JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
J Label Compd Radiopharm 2004; **47**: 523–530.
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jlcr.841

Research Article

BF_3 etherate catalysed formation of [^{11}C]methyl esters: a novel radiolabelling technique

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Summary

A novel way of preparing ^{11}C labelled methyl esters using [^{11}C]methanol and either BF_3 etherate or trimethylsilyl chloride as catalyst was investigated. Radiochemical yields with BF_3 etherate were between 30 and 33% for [^{11}C]methyl benzoate and less than 1% for [^{11}C]methyl thio salicylate. No [^{11}C]methyl ester formation could be observed with trimethylsilyl chloride for all compounds investigated.

This method is an alternative to using [^{11}C]methyl iodide in the presence of a base. It is particularly suited for carboxylic acids bearing functional groups which would compete for [^{11}C]methyl iodide, thus eliminating the need to introduce protecting groups. However, *o*-anisic acid formed [^{11}C]methyl salicylate in 33–30% decay corrected radiochemical yield due to hydrolytic cleavage of the methyl ether, and none of the desired [^{11}C]methyl 2-methoxy benzoate could be obtained. When salicylic acid was used as starting material, [^{11}C]methyl salicylate could only be obtained in 5–8% decay corrected radiochemical yield.

Key Words: radiolabelling; carbon-11 methanol; catalysis

Introduction

The radiolabelling of carboxylic acids via a nucleophilic substitution mechanism using [^{11}C]methyl iodide or [^{11}C]methyl triflate is usually complicated by the presence of other functional groups in the molecule, which compete with the carboxylate ion for the methyl iodide.[1] This leads to labelling at multiple sites of the molecule and is therefore not a very selective strategy. [^{11}C]diazomethane has also been used to synthesize [^{11}C]methyl

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Contract/grant sponsor: Austin Hospital Research Foundation; contract/grant number: 2-1551

Received 31 July 2003
Revised 31 March 2004
Accepted 17 April 2004

esters.[2,3] However, the production of this compound is technically demanding and is not routinely available at most PET centres.

An addition–elimination mechanism, as can be found in esterification reactions, would be an attractive alternative, since few functional groups are able to react in such a manner and the [^{11}C]methanol required for such a reaction can be produced easily. However, due to the low reactivity of carboxylic acids, a catalyst is usually necessary to promote these types of reactions. While Lewis acids are routinely employed for this purpose in organic synthesis[4–8] they have yet to be used in PET radiochemistry. Here we have investigated the potential of the two most commonly available Lewis acids, BF_3 etherate and trimethylsilyl chloride, for the synthesis of ^{11}C labelled methyl esters from [^{11}C]methanol and the corresponding carboxylic acid. The most promising catalyst was employed to investigate the feasibility of this strategy for the one step synthesis of [^{11}C]AG957 (**4**). AG957 is an inhibitor of the tyrosine kinase p210$^{bcr\text{-}abl}$, a protein which is exclusively found in patients suffering from chronic myeloid leukaemia (CML). The synthesis of [^{11}C]AG957 (**4**) currently involves 2 additional synthesis steps after the labelling step (Figure 1) and is difficult to automate.[9] It is also a time consuming process thus making it difficult to prepare [^{11}C]AG957 (**4**) with high specific activity due to the short halflife of ^{11}C. The development of a one-step synthesis for this radiotracer is desirable since it makes automation easier and a shorter synthesis time would also increase the specific activity.

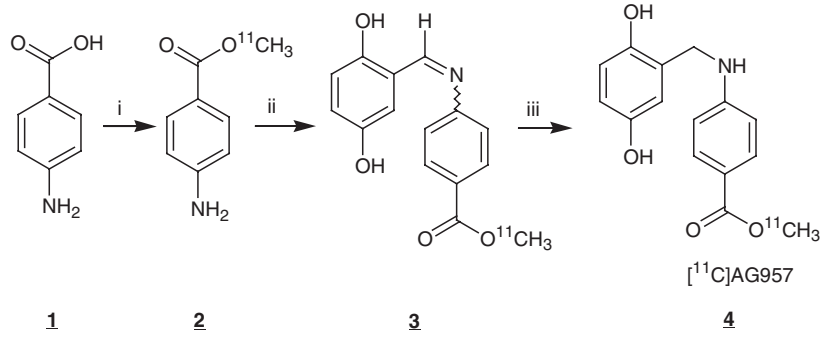


Figure 1. Synthesis and radiolabelling of AG957
Reagents and conditions: (i) [^{11}C]CH_3I, $BnNMe_3OH$, DMF, 5 min, 70°C. (ii) 2,5 dihydroxy benzaldehyde, DMF, TosOH, 5 min, RT. (iii) $NaBH_3CN$, RT, 30 s

Results and discussion

Six different aromatic carboxylic acids have been tested for esterification using BF_3 etherate or trimethylsilyl chloride and [^{11}C]methanol (Figure 2).

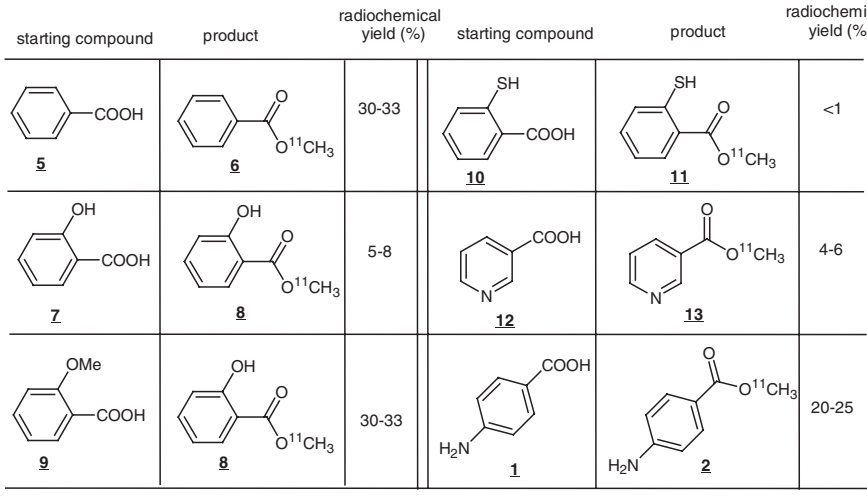


Figure 2. Carboxylic acids tested for esterification using BF_3 etherate and [^{11}C]CH_3OH
Reagents and conditions: neat BF_3 etherate, 150°C, 10 min

Radiochemical yields with BF_3 etherate varied greatly, ranging from 30 to 33% for [^{11}C]methyl benzoate (**6**) to less than 1% for [^{11}C]methyl thio salicylate (**11**). In comparison, labelling was unsuccessful with trimethylsilyl chloride for all compounds investigated. Reacting *o*-anisic acid (**9**) with BF_3 etherate yields [^{11}C]methyl salicylate (**8**) in 30–33% radiochemical yield and does not form any of the desired [^{11}C]methyl 2-methoxy benzoate due to cleavage of the methoxy group under the reaction conditions. Unexpectedly, when using salicylic acid (**7**) as precursor the decay corrected radiochemical yield of [^{11}C]methyl salicylate (**8**) was only 5–8% compared to 30–33% when using *o*-anisic acid as starting material.

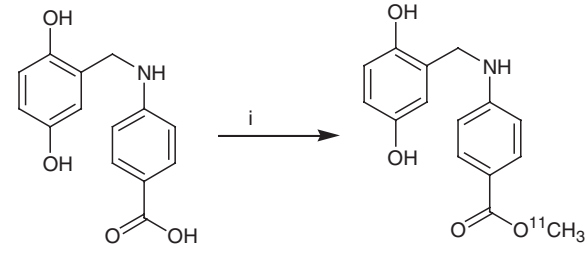
For simple carboxylic acids which do not require the use of protecting groups for labelling, the BF_3 etherate method produces lower radiochemical yields compared to labelling with methyl iodide. For example, the reaction of *p*-amino benzoic acid (**1**) with [^{11}C]methyl iodide gives [^{11}C]methyl *p*-amino benzoate (**2**) in 85–95% decay corrected radiochemical yield,[9] whereas a yield of only 20–25% of this compound could be obtained when using the BF_3 etherate method. On the other hand the labelling of carboxylic acids bearing functional groups that can complicate labelling with [^{11}C]methyl iodide benefits from the BF_3 etherate method. For example, we could not synthesise any [^{11}C]methyl salicylate (**8**) when we reacted salicylic acid (**7**) with [^{11}C]methyl iodide in the presence of benzyl trimethylammonium hydroxide as base. The BF_3 etherate method gave 4–6% decay corrected radiochemical yield of [^{11}C]methyl salicylate (**8**) when salicylic acid (**7**) was used as starting material, and 33–30% when starting from *o*-anisic acid (**9**).

In comparison, radiochemical yields between 20 and 50% using [^{11}C]diazomethane as a labelling reagent have been reported in the literature.[2,3] However, the preparation of [^{11}C]diazomethane takes 10 min and has only been achieved in 30% decay corrected radiochemical yield.[3] Unfortunately, [^{11}C]diazomethane is not available at our centre and we were therefore unable to use this reagent for this study.

We also investigated whether the BF_3 etherate method could be used to synthesize [^{11}C]AG957 (**4**). The carboxylic acid precursor (**14**) of AG957 (**4**) was synthesized as described previously[9] and reacted with [^{11}C]methanol in BF_3 etherate at 150°C (Figure 3). Unfortunately, desmethyl AG957 (**14**) was unstable under these conditions and the decay corrected radiochemical yield of [^{11}C]AG957 (**4**) was only 1%. Figure 4 shows the HPLC trace of the crude reaction mixture. The radioactive peak at 15 min corresponds to [^{11}C]AG957 (**4**). The UV trace shows intact desmethyl AG957 (**14**) at 7.5 min as well as an unidentified compound at 5 min, indicating decomposition of the starting material under the reaction conditions.

Our results demonstrate that ^{11}C labelled methyl esters can be prepared from carboxylic acids and [^{11}C]methanol using neat BF_3 etherate as catalyst. However, the relatively high temperatures necessary to promote the catalytic effect of BF_3 etherate can cleave other functional groups in the molecule. For example, under the reaction conditions, *o*-anisic acid (**9**) yields [^{11}C]methyl salicylate (**8**) in 33% decay corrected radiochemical yield but the desired [^{11}C]methyl 2-methoxy benzoate could not be detected. Also, due to the thermal instability of desmethyl AG957 (**14**), only small amounts of [^{11}C]AG957 (**4**) could be prepared by this method.

In order to circumvent these problems, we are currently investigating a transesterification reaction starting from enol esters[10] and [^{11}C]methanol using 1,3-dichlorotetrabutyldistannoxane as catalyst.[11,12] This transesterification is



desmethyl AG957 **14** [^{11}C]AG957 **4**

Figure 3. Radiolabelling of desmethyl AG957. Reagents and conditions: (i) [^{11}C]CH_3OH, BF_3 etherate 150°C, 1% yield

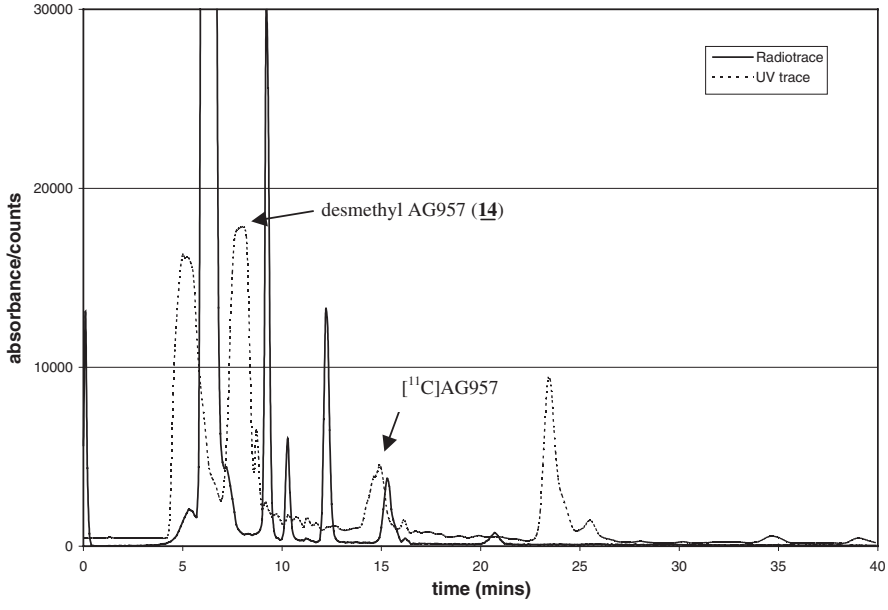


Figure 4. HPLC chromatogram of the crude reaction mixture of AG957 labelling

irreversible and our results with 1-ethoxy vinyl benzoate (**15**), an enol ester of benzoic acid (**5**), show that it proceeds smoothly at 80°C, forming [^{11}C]methyl benzoate (**6**) in 30–45% decay corrected radiochemical yield (Figure 5). Also, this catalyst is not known to cleave any other functional groups.[13]

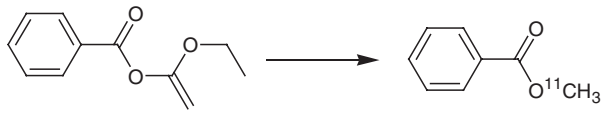
This method might represent a more efficient procedure for the production of [^{11}C]AG957 (**4**).

Experimental

General

[^{11}C]CO_2 was produced by the ^{14}N(p,α)^{11}C nuclear reaction using a target gas that consisted of 98.03% nitrogen and 1.97% oxygen. A 10 MeV proton beam was generated using the IBA Cyclone 10/5 cyclotron at the Austin Health, Centre for PET. Typical irradiation parameters were 40 μA for 30 min, which produced 22.2–25.9 GBq (600–700 mCi) of [^{11}C]CO_2. AG957 (**4**) and desmethyl AG957 (**14**) were synthesized following a published protocol.[9] $LiAlH_4$ (1 M in THF) was obtained in 1 ml vials from ABX advanced biochemical compounds. All other chemicals and solvents, including methyl ester standards, were purchased from Sigma-Aldrich.

The labelling procedure as well as semi-preparative HPLC purification was carried out using an in-house built automated system. A sealed glass vial with silicon septum was used to carry out the radiolabelling at 150°C.



15 **6**

Figure 5. Transesterification of 1-ethoxyvinyl benzoate. Reagents and conditions: $(Bu_4Sn_2OCl_2)_2$, [^{11}C]MeOH, 80°C

1-ethoxy vinyl benzoate (**15**) was synthesized according to a literature procedure and showed analytical data which were consistent with the literature values.[14]

Semi-preparative HPLC was performed using a Shimadzu LC-10AS isocratic pump equipped with a 1 ml injection loop, a reversed phase column (Exsil C-18, 250 mm, I.D. 10 mm), a Shimadzu SPD-6AV UV detector (254 nm) and a Geiger-Müller tube as radiodetector. The retention times of all [^{11}C]methyl esters were identical to their unlabelled standards.

[^{11}C]Methanol. [^{11}C]CO_2 was purged from the target, trapped in a cryogenic loop using liquid nitrogen and then passed through a vessel, containing a 1 M solution of $LiAlH_4$ in THF (150 µl). After completion of the transfer, the THF was evaporated and HCl (300 µl, 1 M) was added. The vessel was heated to 125°C to form [^{11}C]methanol, which was distilled in a stream of nitrogen gas. The decay corrected radiochemical yield of [^{11}C]methanol was 45–55%.

Standard procedure for the labelling of carboxylic acids using BF_3 etherate

[^{11}C]methanol was distilled in a stream of nitrogen gas using a double needle into a septum-sealed glass vial containing 50 µmol of the carboxylic acid in BF_3 etherate (300 µl). After complete transfer of [^{11}C]methanol, the needle was withdrawn and the vial lowered into an oil bath and kept at 150°C for 10 min. For injection into the HPLC, 1 ml of mobile phase was added and the crude product was then purified by semi-preparative HPLC using the above-mentioned set-up with 70% 0.1 M ammonium formate/30% acetonitrile as mobile phase at a flow rate of 4 ml/min.

The radioactive peak corresponding to the respective [^{11}C]methyl ester was collected and the level of radioactivity measured in a Capintec well counter. Decay corrected radiochemical yields are given in Figure 2. Specific activities for all compounds ranged between 0.8 and 1.2 Ci/µmol. Synthesis time from EOB was 40 min.

Standard procedure for the transesterification of (**15**)

[^{11}C]methyl benzoate. [^{11}C]methanol was distilled in a stream of nitrogen gas using a double needle into a septum-sealed glass vial containing 10 mg

(52 μmol) of 1-ethoxy vinyl benzoate (**15**) and 5 mg (4.5 μmol) of 1,3-dichlorotetrabutyldistannoxane in dry DMSO (300 μl). After complete transfer of [^{11}C]methanol, the needle was withdrawn and the vial was lowered into an oil bath and kept at 80°C for 7 min. For injection into the HPLC, 1 ml of mobile phase was added and the crude product was then purified by semi-preparative HPLC using the above-mentioned set-up with 70% 0.1 M ammonium formate/30% acetonitrile as mobile phase at a flow rate of 4 ml/min.

The radioactive peak corresponding to [^{11}C]methyl benzoate (**6**) was collected and the level of radioactivity measured in a Capintec counter. With this method, we produced a decay corrected radiochemical yield of 30–45% of [^{11}C] (**6**) based on [^{11}C]methanol, with a specific activity of 0.9–1.4 Ci/μmol. Synthesis time from EOB was 37 min.

Attempted labelling of salicylic acid (**7**) *with [^{11}C]methyl iodide*

[^{11}C]Methyl iodide. [^{11}C]CO_2 was purged from the target, trapped in a cryogenic loop using liquid nitrogen and then passed through a vessel, containing a 1 M solution of $LiAlH_4$ in THF (150 μl). After completion of the transfer, the THF was evaporated and non-stabilized HI (300 μl, 55–58%) was added. The vessel was heated up to 125°C to form [^{11}C]methyl iodide.

Attempted labelling. [^{11}C]methyl iodide was distilled in a stream of nitrogen gas into a reaction vial containing salicylic acid (**7**) (7 mg, 50 μmol) and benzyl trimethylammonium hydroxide (21 μl of a 40 wt% solution in water, 50 μmol) in 300 μl of DMF. After complete transfer of the methyl iodide, the vial was kept at 70°C for 5 min. For injection into the HPLC, 800 μl of mobile phase was added and the crude product was then analysed by semi-preparative HPLC using the above-mentioned set-up. No radioactive peak corresponding to [^{11}C]methyl salicylate (**8**) could be identified.

Conclusion

This novel strategy allows for efficient labelling of carboxylic acids bearing free amino or hydroxy groups. It is an alternative to the commonly used [^{11}C]methyl iodide since it eliminates the need for protecting groups, thus saving time and increasing specific activity. However, the high temperatures necessary to promote the catalytic effect of the BF_3 etherate exclude some compounds, such as desmethyl AG957 (**14**). A transesterification reaction, starting from enol esters might be a more suitable alternative for the labelling of those compounds.

Acknowledgements

This work was supported by the Austin Hospital Research Foundation, grant no. 2-1551.

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