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An efficient and expedient method for the synthesis of ^{11}C-labeled α-aminoisobutyric acid: A tumor imaging agent potentially useful for cancer diagnosis

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ABSTRACT

We describe the synthesis of ^{11}C-labeled α-aminoisobutyric acid **2** from iodo[^{11}C]methane and methyl *N*-(diphenylmethylen)-D,L-alaniate (**5**). The tetrabutylammonium fluoride (TBAF)-promoted α-[^{11}C]methylation of sterically hindered analog **5** was a key step in our synthesis process. Total radiochemical conversion of **2** was high and a remote-controlled synthesis was carried out. A comparative tumor positron emission tomography (PET) imaging study using the same model mouse showed higher uptake of **2** than with ^{11}C-labeled methionine and [^{18}F] fluorodeoxyglucose (FDG).

Isotopic labeling of amino acids with positron-emitting radionuclides is an important research topic for positron emission tomography (PET), a non-invasive imaging technology.[1] A variety of natural and unnatural amino acids labeled with carbon-11 and fluorine-18 have been synthesized and used for tumor imaging and brain studies.[1,2] Although labeled amino acids are useful probes to perform a large variety of PET studies, they are not readily synthesized by accessible methodologies, except for [*methyl*-^{11}C]methionine (Fig. 1).[1] As a result, only [*methyl*-^{11}C]methionine is used widely for cancer diagnosis; however, this compound is not ideal for tumor imaging because of metabolic [^{11}C]demethylation.[1,2a] The development of convenient synthetic methods for the preparation of labeled amino acids is an important challenge in the area of PET research.

The increase in amino acid transporters activity is found in many tumors. Such transporters are up-regulated during cell growth and division in tumor cells; thus, its assessment in vivo (which can be approached by tracer analysis using radio-labeled amino acids) becomes a useful tool for the clinical diagnosis of cancer.[3] In addition, a PET image of a labeled amino acid can describe tumors more selectively as compared with PET images using 2-deoxy-2-[^{18}F]fluoro-glucose ([^{18}F]FDG).[1,2a,b] Metabolically stable amino acid analogs have become a focus for isotope incorporation, because they can simplify the interpretation of PET images. In this context, ^{11}C-labeled α-aminoisobutyric acid ([^{11}C]AIB, Fig. 1) is a suitable molecule for tumor imaging, because AIB is primarily transported into viable cells via an A-type amino acid transporter system and is metabolically stable in cells.[4,5] The absence of a typical chiral center in amino acids at the α-position is advantageous as this feature facilitates interpretation of PET images.

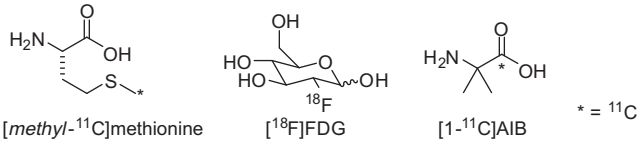

Figure 1. Structures of [*methyl*-^{11}C[methionine, [^{18}F]FDG, and [1-^{11}C]AIB.

The usefulness of ^{11}C-labeled AIB has been demonstrated in human and animal studies in vitro and in vivo; however, its use has not been widely developed because the labeling synthesis approach of this compound is generally inaccessible and not robust. Carbon-11-labeled AIB was first synthesized as [1-^{11}C]AIB by Bucherer–Strecker synthesis using [^{11}C]cyanide ion as a ^{11}C-labeling agent.[4a] Human and animal PET studies were performed using [1-^{11}C]AIB synthesized by this method.[5] However, the [^{11}C]cyanide ion is not a frequently used ^{11}C-labeling agent. Moreover, its synthesis requires the addition of carrier (non radioactive) cyanide to compensate for its low reactivity. As a result, rigorous quality assurance programs to ensure the absence of toxic

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amounts of cyanide are required. The syntheses of ^{11}C-labeled AIB using more convenient ^{11}C-labeling agents such as iodo[^{11}C]methane (**1**) and [^{11}C]carbon dioxide have been reported.[4b–d] However, organolithiums such as lithium diisopropylamide and methyl lithium are used in these methods. Careful handling and stringent stoichiometric requirements of organolithiums under the highly diluted conditions of the labeling reaction limit the practical use of ^{11}C-labeled AIB. As a result, no PET studies have been reported using ^{11}C-labeled AIB synthesized by these methods. Further development of new methodologies for carbon-11 labeling of AIB have stalled, while AIB analogs such as [*N*-methyl-^{11}C]MeAIB and ^{18}F-labeled AIBs have been developed.[6,7] The usefulness of AIB has led to the development of analogous tracers that can be synthesized more conveniently. Thus, the efficient and practical synthesis of ^{11}C-labeled AIB is still necessary for the use of this compound as a diagnostic agent.

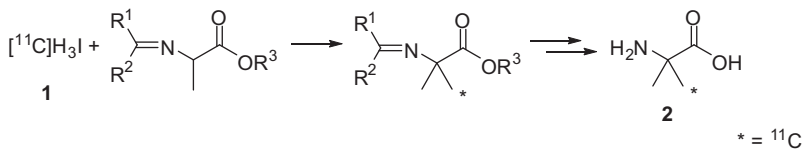
The use of Schiff base activated amino acid analogs is a primary method in the preparation of higher amino acids by α-alkylation under non-radiolabeling conditions.[8,9] Those methods have frequently employed phase-transfer (PT) conditions, and reactions are carried out under milder conditions. The benefit of using Schiff base analogs for the preparation of ^{11}C-labeled amino acids is that the radioactive precursor **1** (a very frequently-used methylating agent in PET chemistry) can be introduced in the synthetic scheme. Consequently, we planned to synthesize 2-amino-[3-^{11}C]isobutyric acid ([3-^{11}C]AIB, **2**) via α-[^{11}C]methylation of Schiff base activated alanine analogs (Scheme 1). Although the isotopic substitution at the 3-position by carbon-11 affords two enantiomers, the influence of a new chiral center is not expected to affect PET analyses.[10]

As compared with non-radiolabeling reactions, there are specific requirements that render the synthetic method suitable for the preparation of widely used PET tracers.[11] First, the use of easily-handled precursors is crucial for the success of a PET tracer synthesis; therefore benzophenone imine analogs (R^1, R^2 = Ph) have been chosen as Schiff base precursors. The pK_a values of benzophenone imine analogs are considerably higher than the ones corresponding *p*-chlorophenyl aldimine analogs (R^1 = *p*-ClPh, R^2 = H), which are frequently-used building blocks for the preparation of dialkylated amino acids under catalytic PT conditions.[8] Thus, dialkylation of benzophenone imine-type glycine derivatives yielding the corresponding α,α-symmetrical amino acids has been found to be difficult.[9b,c] However, the stable properties of benzophenone imine in air at room temperature for extended periods of time are recognized to be more important for a widely-used labeling precursor.[9a] Second, one-pot syntheses processes in which time-consuming manipulation steps are skipped are essential to approach ^{11}C-labeling strategies that involve more than two steps. One-pot methods render remote-controlled PET tracer syntheses more accessible and increase the radioactivity of the products. In this regard, the choice of the reaction solvent is a critical parameter and consequently, we have selected THF, DMF and DMSO as the reaction solvents. These solvents accept the direct addition of an aqueous solution to the reaction mixture for the subsequent hydrolysis of both the imine and ester groups.

In addition to the above mentioned points, there is another crucial point when identifying a suitable base for the incorporation of the [^{11}C]methyl-group into alanine analogs. Due to the difficulties associated with continuous stirring of the reaction mixture, the use of a dissolved base is preferable for the ^{11}C-labeling synthesis. In this regard, we have focused on the use of tetrabutylammonium fluoride (TBAF), because the fluoride ion shows a basic character when dissolved in organic solvents.[12,13] A comparison with tetrabutylammonium hydroxide (TBAOH), an active base of PT conditions, was carried out, because the pK_a values of the alanine analogs (around 23) were in the upper range of where PT conditions should be acceptable.[8,14] Other typical soluble bases such as triethylamine (TEA) and 1,8-diazabicyclo[5,4,0]undec-7-ene DBU were also investigated.

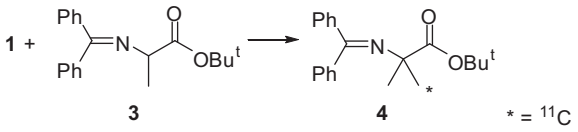
We initially explored the α-[^{11}C]methylation of the *tert*-butyl ester analog **3** by the reaction conditions: **3** (5 μmol), base (10 μmol), solvent (300 μL), reaction time (90 s) and reaction temperature (room temperature, rt). Two different reaction approaches that could be followed by remote-controlled synthesis were introduced. Method **A**: **3** and base were mixed around 10 min before the addition of **1**. Method **B**: base was added to the mixture of **1** and **3**. The results are summarized in Table 1. Treatment of **1** and **3** with TBAF by methods **A** and **B** did not results in α-[^{11}C]methylation neither using THF nor DMF. In contrast, treatment of **1** and **3** with TBAF in DMSO using method **A** yielded **4** with excellent radiochemical conversion (78.1 ± 4.8%). The reaction was carried out using an excess of TBAF; therefore stringent stoichiometry was not required for the fluoride-promoted α-[^{11}C]methylation of **3**. In addition, it was practical to use a commercial solution of TBAF·3H_2O for the α-[^{11}C]methylation reaction. Dimethylsulfoxide was also a suitable solvent for the α-[^{11}C]methylation using TBAOH as a base. Treatment of **1**, **3** and TBAOH in DMSO by method **B** gave **4** with a 34.3 ± 1.0% radiochemical conversion. However, α-[^{11}C]methylation was not accomplished by using DBU or TEA in DMSO, neither following **A** nor **B**.

During the search of α-[^{11}C]methylation in DMSO, remarkable differences were observed between TBAF and TBAOH. The reaction using method **B** gave better results than method **A** using TBAOH in DMSO. The radiochemical conversion of **4** using TBAOH with method **B** was moderate. In contrast, efficient α-[^{11}C]methylation of **3** was achieved using TBAF in DMSO using method **A**, whereas poor radiochemical conversion was obtained by method **B**. When TBAOH was used, the solution containing compound **3** immediately colored following the addition of the base; thus, the fast formation of the corresponding anion of compound **3** (which could contribute to the α-[^{11}C]methylation of **3** when method **B** was used) was assumed in a first step.[8a] However, the color diminished over time and was almost absent before the α-[^{11}C]methylation reaction was initiated by the addition of **1** to the mixture using method **A**. Since a significant amount of benzophenone was observed in the chromatogram of the reaction mixture, the hydrolysis of the imine group must contribute to the appearance of the color. Consequently, the hydrolysis consumed the hydroxide and decreased the amount of the acidic imine analog. In addition, the hydrolysis of **1** by TBAOH retarded the α-[^{11}C]methylation of **3**. These types of hydrolyses were also observed in the reaction using method **B**, and could be main reasons for the observed moderate radiochemical conversion when TBAOH was used. In contrast to TBAOH, the solution containing **3** gradually colored after mixing with TBAF in DMSO. This significant factor suggested the slow gen-



Scheme 1. General strategy for the synthesis of **2**.

Table 1
α-[^{11}C]Methylation of **3**[a]



Entry	Solvent	Base	Method	RCC[b] (%)
1	THF	TBAF	**A**	0
2	THF	TBAF	**B**	0
3	DMF	TBAF	**A**	0
4	DMF	TBAF	**B**	0
5	DMSO	TBAF	**A**	78.1 ± 4.1
6	DMSO	TBAF	**B**	10.0 ± 3.1
7	DMSO	TBAOH	**A**	4.1 ± 0.7
8	DMSO	TBAOH	**B**	39.5 ± 0.2
9	DMSO	TEA	**A**	0
10	DMSO	TEA	**B**	0
11	DMSO	DBU	**A**	0
12	DMSO	DBU	**B**	0

[a] Each reaction was carried out more than three times.
[b] The radiochemical conversion (RCC) was calculated by a radiochromatogram using analytical HPLC following the decay correction.

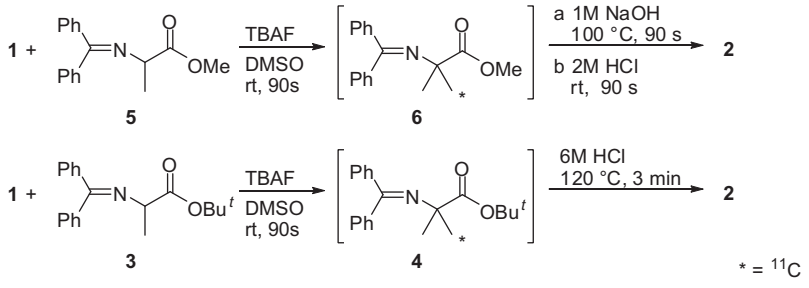
eration of the anion of compound **3** which leaded to a poor radiochemical conversion when method **B** was used. Furthermore, the color of the reaction mixture did not diminish significantly on treatment of **1**, **3** and TBAF in DMSO by method **A** until the α-[^{11}C]methylation reaction was quenched. Therefore, the hydrolysis by TBAF·3H$_2$O was negligible and the less latent activity of TBAF for hydrolysis contributed to the furnishing of the anion, resulting in high radiochemical conversion of **3**.

The advantage of using TBAF is that it provided the opportunity to render the synthesis of **2** more accessible. The lower latent hydrolytic activity of TBAF permitted the α-[^{11}C]methylation reaction of the methyl ester analog **5** without the presence of considerable hydrolysis-mediated side-reactions. The reaction of **1** and **5** with TBAF underwent the α-[^{11}C]methylation using method **A** to yield **6** with a 79.2 ± 6.2% radiochemical conversion (decay-corrected). In this reaction, prompt anion formation of **5** was observed by the addition of TBAF, due to the reduced steric hindrance of the methyl ester. The advantage of using **5** is the introduction of milder reaction conditions for the subsequent deprotection steps. Consequently, the hydrolysis of **6** was performed by the addition of a 1 M NaOH aqueous solution (100 μL) to the reaction mixture containing **6** and heating at 100 °C for 90 s. This was followed by cooling and the addition of a 2 M HCl aqueous solution (150 μL) at room temperature for 90 s. A radiochemical conversion of 72.1 ± 2.2% was obtained for the synthesis of compound **2** in three steps (Scheme 2).[15] In contrast, the addition of a 6 M HCl aqueous solution to the reaction mixture containing **4** and heating at 120 °C for 3 min gave **2** with a 70.0 ± 3.3% conversion started from **3** (Scheme 2).[15] The milder deprotection conditions for **6** enabled the direct injection of the final reaction mixture containing **2** onto the HPLC. Therefore, compound **5** was a more appropriate precursor for the implementation of a remote-controlled synthesis procedure for the preparation of **2**.

While PET tracer synthesis reactions require careful optimization to ensure high yields, particular attention should also be paid to the purification process in the development of an efficient PET tracer synthesis protocol. AIB is a small polar molecule and is therefore not an ideal substrate for HPLC purification using reversed-phase HPLC, which is the most frequently employed method in PET chemistry. The choice of a hydrophilic interaction chromatography (HILIC) mode stationary phase with triazole derivative coated silica provided a suitable method for the semi-preparative HPLC purification of **2** using a mixture of MeCN and ammonium acetate buffer.[16] After implementation of the remote-controlled system, the production of **2** was successfully achieved in a total synthesis time of 30–35 min from the end of bombardment (EOB). The syntheses could be carried out starting from 11.1 to 22.2 GBq of [^{11}C]carbon dioxide (EOB). Final radiochemical yields were in the range from 8% to 11% (decay uncorrected, referred to [^{11}C]carbon dioxide).[17] The amount of radioactivity was sufficient to perform PET studies in animals.

The usefulness of our synthesis was demonstrated by a comparison of PET studies using **2**, [*methyl*-^{11}C]methionine and [^{18}F]FDG for the same mouse bearing SY-tumor, a small cell lung cancer cell line. The PET scans of **2** and [*methyl*-^{11}C]methionine could be carried out on the same day due to the short-lived carbon-11. As seen in Figure 2, high uptake was observed in the tumors with all tracers, but the maximum standardized uptake value (SUV max) of compound **2** into tumor cells was higher than the one obtained for [*methyl*-^{11}C]methionine and [^{18}F]FDG. This is the first comparative tumor imaging study of these three molecules by PET and results strongly suggest the usefulness of **2** as a suitable radiotracer for cancer diagnosis. Further details about the tumor imaging studies by PET will be reported separately.

We have developed an efficient method for the synthesis of **2**. We found that the use of quaternary ammonium fluoride in DMSO was useful for the α-[^{11}C]methylation of highly hindered analogs using **1**. The reaction conditions were not noticeably moisture sensitive and did not require stringent stoichiometry. The established method employed a stable labeling precursor, an expedient ^{11}C-labeling agent and a one-pot approach, and therefore a remote-controlled synthesis could be performed without any technical difficulties. Comparative PET studies using **2** and some well known radiotracers ([*methyl*-^{11}C]methionine and [^{18}F]FDG) could be successfully performed thanks to the reliability of the here reported synthesis process. Future application of this radiotracer might be thus revitalized due to the synthesis methodology presented herein. We expect the synthesis of **2** can be accessible where tumor imaging studies using **2** are useful. Furthermore, we anticipate PET studies using ^{11}C-labeled AIB will be revitalized due to the synthesis methodology presented herein.



Scheme 2. Syntheses of **2**.

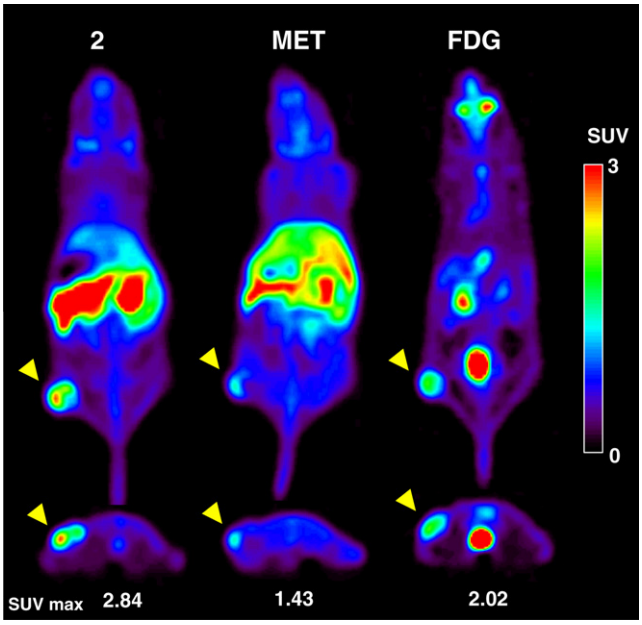


Figure 2. Comparative PET images of **2**, [*methyl*-^{11}C]methionine, and [^{18}F]FDG for a tumor model mouse. Coronal (upper) and transaxial (lower) images of **2**, [*methyl*-^{11}C]methionine (MET, center) and [^{18}F]FDG (FDG, right) in a mouse bearing small cell lung cancer SY-tumor cell lines. The same mouse was injected with 20 MBq of **2** or [*methyl*-^{11}C]methionine, or 200 kBq of [^{18}F]FDG intravenously, and a PET scan was performed for 60 min just after injection. Shown are images at the optimal time point of each PET tracer, 50–60 min for **2** and [^{18}F]FDG and 15–25 min for [*methyl*-^{11}C]methionine. The arrowhead in each image indicates the SY tumor location. The numbers at the bottom of the figure indicate the maximum SUV within the tumor.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.02.065.

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15. The radiochemical conversion was calculated from decay-corrected analytical HPLC profiles.
16. The conditions for HPLC use are described in the Supplementary data.
17. The isolated yield of **2** was determined for the calculated amount of [^{11}C]O_2 obtained by bombardment.