years[2, 15, 16] have dramatically diversified the tritium chemist's lab work, as reagents are now available for almost any synthetic task. LiT derived tritide reagents offer both maximum specific activities (29 Ci/mmol per incorporated tritide) and 100% predictability of the label's position, which is of particular importance for metabolic studies. As stoichiometric quantities of tritiated butane result as a side product from the LiT production, special worker and environmental safety precautions have to be taken. The handling of these reagents in multi Ci quantities asks for dedicated equipment which is available in the form of stainless steel vacuum manifold systems manufactured by RC TRITEC.[17–19] Figure 3 illustrates the use of tritide reducing agents.

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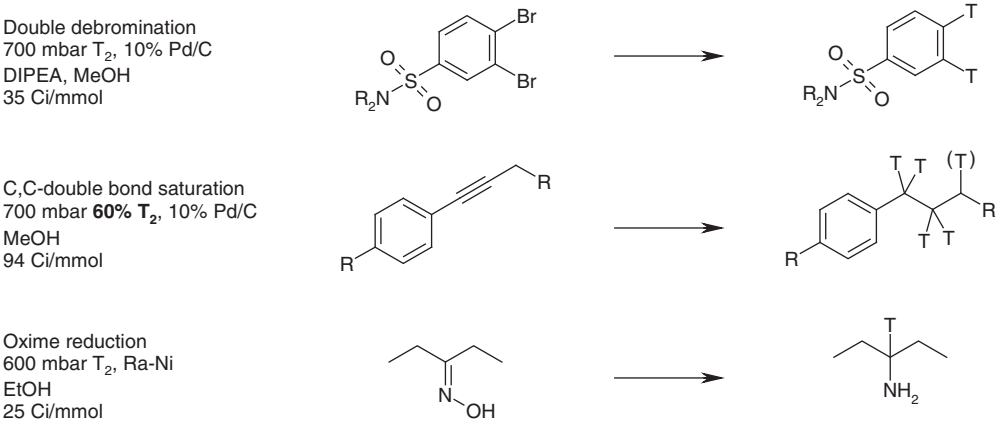

Figure 2. Catalytic gas reductions.

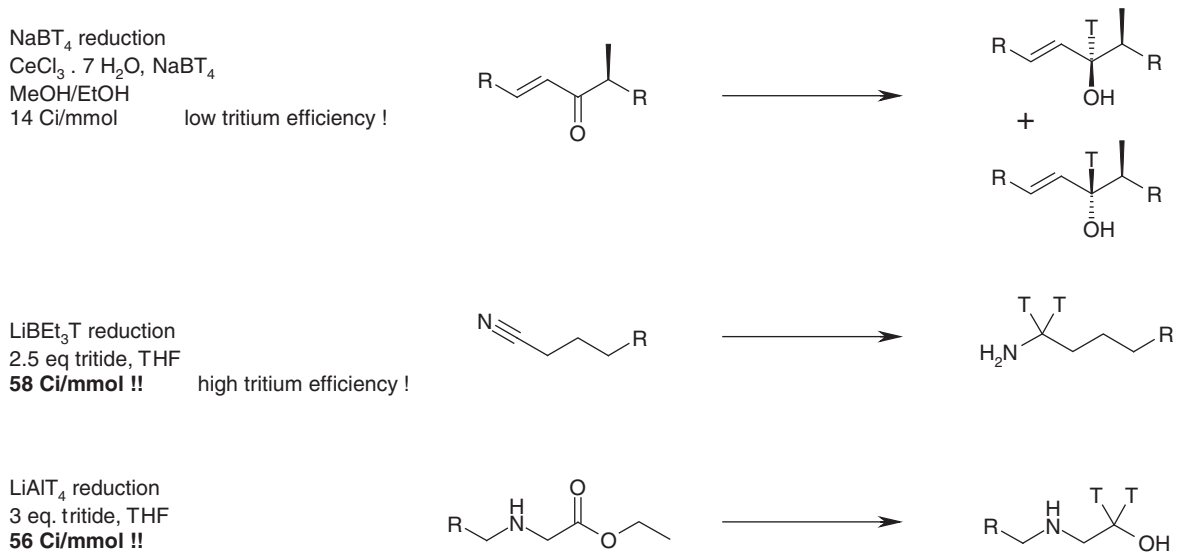

Figure 3. Various applications of tritide reagents.

Class 4: Methylations

The introduction of highly tritiated methyl groups (60–87 Ci/mmol) has become an important option as various target compounds can be prepared by methylation at hydroxy-, amino-, amido-, and thiol-groups. Furthermore, methyl-acetylenes and methyl-aromates are accessible through C-methylation, either by direct methylation of a carbanion as shown in the second example in Figure 4 or by Pd-catalysed coupling reactions. The already intrinsic sensitivity of these reactions is even worsened by the very small scales of tritium synthesis, as 10 μmol of methylating reagent correspond to 600–870 mCi of activity. The disadvantages of methyl iodide, namely its volatility, low stability, and poor UV absorbance, have been overcome by the introduction of non-volatile, stable, and UV-active aromatic methyl sulfonates.[20]

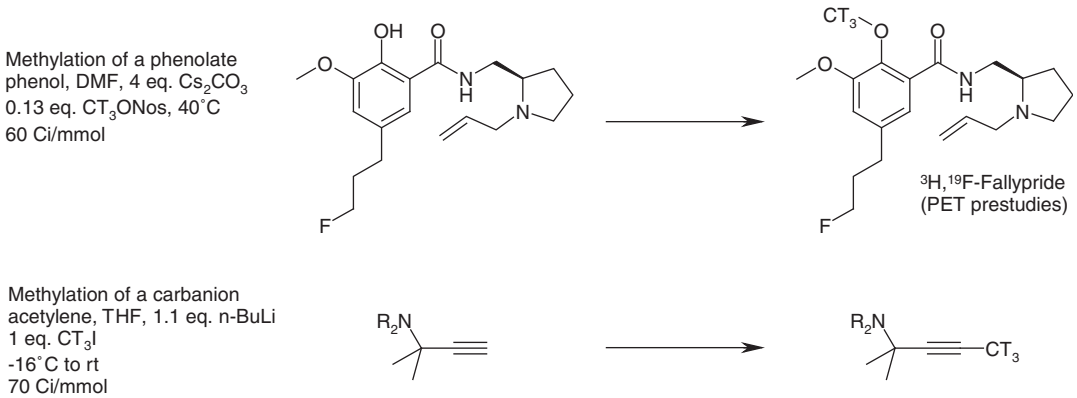

Figure 4. Methylations at phenolic oxygen and acetylenic carbon.

Class 5: Coupling of intermediates

In case the target compound bears a terminal group that can be coupled easily to an available precursor, one may employ tritiated intermediates, such as methyl amine, ethanol amine, carboxylic acids, amino acids etc. The site specific and high degree of labelling has to be paid with multiple active steps and difficult coupling reactions on small scales (often $<10\,\mu$mol), see Figure 5.

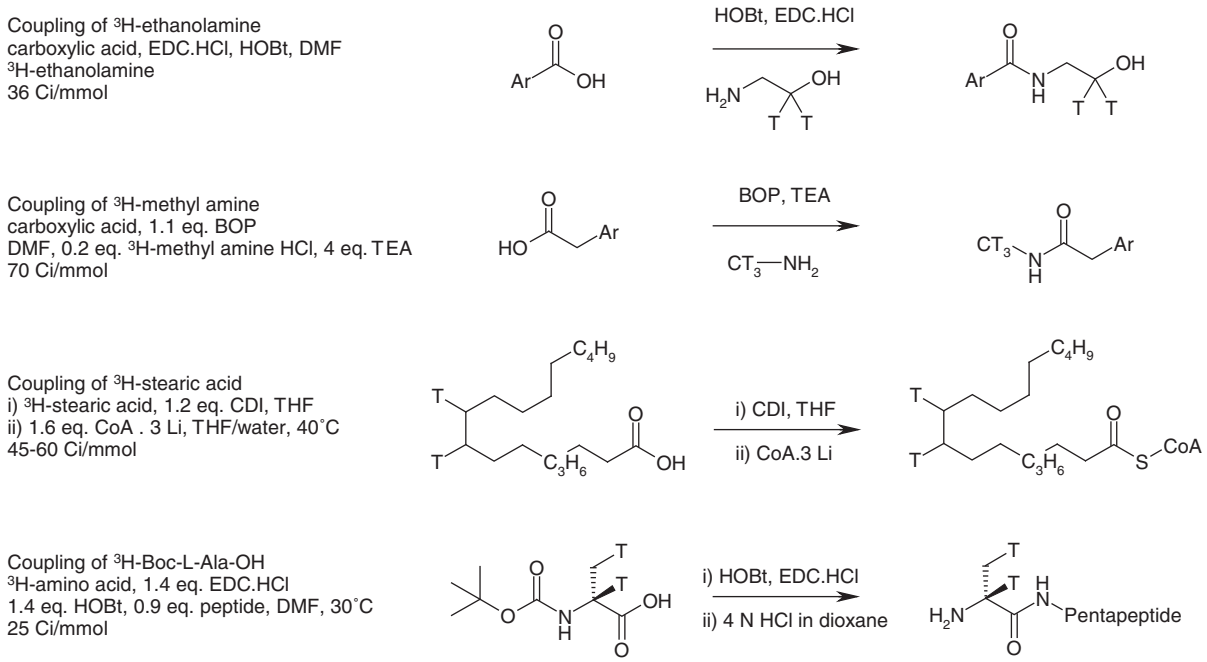

Figure 5. Coupling reactions of selected tritiated intermediates.

Popularity statistics of methods used at RC TRITEC

The statistical analysis of all the tritiations projects carried out at RC TRITEC between 2005 and 2008 revealed the distribution depicted in Figure 6.

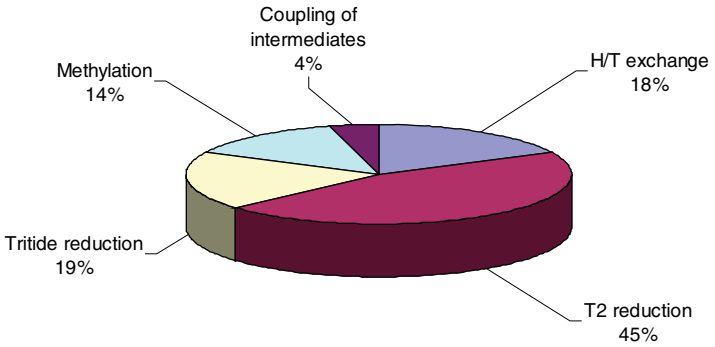

Figure 6. Statistical distribution of the projects over the five method classes.

A surprisingly low 18% of the projects involved an exchange methodology. Among these projects, most were carried out using tritium gas and a homogeneous or heterogeneous catalyst, for the rest a triton source was used. In almost every second project (45%) a gas reduction method was applied, either tritiodehalogenation, C-C-double/triple bond saturation, rarely C-heteroatom-double/triple bond saturation (e.g. nitrile reduction). Tritide chemistry plays a most important role in our facility, as almost every fifth tritiation involved the preparation and use of a tritide reagent, mainly made from lithium tritide (aluminotritides, lithium borotritides, tin tritide), to a lesser extent made from sodium borotritide prepared by thermal exchange of sodium borohydride in tritium gas. Every seventh tritium-labelled product resulted from a methylation, mostly at heteroatoms like oxygen, nitrogen and sulphur, occasionally C-methylation was applied successfully. The remaining 4% of all projects accounted for the production and coupling of tritium-labelled intermediates, a strategy that is rarely used due to its time-consumption and thus costly nature.

Conclusion: We have systematically classified the tritium-labelling methods used at our facility in five different classes and illustrated them with typical examples. The discussion of the main features of the different methods like suitable target compounds, observed specific activities, and site specific labelling should be helpful for researchers to choose a tailor-made tritium-labelling strategy for their labelling candidates. The statistical analysis of all the applications from the past four years done at RC TRITEC gives an idea of the diversity of today's tritium chemistry and its methods.

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THE SYNTHESIS OF 5, 7-DIMETHYL-1, 2, 4-TRIAZOLO[1, 5A(^{14}C)]PYRIMIDINE-2-CARBALDEHYDE FROM [^{14}C]AMINOGUANIDINE: A KEY INTERMEDIATE FOR THE RADIOCHEMICAL GMP SYNTHESIS OF A DRUG CANDIDATE

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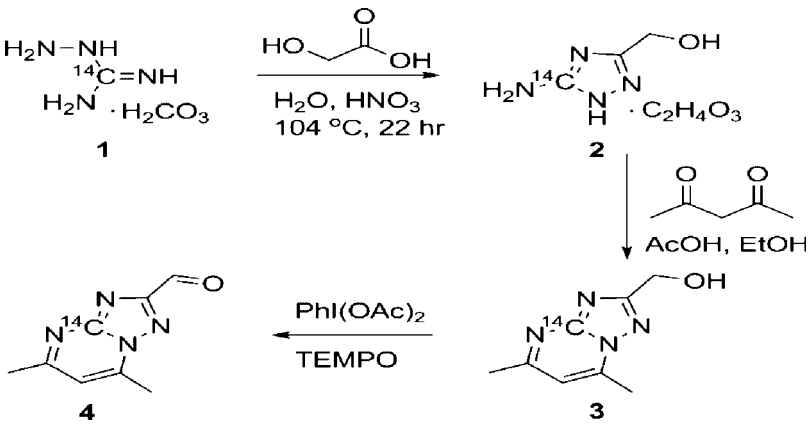
Abstract: Hydrazination of barium [^{14}C]cyanamide and hydrazinolysis of *S*-methylisothio[^{14}C]urea were compared for their ability to efficiently prepare [^{14}C]aminoguanidine bicarbonate. [^{14}C]Aminoguanidine bicarbonate was converted in three steps to 5, 7-dimethyl-1,2,4-triazolo[1,5a(^{14}C)]pyrimidine-2-carbaldehyde, a key radiochemical GMP starting material.

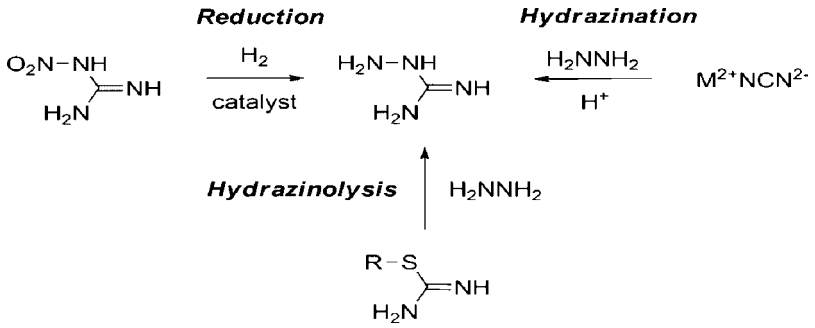
Keywords: Barium [^{14}C]cyanamide, *S*-methylisothio[^{14}C]urea, [^{14}C]aminoguanidine bicarbonate, 5,7-Dimethyl-1,2,4-triazolo[1,5a(^{14}C)]pyrimidine-2-carbaldehyde

Introduction: We required a large quantity of 5,7-dimethyl-1,2,4-triazolo[1,5a(^{14}C)]pyrimidine-2-carbaldehyde (**4**) for use as a radiochemical GMP starting material. The established synthesis of non-labeled **4** started from aminoguanidine bicarbonate[1]–a readily available reagent. However, [^{14}C]aminoguanidine bicarbonate (**1**) was not commercially available and required an efficient synthesis to enable the production of **4** (Scheme 1).



Scheme 1.

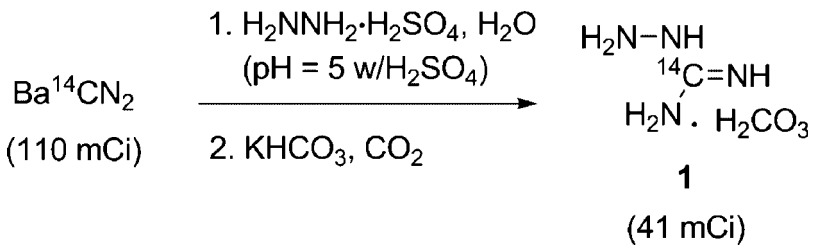
The journal and patent literature describing both non-labeled and labeled syntheses of aminoguanidine[2] can be categorized into three general strategies: reduction of nitroguanidine, hydrazination (hydrazine nucleophilic addition) of cyanamide and hydrazinolysis (hydrazine nucleophilic addition-elimination) of urea derivatives (Scheme 2). Of these strategies, we focused our attention on both hydrazination and hydrazinolysis approaches to the synthesis of **1** because they utilize starting materials ($BaCN_2$ and thiourea, respectively) that are readily available in carbon-14 form.



Scheme 2.

Results and Discussion: Several generalizations can be made from published hydrazination syntheses of non-labeled/labeled aminoguanidine. Firstly, $CaCN_2$ or $NaHCN_2$ are used as cyanamide sources in unlabeled aminoguanidine syntheses and $Ba^{14}CN_2$ is the only reported reagent used in labeled aminoguanidine syntheses. Hydrazine is typically introduced as a hydrate or sulfate salt. Reactions are typically run with excess cyanamide and in water at low concentration to avoid cyanamide dimerization. Reaction pH is often kept at 5-6 to avoid formation of a variety of impurities and maximize product yield. Precipitation of aminoguanidine as its bicarbonate salt (the most commonly sited salt form and the one we desired) is typically accomplished by saturating the product solution with $KHCO_3$ and/or CO_2 gas and adjusting reaction pH with H_2SO_4. Within this body of work, reported yields of aminoguanidine range widely from 30–95%.

After exploring all established hydrazination procedures to prepare aminoguanidine bicarbonate, we were able to confirm several process observations. Maintaining tight control of reaction pH was indeed critical to product formation, but reaction heterogeneity throughout the course of the reaction made pH measurement difficult. Precipitation of the product bicarbonate salt proved difficult if aminoguanidine was not present in high concentration. Additionally, since unlabeled $BaCN_2$ is not commercially available (and $Ba^{14}CN_2$ is the only carbon-14 cyanamide commercially available) we necessarily performed our exploratory work with $CaCN_2$. The dramatic solubility differences between these reagents made adequate development of labeled chemistry impossible. Our optimized hydrazination synthesis of **1**, performed on 110 mCi of $Ba^{14}CN_2$, proved only modestly successful, Scheme 3.



Scheme 3.

In light of our inability to prepare [^{14}C]aminoguanidine in high yield *via* hydrazination of $Ba^{14}CN_2$, we turned our attention to hydrazinolysis methodology. Specifically, *S*-methylisolthiourea **5** was an appealing aminoguanidine precursor because it can be directly prepared from commercially available carbon-14 labeled thiourea, and can also be derived from $Ba^{14}CN_2$. Numerous literature reports showcase the conversion of *S*-methylisolthiurea salts to aminoguanidine in water by reaction with hydrazine sulfates or hydrates in the presence of base.[2a,3] This approach has been used to prepare both $^{13}C/^{15}N_2$ and ^{14}C aminoguanidine.[3]

Using unlabeled thiourea, we quickly confirmed the utility of this approach, Scheme 4. Methylation of this reagent with either methyliodide or dimethylsulfate in ethanol solvent afforded unlabeled *S*-methylisothiourea **5a** and **5b** in good yields as H_2SO_4

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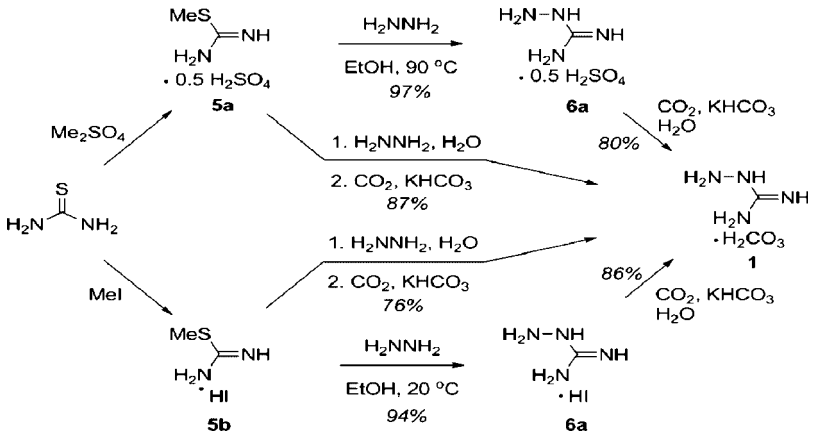
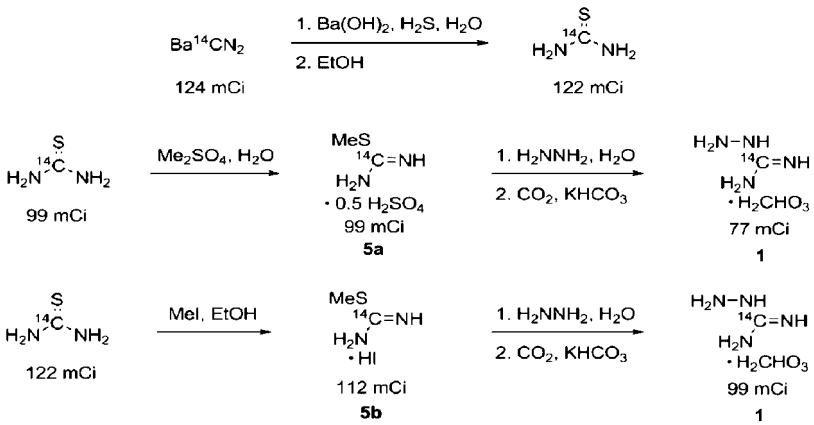
(insoluble) or HI (soluble) salts, respectively. These salts could be converted in high yield to aminoguanidine bicarbonate by either stepwise or one pot reaction with hydrazine hydrate followed by CO_2/$KHCO_3$ treatment.



Scheme 4.

The efficient conversion of barium [^{14}C]cyanamide to *S*-methyliso[^{14}C]thiourea **5** was central to our choice for pursuing a hydrazinolysis approach to **1**. In practice, a suspension of 124 mCi of $Ba^{14}CN_2$ in water in a pressure tube was treated with 3.4 equivalents of $Ba(OH)_2$ (to maintain basic reaction pH and a common counterion) and saturated with H_2S gas. The reaction tube was sealed and heated to affect cyanamide sulfurization.[4] The reaction was then saturated with carbon dioxide to purge excess H_2S gas and to precipitate barium as $BaCO_3$. The reaction was filtered to remove the suspended $BaCO_3$, the aqueous filtrate was concentrated and the residue was diluted with ethanol to suspend remaining traces of the salt. Additional filtration and concentration delivered 122 mCi of chemical and radiochemical pure [^{14}C]thiourea.

Two methylation procedures were then investigated for their ability to efficiently *S*-alkylate [^{14}C]thiourea. Specifically, reaction of [^{14}C]thiourea with dimethyl sulfate[3] in water proved slightly more effective than the same reaction performed with iodomethane[5] in ethanol for formation of *S*-methyliso[^{14}C]thiourea salts, **5a**/**5b**, respectively. Of note, the methylation to form **5a** directly provided high purity product, while the analogous formation of **5b** required purification (Scheme 5).



Scheme 5.

Carbon-14 salts **5a** and **5b** were processed to [^{14}C]aminoguanidine bicarbonate (**1**) separately to determine whether the intermediate HSO_4^- and I^- counterions in any way effected reaction efficiency. To that end, **5a** and **5b** were reacted with hydrazine in water at room temperature–both reactions were deemed complete within 3 hours, and the reaction of **5b** was allowed to continue for an extended time period to demonstrate product stability. Each reaction solution was then saturated with carbon dioxide gas to purge liberated methyl mercaptan and introduce carbonate. Dissolution of solid $KHCO_3$ into each solution initiated precipitation of **1** as white solids that were isolated by filtration. Overall, syntheses of **1** *via* dimethyl sulfate or iodomethane methylations and subsequent hydrazinolyses of the *S*-methyliso[^{14}C]thiourea intermediates proceeded with equal efficiency. The overall yield from hydrazinolysis approach starting from $Ba^{14}CN_2$ was 78% over three steps compared to hydrazination approach which afforded in only 37%.

Having developed high yielding synthesis of **1**, we turned our attention on efficiently constructing aldehyde **4**. First, the reaction of unlabeled **1** with glycolic acid (2 equiv) in water with catalytic HNO_3 at 100–110°C afforded intermediate **5** in moderate yield. Product degradation resulted from unavoidable evaporation during this reaction. Because of this we explored alternate reaction heating methods. Microwave heating and sealed vessel heating proved equally superior to the original open reflux reaction conditions, with the sealed vessel method being more appropriate for processing radioactivity. With this information in hand a solution of 99 mCi of **1**, 2 equivalents of glycolic acid, water and catalytic HNO_3 was heated at 110°C in a sealed vessel for 40 hours to afford high radiochemical purity **2**, Scheme 1. Azeotropic removal of water by ethanol afforded solid **2** that was used without purification in the next step. A suspension of **2** in ethanol was reacted with 1.8 equivalents of acetylacetone and catalytic AcOH at 70°C for 1.5 h to afford 78 mCi of triazolopyrimidine **3** after flash column chromatography. Finally, **3** was oxidized with $PhI(OAc)_2$ in the presence of TEMPO radical to afford 62 mCi of **4** after flash column chromatography.

Summary: [^{14}C]Aminoguanidine bicarbonate (**1**) was initially prepared in low yield by hydrazination of $Ba^{14}CN_2$. A more efficient process for synthesizing **1** was developed that involved converting $Ba^{14}CN_2$ to S-methyliso[^{14}C]thiourea and reacting that intermediate with hydrazine. Reaction of **1** with glycolic acid in a sealed vessel afforded triazole **2** which was directly reacted with acetylacetone to afford triazolopyrimidine **3**. $PhI(OAc)_2$/TEMPO mediated oxidation of **3** delivered 5,7-dimethyl-1,2,4-triazolo[1,5a(^{14}C)]pyrimidine-2-carbaldehyde (**4**) in high overall yield.

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CHALLENGES AND DIFFICULTIES IN SYNTHESIS OF TRITIUM LABELED FLUOCINOLONE ACETONIDE

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Abstract: Tritium labeled fluocinolone acetonide was synthesized through five-step procedure. Many challenges and difficulties were encountered in the synthesis, especially in the regeneration of 1,2 double bond from reaction of oxidative de-hydrogenation of tritium labeled 4-ene analog. Selenium oxide oxidative de-hydrogenation of 11,21-dihydroxy un-protected 4-ene fluocinolone acetonide analog gave a complex product mixture. The reaction gave a clean unlabeled intermediate after protection of 21-hydroxy group by acetylation, but the same procedure failed to give desired tritium labeled intermediate due to fast tritium-hydrogen exchange reaction. The de-hydrogenation proceeded well with DDQ in refluxing benzene for unlabeled intermediate, but no reaction at all for the tritium labeled one. Benzeneseleninic anhydride/toluene refluxed with tritium labeled 4-ene analog was found to be the best de-hydrogenation reaction conditions after exploring suitable conditions. Detailed examination of the de-hydrogenation product, 1,4-diene analog by radio-HPLC, HPLC-MS, proton and tritium NMR found that not only had the de-hydrogenation taken place, but also 11-hydroxy group was oxidized to ketone at the same time. This problem was resolved by acetylation protection of both 11,21-dihydoxy groups. De-protection of tritium labeled 11,21-diacetyl 1,4-diene intermediate in $K_2CO_3/CH_3OH/H_2O$ gave expected final product, [1,2-^{3}H]fluocinolone acetonide with a specific activity of 36.8 Ci/mmol (radiochemical purity: 97%) after purification.

Keywords: fluocinolone acetonide; oxidative de-hydrogenation; tritium labeled, benzeneseleninic anhydride

Introduction: Fluocinolone acetonide is a compound with anti-inflammatory, anti-pruritic properties and it has been used for treatment of various kinds of skin disorders since 1965 for its glucocorticoid activity. In addition, it has also been used for ear, eye, and nose inflammatory disorders.[1] Recent uses of fluocinolone acetonide include treatment of melasma when combined with other components in a cream,[2] treatment of retinopathy[3] and uveitis.[4] Tritium labeled fluocinolone acetonide with high specific activity was needed in receptor binding studies. To our best knowledge, tritium labeling of fluocinolone acetonide has not been reported although carbon-14 labeled version was used in mice[5] and human.[6]

Results and Discussion: Based on literature data[7] and our knowledge of the reactions of this compound class, Scheme 1, shown below, seemed a straightforward approach to tritium labeled fluocinolone acetonide [^{3}H]-1.

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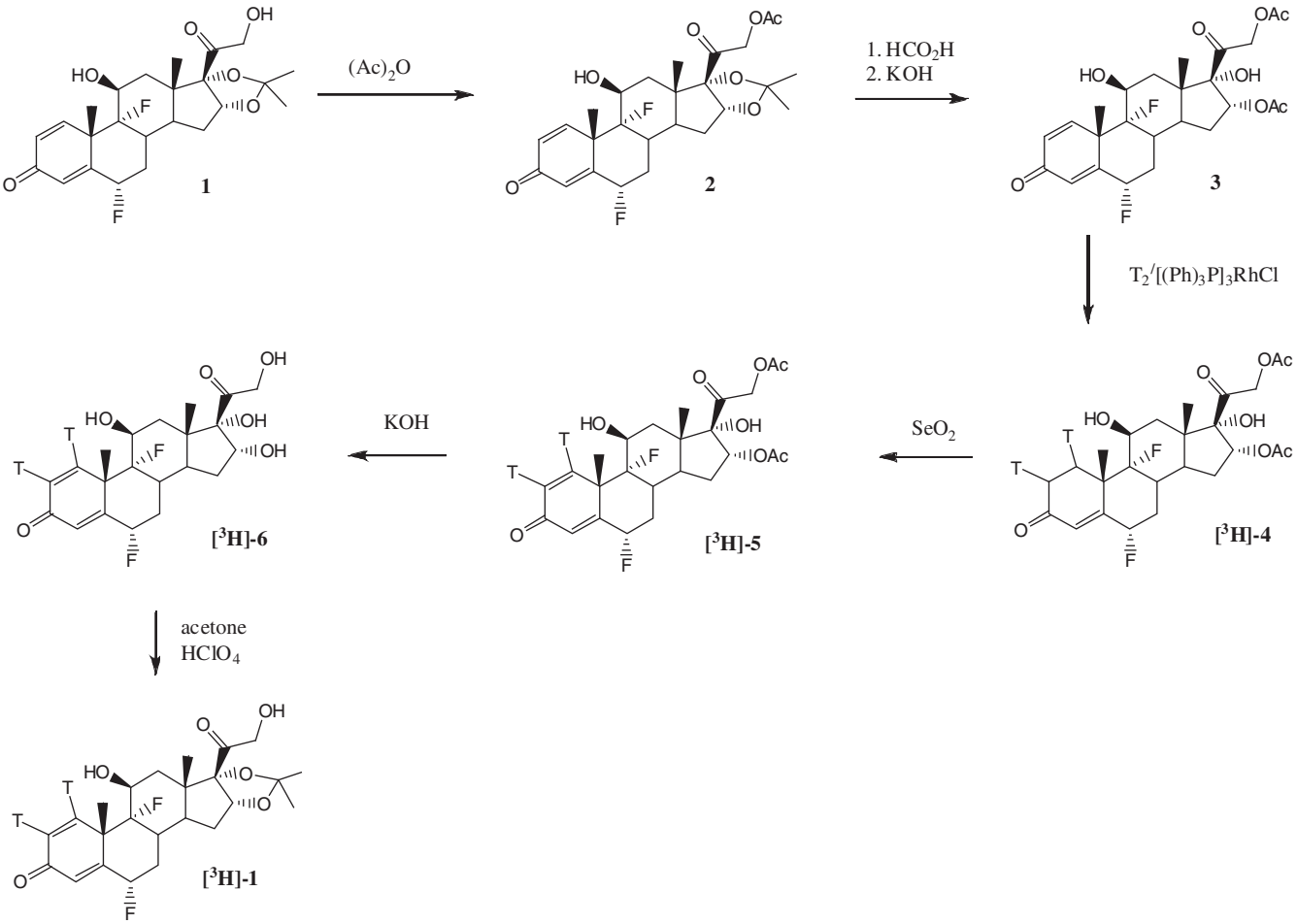
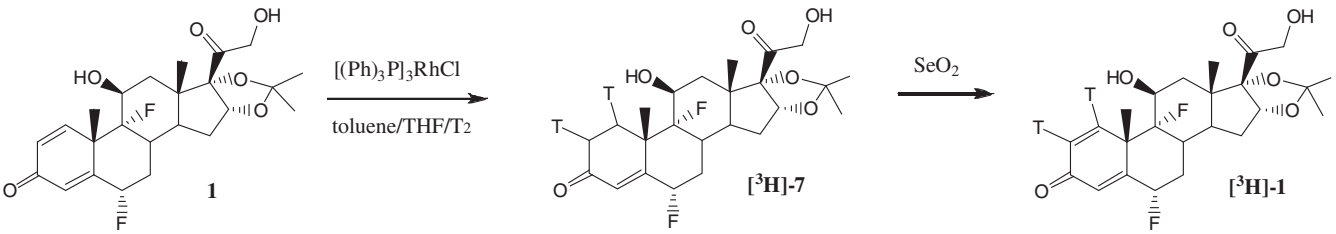


Scheme 1.

The key steps would be the selective catalytic tritiation of 1,2-double bond of unlabeled fluocinolone acetonide and regeneration of the 1,2-double bond by oxidative de-hydrogenation after tritiation reaction.

In order to save time and to improve radiochemical yield, we briefly investigated the shortest synthesis route (Scheme 2, shown below). Hydrogenation of fluocinolone acetonide under the modified literature condition[vii] using Wilkinson's catalyst gave the expected unlabeled intermediate **7**. Oxidative de-hydrogenation of **7** using selenium oxide as oxidizing agent gave a multi-component mixture (HPLC). This result was not totally surprising since the reported[ix] yield for this kind of reaction was only about 10% even when 21-hydroxy group was acetyl protected.

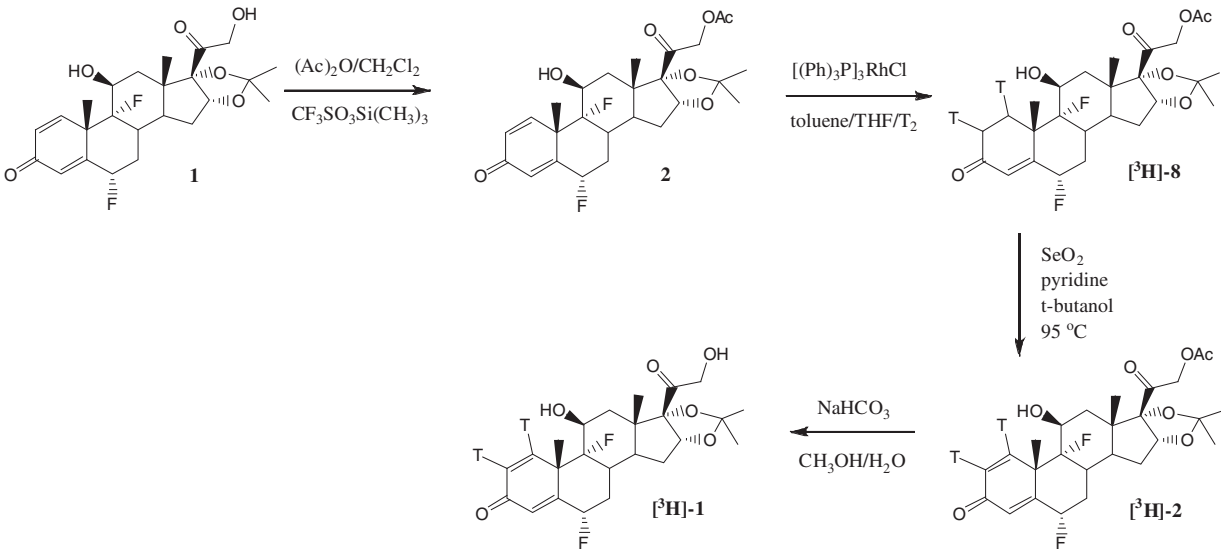


Scheme 2.

It was therefore decided to protect the 21-hydroxy group and obtain tritium labeled fluocinolone acetonide as shown in Scheme 3. Selective protection of the 21-hydroxy group (secondary alcohol) was achieved with acetic anhydride using trimethylsilyltrifluoromethanesulfonate in methylene chloride. Selective hydrogenation of 21-acetate gave the expected 4-ene (**[^{3}H]-8**) analogue.

Oxidative de-hydrogenation of the 4-ene using selenium dioxide in pyridine and t-butanol under reflux (at 95°C) re-generated the 1,2-double bond. Selective de-protection of the 21-acetate without hydrolyzing the acetonide under separately optimized condition ($NaHCO_3/CH_3OH/H_2O$) gave fluocinolone acetonide.

Problems were encountered when the same approach was applied to the radiosynthesis. Selective tritiation of 21-acetate **2** with tritium gas using Wilkinson's catalyst in toluene/THF gave tritiated 4-ene in good yield (317 mCi, 26% radiochemical yield). Refluxing of a mixture of tritiated 4-ene with selenium dioxide in pyridine/t-butanol did not give the expected 1,4-diene **[^{3}H]-2** although the radio-HPLC peak of tritiated 4-ene (**[^{3}H]-8**) disappeared. Instead, most of the radioactivity appeared in the solvent front. This implied that tritium-hydrogen exchange preceded oxidative de-hydrogenation under the reaction condition



Scheme 3.

When oxidative de-hydrogenation of unlabeled 4-ene **8** was tried using DDQ (2,3-dichloro-5,6-dicyano-1,4-benzoquinone) in refluxing benzene[10] for 14 hours, clean unlabeled 1,4-diene **2** was obtained. Surprisingly, no oxidative de-hydrogenation took place when tritium labeled 4-ene **[^{3}H]-8** was treated under the same condition. Instead, most of **[^{3}H]-8** was recovered.

It was concluded that oxidative de-hydrogenation conditions should be explored with tritium labeled 4-ene **[^{3}H]-8**. Different oxidative de-hydrogenation conditions, including refluxing selenium dioxide/dioxane,[11] hν/DIB/I_2/CH_2Cl_2, hν/DIB/I_2/toluene,[12], refluxing iodoxybenzene/benzeneseleninic anhydride/toluene,[13] and refluxing benzeneseleninic anhydride/toluene,[8] were tried to regenerate the 1,2-double bond from tritium labeled 4-ene [^{3}H]-8. Only the last condition (refluxing benzeneseleninic anhydride/toluene), followed by de-acetylation gave a mixture displaying two vinyl resonances and two aliphatic resonances in the tritium NMR spectrum (Figure 1). The chemical shifts of the latter were not consistent with those of the tritiated starting material. Moreover, HPLC co-injection of this material, which was expected to contain the tritium labeled final product [^{3}H]-1, with authentic fluocinolone acetonide (1) generated two very closely eluting UV peaks (Figure 2). Treatment of the product with metal scavengers showed that the differences in the NMR and HPLC profiles of the unlabeled reference and the tritiated product were not caused by complexation of transition metal (from Wilkinson's catalyst) with the product. The different retention time could also be caused by an isotope effect, but this possibility was removed by observing that the proton NMR spectra of purified product (thought-to-be [^{3}H]-1) and the authentic unlabeled fluocinolone acetonide were different. Mass spectrometric analysis of the de-hydrogenation product obtained from unlabeled 4-ene 8 under the same condition followed by de-protection showed that the 11-hydroxy group was oxidized to ketone. In other words, the de-hydrogenation product from tritium labeled 4-ene [^{3}H]-8 using refluxing benzeneseleninic anhydride/toluene was [^{3}H]-9, not expected [^{3}H]-2 (Scheme 4). This is noteworthy since it has been reported that the 11-hydroxy was intact when DDQ was used for the same class of compound.[14,15]

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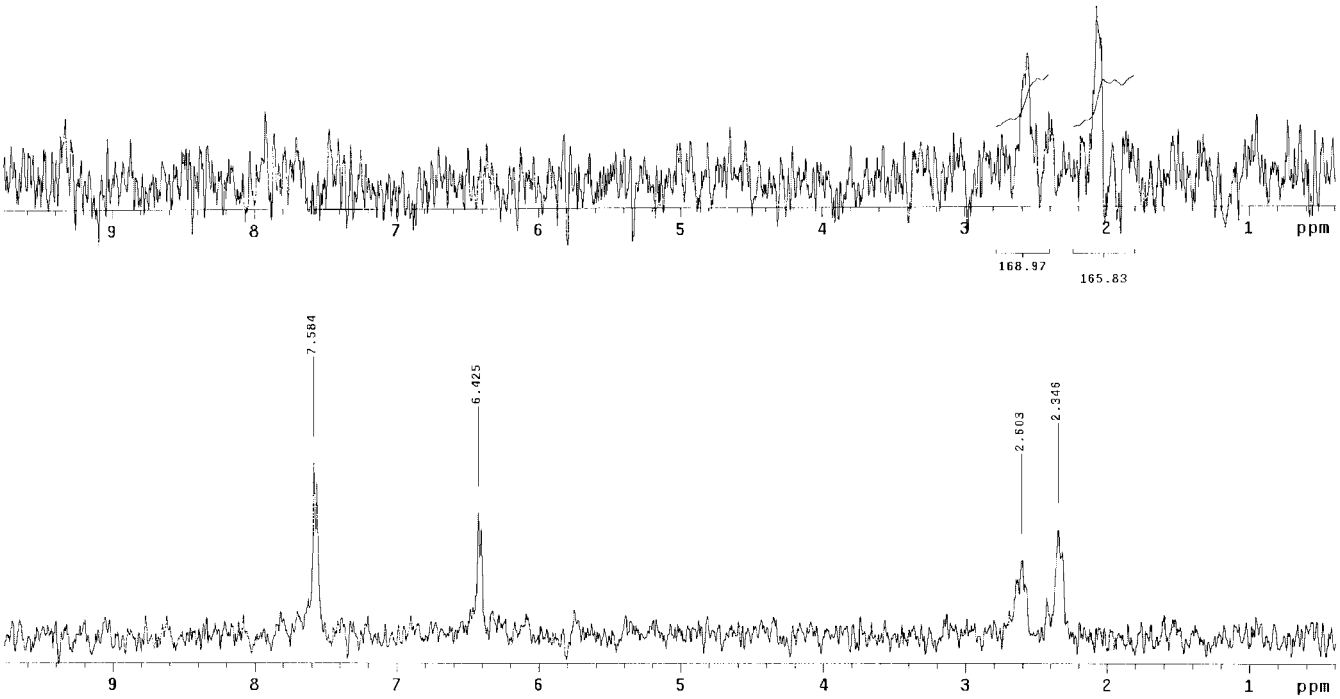


Figure 1. Tritium NMR: top: [^{3}H]-8; bottom: de-protected intermediate from refluxing benzeneseleninic anhydride/toluene.

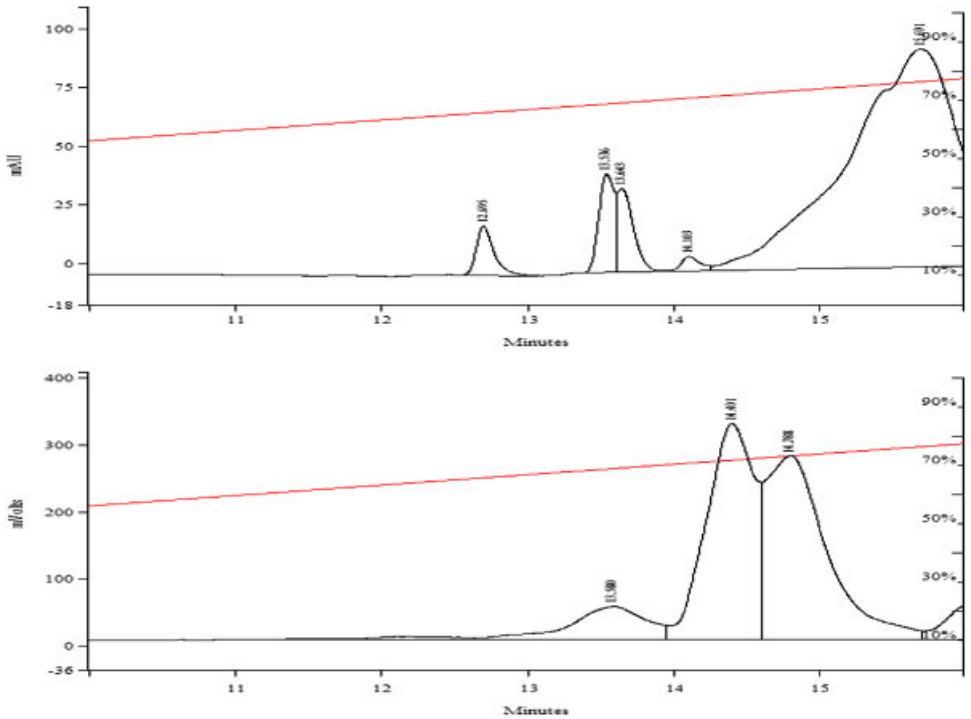
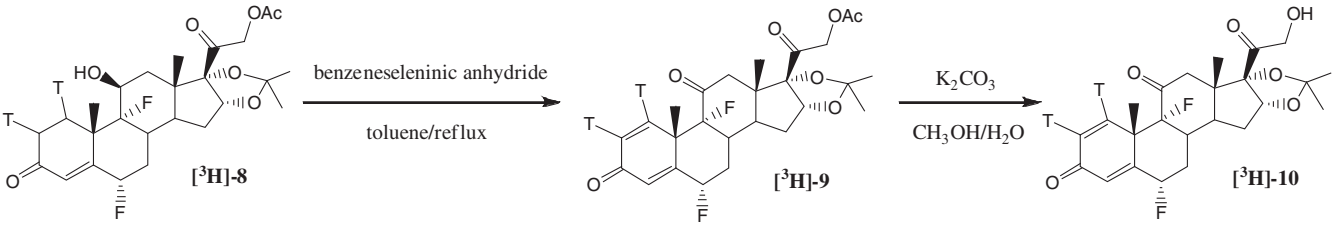


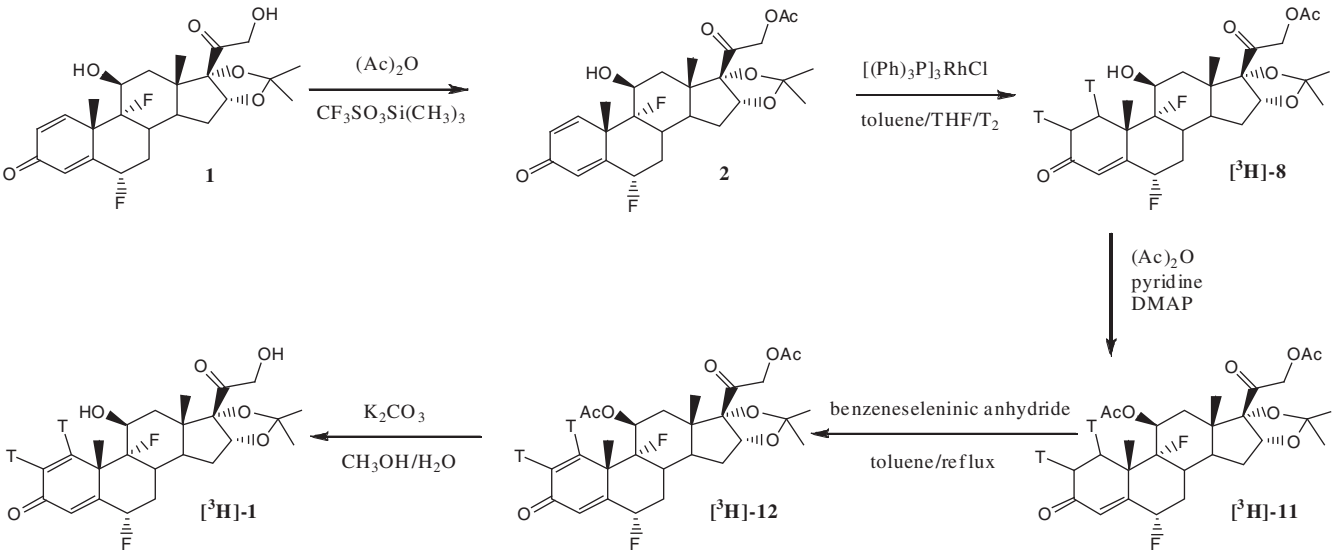
Figure 2. HPLC co-injection of de-protected intermediate from refluxing benzeneseleninic anhydride/toluene with authentic unlabeled fluocinolone acetonide: top: UV channel (235 nm); bottom: β-RAM channel.



Scheme 4.

Based on these findings, the synthetic approach shown in Scheme 5 was used to complete the synthesis of [^{3}H]-11. Reaction conditions were probed using 11,21-diacetate, which was generated by reacting unlabeled 4-ene **8** with acetic anhydride in pyridine and DMAP. Longer reaction time was needed in the oxidative de-hydrogenation of 4-ene-11,21-diacetate **11**. The de-protection condition ($NaHCO_3/CH_3OH/H_2O$) used for 21-acetate **2** was found inappropriate for the 11,21-diacetate as the de-acetylation of 11-acetyl was sluggish and the de-protected product decomposed during longer reaction time. The best de-protection condition found for 11,21-diacetate was $K_2CO_3/CH_3OH/H_2O$.

The radiosynthesis was continued by recovering tritium labeled 11-hydroxy-4-ene- **[^{3}H]-8** (261 mCi) from an unsuccessful DDQ oxidation attempt using an alumina solid phase extraction cartridge. Acetylation of the recovered material with acetic anhydride in pyridine and DMAP gave 11,21-diacetate (238 mCi) and this material was oxidatively de-hydrogenated using refluxing benzeneseleninic anhydride/toluene to afford 1,4-diene **[^{3}H]-12.** This required a much longer period of time (37 hours) than the analogous reaction with unlabeled material (7 hours), but the reaction was clean. After C-18 solid phase extraction cartridge purification, two fractions, 71.3 mCi (98% radiochemical purity), and 21 mCi (76% radiochemical purity), were obtained. Separate de-protection of the two fractions gave crude final product **[^{3}H]-1** (60.4 mCi with 49% radiochemical purity and 15.9 mCi with 49% radiochemical purity, respectively). The pure final product **[^{3}H]-1** (30.8 mCi with 97% radiochemical purity and 95% chemical purity) was obtained from prep-HPLC purification of the combined fractions. The specific activity (36.8 Ci/mmol) was determined using an HPLC calibration curve generated from the authentic unlabeled fluocinolone acetonide (**1**). The product identity was confirmed by HPLC co-injection with the authentic unlabeled fluocinolone acetonide.



Scheme 5.

Conclusion: Tritium labeled fluocinolone acetonide (30.8 mCi) with high specific activity (36.8 Ci/mmol) was synthesized from unlabeled fluocinolone acetonide by selective tritiation of the 1,2-double bond, followed by re-generation of the 1,2-double bond by oxidative de-hydrogenation with benzeneseleninic anhydride in refluxing toluene. Protection of both the 21- and 11-hydroxy functionalities was found to be necessary in the syntheses.

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SYNTHETIC STUDIES OF [M+5] HYDROXYMIDAZOLAM

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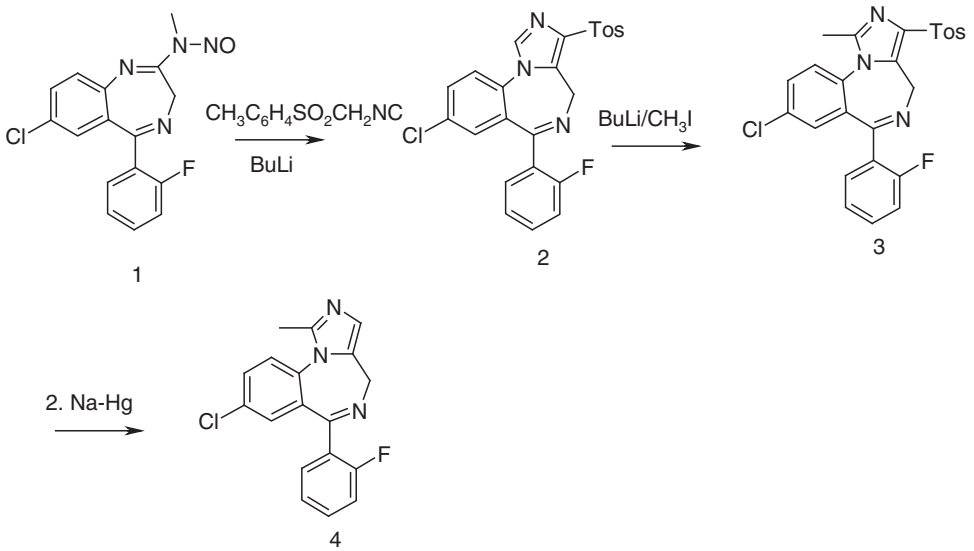
Abstract: Midazolam is often used for drug interaction research and stable isotope labeled hydroxymidazolam is needed for those studies. Although the synthesis of an M+3 hydroxymidazolam has been published, it is much more desirable to have a stable isotope labeled hydroxymidazolam which has a molecular weight of 4 or more mass units higher than the parent compound because of the presence of a chlorine atom in the molecule. Herein we report our efforts on the synthesis of M+5 hydroxymidazolam, in which the key step is the use of 3-(4-tosyl)imidazo[1,5-a][1,4]-benzodiazepines. Details of the synthesis will be discussed.

Keywords: stable isotope labeling; midazolam; hydroxymidazolam; synthesis

Introduction: Hydroxymidazolam is a metabolite of midazolam which is an ultra short-acting benzodiazepine derivative that has found many therapeutical applications including anxiolytics, hypnotic, anticonvulsant, and as a skeletal muscle relaxant[1]. Stable isotope labeled hydroxymidazolam is often used as an internal standard in bio-analytical studies.

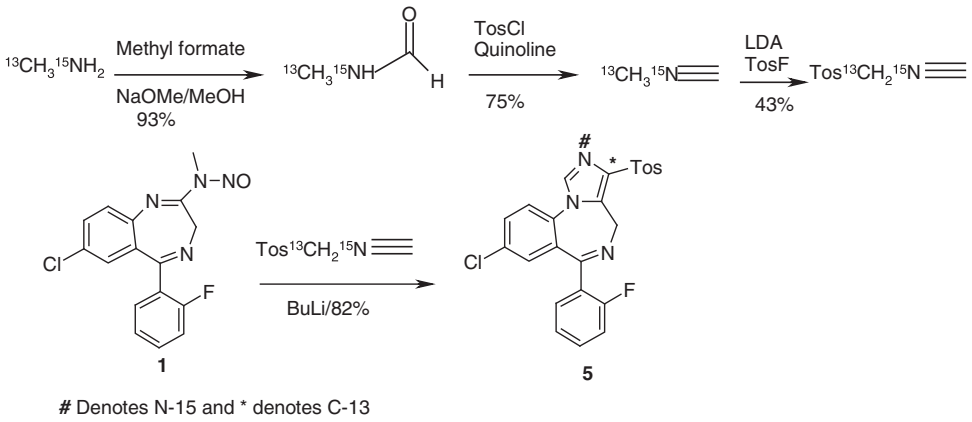
Two chemical syntheses have been reported for hydroxymidazolam, one of which used midazolam as the key intermediate. The oxidation of midazolam and subsequent rearrangement furnished hydroxymidazolam in very low yield.[2] The other reported synthesis of a stable isotope labeled hydroxymidazolam with 3 mass unites higher than the non-labeled parent compound.[3] It avoided the low yield oxidation step and is a better synthetic route from chemical synthesis point of view. But it is much more desirable to have a stable isotope labeled hydroxymidazolam with a molecular weight of 4 or more mass units higher than the parent compound because of the presence of a chlorine atom in the molecule. A recent publication reported the formation of imidazo[1,5-a][1,4]benzodiazepines (**2**) by the reaction of 1,4-benzodiazepinic N-nitrosoamidines (**1**) with tosylmethyl isocyanide (Scheme 1). The treatment of **2** with butyllithium and methyl iodide, followed by the removal of tosyl group gave rise to midazolam (**4**).[4]

This looked attractive to us since the anion of **2** could react with an oxygen carrying precursor to form a compound that could later on be converted to hydroxymidazolam.



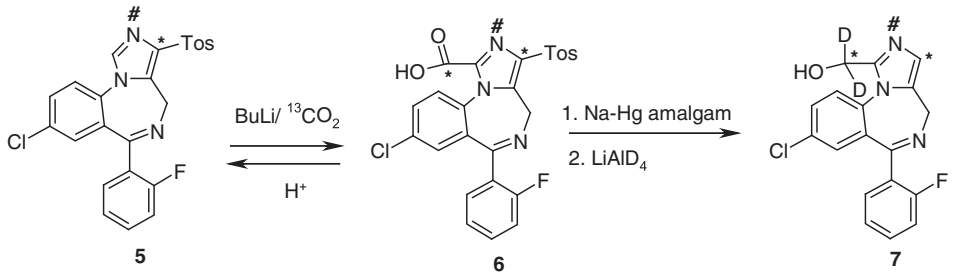
Scheme 1. Synthesis of Midazolam (4)

Results and Discussion: The synthesis of stable isotope labeled key intermediate **5** is outlined in Scheme 2. Thus, [^{13}C, ^{15}N] methylamine was vacuum transferred to a methanol solution of methylformate with a catalytic amount of NaOMe to afford [^{13}C, ^{15}N] methyl formamide, which was then treated with tosyl chloride in quinoline to afford [^{13}C, ^{15}N] methyl isocyanide. [^{13}C, ^{15}N] Methyl isocyanide was converted to [^{13}C, ^{15}N] tosyl methylisocyanide by first treating with n-butyllithium, followed by reacting with tosyl fluoride. The coupling of [^{13}C, ^{15}N] tosyl methylisocyanide with N-nitrosoamidine (**1**) furnished [^{13}C, ^{15}N] imidazo[1,5-a][1,4]benzodiazepines (**5**) in a reasonable overall yield (25%).



Scheme 2. Synthesis of 5.

Our initial plan was to introduce a [^{13}C] carboxylic group in the imidazole ring to form the carboxylic acid (**6**); the removal of the tosyl group and the reduction of the carboxylic group with $LiAlD_4$ should provide [M+5] hydroxymidazolam (**7**) (Scheme 3).



Scheme 3. Planned Synthetic Route for M+5 Hydroxymidazolam (7).

Unfortunately, the carboxylation reaction failed to provide carboxylic acid **6**. Compound **6** was so unstable, due to the presence of a strong electron withdrawing tosyl group in the imidazole ring, that decarboxylation occurred after the reaction mixture was neutralized. LC-MS analysis of the reaction mixture showed the presence of a small amount of desired product (9%) immediately after the reaction mixture was acidified. But no product was detected by LC-MS the next day.

We then decided to synthesize the tosyl hydroxymidazolam directly from [^{13}C, ^{15}N] imidazo[1,5-a][1,4]benzodiazepines (**5**) and [$^{13}CD_2$] paraformaldehyde. Thus, **5** was first treated with one equivalent of n-butyllithium in THF, and then [$^{13}CD_2$] formaldehyde, obtained by heating [$^{13}CD_2$] paraformaldehyde at 180°C, was passed through the reaction mixture with the help of a slow stream of nitrogen gas. The desired product **8** was obtained in moderate yield (about 40%), along with unreacted starting material, and was used directly in the next step. Unfortunately, treatment of **8** with sodium-mercury amalgam did not afford any desired product **7**. Instead, it produced the hydroxydihydromidazolam (**10**) as the major product. LC-MS analysis of incomplete reaction mixture indicated that the reaction occurred in a stepwise manner: the tosyl hydroxydihydromidazolam (**9**) formed first, and subsequent de-tosylation of **9** afforded **10** as the final product (Scheme 4).

The result was a surprise to us. We did not expect the hydroxymethyl group in **8** to have such an effect over the methyl group in compound **3**, both in the imidazole ring, that it induced hydrogenation at a distance carbon-nitrogen double bond prior to the removal of the tosyl group, while compound **3** afforded midazolam (**4**) with the carbon-nitrogen double bond intact, under the exact same reaction conditions.[4]

This prompted us to prepare stable isotope labeled midazolam according to the published procedure.[4] Our intention was to verify that the removal of the tosyl group would not cause saturation of the carbon-nitrogen double bond, as the authors had claimed, and if this was true, to synthesize [M+5] hydroxymidazolam from midazolam according to the published procedure.[2]

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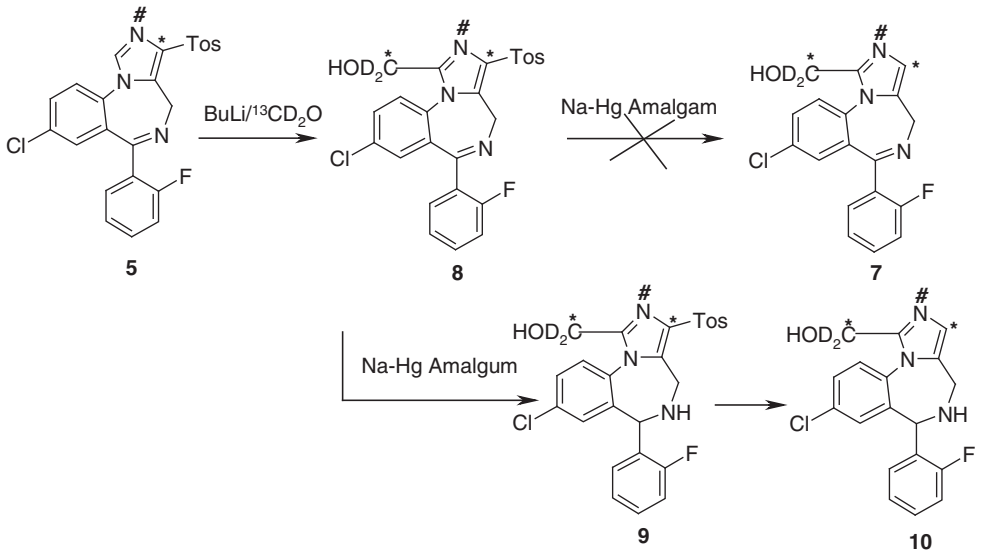
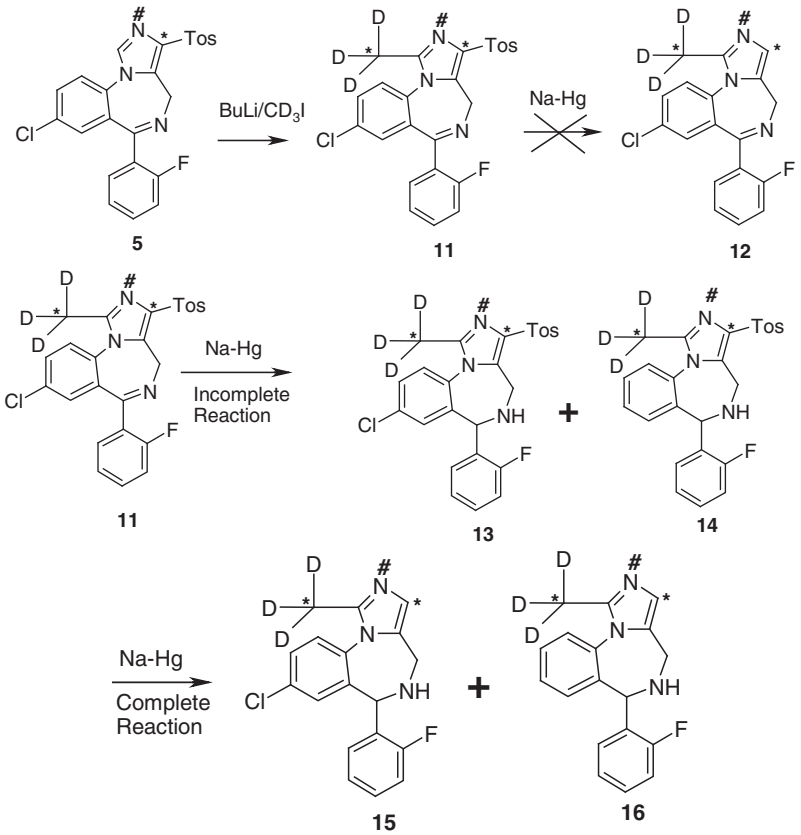


Scheme 4. Attempted Synthesis of M+5 Hydroxymidazolam (7).

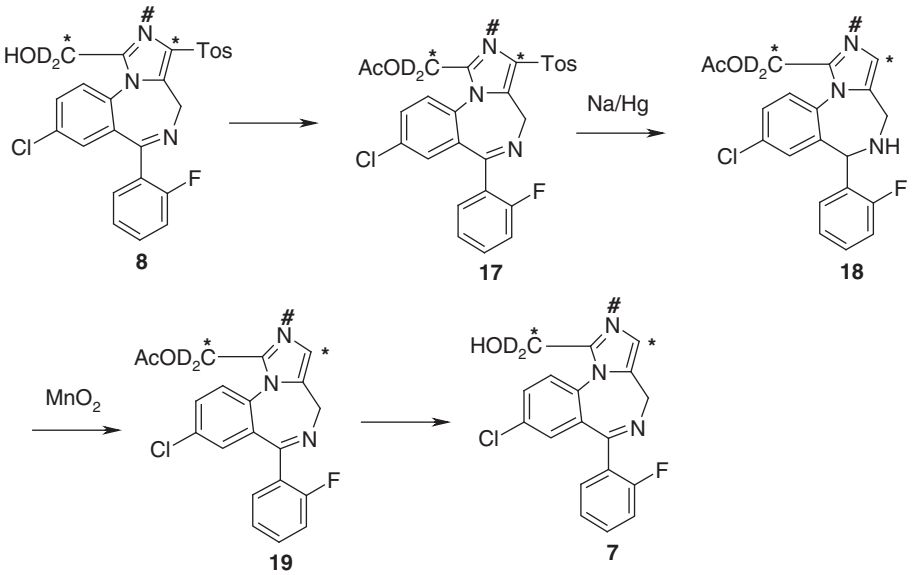
Stable isotope labeled tosyl midazolam (**11**) was synthesized by treating **5** with n-butyllithium and [$^{13}CD_3$] methyl iodide. Contrary to what the authors had claimed[4], no stable isotope labeled midazolam (**12**) was detected when **11** was treated with Na-Hg amalgam. LC-MS analysis of the reaction mixture indicated that tosyl dihydromidazolam (**13**) and the corresponding de-chlorinated product **14** was produced initially, and de-tosylation products **15** and **16** formed afterwards (Scheme 5).



Scheme 5. Attempted Synthesis of M+6 Midazolam (12).

This result was partially consistent with our work with tosyl hydroxymidazolam and clearly indicated that de-tosylation reaction will not lead to the formation of desired product directly.

We are currently pursuing the synthesis of stable isotope labeled hydroxymidazolam through a modified synthetic plan (Scheme 6). The key of the new synthesis is to re-introduce the carbon-nitrogen double bond to form **19** after the removal of the tosyl group in acetyl hydroxydihydromidazolam (**18**). Hydrolysis of **19** should provide [M+5] hydroxymidazolam (**7**).



Scheme 6. Revised Synthetic Plan for M+5 Hydroxymidazolam (7).

Conclusion: The synthesis of [M+5] hydroxymidazolam through a key intermediate of [^{13}C, ^{15}N] imidazo[1,5-a][1,4]benzodiazepines (**5**) was investigated. The de-tosylation reaction of both tosyl hydroxymidazolam (**8**) and tosyl midazolam (**11**), unfortunately, failed to afford [M+5] hydroxymidazolam (**7**) and [M+6] midazolam (**12**) respectively. A modified synthesis of [M+5] hydroxymidazolam through the re-introduction of double bond after the removal of the tosyl group is currently under investigation.

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