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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

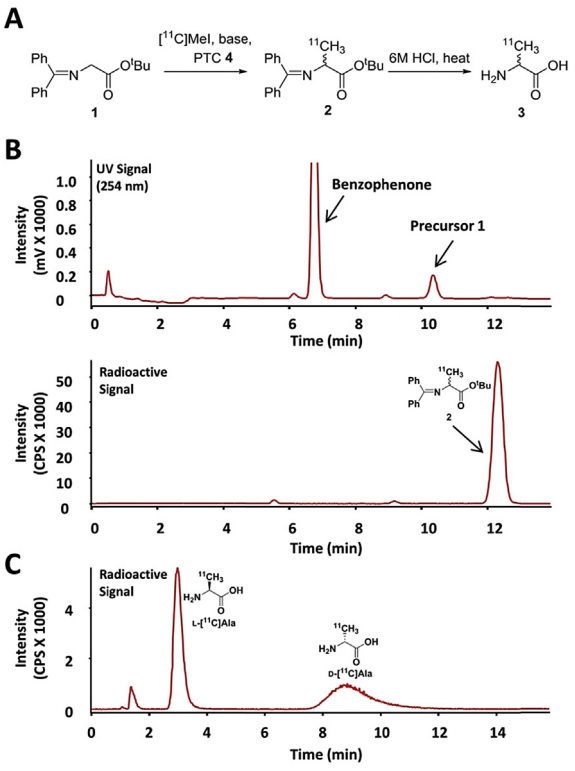
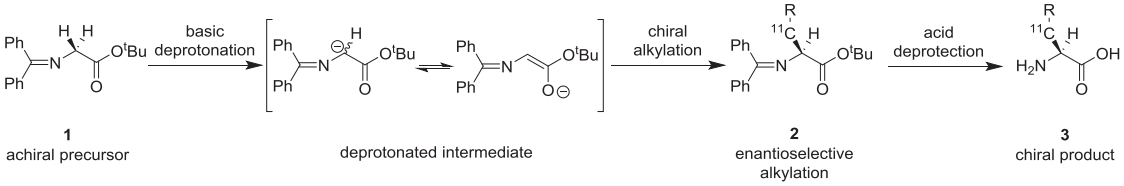


Fig. 1. (**A**) Radiochemical synthesis of [^{11}C]alanine by alkylation of precursor **1** with [^{11}C]MeI and its acidic deprotection; (**B**) HPLC profiles of the UV and radioactive signal of the crude alkylation mixture of **1** with [^{11}C]MeI; (**C**) Analysis of deprotected D/L-[^{11}C]alanine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



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

to deprotonate **1** and perform alkylation reactions of Schiff bases with [^{11}C]MeI and synthesize [^{11}C]alanine. Unfortunately, when using aqueous solutions of NaOH no alkylation was observed to **2** at room temperature. Likewise, only low radiochemical conversions were observed while using CsOH×H_2O as a solid base for the alkylation of **1** with [^{11}C]MeI. An explanation for the low reactivity of precursor **1** towards [^{11}C]MeI is the poor solubility of alkali metal hydroxides in organic solvents, whereas TBAF is soluble in organic solvents and can act as a base more easily. A general observation from all alkylation reactions that were investigated and analysed by HPLC in this study (Fig. 1 B), was the formation of very high concentrations of benzophenone in the reaction mixture coming from precursor **1**. It can be concluded that precursor **1** is clearly unstable during the alkylation reactions, yielding high concentration of benzophenone and unreactive glycine *tert*-butyl ester. Despite these high concentrations of benzophenone, the investigated alkylation reactions with [^{11}C]MeI proved to be successful. This can be explained by the stoichiometry of Schiff base **1** to [^{11}C]MeI in radiochemical alkylation reactions. [^{11}C]MeI is present in nanomolar concentrations, meaning that, despite the high concentrations of benzophenone in the reaction, there is still a large excess of precursor **1**, which is present in μmoles, available for alkylation. Since this instability did not hamper the radioalkylation reactions investigated, no further attention was paid to this observation.

2.1. Chiral alkylation reactions with phase-transfer catalyst 4

These encouraging initial results formed the basis to move forward to the asymmetric synthesis of [^{11}C]alanine to selectively obtain the D- or L-enantiomer. To achieve this, the optimization of catalyst, temperature, solvent and time were taken into account and modified to achieve near quantitative radiochemical yield and as high as possible enantiomeric excess. Executing the reaction conditions as were described in organic literature was a first set-off point in this study.[30] The initial challenge in the application of phase-transfer catalysis reactions in radiochemistry is that in organic chemistry mixtures are reacted for several hours before work-up, which is not possible working with carbon-11, where the maximum time of reaction and analysis of the product is 3 half-lives. This study was set out with the aim of the enantioselective synthesis of L-[^{11}C]alanine and the first set of experiments concentrated on phase-transfer catalyst **4** (Fig. 2). Other PTCs have been investigated in this study as well after optimization of the chiral alkylation with PTC **4**, to determine the influence of the catalyst on the ee of the product (Fig. 2). The reactions were performed at 0–10 °C for 5–10 min with generally 7 μmol of precursor **1** and 10 mol % of catalyst.

Next to TBAF as base, many studies in organic chemistry have performed enantioselective alkylations with alkali metal hydroxides aqueous solution as base.[31] The main advantage of using aqueous solutions of metal hydroxides lays in the more accurate amount of base added to the reaction mixture as otherwise possible with hygroscopic alkali hydroxides. Therefore, we initially examined various amounts of aqueous alkaline bases in the asymmetric alkylation reactions, which was added to the reaction mixture containing **1** and [^{11}C]MeI in toluene as organic solvent. In general, the observed alkylation conversion to obtain compound **2** was low and never exceeded 50%. Despite the low conversion, we carefully analyzed the ee of the obtained product by chiral HPLC (Fig. 3) and discovered that the application of PTC **4** induced the chiral alkylation of Schiff bases with [^{11}C]MeI resulting in moderate to high ee of L-[^{11}C]alanine. As the conversion rates of the alkylation reactions, using these conditions, was unpredictable and too low, a more reactive alkylation reagent, [^{11}C]MeOTf ([^{11}C]methyl trifluoromethanesulfonate) instead of [^{11}C]MeI, was investigated to enhance the alkylation reaction. Unfortunately, only low conversions were observed and [^{11}C]MeOTf was not further used as alkylation reagent in this study. To increase the reaction yields and maintain the high ee's of the reactions (Fig. 3A), mixtures of aqueous CsOH solution and organic bases like TBAF, TBAOH (Tetramethyl ammoniumhydroxide) or $TBAHSO_4$ (Tetrabutylammonium hydrogensulfate) were used, indeed resulting in high conversions of 90%, which is in accordance with previous findings. Unfortunately however, the ee of all reactions dropped to undesirable and low rates.

Since alkali bases proved to be optimal thus far with respect to enantioselectivity of the alkylation reaction, other Cesium bases were examined. Aqueous solutions of 1 M and 10 M concentrations of Cs_2CO_3 were evaluated for the alkylation reaction, but no conversion was observed in any of the attempts. Furthermore, semi-organic base 1 M aqueous Cesium acetate was used in the alkylation, however, no reaction between **1** and [^{11}C]MeI was observed. Presumably this is due to lower basicity of the used Cesium salts compared to CsOH×H_2O.

To further explore the use of CsOH and its application for chiral alkylation reactions, CsOH was used as a dry powder (CsOH solid base). This led to a significant increase in the conversions of the reaction of over 80%, when an excess of base was used compared to precursor **1** (Fig. 3B). Furthermore, the stereoselectivity of the alkylation reaction was influenced dramatically when using CsOH

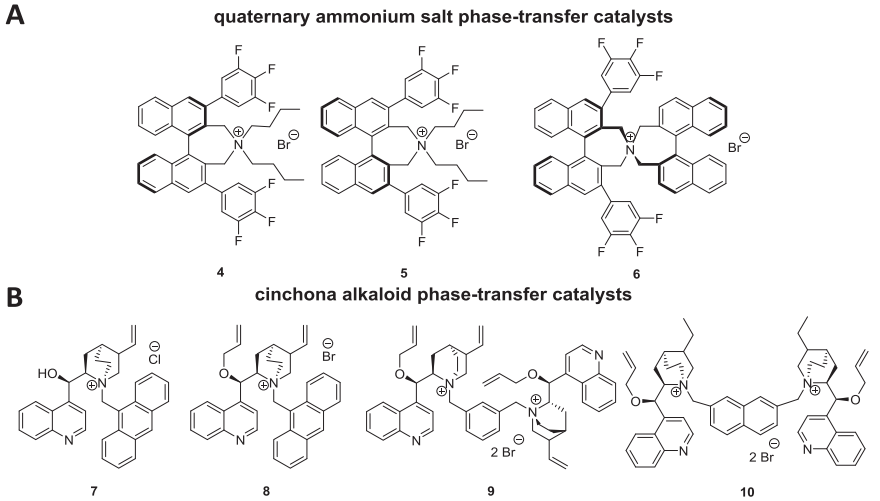


Fig. 2. Chiral phase-transfer catalysts explored for the radiosynthesis of D/L-[^{11}C]alanine with (**A**) quaternary ammonium based catalysts and (**B**) the cinchonidinium based catalysts.

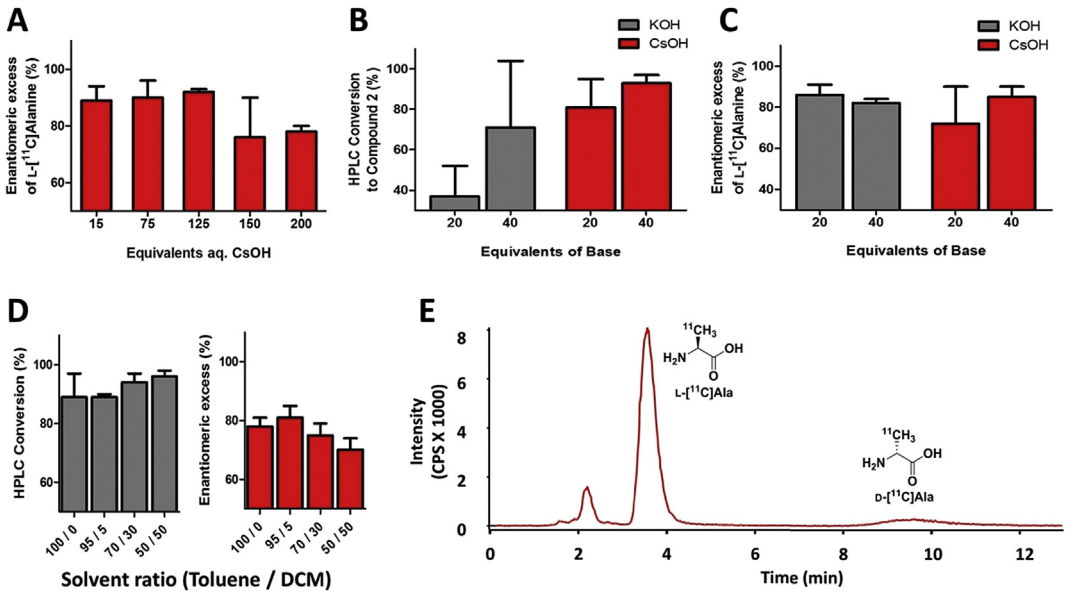


Fig. 3. (**A**) Enantiomeric excess of L-[^{11}C]alanine **3** with different concentrations of aqueous CsOH solutions. (**B**) HPLC conversions for the alkylation reaction of Schiff base **1** with [^{11}C]MeI in the presence of different bases. (**C**) Enantiomeric excess of L-[^{11}C]alanine **3** in the presence of different bases. (**D**) Conversion rates of asymmetric alkylation to compound **2** and ee of the formed product in the presence of different solvent mixtures. (**E**) Analysis of D/L-[^{11}C]alanine by chiral HPLC after deprotection of the enantiomeric product.

solid base and the ee of the obtained [^{11}C]alanine was increased to over 80% when using 40 equiv of CsOH (Fig. 3C). A drawback, of using CsOH as a solid base, is the hygroscopicity and its commercial availability only as CsOH×H_2O. Therefore, one other alkali metal that is less hygroscopic than CsOH hydroxide was investigated.[17,20] Fig. 3B depicts the results for various concentrations of solid KOH in comparison to CsOH, when the reactions were performed at 5 °C.

In comparison to the use of CsOH, the obtained results with KOH were not as desired since the conversion rates of the alkylation reaction dropped and became unreliable. Therefore, it was concluded that only the strongest metal hydroxide base is preferred over the other available metal hydroxides, meaning that CsOH is superior over KOH. These results led us to believe, that the use of solid CsOH base is most promising and we were able to achieve both high conversion and ee. An additional benefit for using solid bases instead of aqueous solutions was the rate of hydrolysis of Schiff base precursor **1**, which was much less compared to aqueous solutions and after completion of the alkylation reaction we had more intact precursor available. To increase the stability of the reaction even further during the alkylation reaction, CsOH×H_2O was azeotropically dried with MeCN beforehand. Unfortunately, the use of azeotropically dried CsOH resulted in a reduction in ee ratios of the obtained [^{11}C]alanine to cca. 70% and therefore these preparations were discontinued.

Finally the impact of the solvent on the alkylation reaction was assessed as well as the ee of the formed product, therefore various solvent mixtures have been used. In a few papers the use of toluene[32] was described, which yielded satisfactory results already if CsOH as a solid base (~20 equiv) was used, however other describe mixtures of toluene/dichloromethane[33] resulting in an improvement in both conversion yield and ee of the obtained product (Fig. 3D). The best result was the use of a small amount of 5% of dichloromethane in toluene. An explanation for this improved alkylation reaction can be found in the fact that it improves the solubility of the catalyst in the reaction mixture.

Thus far, our optimization study showed that with CsOH as solid base in a mixture of toluene/dichloromethane with 10 mol % of PTC **4** yielded the best results with respect to the obtained radiochemical conversion and the ee of L-[^{11}C]alanine.

2.2. Screening of multiple phase-transfer catalysts

With the currently available optimal conditions of the chiral synthesis of L-[^{11}C]alanine, other chiral PTCs have been evaluated to further improve the ee of the obtained products. Therefore classical PTCs, termed first generation cinchonidonium salts **7** and **8** were investigated, as well as dimeric cinchonidinium salt **9** and **10** (Fig. 2B). To complete the series of PTCs quaternary ammonium based compounds were used, namely compound **5**, which is an stereoisomer of **4** and should result in the formation of D-[^{11}C]alanine and catalyst **6** which should, because of its bulkier character, improve the ee of the reaction (Fig. 2A).

All depicted PTCs in Fig. 2 have been applied for the chiral alkylation of Schiff base precursor **1** and its alkylation with [^{11}C]MeI using the optimal conditions that were investigated previously when applying PTC **4**. Results of these alkylation reactions are summarized in Table 1.

PTC **5**, which is the stereoisomer of PTC **4** should yield D-[^{11}C]alanine predominantly and was evaluated to prove that the theory of using inverse catalysts for this kind of alkylation reactions is also valid in radiochemistry resulting in the formation of the opposite enantiomer (Table 1). Nevertheless, alkylation results and the obtained ee of the product, when using PTC **5**, was lower than for PTC **4**. To find and explanation for the less optimal performance of PTC **5** in comparison to its counterpart PTC **4**, the optical and chemical purity of both catalysts was further investigated. Unfortunately,

Table 1
Results from the alkylation reactions (n>3, decay corrected) and the ee of the obtained product using PTC **5** to **10** with the optimal conditions obtained for catalyst **4**

Entry	Applied PTC	Alkylation conversion (%)	ee of [^{11}C]alanine (%)
1	**4**	96.9±1.1	88.2±2.0
2	**5**	47.5±9.4	58.3±9.1 D-ala
3	**6**	6.6±0.5	80.6±8.0
4	**7**	Trace	ND
5	**8**	72.7±5.5	90.4±2.7
6	**9**	73.8±9.8	68.2±3.2
7	**10**	59.7±27.1	52.5±11.3

despite multiple attempts to investigate the optical rotation of the catalyst and the analysis of the purity by HPLC, no full explanation for the observed performance of the catalyst could be found. Therefore, to optimize the performance of PTC **5** and thus the radiosynthesis of D-[^{11}C]alanine, the reaction conditions for the alkylation reactions with this catalyst were slightly modified to yield the best possible conditions. Despite changes in temperature ranging from 5 to 45 °C and the change towards other alkaline bases such as NaOH and KOH, the only real improvement when using PTC **5** was increasing the amount of CsOH to 100 equiv compared to the precursor. Using these conditions the conversion of the alkylation reaction of precursor **1** with [^{11}C]MeI was improved to 97%, but the ee of the obtained product, D-[^{11}C]alanine, never exceeded 66%.

The final chiral quaternary ammonium salt that was investigated in this study was PTC **6**. Due to the bulky character of this PTC **6** it was expected that this could positively influence the enantioselectivity of the alkylation reaction. Unfortunately, with PTC **6** the conversion of the alkylation reaction was less than 5%, even when high concentrations of CsOH were used in the alkylation reaction. As a result of the low conversion in the alkylation reaction, the enantiomeric ratios could not be reliably determined and the use of PTC **6** in this study was discontinued.

Next to the quaternary ammonium salts as PTC, first generation cinchonidinium based PTCs have been investigated as chiral auxiliary for the alkylation of Schiff base **1** with [^{11}C]MeI. When exploring PTCs **7** and **8**,[34–36] two catalysts that only differ in the hydroxyl-position, PTC **8** outperformed **7** and good results were achieved. Also when PTC **8** was applied for the chiral alkylation reactions, CsOH in an excess of 20–40 equiv is optimal in a mixture of toluene and dichloromethane. When using PTC **8** and a temperature of 5 °C, an alkylation conversion of 73% could be achieved with an ee of the reaction of 90%, which was very satisfactory. More recently, dimer PTCs have been described that are based on the first generation cinchonidonium based catalysts. Both PTC **9**[33,37] and **10** have been investigated and it was expected that the more voluminous character of both PTCs would again increase the stereoselectivity of the alkylation reaction. Unfortunately, neither PTC **9** nor **10** did yield any reasonable conversion in the alkylation reaction nor a satisfactory enantiomeric excess. PTC **9** only resulted in traces of the alkylated product, even with the adaption of the reaction conditions in which the amounts of base and the temperature were changed. In the end, PTC **10**, which has been described in the literature as highly promising for organic chemistry[33] proved to be the more successful PTC of the dimer cinchonidinium based PTCs. When using PTC **10** with optimized conditions, that were earlier described for PTC **4**, a conversion could be achieved of 61% with an ee of 53%. Nevertheless, both conversion and ee of the product were lower than obtained in the presence of PTC **4** or **8**.

To summarize all achieved results within this study, phase-transfer catalysis allows the enantioselective synthesis of carbon-11 labeled amino acids as potential PET tracers, which was shown in this study for the radiosynthesis of [^{11}C]alanine. Despite these achievements, it should be noted that there are still uncertainties about the mode of action, origin of the enantioselectivity and enolate binding. A quantum-mechanical analysis provided by Cook gave insights into the lowest energy enantiomeric allylation transition states of cinchonidinium-derived catalysts, which provides us with L-[^{11}C]alanine.[38] Catalyst **4** and **5** are conformational rigid homochiral quaternary ammonium bromides and have been shown to have a substantially higher catalytic activity then their heterochiral diastereomers.[31,39] With this theory it could be explained why PTC **4** was most successful in our study, due to their favorable transition states. Nevertheless, in radiochemistry the alkylating agent is present in a very small amount compared to both the Schiff base and the catalyst. Notably, this is the first report that demonstrates a successful enantioselective radiosynthesis with readily available [^{11}C]MeI as reagent in radiochemistry, the smallest alkylating agent possible, where in organic chemistry most do not even establish reactions with methyl iodide. Taking into account all these results, the best results for the radiosynthesis of [^{11}C]alanine by the alkylation of Schiff base **1** were obtained using catalyst **4** with respect to yield and ee. All in all, a good incorporation was achieved when the alkylation reaction proceeded at 5 °C for 5–10 min in a mixture of toluene/dichloromethane in the presence of 30 equiv of CsOH as base and 0.1 equiv of PTC **4**, respectively.

To conclude, L-[^{11}C]alanine was synthesized within 50 min in high radiochemical yield of 20% (decay corrected) calculated from the end of bombardment (EOB). The specific activity was >50 GBq/μmol at the end of the synthesis (EOS) and the highest ee achieved was >90%. Both chemical and radiochemical purities were >95%.

3. Conclusion

We have synthesized L-[^{11}C]alanine via a new, general applicable, radiochemistry method utilizing a PTC catalyzed enantioselective alkylation with [^{11}C]MeI, followed by acidic deprotection. These findings provide new insights in the suitability of phase-transfer catalyzed reactions in radiochemistry and the potential to synthesize novel amino acid PET tracers using this methodology. Finally, this radiosynthesis strategy would allow the synthesis of carbon-11 radiolabeled peptides as well.

4. Materials and methods

4.1. General

N-(diphenylmethylene)glycine *tert*-butyl ester **1** was purchased from ABCR (Karlsruhe, Germany) and catalysts were purchased from Sigma Aldrich (Zwijndrecht, The Netherlands) and Wako Pure Chemical Industries (Osaka, Japan). All commercially available chemicals were used without further purification. The non-radioactive reference compounds were synthesized according to reported methods and were used to verify the identity of radiolabeled compounds. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were obtained using a Bruker AC 250 and AC 400 (Billerica, USA) and chemical shifts (δ) were defined relative to the signal of the solvent (7.27 ppm for $CDCl_3$, 3.31 ppm for MeOD, 2.50 ppm for DMSO-d_6) and tetramethylsilane as an internal standard (δ=0). High resolution mass spectra (HRMS) were carried out using a Bruker micrOTOF-Q instrument in positive or negative ion mode (capillary potential of 4500 V). Flash chromatography purifications were performed on a Buchi system operated by SepacoreControl system. Analytical HPLC systems used were equipped with: a Waters 600E pump, a manual Rheodyne injector (20–100 μL loop), a Waters PDA and GinaStar software from Raytest (Straubenhardt, Germany). The radioactive profile was monitored with a Raytest 2.5 inch radioactivity detector (Raytest, Germany). Analytical HPLC columns used in this study were a Grace Smart C-18 5 μm, 4.6×250 mm with a mixture consisting of acetonitrile/4 mM sodium formate+4% DMF (70/30, *v*/*v*) at a constant flow rate of 1 mL/min for the analysis of **2**, UV monitoring at 254 nm. Enantiomeric purity of the amino acid was determined using an analytical Reprosil chiral-aa (8 μm; 4.6×250 mm) from Dr. Maisch GmbH (Ammerbuch, Germany) at 214 nm. The product was eluted with methanol/water (70/30, *v*/*v*) at a flow rate of 1 mL/min or as stated otherwise. Radiochemical conversions are based on the AUC of the radioactivity profile of HPLC analysis. The [^{11}C]MeI synthesis was performed before every radiochemical reactions on an in-house built synthesis device and according to procedures described in literature.

4.2. Synthesis of reference compounds and non-commercially available PTCs

*4.2.1. L-N-(diphenyl)alanine tert-butyl ester (**2a**).* L-Alanine *tert*-butyl ester (300 mg, 2.07 mmol) was suspended in dichloromethane (4 mL) and treated with benzophenone imine (381 μL, 1.82 mmol). The reaction was stirred at room temperature overnight. The precipitate was filtered and the filtrate evaporated to dryness. The crude product was purified using flash chromatography (Sepacore® flash system) with 4% ethyl acetate in hexane. The collected fraction was evaporated to give L-*N*-(diphenyl)alanine *tert*-butyl ester as a white solid (Yield: 472 mg, 1.60 mmol, 77%).

^{1}H NMR (250 MHz, $CDCl_3$) δ 7.58 (m, 2H), 7.41–7.36 (m, 3H), 7.30–7.24 (m, 3H), 7.14 (m, 2H), 3.98 (q, 1H, *J*=8 Hz), 1.37 (s, 9H), 1.35 (d, 3H, *J*=8 Hz) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.1, 169.3, 139.8, 136.6, 132.4, 130.4, 128.7, 80.7, 61.3, 28.1, 19.2 ppm; HRMS (ESI) calculated $C_{20}H_{23}NO_2$: 310.1807 ($[M+H]^+$), found 310.1797 ($[M+H]^+$).

*4.2.2. D-N-(diphenyl)alanine tert-butyl ester (**2b**).* Compound **2b** was synthesized analogous to the method used for the synthesis of compound **2a**. **2b** was obtained in a yield of 365 mg (1.24 mmol, 60%) as colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 (m, 2H), 7.41–7.36 (m, 3H), 7.30–7.24 (m, 3H), 7.14 (m, 2H), 3.99 (q, 1H, *J*=8 Hz), 1.37 (s, 9H), 1.34 (d, 3H, *J*=8 Hz) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.1, 169.3, 139.8, 136.6, 132.4, 130.4, 128.7, 80.7, 61.3, 28.1, 19.2 ppm; HRMS (ESI) calculated $C_{20}H_{23}NO_2$: 310.1807 ($[M+H]^+$), found 310.1812 ($[M+H]^+$).

*4.2.3. α,α′-Biscinchonidinium-m-xylene dibromide (pre-PTC **9***).* The synthesis was performed according to described literature procedures.[37] (−)-Cinchonidine (2.00 g, 6.79 mmol) and dibromo-*m*-xylene (0.88 g, 3.33 mmol) were dissolved in EtOH (5 mL), DMF (6 mL) and chloroform (2 mL) and refluxed at 100 °C for 4 h. When all dibromo-*m*-xylene was consumed according to TLC, the reaction mixture was cooled to room temperature, diluted with MeOH and precipitated in cold diethyl ether. The desired product was obtained in a yield of 72% as a pink solid (3.38 g, 4.88 mmol).

^{1}H NMR (400 MHz, DMSO-d_6) δ 9.02 (d, 2H, *J*=2.5 Hz), 8.37 (d, 2H, *J*=10 Hz), 8.15 (d, 3H, *J*=7.5 Hz), 7.94 (d, 2H, *J*=5 Hz), 7.91–7.84 (m, 4H), 7.81–7.75 (m, 3H), 6.79 (d, 2H, *J*=2.5 Hz), 6.61 (s, 2H), 5.79–5.65 (m, 2H), 5.32 (d, 2H, *J*=12.5 Hz), 5.22–5.15 (m, 4H), 5.00 (d, 2H, *J*=10 Hz), 4.38–4.30 (m, 2H), 4.01–3.94 (m, 2H), 3.83–3.78 (m, 2H), 3.60–3.51 (m, 2H), 3.18 (d, 2H, *J*=5 Hz), 2.20–2.10 (m, 3H), 2.07–2.03 (m, 3H), 1.87–1.83 (m, 2H), 1.37–1.30 (m, 2H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ 155.5, 155.4, 152.8, 150.4, 144.1, 143.6, 140.5, 135.1, 134.7, 133.8, 132.6, 129.5, 128.9, 125.4, 121.6, 72.9, 69.4, 67.5, 64.4, 55.8, 42.1, 31.0, 29.4, 26.3 ppm; HRMS (ESI) calculated $C_{46}H_{52}N_4O_2$: 346.7084 ($[M+H]^{2+}$), found 346.2017 ($[M+H]^{2+}$).

*4.2.4. α,α′-Bis[O(9)-allylcinchonidinium]-m-xylene dibromide (PTC **9**).* α,α′-Biscinchonidinium-*m*-xylene dibromide **9*** (300 mg, 0.43 mmol) was suspended in dichloromethane (5 mL) and allyl bromide (1 mL, 11.56 mmol) and 50% aqueous KOH (2 mL, 17.60 mmol) were added at room temperature. The suspension was stirred vigorously for 3 h until the reactants were consumed according to TLC. The mixture was diluted with water and extracted with dichloromethane. The combined organic extracts were evaporated to yield 305 mg of **9** as orange solid (0.39 mmol, 91%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 9.05 (d, 2H, *J*=4 Hz), 8.32 (d, 2H, *J*=8 Hz), 8.17 (d, 2H, *J*=8 Hz), 8.08 (s, 1H), 7.96–7.94 (m, 2H), 7.91–7.87 (m, 2H), 7.82 (t, 3H, *J*=8 Hz), 7.73 (d, 2H, *J*=4 Hz), 6.51 (s, 2H), 6.23–6.13 (m, 2H), 5.79–5.70 (m, 2H), 5.51 (d, 2H, *J*=20 Hz), 5.37–5.28 (m, 4H), 5.19–5.08 (m, 4H), 5.02 (d, 2H, *J*=12 Hz), 4.47 (dd, 2H, *J*=20, 8 Hz), 4.06–3.99 (m, 6H), 3.77–3.73 (m, 2H), 3.66–3.60 (m, 2H), 3.45–3.38 (m, 2H), 2.80–2.73 (m, 2H), 2.36–2.31 (m, 2H), 2.15–2.06 (m, 4H), 1.91–1.84 (m, 2H), 1.53–1.45 (m, 2H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ 150.8, 148.5, 141.7, 138.4, 136.0, 134.7, 134.4, 131.7, 130.2, 128.9, 128.0, 126.4, 125.5, 124.1, 121.3, 120.1, 118.1, 117.1, 69.7, 68.4, 63.5, 59.3, 51.3, 26.4, 24.7, 21.2 ppm; HRMS (ESI) calculated $C_{52}H_{60}N_4O_2$: 386.7379 ($[M+H]^{2+}$), found 386.2387 ($[M+H]^{2+}$).

5. Radiochemistry

5.1. Radiochemical procedure for the production of [^{11}C]MeI

Cyclotron produced [^{11}C]CO_2 was carried in a stream of helium and trapped in 0.1 mL of a 0.1 M lithium aluminium hydride solution in THF in a glass reaction vessel at room temperature. After trapping, the gas flow was increased to 20 mL/min and the THF evaporated at 130 °C. After evaporation to dryness 0.2 mL of 56% hydriodic acid was added and the [^{11}C]MeI was distilled from the reaction vessel under a stream of helium (flow 20 mL/min) to the second reaction vessel for the alkylation reaction.[40]

5.2. Alkylation procedure of Schiff base 1

[^{11}C]MeI is distilled into a closed reaction vessel containing the Schiff base **1**, phase transfer catalyst and base, in a mixture of toluene/dichloromethane (19/1, *v*/*v*). The color changed instantly to yellow, [^{11}C]MeI was trapped in the second reaction vessel prior to heating or cooling of the reaction mixture. At set timepoints, samples were taken from the reaction mixture for analysis by radioHPLC to determine the alkylation conversion rates. For deprotection, 0.1 mL of 6 M HCl was added to the reaction mixture prior to heating to 100 °C for 2 min. After cooling to room temperature, a sample was taken for analysis on chiral radioHPLC to determine the enantiomeric excess of L-[^{11}C]alanine.

5.3. Optimized procedure for the alkylation with [^{11}C]MeI to obtain L-[^{11}C]alanine

In a reaction vessel, Schiff base **1** (1.89 mg, 7 μmol), catalyst **4** (0.5 mg, 0.8 μmol) and $CsOH{\times}H_2O$ (30 mg, 200 μmol) are suspended in a mixture of toluene/dichloromethane (300 μL, 19:1, *v*/*v*) and the color changes instantly to yellow. After distilling [^{11}C]MeI in the reaction vial, the mixture is cooled to 5 °C and stirred for 5 min. A sample is taken for analysis on Grace Smart RP18 column (acetonitrile/sodiumformate 4 mM+4% DMF 70/30, *v*/*v*) with a retention time (t_R) of 8.3 min for product **2**. The deprotection is initialized by the addition of 0.1 mL of 6 M HCl solution and heating to 100 °C for 1.5 min. A second sample is taken for analysis on chiral radioHPLC to determine the enantiomeric excess of L-[^{11}C]alanine with HPLC column Reprosil chiral-aa (methanol/water 70/30, *v*/*v*) with a t_R of 4.5 min for L-[^{11}C]alanine and 10.3 min for D-[^{11}C]alanine.

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Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.tet.2016.08.069.

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