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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 17, Thursday, June 18, 2009

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Abstract: This session is a continuation of Session 1. A number of methods detailing the synthesis of tritiated compounds as well as a discussion on the handling of tritium on a multi-Curie scale are presented. In addition, the descriptions of a number of preparations of individual isotopically labelled compounds are detailed.

Keywords: tritium; [^{3}H]-methyl nosylate; iridium catalysts; polyphenols; Rhodium black; Crabtree's catalyst; Carbon-14; Deuterium; PPAR; CXCR3; [^{3}H]-methyl iodide; Tritec; Carbonylation; Delta-opioid receptor

THE SYNTHESIS OF TRITIUM-LABELED COMPOUNDS

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Abstract: Tritium-labeled compounds are synthesized frequently in our group in order to assess metabolism. Tritium is the label of choice because these compounds can be synthesized rapidly in most circumstances. Tritium gas and tritiated water are common label sources, in addition to other commercially available tritiating reagents. This paper will focus on recent uses of sodium borotritide, [^{3}H]methyl nosylate, and iridium catalysts with tritium gas or tritiated water in our lab.

Keywords: tritium; sodium borotritide; [^{3}H]methyl nosylate; iridium catalysts; tritium gas; tritiated water

Introduction: A number of tritium-labeled compounds are synthesized each year in our lab using a variety of approaches. Because of the need for rapid turn-around, simple labeled starting materials are used. This paper will explore four methods that we commonly use in order to label compounds with tritium. These methods include: 1) [^{3}H]methyl nosylate, 2) the use of iridium catalysts for ortho-directed exchange (specifically Ir(COD)(F_6AcAc) with tritiated water [THO] and Ir($Ph_3P)_2$(COD)BF_4 with tritium gas [T_2]), 3) sodium borotritide used in reduction and dehalogenation reactions, and 4) the use of sodium borotritide/palladium acetate in dehalogenation reactions.

Results and Discussion: *[^{3}H]methyl nosylate*

We have found [^{3}H]methyl nosylate ([^{3}H]methyl 4-nitrobenzenesulfonate) to be a very versatile and 'user-friendly' reagent for oxygen, nitrogen, and sulfur methylation reactions. The compound is supplied at a specific activity of approximately 85 Ci/mmol and is stored as a (1:3) ethyl acetate : hexanes solution at −80°C. We have found that the material is reasonably stable when stored at −80°C. However, it can be easily purified using a small silica gel column and (1:1) hexane : dichloromethane as eluent, if necessary. For example, the radiochemical purity (RCP) of a batch of [^{3}H]methyl nosylate was 78% after 1.5 years of storage. Following purification as described above, the RCP was 97%.

Two examples of the use of [^{3}H]methyl nosylate to synthesize high specific activity tritium-labeled ligands are shown in Figure 1.

In the first example, [^{3}H]methyl nosylate (50 mCi, 85 Ci/mmol) was reacted with approximately four equivalents of the phenol in dimethylformamide (DMF) using sodium tert-pentoxide as base. The RCP of product in the reaction mixture was 66% after 3 h.

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Following purification, [^{3}H]Sch A (22.1 mCi) at a specific activity of 85.2 Ci/mmol resulted. In the second example, [^{3}H]methyl nosylate (55.2 mCi, 85 Ci/mmol) was reacted with approximately four equivalents of the phenol in acetone/DMF using cesium carbonate as base. The RCP of product in the reaction mixture was 85% after 2 h. Following purification, [^{3}H]Sch B (46.8 mCi) at a specific activity of 86.6 Ci/mmol resulted.

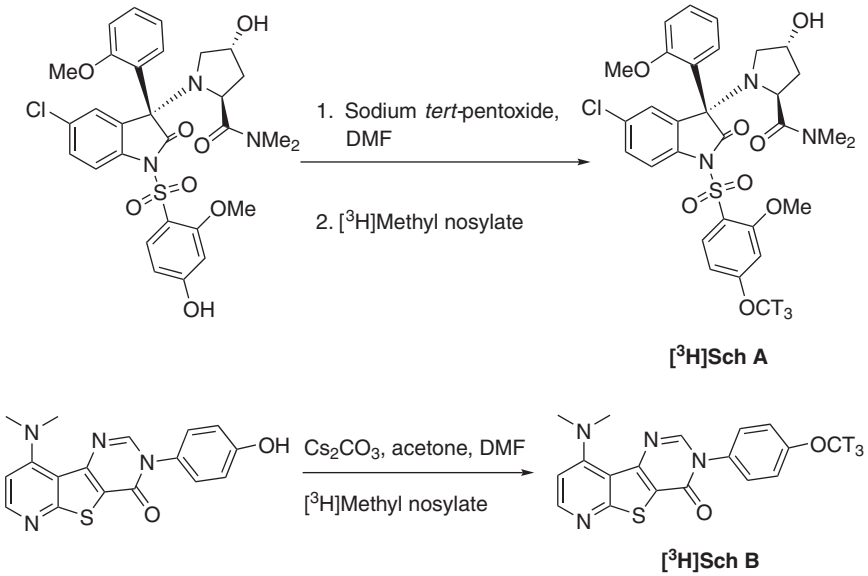

Figure 1. The use of [^{3}H]methyl nosylate to synthesize high specific activity tritium-labeled compounds.

Iridium catalysts and THO or T_2

Crabtree's catalyst [Ir(Py)(PCy_3)(COD)PF_6] is frequently used in our lab for ortho-directed tritium-hydrogen exchange. There are times, however, when either tritium incorporation is lower than we would like or the substrate is not soluble in dichloromethane, the only solvent in which the catalyst is soluble. At these times, we must screen other iridium catalysts for their reactivity and specificity.

The synthesis of Ir(COD)(F_6AcAc) and an example of the use of this catalyst in our lab is shown in Figure 2. Although this catalyst is now commercially available, at the time it was synthesized according to a literature procedure[1] using bis(1,5-cyclooctadiene)diiridium(I) dichloride and hexafluoro 2,4-pentanedione. The catalyst can be used for ortho-directed labeling of aromatics functionalized with nitrogen heterocycles using either THO or T_2 in either DMF or dimethylacetamide (DMA)[1,2]. In the example shown, an oxazole containing substrate (20 mg) was tritiated using Ir(COD)(F_6AcAc) (10 mg) and THO (500 mCi, 50 Ci/mmol) in DMA, resulting in 32 mCi of crude product **1** with a RCP of 27%. Although the tritium incorporation was modest, the results were reproducible so that an adequate amount of the desired compound could be made.

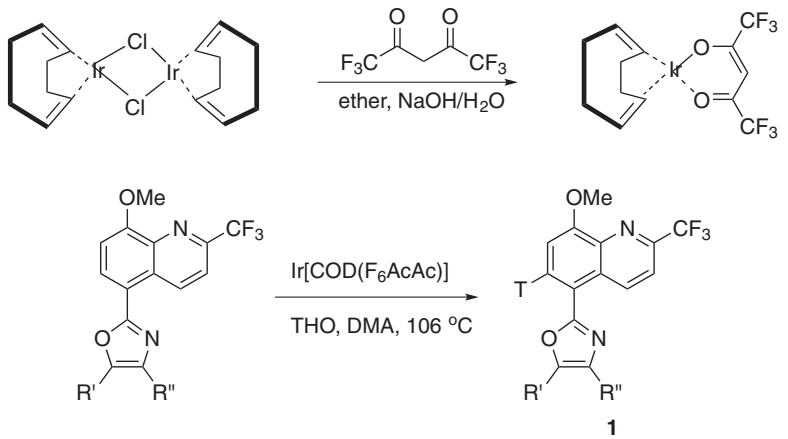

Figure 2. The synthesis of Ir(COD)(F_6AcAc) and its use as a catalyst for tritiation.

When we needed to incorporate tritium into compound **2** ('R' = H, 'acid sensitive group' = CN, Figure 3), our first choice was to use $Ru(PPh_3)_3Cl_2$ and THO (500 mCi) in dioxane at 120°C, but there was very little tritium incorporation. When harsher conditons were used (the reaction was run in DMSO at 150°C), the compound decomposed. Crabtree's catalyst was tried next with deuterium gas, with the results that when 1 eq, 0.25 eq, and 2 eq of catalyst were used, there was a small amount of deuterium incorporation, no deuterium incorporation, and compound decomposition, respectively. Another labeling method needed to be found.

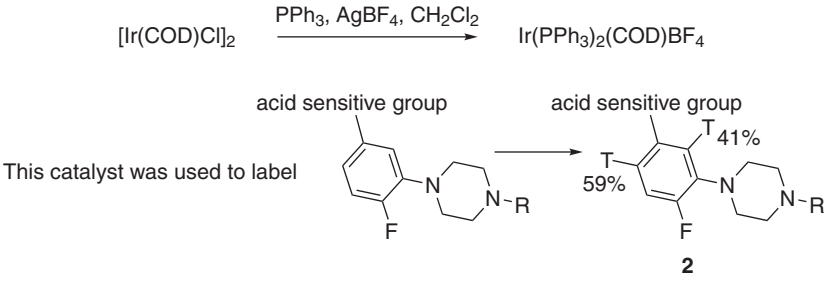

Figure 3. The synthesis of bis(triphenylphosphine)(cyclohexadiene)iridium (I) tetrafluoroborate.

The bis(triphenylphosphine)(cyclooctadiene)iridium(I) tetrafluoroborate catalyst was synthesized according to the literature procedure[3] as shown in Figure 3. This catalyst has been found useful in the incorporation of hydrogen isotopes *alpha* to heteroatom-containing functional groups[3]. When the catalyst (1 eq) was used with substrate **2** ('R' = H) and deuterium gas, there was better deuterium incorporation than when Crabtree's catalyst was used. However, when the reaction was duplicated using tritium gas, 40 mCi of crude product with a RCP of 8% resulted. Although the UV profile of the reaction mixture looked clean, there were many radioactive impurities, suggesting that the conditions needed to be optimized further. When compound **2** ('R' = *tert.*-BOC) was treated with catalyst (0.4 eq) and deuterium gas, there was a lot of deuterium incorporation. This reaction was repeated using tritium gas (850 mCi) to give 155 mCi of crude product at a RCP of 54%. Tritium NMR showed that tritium distribution in the final compound was as shown in Figure 3. This example illustrates that both varying the catalyst and the ratio of substrate to catalyst will affect the results of the experiment, with the outcome dependent on the nature of the substrate.

Sodium Borotritide

Sodium borotritide has been used in our lab for ketone reductions and dehalogenation reactions. It has also been used with palladium acetate for selective dehalogenation reactions in the presence of other reduction-sensitive functional groups. Examples of each of these reactions will be discussed. In general, the sodium borotritide is supplied in a small amber ampoule. The ampoule is cracked open and the reaction is run in the ampoule to minimize sodium borotritide loss and personnel/hood contamination.

A typical ketone reduction reaction using sodium borotritide, for the eventual synthesis of compound **3**, is shown in Figure 4. Benzophenone was treated with sodium borotritide (890 mCi was accounted for in a vial containing '500 mCi;' specific activity = 50 Ci/mmol) to give 762 mCi of [^{3}H]diphenylmethanol. To complete the synthesis of compound **3**, [^{3}H]chlorodiphenylmethane was synthesized by treating the alcohol with thionyl chloride, and the crude [^{3}H]chloro compound (80% RCP) was used in the Finkelstein reaction with a substituted piperazine to give 488 mCi of compound **3** with a RCP of 59%.

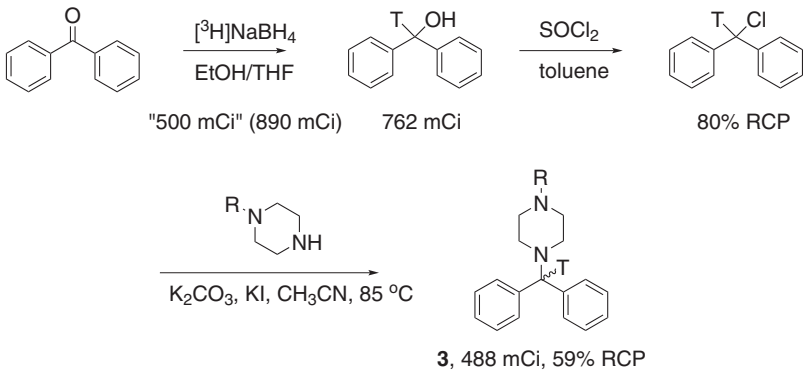

Figure 4. Typical ketone reduction using sodium borotritide.

The use of sodium borotritide in a dehalogenation reaction is shown in Figure 5. In this Suzuki reaction, a vinyl bromide was treated with sodium borotritide (125 mCi, specific activity = 15 Ci/mmol) in the presence of tetrakis(triphenylphosphine)palladium(0). There was a concern about potential side reactions with other functional groups on the molecule so the reaction did not go to completion. However, 11.1 mCi of compound **4** was produced at a specific activity of 2.5 Ci/mmol.

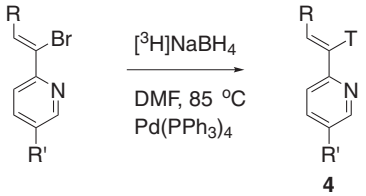

Figure 5. The use of sodium borotritide in dehalogenation in tritiation.

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Finally, we have used sodium borotritide with palladium acetate to carry out selective dehalogenation reactions. This reagent combination is very useful because it can be used to dehalogenate aryl halides in the order of I > Br > Cl > F, double bonds and triple bonds are not reduced, esters are not affected, and secondary hydrogen/tritium exchange does not occur[4]. In the reaction set-up, the substrate is pre-treated with palladium acetate in methanol for 10 min. The resulting mixture is then added to the ampoule containing the sodium borotritide. After one hour, the mixture is filtered and then immediately purified in order to prevent product decomposition.

An example of the use of sodium borotritide/palladium acetate is shown in Figure 6. A substrate containing an alkyne and ester was treated with *N*-iodosuccinimide to give a mono- and di-iodinated product. The di-iodinated product was reacted with the sodium borotritide (500 mCi)/palladium acetate mixture to give 21 mCi of compound **5** at a specific activity of 15.7 Ci/mmol.

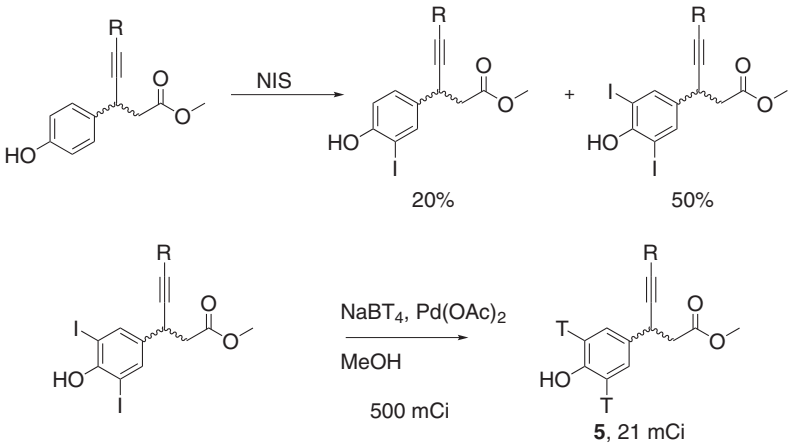

Figure 6. The use of sodium borotritide/palladium acetate in tritiation.

Conclusion: We use a variety of techniques in our lab to label compounds with tritium, and a few of these methods were highlighted in this paper. To summarize, we consider [^{3}H]methyl nosylate to be a very 'user friendly' reagent that can be used to produce high specific activity ligands. The different iridium catalysts that are available complement each other in terms of their reactivity and specificity. Sodium borotritide can be used to label compounds via reduction or dehalogenation reactions, or in conjunction with palladium acetate for dehalogenation reactions on substrates containing other reduction-sensitive functional groups.

Acknowledgements: The authors would like to thank Drs. T. M. Chan and Mary Senior from the Schering-Plough Research Institute Molecular Spectroscopy group for tritium NMR analysis of synthesized compounds. Thanks are also due to SPRI Chemical Research for some starting materials and synthetic procedures.

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TOTAL SYNTHESIS OF ^{14}C-LABELED PROCYANIDIN B2

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Abstract: During the last decades, many *in vitro* and *in vivo* studies have shown the beneficial effects on health of procyanidins. However, their absorption and metabolism is still not fully understood and some aspects are still controversial. In order to have a clearer picture of the metabolism of procyanidins, the use of labelled compounds is essential. In this context, the enantioselective synthesis of ^{14}C-radiolabelled procyanidin B2 was developed in our laboratories. It was achieved in fourteen 'hot' steps, involving

as key steps the Sharpless dihydroxylation of an elaborated alkene, a stereoselective intramolecular cyclization to benzylated (+)-catechin and the condensation of two (−)-epicatechin units. 11 mCi of protected procyanidin B2 were obtained from 524 mCi of potassium [^{14}C]cyanide.

Keywords: carbon-14C; radiolabeled synthesis; procyanidins; catechins; polyphenols

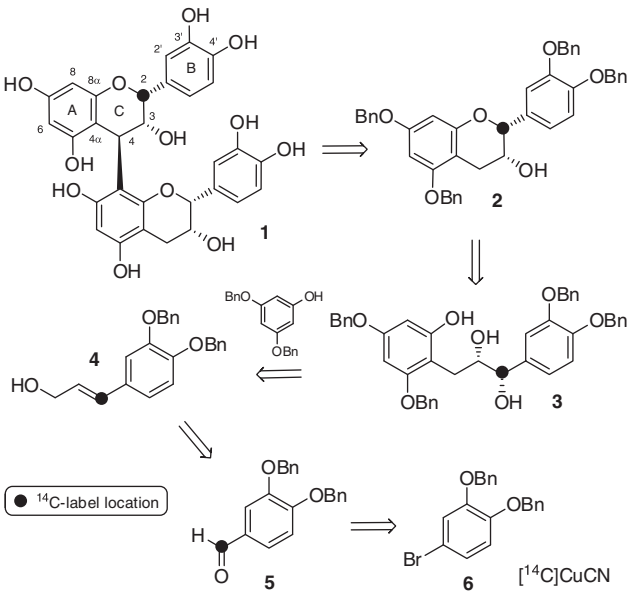
Introduction: Flavan-3-ols and their oligomeric procyanidin forms represent one of the major dietary families of polyphenols; fresh fruits, tea, cocoa and dark chocolate are particularly rich in procyanidins.[1] During the last few decades, many *in vitro* and *in vivo* studies have shown the beneficial effects of procyanidins on health.[2] However, their absorption and metabolism is still not fully understood and some aspects are still controversial.[3]

Radiolabeled compounds have proven invaluable in metabolism studies for tracing parent compounds and their metabolites. Therefore, to support our continuous efforts in studying polyphenols metabolism, we decided to undertake the preparation of radiolabeled procyanidin B2 [^{14}C]-**1**, one of the major procyanidin present in cocoa and chocolate.

Previous syntheses of procyanidins were based on the coupling of readily available flavan-3-ol monomers.[4] However, this strategy was not compatible with the introduction of a ^{14}C-label in the target molecule.[5] Proton/tritium exchange from a labile site of a natural precursor was also discarded so as not to compromise the stability of the target molecule during biological assays.[6]

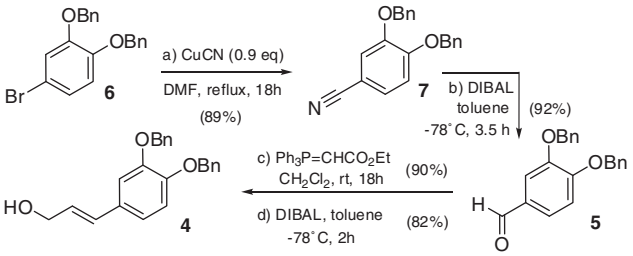
Our rational to delineate a synthetic pathway compatible with the constraints of a radiolabeled synthesis is described here. The key milestones in the development of the first asymmetric total synthesis of procyanidin B2 ((−)-epicatechin-(4β-8)-(−)-epicatechin) and its application to the preparation of a regioselectively radiolabeled ^{14}C–analogue are also summarized.[7]

Results and discussion: Our retrosynthetic analysis of procyanidin B2 is shown in Scheme 1. In terms of a radiolabeling strategy, preliminary work led us to consider position 2 of the upper C–ring moiety as the most feasible position to introduce the radioactive label. Thus, the target ^{14}C-labeled molecule was envisioned to arise from the heterologous coupling of radiolabeled/non-labeled (−)-epicatechin functionalized units. This coupling step is known to require an excess of one of the two units, which precluded labeling of both top and bottom moieties of procyanidin B2.



Scheme 1. Retrosynthetic analysis of ^{14}C-labeled procyanidin B2 [^{14}C]-**1**.

Our work started with securing an efficient and reliable synthetic pathway, compatible with the constraints of a radioactive synthesis. Scheme 2 presents the synthetic approach for the cinnamyl alcohol **4**, starting from aryl bromide **6**.[8] The direct condensation of **6** and CuCN provided in high yield nitrile **7** which was then elaborated into the cinnamyl alcohol intermediate **4** in three steps.



Scheme 2. Synthesis of cinnamyl alcohol intermediate **4**.

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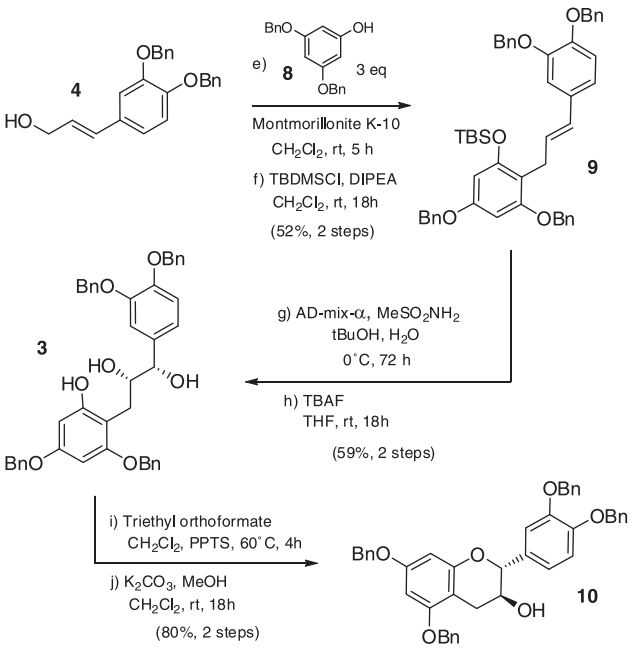
The elaborated *trans* alkene **9** having the necessary C6-C3-C6 skeleton was then prepared from **4** (Scheme 3). In the synthetic route we selected, this coupling step of C6-C3 and C6 units was generally limiting due to 'over-alkylation' of the C6 phenolic unit. This potential issue of coupling alcohol **4** with 1,3–dibenzylphloroglucinol **8**[9] was overcome by ensuring that the phloroglucinol was permanently present in excess in the reaction medium. When using 3 equivalents of **8** and performing a slow addition of **4** the condensation proceeded cleanly and with a satisfactory conversion to afford **9**. This material was then converted into benzylated (+)-catechin **10** using the sequence described by Wan *et al.*[10] Thus, Sharpless asymmetric dihydroxylation of **9** led to diol **3**, which was cyclised to benzylated (+)–catechin **10** using triethyl orthoformate in the presence of PPTS followed by methanolysis.[11] This cyclization proceeded cleanly with inversion of the stereochemistry at carbon C-2, the free phenolic hydroxyl group reacting with the intermediate cyclic orthoacetate.[10]

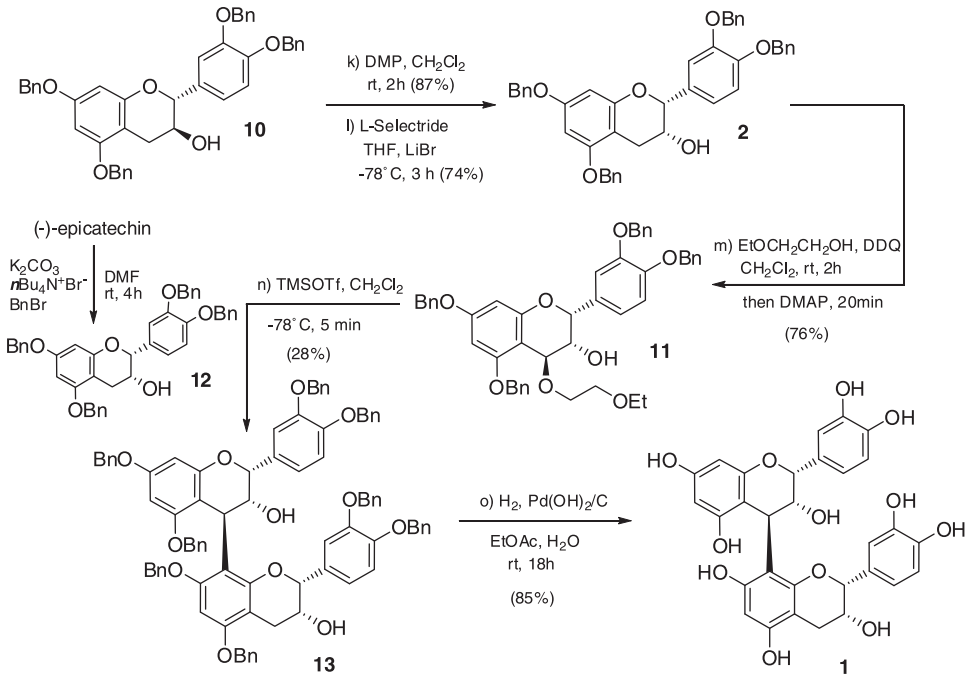


Scheme 3. Synthesis of benzylated (+)-catechin **10**.

10 was then converted into the corresponding (−)-epicatechin derivative **2** as shown in Scheme 4.[10,12] The chiral purity of **2** was assessed to be > 92%.[7]

Having secured benzylated (−)-epicatechin **2** in 12 steps and 9.5% overall yield from aryl bromide **6** and with excellent chiral enrichment, we turned our efforts towards the preparation of the dimer procyanidin B2 (Scheme 4). Treating **2** with DDQ in the presence of 2-ethoxyethanol led to the activated monomer **11**.[13] Condensation of **11** with 4 equivalents of benzylated (−)-epicatechin **12** (prepared from commercially available (−)-epicatechin) led to dimer **13**.[13,14] Finally, debenzylation of **13** into procyanidin B2 **1** was achieved by hydrogenation with palladium hydroxide.[15]

At this point, we felt sufficiently confident in our synthetic pathway to undertake the preparation of the ^{14}C-analogue labeled at position 2, without the need for further optimizations. [^{14}C]CuCN was prepared from 524 mCi of [^{14}C]KCN,[16] and used as the radioactive precursor in the radiolabeled synthesis of [^{14}C]-**1**. In the synthesis of radioactive procyanidin B2, results comparable to the synthesis of non-labeled procyanidin B2 **1** were obtained: radiolabeled [^{14}C]-**2** was obtained in 12 'hot' steps and 8.5% overall yield from [^{14}C]KCN. Finally, the last steps of the radioactive synthesis (electrophilic activation and coupling of (−)-epicatechin monomers) afforded 11.6 mCi (at 55.2 mCi/mmol) of [^{14}C]-**13**. Small amounts of radiolabeled procyanidin B2 [^{14}C]-**1** were obtained after hydrogenation and minor work-up in the form of diluted aqueous solutions, which exhibited satisfactory purity and stability ([^{14}C]-**1** stable under N_2 atmosphere at 4°C in the dark for at least two days). Thus, the radioactive material was kept in the protected form [^{14}C]–**13** for batch to batch deprotections prior to use in biological assays.



Scheme 4. Synthesis of procyanidin B2 **1**.

Conclusion: In conclusion, we have developed the first asymmetric total synthesis of procyanidin B2 and applied this synthetic route to the preparation of a regioselectively ^{14}C-radiolabeled analogue.[7] The ^{14}C–labeled procyanidin B2 has been used to improve our knowledge of procyanidins metabolism and these results will be published in due course.

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[16] Copper [^{14}C]cyanide was prepared using a Selcia procedure in 90% yield.

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TUNING UP RHODIUM BLACK

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Abstract: A new catalytic system based on rhodium black using Crabtree's catalyst as an additive for direct hydrogen isotope exchange in aromatic compounds has been investigated. The level of deuterium incorporation can be improved from 16% to 93%. The new catalyst mixture tolerates a variety of solvents. Other rhodium sources can be used, but the degree of crystallinity of the rhodium (metal, black or on support) plays an important role. Rhodium sources with a low degree of crystallinity had the highest catalytic activity.

Keywords: Rhodium black; Crabtree's catalyst; deuterium; catalytic direct hydrogen isotope exchange.

Introduction: Isotopically labelled compounds are important in the screening of pharmaceutical candidates in drug development programmes. Such compounds facilitate studies of pharmacokinetics and metabolism, as well as other important assays. Due to an increased attention to speed for drug development and the quality of the drug candidate, isotopically labelled compounds have to be prepared, preferably in a fast and efficient way. The synthesis of labelled compounds can often be laborious and synthetically challenging. The use of catalysts for direct exchange of hydrogen isotopes can ameliorate some of these difficulties associated with the preparation of isotopically labelled compounds because no synthesis of appropriate precursor is required.

Recently, Alexakis *et al.*[1] reported the use of heterogeneous systems in THF as suitable catalysts for promoting hydrogen isotope exchange in pyridines and other nitrogen heteroaromatic compounds. These catalytic systems were based on group VIII metals such as rhodium or ruthenium, used as rhodium black, rhodium on alumina or ruthenium black.

Crabtree's catalyst, $[Ir(COD)(PCy_3)(Py)]PF_6$, is a homogeneous catalyst based on iridium, coordinated to pyridine and tricyclohexyl phosphine (Figure 1). Crabtree's catalyst is a well established catalyst used for hydrogen isotope exchange reactions.[2–9] It is able to promote deuterium or tritium incorporation into arenes at positions *ortho* to a directing group such as carbonyl groups.[2–4],[6–9]

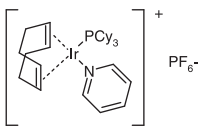


Figure 1. Crabtree's catalyst.

Here we report improvements that can be obtained by adding Crabtree's catalyst to rhodium black.

Results and discussion: As part of a medicinal chemistry programme, we were interested in labelling compound **1**[10] with deuterium. Three experiments were set up, one using Crabtree's catalyst which was expected to promote H/D-exchange in the *ortho*-position to the carbonyl group, a second experiment using rhodium black which was anticipated to effect H/D-exchange in the *ortho*-position to the pyridine nitrogen and finally a third experiment where a mixture of the two catalysts was used to potentially provide the dilabelled form of **1** (Figure 2). To our surprise there was little or no incorporation of deuterium using either Crabtree's catalyst alone or rhodium black (16% incorporation *ortho* to pyridine nitrogen). However, using a mixture of the two, an incorporation level of 93% deuterium was observed in the position *ortho* to the nitrogen in the pyridine ring.

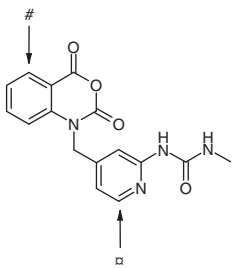


Figure 2. The structure of **1**. Marks indicate sites for anticipated deuteration using Crabtree's catalyst (♯) and Rhodium black (¤).

To further investigate this surprising observation, a set of experiments were undertaken to test the generality of the catalyst mixture in comparison with the single catalysts. We investigated electron rich, electron poor and sterically hindered pyridines (Table 1).

As shown in Table 1 (entries 2, 3, 4, 6, 7, 8 and 15), there is a clear trend towards greater incorporation of deuterium, although not all compounds show increased incorporation of deuterium using the catalyst mixture.

Table 1. Deuteration of substituted pyridines, pyrimidine and benzene using Crabtree's catalyst, rhodium black or the mixture.

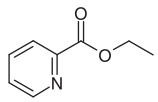
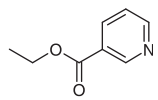
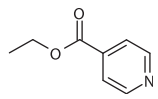
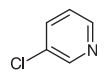
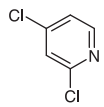
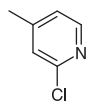
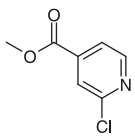
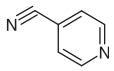
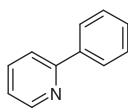
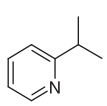
Entry	Substrate	% incorporation of deuterium in the given position														
		Crabtree's catalyst[a]					Rhodium black[b]					Mixture[c]				
		2	3	4	5	6	2	3	4	5	6	2	3	4	5	6
1		–	0	0	0	0	–	28	9	0	55	–	25	16	0	28
2		0	–	0	0	0	23	–	0	0	96	41	–	0	0	96
3		0	0	–	0	0	98	12	–	12	98	98	24	–	24	98
4		–	0	0	0	24	–	0	0	0	67	–	0	0	0	87
5		0	–	0	0	0	93	–	0	0	95	94	–	0	0	93
6		–	23	–	0	31	–	0	–	0	73	–	11	–	0	95
7		–	0	–	0	13	–	0	–	0	69	–	0	–	0	95
8		–	25	–	18	87	–	0	–	0	64	–	25	–	25	96
9		0	0	–	0	0	28	0	–	0	28	29	0	–	0	29
10[d]		–	0	0	0	0	–	0	0	0	71	–	0	0	0	67
11		–	0	0	0	0	–	#[e]	#[e]	#[e]	#[e]	–	0	0	0	80

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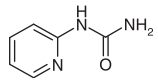
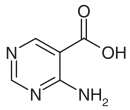
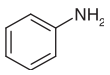
Table 1. Continued

12		–	0	0	0	0	–	0	44	0	81	–	0	21	0	85
13		0	–	0	0	0	97	–	0	0	97	97	–	0	0	97
14		0	–	–	–	0	0	–	–	–	0	60[f]	–	–	–	0
15		0	0	0	0	0	25	0	0	0	25	36	0	0	0	36

[a]Experimental conditions: Substrate (0.016 mmol), Crabtree's catalyst (0.008 mmol), dry DCM (3 mL), D_2 (1342–1590 mbar), RT, 4 h.
[b]Experimental conditions: Substrate (0.016 mmol), Rh black (0.010 mmol), dry THF (3 mL), D_2 (1342–1590 mbar), RT, 4 h.
[c]Experimental conditions: Substrate (0.016 mmol), Crabtree's catalyst (0.008 mmol), Rh black (0.010 mmol), dry DCM:THF (1:1)(3 mL), D_2 (1342–1590 mbar), RT, 4 h.
[d]Incorporation of D in the Ph-ring in the 2- and 6- position (Crabtree's catalyst: 88%; Rhodium black: 32%; Mixture: 75%).
[e]No trace of starting material. Experiment repeated twice with the same outcome.
[f]Experiment repeated 3 times.

In the 2-substituted pyridines the most significant enhancement was observed using the mixture, except entry 1, where a lower incorporation in the 6-position is observed. In 2-phenyl-pyridine (entry 10) incorporation of deuterium in the 2- and 6- position of the phenyl group was also observed. The same deuteration pattern has earlier been reported using rhodium(III) hydride complexes but with lower level of deuterium incorporation (9%) in the pyridine ring.[11] Ellames *et al.*[12] has investigated the use of iridium based catalysts of the general form $[Ir(PR_3)_2(COD)]^+$ for their capability of promoting H/D isotope exchange. Using PCy_3 as ligand, which is the closest catalyst related to Crabtree's catalyst, they found no incorporation of deuterium into the phenyl group but a 50% incorporation of deuterium in the C6-position of the pyridine ring. Surprisingly we found no incorporation of deuterium into the pyridine ring using the iridium based Crabtree's catalyst. No incorporation was observed in the substituent of 2-isopropyl-pyridine (entry 11), but in this compound attempted exchange with rhodium black alone gave a reaction mixture with no trace of starting material. The catalyst mixture, however, gave 80% deuterium incorporation. Whether deuterium was incorporated before or after degradation of 2-isopropyl-pyridine using rhodium black was not determined.

Using electron rich pyridines (entries 12 and 13) the effect of adding Crabtree's catalyst to rhodium black was less noticeable due to the high level of deuterium incorporation using rhodium black alone.

Concerning the electron poor pyridines, no enhanced effect was observed by adding Crabtree's catalyst to rhodium black. In some cases (entries 2 and 3) high levels of deuterium incorporation resulted from using rhodium black alone; in other cases (entries 1 and 9) no significant enhancement was observed.

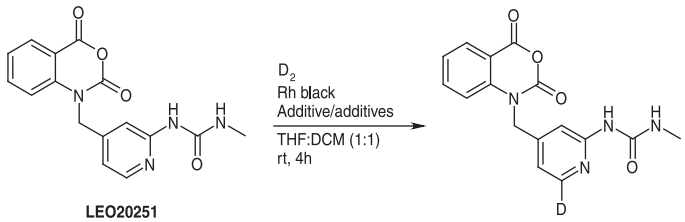
To investigate the scope, two aromatic non-pyridyl compounds, one benzene nucleus and one pyrimidine scaffold were tested. In agreement with the results reported by Ellames *et al.*[2] we found that aniline (entry 15) did not undergo exchange using Crabtree's catalyst. Using rhodium black we found a 51% incorporation of deuterium and using the mixture 72% incorporation of deuterium was obtained. Using Crabtree's catalyst or rhodium black, no incorporation of deuterium in 4-amino-pyrimidine-5-carboxylic acid (entry 14) was obtained. On the other hand using the mixture an incorporation of 60% deuterium was observed.

Sometimes, an additive effect was observed using the catalyst mixture, but less than an additive effect was also observed (entry 1). The effect of the catalyst mixture cannot be predicted and must be determined by experiments. Most important however, is the surprisingly positive outcome that sometimes can be found using the catalyst mixture, and substrates not able to undergo direct hydrogen isotope exchange using Crabtree's catalyst or rhodium black alone can be labelled using the catalyst mixture (entry 14).

The catalytic effect of rhodium black can be enhanced by adding Crabtree's catalyst, while there is no enhanced catalytic effect of Crabtree's catalyst when rhodium black is added. In two cases, only the mixture was able to promote hydrogen isotope exchange (entries 11 and 14), indicating that a new and more active catalytic system is at hand.

To investigate the mechanism further an experiment (mix and split) was undertaken where Crabtree's catalyst and rhodium black were stirred under D_2-atmosphere for 4h without **1** added. The heterogeneous catalyst mixture was then centrifuged and divided into solid and solution and each part was then added **1** and stirred under D_2-atmosphere for 4 h. The solid was able to promote an incorporation of 24% deuterium while no incorporation of deuterium was observed in the solution, supporting that the catalytic exchange takes place exclusively on the rhodium surface and not in solution.

A hypothesis for the enhanced effect may involve a ligand transfer from Crabtree's catalyst to rhodium. To investigate this hypothesis, a set of experiments using **1** as a model compound were undertaken, where one or more of the components of Crabtree's catalyst were added to rhodium black (Scheme 1 and Table 2).



Scheme 1. Setup to investigate a possible ligand transfer.

Table 2. Deuteration of 1 using different catalyst components

Entry	Ir	Ir(COD)Cl	Rh(COD)Cl	PCy_3	Py	%D
1	0.5					16
2	0.5			0.5		35
3	0.5				0.5	26
4	0.5			0.5	0.5	27
5		0.5				0
6		0.5		0.5		20
7		0.5		0.5	0.5	16[a]
8		0.5			0.5	13
9			0.6			11
10			0.6	0.5		17
11			0.6		0.5	34
12			0.6	0.5	0.5	26
13				0.5	0.5	27[b]
14				0.5		23[b]
15					0.5	42
16					1	35[b]
17					10	25[c]
18[d]					0.5	0
19[d]					1	0

Experimental conditions: **1** (0.016 mmol), Rh black (0.010 mmol), catalyst component/components, dry THF:DCM (1:1)(3 mL), D_2 (1457–1639 mbar), RT, 4 h. Numbers in table are numbers of equivalents added.
[a]No deuteration is also observed if 0.5 eq NH_4PF_6 is added.
[b]Same incorporation level observed if 0.5 eq COD is added.
[c]Unknown impurity formed.
[d]No rhodium black added.

It was possible to improve the catalytic effect of rhodium black (Table 2) (from 16% to 42% deuterium incorporation), but not to the same extent as adding Crabtree's catalyst (93% incorporation). The most effective additive was pyridine (entry 15) however the addition of larger amounts of pyridine did not promote further deuterium incorporation (entries 16 and 17). To investigate if the enhanced effect of pyridine was an additive effect to rhodium black, pyridine was tested in absence of catalyst, and, as expected, no incorporation could be detected (entries 18 and 19). These findings support the hypothesis that the enhanced catalytic exchange reaction takes place at the rhodium surface.

Table 3 shows the effect of decreasing the amount of Crabtree's catalyst added to rhodium black. Even a low amount (0.05 eq) of Crabtree's catalyst was able to enhance the catalytic effect of rhodium black noticeable, enhancing the incorporation level from 16 to 32% (entry 3).

Table 4 shows the catalytic effect of different rhodium sources. All the tested sources of rhodium were able to promote catalytic hydrogen isotope exchange except non-amorphous rhodium, which was unable to promote exchange.

A batch variation of the different rhodium black sources was observed, noticeable when Crabtree's catalyst was added. Rhodium on different supports was quite active even without activation with Crabtree's catalyst. An explanation for the high catalytic activity could be that rhodium on support has a larger surface area than non-amorphous rhodium or even rhodium black. This is supported by the powder X-ray diffractograms of the different sources (Figure 3). A sharp and distinguished peak indicates a high degree of crystallinity whereas a blurred peak or non-existing peak indicates low or very low degree of crystallinity. The powder X-rays diffractograms show a big difference between the different sources, and these findings correlate well with the amount of incorporated deuterium. With respect to rhodium, sharp peaks are seen, indicating a high degree of crystallinity and no

Table 3. Incorporation of deuterium using different amounts of Crabtree's catalyst

Entry	Rhodium black	Crabtree's catalyst	%D
1	0.6	0.5	93
2	0.6	0.1	46
3	0.6	0.05	32
4	0.6	0.02	16
5	0.6	0	16

Experimental conditions: **1** (0.016 mmol), Rh black (0.010 mmol), Crabtree's catalyst, dry THF:DCM (1:1)(3 mL), D_2 (1463–1575 mbar), RT, 4 h. Numbers in table are numbers of equivalents added.

Table 4. Incorporation of deuterium observed using different rhodium sources

Entry	Rhodium source	%D	+ 0.5 eq Crabtree's catalyst, %D
1	Rh black (ABCR)	16	57
2	Rh black (Aldrich)	16	91
3	Rh (Acros)	0	0
4	Rh (Fluka)	0	0
5	5wt% Rh/act. alumina[a]	75–92	90
6	5wt% Rh/alumina[a]	71	93
7	5wt% Rh/carbon[a] (Aldrich)	88	85
8	5wt% Rh/carbon[b]	8	16

Experimental conditions: **1** (0.016 mmol), catalyst/catalysts, dry THF:DCM (1:1)(3 mL), D_2 (1550–1665 mbar), RT, 4 h.
[a]Added amount correlates to 0.6 eq rhodium.
[b]Added amount correlates to 0.6 eq rhodium, old batch with unknown origin.

incorporation of deuterium was observed. On the other hand looking at *e.g.* rhodium, 5 wt% on activated alumina, blurred or no peaks are observed, indicating a very low degree of crystallinity and very high degree of incorporation (92%) was observed. The powder X-ray can also clarify the difference in catalytic capacity of an old batch with unknown origin (entry 8) and a newly purchased batch (entry 7) of 5% rhodium on carbon. The old batch (8% incorporation) has a higher degree of crystallinity compared to the new one (88% incorporation).

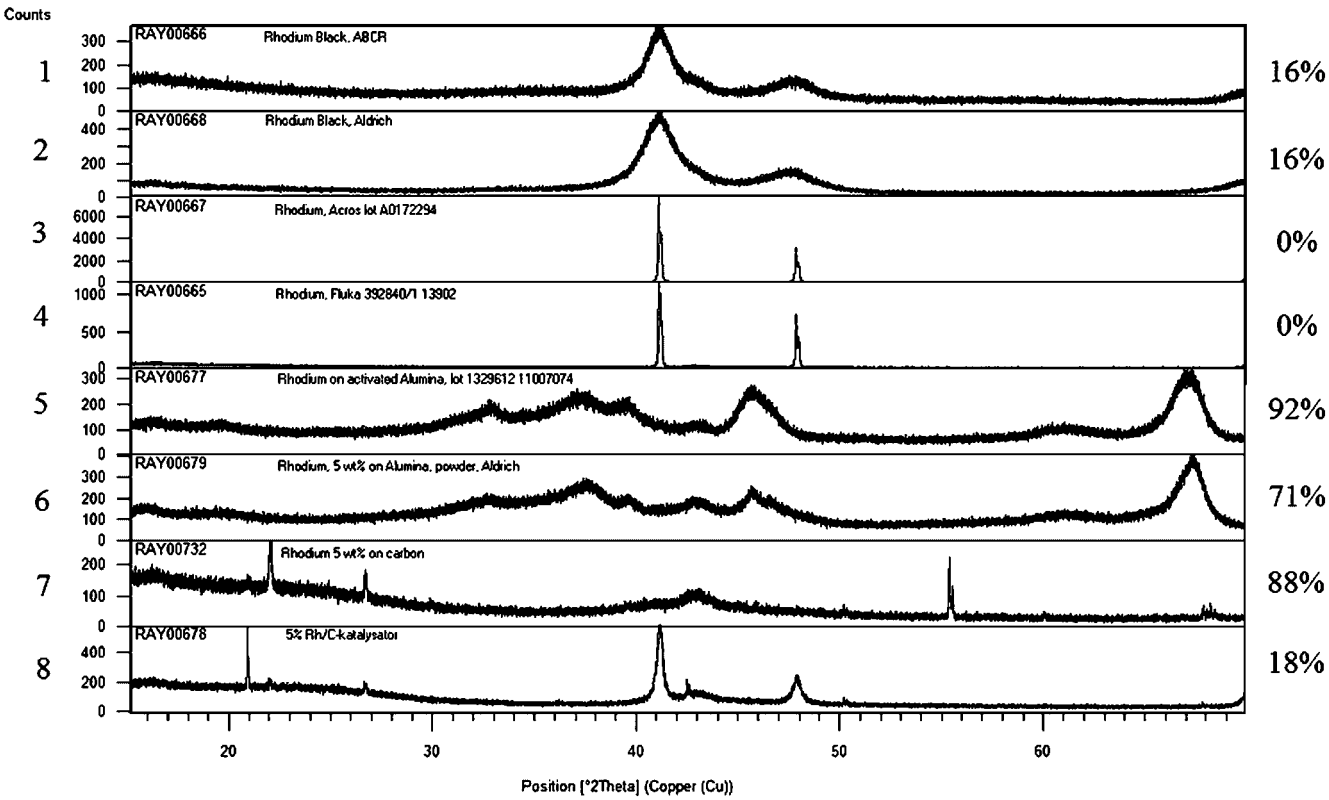


Figure 3. X-Ray powder diffractograms of the different rhodium sources. Numbers to the right are amount of deuterium incorporated (*cf.* Table 4).

To test the robustness of the catalytic mixture, experiments in different solvents were performed (Table 5). The catalytic system is more or less independent of the solvent, and acceptable to high incorporation levels were observed in all solvents except diethyl ether, which could be due to low solubility of **1**.

Table 5. Incorporation of deuterium in different solvents.

Entry	Solvent	% incorporation of deuterium	
		No additive	+ 0.5 eq Crabtree's catalyst
1	Et_2O		0[a]
2	EtOAc		84
3	DCM		60
4	THF		89
5	THF:DCM (1:1)	16[b]	90–93[c]

Experimental conditions: **1** (0.016 mmol), catalyst/catalysts, dry THF:DCM (1:1)(3 mL), D_2 (1418–1666 mbar), RT, 4 h.
[a]**1** is slightly soluble in Et_2O
[b]Experiment performed in THF-d_8:DCM-d_2 show a similar incorporation level (12%).
[c]Experiment performed in THF-d_8:DCM-d_2 show a slightly decreased incorporation level (68%).

Consequently, the high solvent tolerance expands the scope and usefulness for the catalyst mixture, allowing a variety of compounds to be labelled.

Conclusion: A new catalytic system based on rhodium black with Crabtree's catalyst as an additive has been investigated. In general, the mixture has an improved activity, compared to rhodium black alone. In two cases neither Crabtree's catalyst nor rhodium black was able to promote exchange, but using the mixture an incorporation of deuterium was observed. The new catalytic system is active in a variety of solvents, extending its usefulness. This protocol will, of course, also be useful when tritiated compounds are needed.

Experimental: General: All reactions were performed in a stainless steel manifold purchased from RC Tritec, Teufen, Switzerland. D_2 (99.8 atom % D) was purchased from Isotec. Anhydrous solvents were dried over molecular sieves (4 Å). All other solvents and reagents were used as received, purchased from Sigma-Aldrich, ABCR, Fluka, Alfa Aesar and Acros. ^1H and ^{13}C NMR spectra were obtained on a Bruker AV600 spectrometer with a 5 mm TCI-Cryoprobe or a Bruker DRX500 spectrometer with a 5 mm PAPPI-probe. The deuterium content was measured on the basis of integration. Chemical shifts are reported in ppm with tetramethylsilane (TMS, $\delta = 0.00$) as internal reference. The X-Ray Powder Diffractograms were obtained using on a X'pert PRO MPD from PANalytical with Cu Kα radiation and operating at 45 kV and 40 mA. The samples were scanned from 3 to 70 degree 2 theta with a step size of 0.0020 degree 2 theta and at 121.18 s per step.

General procedure for deuterium exchange reaction: Catalyst was weighed into an 8 mL round bottom reaction flask containing a new teflon coated stir bar (3×10 mm), substrate (0.016 mmol) was dissolved in dry solvent (3 mL) and added. The reaction mixture was frozen in liquid N_2 and evacuated (below 3.5×10^{-3} mbar), thawed and then stirred under D_2-atmosphere (1340–1666 mbar) for 4 h at RT. The reaction mixture was filtered through a syringe-filter (Whatman, 0.45 μm) and concentrated *in vacuo*.

NMR-data for non-deuterated compounds are described in the literature.[13]

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SYNTHESIS OF ^{14}C- AND ^{13}C/^{2}H-LABELED SGLT INHIBITORS AVE2268 AND AVE8887

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Abstract: Isotopically labeled analogues of two structurally very similar SGLT inhibitors AVE2268 (**1a**) and AVE8887 (**1b**) have been synthesized by various routes. The radioactive labeled [^{14}C]-AVE2268 was prepared in 5 steps including a Friedel-Crafts acylation as the key step for the ^{14}C-label introduction. For [^{14}C]-AVE8887 the same synthetic approach was not successful and therefore an alternative thiophene metalation/Weinreb amide sequence was developed. This pathway was also applied to obtain stable isotopically labeled analogs of both AVE2268 and AVE8887.

Keywords: SGLT; Lithium; Carbon-14; Deuterium

Introduction: Sodium glucose co-transporters (SGLT-2)[1] are membrane proteins. They play an important role in maintaining glucose equilibrium in the human body by re-absorption of glucose in the kidney. It is therefore expected that the inhibition of SGLT 2 transporters could decrease glucose blood levels by preventing re-absorption from the urine. The control of these transporters could be an effective tool in the normalisation of high blood glucose levels that are associated with diseases such as Type 1 and Type 2 diabetes.[2] Both radiolabeled AVE2268 and AVE8887 were required to measure the ADME (absorption, distribution, metabolism and elimination) profiles of these new drug candidates in animals and humans. In addition stable-isotope labelled analogues of both compounds were also required for use as an internal standard for bioanalytical assay validation. Due to the presence of sulphur in the thiophene ring moieties of **1a** and **1b** at least 5 deuterium atoms were required in both cases.

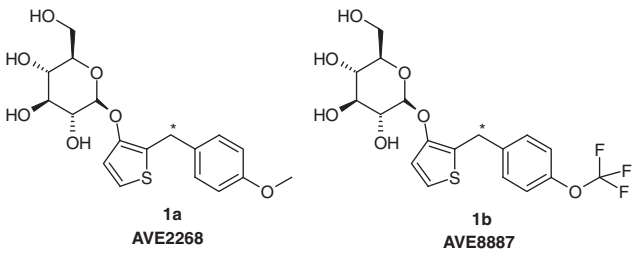
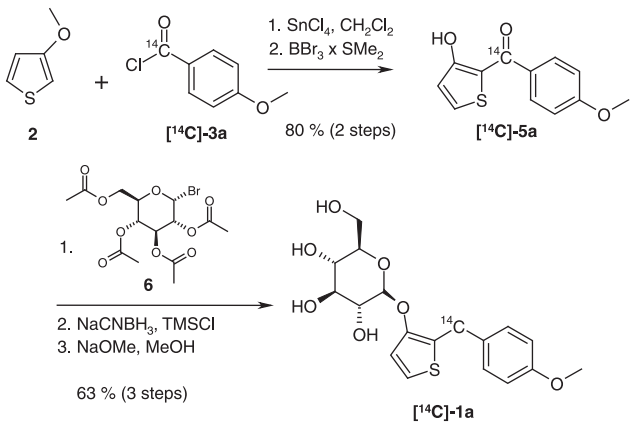


Figure 1. Structures of AVE2268 and AVE8887 (^{14}C-labelling position marked with *).

Results and Discussion: The synthesis of [^{14}C]-AVE2268 (**1a**) was achieved following the simple 5 step synthesis path depicted in scheme 1.[3] Starting from commercially available 3-methoxythiophene (**2**) and 100 mCi [^{14}CO]anisoyl chloride, **[^{14}C]-3a**, purchased from Amersham Biosciences, a completely regioselective Friedel-Crafts acylation followed by a selective methyl ether cleavage of 3-methoxythiophene derivative **4a** gave the hydroxyl-thiophene derivative **[^{14}C]-5a**. Subsequent alkylation with acetobromo-alpha-glucose under phase transfer conditions, carbonyl reduction with $NaCNBH_3$/TMSCl[4] and final basic acyl deprotection afforded the desired compound **[^{14}C]-1a** in a very good overall yield of 50% (50 mCi, 99.9% radiochemical purity, 57 mCi/mmol).

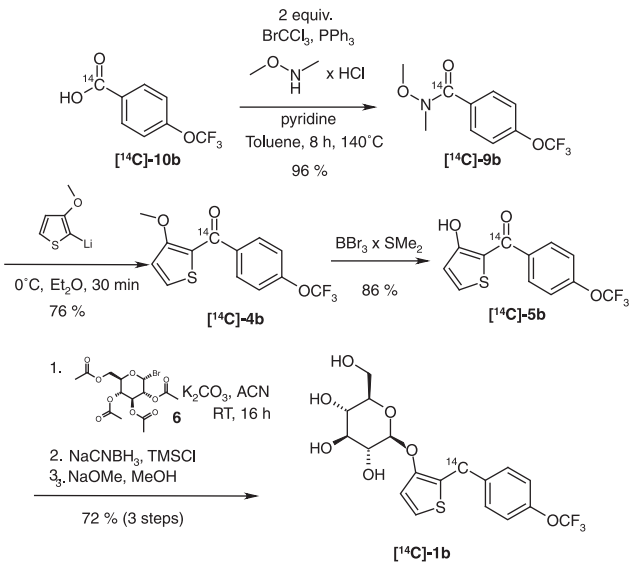


Scheme 1. Synthesis of [^{14}C]-AVE2268 (**1a**).

For ^{14}C-labelling of AVE8887 (**1b**) a very similar pathway was envisioned employing 4-trifluoromethoxy-[^{14}CO]benzoyl chloride **[^{14}C]-3b** instead of **[^{14}C]-3a**. Surprisingly we found in our cold elaboration experiments that more electron deficient benzoyl chlorides, such as (**3b**), reacted much slower with thiophene (**2**) under Friedel-Crafts acylation conditions. Consequently the acid labile thiophene (**2**) started to decompose resulting in low yields for this reaction step. Even under optimised conditions and a stepwise addition (2–3 equiv.) of (**2**), the maximum yield achieved was only 32%. In addition, work-up and purification became difficult due to the numerous side products formed. Therefore, we started to look for alternative strategies for the synthesis of (**5b**) in which greater emphasise was taken in minimising decomposition by increased reactivity of the thiophene (**2**).

Stimulated by results reported by Miller and Yu[5] we examined the regioselective lithiation of 3-methoxythiophene (**2**) in refluxing diethylether over 5 min. However, when quenching the lithiated thiophene (**2**) either with anisoyl chloride (**3a**) or 4-trifluoromethoxy benzoyl chloride **3b** only traces of the desired products **4a** and **4b** respectively could be observed. Besides acyl chlorides the Weinreb amide is well known to be reactive in organometallic reactions and would be readily accessible also in labeled form.[6] After optimisation of the reaction of **9b** with the lithiated thiophene (**2**) we obtained the formation of **4b** in 76% yield.

The ^{14}C-synthesis of **[^{14}C]-1b** was finally accomplished following the pathway shown in Scheme 2. Starting from 100 mCi [^{14}CO]trifluoromethoxy benzoic acid **[^{14}C]-10b,** the corresponding Weinreb amide **[^{14}C]-9b** was formed under Appel conditions.[7] Addition of the lithiated thiophene to an ice-cold solution of the Weinreb amide **[^{14}C]-9b** yielded **[^{14}C]-4b** in 76%. Luckily the rest of the synthesis showed no significant deviation from the synthesis of **[^{14}C]-1a** reported above. The colorless compound was isolated after 6 reaction steps with an overall yield of 45% (45 mCi, 99.5% radiochemical purity, 53.2 mCi/mmol).



Scheme 2. Synthesis of [^{14}C]-AVE8887 (**1b**).

Synthesis of stable isotopically labeled AVE2268 (1a) and AVE8887 (1b): Stable isotopically labeled (**1a**) was synthesised according to the path shown in Scheme 3 in 8 chemical steps with an overall yield of 25%. Starting from 4-hydroxy benzoyl methylester (**11)** the hydroxy group was alkylated with deuterated methyliodide followed by basic ester hydrolysis to give the corresponding acid **[D_3]-10a** in 90% yield over two steps. The subsequent steps were described in the ^{14}C-synthesis above, however sodiumcyanoborodeuteride was employed in the carbonyl reduction to introduce two additional deuterium atoms. A co-injection of AVE2268 and [D_5]-AVE2268 revealed the expected mass difference but identical retention time in the LC-MS.

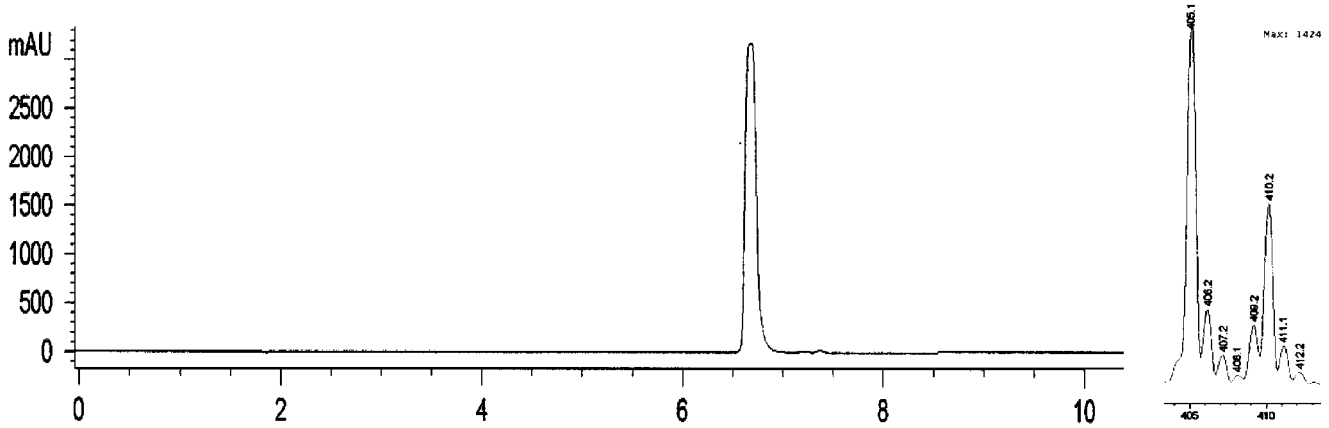


Figure 1. LC-MS-spectra of a 1:1 mixture of AVE2268 and [D_5]-AVE2268 (M+Na).

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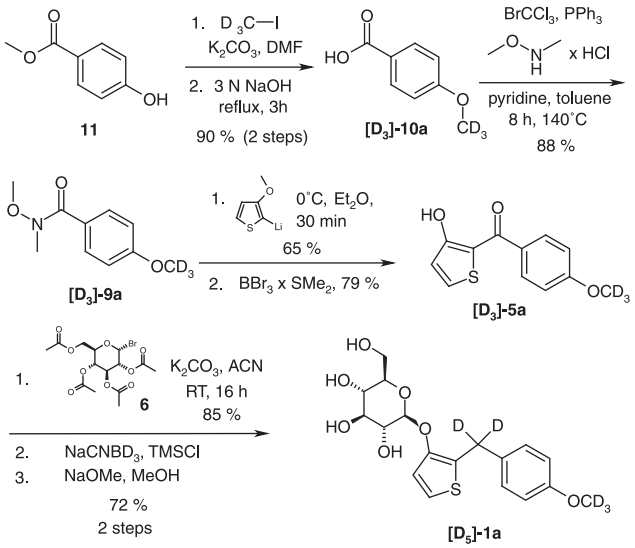

Scheme 3. Synthesis of [D_5]-AVE2268 (**1a**).

The synthesis of stable isotopically labeled AVE8887 (**1b**) was achieved applying exactly the same pathway starting from ^{13}C-labeled-trifluoromethoxy benzoic acid **10b**.

Conclusions: We have described in detail the challenges of the synthesis of isotopically labeled compounds of SGLT inhibitors (**1a**) and (**1b**). Although very similar in their chemical structure, different synthetic pathways had to be optimised.

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STABLE ISOTOPE AND CARBON-14 SYNTHESES OF CP-945,598: A CANNABINOID CB1 RECEPTOR ANTAGONIST

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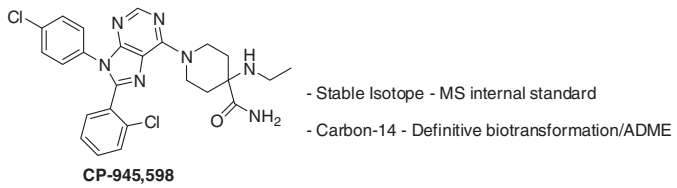
Abstract: CP-945,598 is a cannabinoid CB1 receptor antagonist drug candidate for the treatment of obesity. Stable isotope labeled CP-945,598 was required for use as a clinical assay internal standard, and carbon-14 labeled CP-945,598 was required to perform detailed adsorption, distribution, metabolism and excretion (ADME) studies. A key consideration within the stable isotope synthesis was the incorporation of sufficient H/C/N isotopes to increase the candidate's molecular weight profile beyond that exhibited by the non-labeled parent. The carbon-14 synthesis required unique attention to label position based on metabolism concerns.

Keywords: Stable Isotope; Carbon-14; Cannabinoid receptor

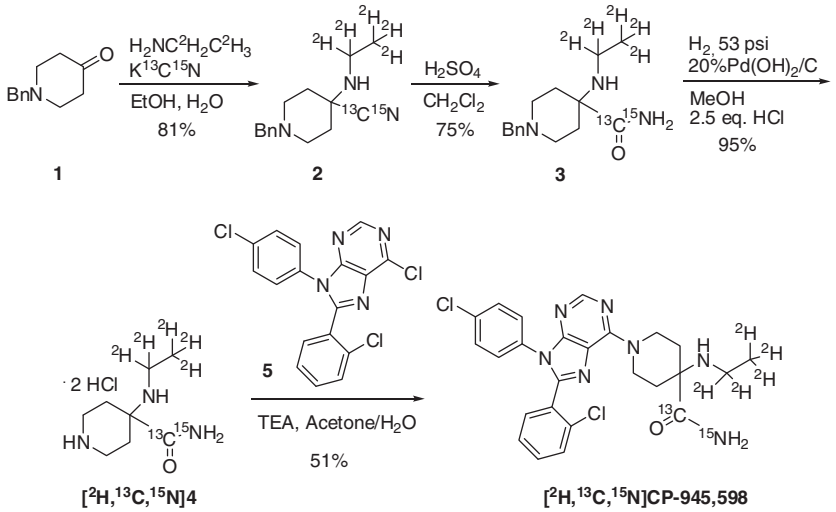
Introduction: **CP-945,598**, Figure 1, is a cannabinoid CB1 receptor antagonist under investigation for the treatment of obesity.[1] Stable isotope labeled **CP-945,598** was required for use as a clinical assay internal standard, and carbon-14 labeled **CP-945,598** was required to perform detailed adsorption, distribution, metabolism and excretion (ADME) studies. A key consideration within the stable isotope synthesis was the incorporation of sufficient H/C/N isotopes to increase the candidate's molecular weight profile beyond that exhibited by the non-labeled parent. The carbon-14 synthesis required unique attention to label position based on metabolism concerns.



CP-945,598

Figure 1. Structure of CP-945,598 and intended isotopic studies.

Results and Discussion: Central to preparing an appropriate **CP-954,598** clinical assay internal standard was to incorporate a sufficient number of stable isotope atoms within the target structure to increase its molecular weight beyond the nonlabeled two chlorine parent molecular ion pattern. The established **CP-945,598** synthesis involved a final coupling of a piperidine aminoamide to a chloropurine core–the piperidine moiety presented the most straightforward target for stable isotope incorporation. To that end, commercially available piperidone **1** was treated with perdeuterated ethylamine and $K^{13}C^{15}N$ in aqueous ethanol to afford the Strecker product **2** containing a total of seven stable isotope atoms, in 81% yield, Scheme 1. Partial nitrile hydrolysis of **2** was affected with concentrated H_2SO_4 in CH_2Cl_2 to give stable isotope labeled aminoamide **3**. Debenzylation of **3** was accomplished by hyrogenolysis of the substrate in acidic methanol to yield piperidine **[^{2}H,^{13}C,^{15}N]4** as an HCl salt. Finally, coupling of this piperidine to chloropurine **5** was performed in aqueous acetone with triethylamine to arrive at **[^{2}H,^{13}C,^{15}N]CP-945,598**. Of note, this coupling was completely regioselective with respect to the two available piperidine secondary amines.



Scheme 1.

Importantly, mass spectral analysis of **[^{2}H,^{13}C,^{15}N]CP-945,598** showed it to be completely void of nonlabeled **CP-945,598**, Figure 2.

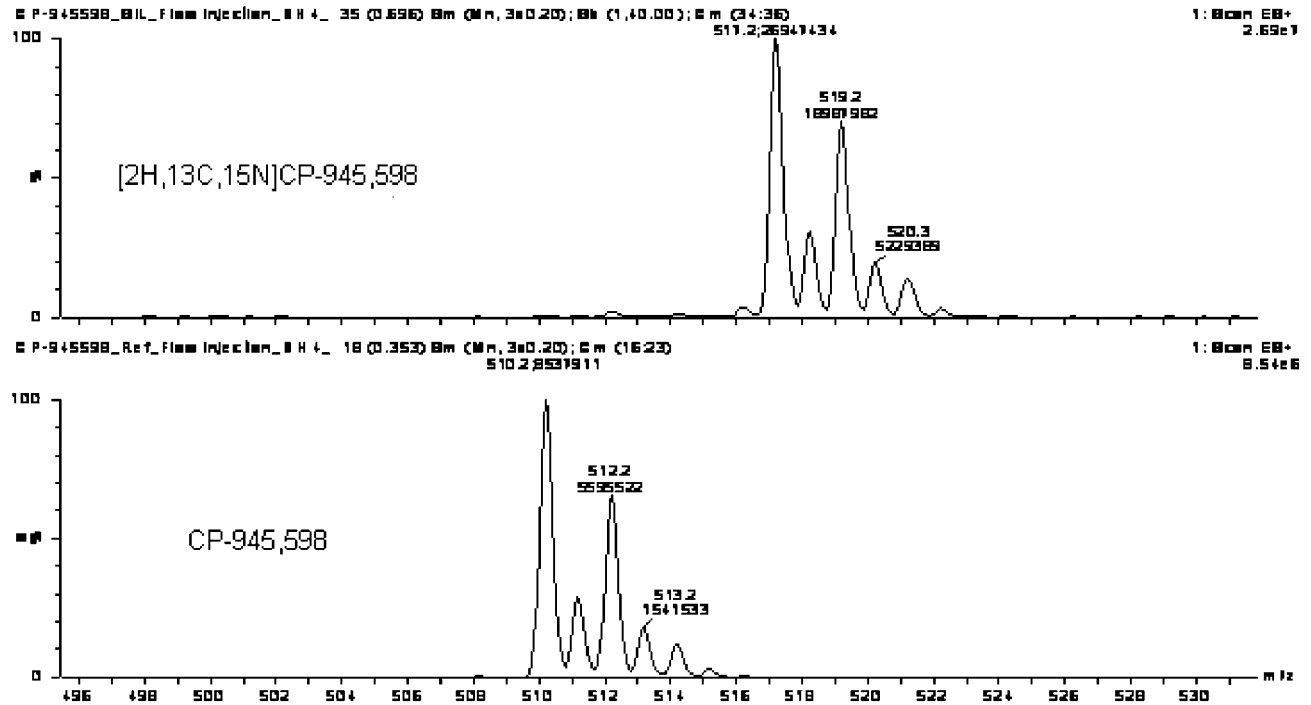


Figure 2. Mass spectral comparison of SIL CP-945,598 and unlabeled reference.

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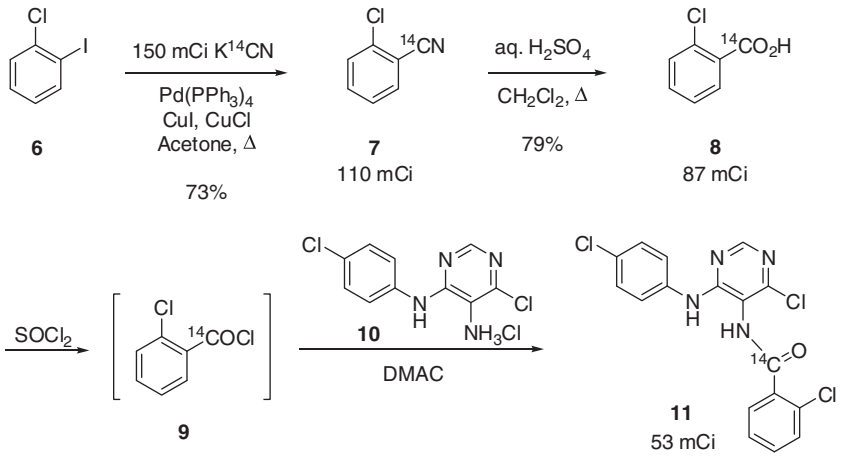
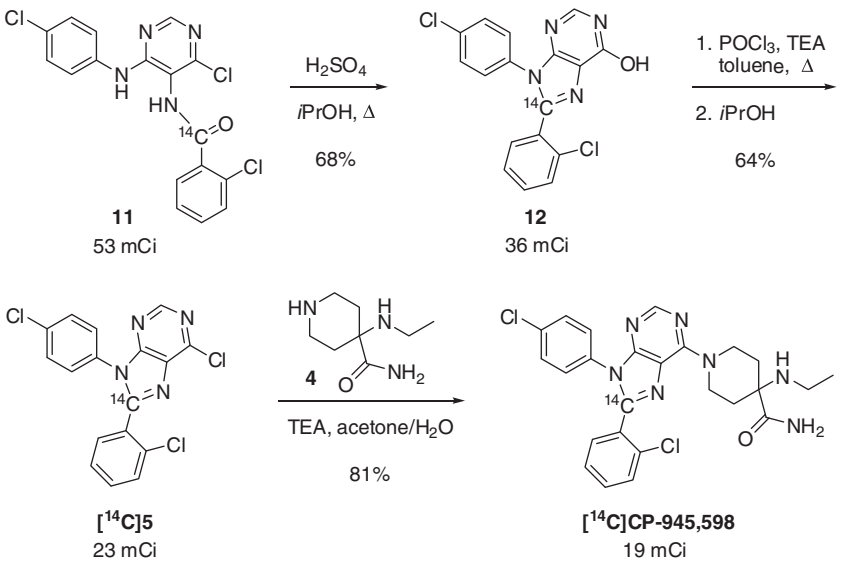
Carbon-14 labeled **CP-945,598** was needed to perform ADME profiling studies. The compound's central purine core was targeted for radiolabeling because it was deemed metabolically stable and presented an attractive opportunity to utilize readily available labeled cyanide as a carbon-14 source.

1-Chloro-2-iodobenzene (**6**) was treated with 150 mCi of $K^{14}CN$ and heated in refluxing acetone with a mixed palladium(0)/copper(1) catalyst system to afford 110 mCi of 2-chlorobenzo[^{14}C]nitrile (**7**), Scheme 2. It is important to note that this cyanation method was completely selective for reaction at the aryliodide bond. In contrast, preliminary experiments involving metallation and carbonation of **6** produced **8** contaminated with a minor quantity of 2-iodobenzoic acid–an impurity that would be difficult to differentiate/purge from desired product. Nitrile **7** was hydrolyzed to carboxylic acid **8** in a biphasic refluxing solution of aqueous sulfuric acid/dichloromethane. Base/acid workup of this reaction delivered 87 mCi of radiochemically pure **8**. Conversion of **8** to its corresponding acid chloride **9** was affected in refluxing thionyl chloride. The product was isolated from this reaction in crude form by distillation of the solvent and then reacted directly with diaminpyrimidine **10** in dimethylacetamide to yield crude amide **11**. Chromatographic purification of the crude product delivered 53 mCi of high radiochemical purity **11** (61% yield from benzoic acid **8**). There was no regioisomeric amide formed in this reaction.



Scheme 2.

Cyclization of amide **11** to purine **12** was completed in a mixture of refluxing sulfuric acid and isopropanol, Scheme 3. The product was easily isolated as a pure crystalline material from this reaction. An expected and unfortunate side reaction from this process involved hydrolysis of the purine 6-chloro substituent. This halide was required as a leaving group for the final piperidine addition to form **[^{14}C]CP-945,598**. To reinstall the chloride, 36 mCi of hydroxypurine **12** was reacted with phosphorus oxychloride and trietlylamine in refluxing toluene. Addition of isopropanol to this reaction precipitated crude product which was further purified by flash column chromatography to afford 23 mCi of radiochemically pure chloropurine **[^{14}C]5**. In similar fashion to the stable isotope labeled synthesis of the target, **[^{14}C]5** was reacted with piperidine **4** in aqueous acetone and triethylamine to give crude final product. This material was further purified by flash column chromatography to afford 19 mCi of high radiochemical purity **[^{14}C]CP-945,598**. The specific activity of this material was measured at 55.1 mCi/mmol by mass spectrometry.



Scheme 3.

Summary: **[^{2}H,^{13}C,^{15}N]CP-945,598** was prepared in 29% yield in 4 steps starting from deuterated ethylamine and K^{13}C^{15}N, and **[^{14}C]CP-945,598** was prepared in 12% yield in 7 steps starting from K^{14}CN.

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SYNTHESIS OF A DUAL CARBON-14 LABELED PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR α AND γ (PPARα/γ) DUAL AGONIST FOR USE IN A HUMAN ADME STUDY

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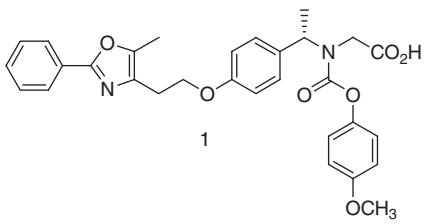
Abstract: A dual carbon-14 labeled peroxisome proliferator activated receptor α and γ (PPAR α and γ) dual agonist (**[^{14}C]-1**) was synthesized for use in a human ADME study. A total of 9.5 mCi of **[^{14}C]-1** was prepared with a specific activity of 10.18 μCi/mg and a radiochemical purity of 99.85%. The title compound **[^{14}C]-1** was synthesized in 6 radioactive steps and a 23% radiochemical yield from the dual carbon-14 labeled intermediate **[^{14}C]-6**. The dual carbon-14 labeled intermediate **[^{14}C]-6** was prepared by mixing equal millicurie amounts of mono labeled **[^{14}C]-6 Label A** and mono labeled **[^{14}C]-6 Label B**. Label A (**[^{14}C]-6 Label A**) was prepared in 4 radioactive steps in a 55% yield from carbon-14 labeled potassium cyanide. Label B (**[^{14}C]-6 Label B**) was prepared in 1 radioactive step in a 96% yield from carbon-14 labeled 4-hydroxyacetophenone.

Key Words: Type 2 diabetes; peroxisome proliferator activated receptors; PPARα; PPARγ; carbon-14 labeling

Introduction: Type 2 diabetes is a metabolic disease that currently affects about 180 million people worldwide and it is estimated to rise to over 360 million by the year 2030.[1] In addition to the characteristic combination of insulin resistance and insulin deficiency, the type 2 diabetic often displays cardiovascular risk factors such as hypertriglyceridemia, low HDL-cholesterol and elevated LDL-cholesterol levels. The peroxisome proliferator activated receptors (PPARα, PPARγ and PPARδ) are ligand-activated transcriptional factors that belong to the nuclear hormone receptor superfamily which are essential in controlling glucose, lipid, and energy homeostasis.[2,3] The dual PPARα/γ agonist appears well-suited as a treatment for type 2 diabetes because of the insulin-sensitizing/glucose-controlling potential of the PPARγ agonists, in combination with the positive lipid and cholesterol modulating activities of the PPARα agonists.[3] Compound **1** is a dual PPARα/γ agonist that is being developed by Bristol-Myers Squibb. An important step in the development of this compound was the synthesis of a carbon-14 labeled analog for use in a human absorption, distribution, metabolism and excretion (ADME) study. This report describes an efficient synthesis of dual carbon-14 labeled **1**.



1

Figure 1. Structure of the PPAR α/γ dual agonist.

Results and discussion: In-vitro metabolism studies with unlabeled **1** revealed metabolites that arose from cleavage of the central ether bond. This was not surprising since this metabolic pathway has been documented previously for a structurally similar PPAR compound.[4] The metabolic fate of the entire molecule needed to be elucidated by conducting a human ADME study with an analog of **1** appropriately labeled with carbon-14. Since metabolic cleavage of the central ether bond would result in two major fragments, a decision was made to prepare a dual carbon-14 labeled analog of **1** with labels on both sides of the central ether moiety. To minimize the redundancy of two complete syntheses of carbon-14 labeled **1**, a labeling strategy was developed in which an early intermediate would be dual labeled. The dual carbon-14 labeled intermediate would then be carried forward to give dual carbon-14 labeled **1**. This labeling strategy would maximize the radiochemical yield and minimize the total number of radioactive steps.

The intermediate **[^{14}C]-6 Label A** was prepared in 4 radioactive steps from carbon-14 labeled potassium cyanide as shown in Scheme 1.[5–7] A total of 73 mCi of **[^{14}C]-6 Label A** was prepared with a radiochemical purity of 99% and a specific activity of 83.9 μCi/mg. The overall yield was 55%.

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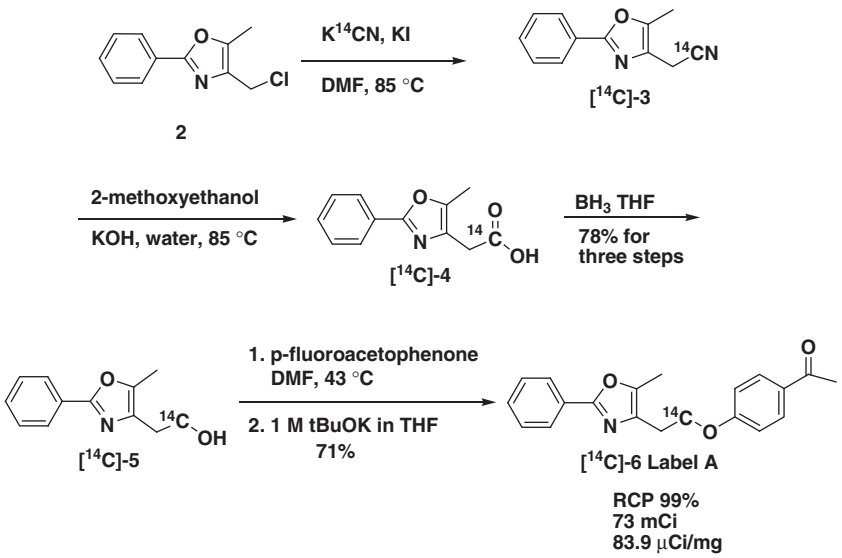
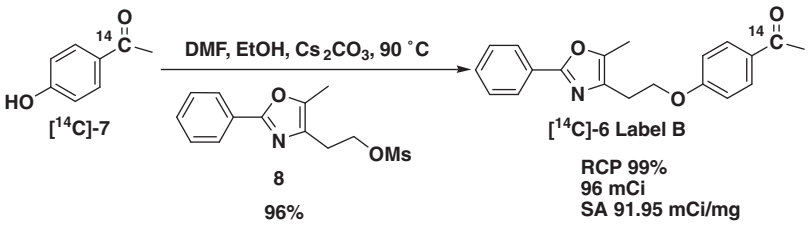
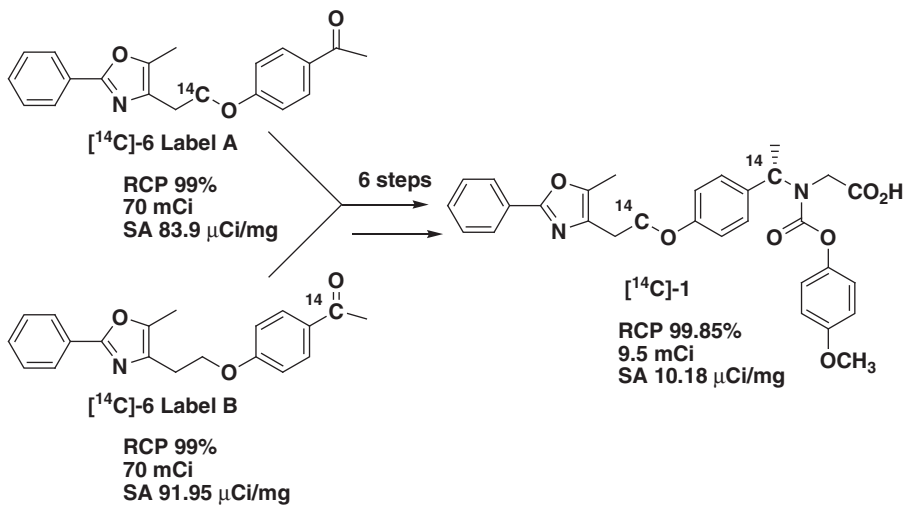


Scheme 1. Synthesis of [^{14}C]-6 Label A.



Scheme 2. Synthesis of [^{14}C]-6 Label B.

The intermediate **[^{14}C]-6 Label B** was prepared in 1 radioactive step from carbon-14 labeled 4-hydroxyacetophenone **([^{14}C]-7)** as shown in Scheme 2.[8–10] A total of 96 mCi of **[^{14}C]-6 Label B** was prepared with a radiochemical purity of 99% and a specific activity of 91.95 μCi/mg. The overall yield was 96%.



Scheme 3. Synthesis of dual carbon-14 labeled of [^{14}C]-1.

Equal millicurie amounts of **[^{14}C]-6 Label A** (70 mCi) and **[^{14}C]-6 Label B** (70 mCi) were mixed to give dual carbon-14 labeled material as shown in Scheme 3. This material **([^{14}C]-6 Label A+[^{14}C]-6 Label B)** was converted to the desired product **[^{14}C]-1** in 6 radioactive steps.[9,10] A total of 9.5 mCi of **[^{14}C]-1** was prepared with a radiochemical purity of 99.85% and a specific activity of 10.18 μCi/mg. The overall yield for the 6 steps was 23%. This material was used successfully in a clinical ADME study to elucidate the human metabolism of **[^{14}C]-1**.

Acknowledgements: The authors would like to thank the Bristol-Myers Squibb Process Research & Development Department, the Medicinal Chemistry Department and the Biotransformation group.

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SYNTHESIS OF [$^{2}H_4$]; [^{3}H]; AND [^{14}C] SCH 900875: A POTENT CXCR3 ANTAGONIST WITH POTENTIAL THERAPEUTIC USE IN THE TREATMENT OF PSORIASIS

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Abstract: $^{2}H_4$, ^{3}H and ^{14}C–syntheses of the potent CXCR3 antagonist Sch 900875 were performed in order to aid the biological evaluation of this compound.

Keywords: pyruvate; tritiation; CXCR3; deuterated; carbon–14

Introduction: Sch 900875 (**1**), (Figure 1) is a potent and selective CXCR3 antagonist with therapeutic potential in the treatment of CXCR3 chemokine-mediated illnesses such as inflammatory disease (including psoriasis or inflammatory bowel disease); as well as auto-immune diseases such as multiple sclerosis and rheumatoid arthritis.[12] $^{2}H_4$, ^{3}H and ^{14}C-analogs were prepared in order to aid the biological evaluation of this compound.

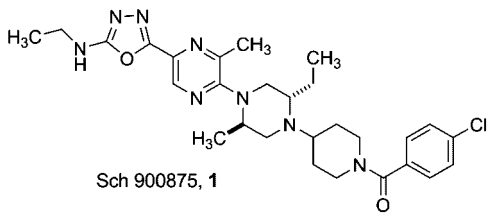

Figure 1. Structure of Sch 900875, **1**.

Results and Discussion: Sch 900875 was synthesized in unlabelled form starting with a Fluoride–promoted nucleophilic aromatic substitution of the commercially available pyrazine (**2**) with *N*-Boc–protected piperazine (**3**) (Scheme 1).[2] After deprotection of the Boc-group, reductive amination of the resultant piperazine with *N*-Boc-4-piperidinone yielded **6** in 73% yield. A second Boc-group deprotection followed by amide bond formation with 4-benzoyl chloride (**9**) generated the advanced ester intermediate (**10**) in 82% yield over three steps. The methyl ester was then elaborated via a three step sequence to the oxadiazole heterocycle furnishing **1** in 64% yield. Deuterated and tritiated intermediates were prepared and incorporated into the existing non-labeled synthesis depicted in Scheme 1.

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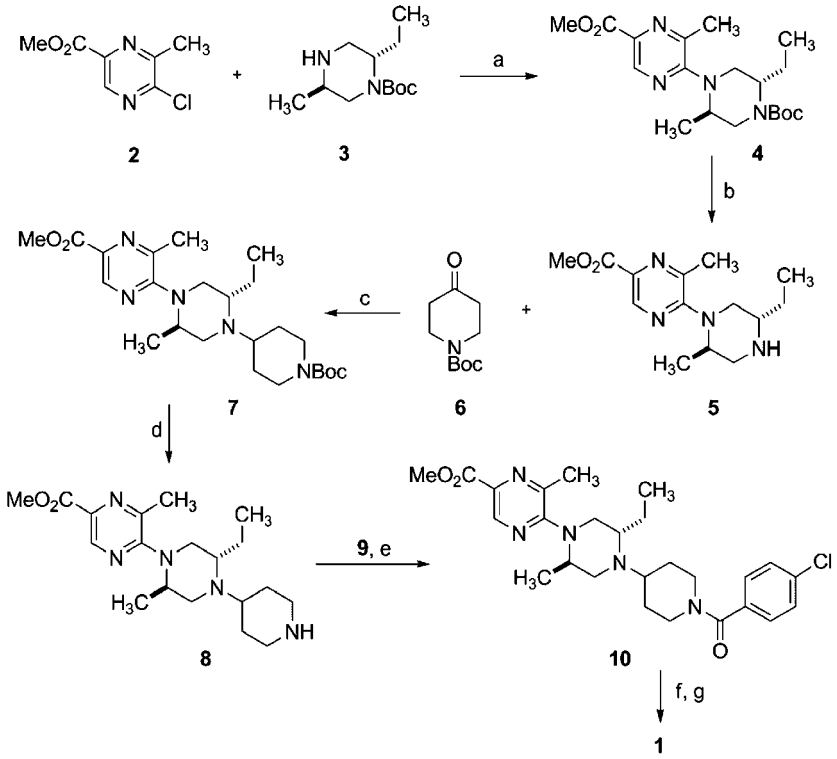
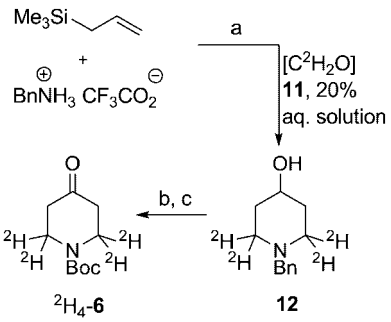


(a) DIPEA, TBAF·H_2O, DMSO 85 °C, 16 h, 75% (b) 1.25 N HCl then 15% v/v Na_3PO_4(aq) *quant*. (c) $NaBH(OAc)_3$, Na_2SO_4, THF, 16 h, 73% (d) 4 N HCl then 15% v/v Na_3PO_4(aq) 95% (e) 4-chlorobenzoyl chloride **9**, *cat*. DMAP, DIPEA, MeCN, 5 h 82% (f) NH_2NH_2·H_2O, MeOH, 65 °C *quant*. (g) EtNCO, CH_2Cl_2, 30 min, then TsCl, DIPEA, 64%

Scheme 1.

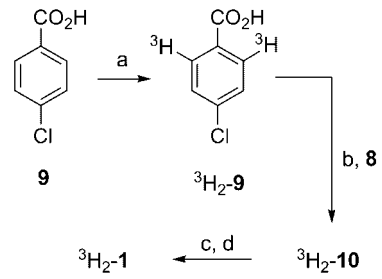
^{2}H Chemistry: Synthesis of the $^2H_4$-labeled analog **6** was accomplished using a modified procedure for the synthesis of *N*-benzyl-4-hydroxypiperidine (**12**) reported by Grieco and co-workers (Scheme 2).[3] Benzylamine trifluoroacetate salt, allyltrimethylsilane and deuterated formaldehyde (employed as a 20% w/v aqueous solution), were heated to 40°C and allowed to react overnight, yielding 57% of the desired *N*-benzyl hydroxypiperidine (**12**). **12** was then converted to the *N*-Boc-protected analog, before smoothly undergoing TPAP-mediated oxidation to the desired labeled precursor $^2H_4$-**6** in 83% yield over two steps.[4] The [$^2H_4$] synthesis was completed according to Scheme 1, providing 503 mg of $^2H_4$-**1** at 97.8% purity. This procedure has been utilized for the synthesis of several labeled-piperidinones in our laboratory, using an aqueous solution of labelled formaldehyde (Scheme 2).[5]



(a) 40 °C, 16 h, 57% (b) 1. Boc_2O, $Pd(OH)_2$, H_2 (40 psi) (c) TPAP/NMO 67% over two steps

Scheme 2.

^{3}H Chemistry: A tritium-label was readily introduced into the same synthetic route via 2,6-(^{3}H)-4-chlorobenzoic acid by an amide bond coupling reaction with **8**. 48 mCi tritiated 4-chlorobenzoic acid ^{3}**H-9** was prepared by tritium exchange with THO using $Ru(acac)_3$ as the catalyst. ^{3}**H-10** was then converted to ^{3}**H-1** according to Scheme 1, yielding 31 mCi of **1** (Scheme 3).



(a) $Ru(acac)_3$, THO, 48 mCi, 96% RCP (b) HOBt, DIPEA (c) $NH_2NH_2{\cdot}H_2O$, MeOH, 65 °C (d) EtNCO, CH_2Cl_2, 30 min, then TsCl, DIPEA, 64% over three steps, 31 mCi, > 99% RCP

Scheme 3.

^{14}C Chemistry: The desired site of labeling was determined to be the aromatic pyrazine heterocycle **2**. The synthesis of this heterocycle has been published by Imai and coworkers, starting from methyl pyruvate and diaminomaleonitrile.[6] Rather than carry the labeled pyrazine through all of the synthetic steps outlined in Scheme 1, we envisaged coupling the whole of the right hand heterocyclic system directly to the pyrazine core, generating ^{14}C-**10** directly and intersecting the existing route, with only a few synthetic operations remaining (Figure 2).

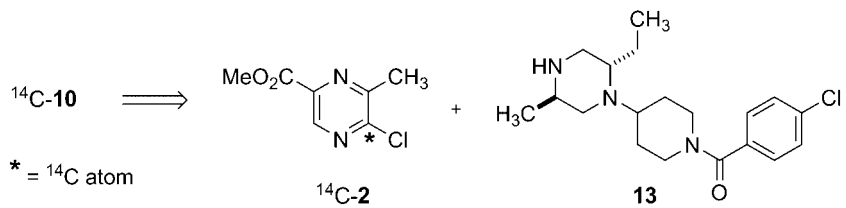
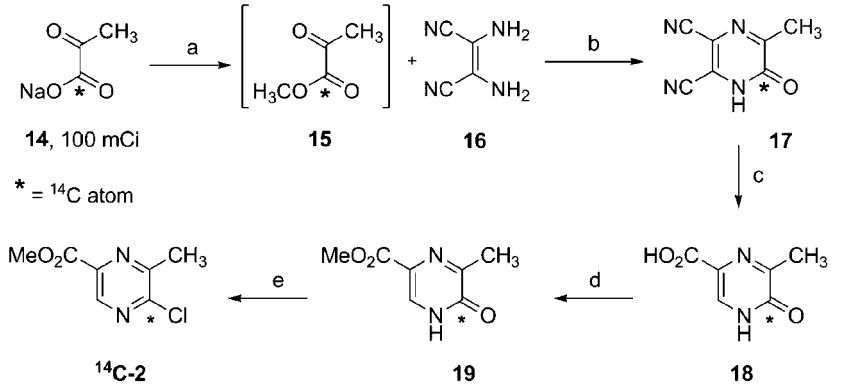


Figure 2. Retrosynthetic analysis of C-14 labeled **10**.

We were attracted to the idea of incorporating a ^{14}C-labeled atom into the piperazine via a derivative of pyruvate. ^{14}C-sodium pyruvate (**8**) was selected as the labeling source, and incorporated into the heterocycle according to Scheme 4:



(a) 7.5 N HCl/MeOH, 4 h (b) H_2O, 2 h (c) conc. HCl(aq), Δ, 16 h, (d) $SOCl_2$-MeOH, 2 h (e) $POCl_3$, 1 drop DMF, reflux, 4 h, 68 mCi

Scheme 4.

Treatment of sodium pyruvate ^{14}C-(**14**) with concentrated HCl in methanol (7.5 N) generated methyl pyruvate ^{14}C-(**15**) in situ. After dilution with water, treatment with diaminomaleonitrile (**16**) led to the formation of heterocycle ^{14}C-**17**. Refluxing aqueous HCl then effected the hydrolysis and regioselective decarboxylation to yield ^{14}C-**18**, which was then subjected to sequential treatment with thionyl chloride and methanol, followed by phosphorus oxychloride giving ^{14}C-**2** in 68% yield at 98% radiochemical purity.

From 100 mCi of sodium pyruvate, 68 mCi of the desired chloropyrazine ester ^{14}C-(**2**) was obtained. To utilize our modified strategy required the synthesis of **13**, which we accomplished in six steps starting from *N*-Boc-piperazine (**3**) (Scheme 5).

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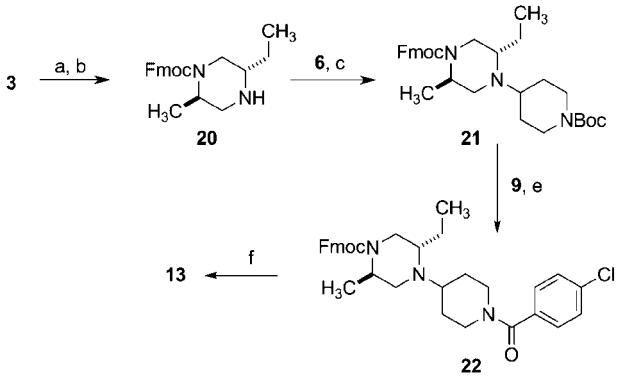

(a) 1. FMocCl, 16 h then 4 N HCl 1 h (b) 15% v/v Na_3PO_4(aq) (c) 1. $NaBH(OAc)_3$, Na_2SO_4, THF (d) 4 N HCl 1 h, then Na_3PO_4(aq) (e) DMAP, TEA, MeCN (f) piperidine, THF, 1 h

Scheme 5.

The key TBAF-promoted coupling of piperazine **13** with chloropyrazine ^{14}C-**2** proceeded smoothly to yield 18.6 mCi after flash chromatography. ^{14}C-**6** was then converted to the target compound in identical fashion to Scheme 1 to yield 11.2 mCi at 98.6% radiochemical purity.

Conclusions: The $^{3}H_2$-Sch 900875-labelled fragment was prepared by ruthenium-catalyzed tritium-hydrogen exchange of 4-chlorobenzoic acid (**9**), which was readily incorporated into the existing synthesis. $^{2}H_4$-Sch 900875 was prepared by introducing a deuterium-labelled 4-piperidone (**6**) into the synthetic sequence. ^{14}C-Sch 900875, with the label located in the pyrazine ring was prepared by modifying the synthesis to introduce the ^{14}C-label at the latest possible stage. 11.2 mCi was prepared starting from 100 mCi of sodium pyruvate. chemical purities of labelled compounds were assessed by HPLC, LC-MS, MS and ^{1}H and ^{13}C NMR.

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[2] **3** can be prepared via a four-step sequence starting with *N*-benzyl-protected alanine methyl ester. Homo-coupling with a 2-amino propionic acid derivative leads to a diketopiperazine, which after reduction and protecting group manipulation gives **3**. For details see: G. N. Anilkumar, Q. Zeng, S. B. Rosenblum, J. A. Kozlowski, B. F. McGuinness, D. W. Hobbs WO/2006/088840.

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HANDLING OF TRITIUM REAGENTS IN MULTI-CURIE SCALE

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Keywords: tritium; tritiated water; lithium tritide; tritiomethyl iodide

Abstract: Synthesis and applications of various tritium reagents (tritiated water, tritide reagents, tritiomethyl iodide) for the chemo- and regioselective labelling are described and examples of products, which show high specific activities, are presented.

Introduction: Tritium-labelled compounds with high specific activities are applied in pharmaceutical research for studies of metabolism (ADME), imaging technologies for the detection of tissue distribution (autoradiography), and experiments to elucidate irreversible covalent binding to proteins.[1] Metabolism studies in particular require chemo- and regioselectively labelled compounds. Besides the standard reactions of halogen/tritium exchange and hydrogenation of unsaturated compounds, labelling with tritiated water with high specific activity (SA), reduction of suitable precursor compounds with tritide reagents and the introduction of a tritium-labelled methyl group have gained in popularity in the past decade.

Results and Discussion: *Applications of tritiated water:* Tritiated water (HTO, T_2O) has been used for decades for H/T exchange.[2] Sometimes the position of the exchange is predictable, but often additional exchange occurs, which is difficult to estimate. This labelling method is of particular interest when no precursor is available for chemical transformations. Tritiated water is synthesized in a well dried vessel using PtO_2 and carrier-free tritium gas according to the literature.[3] Pre-drying of commercially available PtO_2, which is not free of water, leads to an increased specific activity. In this context, aspects of radioprotection have to be considered seriously as a tiny quantity of 7.5 μl (0.35 mmol) of T_2O (assuming a specific activity of 57 Ci/mmol) corresponds to an activity of 20 Ci. It is well known that tritiated water is rapidly incorporated through lungs and skin.[4] Therefore, it is easy to understand that the synthesis and all manipulations with tritiated water have to be carried out in a completely closed system. In case of release and uptake of just a 1/100 of the above mentioned activity, an effective dose of 130 mSv would result. According to the Swiss regulations, this is far above the limit for the effective dose of 20 mSv, the maximum annual exposure for an employee working with radioactivity. Therefore, T_2O is synthesized and handled using a tight tritium manifold system manufactured by RC TRITEC Ltd.,[5,6,7] which allows manipulations without any release of T_2O.

Typical approaches for H/T exchange using tritiated water vary pH or use different metal catalysts. Some of these methods were deduced from acid and base promoted H/D exchange.[8] The difficult radioprotection regulations allow the use of only small quantities of tritiated water due to its high specific activity, whereas in an H/D exchange D_2O can be utilized as neat solvent.

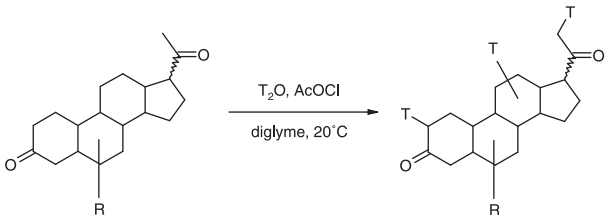

Figure 1. Acetyl chloride promoted H/T exchange.

Figure 1 shows an acid promoted H/T exchange at a steroid derivative yielding the tritium-labelled product with a specific activity of 118 Ci/mmol due to additional exchange at the skeleton. We used tritiated water in twelvefold excess (19.4 Ci), acetyl chloride with 18 equivalents, and produced a crude product with a radiochemical purity of $>80\%$.

Metal catalyzed H/D and H/T exchanges using $RhCl_3 \cdot 3\ H_2O$ and Ru acetylacetonate were found to promote *ortho*-exchange with high regioselectivity. [9,10] The unspecific H/T exchange using PtO_2 and tritium gas mostly gives products with low specific activities ($\leqslant$1 Ci/mmol)[11], and was successfully applied for H/T exchange of biopolymers in our laboratory.

An attractive method for the synthesis of compounds with high specific activities is the quench with tritiated water of carbanions generated by deprotonation with strong lithium bases.

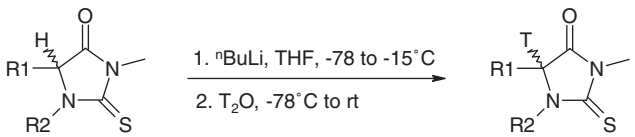

Figure 2. Deprotonation and quench with tritiated water gave a 2-thioxo-4-imidazolidinone derivative at 12 Ci/mmol.

The example in Figure 2 shows the deprotonation of a 2-thioxo-4-imidazolidinone derivative (21 μmol) with an equimolar quantity of *n*-butyllithium and subsequent quench with 24 equivalents of tritiated water (28.5 Ci).

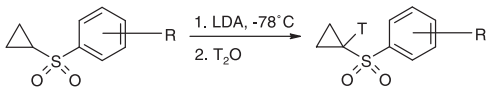

Figure 3. Specific activity of the product was 24 Ci/mmol.

Figure 3 illustrates the H/T exchange of a cyclopropyl derivative, when treated with a tenfold excess of lithium diisopropylamide at -78°C and quenched with 16 equivalents of tritiated water (18.8 Ci). The crude product had a radiochemical purity of 50%.

Applications of LiT: A milestone in regioselective tritium-labelling was the development of various lithium and other metal tritides, all derived from lithium tritide. Than *et al.*[12] used the observation to form finely powdered lithium hydride, obtained from stirring a hexane solution of *n*-butyllithium and 1,2-bis(dimethylamino)ethane (TMEDA) under an atmosphere of hydrogen gas[13] to synthesize lithium tritide. With this method a specific activity of approximately the theoretically value (28.8 Ci/mmol) was achieved. LiT was found to be the key compound for the syntheses of important reducing reagents: $LiBT_4$, $LiAlT_4$, $LiBEt_3T$ and Bu_3SnT.[3,14–16] These compounds allow chemo- and regioselective labelling, making them versatile tools in tritium chemistry. However, the major drawback of synthesizing tritides is the production of tritiated butane as an undesired by-product. The problem of gaseous waste treatment prevents the wide spread use of these valuable reagents, which are known to give products with high specific activities.

The chemoselective reduction of an aldehyde in presence of an amide function was achieved with $LiBT_4$ and the corresponding primary alcohol had a specific activity of 21 Ci/mmol. The reduction of an ester function of sugar derivative with 40 μmol (4.0 Ci) $LiBT_4$ gave the alcohol with a specific activity of 55 Ci/mmol (Figure 4A). We encountered an enhanced specific activity of the products when $LiBT_4$ (estimated specific activity of approx. 100 Ci/mmol)[17] was used compared to $NaBT_4$ (estimated specific activity

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40 Ci/mmol), which is synthesized by thermal H/T exchange with carrier-free tritium gas. In comparison with $NaBT_4$, $LiBT_4$ shows a stronger reduction potential, allowing the reduction of ester and lactone functionalities. Furthermore, the solubility of $LiBT_4$ in THF and diethyl ether is higher. Since the Li cation is a stronger Lewis acid showing increased tendency to form complexes, a higher diastereoselectivity is observed.[17]

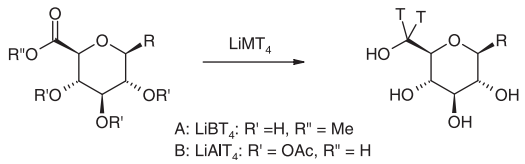

Figure 4. A: Reduction of an ester with $LiBT_4$ gave a primary alcohol with a specific activity of 56 Ci/mmol. B: In a one-step reaction, reduction of a carboxylic acid and cleavage of the protected hydroxy groups was achieved, giving a primary alcohol at 55 Ci/mmol.

The powerful tritide reagents $LiAlT_4$ and $LiBEt_3T$ (Supertritide), synthesized from LiT and $AlCl_3$ or BEt_3 respectively, can be used for the reduction of various substrates and are well known to give products with high specific activities.[3,14–16,18] In Figures 4B and 5, the reduction of a carboxylic acid with $LiAlT_4$ and the reduction of an ester with $LiBEt_3T$ are shown. Reactions were performed in typical scales of 40 μmol of the tritide reagent, corresponding to an activity of 4.0 Ci. Depending on the substrate, the stoichiometry of the tritide can vary as in the first example 6.7 equivalents of $LiAlT_4$, in the second example 1.7 equivalents of Supertritide were used. In both cases the theoretically expected values of the specific activities were almost achieved.

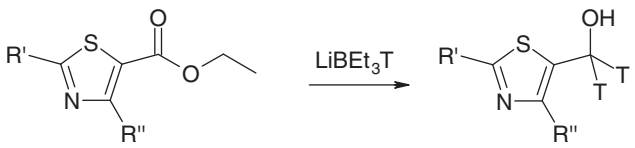

Figure 5. Reduction of an ester with Supertritide giving a primary alcohol at 58 Ci/mmol.

Tributyltin tritide ($^nBu_3SnT$) is a versatile tool to accomplish dehalogenation of aliphatic compounds and deoxygenation of alcohols.[14] The synthesis of the reagent from LiT and $^nBu_3SnCl$ with maximum specific activity of 28.8 Ci/mmol is facile and the reagent can be readily purified by column chromatography prior to use.[19] As $^nBu_3SnT$ shows high chemoselectivity, it can be applied even in presence of sensitive functional groups.[3] Figure 6 illustrates the radical induced dechlorination using 3.28 Ci of tributyltin tritide to give a ketone derivative at 35 Ci/mmol in a radiochemical yield of 20%.

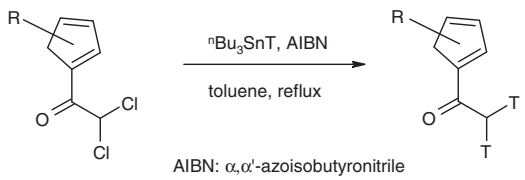

Figure 6. Dechlorination with $^nBu_3SnT$ yielded a ketone derivative at 35 Ci/mmol.

Applications of tritiomethyl iodide (CT_3I): Different starting materials were reported for the syntheses of tritiomethanol with high specific activity, which is converted to CT_3I with hydriodic acid.[20–23] Our approach was inspired by the synthesis of tritiomethanol from carbon dioxide reduced with $LiAlT_4$.[22] Substitution of gaseous carbon dioxide by the non-volatile and commercially available substrate diphenyl carbonate simplified the handling, and the final product CT_3I shows specific activities in the range of 65 to 85 Ci/mmol. With this reagent, methylation of alcohols, phenols, amines, thiols, etc. are accomplished in μmol scale. Due to its volatility and toxicity, careful handling in a completely tight system has to be ensured. A selected example for an S_N2 reaction with an alkynyl derivative, which is generated *in situ* by deprotonation with butyllithium, is presented in Figure 7. The C-C coupling reaction was performed in a 1.6 Ci (25 μmol) scale of CT_3I and a 1.3-fold excess of acetylene derivative in a radiochemical yield of 32%.

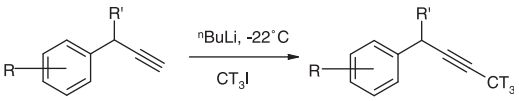

Figure 7. Methylation of an alkynyl carbon gave a tritio-2-butin derivative at 65 Ci/mmol.

Conclusion: The need of tritium-labelled compounds with high specific activities in pharmaceutical research can be covered by application of the reviewed tritium-labelled reagents. The use of tritiated water for H/T exchange should be considered if suitable precursors are not available. While this method often gives rise to additional exchange on the skeleton, treatment of acidic C-H positions with base may provide access to regioselective labelling. If regioselectivity is a prerequisite, the chemoselective reduction of a precursor with a suitable tritide reagent is the method of choice. Methylated compounds can be obtained in a one-step reaction using tritiomethyl iodide and the desmethyl precursor.

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SYNTHESIS OF A C-14 LABELED DELTA-OPIOID RECEPTOR AGONIST BY CARBONYLATION

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Abstract: Opioid drugs have long been known to bind to specific receptors in both the brain and periphery. First identified in the 1970s, these receptors are classified as mu, kappa and delta.[1] While each of these receptors appear to be involved in regulation of pain pathways and of mood, the delta opioid receptor produces a unique pharmacology. AstraZeneca has designed a number of agonists for the receptor, and in order to better understand this receptor's pharmacology, a C14-labeled compound was prepared. The structure of this compound was ideal for a late stage carbonylation using C-14 carbon monoxide to give the C-14 labeled compound an 18% overall radiochemical yield.

Key words: C-14 carbonylation; Delta-Opioid receptor agonist

Introduction: There are 3 distinct opioid receptor subtypes : mu, kappa, and delta. While these G-protein coupled receptors have a relatively high sequence homology, they differ considerably in terms of their pharmacology and distribution both in brain as well as in the periphery.[2]

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Mu receptors underlie the analgesic, sedative, addictive, gastrointestinal, as well as respiratory depressant effects of the prototype agonist, morphine and related drugs. Both enkephalin and endorphin peptides serve as endogenous ligands for mu receptors. Kappa receptors, on the other hand, subserve a different type of analgesia than mu receptors, and agonists additionally are known to produce diuresis, negatively regulate mood and produce hallucinogenic effects.[3] Agonists of delta opioid receptors may confer still a different type of analgesia than mu or kappa agonists, but without the same deleterious side effects associated with the other opioid receptors. Enkephalins are the endogenous ligands for delta receptors, and agonists could therefore be referred to as 'enkephalinergics'. Delta agonists have been demonstrated to produce antidepressant and anxiolytic effects in a variety of animal models, suggesting further therapeutic indications.[4]

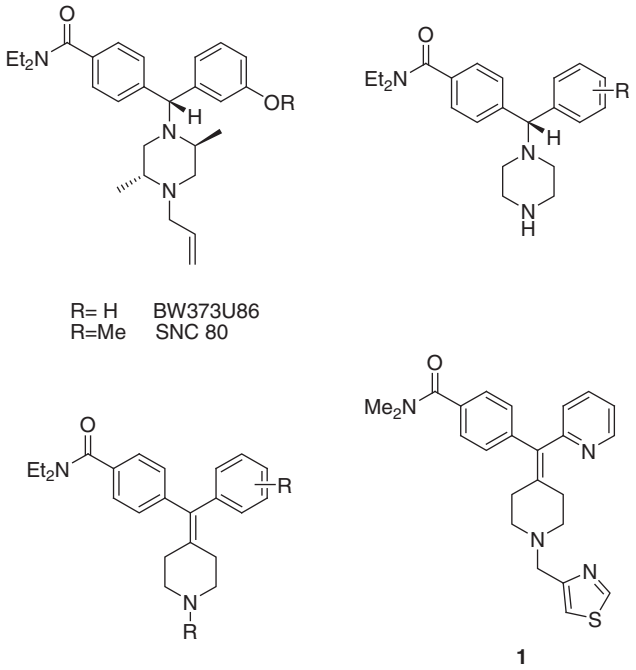
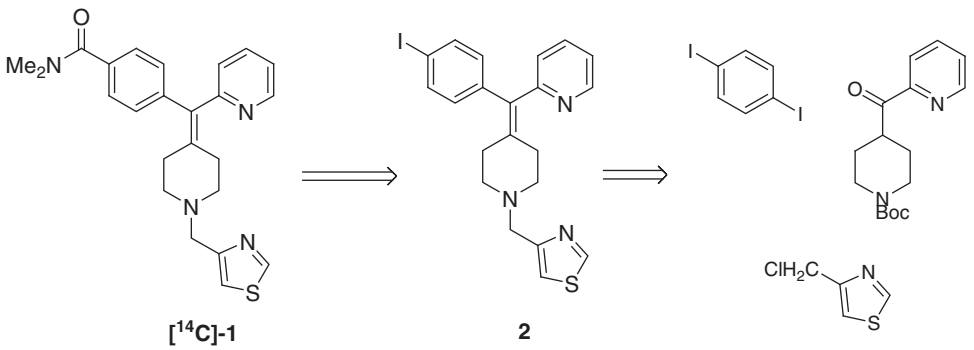


Figure 1. Several non-peptidic, small molecule delta-opioid selective agonists.

Several small molecules have been reported to have high affinity and good selectivity for the delta-opioid receptor (Figure 1). BW373U86 was the first reported non-peptidic delta-opioid selective receptor agonist;[5] it displays high potency at the delta-opioid receptor (1 nM) with > 50 selectivity over the mu-opioid receptor. Methylation of the BW373U86 and resolution of the enantiomers afforded SNC 80 which displayed improved selectivity against mu-opioid binding (> 300 fold) while also maintaining potency (0.5 nM).[6] Further modification of BW373U86 demonstrated that the piperazine methyls were not required for activity[7] and in fact an achiral molecule–substituting an olefin for the benzylic amine - could have high potency and good selectivity.[8] In an effort to find a compound with better drug-like properties, dimethylamide **1** was prepared, and in order to more fully understand its properties, it was required labeled with C-14.

Results: The most obvious site for C-14 labeling of compound **1** was in the amide carbonyl (Scheme 1). We felt that incorporation of the label using either late-stage carbonylation or carbonylation[9] of an appropriate precursor would provide the target compound in one to two steps. The requisite starting iodide **2** could be prepared in a straightforward method similar to that previously reported.[8]

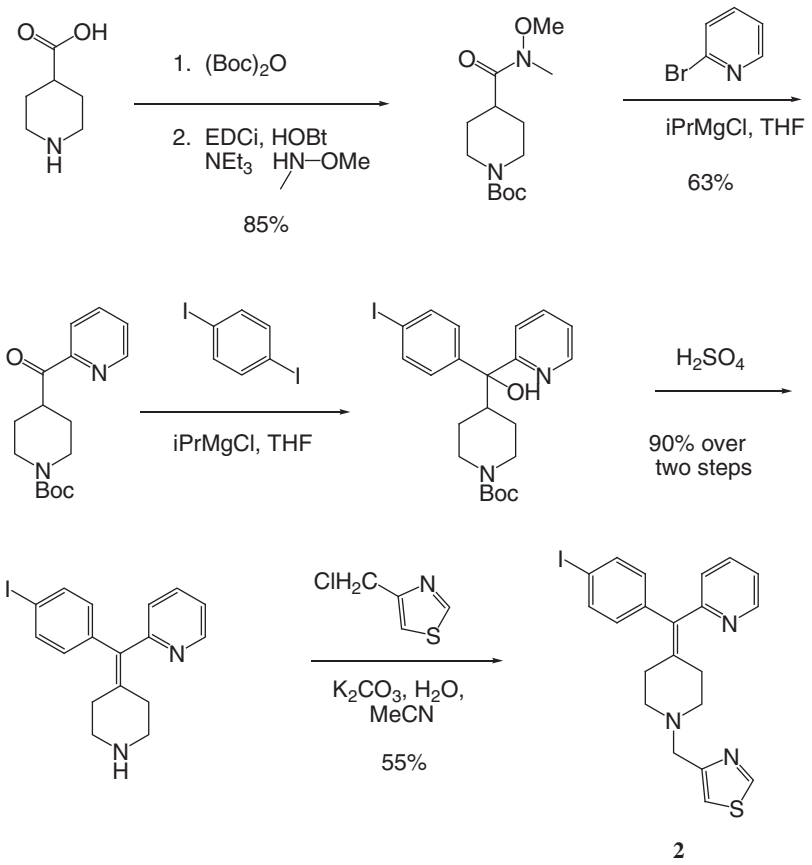


Scheme 1. Retrosynthetic analysis of **1**.

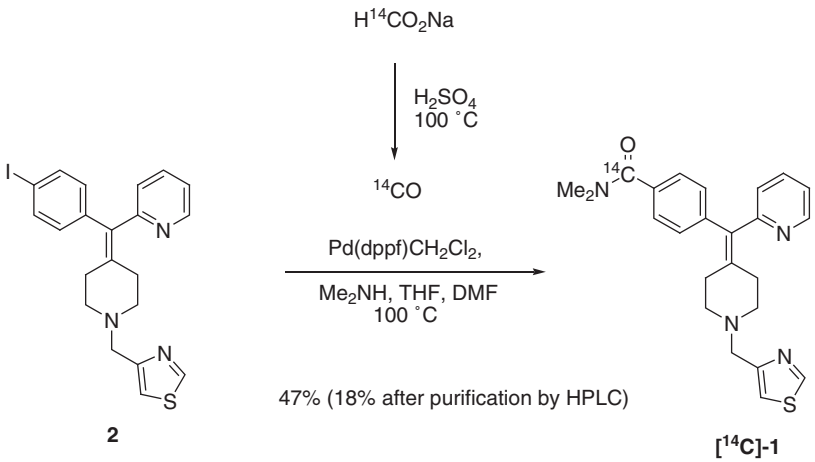
Isonipecotic acid was protected as the *t*-butylcarbamate and a standard peptide coupling was used to prepare the Weinreb amide from the acid (Scheme 2). The amide was then coupled with the Grignard reagent prepared from 2-bromopyridine using the methodology of Knochel to give the ketone in 63% yield.[10] A similar coupling of a Grignard reagent prepared from 1,4-diiodobenzene afforded the tertiary alcohol which was readily eliminated to give the olefin with concomitant removal of the *t*-butylcarbamate in 90% yield over the two step sequence. Coupling of the secondary amine with the corresponding chloride afforded iodide **2** in moderate yield after silica gel chromatography.

Both carbonylation with C-14 carbon monoxide and carboxylation with C-14 carbon dioxide were considered for the label incorporation. Carboxylations are in general higher yielding and less complex to run; however, carbonylation can provide amide **1** directly while carboxylation would necessitate a further step to form the peptide.[11] Additionally, the acidity of the thiazole might complicate the halogen metal exchange reaction. Therefore, carbonylation was the first approach investigated for the synthesis of **[^{14}C]-1**.

One equivilent of [^{14}C]formic acid was dehydrated with sulfuric acid and the resulting ^{14}CO was reacted with two equivilents of iodide **2**, 0.4 eq of Pd(Dppf)Cl_2 and excess dimethylamine in a 1:1 mixture of THF:DMF mixture at 80°C overnight (Scheme 3). After workup, HPLC assay showed a radiochemical purity of 90% and a radiochemical yield of 47%. Purification by preparative HPLC gave 1.5 mCi (18% radiochemical yield) at a radiochemical purity of 97.9% and specific activity of 52 mCi/mmol.



Scheme 2. Preparation of iodide **2**.



Scheme 3. Synthesis of [^{14}C]-1 *via* carbonylation.

Conclusions: Delta-opioid agonist **[^{14}C]-1** was prepared in one radiochemical step from iodide **2**. Close eluting radiochemical impurities necessitated the discarding of several product containing fractions to give an overall radiochemical yield of only 18%.

Experimental: *General*: Pd(Dppf)$Cl_2 \cdot CH_2Cl_2$, 2 M Me_2NH in THF and DMF were obtained from Sigma-Aldrich Chemical Company and were used directly in reaction. Sodium [^{14}C]formate was obtained from American Radiolabeled Chemical Company. ^{1}H NMR spectra were recorded on an Avance 500 and were reference to residual solvent peak (7.26 for $CDCl_3$).

N,N-dimethyl-4-((pyridin-2-yl)(1-((thiazol-4-yl)methyl)piperidin-4-ylidene)methyl)benzamide (**[^{14}C]-1**) A three necked flask containing 13 mg (8.5 mCi, 0.16 mmol) of sodium [^{14}C]formate was fitted with a rubber septum, a vacuum adapter, and a 90° bent adapter. A flask containing 114 mg (0.14 mmol) of Pd(Dppf)$Cl_2 \cdot CH_2Cl_2$, 156 mg (0.33 mmol) of iodide **2**, 4 mL of DMF and 4 mL of 2 M Me_2NH in THF was attached to the other end of the bent adapter (N2 was bubbled through the solution for 2 min). The flask containing the THF/DMF solution was cooled in liquid nitrogen and the apparatus evacuated. The valve leading to the vacuum was closed and 20 mL of concentrated sulfuric acid was injected through the septum. The sulfuric acid solution was warmed to 110°C for 1 h. and the reaction flask was heated and stirred at 80°C for 12 h. The DMF/THF mixture was concentrated to dryness and was then taken up in MeCN to give 4.5 mCi. HPLC analysis (5 to 20% MeCN-0.1% TFA on a Phenomenex Luna C-18(2) column over 20 min) indicated a radiochemical purity of 90%. Preparative HPLC (5 to 15% MeCN-0.1% TFA on 21.2 × 250 mm Phenomenex Luna C-18(2) column over 45 min, 15 mL/min) afford 1.5 mCi of **[^{14}C]-1** as a solution in EtOH with a radiochemical purity of 97.9% (5 to 20% MeCN-0.1% TFA on Phenomenex Luna C-18(2) over 20 min followed by wash with 100% MeCN) and a specific activity of 52 mCi/mmol. ^{1}H NMR (500 MHz, $CDCl_3$) ● ppm 2.7 (m, 4 H), 2.98 (br. s., 3 H), 3.10 (br. s., 3 H), 3.5 (m, 4 H), 4.41 (br. s., 2 H), 7.20 (m, 3 H), 7.39 (m, 3 H), 7.74 (br. s., 1 H), 7.86 (t, J = 7.5 Hz, 1 H), 8.72 (br. s., 1 H), 8.82 (br. s., 1 H). LC/MS (M+H): 421 (100%), 419 (19%), 422 (25%).

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