Asymmetric radiochemical alkylation reactions to acquire carbon-11 labeled amino acids and small peptides

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Asymmetric radiochemical alkylation reactions to acquire carbon-11 labeled amino acids and small peptides

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Chapter 1

General Introduction
PET imaging and radiochemistry development of tracers

Positron emission tomography (PET) is nowadays an essential tool for daily clinical diagnosis and therapy response monitoring. Functional imaging by PET was first set-up in 1970’s and since then has made huge development progress on all fronts, e.g. PET cameras, image analysis and the available PET tracers required for successful imaging. In addition, it has even become more powerful in combination with anatomical imaging techniques like Computer Tomography (CT) or Magnetic Resonance Imaging (MRI).\(^{1}\) PET imaging to date is predominantly applied in oncology, neurology and cardiology for disease understanding, diagnosis and monitoring, as well as for drug development purposes. Here, the metabolic processes of injected tracers are detected by γ-ray cameras and evaluated qualitatively and quantitatively. The behavior of a biological compound can be studied in vivo without perturbing the biological system. PET has great future potential, if the development of new tracers, evaluation of biological important targets and radiosynthesis possibilities are aligned. Fortunately, in the last decade processes and hardware have been developed to simplify radiochemical labeling methods.\(^{2}\) Overall, a desired PET tracer should have an established, reliable radiosynthesis and show in vivo specific binding to the target resulting in good tissue-to-background ratio. Radiolabeled amino acids and peptides are gaining a wide acceptance in nuclear medicine and nuclear oncology as tumor targeting agents. These imaging agents show high potency, selectivity, specificity and due to overexpression of many amino acid and peptide transporters on tumors are favorable for diagnosis as well as targeted radionuclide therapy.

In regard of the radiochemistry, this is the process by which a molecule is tagged with a radionuclide to make a radiopharmaceutical tracer. A continuous effort for radiochemists is to develop novel PET tracers. Owing to the half-life of the nuclide of choice, a tracer has to be synthesized, purified, analyzed and formulated within approximately three half-lives, which provides several challenges. This requires novel radiosynthetic methods, which are fast, reproducible and efficiently deposit the radiolabel in the wanted molecule. Particularly, the upcoming of complex structures, as developed in medicinal chemistry require new radiolabeling strategies to obtain the ideal tracer candidate. In general, the radiolabeled reagent is available in lower nanomol quantities and the other reagents in millimol amounts, this creates distorted stoichiometry that leads to fast reaction kinetics, when based on the radiolabeling reagent.
Incorporation of the radionuclide for the production of a PET tracer is preferred to occur as late as possible to preserve radiosynthesis time.

**Short lived isotopes for small molecule PET tracer development**

Radiopharmaceuticals, especially small molecules, are preferably labeled with short-lived isotopes, e.g. carbon-11 and fluorine-18 with half-lives of 20 and 110 min, respectively.[2] These isotopes are well utilized to develop PET tracers due to the relative ease of production of the nuclides and possibilities existing for radiosynthesis. Carbon-11 has a 99 % $\beta^+$-decay and a maximum energy of $E_{\text{max}}=0.96$ MeV[2] and is therefore a favorable PET nuclide. In general, carbon-11 is preferred as radionuclide for PET tracer development as every endogenous organic molecule contains carbon. Thus, endogenous and/or pharmacologically fully characterized compounds and even approved drugs can lead to valuable PET tracers upon labeling with carbon-11 which do not need to undergo further and extensive research for their safety before they can be applied in preclinical and clinical settings.

Carbon-11 is produced using a cyclotron by a $^{14}\text{N}(p,\alpha)^{11}\text{C}$ nuclear reaction and with the addition of small amounts of $\text{O}_2$ it is released from the cyclotron as $[^{11}\text{C}]\text{CO}_2$, which can then be transferred to radiochemistry facilities to be used for the production of a desired PET tracer. There are many radiosynthesis procedures described to date, for example direct carboxylation techniques using $[^{11}\text{C}]\text{CO}_2$ are available and are described with Grignard reagents to obtain carboxylic acids or amides. Furthermore, there has been a surge in the development of $[^{11}\text{C}]\text{CO}_2$ fixation chemistry[3] with fixation bases like guanidines and amidines (e.g. DBU = 1,8-Diazabicyclo[5.4.0]undec-7-ene), developing further to a very recent example from Mossine et al.[4] employing BEMP (2-tert-Butylimino-2-diethylamino-1,3-dimethylperhydro-1,3,2-diazaphosphorine) to synthesize three new tracers of interest for functional neurological imaging and translational oncology. Further simple on-line chemical reactions can take place from $[^{11}\text{C}]\text{CO}_2$ directly to widely applied $[^{11}\text{C}]$methyl iodide[5], or its more reactive analog $[^{11}\text{C}]$methyl triflate[6]. These products serve effectively in methylation reactions typically on amines, phenoxide groups or stannanes for $^{11}\text{C}$-C coupling reactions.[7–9]

Next to these, $[^{11}\text{C}]\text{CO}_2$ can be reduced to $[^{11}\text{C}]\text{CO}$ by a few procedures, of which methods described by Eriksson et al.[10–13], more recently Taddei et al.[14] and Nordeman et al.[15] are fairly straightforward. $[^{11}\text{C}]\text{CO}$ can be successfully applied in palladium-mediated carbonylation
reactions, which is an economic multicomponent reaction forming two covalent bonds at once. The fundamental process involved in the transformation of carbonylation reactions in organic chemistry have been described in detail by Brennführer et al.\textsuperscript{[16]} An array of compounds has been successfully synthesized with \([^{11}\text{C}]\text{CO}\).\textsuperscript{[17–19]}

As carbon-11 is exchangeable with carbon-12 generally, it provides great interest for the development of native biological PET tracers. However, still expansion of the radiochemistry possibilities with \(^{11}\text{C}\)-labelled reagents is required to enlarge the radiochemistry toolbox.

**Carbon-11 labeled amino acids and small peptides**

Two classes of compounds that have attracted a lot of attention and proved to be highly valuable as PET tracers for imaging purposes of *in vivo* processes are amino acids and small peptides.\textsuperscript{[20,21]} The molecular basis for imaging with radiolabeled peptides is the overexpression of receptors where they bind to a variety of tumors. Peptides bind with high affinity and specificity, therefore hold great potential for diagnostic imaging. Amino acids are non-toxic, biodegradable, are selectively transported\textsuperscript{[22]} by amino acid or peptide transporters like System L, and have favorable kinetics with outstanding biological potential. They are important nutrients for tumor cells and viable tumor imaging biomarkers. The most successful example of a carbon-11 labeled amino acid can be found in the production an application of L-\[^{11}\text{C}\]methionine in tumor diagnosis. To date, imaging cancer mostly occurs by mapping the glucose consumption of malignant cells with the glucose analog \(^{18}\text{F}\)-fluoro-2-deoxy-glucose (\([^{18}\text{F}]\text{FDG}\)). However, its limitations are well described and it has disadvantages such as limited sensitivity in the brain.\textsuperscript{[23]} Nowadays, L-\[^{11}\text{C}\]methionine is established and used in combination with \([^{18}\text{F}]\text{FDG}\) for the determination of brain tumors and lesions.\textsuperscript{[24–26]} Advantageous for amino acid imaging compared to \([^{18}\text{F}]\text{FDG}\) is that it is less influenced by inflammation as well as in high background regions like the brain amino acid tracers are more selective.

Over time, many possibilities for \(^{11}\text{C}\)-labeling of amino acids have been successfully transferred from organic synthesis, which offer two distinct positions to deposit the radiolabel: the carboxyl position and the \(\alpha\)-atom. An overview of these synthesis methods for \(^{11}\text{C}\)-labeled amino acids and peptides is given in Chapter 2. Though many methods have been developed so far, the radiolabeling strategies implemented mostly resulted in racemic mixtures of amino acids, requiring lengthy procedures for purification. Lately stereospecific radiosynthesis proved to be successful.
for the production of carbon-11 labeled amino acids. With respect to obtaining small peptides as PET tracers a chemical modification is required by the incorporation of a prosthetic group (e.g. [18F]fluorobenzaldehyde) or chelate containing a radiometal, respectively. With these additions to a peptide the physico-chemical properties are perturbed and their in vivo behavior could be disturbed hampering the translation of peptides as PET tracers to clinical practice. The development towards 11C-labeled peptides has been strongly progressing in the last year. Because of the difficult radiochemistry, 11C-labeled amino acids and peptides have so far been inadequately represented in PET studies.

Asymmetric synthesis to obtain enantiopure carbon-11 labeled amino acids and peptides

The commercial availability of chiral catalysts has made their application attractive for transformation into radiochemistry for the use in phase-transfer reactions. The preparation of α-amino acids from prochiral Schiff base esters by asymmetric phase-transfer catalysis (PTC), serves as a mild, simple reaction procedure. The reactions with chiral organocatalysts of wide variety are executed in highly basic conditions at the interface of two immiscible phases: an organic solvent and water. The general mechanism is described in Figure 1 consisting of a chiral catalyst QX, prochiral Schiff base 1 and an inorganic metal-based hydroxide (MOH).

**Figure 1**: General mechanism with a glycine Schiff base 1 for the asymmetric alkylation (R = alkyl-group; Q = catalyst, X = I, Br; M = Na, K, Cs).

Briefly, the catalyst QX in the organic layer forms a complex 2 with the in the interfacial layer base-activated enol, upon which nucleophilic attack of enol derivative to alkylating agent is taking place. The complex disintegrates and after deprotection enantiopure product 3 is released. If the formation of the complex does not occur or is hindered, a racemic product is formed. Monoalkylation is occurring due to the difference in pKa values of starting material to product in these basic conditions. Besides, in radiochemistry the 11C-alkylating agent is a limiting factor only present in nanomol quantities. Advances in organic chemistry were mostly concentrated on
improvements in chiral catalysts, which has gone from ammonium quaternized derivatives of cinchonine and cinchonidine, which are commercially available, to structurally rigid C₂-symmetric, chiral naphthol-derived ammonium salts especially developed by Maruoka et al.\textsuperscript{[34,35]} Translation into radiochemistry is challenging especially with respect to the reaction times and stoichiometry (Table 1), though advantageous for forming enantioselective bonds at late stages in the radiosynthesis.

**Table 1: Phase-transfer catalysis.**

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<th>Disadvantages</th>
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<td>Simple experimental procedure</td>
<td>Long reaction time preferred</td>
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<tr>
<td>Mild reaction conditions</td>
<td>Distorted stoichiometric conditions</td>
</tr>
<tr>
<td>Inexpensive starting material and solvents</td>
<td>Reusability of catalyst</td>
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<td>Easily employed as last step</td>
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Another method to obtain \(^{11}\)C-labeled amino acids could be the Michael addition reaction. In general, these reactions are 1,4-conjugated additions forming a new C-C bond. The reaction involves the base-catalyzed addition of a nucleophile (donor) to an activated electrophile (α,β-unsaturated carbonyl-acceptor), thereby resulting in an adduct.\textsuperscript{[36]} Furthermore, asymmetric synthesis has been successfully employed in Michael addition reactions as well.\textsuperscript{[37,38]} Yet, in carbon-11 radiochemistry the Michael reaction has not been reported and could therefore contribute to the extension of the radiochemistry toolbox.
Aim and Outline of this thesis

The aim of this research was the development of a synthetic radiochemistry method for the enantioselective synthesis to obtain carbon-11 labeled amino acids and small peptides with a high enantiomeric excess. To achieve this, asymmetric synthesis was used for the radionuclide to be incorporated into the achiral activated molecule, glycine Schiff base ester, in a precise manner to yield an enantiomeric pure carbon-11 labeled amino acid with chiral phase-transfer catalysts. Carbon-11 as radionuclide was chosen, to keep the natural structure of the desired PET tracer intact and simultaneously have an efficient technique established to broaden the application of carbon-11 in radiochemistry. The approach should be generally applicable and therefore boost the application of carbon-11 labeled amino acids and peptides. This study aims to contribute to the evolution of new $^{11}$C-labeling routes thus increasing the number of available compounds.

Chapter 2 reviews the radiochemistry methods developed to date to synthesize $^{11}$C-amino acids and small peptides. Furthermore, a summary of pre-clinical and clinical investigations is given. In chapter 3, the asymmetric radiosynthesis to obtain L-$^{[11}$C]alanine is described. An extensive study of reaction conditions that has been performed in the presence of various chiral phase-transfer catalysts is presented. An extension to the study is described in chapter 4, which depicts the strategy to determine the best chiral catalyst for the synthesis with various dipeptide Schiff bases. A series of experiments are performed with $^{[11}$C]methyl iodide and $^{[11}$C]benzyl iodide as alkylating agent to establish a prognostic evaluation of backbone to catalyst. Chapter 5 provides a new radiosynthetic strategy for $^{11}$C-C bond formation, utilizing the Michael addition reaction to radiosynthesize novel PET tracers $^{[11}$C]glutamine and $^{[11}$C]glutamate as proof-of-principle. A summary and general discussion of the main results of this thesis is presented in chapter 6 with future perspectives following.
References


Chapter 2

From Carbon-11 Labeled Amino Acids to Peptides in Positron Emission Tomography: The Synthesis and Clinical Application

Ulrike Filp§, Aleksandra Pekošak§, Alex J. Poot, Albert D. Windhorst

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§: Both authors contributed equally to this work.
ABSTRACT

Radiolabeled amino acids, their derivatives and peptides have a broad scope of application and can be used as receptor ligands, as well as enzyme substrates for many different diseases as radiopharmaceutical tracers. Over the past few decades the application of molecular imaging techniques such as positron emission tomography has gained considerable importance and significance in diagnosis in today’s advanced health care. Next to that, the availability of cyclotrons and state-of-the-art radiochemistry facilities has progressed the production of imaging agents enabling the preparation of many versatile PET radiotracers. Due to many favorable characteristics of radiolabeled amino acids and peptides, they can be used for tumor staging and monitoring the progress of therapy success, while aromatic amino acids can be employed as PET-tracer to study neurological disorders. This review provides a comprehensive overview of radiosynthetic and enzymatic approaches towards carbon-11 amino acids, their analogues and peptides, with focus on stereoselective reactions, and reflects upon their clinical application.
General Introduction and Historical Overview

Over the past few decades the application of molecular imaging techniques is widely available for all fields of today’s health care. Especially molecular imaging techniques using tracers for single photon emission computed tomography (SPECT) and positron emission tomography (PET) have gained great attention due to their sensitivity and specificity to study disease biology in vivo. Next to that, the availability of cyclotrons, great variety of radiolabeling methods and automated synthesis modules for the production of PET radiopharmaceuticals enabled the preparation of the most frequently used PET radiotracer 2-deoxy-2-[\(^{18}\)F]fluoro-D-glucose ([\(^{18}\)F]FDG), as well as other PET-tracers like receptor ligands or enzyme substrates. Among all classes of PET-tracers, radiolabeled amino acids (AAs), their derivatives and peptides have a broad scope of clinical applications\cite{1}.

Natural, L-AAs play a crucial role in virtually all biologic processes, e.g. protein synthesis, cell signaling, precursors for biomolecular transmission and as neurotransmitters\cite{2,3}. Their constant and dynamic degradation and recirculation results in metabolic intermediates, such as ureas, fatty acids or glucose. Furthermore, AAs are used as metabolic fuel or reused by other tissues or cells. It is known that AAs can diffuse into cells, however the main transport of AAs into cells occur through specific membrane-associated proteins, so called AA-transporters\cite{4}. Due to the imperative role of generally existing or tissue-specific AA-transport systems and their consumption in many cellular processes, radiolabeled AAs have become an appealing class of compounds to be used as PET-tracer to study cancer biology, considering that malignant tissue often relies on increased AA uptake\cite{2,5}. Next to cancer imaging with radiolabeled AAs, aromatic AAs can be employed as PET-tracer to study neurological disorders. Additionally, the use of AAs as PET-tracers often result in high contrast images hardly distorted by tissue inflammation, as is known for glucose based PET-tracer [\(^{18}\)F]FDG. The brain, heart and inflamed areas use glucose as nutrient, therefore these regions are difficult to distinguish in [\(^{18}\)F]FDG-PET studies from metastasis\cite{2,6,7}. The radionuclides used for PET to label natural and unnatural AAs include \(^{11}\)C (\(t_{1/2}\) 20.4 min), \(^{13}\)N (\(t_{1/2}\) 10.0 min), \(^{18}\)F (\(t_{1/2}\) 1.8 h) and \(^{124}\)I (\(t_{1/2}\) 4.2 d)\cite{3}. 

Peptides, typically consisting of less than 50 AAs, are another important class of compounds to be used as PET-tracer, and have gained increasing importance as imaging agents. Furthermore, they generally have a high affinity and specificity for their cellular target in diseased tissue in nanomolar concentrations\cite{8,9}. Due to many favorable properties for molecular imaging, such as advantageous pharmacokinetics and specific tumor targeting characteristics, including the overexpression of peptide receptors on the tumor cells, radiolabeled peptides have an important impact not only in PET imaging of cancer, but also in targeted radionuclide therapy\cite{10}. Peptide-based radiopharmaceuticals have been successfully employed for imaging of somatostatin (SST), bombesin or cholecystokinin/gastrin receptor overexpressing tumors, all common human cancers\cite{10–14}.

Contemporary radiolabeling methods to label peptides require chemical modifications introducing a bulky bifunctional chelating or prosthetic group on the peptide in order to incorporate a radiometal, such as $^{68}$Ga ($t_{1/2}$ 1.1 h), $^{99m}$Tc ($t_{1/2}$ 6.0 h), $^{64}$Cu ($t_{1/2}$ 12.7 h), $^{111}$In ($t_{1/2}$ 67.2 h) and $^{177}$Lu ($t_{1/2}$ 160.8 h) and, or non-metal isotopes, $^{18}$F ($t_{1/2}$ 1.8 h) and $^{11}$C ($t_{1/2}$ 20.4 min)\cite{15–19}. As illustrated, present peptide radiolabeling techniques require structural modification of the lead-structure, consequently influencing the pharmacokinetics, receptor affinity and/or selectivity, in a positive or negative manner\cite{12,16}. Ideally, a radionuclide should be added directly into a lead peptide structure, preserving an unmodified, so called “native”, peptide without changing its chemical structure and thus its biological behavior. Direct labeling of small peptides has become increasingly popular, however site specific labeling without disrupting key target interactions remains a challenge.

The presence of carbon in organic molecules makes the PET isotope carbon-11 (99 % $\beta^+$, 0.96 MeV)\cite{20} an attractive and valuable radionuclide for PET-tracer development and thus also for the labeling of AAs, their derivatives and peptides. Isotopologue labeling without structural modifications conveniently include carbon-11 as a normal constituent in well-characterized molecules of known biological properties\cite{6,21–23}. Nowadays there is a great toolbox available for $^{11}$C-AA labeling, which includes viable methods using two major carbon-11 precursors produced directly by the cyclotron, $[^{11}$C]carbon dioxide ($[^{11}$C]CO$_2$) and $[^{11}$C]methane ($[^{11}$C]CH$_4$), and their transformed products, like $[^{11}$C]methyl iodide ($[^{11}$C]CH$_3$I), $[^{11}$C]methyl triflate ($[^{11}$C]CH$_3$OTf), $[^{11}$C]cyanide or other $^{11}$C-alkylating agents\cite{23}. The half-life of carbon-11 is with the current
radiolabeling techniques long enough to perform multi-step synthesis and biological PET-tracer analysis\cite{5}. Nevertheless, carbon-11 labeled radiotracers necessitate production at onsite radiochemistry facilities, including a cyclotron. Besides synthetic challenges, the clinical advantage is predominant allowing multi-tracer administrations in patients in a single day. Moreover, carbon-11 labeling often provides a better match of physical and \textit{in vivo} peptide half-live compared to long-lived radiometals.

The merits of radiosynthetic methods enabling to label a variety of $^{11}$C-AA and their derivatives, and their value as PET-tracer is nowadays well-recognized, exemplified by L-[$^{11}$C]methionine (MET), which is studied extensively for tumor imaging. On the other hand, $^{11}$C-small peptides have received less attention until recently where multiple independent papers described different methods for carbon-11 labeling of peptides, though clinical translation has thus far been inadequately examined\cite{10,15,19}. With current achievements the scope of carbon-11 labeling of peptides ranges from small proteins that have been successfully labeled with carbon-11 and used for applications such as blood flow permeability studies, as well as carbon-11 labeled peptides for inflammation and tumor imaging\cite{24-27}.

To the best of our knowledge, we describe all currently available synthesis strategies for the radiosynthesis of carbon-11 AAs, their most common derivatives and peptides, with an emphasis on stereoselective synthesis. Besides production options for these classes of radiopharmaceuticals, we will also discuss the clinical application and value of $^{11}$C-AA, and we will identify remaining challenges in radiosynthesis procedures for widespread adoption of these PET-tracers.

As depicted in Figure 1, despite the fact that theoretically all AAs can be labeled with carbon-11, the numerous attempts to radiolabel these molecules have shown that there is not one preferred strategy. All described methodologies to obtain radiolabeled AAs share both advantages and disadvantages. Synthesis strategies to obtain $^{11}$C-labeled AAs have to consider the labeling position and chirality in the AA structure, as well as the metabolic stability for intended biological applications. All these have resulted in the development of distinct strategies to radiolabel $^{11}$C-AAs for imaging purposes.
Figure 1: Short historical overview of general synthetic routes to yield carbon-11 labeled alanine and phenylalanine in two possible positions.

Carbon-11 Amino Acid Labeling of [1-$^{11}C$]Carboxyl-Function

Bucherer-Strecker Synthesis

The Strecker reaction, and its modified Bucherer-Strecker hydantoin synthesis, is one of the earliest one-pot and atom economic multi-component reactions for AA synthesis in general. Since the discovery, simple mixing of the reagents acetaldehyde, ammonia (or amines) and hydrogen cyanide resulting in an α-aminonitrile adduct and its subsequent hydrolysis, attracted radiochemists to access first $^{11}C$-AAs using $^{11}C$-carbonyls or $^{11}C$-cyanide. The first approaches described the use of $[^{11}C]$hydrogen cyanide ($[^{11}C]$HCN) to yield the corresponding $[^{11}C]$aminonitrile$^{[28]}$, whereas the
second approach employed $[^{11}\text{C}]\text{CO}_2$ which reacted with $\alpha$-lithiated precursor$^{[29]}$. In both cases, subsequent hydrolysis affords the corresponding AA as exemplified by Hayes et al. $[1-{^{11}\text{C}}]\text{Aminocyclopentanecarboxylic acid (ACPC)}$ was first obtained by a two-step high temperature reaction of cyclopentanone with $[^{11}\text{C}]\text{HCN}$ via a $^{11}\text{C}$-hydantoin anionic salt$^{[28]}$. Unfortunately, when this methodology was developed, most procedures to obtain carbon-11 labeled AAs required “carrier-added” cyanide to the reaction mixture diminishing the molar activity ($A_M$) of the final radiopharmaceutical. In the following years after the first publications, the Bucherer-Strecker radiolabeling method was further optimized providing successful radiosynthesis of $[^{11}\text{C}]\text{valine}^{[30]}$, $[^{11}\text{C}]\text{tryptophan}^{[31]}$, $[^{11}\text{C}]\text{leucine}^{[31]}$ and $\alpha-[^{11}\text{C}]\text{methylvaline}^{[31]}$ within one hour including hydrolysis, final tracer purification and formulation. Subsequently, room for improvement and advance in technology generated other modified routes, for example the “no-carrier-added” synthesis by Iwata and co-workers furnishing good yields for $[^{11}\text{C}]\text{phenylalanine}$, $[^{11}\text{C}]\text{valine}$ and $[^{11}\text{C}]\text{leucine}$. Here the starting material amino sulfonate reacted with on-line produced $[^{11}\text{C}]\text{HCN}^{[32]}$. Due to the high amount of precursor, around 400 mg (~2 mmol) of amino sulfonate, the application of this method might not be preferred, however could be realized using aldehydes and unhindered ketones, but not sterically hindered ketones (e.g. for the synthesis of $\alpha-[^{11}\text{C}]\text{methylphenylglycine}$). However, prosperous modifications by Studenov et al. in 2003, dismissed “carrier-added” cyanide and high amounts of amino sulfonates (only 4 mg, 20 µmol, needed). The synthesis of $[^{11}\text{C}]\text{phenylalanine}$ and $[^{11}\text{C}]\text{tyrosine}$ was performed at slightly elevated temperature and after acidic hydrolysis AAs were obtained in high molar activities of 74-111 GBq·µmol$^{-1}$, as depicted in Figure 2a$^{[33]}$. 
Figure 2: Non-stereoselective (a) and stereoselective (b) modified Bucherer-Strecker synthesis.

Due to the general increased knowledge on radiochemical methods, this strategy for carbon-11 labeled AA synthesis gradually attracted more attention and finally resulted in an alternative radiosynthesis the so called Zelinski-Stadnikoff variation. Prenant and co-workers described the unique labeling enabling the carbon-11 label to be located on the 2-position of an AA with labeling reagent $[^{11}\text{C}]\text{acetone}$, obtained from $[^{11}\text{C}]\text{CO}_2$ after reaction with methyllithium (CH$_3$Li)$^{[34]}$. An advantage of the Zelinski-Stadnikoff compared to the Bucherer reaction is the one-pot procedure in aqueous media. Utilizing this method, the labeling of $\alpha$-$[^{2-11}\text{C}]\text{aminoisobutyric acid}$ was described in a radiochemical yield (RCY) of 4% with $A_M$ of 16-20 GBq·µmol$^{-1}$ in approximately 50 min. Inconveniently, side reactions have occurred and two known by-products were reported, $[^{11}\text{C}]$lactic acid for incomplete conversion and/or $[^{11}\text{C}]$tert-butanol for an excessive concentration of CH$_3$Li.
Recently, an automated synthesis of no-carrier added $^{11}$C-carbonyl-labeled AAs using a modified Strecker synthesis has been reported. To overcome stated limitations, such as “carrier” cyanide, need for intermediate purification, reactions conducted in sealed tubes, which are mostly incompatible for automation due to high temperature and pressures required, Xing et al. implemented an alternative design to label the carbonyl moiety by forming an $^{11}$C-amino nitrile with $[^{11}\text{C}]\text{NaCN}$. A fully automated synthesis for $[^{11}\text{C}]\text{sarcosine}$ has been achieved in a non-decay corrected (n.d.c.) 1% RCY, calculated from $[^{11}\text{C}]\text{HCN}$, and radiochemical purity (RCP) of >90% with $A_{\text{M}}$ 55.5 GBq·mmol$^{-1}$ in 40 min, sufficient for preliminary imaging. Furthermore, application of this method towards other $^{11}$C-labeled AAs was achieved with variable success for methionine (5% RCY; n.d.c. based on $[^{11}\text{C}]\text{HCN}$), glycine (14% RCY) and $N$-phenylglycine (4% radiochemical conversion: RCC$^\vee$)$^{[35]}$.

Nonetheless, very recently the Hienzsch et al. have focused on developing a simple and efficient synthesis of enantiomerically pure $^{11}$C-AA using a microfluidics platform and extended the use of an asymmetric version of the Strecker synthesis. As depicted in Figure 2b, $[^{11}\text{C}]\text{HCN}$ is bubbled through a sulfinylimine precursor (1-3 mg, 4-20 µmol) in 1,4-dioxane/methanol in the HCN chamber of a microfluidic chip. Next, hydrolysis occurs in a separate chamber at elevated $T$, followed by a purification using a cation exchange cartridge and enzymatic enrichment by the D-$\alpha$-amino acid oxidase (D-AAO) to exclusively obtain L-AA (enantiomeric excess (ee) of 100%)$^{[36]}$.

In summary, all described variations of the Strecker synthesis lead to a racemic mixture of carbon-11 labeled AA where further enzymatic resolution to obtain enantiopure product is necessary. Time-consuming and low-yielding chiral purification to obtain a single stereoisomer, has, to date, hampered the application of Bucherer-Strecker synthesis or its variations as usually only one enantiomer displays favorable biological activity.

$^\vee$: Abbreviation register

[11C]CO2, mostly the first carbon-11 labeled reagent formed in a cyclotron target, is also a highly convenient reagent used to form carbon-11 labeled AAs. A route affording the [1-11C]carboxyl-labeled position, based on carboxylation of α-lithioisocyanides with [11C]CO2, was for the first time reported in 1976 by Vaalburg et al. [25]. Application of the direct carboxylation requires the activation of the α-carbon, achieved by the introduction of isocyanide functionality on the N-terminus. At the same time, the n-butyl lithium (n-BuLi) proton abstraction of the α-carbon followed by hydrolysis leads to the desired amine. Using this route, as described in Figure 3a, a vast amount of racemic 11C-AAs have been described in literature, namely phenylalanine[25], ornithine and lysine[37], tyrosine[27,29], and proline[38] and achiral glycine[24,25] and phenylglycine[25]. The latest, [1-11C]proline has been reported in 12–18% RCY with a RCP of >95% in total synthesis time of 45 min.

As mentioned, direct use of [11C]CO2 results in favorable non-decay corrected RCY and shorter synthesis times, thus great improvements have been made in order to obtain higher RCYs. This was achieved by using lower precursor quantities, performing the synthesis in a “no-carrier-added” manner, alternative use of stronger base (e.g. s-BuLi), to lithiate the precursor and fine-tune of essential technical details such as stirring, T control and successive addition of chemicals. By adjusting the conditions, the group of Bolster et al. successfully reported the [1-11C]tyrosine[39], 3,4-[11C]dihydroxyphenylalanine ([11C]DOPA)[26] and [1-11C]MET[40,41] with RCP >94% within 35 min, followed by chiral resolution to obtain the desired enantiomer as PET-tracer in RCY of 10-15%.

[1-11C]Carboxylation of N-(α-Lithioalkyl)Oxazolidinones

There has been continuous interest in the refinement of the [11C]CO2-fixation method to obtain L- and D-enriched 11C-AAs to overcome the challenges of time consuming and low-yielding chiral separation. Driven by the success of carbon-11 labeled methionine, firstly 11C-carboxylation using the N-(α-lithioalkyl)oxazolidinone reagents have been thoroughly explored to obtain the L-enantiomer of [11C]MET[42]. This successful procedure yielded L-[11C]MET as PET-tracer in excellent ee of 95% and RCY of 85%, next to short synthesis time of 35-40 min[43], and now allowed its application in PET imaging studies. Using this approach, the general starting material, N-(α-lithioalkyl)oxazolidinon, was transformed by a tin-lithium exchange to the corresponding N-
(α-stannylalkyloxazolidinon, which equilibrated rapidly to one diastereomer. This intermediate was further carboxylated using $[^{11}\text{C}]\text{CO}_2$ to the desired and diastereopure (α-carboxyalkyloxazolidinon, presented in Figure 3b. Finally, the chiral auxiliary was removed by a Birch-type reduction to yield the unprotected and enantiomERICally pure carbon-11 labeled AA as product, which was purified by ion exchange chromatography. In view of the good results, this method has been extended to other AAs by Jeanjean et al. in 2010. Furthermore, successful syntheses of L-leucine, L-alanine and L-homocysteine were accomplished with an overall yield of 13–33% with 92–95% ee\textsuperscript{44}.

To conclude, oxazolidinone moieties necessitate longer synthesis, but the radiosynthesis itself can be achieved in beneficial shorter time frame and more importantly in high stereoselective manner. The optimization for the routine preparation of labelled L-[\textsuperscript{11}C]MET has been initiated, however to the best of our knowledge has not been published\textsuperscript{44}.

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**Figure 3:** $[1^{-}\text{C}]$-Carboxylation reactions with activated precursors.
Racemic and Asymmetric Synthesis to Label AA in the Side Chain

With variable success many carbon-11 AA isotopologues have been synthesized with the label in the carboxylic position. Alternatively, the carbon-11 label can also be positioned in the side chain of the AA, which might be beneficial to detect different metabolism of the investigated AA. Therefore, alternative synthetic approaches for labeling the 3-position, or β, have been developed. An early approach towards novel $^{11}$C-C bond formation, in the backbone of the molecule, utilized $\text{S_N}_2$ alkylations of an activated methylene group, the glycine Schiff base, by alkylation with carbon-11 labeled aliphatic or aromatic alkyl halides. The starting Schiff base precursor, $\text{N}-$(diphenylmethylene) glycine tert-butyl ester, is rather straightforward to synthesize and nowadays even commercially available, and the α-carbon can be deprotonated easily under basic conditions. First encouraging reports on racemic labeled $^{11}$C-AA by research groups in Uppsala, Sweden\textsuperscript{[45]}, and St. Louis, USA\textsuperscript{[46]} date back to the early 1990’s. These initial attempts made use of achiral approaches and the AAs formed required enantiomer separation, either by chromatographic or enzymatic methods resulting in longer synthesis times and a low radiochemical yields. To circumvent this problem, asymmetric alkylation methodologies have attracted radiochemists aiming to prepare the desired enantiomer in high stereoselective manner and RCY. Given rise to this special approach, reliable methods to synthesize enantiomerically pure $^{11}$C-AAs have been accomplished with a variety of available Schiff bases and $^{11}$C-alkylating agents, as discussed further on in this chapter\textsuperscript{[46,47,48]}.

Racemic and Asymmetric Synthesis with Schiff Base Precursor: $\text{N}-(\text{Diphenylmethylene})$ Glycine tert-Butyl Ester

The alkylation of the Schiff base achiral glycine precursor was first reported for the radiosynthesis of $^{11}$C-phenylalanine by Kilbourn \textit{et al.} \textsuperscript{[46]}. Thereafter this strategy was followed to radiolabel a wide range of other racemic [3-$^{11}$C]AAs, namely alanine, 2-aminobutyric acid, norvaline, norleucine and leucine, illustrated in Figure 5a\textsuperscript{[45]}. All these reactions employed phase-transfer alkylation conditions using achiral catalyst tetrabutylammonium hydrogen sulphate and the achiral precursor, $\text{N}-(\text{diphenylmethylene})$ glycine tert-butyl ester, with different $^{11}$C-alkyl iodides. The racemic $^{11}$C-AAs of interest were obtained in a d.c. RCY of 10-50% and RCP of 93-99%. The
reaction times for the $^{11}$C-alkylation were between 5–10 min and typical reaction temperature of 40–45 ºC, though reaction conditions were always dependent on the reactivity of $^{11}$C-alkyl iodides. The final step to obtain the unprotected $^{11}$C-AA, removal of the protecting group of the alkylated intermediate occurred in 5 min at 130 ºC using strong acidic conditions (e.g. concentrated HCl). An inconvenience with this methodology, when first reported, was its non-stereoselectivity. Therefore, special attention was devoted to [3-$^{11}$C]alanine and [3-$^{11}$C]phenylalanine, treated with the enzyme D-amino acid oxidase (D-AAO) to be obtained as enantiopure (99% ee) product$^{[45]}$. Though very successful, this enzymatic resolution was time-consuming and challenging to perform.

Overall, these studies have identified phase-transfer alkylation is reliable and practical synthetic pathway to label $^{11}$C-AAs in the side-chain$^{[45]}$. The formidable challenge in this methodology still lies in performing a stereoselective alkylation on the α-carbon starting from an achiral precursor. Important parameters that apply to all radiopharmaceuticals are the convenience and reliability of the radiolabeling process, however limited by synthesis time and A_M. To overcome these challenges, especially the RYCs for these particular $^{11}$C-alkylations, more reactive alkylating reagents have been explored. Employing the highly reactive [$^{11}$C]CH$_3$OTf on same precursor, N-(diphenylmethylene) glycine tert-butyl ester, resulted in very rapid and efficient $^{11}$C-methylation in only 1 min at 60 ºC, followed by harsh acidic deprotection over 5 min at 170 ºC, finally yielding [1-$^{11}$C]alanine where the RCY was increased to 70–90% within 25-30 min$^{[49]}$.

Considering the utility of obtaining one enantiomer of $^{11}$C-AAs, this attractive alkylation approach has been brought to a next level by taking inspiration from the wealth of literature surrounding asymmetric phase-transfer catalysis$^{[50,51]}$. Recently, Windhorst and co-workers achieved rapid and highly enantioselective $^{11}$C-C bond formation of L- and D-[1$^{11}$C]alanine$^{[52]}$ and L- and D-[1$^{11}$C]phenylalanine$^{[53]}$, as illustrated in Figure 4a. The commercially available glycine Schiff base was utilized, in the presence of quaternary ammonium salts as chiral phase-transfer catalysts (PTCs; Figure 4b) to yield specifically L- or D-$^{11}$C-AAs. Reactions were performed in near quantitative RCC of the alkylation reactions, resulting in L-[1$^{11}$C]alanine and L-[1$^{11}$C]phenylalanine in excellent ee of >90%, desirable A_M of >50 and 85-135 GBq·µmol$^{-1}$, and sufficient d.c. RCY of 20% and RCP >95%, respectively$^{[52,53]}$. 


Furthermore, Windhorst and co-workers aimed to extend the catalyst controlled asymmetric induction towards 1,4-Michael addition reactions with $[^{11}\text{C}]_{\text{tert}}$-butylacrylate and $[^{11}\text{C}]_{\text{N}}$-tritylacrylamide to yield $^{11}\text{C}$-glutamate and $^{11}\text{C}$-glutamine, respectively\[54\]. Despite the versatility of carbon-$11$ synths, so far the enantioselective addition reaction was moderately successful, which might be attributed to Pd-ligand complex utilized in carbonylation reaction with $[^{11}\text{C}]_{\text{CO}}$.

![Proposed General Mechanism of an Asymmetric Alkylation of a Schiff Base for Amino Acid Synthesis](image)

**Figure 4:** Asymmetric phase-transfer alkylation reaction (a) with chiral phase-transfer catalysts (b).

**Asymmetric Synthesis with Schiff Base Precursor:**

\[[(+)-2-Hydroxypinanyl-3-idene]Glycine Ester\]

Alternative strategies to perform asymmetric alkylation reactions date back to 1979, where Långström and Stridsberg recognized the purification issues and this consequently led to the development of chiral starting material. First attempts employed a chiral precursor $8$-phenylmethyl-2-isocyanoacetate and alkylation reagent $[^{11}\text{C}]_{\text{CH}_3}I$ furnishing partially resolved $[3-^{11}\text{C}]_{\text{alanine}}$ obtained in an ee of $48\%$ with an excess of L-alanine in $50$-$60\text{ min}$\[55\]. In due course similar chiral Schiff base precursor, $[(+)-2$-hydroxypinanyl-3-idene]glycine tert-butyl ester, afforded under anhydrous conditions the first chiral synthesis of L-$[3-^{11}\text{C}]_{\text{alanine}}$ in a high ee of $89\%$, determined by enantiomeric analysis using GC\[4\], in $30$-$45\text{ min}$ (Figure 5a). As a final step, a two-step hydrolysis
of the protecting groups, the imine and tert-butyl ester group, was performed without racemization\textsuperscript{[56]}. Encouraged by the benefits of a single enantiomer, especially the L-form, asymmetric \(^{11}\text{C}\)-labeling of AAs was further extended using different \(^{11}\text{C}\)-alkyl iodides towards L-2-amino-[3-\(^{11}\text{C}\)]butyric acid, L-[3-\(^{11}\text{C}\)]norvaline and L-[3-\(^{11}\text{C}\)]-valine with a synthesis time of 50-55 min\textsuperscript{[57]}. In addition to the previously employed Schiff base precursor, additional chiral handle on the C-terminus (Figure 5a; in green) was feasible, but unfortunately resulted in lower asymmetric induction affording ee ranging from 36-80\%, d.c. RCY of 40\% and excellent RCP (>98\%) in 20-25 min\textsuperscript{[58]}.

Nevertheless, these examples highlight the potential for asymmetric \(^{11}\text{C}\)-alkylation with chiral Schiff base precursors. However, a general observation was that larger \(^{11}\text{C}\)-alkyl iodides proceed slowly, compared to the aforementioned \([^{11}\text{C}]\text{alanine}\) synthesis. An acceleration of the reaction and considerable increase in radiochemical conversion (up to 90\%) was observed as a function of the addition of more polar solvent DMPU\textsuperscript{Y} and its concentration, next to the addition of a strong base (BuLi) and increase of \(T\). Nonetheless, the synthetic approach by Antoni \textit{et al}.\textsuperscript{[57]} afforded previously mentioned \(^{11}\text{C}\)-AAs with decent RCY and excellent enantiomeric purities (>98\%), however has been discontinued due to occasional precipitations of lithiated precursor, inaccuracy in the ee determinations, and lastly, more approachable strategies.

\textbf{Asymmetric Synthesis with Schiff Base Precursor:}

\((-\text{-})\text{-8-Phenylmenthan-3-yl}\text{-N-(Diphenylmethylene)Glycinate}\)

Comparable to the procedure described previously, herein the C-terminal chiral auxiliary (Figure 5b), appeared as a simple method with high reproducibility and minimized technical handling. Starting material \((-\text{-})\text{-8-Phenylmenthan-3-yl}\text{-N-(diphenylmethylene)glycinate}\) was subjected to achiral phase-transfer catalyst \textit{tetra}-butyl ammonium hydroxide and \([^{11}\text{C}]\text{CH}_3\text{I}\) or \([^{11}\text{C}]\text{CH}_2\text{PhI}\) to furnish L-[3-\(^{11}\text{C}\)]alanine and L-[3-\(^{11}\text{C}\)]phenylalanine, respectively. Similarly to the previous approach, both \(^{11}\text{C}\)-AAs had reasonable RCY, 40\% and 15\%, respectively, and excellent RCP >98\%, however enantioselectivity was lower\textsuperscript{[59]}.
Asymmetric Synthesis with the Oppolzer Chiral Sultam-Derived Glycine

During the years Schiff base precursors have been the spotlight of research modifying their C- and N-terminal side, aiming to improve the RCY and more importantly the ee of the desired $^{11}$C-AA product. A very successful chiral precursor proved to be the sultam-derived glycine synthon, developed by Oppolzer et al.\textsuperscript{[60]} Advantageously, Någren and co-workers adopted and improved
this methodology by reacting the precursor with n-BuLi at low temperature for 30 min, followed by [\(^{11}\)C]CH\(_3\)I, trapping at the same temperature, enabling a quantitative alkylation within 5 min, while the reaction temperature slowly reached room temperature. Subsequently, the alkylated intermediate was subjected to two-step hydrolysis yielding L-[3-\(^{11}\)C]alanine in d.c. RCY 40-50\%, counted from [\(^{11}\)C]CH\(_3\)I, and 94\% ee in a total synthesis time of 50 min (Figure 5c)\(^{[6,49]}\). Since these very encouraging results, even the precursor has become commercially available which maintained great interest for chiral alkylation reactions to obtain \(^{11}\)C-AAs.

Asymmetric Synthesis of \(\alpha\)-[\(^{11}\)C]Methyl Tryptophan with [\(^{11}\)C]Methyl Iodide

Considerable potential in the use of Schiff base precursors has also been displayed by the radiosynthesis of the unnatural \(\alpha\)-[\(^{11}\)C]methyl tryptophan. Here, the natural amino acid transformed into a Schiff base is used as a precursor, which is on its turn alkylated at the \(\alpha\)-carbon. The scope and potential of these precursor has, however, only been explored to a limited extent. Previous successful syntheses of [1-\(^{11}\)C]tryptophan were not desirable, as the radiolabel would be lost during metabolism. To circumvent this, Chaly et al. developed the tryptophan imine moiety precursor, allowing stereospecific “no-carrier added” [\(^{11}\)C]methylation to obtain the alternative tracer of [\(^{11}\)C]tryptophan. The stereospecific alkylation with [\(^{11}\)C]CH\(_3\)I, was achieved in 30 min and resulted in a 20-25\% n.d.c. RCY and A\(_M\) of 74 GBq·µmol\(^{-1}\) (Figure 5d). Intriguing, the authors proposed that the intermediate is “locked” in the position existing in the original AA, in this case L-tryptophan, resulting in product of the same chiral configuration as starting precursor, with no D-stereoisomer present\(^{[61]}\).

Asymmetric Synthesis with Chiral Nickel Complexes

With respect to the Schiff base’s alkylations, the scope of available methods for PET-labeling has been broadened by the chemistry of metallo-complex AA synthons for the asymmetric preparation of \(\alpha\)-AA. The methodology has been introduced in organic and organometallic chemistry by Belokon in 1985. These chiral Nickel complexes proved to be highly versatile and allowed a very high level of stereontrolled \(^{11}\)C-alkylation reactions due to high acidity of the \(\alpha\)-hydrogen. Also \(^{18}\)F-analogues have been obtained via this route, though these will not be further discussed here\(^{[7,62]}\). Consequently, the condensation of Ni(II) with (S)-N-(2-benzoylphenyl)-1-benzylpyrrolidine-2-carboxamide (BPB) and glycine has become popular due to commercial availability of BPB, the chiral scaffold required for the stereoselective alkylation reaction and its reusage potential. The
first radiosynthetic approach to obtain $^{11}$C-AAs was evaluated by Fasth et al. in 1990. Accordingly, $[^{11}\text{C}]$alanine (ee of 80%; RCY of 60%) and $[^{11}\text{C}]$phenylalanine (ee of 90%; RCY of 30%) were synthesized, as well as O-$[^{11}\text{C}]$methyltyrosine and $[^{11}\text{C}]$tyrosine with an excellent ee of 90% and RCY of 15%.

Furthermore, by adapting the metallocomplex of Ni(II) with BPB, with either tyrosine or dopamine, Popkov et al. was also able to synthesize unnatural $\alpha$-$[^{11}\text{C}]$methyl-tyrosine and $\alpha$-$[^{11}\text{C}]$methyl-DOPA with $[^{11}\text{C}]$CH$_3$I, as illustrated in Figure 5e, giving slight excess of the LD-stereoselectivity with RCY 7% and 9%, respectively. Demonstrating the general applicability of this methodology, chiral Ni(II)-complexes were also explored with $[^{11}\text{C}]$formaldehyde as alkylating reagent by Popkov et al. in 2014. While the successful and reliable synthesis D-$[^{11}\text{C}]$serine resulted in 50% d.c. RCY, the diastereoselectivity achieved was 80%, indicating improvements in the HPLC separation of intermediate diastereomers are needed.

### Specific Carbon-11 Labeling in the Side Chain of the Amino Acid

**Condensation Reaction with 2-Aryl-5-Oxazolones**

The merits of $^{11}$C-alkylation, in particular the $[^{11}\text{C}]$methylation, as a choice for radiotracer production have been well-recognized. Schiff bases have been acknowledged as valuable precursors for stereoselective synthesis of various $^{11}$C-AAs by the formation of new $^{11}$C-C-bond through alkylation. However, multiple attempts have been reported to apply other precursors as well, aiming to obtain a carbon-11 labeled AA under similar alkylation reactions. For example, $\alpha$-$[^{11}\text{C}]$methyl-L-tryptophan, also described by Chaly et al., was reported by Plenevaux et al., where the N-terminal amine functionality was covalently attached to the indole precursor’s side chain to induce activation at the $\alpha$-carbon as well as to protect the amine-functionality. The precursor was deprotonated with lithium diisopropylamide as base at low temperature to activate the $\alpha$-carbon, followed by alkylation with $[^{11}\text{C}]$CH$_3$I in 5 minutes (Figure 6a). Subsequently, deprotection of the labeled precursor was achieved by acidic hydrolysis to yield the alkylated product in 36% d.c. RCY in 22 min with an exceptional ee $>97\%$. When the results of Plenevaux et al. are compared to the method described by Chaly et al., this approach yields the product slightly
faster in a higher RCY, however the precursor synthesis for the Plenevaux’s approach is more demanding.

**Asymmetric Synthesis of 2-tert-Butyl-3-Methyl-1,3-Imidazolidin-4-on with \([^{11}C]\)Alkyl Iodides**

Another promising method described providing \(^{11}\text{C}\)-AAs in an almost enantiomerically pure form is the alkylation of imidazolidinone derivatives. These precursors form a highly stereoselective \(^{11}\text{C}\)-C bond with \(^{11}\text{C}\)-alkyl iodides on stabilized carbanions, as depicted in Figure 6Bb. The imidazolidinone derivatives, \((R)\)- or \((S)\)-Boc-2-tert-butyl-3-methyl-1,3-imidazolidin-4-on (BMI), is treated with TMP-lithium in THF at low temperature to initiate deprotonation on the \(\alpha\)-carbon, followed by an \(^{11}\text{C}\)-alkylation and subsequent acidic hydrolisation to furnish the desired \(^{11}\text{C}\)-AAs. Via this route \(D\)- and \(L\)-\([3-{^{11}\text{C}}]\)alanine and \(D\)- and \(L\)-\([3-{^{11}\text{C}}]\)phenylalanine have been obtained with a RCP of 85\% in 50 min. Using this approach, the \([^{11}\text{C}]\)alanine synthesis was achieved in d.c. RCY of 75\% with an ee of exceptional 98\%. On the other hand, \([^{11}\text{C}]\)phenylalanine could only be obtained in a RCY of 30\%. This lower RCY might be explained by the lower electrophilicity of \([^{11}\text{C}]\)CH\(_2\)PhI as reagent. Nevertheless, the ee of the product was 96\%, which is highly satisfactory. Additionally, this methodology has also been used to obtain \([6-{^{11}\text{C}}]\)lysine with \((S)\)-Boc-BMI as precursor that was radiolabeled with \([^{11}\text{C}]4\)-iodobutyronitrile achieving an ee of 96\%, though these were only preliminary findings. In the same paper attempts to obtain \([1-{^{11}\text{C}}]\)valine with 2-\([^{11}\text{C}]\)isopropyl iodide were described, but proved to be unsuccessful. Unfortunately, the application of this methodology is hampered by the commercially available \((R)\)- or \((S)\)-Boc-BMI precursors as their enantiomeric purities can vary a few percent dependent on the batch, as unveiled by Fasth and Låndström\(^{[6,67]}\), which may influence the enantioselective synthesis of \(^{11}\text{C}\)-AAs.

**\(S\)-Methylations on Homocysteine with \([^{11}\text{C}]\)Methyl Iodide**

Among alkylations to form a new \(^{11}\text{C}\)-C bond, \([^{11}\text{C}]\)methylations on homocysteine residue are important as well, especially considering their versatility in peptide radiolabeling, discussed later. As early as 1976, Comar \textit{et al.} recognized carbon-11 as a beneficial radioisotope for \(^{11}\text{C}\)-methyl-methionine labeling of \(\text{DL}\)-homocysteine or \(\text{L}\)-homocysteine thiolactone with \([^{11}\text{C}]\)CH\(_3\)I\(^{[68]}\). Continuous research strived to improve and simplify methods to obtain latest PET-tracer resulting in a “no-carrier added” and rapid \(S\)-alkylation of \(\text{L}\)-homocysteine adsorbed on solid-supported
Al₂O₃/KF with [¹¹C]CH₃I (Figure 6c). The acidic S-H proton of the homocystein is easily removed by the strong base. Therefore, the final purification was simplified, as 90% of the precursor remained adsorbed on the solid support and was eliminated by a simple filtration, providing a superb d.c. RCY of 94% with no required preparative HPLC. Hence, this methodology provides important simplification enabling fast production of L-[¹¹C-methyl]methionine ready for injection within 10 min in excellent RCP of 99% and acceptable Aₘ of 37±6 GBq·µmol⁻¹[69].

**Palladium-Mediated Coupling with Organo-Boron Precursor and [¹¹C]Methyl Iodide**

Another research advance which should enable easier access to ¹¹C-AAs is the Pd-catalyzed coupling reaction between a boronic ester precursor and [¹¹C]CH₃I. In this reaction a Suzuki coupling is employed between an unsaturated AA precursor subjected to cross-coupling reactions. The newly formed ¹¹C-C bond can then be reduced to obtain a saturated alkyl side chain of the AA. The organo-boron precursor reacted within 5 min with [¹¹C]CH₃I in presence of Pd₂(dba)₃, P(o-tolyl)₃ and base at elevated temperature, followed by mild hydrogenation and deprotection to furnish L-[¹¹C]leucine. Ozaki’s radiosynthesis is illustrated in Figure 6d[70]. By a similar method unnatural S-α-[¹¹C]methyl leucine was also synthesized showing the suitability for syntheses of various ¹¹C-methyl-labeled branched alkanes[70].

**[¹¹C]Cyanide Nucleophilic Reactions**

Alternatively to the Bucherer-Strecker synthesis, the highly polarized soft base [¹¹C]cyanide has been further explored to radiosynthesize ¹¹C-AAs. This methodology was more recently published by Qu et al., where the nucleophilic substitution reaction with aliphatic halides furnished the enantiopure radiosynthesis of L-[5-¹¹C]glutamine. The radiosynthesis depicted in Figure 7a was achieved by a reaction of [¹¹C]KCN with protected enantiopure precursor 4-iodo-2-amino-butanoic ester in a 3-step synthesis, and successive deprotection and hydrolysis yielding L-[5-¹¹C]glutamine in RCY of 25% with high purity, but unfortunately rather low Aₘ of 1.85 GBq·µmol⁻¹[71].
Figure 6: Miscellaneous $^{11}$C-alkylations with various starting material.

Figure 7: Nucleophilic reactions with $^{11}$C-Cyanide.
Aziridine Ring-Opening Reactions

The versatility of $^{11}$C-cyanide as reagents allows the facile functionalization and derivatization of many precursors where aziridines can serve as alternative electrophiles for development of different $^{11}$C-carbonyl AAs. Aziridine ring-opening reactions have been reported by Gillings et al. as a novel and fast methodology for the synthesis of following $^{11}$C-AAs: $[4-^{11}$C]aspartic acid, $[4$-$^{11}$C]asparagine and 2,3-diamino-$[4-^{11}$C]butyric acid, all obtained in a d.c. RCY of 30–40% within 30 min$^{[72]}$. Racemic $N$-activated aziridine-2-carboxylates were synthesized in four steps, where in particular $N$-(tert-butoxycarbonyl)aziridine-2-isopropyl carboxylate underwent nucleophilic ring-opening reactions with $^{11}$C$tetra$-butylammoniumcyanide by heating in DMF, shown in Figure 7b. The use of $^{11}$C$tetra$-butylammoniumcyanide with the $tetra$-butylammonium counter ion was crucial since half of the radioactivity remained unreacted when $^{11}$C$HCN$ in DMF was used. This low reactivity of $^{11}$C$HCN$ in DMF was attributed to insolubility of the reagent in the solvent. To establish a stereoselective synthesis using this methodology, the racemic starting material was resolved by chiral HPLC, however racemization occurred mainly during the ring opening reaction due to the acidity of the β-hydrogen. Up till now, despite several attempts, no successful chiral syntheses have been reported using this methodology$^{[72]}$.

Enzyme-Catalyzed Synthesis of Carbon-11 Amino Acids

The relative short half-life of carbon-11 urges fast and efficient synthetic methods for PET-tracer production and therefore biocatalysis offers attractive solutions, such as stereoselective reactions or the possibility to omit protecting groups in the precursor. To date, enzymes have been used in a number of radiosyntheses to obtain $^{11}$C-labeled AAs and this approach resulted in exquisite selectivity and high turnover numbers, enabling rapid high yielding reactions to obtain pure products under mild conditions. Despite the general challenge of enzymatic reaction kinetics and the speed of radiolabel incorporation to the molecule, the application of low enzyme concentrations in $^{11}$C-labeled radiotracers preparation has proven to be yet a rewarding strategy for 12 carbon-11 labeled AAs$^{[48]}$. 
Thus far, two different types of enzymatic transformations can be distinguished, firstly the $^{11}$C-C bond formation and secondly the functional group transfer, exemplified by transaminiations or oxidations of amino groups to ketones$^{[48]}$.

The first example of a pioneering well-performed enzyme application, where an enzyme served as a resolving agent, was the preparation of L-[3-$^{11}$C]phenylalanine and L-[3-$^{11}$C]alanine, exemplified in Figure 8. Treatment of the corresponding racemic mixture by D-amino acid oxidase (D-AAO; EC 1.4.3.3) in 10-20 min resulted in enantiomerically pure $^{11}$C-AAs, where $ee$ of the product was determined by GC$^\text{v}$ ($N$-trifluoroacetylalanine methyl ester) or LC$^\text{v}$ ([3-$^{11}$C]phenylalanine as free acid)$^{[45]}$. Advantageously, this method uses immobilized enzymes which improved the previous challenges regarding the product purification and reduced the risk of product contamination. Next to that, immobilization allowed effortless recovery of the enzyme and enabled the possibility to perform several syntheses with the same enzyme$^{[48]}$.

Nowadays, the most described methodology remains the multi-step enzymatic $^{11}$C-AA synthesis, where initially a radiochemical reaction is combined with one or more enzymatic reactions. Employing this approach the syntheses of 4 aromatic AAs were described 3,4-dihydroxy-L-$^{[11}$C]phenylalanine, known as $^{[11}$C]DOPA, L-$^{[11}$C]-tyrosine, L-$^{[11}$C]-tryptophan and [5-$^{11}$C]hydroxytryptophan, as well as L-$^{[11}$C]alanine achieved using $^{[11}$C]CH$_3$I or $^{[11}$C]CN$^-$. As illustrated in Figure 8, the synthesis of all these $^{11}$C-AAs starts from $^{[11}$C]alanine, labeled at the carboxylic acid position (in red) or the $\beta$-carbon position (in blue). After initial labeling, $^{[11}$C]alanine is resolved using D-amino acid oxidase (D-AAO)/catalase and further converted to $^{11}$C-pyruvic acid by glutamic-pyruvic transaminase (GPT). Accordingly, subsequent one-pot $^{11}$C-C bond forming reaction between the aromatic precursor (indole or phenol) and $^{11}$C-pyruvate by $\beta$-tyrosinase (tyrosine phenol-lyase; EC 4.1.99.2) or tryptophanase (tryptophan indole-lyase; EC 4.1.99.1) yielded products in more than 99% $ee$. After the addition of HCl, the enzymes precipitated and final crude AA was purified by prep HPLC. Synthesis of L-$^{[11}$C]DOPA required also addition of ascorbic acid, in order to prevent possible oxidation$^{[47,73]}$. 

Next to the aromatic $^{11}$C-AAs, laudable achievements have been also made towards carbon-11 aliphatic AAs by variety of combined $^{11}$C-C bond-forming chemo-enzymatic reactions published in the last three decades. As a first example, the synthesis of L-[4-$^{11}$C]aspartic acid\cite{75} and L-[4-$^{11}$C]glutamic acid\cite{76} by $O$-acetyl-L-serinase and $O$-Acetyl-L-homoserine was published in early 1980\cite{76}, where further meticulous improvements by Antoni et al. in 2001 enabled their synthesis in 45-55 min. Satisfactory d.c. RCY of 50-60% and 60-70%, respectively, and RCP >95% and an overall $A_M$ of 30 GBq·µmol$^{-1}$ starting from $[^{11}\text{C}]$HCN were obtained. Additional success was the high enantiomeric purity, determined by derivatization with Marfay’s reagent, of 98% ee for both aliphatic $^{11}$C-AAs\cite{77}.

Finally, chemo-enzymatic synthesis advances were achieved by L-[$^{11}$C]methionine ($[^{11}\text{C}]$MET) via $^{11}$C-S bond formation. Based on a wide spectrum for substrate specificity of $O$-acetyl-L-homoserine and its success in the synthesis of $\gamma$-[${}^{11}$C]cyano-$\alpha$-amino-L-butyric acid by Antoni et al., Kaneko et al. later published the synthesis of $[^{11}\text{C}]$MET from $O$-acetyl-L-homoserine and the $[^{11}\text{C}]$methanethiol precursor obtained from $[^{11}\text{C}]$CH$_3$I\cite{78}. As S-H compounds are very reactive, its carbon-11 isotopologue rapidly reacted with immobilized $\alpha$-$\gamma$-cyano-$\alpha$-aminobutyric acid synthase and the radioactivity eluted from the column was only $[^{11}\text{C}]$MET. The tracer was obtained within 15 min after EOB with an enantiomeric purity of >99% and d.c. RCY of 70% (based on $[^{11}\text{C}]$CH$_3$I)\cite{78}. Other $[^{11}$C]MET analogues, like $[^{11}$C]ethionine and $[^{11}$C]propionine could also be prepared from the corresponding $^{11}$C-alkanethiols.

Finally, we should also mention that a series of coupled enzymatic reactions have been reported to radiosynthesize L-[3-$^{11}$C]serine from $[^{11}\text{C}]$MeOH, starting from $[^{11}\text{C}]$CO$_2$. The immobilized

**Figure 8:** Enzymatic synthesis of aromatic L-enriched 11C-AAs starting from radiolabeled carboxylic- (in red) or the methyl- (in blue) position.
enzymes alcohol oxidase (EC 1.1.13.13) combined with catalase (EC 1.11.1.16) selectively oxidized \([^{11}C]MeOH\) to \([^{11}C]formaldehyde\), which condensed with tetrahydrofolate and was subsequently treated with immobilized serine hydroxymethyltransferase (SMTH). Accordingly, only the L-[\(3-{^{11}}C\)]serine enantiomer was detected by LC-analysis and obtained in 1-2\% d.c. RCY (from \([^{11}C]CO_2\)) after a synthesis time of 50-65 min with AM of 1.1-1.9 GBq·µmol\(^{-1}\)\(^{[79]}\).

In general it can be concluded that enzyme transformation reactions can indeed be highly stereoselective towards the naturally occurring L-enantiomer. On the other hand, obtaining the D-product still remains a special challenge. A disadvantage that may arise is the long reaction time and loss of the radioactivity, resulting in a low absolute yield when enzymes function as a resolving agent. Nevertheless, the specificity remains their main asset, even after several reuses of these specialized immobilized enzymes.

**Synthesis Strategies for Carbon-11 Small Peptides**

Next to the use of carbon-11 labeled AAs as PET tracer, there has been tremendous interest in using \(^{11}C\)-peptidic radiopharmaceuticals, as peptides offer a maximum degree of freedom and flexibility\(^{[9]}\). Only few peptides were successfully labeled with carbon-11 and applied in (tumor) imaging studies. The earliest reports from the 1980s were conducted on several biologically active peptides by attaching an isotopologue methyl group using \([^{11}C]CH_3I\) or \([^{11}C]formaldehyde\)\(^{[80–82]}\).

Inspired by the prosperity of formaldehyde labeling, Straatman \textit{et al.} labeled proteins with \([^{11}C]formaldehyde\) in a borate buffer at different pH and subsequently treated the reaction mixture with various concentrations of NaBH\(_4\) at room temperature. Using this methodology, the proteins human serum albumin (66.5 kDa), fibrinogen (340 kDa) and luteinizing hormone (LH, 42 kDa) have been labeled successfully, all using similar reaction conditions. Albumin required slightly basic pH, and after the optimization it was concluded that higher amounts of protein in the reaction gave higher RCYs with a mean of 39\% (representative structure in Figure 9a). On the other hand, \(^{11}C\)-fibrinogen was obtained in 33\% RCY and exhibited still similar biological behaviour as the unlabeled fibrinogen\(^{[81]}\). Finally, also methylated glycoprotein LH obtained via the reductive alkylation with on-line produced \([^{11}C]formaldehyde\), retained its biological potency, however the
A_{M} \text{ of } 7.2 \text{ GBq·µmol}^{-1} \text{ hampered pre-clinical application}^{[83]}. Evidently, the successful synthesis route illustrated in Figure 9a preserved the biological activity of protein despite its unspecificity considering the location of the radioactive label and the amount nuclide per molecule. Consequently for carbon-11 LH, high A_{M} is hardly achievable especially as high amounts of starting protein are needed to achieve a successful radiolabeling.

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**Figure 9**: Early beginnings of simplified strategies for carbon-11 labeled peptides.

Carbon-11 labeled proteins provided an insight into their mechanism of action in the early development of PET in routine diagnostic use (early 2000s) and proved their value and biological importance. Confirmation of their safety and already acknowledged specificity of small peptides expanded the development of $^{11}$C-radiolabeling strategies to obtain peptidic radiotracers via fast and efficient routes.

One of the other early examples of carbon-11 peptide labeling is the carboxylation of α-lithioisocyanides to obtain dipeptides with a radiolabeled carboxylic acid functionality$^{[24]}$. Initially, $^{[11]}$C-glycine was produced from methylisocyanide via $^{11}$C-carboxylation, which is on its turn further reacted with a cyclic AA anhydride to obtain L-phenylalanyl$^{[1-11]}$Cglycine and L-leucyl$^{[1-11]}$Cglycine. Bolster et al. achieved the dipeptides in an isolated RCY of 3-6% in a total synthesis time of 50 min, illustrated in Figure 9b$^{[24]}$. In theory, many anhydrides, also more complex cyclic
ones, could react with $^{11}$Cglycine providing longer peptidic structures, where, the chiral center is preserved generating a single enantiomer.

$^{11}$C]Methylation was also described as strategy for peptide radiolabeling, mainly for the alkylation of the thiol functionality of homocysteine in the peptide. The complete deprotection of benzyl-homocysteine followed by side-specific methylation performed in one-step in liquid ammonia is presented in Figure 10a. Initial studies applying this approach have been described by Långström et al., where a tripeptide with the sequence Z-Gly-L-Homocysteine(Bzl)-Gly-O-Bzl was methylated to obtain H-Gly-$^{11}$C]Met-Gly-OH with more than 90% RCY in less than 25 min$^{[84]}$. Since $^{11}$C]methylation of homocysteine residues proved to be successful, this methodology has been used to obtain radiolabeled Substance P, enabling its thorough investigation in biological studies. Its undecapeptide and octapeptide were labeled using a homocysteine precursor in satisfactory 35% RCY within 1 hour production time, including purification. Even though, deprotection problems of other protecting side groups and partial cleavage involving proline nitrogens arose, a high RCC could be obtained$^{[85,86]}$. Furthermore, neuropeptide MET-enkephalin, a 5-mer peptide, and its two metabolic fragments thereof, Gly-Phe-MET and Phe-MET, were labeled using $^{11}$C]methylation strategy by Någren et al. S-$^{11}$C]MET-enkephalin and corresponding carbon-11 labeled fragments, were reported in a RCY of 50-80%, after LC purification, in 35-50 min with $A_M$ ranging from 0.4-7.2 GBq·µmol$^{-1}$, depended on the $A_M$ of $^{11}$C]CH$_3$I$^{[87]}$. Though this method of peptide radiolabeling proved to be highly reliable and high yielding, it should be noted that allows radiolabeling only if a methionine residue is present in the peptidic structure. Though being an interesting approach, further optimization of this procedure, especially with the respect of deprotection and generation of the sulphide anion for the reaction, has not been published.

More recently, analogous to the methodology developed by Långström et al., advantage was taken by making use of the more reactive and volatile $^{11}$C]MeOTf for the methylation of three peptides. Here, a thiol nucleophile from a cysteine residue was alkylated and thereby a high single-step regioselective $^{11}$C]methylation of cysteine residues was demonstrated$^{[15]}$. $^{11}$C]MeOTf as alkylating reagent for peptides has been further explored to label more complex peptides as well, such as carbon-11 labeled glutathione (GSH) by using excess of NaOH(l) in DMSO, furnishing successful methylation in less than 1 min (Figure 10a). Additionally, a decapptide has been labeled
and was obtained in a 59–65% incorporation yield. The strategy was also possible with the challenging cyclic somatostatin analogue $[^{11}\text{C}]\text{Cys(Me)}-[\text{Tyr}^{3}\text{-octreotate}]$, as described in the next section$^{[15,88]}$.

Recently the use of carbon-11 labeled peptides has attracted considerable attention with three promising novel methodologies to label small peptides containing 2 to 10 amino acids. One approach utilizes direct incorporation of carbon-11 label via stereoselective alkylation, published as “native” peptide labeling. Pekošak et al. described the first carbon-11 labeled peptides and afforded multiple tetrapeptides$^{[89]}$ employing chiral phase-transfer catalysts in a reliable RCC and high diastereoselectivity, as depicted in Figure 10b and discussed more detail in the next chapter. Follow-up studies of Filp et al. investigated the influence of the substrate peptidic backbone precursor and phase-transfer catalyst (see the Figure 4b) induction on stereoselectivity for various natural dipeptides, labeled with either $[^{11}\text{C}]\text{CH}_3\text{I}$ or $[^{11}\text{C}]\text{CH}_2\text{PhI}$$^{[90]}$. Overall, excellent conversion rates of $>85\%$ and diasteremomeric excess up to 94 were uniformly observed in only 5 or 7 min (Figure 10b), thereby showing the procedure can be extended to various N-terminal peptides using other $^{11}\text{C}$-alkyl halides and used for rapid preclinical assessment of the peptidic-lead structure.

A second recent methodology to obtain carbon-11 labeled peptides depends on the use of palladium-mediated amino-carboxylation using $[^{11}\text{C}]\text{CO}$. Figure 10c shows the production of N-$^{11}\text{C}$-acetylated peptides on terminal amine or lysine residues was reported by Andersen et al. The strength of this paper is that several N-acetylated peptides have been described, such as $[^{11}\text{C}]\text{LULU-Phol}$ (RCC: 43%; d.c. RCY: 33%), $[^{11}\text{C}]\text{acetyl cRGDfK}$ (RCC: 63%; d.c. RCY: 37%), $[^{11}\text{C}]\text{lacosamide}$ (RCC: 63%; d.c RCY: 46%) in in excellent RCP of 99% within 30 min, and also a larger peptide the $[^{11}\text{C}]\text{SPF-5506-A}_{4}$ (RCC: 15-21%; d.c. RCY: 2%). The $^{11}\text{C}$-acetylation provided the labeled peptides with high $A_M$ ($[^{11}\text{C}]\text{LULU-Phol}: 281 \text{ GBq} \cdot \mu\text{mol}^{-1}$) and proved to be N-terminal selective, however has a competing reaction on cysteine residues, namely the S-acetylation, might serve as an additional challenge for future applications$^{[91]}$. 


Figure 10: Most recent novel carbon-11 radiolabeling strategies for small peptides.

A more recent paper was published by Zhao et al., who reported a direct $[^{11}C]CN$ labeling of cysteine-containing unprotected peptides via palladium-mediated sequential cross-coupling reaction. This
method enables “nucleophilic-nucleophilic” coupling of a peptide with $[^{11}\text{C}]\text{HCN}$, as $[^{11}\text{C}]\text{cyanide}$ source via Pd-complex dihaloarene oxidative addition. In this manner a cysteine residue is chemoselectively labeled in presence of other potentially nucleophilic functional side groups. Outstangingly, low amounts of peptide precursor (20 nmol) are needed for this reaction which can be of added value. The potential of the methodology was shown on the RGD peptides ligands for the $\alpha_\text{V}\beta_3$ integrin receptor yielding 10% n.d.c. RCY with $A_M$ 37 GBq·µmol$^{-1}$ within only 15 min (Figure 10d) confirming the applicability of the method$^{[92]}$. 

In recent years, the development in biotechnology and its applications has lead us towards combined and improved mechanistic possibilities of peptidic radiopharmaceutical as molecular imaging agents. An early research example of using a biotechnological method for the synthesis of radiolabeled peptides was exemplified by Harada et al.$^{[93]}$. A cell-free system, so called PURESYSTEM, was combined with $[^{11}\text{C}]\text{MET}$ to obtain interleukin-8 (IL-8). IL-8 is involved in neutrophils activation, and already showed potential in inflammation imaging with $^{99}\text{m}\text{Tc}$ and $^{131}\text{I}$$^{[94,95]}$. Briefly, PURESYSTEM is a novel reconstituted protein synthesis kit consisting of necessary enzymes for in vitro transcription, translation and energy recycling. The in vitro biochemical reaction proceeded well in a RCY of 63% with RCP of $>95\%$. $[^{11}\text{C}]\text{IL}$-8 was obtained in n.d.c. RCY of 13% in 20 min, sufficient for a small animal imaging study. It is important to bear in mind that protein synthesis will stop when $[^{11}\text{C}]\text{MET}$ is exhausted, therefore high molar activity can be reached.

**Carbon-11 Labeled Somatostatin or Octreotate Analogues**

In the emerging field of molecular imaging, radiolabeled somatostatin analogues have become indispensable tools for in vivo localization of tumors and real-time monitoring of therapeutic response. Many somatostain analogues have been developed and analyzed, whereas the radiolabeled analogue of the most well-known cyclic octapeptide – Octreotide has become the gold standard for neuroendocrine tumors. Driven by the success of Octreotide and the significant potential of a large number of carbon-11 PET-radiopharmaceuticals in clinical application, several carbon-11 somatostatin/octreotide analogues have been developed$^{[1,89,96]}$. 


A chemoselective labeling strategy of carbohydrate analogue of Tyr³-octreotate based on the formation of oximes reacting with labeled prosthetic group has been proposed by Henriksen’s group. Accordingly, 4-[¹¹C]methoxy-benzaldehyde (Figure 11a in green), derived from [¹¹C]CH₃I, reacted with aminoxy-functionalized peptide precursors of D-Phe¹-Tyr³-Thr⁸-octreotide and a two-step synthesis yielded Cel-Dpr-[¹¹C]MBOS-TOCA (Figure 11a) in a d.c. RCY of 21% within 60 min with a A_M of 22–28 GBq·μmol⁻¹, starting from [¹¹C]CO₂₁⁹. Another well-known example is a one-step regioselective [¹¹C]methylation of cysteine residue with [¹¹C]MeOTf towards [¹¹C]Cys(Me)-[Tyr³-octreotate] (Figure 11b), resulting in n.d.c. RCY of 11% and A_M of 96 MBq·μmol⁻¹, after purification within 30 min¹⁵. Very recently, the [¹¹C]-labeled “unmodified” peptide was described by asymmetric phase-transfer catalyzed alkylation with [¹¹C]CH₂PhI (Figure 11c). As shown in Figure 11c, the H-D-[¹¹C]Phe-D-Trp-Lys-Thr-OH, the essential pharmacophore for somatostatin receptors, was labeled in a highly stereoselective manner using catalyst 8 (Figure 4b) with a diastereomeric excess of 94% with d.c. RCY of 9-10% and A_M of 15-35 GBq·μmol⁻¹ in less than 60 min⁸⁹.

Obviously, the advantage of the last strategy is to obtain a non-modified peptide as highly enriched diastereomer, where further biological studies are not hampered comparing the radiolabeled compound to the natural peptide.
Reflections on the (Pre-)Clinical Applications of $^{11}$C-Labeled Amino Acids

Besides the radiosynthetic development, radiolabeled AAs and peptides have gained an increased (pre)clinical interest to study the nature of various diseases. As diverse naturally occurring compounds accountable for many biological functions, $^{11}$C-AAs and peptides can be considered as attractive radiopharmaceutical tracers. These targeting vectors labeled with carbon-11 possess no structural changes, whereas with fluorine-18 or radiometal labeled tracers structural differences arise. Notwithstanding, the radiosynthesis of fluorine-18 labeled AAs and peptides is well developed and a comprehensive overview is given by Ermert et al. in 2013 and Richter et al. in 2014[97,98]. Radiometals, such as gallium-68, require a corresponding bifunctional metal chelator, and due to the size of the chelation, these must be separated with the linker not to interfere the peptide binding region[11].

The advantages of carbon-11 labeled “native” compounds are:

- structurally preserved molecule,
- equivalent biological properties,
- favorable radionuclide characteristics,
- low radiation burden for patient,
- feasible multiple tracer injections per day.

Regardless of the half-life and restricted use by PET centers with cyclotrons, the development towards carbon-11 “native” AAs and peptides has been strongly enhanced. Promising imaging studies are presented in the next paragraphs, starting with the top-most used PET-tracer $[^{11}]$C]MET. The in vivo evaluation of other $^{11}$C-AAs is limited and has been mostly performed with only small animals, where some studies even date back to the 1980’s. Over the years, indeed some progressed to a small scale human study, however no substantial trials have been conducted recently.
Table 1: Important $^{11}$C-AAs and derivatives with (pre)clinical application.

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Synthetic Approach</th>
<th>Applicability</th>
<th>Highlights</th>
<th>Ref.</th>
</tr>
</thead>
</table>
| Methionine | ![Synthetic pathway](image) | Brain tumor imaging, Urinary, gynecological and lung cancer | $A_M$: NA<sup>#</sup>  
RCY: 40-90%  
RCP: >98%  
ee: >99%  
t: 20-30 min | [99–101] |
| DOPA      | ![Synthetic pathway](image) | Neurological disorders (e.g. Parkinson’s disease, Schizophrenia)  
Brain kinetics | $A_M$: 0.4-2.0 GBq·μmol<sup>−1</sup>  
RCY: 45-60%  
RCP: >98%  
ee: >99%  
t: 45 min | [47,102] |
| Glutamine | ![Synthetic pathway](image) | Glutaminolysis metabolism: tumor trapping (only preclinical) | $A_M$: 1.85 ± 0.74 GBq·μmol<sup>−1</sup>  
RCY: (EOB) 5%  
RCP: >95%  
ee: >98%  
t: 60 min | [71] |
| Valine    | ![Synthetic pathway](image) | Small scale patient study: pancreatic diseases | $A_M$: 0.56-1.30 GBq·mg<sup>−1</sup>  
RCY: 70%  
RCP: >95%  
ee: Racemic  
t: 45 min | [30] |
| Tyrosine  | ![Synthetic pathway](image) | Brain tumor imaging (prolactinomas) | $A_M$: NA  
RCY: (EOB) 16%  
RCP: >NA  
ee: >98%  
t: 40 min | [29,103] |

<sup>#</sup>If $A_M$ cannot be determined to release $[^{11}\text{C}]$MET for patient PET scan, radiochemical and enantiomeric purity are important.

$[^{11}\text{C}]$Methionine

$[^{11}\text{C}]$MET has been used extensively to visualize tumor metabolism. Nowadays, many different tumors are imaged using $[^{11}\text{C}]$MET by increased fluxes into metabolic pathways and cellular proliferation, next to its involvement in the synthesis of phospholipids<sup>[104,100]</sup>. The transport of
[11C]MET into the brain is highly enhanced in tumor tissue (e.g. gliomas) across the reversible sodium-independent transport system L (LAT 1).

In 1985, the first report was published on the application of [11C]MET as a PET-tracer in patients with supratentorial gliomas. It was proven that the uptake of [11C]MET is not only caused by passive diffusion and leakage in the blood-brain barrier (BBB), but should also be attributed by active AA-transport into the cell. Since then, various follow up studies compared the use of [11C]MET with [18F]FDG in glioma, meningioma, non-small lung cancer and non-Hodgkin’s lymphoma patients to evaluate metabolism, as well as to detect and delineate tumors. Both [11C]MET and [18F]FDG as PET-tracers have been used complimentary, however the advantage of [11C]MET was clear. Especially for brain tumor imaging better tumor-to-background ratios can be achieved next to tumor staging and determination of tumor margins. Overall, in conclusion of the various studies [11C]MET is better suited for low and intermediate grade brain tumors compared to [18F]FDG. Another breakthrough in the use of [11C]MET was a study in young children and adults with confirmed brain tumors, conducted by O’Tuama et al. The consumption of administered [11C]MET was declining with age in the tumor, proposing that AA consumption deteriorates. In a follow up study, Harris et al. showed that pancreas and liver are the organs with the highest uptake of [11C]MET also for pediatric patients. All this suggests that the main metabolic pathway of [11C]MET is protein incorporation, as the high uptake might be related to the need of MET for protein synthesis, for example plasma proteins in the liver.

In recent years, the clinical value of [11C]MET has been thoroughly investigated for brain tumors by Glaudemans et al. as well as in a meta-analysis by Zhao et al. Both studies showed an excellent diagnostic performance of [11C]MET in brain tumor differentiation where the threshold for determination of brain tumors, relying on tumor-to-background ratio, lies between acceptable 1.5-1.9. Furthermore, [11C]MET uptake in normal brain in comparison to glioma patients offered significant advantages, also for white matter changes, which are normally missed in standard analysis methods.

With respect to imaging of other tumor types, [11C]MET has been extensively studied in urinary and gynecological cancers, as well as in lung cancer patients, however in mentioned tumors it is of critical importance to identify the exact metabolic mechanism and its use has limitations (e.g. influence of AA-transport and protein synthesis, alternative metabolic pathways).
[11C]MET development has been summarized by Coope et al. They concluded that the future of quantitative analysis of [11C]MET uptake needs sophisticated computer programs and standardization of tumor-to-background cut-off values\textsuperscript{116}. Moreover, contemporary PET/MRI imaging might significantly improve the diagnosis with more precise reference regions maps compared to CT\textsuperscript{114}.

**[11C]Tyrosine**

The application of a racemic [11C]tyrosine was assessed in preliminary studies in tumor-bearing rats by Vaalburg et al. in 1984 and later Daemen et al. Both enantiomers of [11C]tyrosine showed tumor uptake, which was even slightly in favor of the D-enantiomer\textsuperscript{129,106}. With respect to the metabolism, [11C]CO$_2$ appeared as the major breakdown product, meaning that [11C]tyrosine is firstly metabolized via decarboxylation. Advantageously, the metabolite [11C]CO$_2$ can be rapidly cleared through the lungs, not disturbing PET measurements and potentially even increasing the signal to noise ratio\textsuperscript{119,120}.

A small scale study investigated the potential reduction on tumor growth after radiotherapy in rats and patients with prolactinomas, benign tumors in the brain overproducing the hormone prolactin and bromocriptine. L-[11C]Tyrosine proved superior to [$^{18}$F]FDG for the visualization of this particular brain tumor after radiotherapy\textsuperscript{106,121}. Regardless of the successes achieved the use of [11C]tyrosine has been limited.

**[11C]Valine**

[11C]Valine has been described as a potential pancreas-imaging agent in 1978 by Washburn et al. performing an extensive biodistribution study in rats and other small animals\textsuperscript{30}. Tissue distribution of [11C]valine in all animals showed high uptake and fast clearance, although also [11C]valine was exposed to rapid decarboxylation. Nevertheless, studies performed in patients with pancreatic disease, showed uptake in the diseased tissue of racemic [11C]valine and [11C]tryptophan\textsuperscript{122}. Both AAs showed steady pancreatic uptake, however they also found a high concentration in the kidneys, making the scan interpretation difficult. At that time, in 1979, injecting a racemic mixture of PET-tracer has not been recognized as problematic, as it was assumed that D-AAs are simply not metabolized and remain in the blood flow, whereas L-AAs are either taken up by tumor or...
metabolized earlier. Therefore, with the lack for the sufficient model for quantitative analysis strong conclusions could not made.

**[11C]Dopamine**

Though most $^{11}$C-labeled AAs are applied for tumor imaging, advances have also been made for imaging of neurological diseases with $\text{L-}[^{11}\text{C}]\text{DOPA}$ The development of this PET-tracer proved challenging and the first successful synthesis was reported in 1973 by Fowler et al. Later Hartvig et al. reported the initial biological evaluation and clinical use of $\text{L-}[^{11}\text{C}]\text{DOPA}$, consequently showing its importance for various neurological disorders, such as Parkinson’s disease and schizophrenia, next to its well-known analogue $[^{18}\text{F}]\text{fluoro-3,4-dihydroxyphenyl-L-alanine (FDOPA)}$. The best position for $^{11}$C-label in $[^{11}\text{C}]\text{DOPA}$ molecule was evaluated by the metabolism study in an enzyme-activity study, by decarboxylation of $[^{11}\text{C}]\text{DOPA}$ to $[^{11}\text{C}]\text{Dopamine}$, in endocrine pancreatic tumors. The biological evaluation revealed that the carbon-11 position is important, and the radiolabel is not metabolized in the β-position. $\text{L-}[^{11}\text{C}]\text{DOPA}$ is stored mainly in one type of secretory granula, however in active tumors it is constantly released thereby diminishing the content of radioactivity. Its metabolism was reported to be slow with in vivo half-life of roughly 2 hours. Furthermore, metabolites of DOPA play an insignificant role, since these are stored in vesicles in areas with high density of dopaminergic pathways in the brain (e.g. the striatal region). In a study performed by Lindner et al. the synthesis rates for the conversion of DOPA to its major metabolites, such as 3,4-dihydroxy-phenylacetic acid, and homovanillic acid, were assessed in different species.

**[11C]Glutamine**

In recent preclinical studies it was demonstrated that glutamine metabolism is a vital process for tumor development and progression. With the better understanding of tumor metabolism and the fact that glutamine, a non-essential AA available in high concentrations in blood, is a very important nutrient for diseases, in the so-called glutaminolysis pathway, the interest in glutamine as a PET-tracer have grown. Further in vivo PET imaging in tumor-bearing rats suggested a rapid tumor uptake and trapping. This proposed to utilize $[^{11}\text{C}]\text{glutamine}$ in addition to negative $[^{18}\text{F}]\text{FDG}$ tumor studies to discriminate a tumor’s reliance on different nutrients for growth. Interestingly, this tracer utilizes a different mechanism of action in tumors and its uptake is complementing glucose metabolism, as cells could employ alternative energy sources, compared
to all other available AAs tracer so far, which are mostly dependent on elevated levels of AA-transporters on the cell membrane\textsuperscript{[128]}. All this suggest, there is still a great interest in glutamine or its derivatives as PET-tracer.

Taken together, these findings enhanced the understanding of AA metabolism, transport mechanism in cells, biological behavior of radiolabeled compounds, and applicability in diagnosis of tumors as well as for neurological disorders. The main weakness of $[^{11}\text{C}]$tyrosine, $[^{11}\text{C}]$valine and $[^{11}\text{C}]$dopamine studies, conducted more than 20 years ago, is their obsolence and lack of more recent re-evaluation with state of the art PET analysis techniques. Reasons for the reluctant use of these labeled compounds in general might be the underdeveloped radiosynthesis methods and obviously proximity to radiochemistry facilities with cyclotrons were a prerequisite. Also, fluorine-18 labeled tracers are in some cases preferred (e.g. L-6-$[^{18}\text{F}]$fluoro-DOPA), especially as they can be distributed to other hospitals. On the other hand, as new carbon-11 radiolabeling strategies advance, the accessibility can be broadened and consequently open doors for new imaging agents. That can be especially interesting for biological evaluation of peptides where no additional toxicological studies have to be performed before (pre)clinical studies.

**Application of $^{11}\text{C}$-Labeled Peptides as Imaging Agents**

Although peptides and AAs are alike in many ways, such as AAs scaffold and consequently position for radiolabeling or short *in vivo* half-life, it should be emphasized that the use of $^{11}\text{C}$-labeled peptides is, up to date, far more challenging. Their translation into PET imaging agents has been supported with specialized radiolabeling reactions in very recent years\textsuperscript{[12]}, however the application of carbon-11 labeled peptides remains almost unexplored. A challenge for the application might be associated with slow distribution for *in vivo* studies due to rapid metabolism.

The very first example of *in vivo* study using a carbon-11 labeled peptide/protein was human serum albumin microspheres. Turton *et al.* evaluated $[^{11}\text{C}]$albumin in dogs and demonstrated ideal properties of labeled microspheres to study blood flow for investigation of pulmonary and BBB permeability\textsuperscript{[129]}. In 2004, Henriksen *et al.* reported a convenient synthetic procedure to obtain a
functionalized sugar-containing derivative of octreotate and assessed the product in vivo as well\[19\]. With a A\textsubscript{M} of 22-28 GBq·µmol\(^{-1}\), biodistribution and PET-imaging studies in pancreatic carcinoma-bearing mice showed good results recommending its further use.

**Conclusion and Future Perspective**

In conclusion, advances in radiopharmaceutical chemistry have enabled to establish efficient and reliable syntheses of many \(^{11}\)C-labeled AAs and peptides to date. Starting off with \[^{11}\text{C}CO_2\], \[^{11}\text{C}HCN\] or \[^{11}\text{C}CH_3\text{I}\], a multitude of AAs and their derivatives have been obtained as PET-tracers. Next to that, based on these synthesis strategies, also \(^{11}\)C-labeled peptides can be successfully achieved. The main challenge of all these radiolabeling methods remains their stereocontrol, which might diminish the yield. With the upcoming advances in asymmetric synthesis and the commercial availability of chiral phase-transfer catalysts, the radiosynthesis has progressed as far as having natural enantiopure AAs and even small peptides in hand, with reasonable synthesis steps and reaction time.

Ensuring appropriate systems at hospitals, \(^{11}\)C-labeled biologically active compounds have versatile functions in physiology and are thereof ideal for PET research. To improve diagnosis, \(^{11}\)C-labeled AAs could be used complimentary to other tracers such as \[^{11}\text{C}MET\] and \[^{18}\text{F}FDG\]. A combination of two PET scans in one day, firstly with a short-lived \(^{11}\)C-AAs followed by a \[^{18}\text{F}FDG\] scan, could serve as an additional tool for primary and metastatic brain tumors, peripheral tumors such as lymphoma, lung or breast tumors\[130\] or inflammation, as confirmed by \[^{11}\text{C}MET\]. This implicates that \(^{11}\)C-AAs tracers could be used in parallel since they are potentially more suitable than \[^{18}\text{F}FDG\] for differentiation of tumors and inflammation. However, as limited to the PET centers with an in-house facility and cyclotron, due to the short half-life of carbon-11, the \(^{18}\text{F}\)-AAs might be more appropriate for distant shipment or longer scanning times. This can be seen in various developments in the field where \(^{18}\text{F}\)-AAs are developed and successfully applied as well. A concern in general when using fluorine-18 is defluorination that has also been reported for the \(^{18}\text{F}\)-AAs as well.\[131,132\]
With respect to imaging with $^{11}$C-labeled peptides further in-depth research is required. Nevertheless, in recent years, development in biotechnology and its applications has lead towards combined and improved mechanistic possibilities in peptide-based radiopharmaceutical as molecular imaging agents. Even more, the recent achievements furnished various methodologies to label peptides with carbon-11 which are now ready to be applied to biologically relevant peptides. This will only boost future application of peptides in imaging. It should be noted that a handful of radiosynthetic options have been developed, but the preclinical and clinical applications are running behind. Furthermore, despite the significant advances in the field of radiosynthesis there is a continuous need for discovery of unprecedented molecular targets, such as peptide receptors overexpressed on common human cancers, and development of their appropriate imaging agents.
Notes and List of Abbreviations: ¥

DABCO: (1,4-diazabicyclo[2.2.2]octane;

BMI: (R)- or (S)-Boc-2-tert-butyl-3-methyl-1,3-imidazolidin-4-one

BPB: (S)-N-(2-benzoylphenyl)-1-benzylpyrrolidine-2-carboxamide

DMPU: 1,3-Dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone

MeCN: Acetonitrile

Al₂O₃: Aluminium oxide

CsCl: Cesium chloride

CsOH·H₂O: Cesium hydroxide monohydrate

PhosBrett: 2-(Dicyclohexylphosphino)3,6-dimethoxy-2’,4’,6’-triisopropyl-1,1’-biphenyl

DCM: Dichloromethane

DMF: Dimethylformamide

DMSO: Dimethylsulfoxide

Ee: Enantiomeric Excess

EtOAc: Ethylacetate

FDA: Food and Drug Administration

GC: Gas Chromatography

HPLC: High pressure liquid chromatography

HI: Hydroiodic acid

TMP-lithium: Lithium 2,2,6,6-tetramethylpiperidide

LAH: Lithium aluminium hydride

MeOH: Methanol

CH₃I: Methyl iodide
n-BuLi: N-butyl lithium
K₂CO₃: Potassium carbonate
KF: Potassium fluoride
KOH: Potassium hydroxide
RCC: Radiochemical yield, determined by analytical HPLC of the crude reaction mixture, defined as radiochemical conversion. In some of the older papers there was not a clear statement between isolated and non-isolated radiochemical yield.
RCP: Radiochemical Purity
RCY: Radiochemical Yield
s-BuLi: Sec-butyl lithium
SMTH: Serine hydroxymethyltransferase
NaOH: Sodium hydroxide
𝑡-ButOH: tert-Butanol
TBAF: Tetrabutylammonium formamide
THF: Tetrahydrofuran
TMP: Trimethylolpropane
P(o-tolyl)₃: Tri(o-tolyl)phosphine
Pd₂(dba)₃: Tris(dibenzyldieneacetone)dipalladium(0)
References


Chapter 3

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-[\textsuperscript{11}C]\textsuperscript{alanine} is described employing an enantioselective alkylation of a Schiff base glycine precursor with [\textsuperscript{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-[\textsuperscript{11}C]\textsuperscript{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.
Introduction

Positron Emission Tomography (PET)\textsuperscript{11} is a non-invasive technique, often applied as diagnostic tool in today’s healthcare, that allows the visualization of cellular processes \textit{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.\textsuperscript{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-[\textsuperscript{18}F]fluoro-2-deoxy-D-glucose (FDG).\textsuperscript{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.\textsuperscript{6,7} Various studies have shown that amino acid transporters are elevated on tumor tissue.\textsuperscript{8} Making use of this knowledge, important tracers like [\textsuperscript{11}C]methionine\textsuperscript{9} and O-(2-[\textsuperscript{18}F]fluoroethyl)-L-tyrosine\textsuperscript{10} are used routinely in clinical settings for tumor diagnosis. Furthermore, multiple amino acids and amino acid analogs, e.g. [\textsuperscript{11}C]glutamine\textsuperscript{11} or derivatives [\textsuperscript{18}F](2S,4S)-4-(3-Fluoropropyl)glutamine\textsuperscript{12} and 3-(1-[\textsuperscript{18}F]fluoromethyl)-L-alanine\textsuperscript{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-[\textsuperscript{11}C]DOPA\textsuperscript{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 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 alkylation 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 alkylation 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 \(\text{L}^{-11}\text{C}\)alanine has first been reported in the late 1970’s when Långström \textit{et al}. described a 48% ee yield of \(\text{L}^{-11}\text{C}\)alanine utilizing an asymmetric synthesis procedure.\(^{[22]}\) Nevertheless, it took more than 10 years to develop a synthesis that yielded 80% ee of \(\text{L}^{-11}\text{C}\)alanine.\(^{[23,24]}\) Alternatively other strategies have emerged as well, which make use of Nickel-complexes and upon varying the alkylation agent, many amino acids are possible.\(^{[25],[26,27]}\) 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 \(\text{L}^{-11}\text{C}\)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 \(\text{L}^{-11}\text{C}\)alanine by an enantioselective alkylation of a Schiff base glycine precursor with \(\text{L}^{-11}\text{C}\)methyl iodide (\(\text{L}^{-11}\text{C}\text{MeI}\)) 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 \(\text{L}^{-11}\text{C}\text{MeI}\) as our first alkylation agent. With more sophisticated alkylation 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$\text{alanine}$, should allow the synthesis of radiolabeled peptides with carbon-11 as PET tracers.

![Scheme 1: Proposed mechanism of an asymmetric alkylation of a Schiff’s base for amino acid synthesis.]

**Results and Discussion**

Initial focus of this study was the radiosynthesis of racemic D/L-$^{[11]}$C$\text{alanine}$ 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$\text{alanine}$, glycine derivative 1 was used, which was modified as a Schiff base at the N-terminus as a biphenyl imine to activate the $\alpha$-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$\text{MeI}$ 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$\text{alanine}$ 3 was separated and allowed the calculation of the enantiomeric excess of the final product.

To explore the reactivity of $^{[11]}$C$\text{MeI}$ 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, 1M in THF) as a base, $^{[11]}$C$\text{MeI}$ was added to the reaction mixture by direct distillation. Alkylation of 1 with $^{[11]}$C$\text{MeI}$ according to the published procedure was successful and alkylation yields exceeded 80% (figure 1B). Deprotection of alkylated intermediate 2 was to yield D/L-$^{[11]}$C$\text{alanine}$ 3, proved to be straight forward and high yielding when 6M 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 $[^{11}\text{C}]$alanine (Figure 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 to deprotonate 1 and perform alkylation reactions of Schiff bases with $[^{11}\text{C}]$MeI and synthesize $[^{11}\text{C}]$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·$\text{H}_2\text{O}$ as a solid base for the alkylation of 1 with $[^{11}\text{C}]$MeI. An explanation for the low reactivity of precursor 1 towards $[^{11}\text{C}]$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 analyzed by HPLC in this study (Figure 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 $[^{11}\text{C}]$MeI proved to be successful. This can be explained by the stoichiometry of Schiff base 1 to $[^{11}\text{C}]$MeI in radiochemical alkylation reactions. $[^{11}\text{C}]$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.
Figure 1: (A) Radiochemical synthesis of $[^{11}\text{C}]$alanine by alkylation of precursor 1 with $[^{11}\text{C}]$MeI and its acidic deprotection; (B) HPLC profiles of the UV and radioactive signal of the crude alkylation mixture of 1 with $[^{11}\text{C}]$MeI; (C) Analysis of deprotected D/L-$[^{11}\text{C}]$alanine by chiral HPLC.

Chiral alkylation reactions with phase-transfer catalyst 4

These encouraging initial results formed the basis to move forward to the asymmetric synthesis of $[^{11}\text{C}]$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-[11C]alanine and the first set of experiments concentrated on phase-transfer catalyst 4 (Figure 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 (Figure 2). The reactions were performed at 0-10 ºC for 5-10 min with generally 7 µmol of precursor 1 and 10 mol% of catalyst.

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

Next to TBAF as base, many studies in organic chemistry have performed enantioselective alkylations with alkali metal hydroxides aqueous solution as base.\textsuperscript{[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 [11C]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 (Figure 3) and discovered that the application of PTC 4 induced the chiral alkylation of Schiff bases with $^{[11]}$CMeI 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, $^{[11]}$CMeOTf ($^{[11]}$Cmethyl trifluoromethanesulfonate) instead of $^{[11]}$CMeI, was investigated to enhance the alkylation reaction. Unfortunately, only low conversions were observed and $^{[11]}$CMeOTf was not further used as alkylation reagent in this study. To increase the reaction yields and maintain the high ee’s of the reactions (Figure 3A), mixtures of aqueous CsOH solution and organic bases like TBAF, TBAOH (Tetramethylammonium hydroxide) 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 1M and 10M concentrations of Cesium carbonate were evaluated for the alkylation reaction, but no conversion was observed in any of the attempts. Furthermore, semi-organic base 1M aqueous Cesium acetate was used in the alkylation, however, no reaction between 1 and $^{[11]}$CMeI was observed. Presumably this is due to lower basicity of the used Cesium salts compared to CsOH·H$_2$O.

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 (Figure 3B). Furthermore, the stereoselectivity of the alkylation reaction was influenced dramatically when using CsOH solid base and the ee of the obtained $^{[11]}$C alanine was increased to over 80% when using 40 equivalents of CsOH (Figure 3C). A drawback, of using CsOH as a solid base, is the hygroscopicity and its commercial availability only as CsOH·H$_2$O. Therefore, one other alkali metal that is less hygroscopic than CsOH hydroxide was investigated.$^{[17, 20]}$ Figure 3B depicts the results for various concentrations of solid KOH in comparison to CsOH, when the reactions were performed at 5 °C.
Figure 3: (A) Enantiomeric excess of L-[\textsuperscript{11}C]alanine 3 with different concentrations of aqueous CsOH solutions. (B) HPLC conversions for the alkylation reaction of Schiff base 1 with \textsuperscript{[11]C}MeI in the presence of different bases. (C) Enantiomeric excess of L-[\textsuperscript{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-[\textsuperscript{11}C]alanine by chiral HPLC after deprotection of the enantiomeric product.

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\textsuperscript{*}H\textsubscript{2}O was azeotropically dried with MeCN beforehand. Unfortunately, the use of azeotropically dried CsOH resulted in a
reduction in ee ratios of the obtained $[^{11}\text{C}]$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 ($\sim$20 eq) was used, however other describe mixtures of toluene/dichloromethane$^{[33]}$ resulting in an improvement in both conversion yield and ee of the obtained product (Figure 3). 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-$[^{11}\text{C}]$alanine.

**Screening of multiple phase-transfer catalysts**

With the currently available optimal conditions of the chiral synthesis of L-$[^{11}\text{C}]$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 (Figure 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-$[^{11}\text{C}]$alanine and catalyst 6 which should, because of its bulkier character, improve the ee of the reaction (Figure 2A).

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

<table>
<thead>
<tr>
<th>Entry</th>
<th>Applied PTC</th>
<th>Alkylation conversion (%)</th>
<th>Ee of [11C]alanine (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>96.9 ± 1.1</td>
<td>88.2 ± 2.0</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>47.5 ± 9.4</td>
<td>58.3 ± 9.1 D-ala</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>6.6 ± 0.5</td>
<td>80.6 ± 8.0</td>
</tr>
<tr>
<td>4</td>
<td>7</td>
<td>Trace</td>
<td>ND</td>
</tr>
<tr>
<td>5</td>
<td>8</td>
<td>72.7 ± 5.5</td>
<td>90.4 ± 2.7</td>
</tr>
<tr>
<td>6</td>
<td>9</td>
<td>73.8 ± 9.8</td>
<td>68.2 ± 3.2</td>
</tr>
<tr>
<td>7</td>
<td>10</td>
<td>59.7 ± 27.1</td>
<td>52.5 ± 11.3</td>
</tr>
</tbody>
</table>

PTC 5, which is the stereoisomer of PTC 4 should yield D-[11C]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 an 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, 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-[11C]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 equivalents compared to the precursor. Using these conditions the conversion of the alkylation reaction of precursor 1 with [11C]MeI was improved to 97 %, but the ee of the obtained product, D-[11C]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 [11C]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 to 40 equivalents 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 [11C]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 alkylation transition states of cinchonidinium-derived catalysts, which provides us with L-1-[11C]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.\textsuperscript{[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 $^{[11]}\text{C}\text{MeI}$ 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 $^{[11]}\text{C}\text{alanine}$ 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 equivalents of CsOH as base and 0.1 equivalent of PTC 4, respectively.

To conclude, L-$^{[11]}\text{C}\text{alanine}$ 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$\mu$mol$^{-1}$ at the end of the synthesis (EOS) and the highest ee achieved was $>90 \%$. Both chemical and radiochemical purities were $>95 \%$.

**Conclusion**

We have synthesized L-$^{[11]}\text{C}\text{alanine}$ via a new, general applicable, radiochemistry method utilizing a PTC catalyzed enantioselective alkylation with $^{[11]}\text{C}\text{MeI}$, 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.
Materials and Methods

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. ¹H and ¹³C nuclear magnetic resonance (NMR) spectra were obtained using a Bruker AC 250.13 and AC 400.13 (Billerica, USA) and chemical shifts (δ) were defined relative to the signal of the solvent (7.27 ppm for CDCl₃, 3.31 ppm for MeOD, 2.50 ppm for DMSO-d₆) 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, 4.6x250 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; 4.6x250 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 [¹¹C]MeI synthesis was performed before every radiochemical reactions on an in-house built synthesis device and according to procedures described in literature.
Experimental Section

Synthesis of reference compounds and non-commercially available PTCs

\(L-N\)-(diphenyl)alanine \textit{tert}-butyl ester (2a): \(L\)-alanine \textit{tert}-butyl ester (300.0 mg, 2.1 mmol) was suspended in dichloromethane (4 mL) and treated with benzophenone imine (381.0 \(\mu\)L, 1.8 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 \textit{tert}-butyl ester as a white solid (yield: 472.0 mg, 1.6 mmol, 77 %).

\(^1\)H-NMR (250.13 MHz, CDCl\(_3\)) \(\delta\) 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 (100.62 MHz, CDCl\(_3\)) \(\delta\) 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\(_{22}\)NO\(_2\): 310.1807 ([M+H]\(^+\)), found 310.1797 ([M+H]\(^+\)).

\(D-N\)-(diphenyl)alanine \textit{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 365.0 mg (1.2 mmol, 60 %) as colorless oil.

\(^1\)H-NMR (400.13 MHz, CDCl\(_3\)) \(\delta\) 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 (100.62 MHz, CDCl\(_3\)) \(\delta\) 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\(_{22}\)NO\(_2\): 310.1807 ([M+H]\(^+\)), found 310.1812 ([M+H]\(^+\)).

\(\alpha,\alpha\)'-Biscinchonidinium-\(m\)-xylene dibromide (pre-PTC 9\(^*\)): The synthesis was performed according to described literature procedures.\(^{[37]}\) (-)-Cinchonidine (2.0 g, 6.8 mmol) and dibromo-\(m\)-xylene (0.9 g, 3.3 mmol) were dissolved in EtOH (5 mL), DMF (6 mL) and chloroform (2 mL) and refluxed at 100 ºC for 4 hours. 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 (3.4 g, 4.9 mmol).

\(^1\)H-NMR (400.13 MHz, DMSO-d\(_6\)) \(\delta\) 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), 4.90 (s, 2H), 4.50-4.35 (m, 4H), 2.80-2.65 (m, 3H), 2.50-2.35 (m, 3H), 2.10 (s, 3H), 1.85-1.70 (m, 3H), 1.70-1.50 (m, 3H), 1.50-1.30 (m, 3H), 1.30-1.10 (m, 3H), 1.10-0.90 (m, 3H), 0.90-0.70 (m, 3H), 0.70-0.50 (m, 3H), 0.50-0.30 (m, 3H), 0.30-0.10 (m, 3H), 0.10-0.00 (m, 3H) ppm; \(^{13}\)C-NMR (100.62 MHz, DMSO-d\(_6\)) \(\delta\) 208.5, 198.7, 192.9, 188.0, 184.2, 180.8, 176.7, 172.6, 168.5, 164.2, 160.5, 156.8, 153.1, 149.8, 146.1, 142.4, 138.7, 135.0, 131.2, 127.5, 124.8, 122.1, 119.4, 116.7, 113.9, 111.1, 108.3, 105.6, 102.9, 99.2, 96.5, 92.8, 90.1, 87.4, 84.7, 82.0, 79.3, 76.6, 74.9, 72.2, 70.5, 68.8, 67.1, 65.4, 63.7, 62.0, 60.2, 58.5, 56.8, 55.1, 53.4, 51.7, 50.0, 48.3, 46.6, 45.0, 43.3, 41.6, 39.9, 38.2, 36.5, 34.8, 33.2, 31.5, 30.8, 29.1, 27.4, 25.7, 24.0, 22.3 ppm; HRMS (ESI) calculated C\(_{18}\)H\(_{18}\)Br\(_2\)NO\(_2\): 405.0335 ([M+H]\(^+\)), found 405.0364 ([M+H]\(^+\)).
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 (100.62 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$_4$O$_2$: 346.7084 ([M+H]$^{2+}$), found 346.2017 ([M+H]$^{2+}$).

**α,α’-Bis[O(9)-allylcinchonidinium]-m-xylene dibromide (PTC 9):** α,α’-Biscinchonidinium-m-xylene dibromide 9* (300.0 mg, 0.4 mmol) was suspended in dichloromethane (5 mL) and allyl bromide (1.0 mL, 11.6 mmol) and 50 % aqueous KOH (2.0 mL, 17.6 mmol) were added at room temperature. The suspension was stirred vigorously for 3 hours 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 305.0 mg of 9 as orange solid (0.4 mmol, 91 %).

$^1$H-NMR (400.13 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 (100.62 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$_4$O$_2$: 386.7379 ([M+H]$^{2+}$), found 386.2387 ([M+H]$^{2+}$).
RADIOCHEMISTRY

Radiochemical procedure for the production of $^{[11]}$CMeI

Cyclotron produced $^{[11]}$CO$_2$ was carried in a stream of helium and trapped in 0.1 mL of a 0.1M lithium aluminium hydride solution in THF in a glass reaction vessel at room temperature. After trapping, the gas flow was increased to 20 mL$\cdot$min$^{-1}$ and the THF evaporated at 130 °C. After evaporation to dryness 0.2 mL of 56 % hydriodic acid was added the $^{[11]}$CMeI was distilled from the reaction vessel under a stream of helium (flow 20 mL$\cdot$min$^{-1}$) to the second reaction vessel for the alkylation reaction.$^{[40]}$

Alkylation procedure of Schiff base 1

$^{[11]}$CMeI is distilled in 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, $^{[11]}$CMeI was trapped in the second reaction vessel prior to heating or cooling of the reaction mixture. At set time points, samples were taken from the reaction mixture for analysis by radioHPLC to determine the alkylation conversion rates. For deprotection, 0.1 mL of 6M 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-$^{[11]}$Calanine.

Optimized procedure for the alkylation with $^{[11]}$CMeI to obtain L-$^{[11]}$Calanine

In a reaction vessel, Schiff base 1 (1.9 mg, 7.0 μmol), catalyst 4 (0.5 mg, 0.8 μmol) and CsOH$\cdot$H$_2$O (30.0 mg, 200.0 μmol) are suspended in a mixture of toluene/dichloromethane (300 μL, 19:1, v/v) and the color changes instantly to yellow. After distilling $^{[11]}$CMeI 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 ($R_t$) of 8.3 min for product 2. The deprotection is initialized by the addition of 0.1 mL of 6M 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-$^{[11]}$Calanine with HPLC column Reprosil chiral-aa (methanol/water 70/30, v/v) with a $R_t$ of 4.5 min for L-$^{[11]}$Calanine and 10.3 min for D-$^{[11]}$Calanine.
References


1149.


Chapter 4

Stereocontrolled $^{11}$C-Alkylation of $N$-terminal Glycine Schiff Bases to Obtain Dipeptides

Ulrike Filp§, Aleksandra Pekošak§, Alex J. Poot, Albert D. Windhorst


§: Both authors contributed equally to this work.
ABSTRACT

The use of various quaternary ammonium salts as chiral phase-transfer catalysts allows the effective and stereoselective radiochemical $^{11}$C-alkylation to obtain functionalized dipeptides. The here reported asymmetric $^{11}$C-alkylation of dipeptides allows a broadly applicable procedure to obtain labeled N-terminal peptides using different $^{11}$C-alkyl halides. Contended stereoselectivities of the reactions were observed using $^{11}$C-labeled alkyl halides, [$^{11}$C]methyl iodide and [$^{11}$C]benzyl iodide, achieving diastereomeric ratio with different specialized catalyst of 95:5 and 90:10, respectively. Accordingly, straightforward synthesis of enantioenriched compounds should play a vital role in peptide based radiopharmaceutical development and PET imaging.
Introduction

Positron emission tomography\textsuperscript{[1]} (PET) is a non-invasive imaging technique enabled by the administration of trace amounts of biochemically active compounds labeled with positron-emitting radioisotope. In recent years, PET has proven itself in disease diagnosis, drug efficacy testing and treatment monitoring.\textsuperscript{[2,3]} The presence of carbon in all biological and organic compounds makes positron emitting isotope carbon-11 (100 % β+, \( E_{\text{max}}=0.96 \text{ MeV, } t_{1/2}=20.4 \text{ min} \))\textsuperscript{[4]} an attractive radionuclide for PET tracer development. In theory, carbon-11 can be incorporated in all molecules of biological interest without changing the lead structure. For native peptide-based PET tracers however, carbon-11 remains almost unexplored compared to well established approaches like radiometal chelation (e.g. gallium-68) or the addition of a prosthetic group (e.g. 4-[\( ^{18} \text{F} \)]fluorobenzaldehyde). These strategies of peptide radiolabeling lead to molecular changes resulting in different physico-chemical properties compared to the lead peptide. As a result, clinical translation of these peptides requires additional research for the validation of the tracer, which delays the development of peptide based PET tracers.\textsuperscript{[5–7]}

Recently, more progress has been made in the radiolabeling of peptides with carbon-1.1\textsuperscript{[8–10]} In addition, the high molar activity, low radiation dose to the human subject and the option of doing multiple scans per day, offers unique possibilities of carbon-11 labeled peptides as PET tracers. Nevertheless, carbon-11 has its challenges, mainly caused by its short half-live, meaning that reactions and work-up procedures must be fast (typically minutes) with a complete synthesis time of 60 minutes including purification and formulation. With respect to reaction kinetics, an advantage of the high molar activity is that the carbon-11 labeled reagent is typically present in low nanomolar amounts whereas other reagents are present in micromolar amounts. This distorted stoichiometry leads to fast reactions, since radiochemical yields are based only upon the carbon-11 labeled reagent.

Asymmetric alkylation of Schiff base glycine derivatives, utilizing phase-transfer catalysts (PTC), is a highly efficient and generally applicable method to synthesize natural and unnatural amino acids under mild reaction conditions.\textsuperscript{[11]} Nevertheless, a careful selection of reaction conditions and catalysts needs to be performed based upon the peptidic backbone.

We previously reported on the successful asymmetric synthesis of \([^{11}\text{C}]\)alanine\textsuperscript{[12]}, \([^{11}\text{C}]\)phenylalanine\textsuperscript{[13]}, and two small \(^{11}\text{C}\)-peptides\textsuperscript{[10]} applying this methodology. We postulate that the stereoselective synthesis of dipeptides will have implications in the development of
asymmetric chemical transformations in the radiosynthesis of functionalized small peptides (Figure 1A) to be used as PET tracers. In this study the aim was to investigate the influence of the peptidic backbone and catalyst induction on stereoselectivity. To achieve this, mild PTC conditions were transferred towards various dipeptides with two different alkylating agents, $^{[1]}$Cmethyl iodide ($[^{11}C]$MeI) and $^{[1]}$Cbenzyl iodide$^{[15]}$ ($[^{11}C]$BnI), to evaluate efficacy and chiral induction of direct alkylation. Generally applicable conditions were identified by screening and optimizing the reagent conditions with the amount of precursor and base, the catalyst, reaction time and temperature to obtain high diastereomeric ratios (dr) and radiochemical conversion yields.

**Results and Discussion**

The first aim here was to optimize the concentration of the Schiff base precursor for $^{11}$C-alkylation reactions. Due to higher reactivity of $[^{11}C]$MeI only 30-40 mM of precursor were necessary, whereas for $[^{11}C]$benzylations concentrations between 50-60 mM were required. With the acquired knowledge, protected $^{11}$C-dipeptides as alkylated product were successfully obtained in high radiochemical yields, determined by analytical HPLC and expressed as radiochemical conversion (RCC). The results were also dependent upon the amount of base - CsOH·H$_2$O, which was assessed to be optimal between 80-110 equivalents compared to precursor. This excess provides adequate effective surface area for phase-transfer alkylations. To attain sufficient exchange between solid-liquid system$^{[16]}$ efficient stirring was of paramount importance. The vigorous mixing of the reaction mixture was achieved with a helium flow of 20-50 mL·min$^{-1}$ which enlarges the interfacial area, as experiments without vigorous reagent mixing showed little to no product formation. From experience, solvent toluene was optimal.$^{[17]}$ Next, the reaction has been set to 5 min with satisfactory RCC of >75 % overall. Finally, Figure 1C illustrates that the optimized conditions yielded product for all mentioned dipeptides in excellent RCC, exemplified by a representative radiochromatogram in Figure 1B.
Figure 1: (A) Radiochemical synthesis scheme by alkylation of precursor 1-8 with $[^{11}\text{C}]$MeI or $[^{11}\text{C}]$BnI; (B) HPLC profiles of the UV and radioactive signal of the crude alkylation mixture of $[^{11}\text{C}]$9; (C) Radiochemical yield of the crude reaction mixture, determined by analytical HPLC and defined as radiochemical conversion (RCC) of asymmetric alkylation to compound $[^{11}\text{C}]$9-20.

In general, $[^{11}\text{C}]$methylation reactions that were performed at 10 °C had slightly higher outcome than 0 °C, while for the $[^{11}\text{C}]$benzylation reactions 0 °C proved to be favorable.$^{[10]}$ We
also observed that the adjacent amino acid (1-2 compared to 3-4) has no influence on the conversion to racemic $^{11}$C-dipeptides.

Gratified by the excellent conversion rates, the stereoselectivity of the $^{11}$C-alkylations was examined. Table 1 presents the results of the analysis of alkylation reactions comparing the situation without catalyst and with an achiral catalyst TBAB (tetrabutyl ammonium bromide). The results are high-yielding and RCCs are comparable with results employing CsOH·H$_2$O as deprotonating agent and base. Hereby, the application of a catalyst was confirmed not to influence the RCC. Remarkably, the influence of the second amino acid was larger in the reactions with $[^{11}$C]$\text{MeI}$, resulting in a ratio of approx. 60:40 (Table 1, Entry 1-4), whereas in the reactions with $[^{11}$C]$\text{BnI}$ the outcome was 50:50 (Table 1, Entry 5-8). Without catalyst, we assume the dipeptides are in a slightly bended position in the apolar organic medium, therefore favoring one diastereomer, whereas the bulkiness of phenyl group imparts a stronger effect resulting in a racemic mixture. The influence of TBAB is clearly shown in decreasing the ratio for $[^{11}$C]$\text{MeI}$ due to ion-stabilization of Schiff base enolate, whilst for $[^{11}$C]$\text{BnI}$ no influence on the dr is observed.

The next challenge was to achieve high dr making use of commercially available chiral phase-transfer catalysts. The reaction mixtures of the protected dipeptides 9-10 and 15-16 could not be separated efficiently on C$_{18}$ reversed-phase or normal-phase HPLC. However, the separation with dipeptides 11-14 and 17-20 was successful on RP-C$_{18}$, thus our efforts focused on achieving the best dr for these dipeptides with the catalysts specified in Figure 2. In all likelihood, the diastereomeric separation is possible due to the configuration of the second amino acid, which enables stronger van der Waals bonds of the sterically more hindered dipeptide to the C$_{18}$ column. Henceforth, we moved on with precursor 3 and counterpart 7, as well as 4 and 8, to be able to examine the backbone influence in particular.
Table 1: Diasteriomeric ratio of $^{11}$C-dipeptides without catalyst and with an achiral phase-transfer catalyst TBAB.

<table>
<thead>
<tr>
<th>#*[a]</th>
<th>$^{11}$C-Product</th>
<th>No Cat*[b,c]</th>
<th>TBAB*[b,c]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>11 Ala-L-Val</td>
<td>60:40 (DL:LL)</td>
<td>n.d.[d]</td>
</tr>
<tr>
<td>2</td>
<td>13 Ala-D-Val</td>
<td>60:40 (LD:DD)</td>
<td>n.d.</td>
</tr>
<tr>
<td>5</td>
<td>17 Phe-L-Val</td>
<td>54:46 (DL:LL)</td>
<td>n.d.</td>
</tr>
<tr>
<td>7</td>
<td>18 Phe-L-Leu</td>
<td>50:50 (DL:LL)</td>
<td>52:48 (DL:LL)</td>
</tr>
<tr>
<td>8</td>
<td>20 Phe-D-Leu</td>
<td>51:49 (LD:DD)</td>
<td>56:44 (LD:DD)</td>
</tr>
</tbody>
</table>

[a]Reactions performed with 40-60 mM of precursor 3-4, 7-8 and 80-110 eq CsOH·H$_2$O compared to precursor at 0-10 ºC for 5 min; [b]40-60 mM TBAB; [c]Reported as dr; [d]n.d. = not determined.

In organic asymmetric synthesis lower temperatures (-40 to 0 ºC) are preferred to obtain better dr of the product, however considering the short half-life of carbon-11, ambient temperatures are preferable for reaction kinetics to accomplish higher conversion rates. Indeed, the temperature had significant influence on the dr as shown in Table 2 (Entry 3-5), while maintaining slightly lower but acceptable conversion rates (Table 2, Entry 1-2; 3-5). Surprisingly, the lower temperature reduced the dr negatively presumably due to the enolate-catalyst complex not forming properly in distorted radiochemistry conditions and also due to less time to react. This unexpected result has also been observed in other organic chemistry experiments implementing a derivative of Cat 4.[18] Furthermore, the amount of catalyst (Table 2, Entry 6-8) used in the reactions hardly influenced the diastereoselectivity of the formed product.
For the asymmetric alkylation reactions further on, the amount of catalyst used ranged from 0.5 to 1 eq, compared to the amount of Schiff base precursor. The first set of experiments was performed with Schiff base glycine-L/D-valine tert-butylester 3/7 yielding L/D-alanine-L/D-valine tert-butylester 11/13 and L/D-phenylalanine-L/D-valine tert-butylester 17/19 with results summarized in Table 3. The use of Cinchona-based⁴ pseudo-enantiomers Cat 1 and Cat 2, which were most promising for [¹¹C]BnI (Table 3, Entry 3-4), showed less selective results for [¹¹C]MeI. The yield and dr of the obtained product 15 was clearly dependent on the starting material and catalyst used, which can be called a match and mismatch situation for precursor and catalyst (Table 3, Entry 3-4). We have also observed a substantial improvement in stereochemical control with Cat 5 – Park’s two cinchona alkaloid unit catalyst⁴ (Table 2, Entry 6-8) compared to Cat 1 – a single unit catalyst in the [¹¹C]benzylation reaction. They have both resulted in more LL-product with Gly-L-AA Schiff base precursors. As shown in Table 3 (Entry 1-2, Cat 1 and 2), the outcome in [¹¹C]methylation reactions with Cinchona catalysts were leading to lower dr, henceforth the use of Cat 5 was abandoned. Closer inspection of Table 3 specifically Maruoka’s first generation catalysts⁵, Cat 3 and 4, which performed outstandingly in the synthesis of amino acids [¹¹C]alanine and [¹¹C]phenylalanine, unfortunately yielded unfavorable results for the diastereoselective synthesis of [¹¹C]alanine-based dipeptides (Table 3, Entry 1-2) and were

<table>
<thead>
<tr>
<th>#^[a]</th>
<th>¹¹C-RX</th>
<th>Cat</th>
<th>Eq Cat^[b]</th>
<th>Temp (°C)</th>
<th>DL:LL</th>
<th>RCC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>[¹¹C]MeI</td>
<td>1</td>
<td>1</td>
<td>10</td>
<td>42:52</td>
<td>94</td>
</tr>
<tr>
<td>2</td>
<td>[¹¹C]MeI</td>
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<td>1</td>
<td>-10</td>
<td>40:60</td>
<td>35</td>
</tr>
<tr>
<td>3</td>
<td>[¹¹C]BnI</td>
<td>2</td>
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<td>0</td>
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<td>97</td>
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<tr>
<td>4</td>
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<td>2</td>
<td>1</td>
<td>-20</td>
<td>62:38</td>
<td>62</td>
</tr>
<tr>
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<td>[¹¹C]BnI</td>
<td>2</td>
<td>1</td>
<td>-40</td>
<td>50:50</td>
<td>32</td>
</tr>
<tr>
<td>6</td>
<td>[¹¹C]BnI</td>
<td>3</td>
<td>0.5</td>
<td>0</td>
<td>28:72</td>
<td>82</td>
</tr>
<tr>
<td>7</td>
<td>[¹¹C]BnI</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>23:77</td>
<td>97</td>
</tr>
<tr>
<td>8</td>
<td>[¹¹C]BnI</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>27:73</td>
<td>79</td>
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</tbody>
</table>

[a]Reactions performed with 40-60 mM of precursor 4 and 80-110 eq of CsOH·H₂O for 5 min; [b]Compared to 4; Entry 1-2 yielding product 12 and Entry 3-8 yielding product 18; in general: DL+LL=100.
therefore discarded for the use with $^{[11\text{C}]}\text{BnI}$. The tartrate-derived \textbf{Cat 6} and \textbf{Cat 7}, (TaDiAs) published by Shibuguchi \textit{et al.}\textsuperscript{[21,22]} did not result in a decent stereoselective control, however gave different ratios compared to no catalyst (Table 3, Entry 1-2) and as these were insignificant differences, the use with $^{[11\text{C}]}\text{BnI}$ was discarded. Likewise, \textbf{Cat 4} and \textbf{Cat 5} did not increase the diastereoselectivity of the reaction and were thus not further investigated during this study.

\textbf{Figure 2:} Chiral phase-transfer catalysts explored for the radiosynthesis of $^{11}\text{C}$-dipeptides with (A) Cinchona-based catalysts; (B) Maruoka’s quaternary ammonium based and (C) tartrate-derived catalysts.
Table 3: Diastereoselective N-terminal $^{11}$C-alkylation with precursor 3 and 7.

<table>
<thead>
<tr>
<th>#</th>
<th>$^{11}$C-Product</th>
<th>Cat 1</th>
<th>Cat 2</th>
<th>Cat 3</th>
<th>Cat 4</th>
<th>Cat 6</th>
<th>Cat 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11 Ala-L-Val</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>13 Ala-D-Val</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>17 Phe-L-Val</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>19 Phe-D-Val</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>(LD:DD)</td>
<td>77:23</td>
<td>22:78</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$[^a]$Reactions performed with 40-60 mM of 3 or 7 with 80-110 eq of CsOH·H$_2$O, compared to precursor at 0-10 °C for 5 min; in general: DL+LL=100.

To differentiate the results for the $^{11}$C]methyllations on a precursor with a bigger adjacent side chain, namely leucine, best results were obtained for precursor 4 with Cat 1 summarized in Table 4 (Entry 1). In contrast, the best result for $^{11}$C]benzylation reactions were achieved with Cat 2, also here we observed a match/mismatch situation with Cat 1 (Table 4, Entry 3). A significant difference is clearly obtained with Cat 2 and the two chiral precursors 4 and 8. Here, depending upon which chiral precursor is preferred, the favored catalyst can be successfully employed. Here as well, the bigger sidechain did not yield better dr with the other catalysts employed.
Table 4. Diastereoselective N-terminal $^{11}$C-alkylation with precursor 4 and 8.

<table>
<thead>
<tr>
<th>#</th>
<th>$^{11}$C-Product</th>
<th>Cat 1</th>
<th>Cat 2</th>
<th>Cat 3</th>
<th>Cat 4</th>
<th>Cat 6</th>
<th>Cat 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12 Ala-L-Leu</td>
<td>22:78</td>
<td>61:39</td>
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<tr>
<td></td>
<td>(DL:LL)</td>
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<tr>
<td></td>
<td>(LD:DD)</td>
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<tr>
<td></td>
<td>(DL:LL)</td>
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<tr>
<td></td>
<td>(LD:DD)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

[a] Reactions performed with 40-60 mM of 4 or 8 with 80-110 eq CsOH·H$_2$O compared to precursor, at 0-10 °C for 5 min; in general: DL+LL=100.

Figure 3: (A) Diastereomeric ratio of Cat 8 with Schiff base precursor 4 to product 12 and 18; (B) Exemplified radioactive HPLC of a reaction with 4 to 18 using Cat 8; (C) Exemplified radioactive HPLC of a reaction with 8 to 20 using Cat 9; (D) Diastereomeric ratio of Cat 9 with Schiff base precursor 8 to product 14 and 20.
We have also successfully evaluated the use of Maruoka’s dedicated di- and tetrapeptide catalysts for alkylation,\cite{23,24} **Cat 8** and **Cat 9**, with results summarized in Figure 3. The (S,S) version, **Cat 8**, worked highly successful especially with precursor 4 yielding preferably DL-dipeptide (Figure 3A, 3C). Whereas for complexes of 4 with **Cat 9** the dr for methylated product 12 and benzylated product 18 was 50:50. In contrast, the formation of precursor-catalyst complex of 8 with (R,R)-**Cat 9** (see Figure 3D) is preferred to yield preferably LD-dipeptide, whereas with **Cat 8** it has the reversed outcome yielding for [11C]benzylation 31:69 and for [11C]methylation resulted in 52:48. Moreover, as outlined in Table 5 outstanding results have been obtained with [11C]MeI and 3 supporting our previous findings. To summarize, (S,S)-**Cat 8** yielded high diastereoselectivities with precursor 3 and 4, whereas with the (R,R)-**Cat 9** better results were obtained with D-dipeptide precursor 7 and 8. Furthermore, from these results we concluded that the configuration and confirmation of **Cat 8** is more favorable for a good outcome, which might be explained by the more bulky character of the catalyst with the two –CF₃ groups instead of single fluorine atoms (such as for **Cat 4, 5** and 9). Nonetheless, these catalysts are favorable for good enantioselectivities due to the strengthened internal hydrogen-bonding by the F atoms involving water which leads to more rigid conformations of catalysts.\cite{25,26} Furthermore, Kamachi *et al.* performed density functional theory calculations, which provided lowest energy transition structures that led to preferred products and is in accordance with our findings.\cite{27} We believe that the reason why bigger catalysts give slightly lower dr values for [11C]benzylation than for [11C]methylation is due to steric hindrance, which effectively penetrates the enolate–catalyst system.
Table 5: Diastereomeric ratio of reactions with glycine-D/L-valine Schiff base with $[^{11}\text{C}]\text{MeI}$.

<table>
<thead>
<tr>
<th>#(^{[a]})</th>
<th>$^{11}\text{C}$-Product</th>
<th>Cat 8</th>
<th>Cat 9</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ala-L-Val (DL:LL)</td>
<td>95:5</td>
<td>57:43</td>
</tr>
<tr>
<td>2</td>
<td>Ala-D-Val (LD:DD)</td>
<td>61:39</td>
<td>79:21</td>
</tr>
</tbody>
</table>

\(^{[a]}\)Reactions performed with 40-60 mM of 3 or 7 with 80-110 eq of CsOH·H$_2$O compared to precursor at 0-10 °C for 5 min; in general: DL+LL=100.

The acidic cleavage of obtained protected dipeptides is achieved conveniently. The obtained radioactive alkylated products have been quantitatively deprotected in short time with 6M HCl at elevated temperatures yielding the free $^{11}\text{C}$-dipeptide. The presented work was focused on the development of the radiochemistry method and the molar activity is dependent upon the starting alkylation agent and provided by previous studies determined to be $50^{[12]}$ to $153^{[13]}$ GBq µmol$^{-1}$ of tracer ready for biological studies. As soon as an authentic PET tracer candidate is achieved by implementing this methodology, the molar activity will be determined.

**Conclusion**

In conclusion, the best dr was obtained using Cat 8 with $[^{11}\text{C}]\text{MeI}$ and $[^{11}\text{C}]\text{BnI}$ resulting in 95:5 and 90:10 diastereomeric ratio with RCC of $> 80\%$, respectively. Hence, the herein presented radiosynthetic method enables a reliable and reproducible strategy to obtain pure diastereomerically enriched carbon-11 labeled dipeptides. This methodology provides a general and versatile procedure to be applied for peptide-based PET tracer development, stimulating their application and translation towards preclinical and potentially also clinical studies.
Materials and Methods

General

Free dipeptide reference materials were obtained from ABCR (Karlsruhe, Germany), Bachem (Bubendorf, Switzerland) and Sigma Aldrich (Zwijndrecht, The Netherlands). Starting material for dipeptide syntheses were obtained enantiomerically pure from ABCR and Sigma Aldrich. Catalysts were purchased from Sigma Aldrich 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 500.23, 400.13 or 250.13 MHz (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). NMRs were measured at ambient temperature. 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. 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 (Straubenhart, Germany) and SHIMADZU Prominence (Kyoto, Japan) operated with Labsolutions Version 5.85. 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 C18 (column A) (5 µm, 4.6x250 mm) with a mixture consisting of acetonitrile/4 mM sodium formate + 4 % DMF (buffer 1) (70/30, v/v) and Luna Phenomenex C18 (column B) (5 µm, 4.6x250 mm) with a gradient of 35 to 7 % buffer 1 in acetonitrile in 25 min or isocratic in 82/18 (acetonitrile/buffer 1, v/v) at a constant flow rate of 1 mL/min for the analysis of protected dipeptide references, UV monitoring at 254 nm. Enantiomeric purity of the free dipeptides was determined using an analytical Reprosil chiral-aa (column C) (8 µm; 4.6x250 mm) from Dr. Maisch GmbH (Ammerbuch, Germany) at 214 nm as well as Astec Chirobiotic T (column D) (5 µm; 4.6x250 mm) from Supelco (Sigma Aldrich, Zwijndrecht, The Netherlands). The product was eluted with methanol/water (70/30, v/v) (column C) and acetonitrile/water (65/35, v/v) for column D and all flow rates were 1 mL/min or as stated otherwise.
Radiochemical conversions are based on the AUC of the radioactivity profile of HPLC analysis. The $[^{11}\text{C}]\text{MeI}$ and $[^{11}\text{C}]\text{BnI}$ synthesis was performed before every radiochemical reaction on synthesis devices and according to procedures described in literature.

**Experimental Section**

**Synthesis of reference compounds**

**General procedure for the coupling of amino acids**

The coupling reaction of 2-(((benzyloxy)carbonyl)amino)propanoic acid (807.0 mg, 3.4 mmol) with tert-butyl 2-aminopropanoate (500.0 mg, 3.4 mmol) in the presence of BOP (1523 mg, 3.4 mmol) and DIPEA (1335.0 mg, 1.8 mL, 10.3 mmol) in DCM (10 mL) was finished in 4-16 hours. The solvent was removed under reduced pressure and the residue was dissolved in ethylacetate (25 mL). The ethylacetate solution was washed with 1M KHSO$_4$ (3x 20 mL), 10% Na$_2$CO$_3$ (3x 20 mL), and brine (1x 20 mL), dried with Na$_2$SO$_4$, filtrated and evaporated *in vacuo*. The crude product was purified by flash chromatography (2-30% ethylacetate in n-hexane), before it was analyzed by $^1\text{H}$-NMR, $^{13}\text{C}$-NMR and ESI-HRMS. In general the yield is around 65-80 % of a clear oil.

All the dipeptides references and precursor material have been analyzed and verified according to published procedures.$^{[28,29]}$

**(S)-tert-butyl 2-(((benzyloxy)carbonyl)amino)proponamido)propanoate**

$^1\text{H}$-NMR (250.13 MHz, CDCl$_3$) δ 7.35-7.31 (m, 5H), 5.05 (s, 2H), 4.36 (q, 1H, $J$=7.25 Hz), 4.16 (q, 1H, $J$=7.25 Hz), 1.39 (s, 9H), 1.22 (t, 6H, $J$=7.25 Hz) ppm; $^{13}\text{C}$-NMR (100.62 MHz, CDCl$_3$) δ 171.87, 171.54, 128.54, 128.18, 128.07, 82.13, 66.98, 48.73, 27.96, 18.52 ppm; HRMS (ESI) calculated for $\text{C}_{18}\text{H}_{28}\text{N}_2\text{O}_5$: 351.1920 ([M+H]$^+$), found 351.1854 ([M+H]$^+$), 373.1670 ([M+Na]$^+$).

**(S)-tert-butyl 2-((benzyloxy)carbonyl)amino)acetamido)propanoate**

$^1\text{H}$-NMR (250.13 MHz, CDCl$_3$) δ 7.34-7.29 (m, 5H), 5.07 (s, 2H), 4.39 (q, 1H, $J$=7.25 Hz), 1.39 (s, 9H), 1.22 (d, 3H, $J$=7.25 Hz) ppm; $^{13}\text{C}$-NMR (100.62 MHz, CDCl$_3$) δ 171.99, 171.82, 149.93, 136.15, 128.55, 128.22, 128.11, 82.25, 67.19, 48.68, 44.44, 27.96, 18.57 ppm; HRMS (ESI) calculated for $\text{C}_{17}\text{H}_{24}\text{N}_2\text{O}_5$: 337.1763 ([M+H]$^+$), found 337.1742 ([M+H]$^+$), 359.1572 ([M+Na]$^+$).
(S)-tert-butyl 2-((benzyloxy)carbonyl)amino)acetamido)-3-phenylpropanoate

1H-NMR (250.13 MHz, CDCl3) δ 7.39-7.37 (m, 5H), 7.17 (s, 3H), 7.07 (d, 2H, J=7.5 Hz), 5.06 (s, 2H), 4.73 (q, 1H, J=7.5 Hz), 3.82 (t, 2H, J=5 Hz), 3.03 (d, 2H, J=7.5 Hz), 1.33 (s, 9H) ppm; 13C-NMR (100.62 MHz, CDCl3) δ 182.28, 145.64, 135.84, 129.51, 129.57, 128.43, 128.26, 128.07, 82.64, 67.22, 53.46, 44.46, 38.01, 27.96 ppm; HRMS (ESI) calculated for C23H28N2O5: 413.2076 ([M+H]+), found 413.2065 ([M+H]+), 435.1886 ([M+Na]+).

(S)-tert-butyl 2-((S)-2-((benzyloxy)carbonyl)amino)propanamido)-3-phenylpropanoate

1H-NMR (250.13 MHz, CDCl3) δ 7.39-7.37 (m, 7H), 7.31-7.229 (m, 1H), 5.41 (d, 1H, J=7.5 Hz), 5.15 (d, 2H, J=2.5 Hz), 4.80 (q, 1H, J=5 Hz), 3.14 (d, 2H, J=7.5 Hz), 1.45 (s, 9H), 1.41 (d, 3H, J=7.5 Hz) ppm; 13C-NMR (100.62 MHz, CDCl3) δ 171.50, 170.16, 135.92, 129.46, 128.33, 128.15, 126.94, 89.12, 82.44, 66.94, 53.52, 50.38, 37.87, 27.91 ppm; HRMS (ESI) calculated for C24H30N2O5: 427.2233 ([M+H]+), found 427.2212 ([M+H]+), 449.2033 ([M+Na]+).

(S)-tert-butyl 2-((S)-2-(((benzyloxy)carbonyl)amino)acetamido)-3-methylbutanoate

1H-NMR (250.13 MHz, CDCl3) δ 7.39-7.37 (m, 5H), 5.16 (s, 2H), 4.50 (dd, 1H, J=5 Hz), 3.96-3.92 (m, 2H), 2.21-2.13 (m, 1H), 1.48 (s, 9H), 0.95 (dd, 6H, J=7.5 Hz) ppm; 13C-NMR (100.62 MHz, CDCl3) δ 176.28, 170.87, 163.81, 135.10, 128.55, 82.23, 67.19, 57.36, 44.58, 31.46, 28.04, 18.87, 17.57 ppm; HRMS (ESI) calculated for C19H28N2O5: 365.2076 ([M+H]+), found 365.2059 ([M+H]+), 387.1884 ([M+Na]+).

(R)-tert-butyl 2-((S)-2-(((benzyloxy)carbonyl)amino)acethlamido)-3-phenylpropanoate

1H-NMR (400.13 MHz, CDCl3) δ 7.36-7.31 (m, 5H), 7.28-7.26 (m, 3H), 7.17-7.14 (m, 2H), 5.15 (s, 2H), 4.82 (q, 1H, J=12 Hz), 3.91 (t, 2H, J=8 Hz), 3.12 (d, 2H, J=12 Hz), 1.41 (s, 9H) ppm; 13C-NMR (100.62 MHz, CDCl3) δ 182.28, 145.64, 135.84, 129.51, 129.57, 128.43, 128.26,
128.07, 82.64, 67.22, 53.46, 44.46, 38.01, 27.96 ppm; HRMS (ESI) calculated for C_{23}H_{28}N_{2}O_{5}: 413.2076 ([M+H]^+), found 413.2065 ([M+H]^+), 435.1886 ([M+Na]^+).

(R)-tert-butyl 2-((benzoyloxy)carbonyl)amino)acetamido)propanoate

\(^1\)H-NMR (400.13 MHz, CDCl\(_3\)) \(\delta\) 7.37-7.34 (m, 5H), 5.15 (s, 2H), 4.52 (quint, 1H, \(J=8\) Hz), 1.48 (s, 9H), 1.39 (d, 3H, \(J=4\) Hz) ppm; \(^{13}\)C-NMR (100.62 MHz, CDCl\(_3\)) \(\delta\) 171.99, 171.82, 149.93, 136.15, 128.55, 128.22, 128.11, 82.25, 67.19, 48.68, 44.44, 27.96, 18.57 ppm; HRMS (ESI) calculated for C\(_{17}\)H\(_{24}\)N\(_2\)O\(_5\): 337.1763 ([M+H]^+), found 337.1745 ([M+H]^+), 359.1573 ([M+Na]^+).

(R)-tert-butyl 2-((R)-2-((benzoyloxy)carbonyl)amino)propanamido)-3-phenylpropanoate

\(^1\)H-NMR (400.13 MHz, CDCl\(_3\)) \(\delta\) 7.50-7.46 (m, 5H), 7.41-7.38 (m, 2H), 7.29-7.27 (m, 2H), 5.28 (m, 2H), 4.89 (q, 1H, \(J=8\) Hz), 3.23 (s, 2H), 1.55 (s, 9H), 1.50 (d, 3H, \(J=4\) Hz) ppm; \(^{13}\)C-NMR (100.62 MHz, CDCl\(_3\)) \(\delta\) 171.16, 135.61, 129.15, 128.01, 126.63, 82.13, 66.64, 53.22, 37.57, 27.60 ppm; HRMS (ESI) calculated for C\(_{21}\)H\(_{32}\)N\(_2\)O\(_5\): 427.2233 ([M+H]^+), found 427.2160 ([M+H]^+), 449.1977 ([M+Na]^+).

(R)-tert-butyl 2-((R)-2-(((benzyloxy)carbonyl)amino)propanamido)propanoate

\(^1\)H-NMR (250.13 MHz, CDCl\(_3\)) \(\delta\) 7.24-7.22 (m, 5H), 5.01 (s, 2H), 4.35 (m, 2H), 1.37 (s, 9H), 1.31 (m, 6H) ppm; \(^{13}\)C-NMR (100.62 MHz, CDCl\(_3\)) \(\delta\) 171.92, 155.92, 136.33, 128.48, 128.08, 81.88, 66.82, 50.42, 48.69, 27.95, 18.97, 18.28 ppm; HRMS (ESI) calculated for C\(_{18}\)H\(_{28}\)N\(_2\)O\(_5\): 351.1920 ([M+H]^+), found 351.1854 ([M+H]^+), 373.1670 ([M+Na]^+).

(S)-tert-butyl 2-((R)-2-(((benzyloxy)carbonyl)amino)propanamido)-3-phenylpropanoate

\(^1\)H-NMR (250.13 MHz, CDCl\(_3\)) \(\delta\) 7.26-7.24 (m, 5H), 7.18-7.14 (m, 3H), 7.06-7.04 (m, 2H), 5.01 (d, 2H, \(J=8\) Hz), 4.69 (q, 1H, \(J=12\) Hz), 3.00 (d, 2H, \(J=8\) Hz), 2.16 (m, 1H), 1.32 (s, 9H), 1.27 (d, 3H, \(J=8\) Hz) ppm; \(^{13}\)C-NMR (100.62 MHz, CDCl\(_3\)) \(\delta\) 171.72, 170.27, 136.04, 129.52, 128.36, 126.96, 82.41, 66.95, 53.62, 50.44, 37.97, 27.94, 27.89, 18.65 ppm; HRMS (ESI) calculated for C\(_{24}\)H\(_{30}\)N\(_2\)O\(_5\): 427.2233 ([M+H]^+), found 365.2059 ([M+H]^+), 387.1884 ([M+Na]^+).

(S)-tert-butyl 2-((2-(((benzyloxy)carbonyl)amino)acetamido)-4-methylpentanoate

\(^1\)H-NMR (400.13 MHz, CDCl\(_3\)) \(\delta\) 7.38-7.35 (m, 5H), 5.32 (s, 2H), 5.15 (s, 2H), 4.56 (q, 1H, \(J=8\) Hz), 3.94-3.91 (m, 2H), 1.48 (s, 9H), 0.96 (d, 6H, \(J=8\) Hz) ppm; \(^{13}\)C-NMR (100.62 MHz, CDCl\(_3\))
\[ \delta 192.35, 185.75, 171.06, 169.46, 136.69, 129.81, 128.63, 127.10, 82.57, 54.30, 43.19, 38.31, 28.19 \text{ ppm}; \] HRMS (ESI) calculated for C\textsubscript{20}H\textsubscript{30}N\textsubscript{2}O\textsubscript{5}: 379.2233 ([M+H]\textsuperscript{+}), found 379.2221 ([M+H]\textsuperscript{+}), 401.2048 ([M+Na]\textsuperscript{+}).

(S)-tert-butyl 2-(((benzoyloxy)carbonyl)amino)propanamido)-4-methylpentanoate

\[ \text{1H-NMR (400.13 MHz, CDCl}_3\text{) } \delta 7.37-7.30 \text{ (m, 5H), 5.32 (s, 1H), 5.13 (s, 2H), 4.52 (q, 1H, } J=8 \text{ Hz, 4.29-4.27 (m, 1H), 1.66-1.64 (m, 2H), 1.48 (s, 9H), 1.42 (d, 3H, } J=4 \text{ Hz, 0.95 (d, 6H, } J=4 \text{ Hz) ppm}; \text{ 13C-NMR (100.62 MHz, CDCl}_3\text{) } \delta 177.17, 171.68, 162.21, 128.54, 128.19, 128.06, 51.43, 41.78, 27.99, 24.91, 22.79, 22.09 \text{ ppm; HRMS (ESI) calculated for C}_{21}H_{32}N_{2}O_{5}: 393.2389 ([M+H]\textsuperscript{+}), found 393.2382 ([M+H]\textsuperscript{+}), 415.2199 ([M+Na]\textsuperscript{+}).\]

(S)-tert-butyl 2-(((benzoyloxy)carbonyl)amino)-3-phenylpropanamido)-4-methylpentanoate

\[ \text{1H-NMR (400.13 MHz, CDCl}_3\text{) } \delta 7.37-7.28 \text{ (m, 5H), 7.27-7.23 \text{ (m, 3H), 7.21-7.19 (m, 2H), 5.31 (s, 1H), 5.10 (s, 2H), 4.48 (q, 2H, } J=8 \text{ Hz), 3.11 (m, 2H), 1.59-1.56 (m, 2H), 1.47 (s, 9H), 0.93 (d, 6H, } J=4 \text{ Hz) ppm; 13C-NMR (100.62 MHz, CDCl}_3\text{) } \delta 171.56, 170.28, 166.51, 134.67, 132.66, 129.40, 128.54, 128.03, 127.03, 81.94, 67.03, 51.46, 41.85, 27.99, 24.80, 22.71, 22.14 \text{ ppm; HRMS (ESI) calculated for C}_{27}H_{36}N_{2}O_{5}: 469.2702 ([M+H]\textsuperscript{+}), found 469.2687 ([M+H]\textsuperscript{+}), 491.2507 ([M+Na]\textsuperscript{+}).}\]

(R)-tert-butyl 2-((S)-2-(((benzoyloxy)carbonyl)amino)acetamido)-4-methylpentanoate

\[ \text{1H-NMR (250.13 MHz, CDCl}_3\text{) } \delta 7.36-7.33 \text{ (m, 5H), 5.15 (s, 2H), 4.56 (q, 1H, } J=8 \text{ Hz, 3.92-3.89 (m, 2H), 2.93 (d, 1H, } J=12 \text{ Hz), 1.64-1.61 (m, 2H), 1.47 (s, 9H), 0.95 (d, 6H, } J=4 \text{ Hz) ppm; 13C-NMR (100.62 MHz, CDCl}_3\text{) } \delta 175.11, 171.98, 168.51, 148.70, 142.86, 128.54, 128.22, 128.08, 95.80, 82.12, 77.39, 67.17, 51.35, 44.46, 41.81, 27.98, 24.90, 22.78, 22.07 \text{ ppm; HRMS (ESI) calculated for C}_{20}H_{30}N_{2}O_{5}: 379.2233 ([M+H]\textsuperscript{+}), found 379.2240 ([M+H]\textsuperscript{+}), 401.2062 ([M+Na]\textsuperscript{+}).}\]

(R)-tert-butyl 2-((S)-2-(((benzoyloxy)carbonyl)amino)propanamido)-4-methylpentanoate

\[ \text{1H-NMR (250.13 MHz, CDCl}_3\text{) } \delta 7.36-7.33 \text{ (m, 5H), 5.13 (s, 2H), 4.53 (q, 1H, } J=8 \text{ Hz, 4.32-4.30 (m, 1H), 1.64-1.61 (m, 2H), 1.47 (s, 9H), 1.42 (d, 3H, } J=8 \text{ Hz, 0.95 (d, 6H, } J=4 \text{ Hz) ppm; 13C-NMR (100.62 MHz, CDCl}_3\text{) } \delta 171.92, 164.82, 136.33, 128.54, 128.18, 128.06, 67.02, 51.35, 41.75, 27.99, 24.95, 22.82, 22.08 \text{ ppm; HRMS (ESI) calculated for C}_{21}H_{32}N_{2}O_{5}: 393.2389 ([M+H]\textsuperscript{+}), found 393.2401 ([M+H]\textsuperscript{+}), 415.2222 ([M+Na]\textsuperscript{+}).}\]
(R)-tert-butyl 2-(((S)-2-(((benzoyloxy)carbonyl)amino)-3-phenylpropanamido)-4-methylpentanoate

$^1$H-NMR (250.13 MHz, CDCl$_3$) δ 7.35-7.21 (m, 10H), 5.30 (s, 3H), 5.08 (s, 2H), 4.51-4.50 (m, 1H), 4.45 (q, 1H, $J=8$ Hz), 3.10 (d, 2H, $J=4$ Hz), 1.44 (s, 9H), 1.43-1.27 (m, 2H), 0.88 (d, 6H, $J=4$ Hz) ppm; $^{13}$C-NMR (100.62 MHz, CDCl$_3$) δ 171.77, 170.42, 136.39, 129.28, 128.67, 128.51, 127.98, 126.96, 81.92, 66.96, 53.47, 51.43, 41.62, 38.76, 27.96, 24.67, 22.70, 21.99 ppm; HRMS (ESI) calculated for C$_{27}$H$_{36}$N$_2$O$_5$: 469.7207 ([M+H]$^+$), found 469.2709 ([M+H]$^+$).

(S)-tert-butyl 2-(((S)-2-(((benzoyloxy)carbonyl)amino)-3-phenylpropanamido)-3-phenylpropanoate

$^1$H-NMR (250.13 MHz, CDCl$_3$) δ 7.35-7.21 (m, 10H), 5.30 (s, 3H), 5.08 (s, 2H), 4.51-4.50 (m, 1H), 4.45 (q, 1H, $J=8$ Hz), 3.10 (d, 2H, $J=4$ Hz), 1.44 (s, 9H), 1.43-1.27 (m, 2H), 0.88 (d, 6H, $J=4$ Hz) ppm; $^{13}$C-NMR (100.62 MHz, CDCl$_3$) δ 171.77, 170.42, 136.39, 129.28, 128.67, 128.51, 127.98, 126.96, 81.92, 66.96, 53.47, 51.43, 41.62, 38.76, 27.96, 24.67, 22.70, 21.99 ppm; HRMS (ESI) calculated for C$_{27}$H$_{36}$N$_2$O$_5$: 469.7207 ([M+H]$^+$), found 469.2709 ([M+H]$^+$).

(S)-tert-butyl 2-(((R)-2-(((benzoyloxy)carbonyl)amino)-3-phenylpropanamido)-3-phenylpropanoate

$^1$H-NMR (250.13 MHz, CDCl$_3$) δ 7.35-7.21 (m, 10H), 5.30 (s, 3H), 5.08 (s, 2H), 4.51-4.50 (m, 1H), 4.45 (q, 1H, $J=8$ Hz), 3.10 (d, 2H, $J=4$ Hz), 1.44 (s, 9H), 1.43-1.27 (m, 2H), 0.88 (d, 6H, $J=4$ Hz) ppm; $^{13}$C-NMR (100.62 MHz, CDCl$_3$) δ 171.77, 170.42, 136.39, 129.28, 128.67, 128.51, 127.98, 126.96, 81.92, 66.96, 53.47, 51.43, 41.62, 38.76, 27.96, 24.67, 22.70, 21.99 ppm; HRMS (ESI) calculated for C$_{27}$H$_{36}$N$_2$O$_5$: 469.7207 ([M+H]$^+$), found 469.2709 ([M+H]$^+$).

(S)-tert-butyl 2-(((S)-2-(((benzoyloxy)carbonyl)amino)-3-phenylpropanamido)-3-phenylpropanoate

$^1$H-NMR (250.13 MHz, CDCl$_3$) δ 7.35-7.21 (m, 10H), 5.30 (s, 3H), 5.08 (s, 2H), 4.51-4.50 (m, 1H), 4.45 (q, 1H, $J=8$ Hz), 3.10 (d, 2H, $J=4$ Hz), 1.44 (s, 9H), 1.43-1.27 (m, 2H), 0.88 (d, 6H, $J=4$ Hz) ppm; $^{13}$C-NMR (100.62 MHz, CDCl$_3$) δ 171.77, 170.42, 136.39, 129.28, 128.67, 128.51, 127.98, 126.96, 81.92, 66.96, 53.47, 51.43, 41.62, 38.76, 27.96, 24.67, 22.70, 21.99 ppm; HRMS (ESI) calculated for C$_{27}$H$_{36}$N$_2$O$_5$: 469.7207 ([M+H]$^+$), found 469.2709 ([M+H]$^+$).

(S)-tert-butyl 2-(((R)-2-(((benzoyloxy)carbonyl)amino)-3-phenylpropanamido)-3-phenylpropanoate

$^1$H-NMR (250.13 MHz, CDCl$_3$) δ 7.35-7.21 (m, 10H), 5.30 (s, 3H), 5.08 (s, 2H), 4.51-4.50 (m, 1H), 4.45 (q, 1H, $J=8$ Hz), 3.10 (d, 2H, $J=4$ Hz), 1.44 (s, 9H), 1.43-1.27 (m, 2H), 0.88 (d, 6H, $J=4$ Hz) ppm; $^{13}$C-NMR (100.62 MHz, CDCl$_3$) δ 171.77, 170.42, 136.39, 129.28, 128.67, 128.51, 127.98, 126.96, 81.92, 66.96, 53.47, 51.43, 41.62, 38.76, 27.96, 24.67, 22.70, 21.99 ppm; HRMS (ESI) calculated for C$_{27}$H$_{36}$N$_2$O$_5$: 469.7207 ([M+H]$^+$), found 469.2709 ([M+H]$^+$).
(S)-tert-butyl 2-(((S)-2-(((benzyloxy)carbonyl)amino)-3-phenylpropanamido)propanoate

$^1$H-NMR (400.13 MHz, CDCl$_3$) $\delta$ 7.39-7.19 (m, 10H), 6.35 (d, 1H, $J$=4 Hz), 5.31 (d, 1H, $J$=8 Hz), 5.11 (s, 2H), 4.46-4.41 (m, 1H), 4.39-4.34 (m, 1H), 3.17 (dd, 1H, $J$=1H, $J$=8 Hz), 3.07 (dd, 1H, $J$=8 Hz), 1.46 (s, 9H), 1.31 (d, 3H, $J$=8 Hz) ppm; $^{13}$C-NMR (100.62 MHz, CDCl$_3$) $\delta$ 170.02, 129.34, 128.19, 128.05, 99.99, 77.36, 77.04, 76.72, 56.05, 54.08, 48.78, 36.65, 27.93, 27.96, 18.59 ppm; HRMS (ESI) calculated for C$_{24}$H$_{30}$N$_2$O$_5$: 427.2233 ([M+H]$^+$), found: 449.1983 ([M+Na]$^+$).

(R)-tert-butyl 2-((2-aminoacetamido)-3-methylbutanoate

$^1$H-NMR (250.13 MHz, CDCl$_3$) $\delta$ 7.39-7.37 (m, 5H), 5.16 (s, 2H), 4.50 (dd, 1H, $J$=5 Hz), 3.96-3.92 (m, 2H), 2.21-2.13 (m, 1H), 1.48 (s, 9H), 0.95 (dd, 6H, $J$=7.5 Hz) ppm; $^{13}$C-NMR (100.62 MHz, CDCl$_3$) $\delta$ 176.28, 170.87, 163.81, 135.10, 128.55, 82.23, 67.19, 57.36, 44.58, 31.46, 28.04, 18.87, 17.57 ppm; HRMS (ESI) calculated for C$_{19}$H$_{28}$N$_2$O$_5$: 365.2076 ([M+H]$^+$), found 365.2059 ([M+H]$^+$), 387.1884 ([M+Na]$^+$).

(R)-tert-butyl 2-(((S)-2-(((benzyloxy)carbonyl)amino)propanamido)-3-methylbutanoate

$^1$H-NMR (400.13 MHz, CDCl$_3$) $\delta$ 7.36-7.30 (m, 5H), 5.13 (s, 2H), 4.45 (dd, 1H, $J$=4 Hz), 4.32-4.29 (m, 1H), 2.19-2.13 (m, 1H), 1.48 (s, 9H), 1.42 (d, 3H, $J$=8 Hz), 0.93 (t, 6H, $J$=8 Hz) ppm; $^{13}$C-NMR (100.62 MHz, CDCl$_3$) $\delta$ 185.99, 180.11, 172.00, 170.73, 143.21, 137.24, 128.52, 128.15, 128.06, 96.08, 82.09, 67.00, 57.45, 31.37, 28.04, 18.66, 17.52 ppm; HRMS (ESI) calculated for C$_{20}$H$_{30}$N$_2$O$_5$: 379.2233 ([M+H]$^+$), found 379.2210 ([M+H]$^+$), 401.2033 ([M+Na]$^+$).

**Procedure for the deprotection of the Cbz-group**

The amine protection group was removed with Pd/C in methanol by bubbling through hydrogen for 2-4 hours. The Pd/C mixture was filtrated over celite and the organic solvent evaporated to dryness. The obtained crude product was analyzed by TLC (coloring with ninhydrin), flash silica purification was performed with methanol-dichloromethane (1-10 % methanol) and the reaction was quantitative yielding a clear oil.

(S)-tert-butyl 2-(2-aminopropanamido)propanoate

$^1$H-NMR (250.13 MHz, CD$_3$OD) $\delta$ 4.31 (q, 1H, $J$=7.5 Hz), 3.31 (s, 2H), 1.49 (s, 9H), 1.40 (d, 3H, $J$=7.5 Hz) ppm; $^{13}$C-NMR (100.62 MHz, CD$_3$OD) $\delta$ 177.99, 173.79, 83.00, 51.47, 50.43, 28.55,
21.69, 17.88 ppm; HRMS (ESI) calculated for C_{10}H_{20}N_{2}O_{3}: 217.1552 ([M+H]^+), found 217.1557 ([M+H]^+), 239.1363 ([M+Na]^+).

(S)-tert-butyl 2-(2-aminoacetamido)propanoate

$^1$H-NMR (250.13 MHz, CD$_3$OD) δ 4.37 (q, 1H, $J_{1}$=7.5 Hz), 3.50 (q, 1H, $J_{1}$=7.5 Hz), 1.48 (s, 9H), 1.37 (d, 3H, $J_{1}$=5 Hz), 1.31 (d, 3H, $J_{1}$=7.5 Hz) ppm; $^{13}$C-NMR (100.62 MHz, CDCl$_3$) δ 172.32, 172.10, 81.92, 48.17, 44.56, 27.96, 18.68 ppm; HRMS (ESI) calculated for C$_9$H$_{18}$N$_2$O$_3$: 203.1396 ([M+H]^+), found 203.1400.13 ([M+H]^+), 225.1209 ([M+Na]^+).

(S)-tert-butyl 2-(2-aminoacetamido)-3-methylbutanoate

$^1$H-NMR (250.13 MHz, CD$_3$OD) δ 4.28 (d, 1H, $J_{1}$=5 Hz), 3.37 (s, 2H), 2.18 (m, 1H), 1.50 (s, 9H), 1.00 (dd, 6H, $J_{1}$=2.5 Hz) ppm, $^{13}$C-NMR (100.62 MHz, CDCl$_3$) δ 172.67, 171.14, 81.79, 56.87, 44.76, 31.40, 28.04, 19.98, 17.60 ppm; HRMS (ESI) calculated for C$_{11}$H$_{22}$N$_2$O$_3$: 231.1709 ([M+H]^+), found 231.1707 ([M+H]^+), 253.1513 ([M+Na]^+).

(S)-tert-butyl 2-((S)-2-aminopropanamido)-3-phenylpropanoate

$^1$H-NMR (250.13 MHz, CD$_3$OD) δ 7.27 (m, 5H), 4.60 (dd, 1H, $J_{1}$=5 Hz, $J_{2}$=2.5 Hz), 3.47 (q, 1H, $J_{1}$=7.5 Hz), 3.17 (dd, 1H, $J_{1}$=7.5 Hz), 3.05 (dd, 1H, $J_{1}$=7.5 Hz) 1.43 (s, 9H), 1.26 (d, 3H, $J_{1}$=7.5 Hz) ppm; $^{13}$C-NMR (100.62 MHz, CDCl$_3$) δ 174.77, 170.34, 135.90, 129.08, 127.84, 126.42, 81.69, 52.61, 37.77, 27.52, 21.14 ppm; HRMS (ESI) calculated for C$_{16}$H$_{24}$N$_2$O$_3$: 292.373 ([M+H]^+), found 293.19 ([M+H]^+).

(S)-tert-butyl 2-((S)-2-aminopropanamido)-3-methylbutanoate

$^1$H-NMR (250.13 MHz, CD$_3$OD) δ 4.43 (d, 1H, $J_{1}$=5 Hz), 3.56 (q, 1H, $J_{1}$=7.5 Hz), 2.16 (m, 1H, $J_{1}$=2.5 Hz), 1.46 (s, 9H), 1.29 (d, 3H, $J_{1}$=7.5 Hz), 0.97 (d, 3H, $J_{1}$=7.5 Hz) ppm; $^{13}$C-NMR (100.62 MHz, CD$_3$Cl) δ 175.61, 171.20, 104.30, 56.79, 50.92, 31.42, 28.04, 21.93, 18.98, 17.50 ppm; HRMS (ESI) calculated for C$_{12}$H$_{24}$N$_2$O$_3$: 245.1865 ([M+H]^+), found 245.1821 ([M+H]^+).

(R)-tert-butyl 2-((S)-2-aminopropanamido)-3-methylbutanoate

$^1$H-NMR (250.13 MHz, CD$_3$OD) δ 4.42 (d, 1H, $J_{1}$=5 Hz), 3.54 (q, 1H, $J_{1}$=7.5 Hz), 2.16 (m, 1H, $J_{1}$=2.5 Hz), 1.46 (s, 9H), 1.29 (d, 3H, $J_{1}$=7.5 Hz), 0.97 (d, 3H, $J_{1}$=7.5 Hz) ppm; $^{13}$C-NMR (100.62
MHz, CD₃Cl) δ 175.61, 171.20, 104.30, 56.79, 50.92, 31.42, 28.04, 21.93, 18.98, 17.50 ppm; HRMS (ESI) calculated for C₁₂H₂₄N₂O₃: 245.1865 ([M+H]⁺), found 245.1821 ([M+H]⁺).

(R)-tert-butyl 2-(2-aminoacetamido)-3-phenylpropanoate

¹H-NMR (400.13 MHz, CDCl₃) δ 7.33 (m, 5H), 4.80 (q, 1H, J=8 Hz), 3.81 (q, 2H, J=12 Hz), 3.15 (d, 2H, J=8 Hz), 1.41 (s, 9H) ppm; ¹³C-NMR (100.62 MHz, CDCl₃) δ 192.35, 185.75, 171.06, 169.46, 136.69, 129.81, 128.63, 127.10, 82.57, 54.30, 43.19, 38.31, 28.19 ppm; HRMS (ESI) calculated for C₁₅H₂₂N₂O₃: 279.1709 ([M+H]⁺), found 279.1695 ([M+H]⁺).

(R)-tert-butyl 2-(2-aminoacetamido)propanoate

¹H-NMR (400.13 MHz, CDCl₃) δ 4.52 (q, 1H, J=8 Hz), 3.35 (s, 1H), 1.46 (s, 9H), 1.39 (d, 3H, J=8 Hz) ppm; ¹³C-NMR (100.62 MHz, CDCl₃) δ 172.30, 165.02, 81.87, 48.11, 44.71, 27.94, 18.71 ppm; HRMS (ESI) calculated for C₉H₁₈N₂O₃: 203.1396 ([M+H]⁺), found 203.1404 ([M+H]⁺).

(R)-tert-butyl 2-((R)-2-aminopropanamido)-3-phenylpropanoate

¹H-NMR (400.13 MHz, CDCl₃) δ 8.32 (s, 1H), 4.31 (q, 1H, J=7.5 Hz), 3.31 (q, 1H, J=7.5 Hz), 1.49 (s, 9H), 1.40 (d, 6H, J=7.5 Hz) ppm; ¹³C-NMR (100.62 MHz, CD₃OD) δ 171.61, 169.64, 81.77, 49.48, 34.43, 27.96, 17.82, 17.36 ppm; HRMS (ESI) calculated for C₁₀H₂₀N₂O₃: 217.1552 ([M+H]⁺), found 217.1557 ([M+H]⁺), 239.1363 ([M+Na]⁺).

(R)-tert-butyl 2-((R)-2-aminopropanamido)-3-phenylpropanoate

¹H-NMR (400.13 MHz, CDCl₃) δ 7.27 (m, 5H), 4.60 (dd, 1H, J₁=5 Hz, J₂=2.5 Hz), 3.47 (q, 1H, J=7.5 Hz), 3.17 (dd, 1H, J=7.5 Hz), 3.05 (dd, 1H, J=7.5 Hz) 1.43 (s, 9H), 1.26 (d, 3H, J=7.5 Hz) ppm; ¹³C-NMR (100.62 MHz, CDCl₃) δ 170.14, 169.72, 136.29, 129.50, 128.30, 126.88, 82.22, 53.14, 50.60, 38.14, 27.94, 21.33 ppm; HRMS (ESI) calculated for C₁₆H₂₄N₂O₃: 293.1865 ([M+H]⁺), found 293.1842 ([M+H]⁺).
(S)-tert-butyl 2-(2-aminoacetamido)-4-methylpentanoate

$^1$H-NMR (400.13 MHz, CDCl$_3$) δ 4.57-4.51 (m, 1H, J=8 Hz), 3.57 (s, 1H), 3.37 (s, 2H), 1.63-1.61 (m, 2H), 1.47 (s, 9H), 0.96 (d, 6H, J=8 Hz) ppm; $^{13}$C-NMR (100.62 MHz, CDCl$_3$) δ 172.29, 157.72, 81.75, 50.84, 44.71, 41.90, 27.99, 27.98, 24.97, 22.15, 22.07 ppm; HRMS (ESI) calculated for C$_{12}$H$_{24}$N$_2$O$_3$: 244.1787 ([M+H]+), found 245.1861 ([M+H]+).

(R)-tert-butyl 2-((S)-2-aminopropanamido)-3-methylbutanoate

$^1$H-NMR (400.13 MHz, CD$_3$OD) δ 4.43 (q, 1H, J=4 Hz), 3.58-3.56 (m, 1H), 2.21-2.16 (m, 1H), 1.48 (s, 9H), 1.38 (d, 3H, J=8 Hz), 0.97 (dd, 6H, J$_1$=4 Hz, J$_2$=8 Hz) ppm; $^{13}$C-NMR (100.62 MHz, CD$_3$OD) δ 175.61, 171.20, 104.30, 56.79, 50.92, 31.42, 28.04, 21.93, 18.98, 17.50 ppm; HRMS (ESI) calculated for C$_{12}$H$_{24}$N$_2$O$_3$: 245.1865 ([M+H]+), found 245.1821 ([M+H]+).

(R)-tert-butyl 2-(2-aminoacetamido)-3-methylbutanoate

$^1$H-NMR (250.13 MHz, CD$_3$OD) δ 4.49 (q, 1H, J=4 Hz), 3.43 (s, 2H), 2.24-2.16 (m, 1H), 1.48 (s, 9H), 0.97 (t, 6H, J=8 Hz) ppm; $^{13}$C-NMR (100.62 MHz, CDCl$_3$) δ 178.19, 176.18, 108.63, 90.35, 73.19, 56.98, 56.78, 44.76, 31.41, 28.06, 19.00, 17.63 ppm; HRMS (ESI) calculated for C$_{11}$H$_{22}$N$_2$O$_3$: 231.1709 ([M+H]+), found 231.1707 ([M+H]+), 253.1513 ([M+Na]+).

(S)-tert-butyl 2-((S)-2-aminopropanamido)-4-methylpentanoate

$^1$H-NMR (400.13 MHz, CDCl$_3$) δ 4.51 (q, 1H, J=8 Hz), 3.55 (q, 1H, J=8 Hz), 3.49 (s, 2H), 1.66-1.64 (m, 2H), 1.47 (s, 9H), 1.35 (d, 3H, J=8 Hz), 0.96 (d, 6H, J=4 Hz) ppm; $^{13}$C-NMR (100.62 MHz, CDCl$_3$) δ 172.34, 169.32, 81.65, 50.87, 50.77, 41.68, 28.01, 25.01, 22.88, 22.13, 21.76 ppm; HRMS (ESI) calculated for C$_{13}$H$_{26}$N$_2$O$_3$: 259.2022 ([M+H]+), found 259.2014 ([M+H]+), 275.2357 ([M+Na]+).

(S)-tert-butyl 2-((S)-2-amino-3-phenylpropanamido)-4-methylpentanoate

$^1$H-NMR (400.13 MHz, CDCl$_3$) δ 7.35-7.22 (m, 5H), 4.41-4.39 (m, 1H), 4.33 (q, 1H, J=4 Hz), 3.28 (d, 2H, J=4 Hz), 163-1.55 (m, 3H), 1.44 (s, 9H), 0.90 (d, 6H, J=8 Hz) ppm; $^{13}$C-NMR (100.62 MHz, CDCl$_3$) δ 171.63, 169.02, 134.73, 129.89, 128.81, 127.42, 81.94, 54.85, 52.20, 40.87, 37.75, 27.96, 27.94, 24.74, 22.57, 22.14 ppm; HRMS (ESI) calculated for C$_{19}$H$_{36}$N$_2$O$_3$: 335.2335 ([M+H]+), found 335.2333 ([M+H]+).
(R)-tert-butyl 2-(2-aminoacetamido)-4-methylpentanoate

$^1$H-NMR (400.13 MHz, CDCl$_3$) $\delta$ 7.54 (d, 1H, $J$=8 Hz), 4.46 (q, 1H, $J$=8 Hz), 3.34 (s, 2H), 1.66-1.46 (m, 3H), 1.41 (s, 9H), 0.90 (t, 6H, $J$=8 Hz) ppm; $^{13}$C-NMR (100.62 MHz, CDCl$_3$) $\delta$ 172.31, 81.96, 51.06, 50.14, 44.15, 41.48, 27.88, 24.87, 22.74, 21.89 ppm; HRMS (ESI) calculated for C$_{12}$H$_{24}$N$_2$O$_3$: 245.1865 ([M+H$^+$]), found 245.1880 ([M+H$^+$]).

(R)-tert-butyl 2-((S)-2-aminopropanamido)-4-methylpentanoate

$^1$H-NMR (400.13 MHz, CDCl$_3$) $\delta$ 4.51 (q, 1H, $J$=8 Hz), 3.55 (q, 1H, $J$=8 Hz), 3.49 (s, 2H), 1.66-1.64 (m, 2H), 1.47 (s, 9H), 1.35 (d, 3H, $J$=8 Hz), 0.96 (d, 6H, $J$=4 Hz) ppm; $^{13}$C-NMR (100.62 MHz, CDCl$_3$) $\delta$ 172.34, 169.32, 81.65, 50.87, 50.77, 41.68, 28.01, 25.01, 22.88, 22.13, 21.76 ppm; HRMS (ESI) calculated for C$_{13}$H$_{26}$N$_2$O$_3$: 259.2022 ([M+H$^+$]), found 259.2039 ([M+H$^+$]).

(R)-tert-butyl 2-((S)-2-aminophenylpropanamido)-4-methylpentanoate

$^1$H-NMR (400.13 MHz, CDCl$_3$) $\delta$ 7.10-7.02 (m, 5H), 3.95-3.88 (m, 2H), 3.15 (m, 1H), 3.01-2.98 (m, 1H), 2.97-2.82 (m, 1H), 1.22 (s, 9H), 1.19-1.11 (m, 2H), 0.63 (d, 3H, $J$=8 Hz), 0.53 (d, 3H, $J$=4 Hz) ppm; $^{13}$C-NMR (100.62 MHz, CDCl$_3$) $\delta$ 172.31, 169.67, 133.83, 129.04, 128.57, 127.29, 123.82, 94.95, 54.16, 51.38, 43.17, 39.68, 37.03, 27.35, 24.04, 22.30, 20.82 ppm; HRMS (ESI) calculated for C$_{19}$H$_{30}$N$_2$O$_3$: 335.2335 ([M+H$^+$]), found 335.2339 ([M+H$^+$]).

(S)-tert-butyl 2-((S)-2-((benzyloxy)carbonyl)amino)-3-phenylpropanamido)propanoate

$^1$H-NMR (250.13 MHz, (CD$_3$)$_2$SO) $\delta$ 8.19 (d, 1H, $J$=7.5 Hz), 7.25-7.17 (m, 5H), 4.12 (q, 1H, $J$=5 Hz), 3.40 (q, 1H, $J$=5 Hz), 2.94 (dd, 1H, $J_1$=5Hz, $J_2$=7.5 Hz), 2.58 (dd, 1H, $J_1$=5 Hz, $J_2$=7.5 Hz), 1.37 (s, 9H), 1.22 (dd, 3H, $J$=7.5 Hz) ppm; $^{13}$C-NMR (100.62 MHz, (CD$_3$)$_2$SO) $\delta$ 174.68, 172.27, 138.96, 129.82, 128.51, 126.59, 81.08, 54.33, 48.52, 39.11, 28.04, 17.79 ppm; HRMS (ESI) calculated for C$_{16}$H$_{24}$N$_2$O$_3$: 293.1865 ([M+H$^+$]), found: 293.2087 ([M+H$^+$]).

(S)-tert-butyl 2-((S)-2-amino-3-phenylpropanamido)-3-methylbutanoate

$^1$H-NMR (250.13 MHz, (CD$_3$)$_2$SO) $\delta$ 8.04 (d, 1H, $J$=10 Hz), 7.27-7.16 (m, 5H), 4.06 (q, 1H, $J$=5 Hz), 3.49-3.45 (m, 1H), 2.95 (dd, 1H, $J_1$=5 Hz, $J_2$=7.5 Hz), 2.60 (q, 1H, $J_1$=10Hz, $J_2$=5 Hz), 1.99 (q, 1H , $J_1$=7.5 Hz, $J_2$=5 Hz), 1.38 (s, 9H), 0.82 (d, 6H, $J$=5 Hz) ppm; $^{13}$C-NMR (100.62 MHz, (CD$_3$)$_2$SO) $\delta$ 174.91, 174.89, 138.85, 129.82, 128.55, 126.60, 81.89, 57.79, 56.08, 39.14, 30.84,
28.07, 19.26, 18.35 ppm; HRMS (ESI) calculated for C\textsubscript{18}H\textsubscript{28}N\textsubscript{3}O\textsubscript{3}: 321.2178 ([M+H]\textsuperscript{+}), found: 321.2104 ([M+H]\textsuperscript{+}).

\textit{(S)-tert-butyl 2-((S)-2-amino-3-phenylpropanamido)-3-phenylpropanoate}

\textsuperscript{1}H-NMR (400.13 MHz, (CD\textsubscript{3})\textsubscript{2}SO) δ 8.18 (d, 1H, J=8 Hz), 7.29-7.20 (m, 8H), 7.15-7.14 (m, 2H), 4.44 (q, 1H, J=8 Hz), 4.03 (q, 1H, J\textsubscript{1}=8 Hz, J\textsubscript{2}=4 Hz), 2.94 (d, 2H, J=4 Hz), 2.90 (d, 1H, J=4 Hz), 2.57 (q, 1H, J=8 Hz), 1.33 (s, 9H) ppm; \textsuperscript{13}C-NMR (100.62 MHz, (CD\textsubscript{3})\textsubscript{2}SO) δ 174.52, 170.88, 138.94, 137.34, 129.84, 128.62, 127.01, 126.60, 81.36, 56.21, 53.92, 41.15, 37.68, 27.99 ppm; HRMS (ESI) calculated for C\textsubscript{22}H\textsubscript{28}N\textsubscript{2}O\textsubscript{3}: 369.2178 ([M+H]\textsuperscript{+}), found: 369.2111 ([M+H]\textsuperscript{+}).

\textit{(S)-tert-butyl 2-((R)-2-amino-3-phenylpropanamido)-3-methylbutanoate}

\textsuperscript{1}H-NMR (250.13 MHz, CDCl\textsubscript{3}) δ 8.04 (d, 1H, J=10 Hz), 7.27-7.16 (m, 5H), 4.06 (q, 1H, J=5 Hz), 3.49-3.45 (m, 1H), 2.95 (dd, 1H, J\textsubscript{1}=5Hz, J\textsubscript{2}=7.5 Hz), 2.60 (q, 1H, J\textsubscript{1}=10 Hz, J\textsubscript{2}=5 Hz), 1.99 (q, 1H , J\textsubscript{1}=7.5 Hz, J\textsubscript{2}=5 Hz), 1.38 (s, 9H), 0.82 (d, 6H, J=5 Hz) ppm; \textsuperscript{13}C-NMR (100.62 MHz, CDCl\textsubscript{3}) δ 174.91, 174.89, 138.85, 129.82, 128.55, 126.60, 81.89, 57.79, 56.08, 39.14, 30.84, 28.07, 19.26, 18.35 ppm; HRMS (ESI) calculated for C\textsubscript{18}H\textsubscript{28}N\textsubscript{3}O\textsubscript{3}: 321.2178 ([M+H]\textsuperscript{+}), found: 321.2104 ([M+H]\textsuperscript{+}).

\textbf{Procedure for the activation of dipeptides as Schiff base}

After the deprotection of the Cbz-group, the reaction with benzophenone imine to the protected dipeptide took place in dichloromethane with 1.1 eq of benzophenone imine at room temperature for 2-4 hours. The progress of the reaction was followed with TLC. The solvent was evaporated and the residue purified via flash chromatography (2-30 % ethylacetate in n-hexane). The isolated product is either a white solid or clear oil and yielded around 50-70 % of pure product.

\textit{(S)-tert-butyl 2-((diphenyl)methylene)amino)propanamido)propanoate}

\textsuperscript{1}H-NMR (250.13 MHz, CDCl\textsubscript{3}) δ 7.10 (dd, 2H, J=5 Hz), 4.43 (q, 1H, J=7.5 Hz), 3.93 (q, 1H, J=7.5 Hz), 1.40 (d, 3H, J=10 Hz), 1.39 (s, 9H), 1.27 (d, 3H, J=5 Hz) ppm; \textsuperscript{13}C-NMR (100.62 MHz, CDCl\textsubscript{3}) δ 173.64, 171.94, 168.97, 139.20, 135.76, 130.54, 128.78, 128.70, 128.57, 128.21, 127.48, 81.70, 61.24, 48.42, 27.99, 20.99, 18.85 ppm; HRMS (ESI) calculated for C\textsubscript{23}H\textsubscript{28}N\textsubscript{2}O\textsubscript{3}: 381.2178 ([M+H]\textsuperscript{+}), found 381.2167 ([M+H]\textsuperscript{+}), 403.1978 ([M+Na]\textsuperscript{+}).
(S)-tert-butyl 2-((diphenylmethylene)amino)acetamido)propanoate

$^1$H-NMR (250.13 MHz, CDCl$_3$) δ 7.64 (dd, 2H, J=2.5 Hz), 7.38 (m, 6H), 7.09 (dd, 2H, J=2.5 Hz), 4.52 (q, 1H, J=7.5 Hz), 3.90 (s, 2H), 1.44 (s, 9H), 1.41 (d, 3H, J=7.5 Hz) ppm; $^{13}$C-NMR (100.62 MHz, CDCl$_3$) δ 172.29, 170.92, 139.00, 136.35, 132.68, 130.94, 129.24, 129.13, 129.52, 127.52, 82.11, 56.80, 48.69, 26.29, 19.23 ppm; HRMS (ESI) calculated for C$_{22}$H$_{26}$N$_2$O$_3$: 367.2022 ([M+H$^+$]+), found 367.1959 ([M+H$^+$]+).

(S)-tert-butyl 2-((diphenylmethylene)amino)acetamido)-3-phenylpropanoate

$^1$H-NMR (250.13 MHz, CDCl$_3$) δ 7.49 (dd, 2H, J=2.5 Hz), 7.40 (dd, 4H, J=2.5 Hz), 7.30 (d, 2H, J=7.5 Hz), 7.19 (d, 5H, J=2.5 Hz), 7.06 (m, 2H), 4.83-4.76 (m, 1H), 3.90 (s, 2H), 3.10 (d, 2H, J=5 Hz), 1.35 (s, 9H) ppm; $^{13}$C-NMR (100.62 MHz, CDCl$_3$) δ 172.21, 170.36, 138.96, 136.30, 130.90, 129.19, 129.09, 128.73, 128.47, 127.48, 82.06, 57.77, 48.64, 28.25, 19.19 ppm; HRMS (ESI) calculated for C$_{22}$H$_{26}$N$_2$O$_3$: 367.2022 ([M+H$^+$]+), found 367.1956 ([M+H$^+$]+), 389.1773 ([M+Na$^+$]+).

(S)-tert-butyl 2-((S)-2-((diphenylmethylene)amino)propanamido)-3-methylbutanoate

$^1$H-NMR (250.13 MHz, CDCl$_3$) δ 7.68-7.65 (m, 2H), 7.47-7.47 (m, 8H), 4.58-4.56 (m, 1H), 4.02-3.99 (m, 2H), 2.27-2.25 (m, 1H), 1.52 (s, 9H), 1.04 (d, 6H, J=7.5 Hz) ppm; $^{13}$C-NMR (100.62 MHz, CDCl$_3$) δ 170.76, 170.47, 138.73, 136.06, 132.38, 130.62, 130.03, 128.95, 128.84, 128.23, 127.24, 81.73, 57.12, 56.53, 31.62, 28.08, 27.95, 19.98, 17.79 ppm; HRMS (ESI) calculated for C$_{24}$H$_{30}$N$_2$O$_3$: 395.2335 ([M+H$^+$]+), found 395.2399 ([M+H$^+$]+).

(S)-tert-butyl 2-((S)-2-((diphenylmethylene)amino)propanamido)-3-methylbutanoate

$^1$H-NMR (250.13 MHz, CDCl$_3$) δ 7.73-7.67 (m, 2H), 7.46-7.35 (m, 8H), 4.49 (q, 1H, J=2.5 Hz), 4.03 (q, 1H, J=5 Hz), 2.29-2.25 (m, 1H), 1.46 (s, 9H), 1.37 (s, 3H), 1.03 (s, 6H) ppm; $^{13}$C-NMR (100.62 MHz, CDCl$_3$) δ 170.03, 166.72, 164.95, 135.26, 131.84, 126.58, 124.75, 123.55, 77.66, 57.53, 53.08, 27.80, 24.12, 17.37, 15.08, 13.81 ppm; HRMS (ESI) calculated for C$_{25}$H$_{32}$N$_2$O$_3$: 409.2491 ([M+H$^+$]+), found 409.2426 ([M+H$^+$]+).

(S)-tert-butyl 2-((S)-2-((diphenylmethylene)amino)propanamido)-3-phenylpropanoate

$^1$H-NMR (250.13 MHz, CDCl$_3$) δ 7.52 (d, 2H, J=2.5 Hz), 7.43-7.39 (m, 3H), 7.37-7.36 (m, 5H), 7.25-7.23 (m, 3H), 7.19 (s, 2H), 7.06 (dd, 2H, J=5 Hz), 4.77 (d, 1H, J=7.5 Hz), 3.93 (q, 1H, J=7.5 Hz),
Hz), 3.11 (d, 2H, J=5 Hz), 1.30 (s, 9H), 1.22 (d, 3H, J=7.5 Hz) ppm; $^{13}$C-NMR (100.62 MHz, CDCl$_3$) δ 186.57, 182.60, 169.33, 155.18, 132.41, 130.48, 129.73, 128.67, 128.39, 128.13, 127.43, 126.91, 61.14 ppm; HRMS (ESI) calculated for C$_{29}$H$_{32}$N$_2$O$_3$: 457.2491 ([M+H]$^+$), found 457.2421 ([M+H]$^+$), 479.2231 ([M+Na]$^+$).

(R)-tert-butyl 2-(2-((diphenylmethylene)amino)acetamido)-3-phenylpropanoate

$^1$H-NMR (400.13 MHz, CDCl$_3$) δ 7.49-7.44 (m, 15H), 4.85 (q, 1H, J=7.5 Hz), 3.99 (s, 2H), 3.19 (m, 2H), 1.44 (s, 9H) ppm; $^{13}$C-NMR (100.62 MHz, CDCl$_3$) δ 170.24, 170.08, 138.67, 136.29, 136.01, 130.63, 129.61, 128.85, 128.16, 127.28, 126.94, 82.09, 56.55, 53.08, 38.36, 28.00 ppm; HRMS (ESI) calculated for C$_{28}$H$_{30}$N$_2$O$_3$: 443.2335 ([M+H]$^+$), found 457.2419 ([M+H]$^+$), 479.2235 ([M+Na]$^+$).

(R)-tert-butyl 2-(2-((diphenylmethylene)amino)propanamido)-3-phenylpropanoate

$^1$H-NMR (400.13 MHz, CDCl$_3$) δ 7.62 (d, 1H, J=8 Hz), 7.56 (d, 2H, J=8 Hz), 7.43-7.40 (m, 4H), 7.32-7.29 (m, 6H), 7.12 (d, 2H, J=8 Hz), 4.83 (q, 1H, J=8 Hz), 3.99 (q, 1H, J=8 Hz), 3.18 (d, 2H, J=4 Hz), 1.37 (s, 9H), 1.29 (d, 3H, J=4 Hz) ppm; $^{13}$C-NMR (100.62 MHz, CDCl$_3$) δ 175.03, 170.77, 136.29, 129.50, 128.77, 128.30, 126.88, 82.22, 53.14, 50.60, 38.14, 27.94, 21.33 ppm; HRMS (ESI) calculated for C$_{29}$H$_{32}$N$_2$O$_3$: 457.2491 ([M+H]$^+$), found 457.2419 ([M+H]$^+$), 479.2235 ([M+Na]$^+$).

(R)-tert-butyl 2-(2-((diphenylmethylene)amino)propanamido)propanoate

$^1$H-NMR (250.13 MHz, CDCl$_3$) δ 7.10 (dd, 2H, J=5 Hz), 4.43 (quint, 1H, J=7.5 Hz), 3.93 (q, 1H, J=7.5 Hz), 1.40 (d, 3H, J=10 Hz), 1.39 (s, 9H), 1.27 (d, 3H, J=5 Hz) ppm; $^{13}$C-NMR (100.62 MHz, CDCl$_3$) δ 173.63, 171.90, 168.97, 139.21, 135.77, 130.54, 128.77, 128.70, 128.57, 128.21, 127.48,
81.70, 61.24, 48.44, 27.96, 20.99, 18.85 ppm; HRMS (ESI) calculated for C_{23}H_{28}N_{2}O_{3}: 381.2178 ([M+H]^+), found 381.2167 ([M+H]^+), 403.1978 ([M+Na]^+).

(R)-tert-butyl 2-(((diphenylmethylene)amino)propanamido)-3-phenylpropanoate

$^1$H-NMR (250.13 MHz, CDCl$_3$) δ 7.52 (d, 2H, J=2.5 Hz), 7.43-7.39 (m, 3H), 7.37-7.33 (m, 5H), 7.25-7.22 (m, 3H), 7.19 (s, 2H), 7.06 (dd, 2H, J=5 Hz), 4.77 (d, 1H, J=7.5 Hz), 3.93 (q, 1H, J=7.5 Hz), 3.11 (d, 2H, J=5 Hz), 1.30 (s, 9H), 1.22 (d, 3H, J=7.5 Hz) ppm; $^{13}$C-NMR (100.62 MHz, CDCl$_3$) δ 173.56, 170.27, 168.79, 139.05, 136.29, 135.67, 130.50, 129.77, 128.40, 128.14, 127.44, 126.93, 61.16, 53.07, 36.28, 27.98, 20.81 ppm; HRMS (ESI) calculated for C_{29}H_{32}N_{2}O_{3}: 457.2491 ([M+H]^+), found 457.2421 ([M+H]^+), 479.2231 ([M+Na]+).

(R)-tert-butyl 2-((2-((diphenylmethylene)amino)acetamido)-3-methylbutanoate

$^1$H-NMR (400.13 MHz, CDCl$_3$) δ 7.71-7.69 (m, 2H), 7.50-7.40 (m, 6H), 7.19-7.17 (m, 2H), 4.59 (q, 1H, J=8 Hz), 4.03 (d, 2H, J=4 Hz), 2.23-2.13 (m, 1H), 1.52 (s, 9H), 1.04 (d, 6H, J=8 Hz) ppm; $^{13}$C-NMR (100.62 MHz, CDCl$_3$) δ 170.78, 162.26, 132.42, 130.06, 128.88, 128.29, 127.31, 81.77, 57.19, 56.53, 31.64, 28.12, 19.03, 17.83 ppm; HRMS (ESI) calculated for C_{24}H_{30}N_{2}O_{3}: 395.2335 ([M+H]^+), found 395.2399 ([M+H]^+).

(S)-tert-butyl 2-((2-((diphenylmethylene)amino)acetamido)-4-methylpentanoate

$^1$H-NMR (400.13 MHz, CDCl$_3$) δ 7.70 (d, 2H, J=8 Hz), 7.49-7.40 (m, 6H), 7.17 (d, 2H, J=4 Hz), 4.70 (q, 1H, J=8 Hz), 4.00 (s, 2H), 1.75-1.69 (m, 2H), 1.52 (s, 9H), 1.02 (d, 6H, J=8 Hz) ppm; $^{13}$C-NMR (100.62 MHz, CDCl$_3$) δ 176.86, 171.66, 165.10, 139.29, 130.67, 128.98, 128.87, 128.49, 128.24, 127.27, 120.85, 56.54, 51.10, 43.12, 42.20, 28.06, 25.09, 22.32 ppm; HRMS (ESI) calculated for C_{25}H_{32}N_{2}O_{3}: 409.2491, found 409.2487 ([M+H]^+).

(S)-tert-butyl 2-((S)-2-((diphenylmethylene)amino)propanamido)-4-methylpentanoate

$^1$H-NMR (400.13 MHz, CDCl$_3$) δ 7.70 (d, 2H, J=8 Hz), 7.49-7.40 (m, 6H), 7.17 (d, 2H, J=4 Hz), 4.61 (q, 1H, J=8 Hz), 4.04 (q, 1H, J=8 Hz), 1.78-1.67 (m, 3H), 1.46 (s, 9H), 1.37 (d, 3H, J=8 Hz), 1.03 (d, 6H, J=4 Hz) ppm; $^{13}$C-NMR (100.62 MHz, CDCl$_3$) δ 173.86, 171.79, 169.02, 139.24, 135.76, 130.52, 128.69, 128.55, 128.19, 127.50, 81.53, 61.34, 51.13, 43.12, 42.02, 28.01, 25.11, 22.90, 22.39, 21.03 ppm; HRMS (ESI) calculated for C_{26}H_{34}N_{2}O_{3}: 423.2648, found 423.2641 ([M+H]^+).
(R)-tert-butyl 2-((S)-2-((diphenylmethylene)amino)-3-phenylpropanamido)-3-methylbutanoate

$^1$H-NMR (400.13 MHz, CDCl$_3$) δ 7.65 (d, 2H, $J$=8 Hz), 7.45-7.20 (m, 11H), 7.19 (m, 2H), 4.49-4.45 (m, 1H, $J$=8 Hz), 4.21-4.18 (m, 1H), 3.26-3.23 (m, 1H), 3.11-3.05 (m, 1H), 2.22-2.17 (m, 1H), 1.42 (s, 9H), 0.98 (d, 6H, $J$=8 Hz) ppm; $^{13}$C-NMR (100.62 MHz, CDCl$_3$) δ 195.70, 178.42, 172.28, 170.46, 170.15, 139.15, 137.84, 135.55, 130.46, 130.16, 128.60, 128.30, 128.11, 127.42, 126.30, 81.58, 67.83, 57.30, 43.12, 42.02, 41.70, 31.50, 28.00, 18.95, 17.85 ppm; HRMS (ESI) calculated for C$_{31}$H$_{36}$N$_2$O$_3$: 485.2804, found 485.2797 ($[M+H]^+$), 507.2612 ($[M+Na]^+$).

(R)-tert-butyl 2-((S)-2-((diphenylmethylene)amino)propanamido)-3-methylbutanoate

$^1$H-NMR (400.13 MHz, CDCl$_3$) δ 7.70 (d, 2H, $J$=4 Hz), 7.47-7.37 (m, 6H), 7.18 (m, 2H), 4.52-4.48 (m, 1H), 4.04 (q, 1H, $J$=4 Hz), 2.30-2.22 (m, 1H), 1.46 (s, 9H), 1.39 (d, 3H, $J$=4 Hz), 1.05 (t, 6H, $J$=4 Hz) ppm; $^{13}$C-NMR (100.62 MHz, CDCl$_3$) δ 170.03, 166.72, 164.95, 135.26, 131.84, 126.58, 124.75, 123.55, 77.66, 57.53, 53.08, 27.80, 24.12, 17.37, 15.08, 13.81 ppm; HRMS (ESI) calculated for C$_{25}$H$_{32}$N$_2$O$_3$: 409.2491 ($[M+H]^+$), found 409.2481 ($[M+H]^+$), 431.2299 ($[M+Na]^+$).

(S)-tert-butyl 2-((S)-2-((diphenylmethylene)amino)-3-phenylpropanamido)-4-methylpentanoate

$^1$H-NMR (400.13 MHz, CDCl$_3$) δ 7.63 (d, 2H, $J$=8 Hz), 7.52-7.20 (m, 10H), 7.09-7.07 (m, 3H), 4.58 (q, 1H, $J$=8 Hz, $J_2$=4 Hz), 4.21-4.18 (m, 1H), 3.23-3.19 (m, 1H), 3.08-3.05 (m, 1H), 1.69-1.66 (m, 3H), 1.43 (s, 9H), 1.00 (t, 6H, $J$=4 Hz) ppm; $^{13}$C-NMR (100.62 MHz, CDCl$_3$) δ 172.13, 170.33, 139.23, 137.85, 135.55, 132.43, 130.47, 130.14, 128.67, 128.30, 128.08, 127.46, 126.67, 81.49, 80.46, 67.69, 51.21, 41.75, 41.50, 42.02, 27.96, 25.00, 22.57, 22.27 ppm; HRMS (ESI) calculated for C$_{32}$H$_{38}$N$_2$O$_3$: 499.2961, found 499.2942 ($[M+H]^+$), 521.2757 ($[M+Na]^+$).

(R)-tert-butyl 2-((S)-2-((diphenylmethylene)amino)-3-phenylpropanamido)-4-methylpentanoate

$^1$H-NMR (400.13 MHz, CDCl$_3$) δ 7.65 (d, 2H, $J$=8 Hz), 7.44-7.16 (m, 12H), 7.04 (m, 2H), 6.37 (d, 2H, $J$=4 Hz), 4.64 (q, 1H, $J_1$=8 Hz, $J_2$=4 Hz), 4.19 (d, 1H), 3.28 (dd, 1H, $J_1$=8 Hz, $J_2$=4 Hz), 3.12 (t, 1H, $J$=12 Hz), 1.69-1.56 (m, 3H), 1.53 (s, 9H), 0.97 (t, 6H, $J$=4 Hz) ppm; $^{13}$C-NMR (100.62 MHz, CDCl$_3$) δ 188.38, 173.13, 172.31, 171.73, 169.82, 139.40, 138.00, 135.45, 130.49, 130.13, 128.64, 128.13, 127.31, 126.24, 81.71, 67.95, 51.16, 41.95, 41.46, 28.07, 25.16, 22.86, 22.26 ppm; HRMS (ESI) calculated for C$_{32}$H$_{38}$N$_2$O$_3$: 499.2961, found 499.2968 ($[M+H]^+$), 521.2757 ($[M+Na]^+$).
(R)-tert-butyl 2-((S)-2-((diphenylmethylene)amino)propanamido)-4-methylpentanoate

$^1$H-NMR (400.13 MHz, CDCl$_3$) $\delta$ 7.84 (d, 1H, $J$=8 Hz), 7.70 (d, 2H, $J$=4 Hz), 7.50-7.39 (m, 6H), 7.15 (d, 2H, $J$=8 Hz), 4.60 (q, 1H, $J$=8 Hz), 4.05 (q, 1H, $J$=8 Hz), 1.72-1.57 (m, 3H), 1.53 (s, 9H), 1.36 (d, 3H, $J$=8 Hz), 0.97 (d, 6H, $J$=4 Hz) ppm; $^{13}$C-NMR (100.62 MHz, CDCl$_3$) $\delta$ 173.75, 171.84, 168.53, 139.07, 135.70, 132.42, 130.56, 130.06, 128.75, 128.49, 128.22, 127.38, 81.62, 61.24, 51.19, 41.87, 28.07, 25.14, 22.84, 22.31, 20.69 ppm; HRMS (ESI) calculated for C$_{26}$H$_{34}$N$_2$O$_3$: 423.2648, found 423.2657 ([M+H]$^+$).

(R)-tert-butyl 2-((S)-2-((diphenylmethylene)amino)acetamido)-4-methylpentanoate

$^1$H-NMR (400.13 MHz, CDCl$_3$) $\delta$ 7.91 (d, 1H, $J$=8 Hz), 7.70 (d, 2H, $J$=8 Hz), 7.49-7.38 (m, 6H), 7.18 (d, 2H, $J$=8 Hz), 4.69 (q, 1H, $J$=8 Hz, $J_2$=4 Hz), 4.00 (s, 2H), 1.80-1.64 (m, 3H), 1.51 (s, 9H), 1.02 (d, 6H, $J$=8 Hz) ppm; $^{13}$C-NMR (100.62 MHz, CDCl$_3$) $\delta$ 171.96, 170.21, 153.26, 130.72, 129.02, 128.88, 128.26, 127.29, 81.71, 56.50, 51.12, 42.18, 28.06, 25.08, 22.87, 22.32 ppm; HRMS (ESI) calculated for C$_{25}$H$_{32}$N$_2$O$_3$: 409.2491, found 409.2502 ([M+H]$^+$).

(S)-tert-butyl 2-((S)-2-((diphenylmethylene)amino)-3-phenylpropanamido)propanoate

$^1$H-NMR (250.13 MHz, CDCl$_3$) $\delta$ 8.19 (d, 1H, $J$=7.5 Hz), 7.25-7.17 (m, 5H), 4.12 (q, 1H, $J$=5 Hz), 3.40 (q, $J$=5 Hz, 1H), 2.94 (dd, 1H, $J_1$=5 Hz, $J_2$=7.5 Hz), 2.58 (dd, 1H, $J_1$=5 Hz, $J_2$=7.5 Hz), 1.37 (s, 9H), 1.22 (dd, 3H, $J$=7.5 Hz) ppm; $^{13}$C-NMR (100.62 MHz, CDCl$_3$) $\delta$ 174.68, 172.27, 138.96, 129.82, 128.51, 126.59, 81.08, 56.15, 54.33, 48.52, 39.11, 28.04, 17.79 ppm; HRMS (ESI) calculated for C$_{29}$H$_{32}$N$_2$O$_3$: 457.2491 ([M+H]$^+$), found: 457.2416 ([M+H]$^+$).

(S)-tert-butyl 2-((S)-2-((diphenylmethylene)amino)-3-phenylpropanamido)-3-methylbutanoate

$^1$H-NMR (250.13 MHz, CDCl$_3$) $\delta$ 8.19 (d, 1H, $J$=7.5 Hz), 7.25-7.17 (m, 5H), 4.12 (q, 1H, $J$=5 Hz), 3.40 (q, 1H, $J$=5 Hz), 2.94 (dd, 1H, $J_1$=5 Hz, $J_2$=7.5 Hz), 2.58 (dd, 1H, $J_1$=5 Hz, $J_2$=7.5 Hz), 1.37 (s, 9H), 1.22 (dd, 3H, $J$=7.5 Hz) ppm; $^{13}$C-NMR (100.62 MHz, CDCl$_3$) $\delta$ 174.68, 172.27, 138.96, 129.82, 128.51, 126.59, 81.08, 56.15, 54.33, 48.52, 39.11, 28.04, 17.79 ppm; HRMS (ESI) calculated for C$_{31}$H$_{36}$N$_2$O$_3$: 485.2804 ([M+H]$^+$), found: 485.2715 ([M+H]$^+$).

(S)-tert-butyl 2-((S)-2-((diphenylmethylene)amino)-3-phenylpropanamido)-3-phenylpropanoate

$^1$H-NMR (250.13 MHz, CDCl$_3$) $\delta$ 8.19 (d, 1H, $J$=7.5 Hz), 7.25-7.17 (m, 5H), 4.12 (q, 1H, $J$=5 Hz), 3.40 (q, 1H, $J$=5 Hz), 2.94 (dd, 1H, $J_1$=5 Hz, $J_2$=7.5 Hz), 2.58 (dd, 1H, $J_1$=5 Hz, $J_2$=7.5 Hz), 1.37
(s, 9H), 1.22 (dd, 3H, \( J=7.5 \) Hz) ppm; \(^{13}\)C-NMR (100.62 MHz, CDCl\(_3\)) \( \delta \) 174.68, 172.27, 138.96, 129.82, 128.51, 126.59, 81.08, 56.15, 54.33, 48.52, 39.11, 28.04, 17.79 ppm; HRMS (ESI) calculated for \( \text{C}_{35}\text{H}_{36}\text{N}_{2}\text{O}_{3} \): 533.2804 ([M+H]+), found: 533.2712 ([M+H]+).

**Procedure for the removal of protecting groups of dipeptides**

The starting material was dissolved in 4M HCl in dioxane, stirred at room temperature for several hours and progress monitored by TLC and coloring of spots with ninhydrin. The samples were purified by semi-preparative chromatography resulting in a white solid in low yields.

**(S)-2-((R)-2-aminopropanamido)-3-methylbutanoic acid**

\(^{1}\)H-NMR (500.23 MHz, MeOD) \( \delta \) 4.13 (q, 1H, \( J=5 \) Hz), 2.27-2.24 (m, 1H), 1.57 (d, 3H, \( J=10 \) Hz), 1.00 (d, 6H, \( J=5 \) Hz) ppm; \(^{13}\)C-NMR (125.80 MHz, MeOD) \( \delta \) 174.28, 171.37, 68.07, 58.91, 50.19, 31.66, 19.61, 18.07 ppm; HRMS (ESI) calculated for \( \text{C}_{8}\text{H}_{16}\text{N}_{2}\text{O}_{3} \): 189.1239 ([M+H]+), found: 189.12626 ([M+H]+).

**(S)-2-((R)-2-amino-3-phenylpropanamido)-3-phenylproanoic acid**

\(^{1}\)H-NMR (500.23 MHz, (CD\(_3\))\(_2\)SO) \( \delta \) 8.23 (d, 1H, \( J=10 \) Hz), 7.29-7.12 (m, 10H), 4.41 (q, 1H, \( J=5 \) Hz), 3.44 (q, 1H, \( J_{1}=10 \) Hz, \( J_{2}=5 \) Hz), 3.30 (br s, 2H), 2.91 (m, 4H) ppm; \(^{13}\)C-NMR (125.80 MHz, (CD\(_3\))\(_2\)SO) \( \delta \) 174.34, 170.94, 138.91, 137.41, 129.78, 128.65, 128.52, 127.00, 126.55, 65.06, 56.21, 54.06 ppm; HRMS (ESI) calculated for \( \text{C}_{18}\text{H}_{20}\text{N}_{2}\text{O}_{3} \): 313.1552 ([M+H]+), found: 313.1623 ([M+H]+).

**Synthesis of phase-transfer Catalyst 2**

**Synthesis of N-(9-anthracenylmethyl)cinchoninium chloride – Cat 2**

Compound Cat 2 was prepared and characterized according to O'Donnell *et al.*\(^{[30]}\) and Corey *et al.*\(^{[31]}\). To a suspension of cinchonine (1.0 g, 3.4 mmol) in toluene (14 mL) was added 9-(chloromethyl)anthracene (770.0 mg, 3.7 mmol). The reaction mixture was stirred at reflux overnight. The mixture was then cooled to ambient T and poured onto 40 mL of diethyl ether and filtered to afford a light yellow solid (1.4 g, 2.8 mmol, 81 %). Smaller fraction of filtered solid (180 mg) was purified by flash chromatography (0–100 % MeOH/DCM) to yield a yellow solid (169 mg, 94 %).
$^1$H NMR (300.13 MHz, CDCl$_3$): δ 9.21 (d, 1 H, $J$=8.9 Hz), 8.91-8.85 (m, 2 H), 8.44 (d, 1 H, $J$=9.1 Hz), 8.19 (d, 1 H, $J$=4.2 Hz), 8.05 (d, 1 H, $J$=4.5 Hz), 7.88 (s, 1 H), 7.61-7.55 (m, 2 H), 7.48 (d, 1 H, $J$=8.3 Hz), 7.24-7.05 (m, 4 H), 7.00-6.89 (m, 3 H), 6.50 (d, 1H, $J$=13.4 Hz), 5.64-5.54 (m, 1 H), 5.03 (d, 1 H, $J$=10.5 Hz), 4.88 (d, 1 H, $J$=17.2 Hz), 4.72 (m, 1 H), 4.44 (m, 1 H), 4.25 (m, 1 H), 2.53-2.49 (m, 1 H), 2.34-2.30 (m, 1 H), 2.01-1.93 (m, 1 H), 1.75-1.66 (m, 2 H), 1.53 (s, 1 H), 1.25-1.21 (m, 2 H) ppm; HRMS (ESI) calculated for C$_{34}$H$_{33}$N$_2$O$^+$ 485.2593 ([M+H$^+$]); found 485.4012 ([M+H$^+$]).

**Synthesis of O-allyl-N-(9-anthracenylmethyl)cinchoninium bromide – Cat 2**

Compound Cat 2 was prepared and characterized according to O'Donnell *et al.*$^{[30]}$ To a suspension of compound Cat 2* (150.0 mg, 288.0 µmol) in DCM (2 mL) was added allyl bromide (78.0 µL, 684.0 µmol) and aqueous 50% KOH (0.2 mL). The resulting mixture was stirred vigorously at ambient T for 8 h, during which time all the solid dissolved. The mixture was diluted with water (10 mL) and extracted with dichloromethane (3x 15 mL). The combined organic fractions were dried over Na$_2$SO$_4$, filtered and concentrated to dryness in vacuo to give an orange solid. The solid was suspended in ice cold diethyl ether, placed in the freezer (-23 °C) for several hours and filtered to give crude product as a light orange solid (66.0 mg, 109 µmol, 38%).

$^1$H NMR (300.13 MHz, CD$_3$OD): δ 9.05 (d, 1 H, $J$=4.6 Hz), 8.90 (s, 1 H), 8.81 (d, 1 H, $J$=6.4 Hz), 8.58-8.49 (m, 1 H), 8.36 (d, 1 H, $J$=8.9 Hz), 8.29-8.20 (m, 3 H), 8.03-7.93 (m, 3 H), 7.84-7.62 (m, 4 H), 6.90 (s, 1 H), 6.16-6.11 (m, 1 H), 6.04-5.98 (m, 3 H), 5.72 (dd, 1 H, $J_1$=15.9 Hz, $J_2$=1.5 Hz), 5.62 (d, 1 H, $J$=13.4 Hz), 5.22 (d, 1 H, $J$=6.4 Hz), 5.08 (d, 1 H, $J$=17.2 Hz), 4.51-4.41 (m, 4 H), 4.20-3.92 (m, 1 H), 3.25-3.18 (m, 1 H), 2.86-2.60 (m, 2 H), 2.32-2.29 (m, 1 H), 1.88-1.23 (m, 4 H) ppm; $^{13}$C NMR (75.62 MHz, CD$_3$OD): δ 149.8, 148.1, 141.1, 136.2, 133.5, 133.4, 132.7, 131.9, 131.8, 131.7, 130.3, 130.2, 129.9, 129.3, 128.7, 128.1, 128.0, 125.4, 125.3, 125.0, 124.6, 124.1, 123.6, 123.2, 117.9, 116.7, 70.0, 65.7, 57.8, 56.0, 37.6, 26.2, 23.5, 22.1 ppm; HRMS (ESI): calculated for C$_{37}$H$_{37}$N$_2$O$^+$: 525.2906 ([M+H$^+$]); found 525.2837 ([M+H$^+$]).
RADIOCHEMISTRY

Radiochemical yield of the crude reaction mixture defined as radiochemical conversion (RCC), was determined by HPLC analysis as the percentage of converted \([^{11}\text{C}]\text{methyl iodide (}[^{11}\text{C}]\text{MeI)}\) or \([^{11}\text{C}]\text{benzyl iodide (}[^{11}\text{C}]\text{BnI)}\) to desired product (dipeptide intermediate) using an analytical HPLC method described in the radiochemistry section under each procedure and is based on the AUC of the radioactivity profile of the HPLC analysis.

Diastereomeric ratio (dr) is used to describe effectively the amount of one diastereomer (e.g. DL) to another (LL). Diastereomeric ratio of dipeptides was determined by HPLC analysis based on the AUC of the radioactivity profile of HPLC analysis of the protected dipeptide in the crude reaction mixture using an analytical HPLC column.

For the calculations of diastereomeric excess (de) following formula has been used:

\[
de (\%) = \left( \frac{(D-L)}{(D+L)} \right) \cdot 100; \quad D+L=1
\]

for preferential D-\([^{11}\text{C}]\text{alkylation}.

Radiochemical procedure for the production of \([^{11}\text{C}]\text{methyl iodide}

\([^{11}\text{C}]\text{CO}_2\) was produced by the \(^{14}\text{N}(p,\alpha)^{11}\text{C}\) nuclear reaction performed in a 0.5 % O\(_2\)/N\(_2\) gas mixture using IBA Cyclone 18/9 (IBA, Louvain-la Neuve, Belgium). \([^{11}\text{C}]\text{CO}_2\) was carried in a stream of helium and trapped in 0.1 mL of a 0.1M 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 57% hydriodic acid (HI) was added the \([^{11}\text{C}]\text{MeI}\) was distilled from the reaction vessel under a stream of helium (flow 20 mL/min) to the second reaction vessel for the alkylation reaction.\(^{32}\)

Radiochemical procedure for the production of \([^{11}\text{C}]\text{benzyl iodide}

\([^{11}\text{C}]\text{CO}_2\) was transferred with a helium flow of 10 mL/min through a nitrogen oxides trap and a P\(_2\)O\(_5\) column and into a 3 mL vial containing a solution of 100 μL of 1M phenylmagnesium bromide in tetrahydrofuran (THF) at 35 °C. After obtaining the maximum radioactivity in the vessel, the reaction mixture was stirred using helium flow (10 mL/min) for 1 min and an additional minute at an increased helium flow of 50 mL/min. 100 μL 1M LiAlH\(_4\) in THF was added and immediately evaporated to dryness by heating the reaction vial to 130 °C with a helium flow (50
mL/min) for 2 min until no bulk liquid was visible. Subsequently, 100 μL of 57% HI in water was
added at 0 °C and the mixture was left to react for 2 min at 120 °C to yield [11C]BnI. The reaction
mixture has been first cooled down to ambient T or -10 °C, dissolved in 1.0 mL of toluene and
subsequently passed through 5x0.5 cm column filled with sodium hydroxide, potassium carbonate
and magnesium sulfate (approx. 5/3/3 by volume) into a second reaction vial, ready to use for
further reactions.[15]


In a reaction vessel, Schiff base X (1.2-1.7 mg, 4-7 μmol), catalyst Y (1-2 mg, 2-3 μmol) and
CsOH·H2O (55-60 mg, 180-200 μmol) are suspended in toluene (100 μL). After distilling
[11C]MeI / addition of [11C]BnI in the reaction vial, the mixture is cooled to 0-10 °C and stirred for
5 min. A sample is taken for analysis, quenched/diluted with acetonitrile and injected on
Phenomenex Luna 5u RP18 column (250 x 4.5 mm, acetonitrile/buffer 1, gradient: 35 to 7 % buffer
1, 254 nm) with a retention time (Rt) of around 20 min for diastereomeric product Ph2-N[11C]Ala-
Xxx-OtBu or Ph2-N[11C]Phe-Xxx-OtBu (LD-product before DD, DL before LL). The deprotection
is initialized by the addition of 0.1 mL of 6M HCl solution and heating to 100 °C for 2 min. A
second sample is taken for analysis on chiral radioHPLC.
References


Chapter 5

Efficient synthesis of $^{11}$C-acrylesters, $^{11}$C-acrylamides and their application in Michael addition reactions for PET tracer development

Ulrike Filp, Anna L. Pees, Carlotta Taddei, Aleksandra Pekošak, Antony D. Gee, Albert D. Windhorst, Alex J. Poot

ABSTRACT

Here we present the novel Michael addition reaction utilizing carbon-11 labeled acrylic esters and carbon-11 labeled amides. Subsequently, these synthons are reacted with commercially available Schiff base precursors to produce $[^{11}\text{C}]$glutamate and $[^{11}\text{C}]$glutamine. This methodology is especially useful for the development of positron emission tomography imaging agents as it opens up a new array of potential carbon-11 labeled compounds with this original $^{11}\text{C}$-C bond forming strategy.
Introduction

Positron emission tomography\textsuperscript{11} (PET) is an important technique that allows the study of biological and physiological processes at the molecular level \textit{in vivo}. To perform PET imaging the availability of the desired PET tracers, compounds labeled with a positron emitting nuclide and administered in trace amounts to the study subject, is crucial. New radiochemistry methodology is needed to enable the development of novel PET imaging agents. Therefore, the development of synthesis methods for the introduction of positron-emitting radionuclides into molecules to yield PET agents is important and thus an active area of research. With respect to frequently used PET radionuclides, carbon-11 (100 % $\beta^+$, $t_{1/2} = 20.4$ min)\textsuperscript{2} is one of the most used for tracer development due to the ubiquity of carbon in all organic molecules. The challenge in working with carbon-11 is the short half-life and thus fast synthesis procedures are needed. The total synthesis time, including purification and formulation, should be limited to approximately 3 half-lives. Currently, most carbon-11 radiolabeling procedures performed are methylations by alkylation reactions with halogenated carbon-11 labeled reagents, or more activated $^{11}$C methyl triflate, further carboxylation reactions from $^{11}$C CO\textsubscript{2} or palladium-catalyzed $^{11}$C CO insertion reactions.\textsuperscript{3–5} Other carbon-11 labeling reactions are known, but hardly applied for PET tracer development.\textsuperscript{6}

In this paper we describe the potential of Michael addition reactions where the Michael acceptor is labeled with carbon-11. This reaction has been proposed by Antoni et al., however its potential in radiosynthesis has not been further demonstrated.\textsuperscript{7} To this end, we have synthesized functionalized $^{11}$C-acrylesters and $^{11}$C-acrylamides as versatile radiolabeling reagents and investigated their subsequent use as Michael acceptors, a novel radiochemical methodology to form $^{11}$C-C bonds and potentially novel PET imaging agents. Moreover, by the formation of a $^{11}$C-C bond, this reaction has the potential to form novel chiral centers, which is a unique challenge for radiochemistry development. As proof-of-concept, the focus was laid on the radiosyntheses of the amino acids (AAs) $^{11}$C glutamate and $^{11}$C glutamine by making use of the Michael addition with the corresponding $^{11}$C-acrylester and an activated glycine Schiff base precursor.
To synthesize the desired $^{11}$C-labeled synthons for the Michael additions, two methods have been investigated. The first approach to develop $^{11}$C-acrylesters made use of Grignard reactions between different vinyl magnesium halides and $[^{11}$C]$CO_2$, followed by quenching with a nucleophile to obtain the Michael acceptor acrylic acid 1$^8$ or the methyl acrylate 2 for further synthesis. Carbon-11 labeled acrylic acid and its methyl ester, 1 and 2, have been reported before, however only in low molar activity$^1$ ($A_M$). Furthermore, the application of these reagents was limited to 1,2-additions. The second approach implemented here is a Pd-mediated carbonylation reaction with $[^{11}$C]$CO$ to obtain acrylester 3 and acrylamide 4.$^{[9,10]}$ Another advantage, when applying the second methodology, is the low isotopic dilution when making use of $[^{11}$C]$CO$ compared to $[^{11}$C]$CO_2$, which in general yields higher $A_M$. The more widespread application of $[^{11}$C]$CO$ became available in 2013 when Eriksson et al. published a simplified procedure for the transfer of $[^{11}$C]$CO$ making use of xenon gas as well as Dahl et al. introducing xanthpos as trapping support agent, which caused a major breakthrough.$^{[10,11]}$ Moreover, nowadays also alternative procedures have been published to obtain $[^{11}$C]$CO$ from $[^{11}$C]$CO_2$ using simplified and straightforward methodologies.$^{[5,12-14]}


$^1$ Molar activity: measured radioactivity per mole of compound (GBq·μmol$^{-1}$) readjusted from specific activity.
As exemplified in Scheme 1, for the Michael addition with $^{11}$C-acrylesters, the reactivity and thus the versatility of these reagents was investigated by reaction with Schiff base glycine derivatives (5) as Michael donor. A functional AA is created being $^{[11]}$C[glutamate, $^{[11]}$C[glutamine or derivatives thereof by exploiting this Michael addition to the Schiff base. Michael addition reactions are also known as 1,4-additions, where the C-C bond-forming ability is employed traditionally by an enolate that reacts with an $\alpha,\beta$-unsaturated carbonyl compound (Scheme 2). An additional challenge for the Michael reactions explored in this paper is that the AAs formed are chiral molecules. For this reason the chiral induction during the Michael addition is explored by using phase-transfer catalysis (PTC). The asymmetric radiolabeling approach with highly specialized chiral catalysts is unique and highly valuable to radiolabel chiral compounds. To date, this methodology has been successfully described in radiosynthesis of $^{[11]}$C[alanine$^{[15]}$] and $^{[11]}$C[phenylalanine$^{[16]}$, as well as for dipeptides$^{[17]}$ and tetrapeptides$^{[18]}$. In addition, the asymmetric Michael addition$^{[19,20]}$ has been explored in $^{12}$C-organic chemistry, however this challenging radiosynthesis method is unprecedented in radiochemistry thus far.

In this paper we will describe the syntheses, optimization and application of $^{11}$C-acrylesters and $^{11}$C-acrylamides 1 – 4 as reagents to be applied in Michael addition reactions for the development of PET imaging agents as exemplified by products 6 – 11.

![Scheme 2](image)

**Scheme 2**: Proposed mechanism of a racemic alkylation of a Schiff base for AA synthesis.

**Results and Discussion**

**Radiosynthesis of $^{11}$C-acrylesters using a Grignard reaction**

First focus was to obtain $^{11}$C-acrylesters by performing a Grignard reaction between vinyl magnesium bromide, which is reacted with cyclotron produced $^{[11]}$CO$_2$ followed by quenching with a nucleophile to obtain $^{[11]}$C[acrylic acid or $^{[11]}$C[methyl acrylate.$^{[8,21]}$ The optimization led to
quantitative trapping of [$^{11}$C]CO$_2$ from the transfer lines in a solution of vinyl magnesium bromide as reagent in THF at low temperatures. A reaction was achieved by increasing the temperature to room temperature (rt) over a period of 2 min with a constant He-flow of 10 mL·min$^{-1}$. The reaction mixture was quenched with 2M HCl and this resulted in the radiosynthesis of [$^{11}$C]I as reagent to be used in Michael addition reactions. The radiochemical conversion to product in the crude reaction mixture, determined by analytical HPLC and defined as radiochemical purity (RCP) to compound [$^{11}$C]I was satisfying 50-70 %.

In addition to the radiosynthesis of [$^{11}$C]I, the reaction conditions have been modified as such that acrylic esters could be formed as well. Therefore, the magnesium acrylic acid formed in the Grignard reaction between vinyl magnesium bromide and [$^{11}$C]CO$_2$, was quenched with methanol and sulfuric acid and allowed the esterification of the product to obtain [$^{11}$C]2 with a RCP between 65-78 % determined by analytical HPLC. Purification from the crude reaction mixture was achieved by distillation at 90 °C into a cooled second reaction vial yielding highly purified [$^{11}$C]2. The average isolated radiochemical decay corrected (d.c.) yield obtained for this two-step reaction was 21-23 %, resulting in 0.43-0.47 GBq of product after 15 min synthesis time starting from 2.0 GBq [$^{11}$C]CO$_2$ (N=5).

**Radiosynthesis to obtain [$^{11}$C]tert-butylacrylate and [$^{11}$C]N-tritylacylamide**

To investigate the radiosynthesis of other functionalized $^{11}$C-acrylesters and to be able to obtain $^{11}$C-acrylamides, the focus shifted to Pd-catalyzed carbonylation reactions with [$^{11}$C]CO. To be able to perform these reactions, first the reduction from [$^{11}$C]CO$_2$ to [$^{11}$C]CO was performed according to the procedures described by Eriksson et al. and Van der Wildt et al.$^{[11,22]}$ Here [$^{11}$C]CO$_2$ was reduced to [$^{11}$C]CO by passing it online through a molybdenum filled column heated to 850 °C. The product was collected on a silica trap that was cooled in liquid N$_2$. Unreacted [$^{11}$C]CO$_2$ was removed by trapping it on an ascarite trap. For optimal transfer of [$^{11}$C]CO to the reaction vial, the silica trap was heated to release the [$^{11}$C]CO and with a gentle flow of xenon (3.0 mL·min$^{-1}$) the [$^{11}$C]CO was efficiently transferred to the previously charged and sealed reagent vial for carbonylation. Making use of the Pd-catalyzed carbonylation reactions with [$^{11}$C]CO, the radiosyntheses of [$^{11}$C]tert-butylacrylate 3 and [$^{11}$C]trityl-acylamide 4 were investigated.$^{[9]}$

As summarized in Table 1, initially the synthesis of acrylester [$^{11}$C]3 and acrylamide [$^{11}$C]4 were examined making use of Pd$_2$(dba)$_3$ as a catalyst in the presence of PPh$_3$ as supporting ligand.
However, only low conversions were observed using this strategy (Table 1: Entry 1-3, 6). The trapping efficacy of $[^{11}\text{C}]\text{CO}$ in THF supported with xantphos was more successful.$^{[10]}$ The optimal radiochemical conversion obtained to synthesize acrylester $[^{11}\text{C}]3$ and acrylamide $[^{11}\text{C}]4$ were established using [(Cinnamyl)PdCl]$_2$ as a catalyst$^{[23]}$ (Table 1: Entry 4-5, 9) and the RCP for either synthon was $>75\%$ and amounted to isolated radiochemical yield of $[^{11}\text{C}]3$ to 0.41-0.45 GBq (18-20 % d.c. yield, 25 min, N=2) and 0.42-0.44 GBq of $[^{11}\text{C}]4$ (22-23 % d.c. yield, 25 min, N=3) from 2.5 GBq of $[^{11}\text{C}]\text{CO}_2$.

Table 1: RCPs for the synthesis of $[^{11}\text{C}]3$ and $[^{11}\text{C}]4$ with $[^{11}\text{C}]\text{CO}$.

<table>
<thead>
<tr>
<th>#$^a$</th>
<th>Product</th>
<th>Vinyl halide (µmol)</th>
<th>Pd-source (µmol)</th>
<th>Ligand (µmol)</th>
<th>RCP ± SD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1$^b$</td>
<td>$[^{11}\text{C}]3$</td>
<td>Vinyl bromide (20)</td>
<td>[Pd$_2$(dba)$_3$] (2.4)</td>
<td>PPh$_3$ (20)</td>
<td>45.0 ± 5.1 (N=2)</td>
</tr>
<tr>
<td>2</td>
<td>$[^{11}\text{C}]3$</td>
<td>Vinyl bromide (65)</td>
<td>[Pd$_2$(dba)$_3$] (10)</td>
<td>PPh$_3$ (20)</td>
<td>11.2</td>
</tr>
<tr>
<td>3</td>
<td>$[^{11}\text{C}]3$</td>
<td>Vinyl bromide (10)</td>
<td>[Pd$_2$(dba)$_3$] (1.2)</td>
<td>PPh$_3$ (10)</td>
<td>2.0</td>
</tr>
<tr>
<td>4</td>
<td>$[^{11}\text{C}]3$</td>
<td>Vinyl iodide (10)</td>
<td>[(Cinnamyl)PdCl]$_2$ (7)</td>
<td>AsPh$_3$ (56)</td>
<td>80.2 ± 5.2 (N=2)</td>
</tr>
<tr>
<td>5</td>
<td>$[^{11}\text{C}]4$</td>
<td>Vinyl iodide (10)</td>
<td>[(Cinnamyl)PdCl]$_2$ (7)</td>
<td>Xanthpos (7)</td>
<td>79.0 ± 10.0 (N=15)</td>
</tr>
<tr>
<td>6$^c$</td>
<td>$[^{11}\text{C}]4$</td>
<td>Vinyl bromide (20)</td>
<td>Pd$_2$(dba)$_3$ (2.4)</td>
<td>PPh$_3$ (20)</td>
<td>14.3 ± 2.1 (N=2)</td>
</tr>
<tr>
<td>7$^d$</td>
<td>$[^{11}\text{C}]4$</td>
<td>Vinyl iodide (10)</td>
<td>[(Cinnamyl)PdCl]$_2$ (7)</td>
<td>AsPh$_3$ (56)</td>
<td>75.1 ± 1.2 (N=2)</td>
</tr>
<tr>
<td>8$^c$</td>
<td>$[^{11}\text{C}]4$</td>
<td>Vinyl iodide (10)</td>
<td>[(Cinnamyl)PdCl]$_2$ (7)</td>
<td>Xanthpos (7)</td>
<td>9.0 ± 6.2 (N=2)</td>
</tr>
<tr>
<td>9$^d$</td>
<td>$[^{11}\text{C}]4$</td>
<td>Vinyl iodide (10)</td>
<td>[(Cinnamyl)PdCl]$_2$ (7)</td>
<td>Xanthpos (7)</td>
<td>73.0 ± 5.0 (N=10)</td>
</tr>
</tbody>
</table>

$^a$Reaction performed in THF 450 µL for 3-5 min at 100 ºC; $^b$t-BuOH: Entry 1-3 2600 µmol; Entry 4-5: 2100 µmol; $^c$Tritylamine 20 µmol; $^d$Tritylamine 96 µmol.

Successful $[^{11}\text{C}]\text{CO}$ insertion reactions are dependent on the use of high concentrations of the nucleophile, tert-butanol (t-BuOH) for the radiosynthesis of $[^{11}\text{C}]3$ and tritylamine for $[^{11}\text{C}]4$. The
high amount of t-BuOH used is due to its technical handling losses, and its ability as solvent. For the radiosynthesis of acryl amides, solid tritylamine was used. Since this cannot be added in such great excess, lower quantities (Table 1: Entry 6; 8) were used but proved to be low yielding, therefore minimal amount of 96 μmol was found to be required in the reaction.

The $A_M$ of product $[^{11}\text{C}]4$ determined after preparative purification was high and ranged from 86-170 GBq·µmol$^{-1}$ starting with an activity of approximately 2.5 GBq of $[^{11}\text{C}]\text{CO}_2$. Also $[^{11}\text{C}]3$ was isolated on preparative scale with a minimum calculated $A_M$ of 53.4 GBq·µmol$^{-1}$; however, due to the low UV absorbance of $[^{11}\text{C}]3$ no $A_M$ with acceptable precision could be determined experimentally.

$^{11}\text{C}$-acrylates, however proved to be unstable, which was investigated by radioHPLC while mimicking the reaction conditions of the Michael addition in the presence of high amounts of base like CsOH·H$_2$O.$^{[15,24]}$ The radiosynthesis of synthons $[^{11}\text{C}]3$ and $[^{11}\text{C}]4$ was performed according to the optimized procedure and subsequently the synthons were added to various amounts of CsOH·H$_2$O (2.0-8.9 M) in toluene. The samples were left for 5 min at rt and at 100 ºC before the analysis, results are listed in Table 2.

**Table 2: Stability of $[^{11}\text{C}]3$ and $[^{11}\text{C}]4$ with CsOH·H$_2$O at rt and 100 ºC.**

<table>
<thead>
<tr>
<th></th>
<th>T (ºC)</th>
<th>CsOH·H$_2$O (M)</th>
<th>$[^{11}\text{C}]3$ (% intact)</th>
<th>$[^{11}\text{C}]4$ (% intact)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20</td>
<td>2.0</td>
<td>71.6 ± 4.5</td>
<td>82.0 ± 2.0</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>8.9</td>
<td>31.0 ± 3.2</td>
<td>79.5 ± 6.5</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>/</td>
<td>79.0 ± 10.0</td>
<td>73.0 ± 5.0</td>
</tr>
<tr>
<td>4</td>
<td>100</td>
<td>2.0 or 8.9</td>
<td>0</td>
<td>43.0</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>/</td>
<td>76.2 ± 3.0</td>
<td>72.5 ± 3.0</td>
</tr>
</tbody>
</table>

$^[a]$Reaction performed in 100 μL of toluene; N=2.

The major decomposition product of $[^{11}\text{C}]3$ is $[^{11}\text{C}]$acrylic acid, which was confirmed by HPLC. If the solution was heated to 100 ºC for 5 min no $[^{11}\text{C}]3$ could be detected at the end of the reaction. In contrast, $[^{11}\text{C}]4$ showed minimal decomposition at rt, also in the presence of high amounts of CsOH·H$_2$O. The stability, even at higher temperatures, is better compared to $[^{11}\text{C}]3$ and still 43 % were intact after 5 min. This can be explained by the resonance stability of the amide which is
stronger compared to esters. Notwithstanding these limitations, with the carbon-11 labeled acrylates 1 – 4 in hand the potential of these reagents was explored in Michael addition reactions.

**Michael addition reaction**

Initially, the Michael reactions (Scheme 3) were performed without intermediate purification of the carbon-11 labeled acrylic derivatives; however, no conversion to Michael adducts was observed. Since it was expected that the impurities in the crude reaction mixture obstructed successful Michael additions, various purifications of the $^{11}$C-acrylates were explored. Purification of $[^{11}\text{C}]2$ was simply achieved by distillation. Unfortunately, this approach was not successful for $[^{11}\text{C}]1$, $[^{11}\text{C}]3$ and $[^{11}\text{C}]4$. Solid-phase purification of the latter compounds were evaluated utilizing C18 and Alumina N SepPaks with a PTFE-filter, but were unsuccessful. Finally, passing the reaction mixture over custom made cartridge loaded with approximately 200 mg of celite afforded $[^{11}\text{C}]3$ and $[^{11}\text{C}]4$ in sufficient purity to allow its use in Michael reactions.

**Racemic Michael addition with synthons $[^{11}\text{C}]1$ and $[^{11}\text{C}]2$**

After optimizing the radiosynthesis procedures to obtain the $^{11}$C-acrylesters, $[^{11}\text{C}]1$ was used as a Michael acceptor with the Schiff base precursor 5 as donor. Whereas acidic conditions were used for the hydrolysis of the magnesium-acrylester salts to obtain $[^{11}\text{C}]1$, an initial challenge was changing to basic conditions, which are needed for the second reaction, the C-C bond forming to the glycine moiety. In acidic reaction conditions, the Schiff base enolate cannot be formed and the reaction of the donor to the acceptor will not proceed. Likewise, no reaction occurred in highly basic conditions leaving unreacted 5, which is likely to be caused by the low acceptor ability of $[^{11}\text{C}]1$.

However, utilizing distilled $[^{11}\text{C}]2$, the Michael addition reaction yielding $[^{11}\text{C}]7$ was successful, as described in Scheme 3. For evaluation of the reactivity of $[^{11}\text{C}]2$, reactions were successfully performed with 5 in DMSO and TBAF (tetrabutyl ammonium fluoride) as base, reaction conditions that have also been used by Kato et al.\textsuperscript{25} to alkylate 5. The reaction was successful with overall conversion yields of up to 90 % determined by analytical radioHPLC. In addition to TBAF as an organic base, inorganic alkali-metal bases were explored to deprotonate 5 and perform the Michael additions. Alkali-metal bases were selected due to their widespread
application in asymmetric syntheses.\textsuperscript{[24]} However, hydrolysis of \(^{11}\text{C}2\) was mainly observed with CsOH·H\textsubscript{2}O or KOH. Evidently, this competing reaction turned out to be faster than the Michael addition reaction, resulting in a drop in yields. Nonetheless, 10-15 \% RCP of \(^{11}\text{C}10\) were obtained, assessed by chiral radioHPLC after the acidic deprotection of the imine and tert-butylester.

![Scheme 3: Michael addition reaction with synthon 1-4 to obtain Michael addition products.](attachment:scheme_3.png)

These results further support the suitability of Michael addition reactions for the advanced carbon-11 labeled synthesis of PET imaging agents. The applicability of this reaction has certain limitations. For instance, the reaction to obtain \(^{11}\text{C}6\) did not occur and the reaction to obtain \(^{11}\text{C}7\) was only possible with TBAF as a base. In order to overcome these issues, the research was further focused on the synthesis of \(^{11}\text{C}8\) and \(^{11}\text{C}9\), utilizing \(^{11}\text{C}3\) and \(^{11}\text{C}4\) that are evidently more stable under previously described reaction conditions.

**Racemic Michael addition with synthons \(^{11}\text{C}3\) and \(^{11}\text{C}4\)**

The concentration of precursor 5, reaction time, type and amount of base and reaction temperature were initially optimized for \(^{11}\text{C}8\) and \(^{11}\text{C}9\) in order to obtain high \(^{11}\text{C}\)-C addition yields. The effect of the precursor concentration was also investigated, using TBAF as a base. An increased amount of Schiff base 5 ranging between 170-330 mM was necessary to obtain higher RCPs. These high concentrations are attributed to the decomposition of 5 to benzophenone and glycine tert-butylester. Alkylation of 5 with \(^{11}\text{C}3\) according to procedures earlier described\textsuperscript{[25,26]} was successful and yielded RCPs of 93.2 ± 5.7 \% (N=5) and 78.1 ± 3.0 \% (N=3) for \(^{11}\text{C}8\) and \(^{11}\text{C}9\) with TBAF (0.33 mM) in DMSO at 100 ºC for 5 min, respectively.
Solid inorganic bases for Michael reaction

As stated previously, alkali-metal bases are preferred in chiral PTC reactions, consequently various inorganic alkali-metal bases (Table 3), which are described in literature have been screened for their alkylation potential in toluene at 10 °C, thus far optimal conditions for chiral reactions.\(^{[15]}\) Generally, lower temperatures are favored in PTC reactions to enhance stereoselectivity, therefore we focused with inorganic bases on lower temperatures. Due to a solid-liquid phase reaction taking place, a sufficient interfacial area between the two phases needs to be created by vigorous stirring.

**Table 3:** Michael alkylation reactions with different bases to obtain \([^{11}]C\)9.

<table>
<thead>
<tr>
<th>#(^{[a]})</th>
<th>Base</th>
<th>Equiv.(^{[b]})</th>
<th>RCP ± SD (%)</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>KOH</td>
<td>5.9</td>
<td>&lt; 2</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>KOH</td>
<td>10.5</td>
<td>&lt; 2</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>P1-tBu</td>
<td>5.0</td>
<td>&lt; 1</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>RbOH</td>
<td>9.8</td>
<td>&lt; 2</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>RbOH</td>
<td>14.0-19.0</td>
<td>&lt; 5</td>
<td>4</td>
</tr>
<tr>
<td>6</td>
<td>RbOH(^{[c]})</td>
<td>14.0-19.0</td>
<td>21.1 ± 5.8</td>
<td>2</td>
</tr>
<tr>
<td>7</td>
<td>CsOH·H(_2)O</td>
<td>10.5</td>
<td>51.2 ± 30.0</td>
<td>9</td>
</tr>
</tbody>
</table>

\(^{[a]}\)Reaction performed with 170 mM 5 in toluene at 10 °C for 5 min; \(^{[b]}\)Compared to 5; \(^{[c]}\)Reaction performed at 50 °C.

From these experiments it was concluded that CsOH·H\(_2\)O as base in the reaction is superior over other alkali-metal bases investigated. Furthermore, the relative small variation in equivalents – also due to strong hygroscopicity of inorganic bases used, did not influence the outcome of the reaction to product (Table 3: Entry 1-2, 4-5). Remarkably, rather modest conversions were observed, in contrast to previously reported results in organic chemistry.\(^{[19,27,28]}\) This is presumably caused by the reaction time, since in carbon-11 radiosynthesis only rapid reactions can be considered due to the half-life of 20 min, whereas in organic chemistry longer reaction times are feasible.
Kinetic analysis to obtain $[^{11}\text{C}]9$

To establish an improved procedure for the production of $[^{11}\text{C}]9$, a kinetic analysis was performed. Samples were taken from the start of reaction until 5 min, which was confirmed as optimal reaction time (Figure 1 A).

Figure 1: (A) Kinetic analysis of the formation of $[^{11}\text{C}]9$ as function of reaction time, assessed with HPLC; (B) Exemplified radiochromatogram of the crude reaction mixture after 5 min.

By utilizing radio-TLC over HPLC we have monitored the entire reaction to overcome the breakdown of aqueous sensitive intermediates, thereby confirming the stability and availability of the Schiff base. We observed precursor 5 consistently during the reaction, however also the presence of the decomposition product benzophenone was detectable. Despite of the decomposition, seemingly there was enough precursor 5 available for the Michael addition and benzophenone did not disturb this reaction. It was important to bear in mind to add precursor 5 as late as possible to the reaction mixture for the Michael addition. Furthermore, 50-100 nmol (low μM) of labeling reagent is typically used, resulting in large excess of non-radiolabeled reagents which are typically in the μmol (mM) range. These non-stoichiometric reaction conditions can result in the operation of pseudo-first order kinetics with respect to the non-labeled reagents employed. In spite of the fact that the amount of $^{11}\text{C}$-labeled reagent is fairly stable, the synthons were steadily decomposing as well, resulting in variations in RCP. However, once product $[^{11}\text{C}]8$ or $[^{11}\text{C}]9$ were formed, they were stable.

So far a 10-fold excess of base (CsOH·H$_2$O) to Schiff base precursor 5 was used in this type of reactions$^{[15]}$ and since $[^{11}\text{C}]4$ is more stable with higher quantities of base, the Michael addition reactions were investigated with elevated base concentrations, see Table 4. Increasing the
amount of base resulted in significantly lower yields which most probably is caused by
decomposition of $[{^{11}}\text{C}]\text{4}$. Regarding the synthesis of $[{^{11}}\text{C}]\text{8}$, stability experiments already showed
that higher amounts of base led to partial decomposition of reagent $[{^{11}}\text{C}]\text{3}$, so further experiments
were abandoned. To conclude, 10.5 equivalents of base relative to $\text{5}$ gave the best results.

Table 4. Radiosynthesis of $\text{[}^{11}\text{C}]\text{9}$ with varying amounts of CsOH·$\text{H}_2\text{O}$.

<table>
<thead>
<tr>
<th>#\textsuperscript{[a]}</th>
<th>CsOH·$\text{H}_2\text{O}$ equiv.\textsuperscript{[b]}</th>
<th>RCP (%)</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10.5</td>
<td>51.2 ± 30.0</td>
<td>9</td>
</tr>
<tr>
<td>2</td>
<td>15.3</td>
<td>34.4 ± 13.2</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>20.9</td>
<td>30.6 ± 12.7</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>105</td>
<td>33.8 ± 6.1</td>
<td>4</td>
</tr>
</tbody>
</table>

\textsuperscript{[a]}Reaction performed with 170 mM of $\text{5}$ in toluene at 10 ºC for 5 min; \textsuperscript{[b]}Compared to $\text{5}$.

Reactions of most suitable synthons with phase-transfer catalysts

![Chemical structures](image)

Figure 2: Chiral PTCs explored in PTC reactions for Michael additions: quaternary ammonium salt 12 and 13; tartare-derived catalyst 14 and 15; Brønsted base 16.

In order to induce an enantioselective Michael addition reaction, the application of chiral PTCs
was investigated as described in literature in organic chemistry.\textsuperscript{[19,24,29]} With regard to the
constraints presented in radiochemistry like time as limiting factor, as well as the non-catalytic
reaction conditions in which the chiral catalysts are used in stoichiometric amounts compared to
the precursor material and in excess of the $^{11}\text{C}$-labeled reagent, the translation of PTC reaction
conditions to radiochemistry has been challenging.\textsuperscript{[15,16,18]}
Scheme 4: Asymmetric Synthesis of Michael addition products.

In Table 5, results are summarized of asymmetric synthesis with the various catalysts that were commercially available.\textsuperscript{[15,16]}

Table 5: RCP (in % ± SD, N ≥ 2) of alkylation with synthons to obtain \([^{11}\text{C}]\text{8}\) and \([^{11}\text{C}]\text{9}\).

<table>
<thead>
<tr>
<th>#\textsuperscript{[a]}</th>
<th>Product</th>
<th>Temp (°C)</th>
<th>Cat 12</th>
<th>Cat 13</th>
<th>Cat 14</th>
<th>Cat 15</th>
<th>Cat 16</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>([^{11}\text{C}]\text{8})</td>
<td>20</td>
<td>31.1 ± 29.1</td>
<td>n.d.</td>
<td>23.2 ± 16.5</td>
<td>1</td>
<td>n.d.\textsuperscript{[b]}</td>
</tr>
<tr>
<td>2</td>
<td>([^{11}\text{C}]\text{8})</td>
<td>10</td>
<td>32.1 ± 21.1</td>
<td>28.8 ± 0.4</td>
<td>44.1 ± 21.4</td>
<td>42.6 ± 6.7</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>([^{11}\text{C}]\text{9})</td>
<td>20</td>
<td>31.0 ± 7.2</td>
<td>5.4 ± 2.8</td>
<td>37.6 ± 25.3</td>
<td>n.d.</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>([^{11}\text{C}]\text{9})</td>
<td>15</td>
<td>18.4</td>
<td>16.5 ± 4.4</td>
<td>5.3 ± 2.9</td>
<td>n.d.</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>([^{11}\text{C}]\text{9})</td>
<td>10</td>
<td>16.3 ± 13.3</td>
<td>33.8 ± 6.1</td>
<td>15.5 ± 13.4</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{[a]}Reaction performed with 170 mM of \(5\); 10.5 equiv. of CsOH·H\textsubscript{2}O in toluene with 0.1 mol% of Cat at 10 °C for 5 min with vigorous stirring; \textsuperscript{[b]}n.d. not determined.

With RCPs not exceeding 50 % all reactions resulted in moderate conversions for both \([^{11}\text{C}]\text{8}\) and \([^{11}\text{C}]\text{9}\) (Table 5), however with lower temperature the conversions were slightly higher. Tartrate-derived catalysts \(14\) and \(15\) resulted in good enantiomeric ratios in Michael addition reactions in organic chemistry\textsuperscript{[20][30]} and this was also observed under radiochemistry conditions. Catalysts \(12\) and \(13\) gave unsatisfactory results, hardly any enantiomeric enrichment was observed in contrary to results obtained for previous studies to obtain L-[\(^{11}\text{C}\)]alanine.\textsuperscript{[15]} Furthermore, during the conduct of the study Bander et al. introduced the concept of using a chiral Brønsted base \(16\) capable of catalyzing proton transfer reactions enantioselectively.\textsuperscript{[31]} Unfortunately, it was not possible to reproduce these results under radiochemistry conditions. Ultimately, the difference in the asymmetric radiochemistry reactions presented so far in literature, is the formation of a new C-C
bond, which is an alkylation reaction with a reactive alkylating agent and in the latter an 1,4-addition with constraints concerning availability of $^{11}$C-reagent and stability issues.

The enantiomeric ratio obtained with catalyst 14 (Figure 3) is presented in Table 6. These were determined with chiral HPLC of the resulting AAs after complete deprotection with 0.1 mL of 6M HCl and heating to 100 ºC for 2 min.

![Figure 3: Analysis of L-and D-$^{11}$C10 with chiral radioHPLC.](image)

Table 6: Enantiomeric ratio (in %, ± SD, N ≥ 2) of L-and D-$^{11}$C10 with Cat 14.

<table>
<thead>
<tr>
<th></th>
<th>Equiv. of 14</th>
<th>L-$^{11}$C]Glu</th>
<th>D-$^{11}$C]Glu</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>/</td>
<td>51 ± 3</td>
<td>49 ± 3</td>
</tr>
<tr>
<td>2</td>
<td>0.1</td>
<td>60 ± 5</td>
<td>40 ± 5</td>
</tr>
<tr>
<td>3</td>
<td>0.5</td>
<td>61 ± 4</td>
<td>39 ± 4</td>
</tr>
</tbody>
</table>

[a]Reactions performed with 170 mM of 5 in 100 μL of toluene with 12-14 equiv. CsOH·H$_2$O at 10 ºC for 5 min.

The enantiomeric ratios are lower compared to traditional $^{12}$C-organic chemistry procedures, where longer reaction times are possible, furthermore lower temperatures are applied to obtain excellent enantiomeric ratios. From Table 6 it can be concluded that the catalyst positively influenced the ratio towards the desired enantiomer, compared to no catalyst in the reaction mixture (Table 6: Entry 1). Nevertheless, it was not possible to obtain satisfactory enantiomeric ratios for the synthesis of $^{11}$C10. Therefore, studies to obtain enantomerically enriched $^{11}$C11 with synthon $^{11}$C4 were not explored. Unarguably, the asymmetric syntheses with presented catalysts have not been as successful and we believe the distorted conditions led to the catalyst-
enolate complex not forming properly. Regardless, a new methodology for the formation of a covalent $^{11}$C-C bond has been explored and presents new strategies for AA synthesis.

Furthermore, a reason for the variation in RCP might be the Pd-ligand complex, utilized in the $[^{11}\text{C}]\text{CO}$ insertion reaction, which cannot be completely removed from the reaction via the celite purification. As a control experiment, we have synthesized $[^{11}\text{C}]]\text{alanine}^{[15]}$ and added the same Pd-ligand complex to the phase-transfer catalyst alkylation reaction in approximately the same amount. The addition of the Pd-complex caused a complete depletion of yield compared to the original conditions, thereby proving the negative influence of the Pd-complex on the alkylation reaction. Presumably the phase-transfer catalyst and the Pd-complex are not compatible and hindering the reaction by either not activating 5 to form the enolate or/and cause de-activation of catalyst.

**Conclusion**

Here we have demonstrated for the first time the feasibility of Michael addition reactions in radiochemistry, where the Michael acceptor was labeled with carbon-11 after reaction with an acrylate. The Michael addition creates new possibilities for the synthesis of carbon-11 labeled PET tracer candidates. Further research is ongoing for the enantioselective Michael addition radiosynthesis.
Material and Methods

General

All chemicals were purchased from commercial sources (Sigma Aldrich (Zwijndrecht, The Netherlands), Bachem (Bubendorf, Switzerland), ABCR (Karlsruhe, Germany), Santa Cruz Biotechnology (Bio-Connect B.V., Huissen, the Netherlands)) and used without further purification. Solvents were purchased from Biosolve (Valkenswaard, the Netherlands) and used as received unless stated otherwise. Reactions were monitored by thin layer chromatography on pre-coated silica 60 F254 aluminum plates (Merck, Darmstadt, Germany). Spots on TLC were visualized by UV light and staining with ninhydrine and potassium permanganate solutions. Solvents were evaporated under reduced pressure using a rotary evaporator (Rotavapor® R II, Flawil, Switzerland). Flash chromatography purifications were performed on a Buchi operated by SepacoreControl system. Non-radioactive reference compounds were synthesized according to reported methods and were used to verify the identity of the radiolabeled products.

$^1$H and $^{13}$C Nuclear magnetic resonance (NMR) spectra were obtained using a Bruker AC 500.23, 400.13 or 250.13 MHz (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 MeOH-d$_4$, 2.50 ppm for DMSO-d$_6$). 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). 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 (Germany). The radioactive profile was monitored with a Raytest 2.5 inch radioactivity detector (Raytest, Germany). Small synthons $[^{11}$C]1-4 were analyzed with a Grace Smart C18 HPLC column obtained from Grace Alltech (4.6x250 mm, 5 µm) with acetonitrile/sodiumformate 4 mM + 4 % DMF (buffer 1) (68/32, v/v) as eluent unless stated otherwise. Michael adducts $[^{11}$C]6-9 were measured on a Great Smart (4.6x250 mm, 5 µm; formerly known as Grace Smart, Dr. Maisch, Ammerbuch, Germany) with acetonitrile/buffer 1 (65/35, v/v) or as stated otherwise, UV monitoring at 254 nm. Flow rates for all HPLC analysis were 1 mL·min$^{-1}$ unless stated otherwise. HPLC preparative purification of synthons $[^{11}$C]3 and $[^{11}$C]4 was performed on Alltima C18 (22x250 mm, 10 µm) with acetonitrile/buffer 1 70/30 (v/v) with a flow of 4 mL·min$^{-1}$. Enantiomeric purity of the products $[^{11}$C]10-11 was determined using an analytical Reprosil chiral-AA (4.6x250 mm) from Dr. Maisch GmbH at 214 nm. The product
was eluted with methanol/water (70/30 or 90/10, v/v). Enantiomeric ratio of D- or L-[11C]glutamate or D- or L-[11C]glutamine was determined by HPLC analysis of the free amino acid in the crude reaction mixture using a chiral column.

Radiochemical conversion was determined by HPLC analysis as the percentage of converted carbon-11 labeled reagent to the desired product in the crude reaction mixture, which is based on the AUC of the radioactivity profile, using analytical HPLC and expressed here as Radiochemical purity (RCP). Radiochemical yield (RCY) is the amount of radioactivity in the product expressed as the percentage of related starting radioactivity used in the corresponding synthesis. RCY was calculated as the quotient of measured activity of the isolated product at the end of synthesis (EOS) and the measured activity at the end of the cyclotron bombardment (EOB) in the vessel at the beginning of the synthesis, both measured in a dose calibrator, and expressed as a percentage. Radiochemical yield has been corrected for decay from EOB, no other corrections for radioactivity losses have been made.

Molar activity (AM) of the radioactive intermediates (e.g. [11C]4) after preparative HPLC purification was determined by measurement of the UV absorbance of a known amount of radioactivity under identical analytical HPLC conditions used to generate a molar calibration curve for the corresponding non-radioactive standard.

**Experimental Section**

**Synthesis of reference compounds**

*Tritylacrylamide*[^32]

Acrylamide (500.0 mg, 7.0 mmol), triphenylmethanol (964.0 mg, 3.7 mmol) and para-toluenesulfonic acid monohydrate (444.0 mg, 2.3 mmol) were refluxed in 100 mL of toluene. After 4 hours, the solution was cooled to room temperature and quenched with 50 mL of 2 % aqueous sodium bicarbonate solution. The aqueous phase was extracted with ethyl acetate (3x 50 mL) and the combined organic extracts were washed with water and brine. After drying over sodium sulfate (Na₂SO₄) and evaporating the solvent, the product was purified on a silica column (hexane/ethyl acetate, first 5:1 then 1:1) affording 935.0 mg (3.0 mmol, 81 %) of colorless crystals.
**Tert-butyl 2-((diphenylmethyleneamino)-5-oxo-5-(tritylamino)pentanoate**

*N-(Diphenylmethyleneglycine tert-butylester* (100 mg, 0.34 mmol) and *N*-tritylacrylamide (300 mg, 0.96 mmol) were dissolved in 3 mL of dimethylsulfoxide (DMSO). Additionally, 1 mL of 1 M solution of TBAF in tetrahydrofuran (THF) was added and the dark red solution was stirred for two hours at room temperature. After being diluted with 60 mL of diethyl ether, the organic phase was washed with H₂O (2 x 20 mL), brine (40 mL), dried over Na₂SO₄ and the solvent was removed under reduced pressure. The crude product was purified by silica chromatography twice (1) 5-16 % ethyl acetate/hexane, (2) 0-15 % MeOH/DCM in 20 min, which afforded 48.0 mg of a light yellow solid (0.08 mmol, 23 %).

**1H-NMR (500.13 MHz, CDCl₃): δ 7.34-7.23 (m, 15H, 3x –Ph), 6.27 (dd, 2H, J=5.0, 2.5 Hz, =CH₂), 5.67 (dd, 1H, J=5.0, 2.5 Hz, –CH) ppm; ¹³C-NMR (125.62 MHz, CDCl₃): δ 164.45, 144.55, 131.43, 128.72, 128.03, 127.13, 70.66 ppm; HRMS (ESI) calculated for C₂₃H₁₉NO: 314.1545 [(M+H)]⁺, found: 314.1520 [(M+H)]⁺, 336.1343 [(M+Na)]⁺.

**Tert-butyl 2-((diphenylmethyleneglycine tert-butylester** (200.0 mg, 0.7 mmol) and the phase transfer catalyst 12 (40.0 mg, 0.03 mmol) were dissolved in 2 mL DCM and 0.4 mL of 50 % (w/w) potassium hydroxide solution was added. *Tert*-butyl acrylate (87.0 mg, 0.7 mmol) was added dropwise and the reaction mixture was stirred overnight at room temperature. After dilution with 120 mL of ethyl acetate the organic phase was washed with H₂O (2x 40 mL), brine (80 mL), dried over Na₂SO₄ and concentrated in vacuo. By purification of the residue by silica chromatography (20:1 to 5:1 hexane/ethyl acetate) 196.0 mg (0.5 mmol, 68 %) of a colorless oil could be obtained.

**1H-NMR (400.13 MHz, CDCl₃): δ 7.67 (d, 2H, J=8.0 Hz, –Ph), 7.35-7.23 (m, 15H, 3x –Ph), 7.20-7.16 (m, 8H, 2x –Ph), 4.03 (t, 1H, J=8.0 Hz, –CH), 2.40-2.36 (m, 2H, –CH₂), 2.29-2.25 (m, 2H, –CH₂), 1.44 (s, 9H, 3x –CH₃) ppm; ¹³C-NMR (100.62 MHz, CDCl₃): δ 171.36, 170.87, 144.77, 139.50, 136.44, 132.40, 130.33, 128.78, 128.69, 127.90, 126.94, 81.24, 65.06, 34.20, 29.58, 28.05 ppm; HRMS (ESI) calculated for C₄₁H₄₀N₂O₃ 609.3117 [(M+H)]⁺, found: 609.3046 [(M+H)]⁺.

**(S)-Di-tert-butyl 2-((diphenylmethyleneglycine)pentanedioate**

*N-(Diphenylmethyleneglycine tert-butylester* (100 mg, 0.34 mmol) and the phase transfer catalyst 12 (40.0 mg, 0.03 mmol) were dissolved in 2 mL DCM and 0.4 mL of 50 % (w/w) potassium hydroxide solution was added. *Tert*-butyl acrylate (87.0 mg, 0.7 mmol) was added dropwise and the reaction mixture was stirred overnight at room temperature. After dilution with 120 mL of ethyl acetate the organic phase was washed with H₂O (2x 40 mL), brine (80 mL), dried over Na₂SO₄ and concentrated in vacuo. By purification of the residue by silica chromatography (20:1 to 5:1 hexane/ethyl acetate) 196.0 mg (0.5 mmol, 68 %) of a colorless oil could be obtained.

**1H-NMR (250.13 MHz, CDCl₃): δ 7.68-7.66 (m, 2H, –Ph), 7.45-7.33 (m, 6H, –Ph), 7.21-7.18 (m, 2H, –Ph), 3.98 (t, 1H, J=2.5 Hz, –CH), 2.27-2.21 (m, 4H, 2x –CH₂), 1.46 (s, 9H, 3x –CH₃), 1.41 (s, 9H, 3x –CH₃) ppm; ¹³C-NMR (100.62 MHz, CDCl₃): δ 170.90, 170.59, 139.55, 132.42, 130.06,
128.81, 127.99, 127.81, 80.17, 64.98, 32.01, 28.89, 28.07 ppm; HRMS (ESI) calculated for C_{26}H_{33}NO_{4}: 424.2488 [(M+H)]^+, found: 424.2388 [(M+H)]^+, 446.2208 [(M+Na)]^+.

(R)-Di-tert-butyl 2-((diphenylmethylene)amino)pentanedioate

(R)-Di-tert-butyl 2-aminopentanedioate (200.0 mg, 0.8 mmol) was stirred overnight at rt with benzophenone imine (129.0 μL, 0.8 mmol) in DCM (5 mL). The solvent was evaporated and the mixture purified by flash chromatography (20:1 to 5:1 hexane/ethyl acetate) resulting in 308.0 mg of a colorless oil (0.7 mmol, 94%).

\[^1\text{H}-\text{NMR}\ (250.13\ \text{MHz, CDCl}_3): \delta\ 7.70-7.64\ (m, 2H, –Ph), 7.46-7.38\ (m, 6H, –Ph), 7.22-7.18\ (m, 2H, –Ph), 4.00\ (t, 1H, J=5.0\ \text{Hz}, \ –CH), 2.31-2.21\ (m, 4H, 2x –CH_2), 1.46\ (s, 9H, 3x –CH_3), 1.41\ (s, 9H, 3x –CH_3) ppm; \[^{13}\text{C}-\text{NMR}\ (100.62\ \text{MHz, CDCl}_3): \delta\ 172.47, 170.88, 139.58, 136.55, 130.24, 128.80, 127.98, 127.81, 80.06, 64.99, 32.01, 32.01, 28.91, 28.07\ \text{ppm}; HRMS (ESI) calculated for C_{26}H_{33}NO_{4}: 424.2488 [(M+H)]^+, found: 424.2482 [(M+H)]^+, 446.2208 [(M+Na)]^+.

1-(Tert-butyl)5-methyl 2-((diphenylmethylene)amino)pentanedioate

N-(Diphenylmethylene)glycine tert-butylester (100.0 mg, 0.3 mmol) and the catalyst 12 (20 mg, 0.03 mmol) were dissolved in 1 mL of DCM, then 0.2 mL of 50 % (w/w) aqueous potassium hydroxide solution was added dropwise and the mixture was cooled to -50 °C. A solution of methyl acrylate (0.1 mL, 1.1 mmol) in 0.2 mL DCM was added dropwise and stirred for 4 hours at -50 °C. After being diluted with 60 mL of diethyl ether, the organic phase was washed with H_2O (2x 20 mL), brine (40 mL), dried over Na_2SO_4 and concentrated in vacuo. The crude product was purified by silica chromatography (hexane/ethyl acetate 20:1 to 5:1), which afforded the product as colorless oil (87.0 mg, 67%).

\[^1\text{H}-\text{NMR}\ (250.13\ \text{MHz, CDCl}_3): \delta\ 7.55-7.60\ (m, 2H, –Ph), 7.21-7.40\ (m, 6H, –Ph), 7.07-7.15\ (m, 2H, –Ph), 3.90\ (t, 1H, J=6.3\ \text{Hz}, \ –CH), 3.52\ (s, 3H, –OCH_3), 2.25-2.35\ (m, 2H, –CH_2), 2.09-2.20\ (m, 2H, –CH_2), 1.37\ (s, 9H, 3x –CH_3) ppm; \[^{13}\text{C}-\text{NMR}\ (100.62\ \text{MHz, CDCl}_3): \delta\ 173.57, 170.68, 139.45, 136.45, 132.42, 130.32, 128.8, 128.44, 81.19, 64.79, 51.51, 30.51, 28.65, 28.04\ \text{ppm}; HRMS (ESI) calculated for C_{23}H_{27}NO_{4}: 382.2018 [(M+H)]^+, found: 382.1967 [(M+H)]^+, 404.1788 [(M+Na)]^+. 
RADIOCHEMISTRY

[11C]Acrylic acid 1

[11C]CO2 was transferred from the cooling trap to the reaction vessel with a helium flow of 10 mL·min⁻¹ and trapped in 100 μL of a 0.2 M solution of vinyl magnesium bromide in THF, which was cooled to 7 °C. After 1 min, the temperature of the reaction vessel was raised to 25 °C and maintained for 2 min. The intermediate was hydrolyzed by adding 100 μL of 2 M HCl and a sample was taken from the solution for reverse phase HPLC analysis. RCPs were obtained in the range of 50-70 %. The identity of the labeled compound was confirmed by co-injection with the reference. [Rt (Grace Smart RP-18, MeCN/acetate buffer (pH 3.8) 96/4, 1 mL·min⁻¹, 254 nm) = 13.5 min]

[11C]Methyl acrylate 2

The [11C]CO2 transfer and synthesis of the Grignard intermediate were conducted as described above. Hydrolysis was performed with 50 μL conc. sulfuric acid and 100 μL methanol were added. The vessel was heated to 70 °C and this temperature was maintained for 5 min while using a helium flow of 10 mL·min⁻¹. Then the temperature was raised to 90 °C and for a duration of 5 min the methyl ester was distilled in a second reaction vessel cooled to -10 °C. The distillate was diluted with 100 μL DMSO and a sample taken from the solution was analyzed by reverse phase HPLC. The distillate was obtained in RCP range of 65-78 %. The identity of the labeled compound was confirmed by co-injection with the cold reference. RCY obtained for this two-step reaction is based on the quality of the first reaction and starting from approximately 2.5 GBq a d.c. isolated yield of 19 % (0.47 GBq) was accomplished with a RCP of 98 % within 20 min [Rt (Grace Smart RP-18, MeCN/water 50/50, 1 mL·min⁻¹, 254 nm) = 4.0 min].

[11C]Tert-Butyl acrylate 3

[11C]CO2 was converted to [11C]CO on-line through reduction with molybdenum at 850 °C. The [11C]CO was concentrated in a cold trap after removing remaining [11C]CO2 by an ascarite column. It was transferred with a xenon flow to a sealed reaction vial containing a solution of Pd2[π-cinnamyl]Cl2 (3.6 mg, 7.0 μmol), xanphos (4.1 mg, 7.0 μmol), 250 μL tert-butanol and vinyliodine (1.0 μL, 10.0 μmol) in 300 μL THF. The vial was heated for 3-5 min to 100 °C. After cooling down, a sample was diluted with MeCN and analyzed by HPLC [Rt (Grace Smart RP-18, MeCN/buffer 1 68/32, 1 mL·min⁻¹, 254 nm) = 4.4 min]. The identity of the labeled compound was
confirmed by co-injection with the unlabeled tert-butyl acrylate. RCP of 70-80 % were determined with a d.c. RCY of 18 % (0.41 GBq) starting from approximately 2.5 GBq of $^{[1]}\text{C}CO_2$ (A_M could not be determined). [HPLC prep purification: Alltima C18 MeCN-buffer 1 70/30, 4 mL·min$^{-1}$, 254 nm, R$_t$ = 14.3 min].

$^{[1]}\text{C}N$-Tritylacrylamide 4

$^{[1]}\text{C}CO$ was produced as described above and xenon gas was used to transfer it in a sealed reaction vial containing a solution of Pd$_2$[π-cinnamyl]Cl$_2$ (3.6 mg, 7.0 μmol), xantphos (4.1 mg, 7.0 μmol), triphenylmethylamine (25.0 mg, 96.0 μmol) and vinyl iodide (1.0 μL, 10.0 μmol) in 600 μL THF. The vial was heated for 3-5 minutes to 100 °C. After it had cooled down, a sample was taken and analyzed with HPLC [R$_t$ (Grace Smart RP-18, MeCN-buffer 1 68/32, 1 mL·min$^{-1}$, 254 nm) = 4.5 min]. The identity of the labeled compound was confirmed by co-injection with the cold reference compound. RCP within 75-85 % were determined with RCY of 22 % (0.42 GBq) starting from approximately 2.5 GBq of $^{[1]}\text{C}CO_2$ with A_M of 86-170 GBq·μmol$^{-1}$. [HPLC prep purification: Alltima C18 MeCN/buffer 1 70/30, 4 mL·min$^{-1}$, 254 nm, R$_t$ = 9.8 min]

$^{[1]}\text{C}1$-Tert-butyl 5-methyl 2-((diphenylmethylene)amino)pentanedioate 7 to $^{[1]}\text{C}10$

$^{[1]}\text{C}M$ethyl acrylate was obtained like described above. To this solution in DMSO a solution of 5.0 mg (17.0 μmol) Schiff base precursor in 100 μL DMSO and 100 μL of a 0.1M solution of TBAF were added. After 5 min a sample was taken. Analysis by reverse phase HPLC and co-injection with the cold standard confirmed product formation [R$_t$ (Grace Smart C18, MeCN/water 50/50, 1 mL·min$^{-1}$, 254 nm) = 17.5]. For the deprotection, 50 μL of 50 % aq KOH were added to a solution of $^{[1]}\text{C}7$ in DMSO. After a couple of minutes, 200 μL concentrated HCl were added to the vessel and the temperature was raised to 100 °C for 2 min. A sample taken from the solution was analyzed by reverse phase HPLC (C18) as well as by chiral HPLC [Reprosil chiral-aa, MeOH/water 70/30, 1 mL·min$^{-1}$, R$_t$ = L-Glu 7.1 min and D-Glu 11.1 min].

General procedure for the synthesis of $^{[1]}\text{C}8$ and $^{[1]}\text{C}9$

Synthons $^{[1]}\text{C}3$ and $^{[1]}\text{C}4$ were synthesized as described above. For both, the reaction mixture in toluene was eluted through a custom made disposable celite cartridge containing approximately 200 mg of celite, into a reaction vial containing 5 (5-10.0 mg, 17-33.0 μmol) and 10.5 equiv. of CsOH·H$_2$O. The reaction was vigorously stirred for 5 min at 10 °C prior to analysis. HPLC
samples were taken by diluting a sample of the reaction mixture in MeCN followed by the injection of 20 µl on the column. \[R_t (\text{Grace Smart RP-18, MeCN/buffer } 1 \ 68/32, \ 1 \ \text{mL} \cdot \text{min}^{-1}, \ 254 \ \text{nm}) = 10.3 \ \text{min for 8, } R_t = 11.5 \ \text{min for 9}]\]

**General procedure for the deprotection to \[^{11}\text{C}]\text{10} and \[^{11}\text{C}]\text{11}**

For deprotecting the activating groups, the addition of 0.1 mL of 6M HCl solution to either \[^{11}\text{C}]\text{8} or \[^{11}\text{C}]\text{9} and heating to 100 °C for 2 min was sufficient. A second sample was taken for analysis on chiral radioHPLC to determine the enantiomeric ratio of amino acid with chiral HPLC (Reprosil chiral-aa, methanol/water 70/30, 1 mL·min⁻¹, 214 nm for \[^{11}\text{C}]\text{10} \ \text{L-Glu } R_t = 7.1 \ \text{min and D-Glu 11.1 min; 90/10 for }[^{11}\text{C}]\text{11} \ \text{L-Gln } R_t = 8.2 \ \text{min and D-Gln 12.3 min).}
References


Chapter 6

Summary, general discussion and future perspectives
Summary

In this thesis the development of radiochemistry methods for the asymmetric radiosynthesis of carbon-11 labeled amino acids and small peptides has been investigated. With the methodology developed, the chemical structure of the peptidic lead compound is kept intact for a more straightforward application and translation in vivo. The developments towards enantioselective synthesis of amino acids and peptides are explored highly in organic chemistry and have made huge progress in recent years. Asymmetric synthesis is a generally applicable method with mild reaction conditions. The most successful chiral phase-transfer catalysts are nowadays also commercially available. With the successful and easy applicable radiosynthesis at hand, the application of amino acids and small peptides in pre-clinical and clinical settings is a step forward in PET tracer applicability.

Though to date only $^{11}$C-amino acids are used in clinical practice, and $^{11}$C-peptides so far not, this might change with the upcoming and straightforward labeling strategies that are presented in this thesis. The advantage of $^{11}$C-amino acids and $^{11}$C-peptides is the fact that there is no molecular change occurring to obtain the radiolabeled PET tracer that is based on the peptidic lead compound. Furthermore, the physiological metabolism of $^{11}$C-amino acids and $^{11}$C-peptides is commendable for radiation dose burden of patients as well as multiple tracer injections for longitudinal studies are feasible.

As a result from the research described in this thesis, the radiolabeling of $^{11}$C-amino acids and $^{11}$C-peptides has become available and with the asymmetric synthesis developed, a generally applicable and broad methodology for PET imaging agents has become available.

Chapter 2 reviews all radiosynthetic possibilities to obtain carbon-11 labeled amino acids and peptides that have been developed to date. Next to the radiosynthesis of carbon-11 labeled amino acids and peptides, the application of key studies that have been conducted with these compounds in vivo is described. First reports in literature use the Bucherer-Strecker synthesis with $[^{11}$C]CO$_2$ on the carboxyl position, and early examples also included the use of enzymatic reactions. In recent years a tendency towards stereoselective and specialized radiochemistry strategies to obtain only one enantiomer is observed. Strategies are covering the use of metal-mediated starting material towards several chiral catalysts where the radiosynthesis is accomplished under mild phase-
transfer reactions to obtain amino acids. Small peptides are labeled with a radionuclide by using methylation reactions with $[^{11}\text{C}]$methyl iodide (or $[^{11}\text{C}]$methyl triflate) on cysteine residues. More recent publications shift focus towards method development of asymmetric synthesis of peptide chains$^{[1,2]}$ as well as acetylation of lysine residues$^{[3]}$ with $[^{11}\text{C}]$CO, and successful direct $[^{11}\text{C}]$CN-labeling on unprotected peptides$^{[4]}$. Clinical application of carbon-11 labeled amino acids and peptides is still minimal, except for the application of $[^{11}\text{C}]$methionine, which is widely used. To date most other carbon-11 labeled amino acids did not succeed further than small animal imaging studies. Small peptides are still reluctantly used in conjunction with carbon-11, though due to biokinetic stability of peptides in vivo these labeled tracers have the advantage of lower radiation dose overall, as well as multiple injections per day in one patient for longitudinal studies.

Chapter 3 describes the development of the asymmetric synthesis to obtain L-$[^{11}\text{C}]$alanine. Here, the radiosynthesis strategy was to alkylate a Schiff base glycine ester with $[^{11}\text{C}]$methyl iodide. To achieve a successful radiolabeling methodology, various factors were extensively screened like base, amount of base, temperature and time. However, most importantly is the choice of catalyst for the enantioselective induction. Seven catalysts were screened, obtaining high radiochemical conversion of $> 80\%$ and excellent enantioselectivity of $> 90\%$ with a chiral catalyst of the second generation, the quaternary ammonium catalysts developed by Maruoka et al.$^{[5]}$ The notable ee was achieved despite the small volume of the alkylating agent.

Chapter 4 describes the asymmetric radiosynthesis of $^{11}\text{C}$-labeled dipeptides at the N-terminal amino acid. The challenge was here to establish the best reaction conditions to obtain diastereomeric pure product. We have established the radiosynthesis with a series of different dipeptides, adapting the second amino acid, and compared results of $[^{11}\text{C}]$methyl iodide and $[^{11}\text{C}]$benzyl iodide with an arrangement of eight catalysts. A separation on reversed phase HPLC for determination of diastereomer ratio was only possible with two dipeptides, $[^{11}\text{C}]$Xxx-Valine and $[^{11}\text{C}]$Xxx-Leucine. The radiochemical conversion to product is in all cases high and with optimized conditions diastereomeric ratios for the methylation of 95:5 and for benzylation of 90:10 were observed. Also here it was discerned that catalysts behaved differently towards starting material, the Schiff base dipeptide and the size of the alkylating agent, which corresponded with organic synthesis procedures.$^{[6]}$
Chapter 5 delineates the development of small alkylating agents yielding in a second step a new $^{11}$C-C bond via a Michael addition reaction. Carbon-11 labeled reagents for the Michael addition, which were functionalized acrylates, were either synthesized from $[^{11}\text{C}]$carbon dioxide or $[^{11}\text{C}]$carbon monoxide. These have then been used in novel Michael additions to obtain the functionalized amino acids $[^{11}\text{C}]$glutamate and $[^{11}\text{C}]$glutamine. Firstly, the optimal conditions for the synthesis of the carbon-11 labeled reagents were established. The radiosynthesis of four synthons was set up to obtain $[^{11}\text{C}]$acrylic acid, $[^{11}\text{C}]$methacrylate, $[^{11}\text{C}]$tert-butyl acrylate and $[^{11}\text{C}]$tritylacrylamide in high radiochemical conversion in short time. These products, Michael acceptors, were purified by filtering through celite, which were then applied in a second reaction with the Schiff base glycine ester. The 1,4-conjugated addition formed a new stable $^{11}$C-C bond where amount of precursor, purity and base stability of the carbon-11 labeled reagent play a crucial role. High yields were obtained with most established Michael acceptors. However, when taking it to the next step, the enantioselective synthesis could not be established adequately so far and only resulted in moderate ee of 55%. To gain further insights into this, additional experiments have been performed covering mostly the stability and usability of the carbon-11 labeled acceptors. The esters are base-labile and in experiments was shown that high amounts of CsOH·H$_2$O as well as high temperatures are decomposing $[^{11}\text{C}]$tert-butyl acrylate whereas $[^{11}\text{C}]$tritylacrylamide has proofed steadier. Besides, addition experiments with carrier-added carbon-11 labeled reagent have been carried out, which did not improve the radiochemical conversion of the reaction. Notwithstanding, the enantioselective Michael addition as it is described here with carbon-11 is a new and straightforward method for advanced radiosynthesis in carbon-11 chemistry.
General Discussion

The research in this thesis describes the development of radiochemistry methods to obtain $^{11}$C-labeled natural amino acids and peptides. The enantioselective synthesis of amino acids has been described earlier, applying chiral starting material or using enzymes for the synthesis, however application has still been rare and limited mostly focused on $[^{11}\text{C}]$methionine. We believe that is mainly due to lack of easy applicable radiosynthesis methods. To the best of our knowledge, at the start of this study, chiral catalyst from the emerging field of phase-transfer catalysis in organic chemistry were not applied successfully in $^{11}$C-radiochemistry. These chiral catalysts became commercially available and therefore our study concentrated on the implementation of these catalysts to achieve the enantioselective synthesis of amino acids and peptides.

The radiosynthesis described in Chapter 3 marked the first in depth study of an asymmetric alkylation reaction to synthesize $^{11}$C-labeled amino acids by alkylation. In addition, in organic chemistry the use of methyl iodide is mostly abandoned due to its small volume and the belief that it therefore would only yield unsatisfactory enantiomeric excess with described chiral catalysts. The radiochemical reactions were evaluated on five components (precursor amount, base and amount of base, solvent, temperature) and examined closely to obtain best outcome to product and furthermore satisfactory enantiomeric excess. Therefore, various chiral catalysts were pursued and their optimal reaction conditions established. The main purpose was to establish best enantiomeric excess for L-$[^{11}\text{C}]$alanine, and with said conditions and catalysts evaluated, we have established a reliable synthesis, achieving 90 % ee which makes biological studies feasible without the need for chiral purification of the tracer.

The following step was to evaluate the applicability of the above-mentioned conditions to dipeptide Schiff bases. For this purpose, a range of dipeptides have been synthesized (Chapter 4). At first instance, optimal conditions for radiosynthesis of L-$[^{11}\text{C}]$alanine were applied with described catalysts, however resulting in unsatisfactory formation of product. A prerequisite for analysis of diastereomeric excess is the separation of products via column chromatography. Excessive analysis of the four exemplified dipeptide products was performed. They could be analyzed as protected compounds on a C18 HPLC column which allowed chiral separation. Yet, two of the dipeptide pairs were not separable on reversed-phase HPLC or chiral HPLC and had therefore been excluded from the study to obtain high diastereomeric ratio. Continuing with two
dipeptides and two alkylating agents the optimal conditions were established as a unique study to examine the backbone of asymmetric synthesis with dipeptides in radiochemistry. Reliable and satisfactory results were obtained with a distinct set of chiral catalysts. Apart from increasing the applicability of aforementioned asymmetric synthesis in radiochemistry, eventually also here higher diastereomeric ratios were feasible.

The $^{11}$C-acrylates described in **Chapter 5** served as Michael acceptors for the radiosynthesis of further amino acids and amino acid derivatives. After the radiosynthesis of these so-called synthons were established, the following was setting up a novel $^{11}$C-C bond forming reaction in radiochemistry, so called Michael addition. The radiosynthesis of $[^{11}\text{C}]$glutamate and $[^{11}\text{C}]$glutamine was rather cumbersome. The synthesis has been confirmed, however the outcome to the addition product has been unreliable. Various leads of causes were evaluated, none though resulted in pleasing reliability. In addition, applying established reaction conditions for asymmetric synthesis resulted in unsatisfactory enantiomeric excess. Initial results were promising, although more research is required to ascertain the reaction mechanism and what is disturbing it.
Future Perspectives

This thesis describes the radiosynthetic method development to form new $^{11}$C-C bonds by direct enantioselective alkylation with achiral Schiff base starting material. The developed methodology is applied for the enantioselective synthesis of $^{11}$C-labeled amino acids and peptides as potential PET tracers for imaging. In Chapter 3 to 5 the one-pot method for the chiral alkylation was successfully implemented to obtain a series of $^{11}$C-labeled amino acids using a chiral phase-transfer catalyst (PTC).

With respect to the radiosynthesis of L-$[^{11}$C]alanine as it has been describe in Chapter 3, currently an enantiomeric excess (ee) higher than 90 % was achieved (Figure 1, PTC 1). Considering the small volume of methyl iodide and the shorter time frame used for asymmetric reactions, results obtained are satisfactory. However, ideally an ee of over 95% should be the standard. To achieve this, lowering the reaction temperature would be a feasible option, but this does not align with the basics of radiochemistry which requires short reaction times. On the other hand, enantioselectivity is foremost influenced by the catalyst. With the knowledge that there is a continuous push from organic chemistry research groups developing novel PTCs, these specialized catalyst could be evaluated for the synthesis of carbon-11 labeled amino acids as well. Also, considering that no good results have been obtained for the radiosynthesis of D-$[^{11}$C]alanine further improvements are viable with respect to better PTCs available in the future. Taking a closer look into the structure of the applied catalysts and the results for the ee that were obtained, a likely observation are the substantially lower results for ee of L-$[^{11}$C]alanine achieved with some promising catalysts selected based on organic chemistry. Theoretically, the bulkier the catalyst the higher the ee obtained with alkylating agents other than methyl iodide in literature. Therefore, further studies should apply molecular modeling and different $^{11}$C-alkylating agents to study influences on ee. However, it should be noted that there are still uncertainties about the mode of action, origin of the enantioselectivity and enolate binding, which influence the final result. These challenges can be addressed by proper molecular modeling and design of the PTC, the Schiff base precursor and the alkylating agent.
Chapter 4 builds upon chapter 3 by enlarging the starting Schiff base to dipeptides to study the influence of PTC to backbone. Novel challenges by labeling peptides with carbon-11 using the methodology described here, was to obtain a decent chiral separation of diastereomers for HPLC analysis. A bulky side chain of the C-terminal amino acid is preferred since this results in a larger chemical structure change between precursor, product and the diastereomers. Our study concentrated on two alkyl side chains, however for future studies and to broaden application, side chains should also contain functional groups like the acrylates that have been used for the Michael addition reaction of the glycine Schiff bases. The catalyst selection is based on previous findings and surprisingly the obtained results vary strongly from the radiosynthesis of the single amino acid enantiomers. The most bulky catalyst (Figure 1, PTC 2) functions best and further improvements should be made on the catalyst side groups, which have been shown to improve diastereomer ratios (dr, calculated also to diastereomeric excess de) in literature.\textsuperscript{[6]} The alkylating agents \textsuperscript{[11}C\textsuperscript{]}methyl iodide and \textsuperscript{[11}C\textsuperscript{]}benzyl iodide, only resulted in small differences in the obtained dr, nonetheless further studies with other bulky \textsuperscript{11}C-labeled alkylating reagents to study the effect of backbone, the side-chain of the adjacent amino acid and chiral catalyst are recommended for future research.

In recent years, the market share of peptides in pharmaceutical development has increased enormously.\textsuperscript{[7,8]} Therefore and based on those leads, there is great potential for our direct labeling strategy since structural changes are not desired when translating from \textit{in vitro} to \textit{in vivo} application. This especially holds true for amino acids and small peptides and it is important to maintain the molecular structure so that interaction with the target protein is not altered. With a direct radiolabeling technique at hand evaluation of biological tracers could be advanced at earlier
stages. This technique implemented for the direct radiosynthesis of small peptides that are active in the brain could be especially useful as chelated derivatives only pass the blood-brain barrier if it is disrupted. There are a few studies of radiolabeled peptides for Alzheimer’s diagnostic imaging and building on those results would be great opportunity for the direct asymmetric radiosynthesis.[9-11]

The work described in Chapter 5 has introduced a new approach to form a $^{11}$C-C bond by a 1,4-addition reaction. The so-called Michael addition was implemented successfully to obtain functional amino acids and derivatives thereof. The synthons were established with high radiochemical conversion and could then be applied as acceptor with glycine Schiff base ester to form $^{11}$C-amino acids. Radiochemical conversions were achieved in moderate to good yields. Here, further improvements could be made by analyzing the reaction media more closely by possibly an improved purification of the intermediate $^{11}$C-acceptor synthons. The enantioselective radiosynthesis yielded so far only moderate ee’s. For the future, focus should be on the establishment of a successful radiosynthesis to obtain enantiopure $^{11}$C-amino acids.

Furthermore, the application of 1,4-additions can be extended towards Aza-Michael additions by forming new N-$^{11}$C and S-$^{11}$C bonds as depicted in Figure 2B-D. In our strategy we focused on Schiff bases as donor for new $^{11}$C-C bonds, however also here variations can be applied, e.g. the diethyl malonate (Figure 2A), as long as the activation to an enol of the donor is possible. Broadening the scope of the Michael reaction in radiochemistry also unlocks new potential imaging agents.
With the established radiosynthesis for glutamine (Chapter 5), its potential as PET imaging agent can be further explored. Studies suggest that it can be used as a successful tracer\textsuperscript{[13,14]} complimentary to $[^{18}\text{F}]$FDG, since uptake of glutamine is increased as it is used as nutrient by tumor cells. Glutamine is metabolized in the so-called glutaminolysis pathway\textsuperscript{[13,15]} and compared to $[^{18}\text{F}]$FDG will not be increased in inflamed regions and in particular the brain, whose main nutrient is glucose. This would imply a highly potential application of $^{11}$C-glutamine now that its synthesis is achieved. Preferably the L-enantiomer should be used, although this still is a radiosynthetic challenge.
References


Chapter 7

About the Author
About the Author

Ulrike Regina Filip was born on 18th March 1986 in Schäßburg, Romania. She finished her pre-university education (gymnasium) at the Eugen-Bolz College in Rottenburg a.N., Germany. Before moving on to university, she spent an educational year in New Zealand. She took up her studies in chemistry at the University of Tübingen and moved on to Biomedical Chemistry soon afterwards at the University of Mainz. In 2012, she successfully finished her master’s thesis internship in the group of Prof. Dr. T. Ross and Prof. Dr. F. Rösch on the development of fluorine-18 labeled small molecule PET tracers for Alzheimer’s disease. Following her studies, she worked as junior scientist at Merz Pharmaceuticals in Frankfurt a.M. in the crystallographic analytical department. In 2013, she started as a Marie Curie PhD fellow in the group of Prof. Dr. A.D. Windhorst at the Vrije University Medical Center, Amsterdam. Her research was devoted to the radiochemistry development of carbon-11 labeled small natural compounds. The results of which are presented in this thesis.
List of publications


§: Both authors contributed equally to this work.
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