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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.

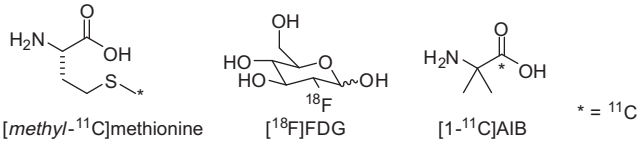

Figure 1. Structures of [*methyl*-^{11}C[methionine, [^{18}F]FDG, and [1-^{11}C]AIB.

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.

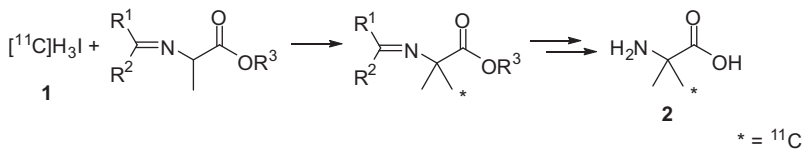
The use of Schiff base activated amino acid analogs is a primary method in the preparation of higher amino acids by α-alkylation under non-radiolabeling conditions.[8,9] Those methods have frequently employed phase-transfer (PT) conditions, and reactions are carried out under milder conditions. The benefit 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**.