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Enantioselective synthesis of carbon-11 labeled L-alanine using phase transfer catalysis of Schiff bases

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ABSTRACT

Radiolabeled amino acids are an important class of compounds that can be used for Positron Emission Tomography (PET) imaging of the amino acid transporter status of various diseases e.g., cancer. Current radiochemistry techniques do not offer synthesis approaches that are generally applicable and result in high yields and enantiomeric purity. Here, the radiosynthesis of L-[^{11}C]alanine is described employing an enantioselective alkylation of a Schiff base glycine precursor with [^{11}C]methyl iodide. By conducting a comprehensive reaction conditions optimization and a strategic analysis of several phase-transfer catalysts that facilitate enantioselective alkylation, the radiosynthesis of L-[^{11}C]alanine was achieved in good radiochemical conversion, short reaction times and above 90% enantiomeric excess. This new methodology is broadly applicable and could also be used for the radiolabeling of other amino acids with carbon-11.

1. Introduction

Positron Emission Tomography (PET)[1] is a non-invasive technique, often applied as diagnostic tool in today's healthcare, that allows the visualization of cellular processes in vivo in real time to study diseases like cancer or neurological disorders. Two important classes of compounds in molecular imaging are amino acids and peptides, which are used to establish new biological targets and tools for a better disease diagnosis and treatment strategies.[2–4] As diagnostic agents for oncology imaging, radiolabeled amino acids often have improved sensitivity and specificity over other PET tracers for oncology imaging, like 2-[^{18}F]fluoro-2-deoxy-D-glucose (FDG).[5] Amino acid uptake is increased to support the rapid growth and proliferation of tumor cells, by which amino acids are used as nutrients or for protein synthesis.[6,7] Various studies have shown that amino acid transporters are elevated on tumor tissue.[8] Making use of this knowledge, important tracers like [^{11}C]methionine[9] and *O*-(2-[^{18}F]fluoroethyl)-L-tyrosine[10] are used routinely in clinical settings for tumor diagnosis. Furthermore, multiple amino acids and amino acid analogs, e.g., [^{11}C]glutamine[11] or derivatives [^{18}F](2*S*,4*S*)-4-(3-Fluoropropyl)glutamine[12] and 3-(1-[^{18}F]fluoromethyl)-L-alanine[13] are under preclinical development. Next to oncologic disorders, amino acids can be used to study neurological disorders as well, which is proven by the application of L-[^{11}C]DOPA[14,15] as important radiolabeled neurotransmitter.

Since current amino acid based PET tracers show good results, there is need for improved and general methods for the radiosynthesis of this class of PET tracers. Optimally, for amino acid based PET tracers it is desired that the native structure of the amino acid is not changed, hence properties of amino acids by exchanging a carbon-12 atom for a carbon-11 is beneficial for guaranteeing real natural behavior. Thereby, carbon-11 labeled amino acids can be tracked into the metabolic pathways in which they are involved. Current precursor molecules for the synthesis of radiolabeled amino acids and amino acid derivatives already contain the chirality of the desired radiolabeled product and this involves challenging precursor synthesis. Furthermore, using chiral starting materials have the uncertainty if chirality is maintained during radiosynthesis, since these reactions often require harsh conditions. Nevertheless, amino acids are chiral molecules and consequently a synthesis is needed that results in an enantiomeric pure product to avoid chiral separation and a 50% loss of the final radiolabeled product resulting in a low yield. Final challenges in the synthesis of radiolabeled amino acids with carbon-11 is the synthesis time, since carbon-11 is a short-lived radioisotope with a half-life of 20.4 min and therefore reactions are required to proceed within minutes instead of hours that are reported for non-radioactive synthesis.[16]

With respect to the asymmetric synthesis of amino acids, chiral alkylation of Schiff base glycine derivatives using Phase-Transfer

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Catalysis (PTC) (Scheme 1)[17] is an ideal and general applicable method to synthesize natural and unnatural amino acids. Though rarely reported, the synthesis of alanine has been described utilizing methyl iodide as alkylating reagent (O'Donnell:[18] ee (enantiomeric excess) not reported; Corey:[19] ee of 97%). Due to the small size of methyl iodide compared to more bulky alkylating agents ee is mostly lower or is not evaluated at all. Nowadays, asymmetric synthesis is possible for many amino acids and small peptides.[17,20,21]

The radiosynthesis of [^{11}C]alanine has first been reported in the late 1970's when Långström et al. described a 48% ee yield of [^{11}C]alanine utilizing an asymmetric synthesis procedure.[22] Nevertheless, it took more than 10 years to develop a synthesis that yielded 80% ee of L-[^{11}C]alanine.[23,24] Alternatively other strategies have emerged as well, which make use of Nickel-complexes and upon varying the alkylating agent, many amino acids are possible.[25–27] However, the synthesis of these Nickel-reagents is considered cumbersome and the release of the unprotected amino acid by hydrolysis of the complex is tedious. Another drawback of the use of Ni-complexes in radiochemistry is that it only allows the synthesis of single radiolabeled amino acids, whereas the methodology that we developed should allow translation towards peptide radiolabeling with carbon-11.

In this paper we have adopted the use of PTC for the chiral radiosynthesis of L-[^{11}C]alanine to demonstrate the use of this method for the enantioselective radiolabeling of amino acids and as potential strategy for PET tracer development. An improved asymmetric synthesis of L-[^{11}C]alanine by an enantioselective alkylation of a Schiff base glycine precursor with [^{11}C]methyl iodide ([^{11}C]MeI) is here described. We focused our radiolabeling approach on asymmetric synthesis (Scheme 1) with highly specialized chiral catalysts, as it uses an accessible precursor, low amounts of catalyst and we could implement [^{11}C]MeI as our first alkylating agent. With more sophisticated alkylating agents this methodology is applicable as well to acquire other amino acids. Ultimately, future research with the methodology presented in this paper to synthesize L-[^{11}C]alanine, should allow the synthesis of radiolabeled peptides with carbon-11 as PET tracers.

2. Results and discussion

Initial focus of this study was the radiosynthesis of racemic D/L-[^{11}C]alanine to study the reactivity of carbon-11 labeled alkylating reagents towards the Schiff base (**1**) and the required reaction conditions for these reactions. As a precursor for the synthesis of [^{11}C]alanine, glycine derivative **1** was used, which was modified as a Schiff base at the N-terminus as a biphenyl imine to activate the α-carbon of glycine for alkylation. Furthermore, the *C*-terminal carboxylic acid was protected as a *tert*-butyl ester during the alkylation reactions. To thoroughly study the radiochemical conversion of the alkylation reaction of precursor **1** with [^{11}C]MeI and the following deprotection under acidic conditions and the enantiomeric excess of the final product, analysis was performed with High Performance Liquid Chromatography (HPLC) of both reactions independently. The analysis of the alkylation reaction was performed on a reverse-phase analytical column. The deprotected reaction mixture check was performed using a chiral column to determine in which D/L-[^{11}C]alanine **3** was separated and allowed the calculation of the enantiomeric excess of the final product.

To explore the reactivity of [^{11}C]MeI towards precursor **1**, the procedure as was described by Kato et al., was investigated.[28,29] Schiff base **1** was suspended in DMSO and in the presence of TBAF-solution (Tetrabutylammonium fluoride, 1 M in THF) as a base, [^{11}C]MeI was added to the reaction mixture by direct distillation. Alkylation of **1** with [^{11}C]MeI according to the published procedure was successful and alkylation yields exceeded 80% (Fig. 1B). Deprotection of alkylated intermediate **2** was to yield D/L-[^{11}C]alanine **3**, proved to be straight forward and high yielding when 6 M solution of HCl was added to the reaction mixture and heated shortly. As anticipated for this part of the study, no enantiomeric selectivity was obtained in the alkylation reactions, which was also demonstrated by the obtained chiral HPLC chromatograms for [^{11}C]alanine (Fig. 1C). Besides the use of TBAF to synthesize amino acids, also inorganic alkali-metal bases are often described in the chiral synthesis of amino acids by alkylation. Therefore, next to the use of TBAF as organic base, inorganic alkali-metal bases were investigated as well to evaluate the suitability of these bases

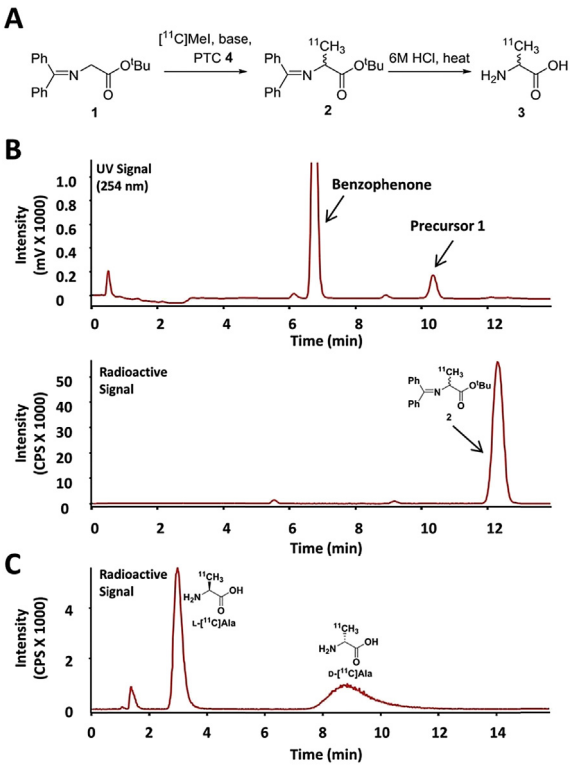
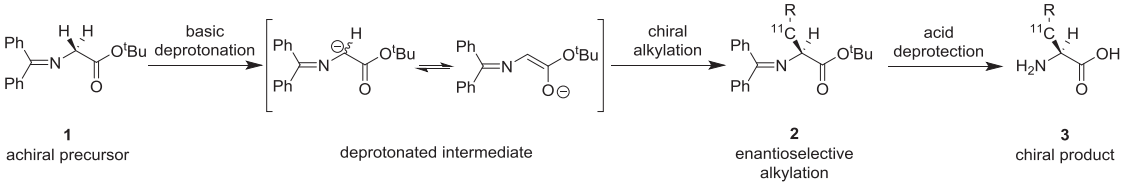


Fig. 1. (**A**) Radiochemical synthesis of [^{11}C]alanine by alkylation of precursor **1** with [^{11}C]MeI and its acidic deprotection; (**B**) HPLC profiles of the UV and radioactive signal of the crude alkylation mixture of **1** with [^{11}C]MeI; (**C**) Analysis of deprotected D/L-[^{11}C]alanine by chiral HPLC.



Scheme 1. Proposed mechanism of an asymmetric alkylation of a Schiff's base for amino acid synthesis.