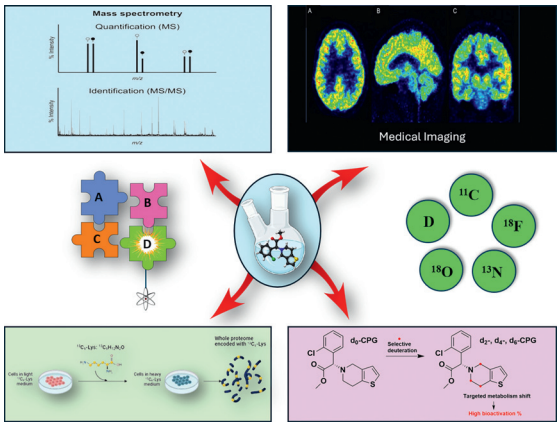
Synlett S. Xiao et al. THIEME OPEN ACCESS Account

Multicomponent Reactions: A Promising Approach to Isotope Labeling

Siyu Xiao[a]
Antonio Conte[b]
Bart T. Cornelissen[a]
Alexander Domling[*b]
Philip H. Elsinga[*a]

[a] University Medical Center Groningen, University of Groningen, 9713 GZ Groningen, The Netherlands
p.h.elsinga@umcg.nl
[b] Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry and Czech Advanced Technology and Research Institute, Palacký University in Olomouc, Olomouc, Czech Republic
alexander.domling@upol.cz

This article is part of the special issue on Isotopic Labeling.



Received: 11.02.2024
Accepted after revision: 22.05.2024
Published online: 22.05.2024 (Accepted Manuscript), 18.09.2024 (Version of Record)
DOI: 10.1055/a-2331-6399; Art ID: ST-2024-02-0035-A

Abstract Isotopic labeling is an attractive modality that has been widely used in many aspects of chemistry, the life sciences, and medical research; especially deuterated drugs and radioactive molecules have been used in the diagnosis and treatment of cancer and neurodegenerative diseases. The widespread application and rapid development of isotopically labeled molecules has led to an increased demand for new isotopic labeling chemical methods to synthesize highly specific molecules bearing defined nuclides. Multicomponent reactions (MCRs) are modular build-up approaches for the rapid generation of complex molecules often containing biologically relevant scaffold structures. There is great potential to use MCRs to construct isotopically labeled molecules because assembly speed and reaction diversity are key advantages of MCR. In this review, we provide an overview of the recent literature on this topic that can provide insight into the application of MCRs in the field of isotopic labeling.

Key words MCR, multicomponent reaction, Ugi, Passerini, isotope, PET, deuterium, isocyanide

1 Introduction

Multi-component reactions (MCRs) stand out as powerful and efficient synthetic strategies in organic chemistry, involving the simultaneous reaction of three or more reactants to produce a single product, often in a one-pot fashion.[1] This approach offers several compelling advantages that contribute to its widespread utilization.

Firstly, MCRs enhance efficiency by allowing the synthesis of complex molecules in a single reaction vessel, minimizing the number of steps, and reducing waste, thereby promoting sustainability. The diversity achieved through MCRs is remarkable; they enable the incorporation of multiple functionalities in a single step, rapidly generating diverse chemical structures.

Additionally, MCRs exhibit high atom economy, are environmentally friendly and economically advantageous, as a large percentage of reactants are incorporated into the final product.[2] The synthetic process is simplified by reducing reaction steps, purification procedures, and the need for multiple reaction vessels, making MCRs attractive for both academic and industrial applications.[3] Many MCRs also provide high yields, proving efficient and practical for large-scale synthesis.[4]

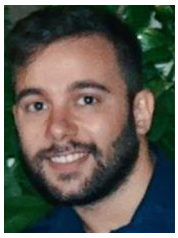
Furthermore, the study and development of MCRs have led to the discovery of new reaction mechanisms and novel chemical transformations, contributing to the advancement of synthetic chemistry. Their versatility allows application to various types of reactions, including carbon–carbon and carbon–heteroatom bond-forming reactions.[5]

In the context of isotopes, which are distinct nuclear species of the same chemical element, MCRs offer an efficient modular build-up approach to synthesize complex drug molecules. Isotopically labeled compounds find widespread use in science and technology, serving to track the passage of isotopes through reactions, metabolic pathways, or cells. Stable isotopes, crucial in mass spectrometry studies, protein folding, and exploring chemical reaction mechanisms, also play a vital role in medicine, particularly in the development of deuterated drugs (Figure 1).

Deuterated drugs, which often exhibit comparable physicochemical properties to their unlabeled counterparts, offer improved safety, enhanced tolerability, and increased bioavailability. For example, deucravacitinib (SOTYKTU™) (Figure 2) is a groundbreaking oral inhibitor of tyrosine kinase 2 (TYK2), earning its first approval in the USA on September 9, 2022, for adults with moderate-to-severe plaque psoriasis. Unlike traditional tyrosine kinase inhibitors targeting the active kinase domain, deucravacitinib

Biographical Sketches

Siyu Xiao obtained his Bachelor of Science degree and Master of Science degree in Southwest University. He is presently studying for a PhD under the guidance of Prof. Dr. P.H. (Philip) Elsinga at the Department of Nuclear Medicine and Molecular Imaging at the University Medical Center Groningen. His current research interests include organic synthesis and radiolabeling.



Antonio Conte was born in Caserta, Italy. He graduated from the University of Naples Federico II with a thesis developed in collaboration with the University of Groningen under the supervision of Professor Alexander Dömling. Since June 2023, he has been working as a researcher at Palacký University in Olomouc, Czech Republic. His PhD studies focus on Medicinal Chemistry within the group led by Professor Alexander Dömling at the Czech Advanced Technology and Research Institute (CATRIN) in Olomouc.

Bart Cornelissen was born in Turnhout, Belgium. He graduated from the Universities of Hasselt and Ghent with a thesis in radiochemistry and Radiopharmacy. He is now Associate Professor at the University Medical Center in Groningen, with a visitor appointment at University of Oxford's Department of Oncology, and the Nuffield Department of Surgical sciences. His research focuses on the development of novel radiopharmaceuticals for imaging and radionuclide therapy, mainly for oncology applications.

Prof. Dr. Alexander Dömling has been ERA Chair at the Palacky University, Olomouc since 2023. Before, he held professor positions at the University of Groningen, and University of Pittsburgh and performed his Habilitation at the Technische Universität München (TUM). He obtained his PhD with Ivar Ugi at the TUM in 1992, and, thereafter, joined Barry Sharpless' lab at Scripps Research Institute as a Feodor Lynen postdoctoral researcher funded by the Alexander von Humboldt society. He is co-founder of several start-up companies. Recently, he received the prestigious ERC Advanced grant AMADEUS. His interests include all aspects of multicomponent reaction chemistry in basic and applied science and he is supported by more than 400 publications and 70 patent applications.

Prof. Dr. Philip H. Elsinga is an organic chemist by training and obtained his PhD in 1995 at the University of Groningen. In 2016 he was certified as a Clinical Radiochemist. He is involved in PET-related radiopharmaceutical research and translation of radiopharmaceuticals to the clinic. His main focus was firstly directed to PET-labeled amino acids, receptor ligands for the beta-adrenergic receptor and substrates for P-glycoprotein. Later on, his interest broadened to other PET-radiopharmaceuticals, such as ^{18}F-labelled peptides, receptor antagonists and artificial amino acids for neuroendocrine tumors. In 2011 Philip Elsinga was appointed as full professor in PET-radiochemistry. He is the author of >200 peer-reviewed articles. He supervised >30 PhD-students including collaboration with Peking University. International activities are (amongst others) Chairman of the EANM Radiopharmacy Committee, member of Board of Directors of Society of Radiopharmaceutical Sciences, Editor-in-Chief of the European Journal of Nuclear Medicine and Molecular Imaging Radiopharmacy and Chemistry and invited expert for IAEA. Current scientific projects involve (immune)oncology, neurology and bacterial infection imaging with PET and in combination with optical and MRI-imaging.

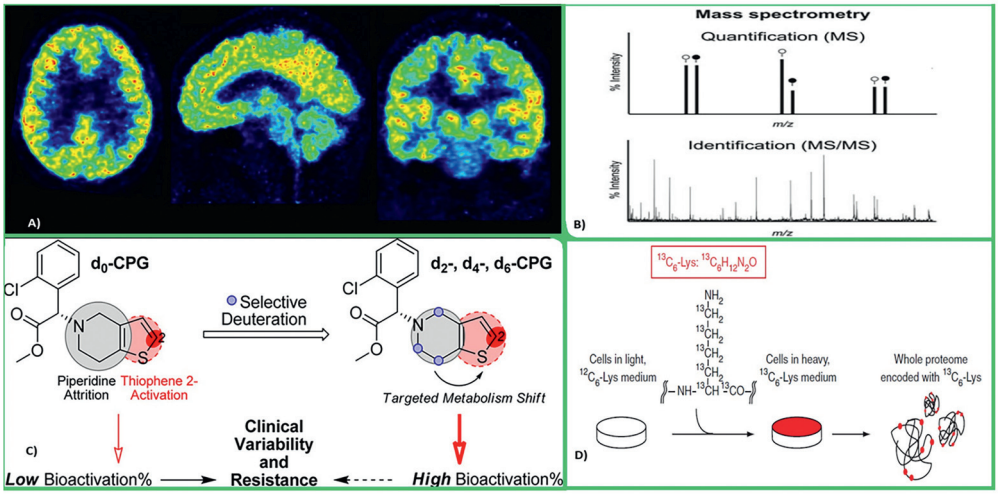


Figure 1 Different application of isotopes in: (A) PET scanning,[6] (B) mass spectrometry,[7] (C) isotopically labeled drugs displaying enhanced bioactivation,[8] (D) SILAC.[9]

acts via allosteric inhibition, binding specifically to TYK2's catalytically inactive pseudo kinase regulatory domain (JH2). This binding stabilizes an inhibitory interaction between the regulatory and catalytic domains of TYK2, effectively preventing receptor-mediated TYK2 activation and downstream signaling. Furthermore, deuteration of deucravacitinib's N-methyl group inhibits the formation of a less selective primary amide metabolite in vivo by suppressing the N-demethylation metabolic pathway via a deuterium kinetic isotope effect. This deuteration process underscores the significance of pharmaceutical optimization strategies, highlighting its pivotal role in enhancing drug selectivity and efficacy.[10]

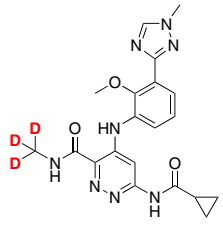


Figure 2 Deucravacitinib (SOTYKTU™) used for adults with moderate-to-severe plaque psoriasis

The deuterated position or group of a drug is usually specific. Introducing a defined number of deuterium atoms at specific locations and ensuring optimal deuterium incorporation poses a formidable synthetic challenge. The analytical scrutiny of isotopic impurities within deuterated active pharmaceutical ingredients presents a technical hurdle of considerable magnitude. Furthermore, the elusive predictability of the effects stemming from deuterium modification amplifies the intricacy of this synthetic endeavor.[1] However, deuterating small molecules is relatively easy, taking into account the availability of commercially deuterated solvents. There is great potential to synthesize complex drug molecules from deuterated small molecules with modular approaches. Examples of approved drugs that can be made by an MCR route but are not necessarily produced by an MCR include atorvastatin,[11] praziquantel,[12] ivosidenib,[13] lidocaine,[14] telaprevir,[14] olanzapine,[15] clopidogrel,[15] lacosamide,[4] carfentanil,[16] nirmatrelvir,[17] amenamevir,[18] and levetiracetam[19] (Figure 3). Olanzapine is a particularly intriguing example on the potential impact of MCR in isotope labeling, since it has been shown to be producible by several convergent MCR routes. It was proposed that 5–10% of the marketed drugs contain substructures that can be made advantageously by MCR.[3] Examples of isotope labeled building blocks useful in MCR chemistry to precisely label specific positions in target molecules are shown in Table 1. For instance, a series of imidazole-linked covalent organic frameworks were robustly constructed through the Debus–Radziszewski MCR from aldehydes, ketones, and ammonia. Among them, [^{15}N]NH_3 was used to synthesize the frameworks to study the reaction mechanism.[20]

With the development of positron emission tomography (PET) technology, various radiolabeled drugs have been developed and widely used in clinical diagnosis of various diseases in recent years (Figure 4). As a powerful non-invasive method, PET is able to monitor pathophysiological processes and evaluate drug treatment.[21] PET imaging shows binding of radiolabeled tracers to biological targets of interest, such as receptors, enzymes, and ion channels.[22] Moreover, PET can be used to accelerate drug development by providing key information of pharmacokinetic and pharmacodynamics properties of novel drugs to the pharmaceu-

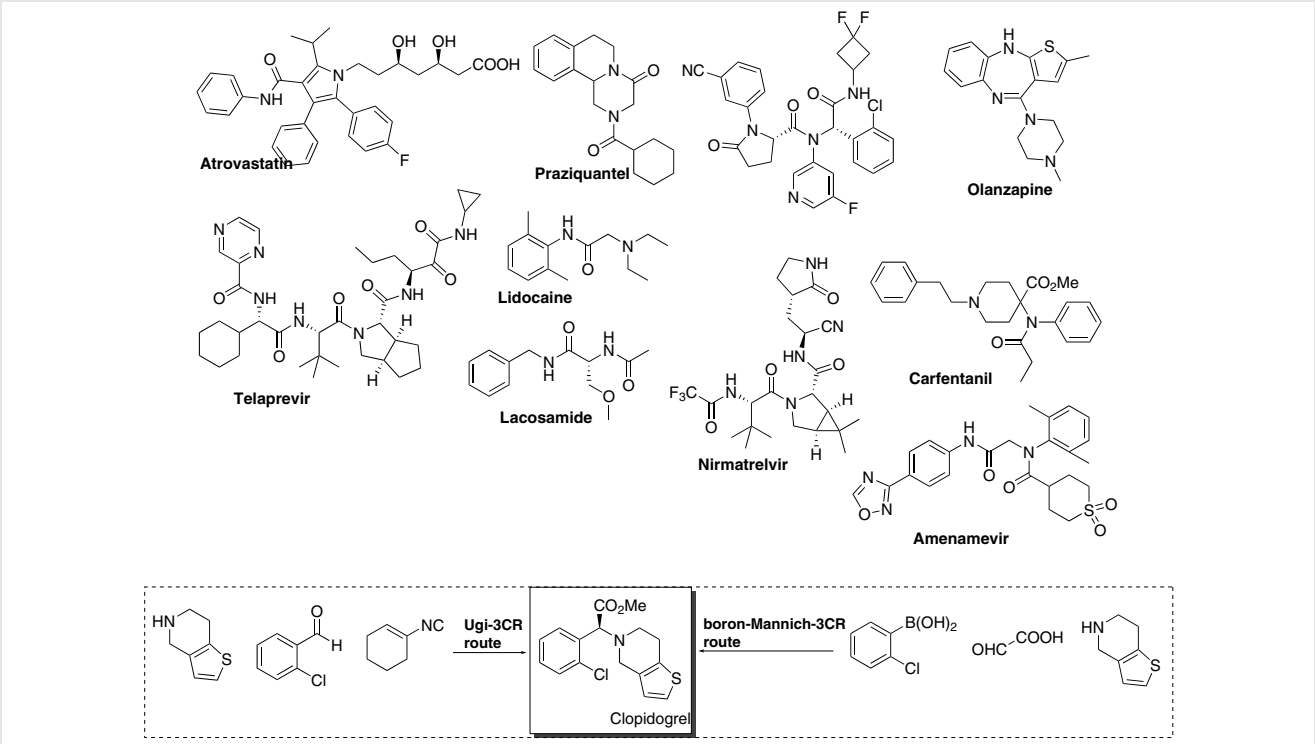
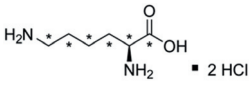


Figure 3 Examples of drugs accessible by MCRs and synthesis of Clopidogrel by two orthogonal MCR approaches

tical industry.[23] Innovations in chemistry to synthesize radiotracers play a crucial role to the point where chemistry may well be the rate-limiting step in the development of PET-tracers.[24] Successful drug development for PET depends on short-cycle, low-cost, and efficient development methods to discover lead compounds.[25] Thus, using PET for drug discovery and patient diagnostics requires an efficient and varied toolbox for PET-tracer synthesis.

The synthetic efficiency of PET-tracers depends not only on synthesis methodology but also on the chemical properties and half-life of the radionuclide.[26] ^{18}F and ^{11}C are the two most commonly used radionuclides for PET. For PET-tracers with complex structures, there are two commonly used synthesis strategies. The first method is to synthesize precursors of PET-tracers step by step, after which the precursors are radiolabeled (Figure 5A). A second method entails the radiolabeling of a small molecule synthon first,

Table 1 Commercial Availability of Isotope Labeled Building Blocks of Potential use in MCRs

Name	Structure	Price ($)	Potential MCR application
Deuterated water	D_2O	1,700 / 1 kg	
Rich(^{18}O) Water	$H_2{}^{18}O$	1,700 / 1 g	U-3CR to label amide
Isopropanol-D_8		168 / 1 g	
Acetic acid-D	CH_3COOD	134 / 50 g	Synthesize deuterated drugs and study reaction mechanisms
Acetone-D_6	CD_3COCD_3	51 / 10 g	
Acetaldehyde-D_4	CD_3CDO	139 / 1 g	
Ammonium chloride (^{15}N)	$^{15}NH_4Cl$	102 / 1 g	Study reaction mechanisms
L-Lysine·2HCl ($^{13}C_6$)		4,272 / 1 g	

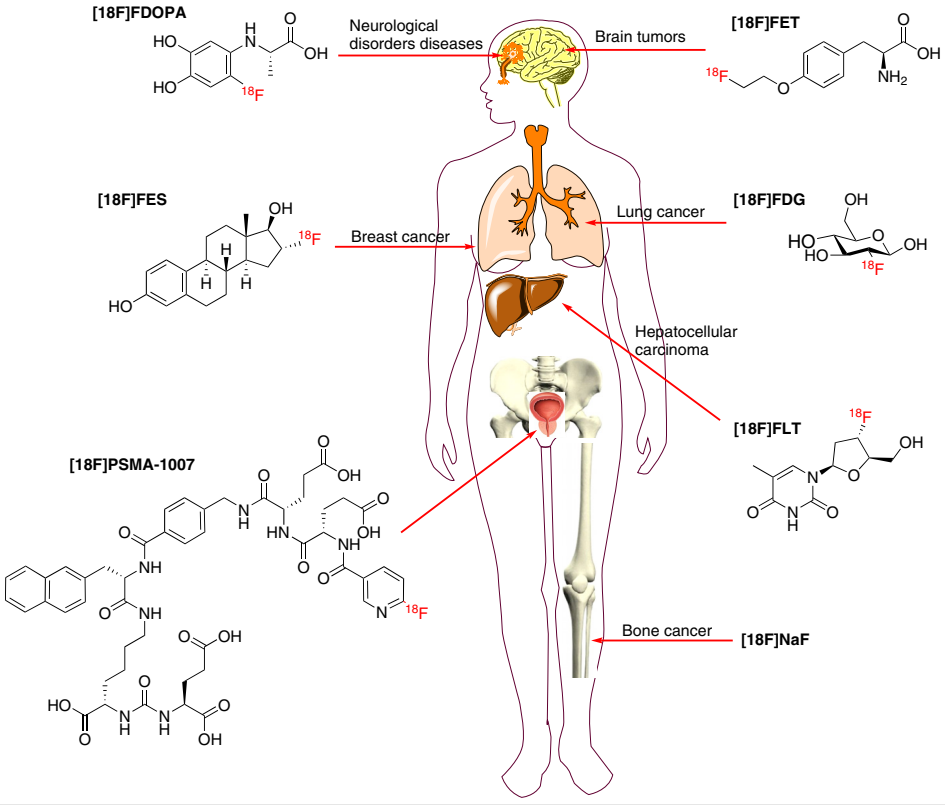


Figure 4 Some clinically available ^{18}F-labeled radiotracers and their related applications.

which is then used as a reactant to synthesize PET-tracers (Figure 5B). However, for the former method, synthesizing precursors step by step inevitably leads to low efficiency, huge waste, and low overall yields. For example, it requires four steps to synthesize the precursor of lacosamide with an overall yield of 23% (Figure 11A) and it requires eight steps to synthesize the ezetimibe with an overall yield of 5% (Figure 14A). For the latter, PET-tracers with complex structures are generally difficult to obtain through one-step reactions. For example, a quaternary ammonium salt group has been used to obtain the L-3,4-dihydroxy-6-[^{18}F]-fluorophenylalanine. It took four more steps to obtain the radio-

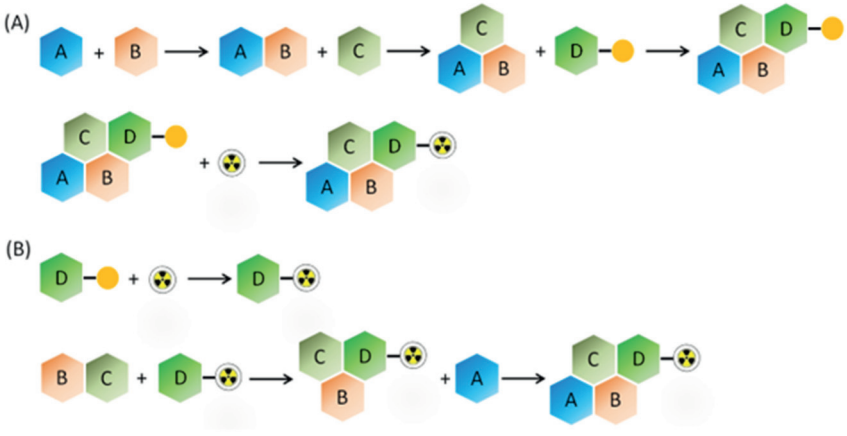


Figure 5 Two orthogonal methods to develop PET-tracers. (A) The precursors of the corresponding versions of PET-tracers are synthesized step by step, and then the precursors are radiolabeled. (B) A small molecule is radiolabeled and then used as a reactant to synthesize PET-tracers.

active product after introducing the ^{18}F.[27] In addition, long reaction times needed for some reactions will cause the radionuclide to significantly decay.

Although research into and application of radioisotopic labeling of small molecule drugs have gradually increased over the years and a significant range of radiolabeling methods have been developed, most of these methods are carried out using small molecules with one reaction site as templates.[28] These methods are not suitable for structurally complex drug molecules that have multiple chiral centers and contain multiple reaction sites.[29] Here, we will explore the applicability of multi-component reactions (MCRs) as a synthetic tool to speed up discovery of radiolabeled compounds containing multiple functional groups .

MCRs are considered an effective solution to the above problems.[25] The products of MCRs are formed in one step, instead of multiple sequential steps, which means that MCRs are efficient, economic, and have high overall yields.[30] The structures of the compounds produced by MCRs include high exploratory chemical space and can be easily diversified through systematic changes in each reactant.[31] Therefore, MCRs are well suited for generating complex precursor libraries (Figure 6A).

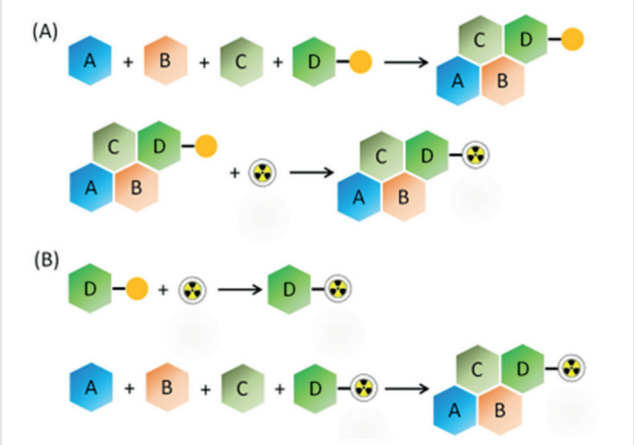


Figure 6 Two different MCRs methods to develop PET-tracers. (A) Synthesized precursors first, followed by radiolabeling. (B) A small molecule is radiolabeled as a synthon, and then MCRs is used to synthesize PET-tracers.

As for the second method (Figure 6B), traditional MCRs usually take hours to several days to complete depending on the reaction type and substituents. Such characteristics of MCRs do not allow the use of most of the radionuclides as long reaction times will lead to a substantial decay of the radionuclide. However, the radioactive reactant in MCRs is always burdened by a severe sub-stoichiometric amount compared to the non-radioactivity reactants. This may result in different reaction kinetics due to stoichiometric amounts of reactants. MCRs could give high radiochemical yields (RCY) with relatively short reaction times because of favorable reaction kinetics combined with elevated temperatures. For example, it takes 24 hours for the yield of Groebke product to reach 73%, but it only takes 15 minutes for the RCY of the same MCR yielding the corresponding ^{18}F-labeled product to reach 85%.[32]

Thus, MCRs not only may allow for more efficient synthesis of diverse precursors, but also for rapid synthesis of structurally complex tracers. This could provide new PET-tracers that are not available through linear synthesis. So MCRs have the potential to greatly expand the range of radiotracers available for PET research.

In this mini-review, we focus on the use of MCRs for the synthesis of organic isotopically labeled compounds. We describe and discuss the principles of MCR usage in isotopic labeling and highlight specific examples, in a non-exhaustive manner, while pointing out some challenges.

2 Labeling with Stable Isotopes Using MCRs

2.1 Deuterium Labeling in MCR

Deuteration, the substitution of deuterium (D) for hydrogen (H) in chemical bonds, offers a distinct opportunity to enhance several critical aspects of drugs. When the cleavage of the C–H/D bond is rate-limiting, this substitution induces a primary deuterium kinetic isotope effect (DKIE), resulting in a slight increase in activation energy (EA) for bond cleavage and a lower reaction rate.[33] Despite the seemingly negligible EA increase, deuterium substitution significantly impacts the metabolic profiles of compounds dependent on C–H bond cleavage.[34] Specifically, drugs metabolized by enzymes such as cytochrome P450s or aldehyde oxidases can benefit from deuteration, leading to pharmaceutical compounds with improved pharmacokinetics, reduced toxicity,[35] and equal potency to the parent drug.[36,37] The advantages of deuteration are evident in its capacity to improve three major characteristics of drugs: (1) Safety: Deuterium incorporation enables 'metabolic shunting', generating fewer toxic metabolites and thereby enhancing the overall safety of the drug. (2) Tolerability: Deuterated drugs exhibit higher tolerability, allowing for administration at higher dosages. The inclusion of deuterium decreases the metabolic activity of specific drugs that undergo breakdown processes involving the cleavage of hydrogen–carbon bonds; this results in maintaining a more stable blood plasma concentration, resulting in increased half-life. (3) Bioavailability: Deuteration contributes to improved drug bioavailability, enhancing the drug's effectiveness and potential therapeutic impact. While maintaining spatial and charge distribution similarities with non-deuterated analogues, deuterium labeling can have complex effects on intermolecular interactions and enzyme binding, underscoring the nuanced advantages presented by deuterated drugs.[37]

Table 2 Properties of PET Radionuclides

Radioisotope	Half-life (min)	Decay mode	Nuclear reaction
^{11}C	20.34	β^+ (99%), EC (<1%)	$^{14}N + p \rightarrow {}^{11}C + {}^{4}He$
^{18}F	109.73	β^+ (97%), EC (3%)	$^{18}O + p \rightarrow {}^{18}F + n$
^{13}N	9.96	β^+ (100%)	$^{16}O + p \rightarrow {}^{13}N + {}^{4}He$
^{15}O	2	β^+ (100%)	$^{14}N + {}^{2}H \rightarrow {}^{15}O + n$

An example of the important role of deuterium substitution is with the antiviral M^{pro} inhibitor used in COVID-19 treatment. Coronavirus disease 2019 (COVID-19) is a highly contagious disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Although vaccination provides extra immunity toward SARS-CoV-2, there has been an urgent need to develop treatments for COVID-19 to alleviate symptoms for carriers of the disease. M^{pro} inhibitors are promising candidates for the treatment of COVID-19 because M^{pro} is a protease that plays a crucial role in viral replication. The majority of M^{pro} inhibitors belong to a class of compounds known as peptidomimetics, and often possess poor pharmacokinetic properties and oral bioavailability. A promising deuterated orally available covalent M^{pro} inhibitor with potent in vivo antiviral activity against emerging variants of SARS-CoV-2, Y180,[38] based on the general structure of the inhibitor Y180 (Figure 7c) proved to be the most effective among the tested inhibitors with the lowest rate of epimerization and an IC_{50} of 8.1 nM against SARS-CoV-2 M^{pro} (compared with ca. 13.3 μM for its non-deuterated analogue). This is a commendable example where deuterium acts to prevent epimerization without affecting the metabolism of the molecule, thus enabling the preparation of a single, more efficient epimer. This approach is also known as deuterium-enabled chiral switching (DECS),[39] which is defined as a strategy to enhance the therapeutic potential of chemically unstable racemic drugs, often resulting in pharmacological agents with improved efficacy and stability and reduced toxicity.

2.2 [^{18}O] Labeling in MCR

The bicyclic octahydro-2*H*-indol-2-one scaffold 4 was produced by an enantioselective three-component reaction between a ketone (1), a carboxylic acid (2), and a nitroalkene (3) (Figure 8).[40] As a chiral organic catalyst, densely substituted proline ester XL was employed, leading to stereoselective formation of three stereocenters. To elucidate the reaction mechanism, double ^{18}O-labeled carboxylic acid was used. The two ^{18}O-labels were found to be introduced in the lactam and ester carbonyl oxygen. The doubly labeled acid [^{18}O]$O_2$2 was synthesized from [^{18}O]OH_2 and (trichloromethyl)benzene. The new stereoselective 3-CR was used for a concise synthesis of the alkaloid (+)-pancracine.

3 Labeling with Radioactive Isotopes Using MCRs

The radioactive isotopes used in PET mainly include carbon-11, fluorine-18, nitrogen-13, oxygen-15 (Table 2), and some radiometal elements, such as copper-64, zirconium-89, gallium-68, etc.[41] The half-life of oxygen-15 is so short that it is rarely used in radiolabeling organic compounds. Therefore, the isotopes we focus on are carbon-11, fluorine-18, and nitrogen-13.

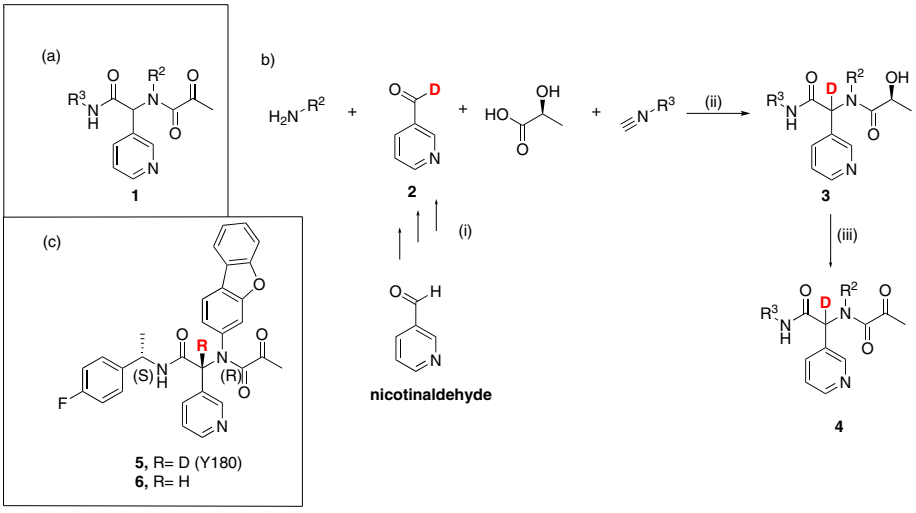
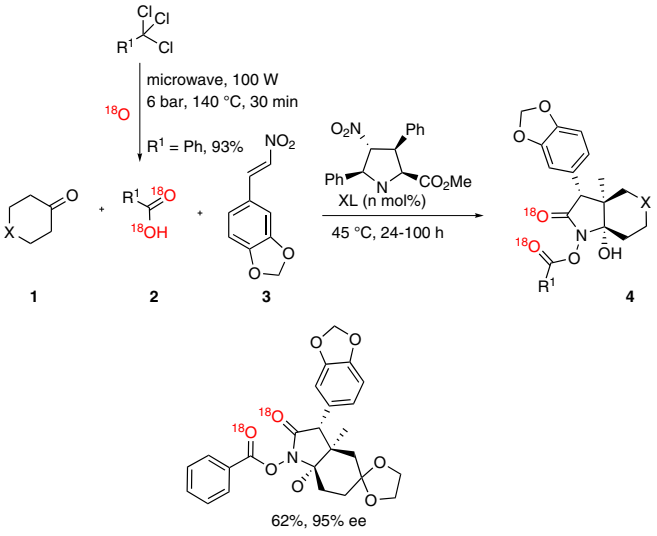


Figure 7 The synthesis of compound (c) can be obtained with a UGI 4-CR using a deuterated analogue of the nicotine aldehyde (**2**). This can be obtained from the non-deuterated nicotinaldehyde by a repeated reduction with $NaBD_4$ (to the deuterated pyridin-3-ylmethanol) followed by Dess–Martin oxidation to the aldehyde three times, sufficient to generate compound **2** with D incorporation >98%.

3.1 Labeling with Carbon-11

There is a great advantage to using ^{11}C to synthesize PET-tracers: it offers the opportunity to prepare PET-tracers with the native molecular structure unchanged. Currently, there are some methods to obtain ^{11}C-labeled compounds, such as isotope exchange reactions and functional group interconversion.[42] However, the half-life of ^{11}C is only 20.3 min, which is too short to be used in the multi-step synthesis of complex products.[40] Thus, ^{11}C requires a highly effi-



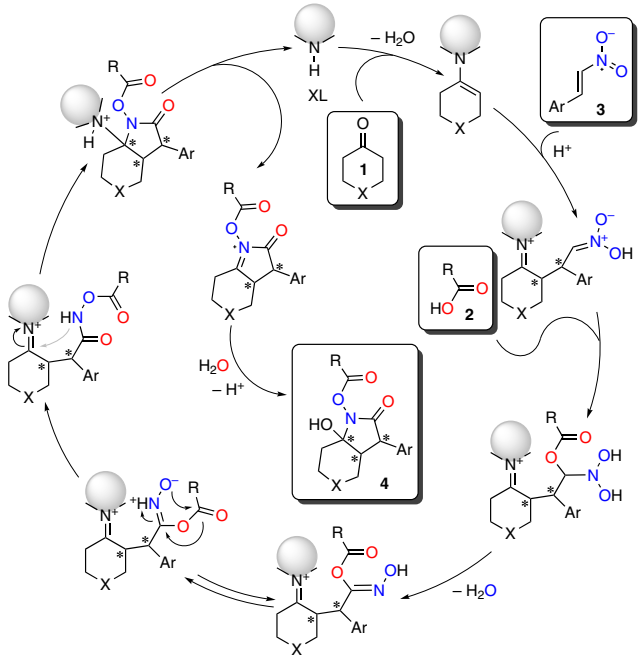


Figure 8 ^{18}O-labeled carboxylic acid was used to elucidate the mechanism of the enantioselective three-component reaction

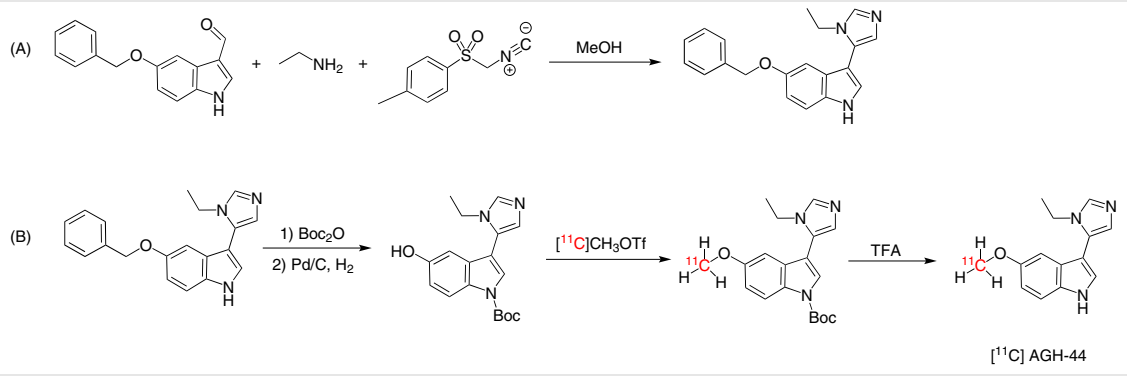


Figure 9 (A) Using van Leusen 3CR to synthesize the precursor. (B) Radiolabeling of [^{11}C]AGH-44.

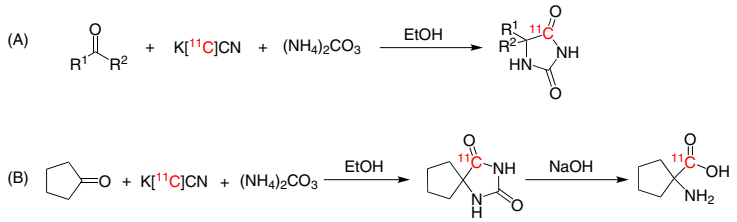


Figure 10 Using Bucherer–Bergs reaction synthesized (A) [^{11}C]hydantoins, and (B) [^{11}C]α-amino acids

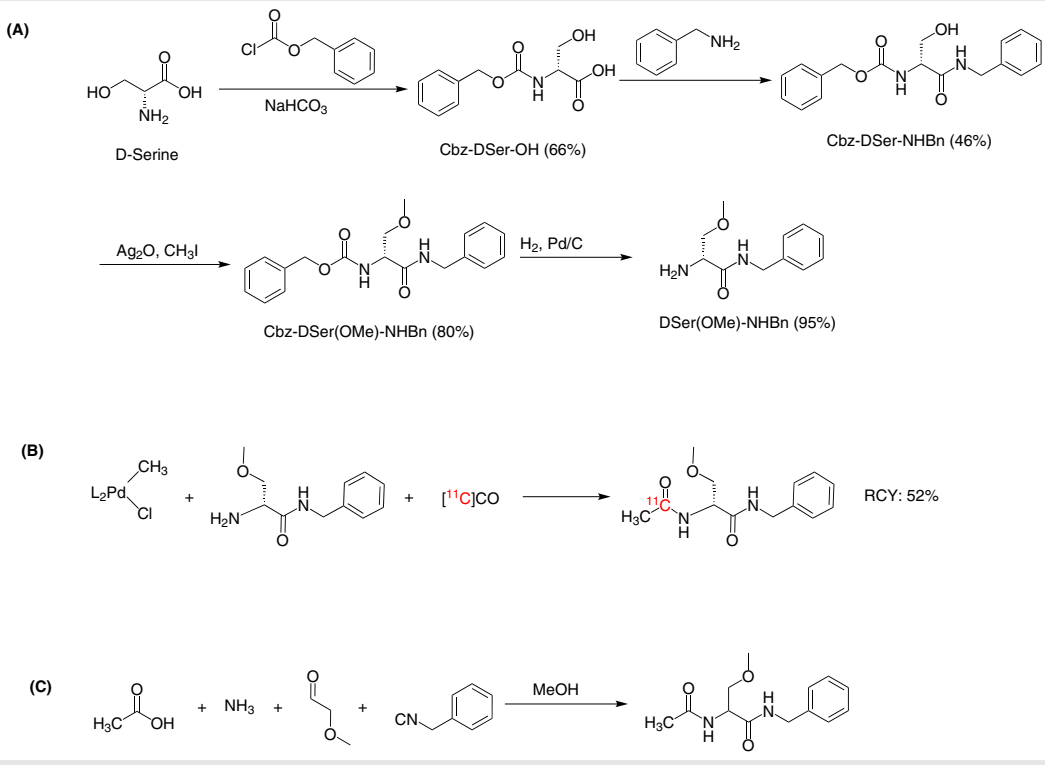


Figure 11 Lacosamide precursor synthesis: (A) stepwise by step; (B) Radiolabeling the precursor with ^{11}C. (C) Production of the lacosamide by MCR.

cient radiolabeling method, which should occur in the last or penultimate step to avoid the radioactive product from decaying completely.

An MCR was used to synthesize a precursor for [^{11}C]AGH-44, a selective serotonin 7 receptor (5-HT$_7$R) agonist. Carbon-11 was used to radiolabel AGH-44 in a two-step procedure, and evaluate its biodistribution and metabolism.[44] First, using the van Leusen imidazole synthesis, a precursor was synthesized (Figure 9A). Then, [^{11}C]methyl trifluoromethanesulfonate was used as a methylation agent to afford [^{11}C]AGH-44 (Figure 9B). The total synthesis time for radiolabeling and purification using HPLC was under 20 minutes, which is less than a half-life of ^{11}C.

In addition to being used for precursor synthesis, MCRs can also produce ^{11}C-labeled products directly. K[^{11}C]CN was used to synthesize [^{11}C]hydantoins using the Bucherer–Bergs reaction (Figure 10A).[45] Using this radiolabeling method, about 9.02 mCi radioactive product can be obtained within one half-life.[46] This shows the great advantage of using MCRs to radiolabel heterocyclic compounds. Various ^{11}C-labeled α-amino acids can also be produced according to this method (Figure 10B).[47] First, [^{11}C]hydantoins can be obtained by MCRs. Those products can then be hydrolyzed to afford the expected α-amino acids. Recently, the new methodology has been used to obtain α-amino acids through isotopic carboxylate exchange with a good RCY.[48]

Lacosamide is a drug that is believed to act through voltage-gated sodium channels for treating focal epileptic seizures.[49] The radiolabeled version could be used for pharmacological research. The precursor is synthesized in four steps and the overall yield is less than 25% (Figure 11A).[50] This is then radiolabeled using [^{11}C]CO (Figure 11B). In fact, lacosamide has been synthesized by MCR (Ugi-4CR) in a single step (Figure 11C).[51] Although the product is racemic, sufficient amounts of chiral products have been obtained after purification by chiral HPLC due to the high yield of the Ugi reaction, which exceeds 95%. The radioactive lacosamide could be synthesized in the same way by replacing common blocks with radioactive blocks; for example, [^{11}C]acetic acid or [^{13}N]NH$_3$. Moreover, this hypothetical

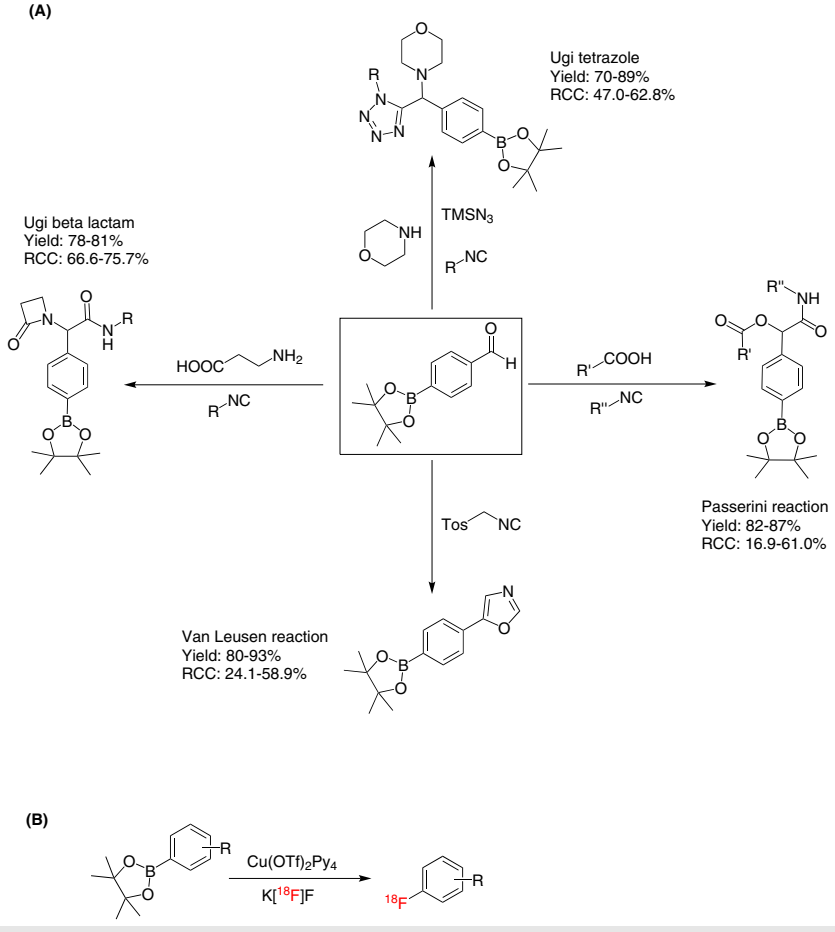


Figure 12 (A) MCRs starting from 4-formylphenylboronic acid pinacol ester. (B) Radiolabeling the precursor with fluorine-18.

method would be even more advantageous if suitable chiral catalysts are used. This shows the great potential of MCRs to improve radiolabeling efficiency.

3.2 Labeling with Fluorine-18 using MCRs

Fluorine-18 is the most widely used isotope for PET imaging because of its short positron range and favorable half-life of 110 min, which means ^{18}F-labeled compounds allow for more complex multistep synthesis, and products can be transported over longer distances to places outside the production site, or used for PET imaging of slower physiological processes, for up to a few hours. Currently, there are some methods to obtain fluorine-18 labeled compounds, such as using [^{18}F]difluorocarbene reagent.[52] Although ^{18}F has a sufficiently long half-life, it is still important to add ^{18}F as late as possible in the synthesis process to avoid unnecessary losses due to decay.

Using 4-formylphenylboronic acid pinacol ester as starting material, several types of MCRs have been used to obtain a panel of PET-precursors (Figure 12A and following the strategy in Figure 6A).[53] The Ugi beta lactam, the Ugi tetrazole, the Passerini reaction, and the van Leusen reaction provide a library of precursors with good yields. Precursors are then radiolabeled with ^{18}F using copper catalysis (Figure 12B). The value of radiochemical conversion (RCC) is the average conversion rate of radiolabeling of precursors using DMA as solvent. With this radiolabeling strategy, not only was the precursor library efficiently constructed, but also the radioactive products were obtained with a high RCC.

Another strategy with MCRs to obtain ^{18}F-labeled products is using 4-[^{18}F]fluorobenzaldehyde as the starting material following the strategy shown in Figure 6B. This affords ^{18}F-products directly through different types of MCRs.[32] Simple radioactive molecules such as 4-[^{18}F]fluorobenzaldehyde can be easily obtained with a high RCY of more than 80%. Using MCRs as the key step, various ^{18}F-labeled 3,4-dihydropyrimidin-2-(1*H*)-ones, imidazo[1,2-*a*]-pyridines, α-acyloxyamides and peptide-type products were produced from 4-[^{18}F]fluorobenzaldehyde (Figure 13). Using MCR to synthesize radioactive compounds, the target compounds can be synthesized with higher RCY in a relatively short time (Table 3). This strategy demonstrates that MCRs can be effectively employed to synthesize PET tracers.

Ezetimibe is a drug used to treat high blood cholesterol levels and certain other lipid abnormalities. Generally, this compound is synthesized stepwise with the overall yield about 5–22% (Figure 14A),[54] after which ^{18}F-radiolabeling is performed (Figure 14B).[55] Notably, the racemic acetylated ezetimibe compound was reportedly synthesized in one step using MCR (Staudinger 3CR). Then, chiral pure acetylated ezetimibe can be obtained by purification with a chiral OD column (Figure 14C).[5,56] In order to get the radioactive ezetimibe, radioactive building blocks such as 4-[^{18}F]fluoro-

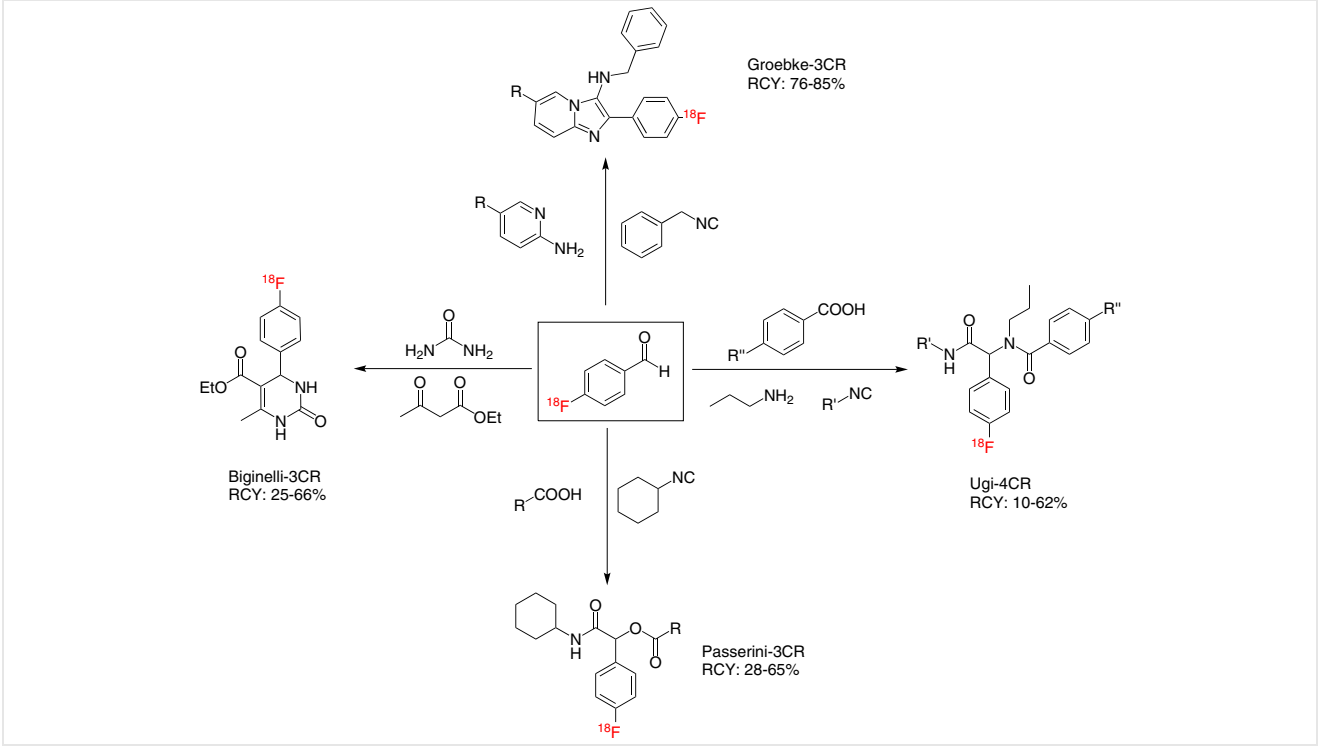


Figure 13 Radiolabeling MCRs starting from 4-[^{18}F]fluorobenzaldehyde

aniline or 4-[^{18}F]fluorobenzaldehyde could be used to replace the common building blocks. Then chiral purification is performed to obtain the radioactive product. This hypothetical approach has greater advantages compared to complex precursor synthesis.

3.3 Labeling with Nitrogen-13 Using MCRs

The half-life of ^{13}N is very short (9.96 min). This necessitates its immediate use after production.[57] It is challenging to use ^{13}N for radiolabeling of even simple compounds. However, combined with MCRs, it becomes possible to synthesize more structurally complex radioactive molecules with ^{13}N. NH_3 is a commonly used reactant in a variety of MCRs. [^{13}N]NH_3 was produced by proton irradiation of wa-

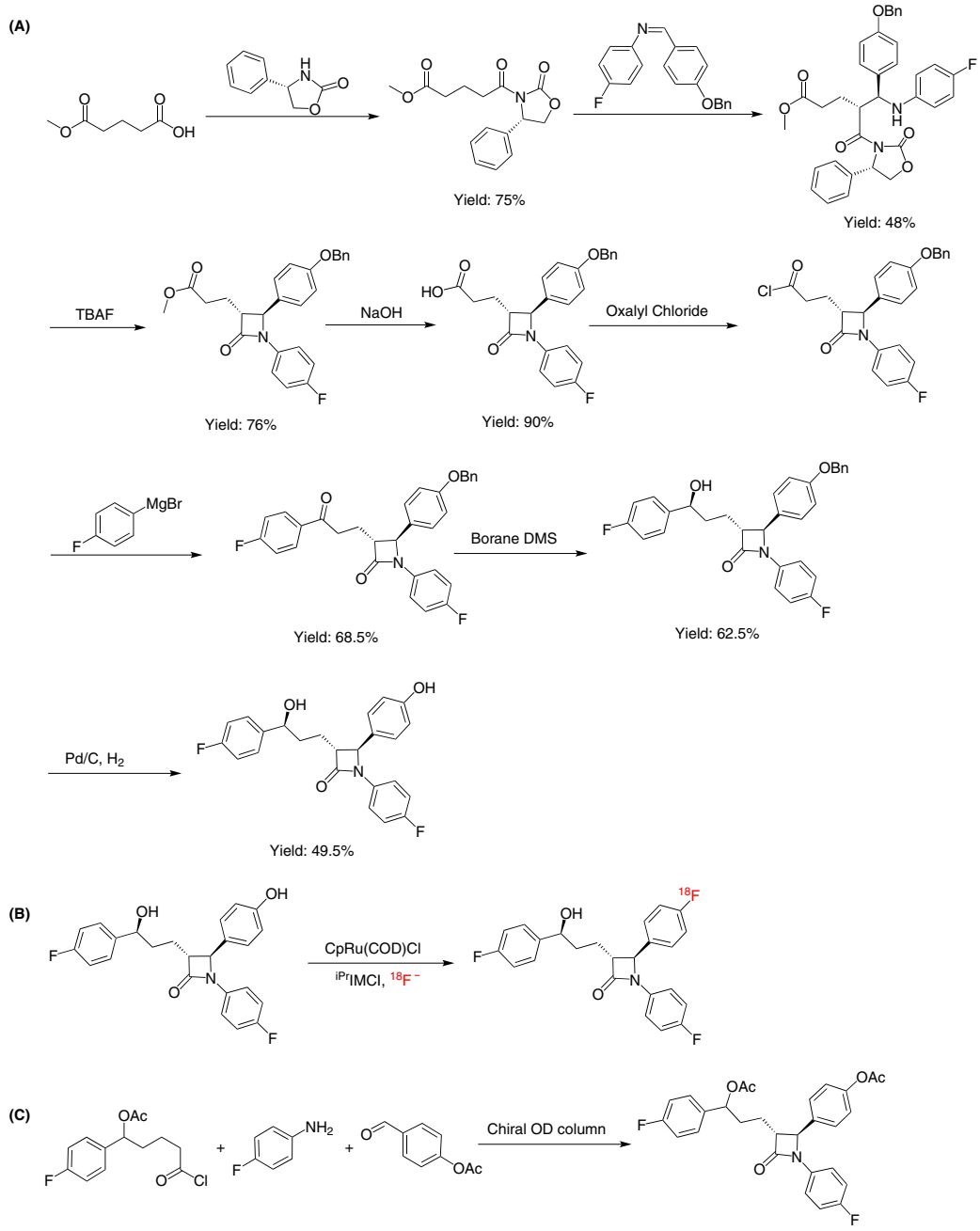
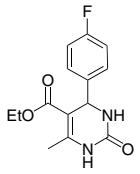
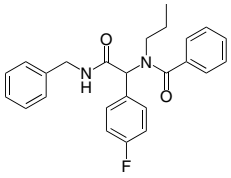
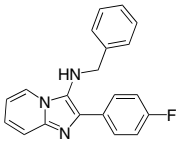
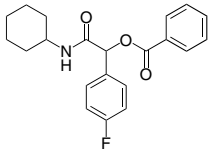


Figure 14 Ezetimibe synthesis: (A) Stepwise. (B) Radiolabeling the ezetimibe with ^{18}F. (C) Production of acetylated ezetimibe by MCR.

Table 3 Comparison of Reaction Time and Yield for Synthesizing ^{18}F and ^{19}F Compounds Using the Same Method

Compound	MCR	Isotope	Reaction time	Yield / RCY (%)
	Biginelli	^{19}F	5 h	80
		^{18}F	30 min	66 (n = 3)
	Ugi	^{19}F	48 h	78
		^{18}F	30 min	62 (n = 3)
	Groebke	^{19}F	24 h	73
		^{18}F	15 min	85 (n = 4)
	Passerini	^{19}F	8 h	98
		^{18}F	30 min	65 (n = 3)

ter and ethanol (nuclear reaction: $^{16}O(p,\alpha)^{13}N$) by cyclotron. Therefore, $[^{13}N]NH_3$ is recovered directly from the cyclotron target.

1,4-Dihydropyridines (1,4-DHPs) and its derivatives are employed as vasodilators, anti-atherosclerotics, anti-diabetics, anti-inflammatories, anti-malarials, and anti-bacterial drugs. The most widely used is nifedipine. Radioactive 1,4-DHPs could be used to monitor and evaluate cardiac abnormalities by PET imaging the function of calcium channels *in vivo*. Gee's group established a radiosynthetic method using aqueous $[^{13}N]NH_3$ for rapid synthesis of 1,4-DHPs and its derivatives with a Hantzsch MCR radiolabeling strategy (Figure 15).[58] The optimized radiolabeling conditions are 100 °C, 5 min, 1 M NaOH in DMF and the radiochemical yield is 85%. This is very meaningful for early clinical development.

$[^{13}N]NH_3$ also can be used to radiolabel peptide-like compounds through an Ugi-4CR reaction using a microwave synthesis method (Figure 16).[59] The RCY of a small library of ^{13}N-labeled α-aminoacyl amide derivatives and γ-lactams was reported to be 11–23% after 10 min in a microwave reactor. This is an efficient strategy to quickly obtain a diverse ^{13}N-labeled peptidomimetics library.

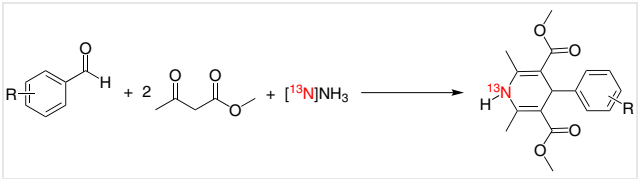


Figure 15 Synthesis of ^{13}N-labeled 1,4-dihydropyridine

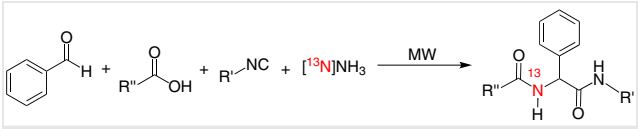


Figure 16 Reaction for the synthesis of ^{13}N-labeled peptides with Ugi-4CR

3.4 Synthesis of a Chelator Using MCRs

Dodecane tetra acetic acid (DOTA) also known as tetraxetan is a widely used metal chelator, especially for lanthanide ions with applications in medicine.[60] DOTA-chelated metals are used as a contrast agent and for cancer diagnosis and treatment. DOTA derivatives were also used in positron emission tomography.[61]

The complex of Gd^{3+} and DOTA is used as a gadolinium-based MRI contrast agent under the name gadoteric acid.[62] Recently, the DOTA tetrazole isosteric TEMDO was de-

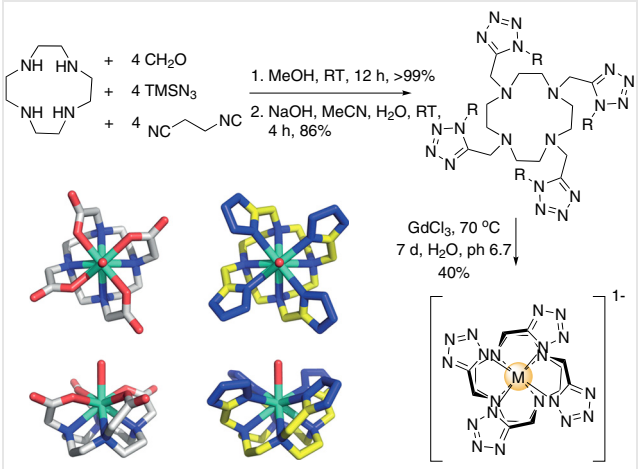


Figure 17 Synthesis of the DOTA-isosteric TEMDO ligand and its Gd complex. Comparison of the solid-state structure of Gd-DOTA (left column) and Gd-TEMDO (right column) with approximate C_4 symmetry. Stick representation of the crystal structures including the coordinated water, shown in red (unbound crystal waters and counter-ions are omitted for clarity).

scribed.[63] The synthesis involves a convergent MCR approach employing the Ugi tetrazole reaction. The ligand was prepared in just two steps in very good yields under mild conditions (Figure 17). The Gd^{3+} complex of TEMDO was prepared and characterized and used in MRI experiments in a left-coronary-artery occlusion murine animal model to image the myocardial infarcted tissue. It is conceivable that many more cyclic or acyclic chelating tetrazole derivatives can be synthesized using Ugi's MCR, tailored to different metal isotopes and applications.

4 Final Remarks

The integration of convergent MCRs for the synthesis of small molecules has garnered momentum over the past decade, leveraging its inherent compatibility with automation[64] and its efficacy in the streamlined synthesis and detection of compounds. MCRs are an efficient synthesis strategy for complex molecules with multiple functional groups and have the potential to improve radiolabeling efficiency and greatly expand the range of radiotracers. We show some examples of the synthesis of PET-tracers using MCRs in Table 4. Additionally, for any new radiolabeling method, purification, automation, quality control, and most importantly GMP feasibility need to be considered to ensure entry into the clinic. The by-products of MCRs are predictable, which enables robust quality control methods for radioactive products. The products of MCRs are racemic and this is not conducive to drug development. With impending progress in asymmetric synthesis and the commercialization of chiral phase-transfer catalysts, this problem will be solved in the future. Therefore, the combination of MCR and radiochemistry will greatly advance the development of PET chemistry and is expected to cause a paradigm shift in radiolabeling.

Table 4 Examples of Using MCRs in PET-Tracers

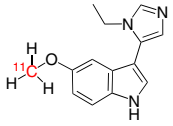
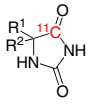
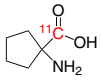
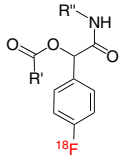
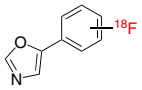
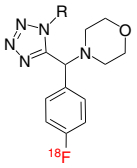
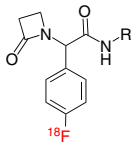
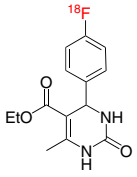
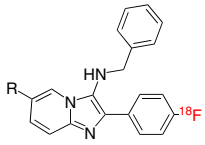
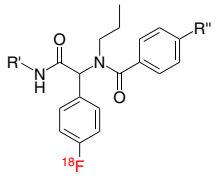
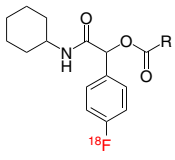
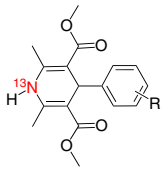
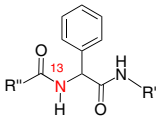
Isotope	Compound	Synthesis method	Application	Ref.
^{11}C		van Leusen imidazole synthesis	pharmacological research	43,44
		Bucherer-Bergs reaction	pharmacological research	45
		Bucherer-Bergs reaction	–	46
^{18}F		Passerini reaction	–	53
		van Leusen reaction	–	53
		Ugi tetrazole	–	53

Table 4 (continued)

Isotope	Compound	Synthesis method	Application	Ref.
		Ugi beta lactam	–	53
		Biginelli reaction	–	32
		Groebke-Blackburn-Bienayme	–	32
		Ugi-4CR	–	32
		Passerini reaction	–	32
^{13}N		Hantzsch reaction	pharmacological research	58
		Ugi-4CR	–	59

The current use of MCRs in isotope labeling, while still limited, holds significant potential due to their unique advantages: (1) Availability of labeled building blocks: MCRs in isotope labeling rely on the accessibility of labeled building blocks or precursors, a factor facilitated by a growing catalog of commercially available isotopically labeled compounds. (2) MCR in PET labeling: The extensive range of MCR-compatible boronic acids, both available and synthesizable, along with the introduction of boronic acid isocyanides, underlines the efficiency of MCRs in ^{18}F-PET labeling. (3) Versatile labeling approaches: MCRs can incorporate a variety of simple isotope-labeled building blocks, making them versatile for applications in medical imaging and diagnostics. (4) Advancement in research: MCRs play a vital

role in research, particularly in developing new drugs and diagnostic agents, by efficiently labeling molecules with isotopes. In fact, many marketed drugs or drugs in development, can be synthesized more conveniently, faster, and more economically by MCR. (5) Cost-effectiveness and scalability: MCRs potentially offer a cost-effective and scalable approach to isotope labeling, which is crucial for large-scale production in clinical research and pharmaceutical development. (6) Environmental sustainability: Aligning with green chemistry principles, MCRs in isotope labeling show high atom economy and reduced waste. Also waste streams in the isotope labeling area are minor compared to drug discovery or production, isotope labeled compounds often comprise a considerable environmental hazard due to their radioactivity. (7) Convergence for short-lived Isotopes: The convergent nature of MCRs is a key to synthesizing compounds with short-lived isotopes or those challenging to handle due to radioactivity. This convergence is crucial for handling isotopes with short half-lives, enhancing safety in handling radioactive materials, streamlining synthesis procedures, and increasing reliability and reproducibility in the synthesis of isotopically labeled compounds.

While isotopic labeling in MCRs presents certain limitations, such as the decay of isotopes and availability of starting materials, these challenges are not insurmountable. The rapid execution of some MCRs mitigates the issue of isotope decay, ensuring efficient labeling processes. Furthermore, ongoing advancements in synthetic methodologies are expanding the repertoire of available starting materials for MCR-based isotopic labeling. Additionally, although not all MCR reactions may be amenable to isotopic labeling, the growing body of evidence, including promising drug candidates undergoing clinical trials, underscores the potential of MCRs coupled with isotopic labeling in pharmaceutical research and beyond. Therefore, despite the current constraints, the synergistic combination of MCRs and isotopic labeling holds significant promise for advancing both chemical synthesis and molecular research endeavors.

In summary, the ability of MCRs to quickly and convergently assemble target compounds and to handle short-lived isotopes safely and efficiently makes them interesting in synthetic chemistry, particularly for medical diagnostics and pharmacological research. This review aims to encourage scientists to consider MCRs as a future alternative for isotope labeling.

Conflict of Interest

The authors declare no conflict of interest.

Funding Information

This research has been supported (to AD) through the ERA Chair grant ACCELERATOR (101087318), the ERC Advanced grant AMADEUS (101098001), and the VIDEC grant (872195). This study was supported (AD) by the National Institute for Cancer Research—Programme EXCELES (ID Project No. LX22NPO5102), funded by the Cancer Research Czech Republic, and the Dutch Cancer Society (KWF Kankerbestrijding, KWF) grant (14712).

References

(1) Dömling, A.; Ugi, I. *Angew. Chem. Int. Ed.* **2000**, *39*, 3168.
(2) Cioc, R. C.; Ruijter, E.; Orru, R. V. *Green Chem.* **2014**, *16*, 2958.
(3) Zarganes-Tzitzikas, T.; Dömling, A. *Org. Chem. Front.* **2014**, *1*, 834.
(4) Wehlan, H.; Oehme, J.; Schäfer, A.; Rossen, K. *Org. Process Res. Dev.* **2015**, *19*, 1980.
(5) Zarganes-Tzitzikas, T.; Chandgude, A. L.; Dömling, A. *Chem. Rec.* **2015**, *15*, 981.
(6) Cervenka, S.; Frick, A.; Bodén, R.; Lubberink, M. *Transl. Psychiatry* **2022**, *12*, 248.
(7) Kellermann, J. *2D PAGE: Sample Preparation and Fractionation, Vol. 1*, In *Methods in Molecular Biology, Vol. 424*; Posch, A., Ed.; Humana Press: New Jersey, **2008**, DOI: 10.1007/978-1-60327-064-9.
(8) Zhu, Y.; Zhou, J.; Jiao, B. *ACS Med. Chem. Lett.* **2013**, *4*, 349.
(9) Ong, S.-E.; Mann, M. *Nat. Protoc.* **2006**, *1*, 2650.
(10) Hoy, S. M. *Drugs* **2022**, *82*, 1671.
(11) Zarganes-Tzitzikas, T.; Neochoritis, C. G.; Dömling, A. *ACS Med. Chem. Lett.* **2019**, *10*, 389.
(12) Cao, H.; Liu, H.; Dömling, A. *Chem. Eur. J.* **2010**, *16*, 12296.
(13) Popovici-Muller, J.; Lemieux, R. M.; Artin, E.; Saunders, J. O.; Salituro, F. G.; Travins, J.; Cianchetta, G.; Cai, Z.; Zhou, D.; Cui, D. *ACS Med. Chem. Lett.* **2018**, *9*, 300.
(14) Znabet, A.; Polak, M. M.; Janssen, E.; de Kanter, F. J.; Turner, N. J.; Orru, R. V.; Ruijter, E. *Chem. Commun.* **2010**, *46*, 7918.
(15) Kalinski, C.; Lemoine, H.; Schmidt, J.; Burdack, C.; Kolb, J.; Umkehrer, M.; Ross, G. *Synthesis* **2008**, 4007.
(16) (a) Váradi, A.; Palmer, T. C.; Haselton, N.; Afonin, D.; Subrath, J. J.; Le Rouzic, V.; Hunkele, A.; Pasternak, G. W.; Marrone, G. F.; Borics, A. *ACS Chem. Neurosci.* **2015**, *6*, 1570. (b) Malaquin, S.; Jida, M.; Gesquiere, J.-C.; Deprez-Poulain, R.; Deprez, B.; Laconde, G. *Tetrahedron Lett.* **2010**, *51*, 2983.
(17) Preschel, H. D.; Otte, R. T.; Zhuo, Y.; Ruscoe, R. E.; Burke, A. J.; Kellerhals, R.; Horst, B.; Hennig, S.; Janssen, E.; Green, A. P. *J. Org. Chem.* **2023**, *88*, 12565.
(18) Li, X.; Zarganes-Tzitzikas, T.; Kurpiewska, K.; Dömling, A. *Green Chem.* **2023**, *25*, 4137.
(19) Cioc, R. C.; van Riempst, L. S.; Schuckman, P.; Ruijter, E.; Orru, R. V. *Synthesis* **2017**, *49*, 1664.
(20) Wang, P.-L.; Ding, S.-Y.; Zhang, Z.-C.; Wang, Z.-P.; Wang, W. *J. Am. Chem. Soc.* **2019**, *141*, 18004.
(21) (a) Willmann, J. K.; van Bruggen, N.; Dinkelborg, L. M.; Gambhir, S. S. *Nat. Rev. Drug Discovery* **2008**, *7*, 591. (b) Zhang, J.-J.; Fu, H.; Lin, R.; Zhou, J.; Haider, A.; Fang, W.; Elghazawy, N. H.; Rong, J.; Chen, J.; Li, Y. *J. Med. Chem.* **2023**, *66*, 10889.
(22) (a) Pike, V. W. *Curr. Med. Chem.* **2016**, *23*, 1818. (b) Micheli, F. *ChemMedChem* **2011**, *6*, 1152.
(23) Matthews, P. M.; Rabiner, E. A.; Passchier, J.; Gunn, R. N. *Br. J. Clin. Pharmacol.* **2012**, *73*, 175.

(24) Nolting, D. D.; Nickels, M. L.; Guo, N.; Pham, W. *Am. J. Nucl. Med. Mol. Imaging* **2012**, *2*, 273.
(25) Slobbe, P.; Ruijter, E.; Orru, R. V. *MedChemComm* **2012**, *3*, 1189.
(26) Deng, X.; Rong, J.; Wang, L.; Vasdev, N.; Zhang, L.; Josephson, L.; Liang, S. H. *Angew. Chem. Int. Ed.* **2019**, *58*, 2580.
(27) Pretze, M.; Franck, D.; Kunkel, F.; Foßhag, E.; Wängler, C.; Wängler, B. *Nucl. Med. Biol.* **2017**, *45*, 35.
(28) (a) Schirrmacher, R.; Wangler, C.; Schirrmacher, E. *Mini-Rev. Org. Chem.* **2007**, *4*, 317. (b) Xu, Y.; Qu, W. *Eur. J. Org. Chem.* **2021**, 4653.
(29) Zhang, M.; Li, S.; Zhang, H.; Xu, H. *Eur. J. Med. Chem.* **2020**, *205*, 112629.
(30) Domling, A.; Wang, W.; Wang, K. *Chem. Rev.* **2012**, *112*, 3083.
(31) Dömling, A. *Chem. Rev.* **2006**, *106*, 17.
(32) Li, L.; Hopkinson, M. N.; Yona, R. L.; Bejot, R.; Gee, A. D.; Gouverneur, V. *Chem. Sci.* **2011**, *2*, 123.
(33) Harbeson, S. L.; Tung, R. D. *Annu. Rep. Med. Chem.* **2011**, *46*, 403.
(34) Pirali, T.; Serafini, M.; Cargnin, S.; Genazzani, A. A. *J. Med. Chem.* **2019**, *62*, 5276.
(35) Zhan, Z.; Peng, X.; Sun, Y.; Ai, J.; Duan, W. *Chem. Res. Toxicol.* **2018**, *31*, 1213.
(36) Atzrodt, J.; Derdau, V.; Kerr, W. J.; Reid, M. *Angew. Chem. Int. Ed.* **2018**, *57*, 1758.
(37) (a) Sun, H.; Piotrowski, D. W.; Orr, S. T.; Warmus, J. S.; Wolford, A. C.; Coffey, S. B.; Futatsugi, K.; Zhang, Y.; Vaz, A. D. *PLoS ONE* **2018**, *13*, e0206279. (b) Gant, T. G. *J. Med. Chem.* **2014**, *57*, 3595. (c) Knutson, D. E.; Kodali, R.; Divović, B.; Treven, M.; Stephen, M. R.; Zahn, N. M.; Dobričić, V.; Huber, A. T.; Meirelles, M. A.; Verma, R. S. *J. Med. Chem.* **2018**, *61*, 2422.
(38) Jansen-van Vuuren, R. D.; Jedlovčnik, L.; Košmrlj, J.; Massey, T. E.; Derdau, V. *ACS Omega* **2022**, *7*, 41840.
(39) DeWitt, S.; Czarnik, A. W.; Jacques, V. *ACS Med. Chem. Lett.* **2020**, *11*, 1789.
(40) de Gracia Retamosa, M.; Ruiz-Olalla, A.; Bello, T.; de Cózar, A.; Cossío, F. P. *Angew. Chem.* **2018**, *130*, 676.
(41) Mikulová, M. B.; Mikuš, P. *Pharmaceuticals* **2021**, *14*, 167.
(42) Labiche, A.; Malandain, A.; Molins, M.; Taran, F.; Audisio, D. *Angew. Chem. Int. Ed.* **2023**, *62*, e202303535.
(43) Cole, E. L.; Stewart, M. N.; Littich, R. M.; Hoareau, R.; Scott, P. J. H. *Curr. Top. Med. Chem.* **2014**, *14*, 875.
(44) (a) L'Estrade, E. T.; Petersen, I. N.; Xiong, M.; Hogendorf, A. S.; Hogendorf, A.; Kristensen, J. L.; Kjær, A.; Bojarski, A. J.; Erlandsson, M.; Ohlsson, T. *J. Radioanal. Nucl. Chem.* **2019**, 322, 847. (b) Hogendorf, A. S.; Hogendorf, A.; Kurczab, R.; Satała, G.; Lenda, T.; Walczak, M.; Latacz, G.; Handzlik, J.; Kieć-Kononowicz, K.; Wierońska, J. M. *Sci. Rep.* **2017**, *7*, 1444.
(45) Winstead, M. B.; Parr, S. J.; Rogal, M. J.; Brockman, P. S.; Lubcher, R.; Khentigan, A.; Lin, T.-H.; Lamb, J. F.; Winchell, H. S. *J. Med. Chem.* **1976**, *19*, 279.
(46) Stavchansky, S.; Tilbury, R.; McDonald, J.; Ting, C.; Kostenbauder, H. *J. Nucl. Med.* **1978**, *19*, 936.
(47) Sambre, J.; Vandecasteele, C.; Goethals, P.; Rabi, N.; Van Haver, D.; Slegers, G. *Int. J. Appl. Radiat. Isot.* **1985**, *36*, 275.
(48) Bsharat, O.; Doyle, M. G. J.; Munch, M.; Mair, B. A.; Cooze, C. J. C.; Derdau, V.; Bauer, A.; Kong, D.; Rotstein, B. H.; Lundgren, R. J. *Nat. Chem.* **2022**, *14*, 1367.
(49) Rogawski, M. A.; Tofighy, A.; White, H. S.; Matagne, A.; Wolff, C. *Epilepsy Res.* **2015**, *110*, 189.
(50) (a) Andersen, T. L.; Nordeman, P.; Christoffersen, H. F.; Audrain, H.; Antoni, G.; Skrydstrup, T. *Angew. Chem.* **2017**, *129*, 4620. (b) Dahl, K.; Nordeman, P. *Eur. J. Org. Chem.* **2017**, 5785.
(51) Wehlan, H.; Rossen, K.; Oehme, J.; Kral, V. International Patents No. WO2013072330A1, **2013**.
(52) Sap, J. B. I.; Meyer, C. F.; Ford, J.; Straathof, N. J. W.; Dürr, A. B.; Lelos, M. J.; Paisey, S. J.; Mollner, T. A.; Hell, S. M.; Trabanco, A. A.; Genicot, C.; am Ende, C. W.; Paton, R. S.; Tredwell, M.; Gouverneur, V. *Nature* **2022**, *606*, 102.
(53) Zarganes-Tzitzikas, T.; Clemente, G. S.; Elsinga, P. H.; Dömling, A. *Molecules* **2019**, *24*, 1327.
(54) (a) Sasikala, C.; Reddy Padi, P.; Sunkara, V.; Ramayya, P.; Dubey, P.; Bhaskar Rao Uppala, V.; Praveen, C. *Org. Process Res. Dev.* **2009**, *13*, 907. (b) Zhu, Y.; Pan, J.; Zhang, S.; Liu, Z.; Ye, D.; Zhou, W. *Synth. Commun.* **2016**, *46*, 1687.
(55) Beyzavi, H.; Mandal, D.; Strebl, M. G.; Neumann, C. N.; D'Amato, E. M.; Chen, J.; Hooker, J. M.; Ritter, T. *ACS Cent. Sci.* **2017**, *3*, 944.
(56) (a) Rosenblum, S. B.; Huynh, T.; Afonso, A.; Davis, H. R.; Yumibe, N.; Clader, J. W.; Burnett, D. A. *J. Med. Chem.* **1998**, *41*, 973. (b) Palomo, C.; Aizpurua, J. M.; Ganboa, I.; Oiarbide, M. *Eur. J. Org. Chem.* **1999**, 3223.
(57) Rong, J.; Haider, A.; Jeppesen, T. E.; Josephson, L.; Liang, S. H. *Nat. Commun.* **2023**, *14*, 3257.
(58) Blower, J. E.; Ma, M. T.; Al-Salemee, F. A.; Gee, A. D. *Chem. Commun.* **2021**, *57*, 4962.
(59) Blower, J. E.; Cousin, S. F.; Gee, A. D. *EJNMMI Radiopharm. Chem.* **2017**, *2*, 1.
(60) (a) Schwarz, G.; Mueller, L.; Beck, S.; Linscheid, M. W. *J. Anal. At. Spectrom.* **2014**, *29*, 221. (b) Sneddon, D.; Cornelissen, B. *Curr. Opin. Chem. Biol.* **2021**, *63*, 152.
(61) (a) Pierri, G.; Schettini, R. *J. Pept. Sci.* **2023**, e3544. (b) Rashid, H. U.; Martines, M. A. U.; Jorge, J.; de Moraes, P. M.; Umar, M. N.; Khan, K.; Rehman, H. U. *Bioorg. Med. Chem.* **2016**, *24*, 5663.
(62) Roxin, Á.; Zhang, C.; Huh, S.; Lepage, M.; Zhang, Z.; Lin, K.-S.; Bénard, F.; Perrin, D. M. *Bioconjugate Chem.* **2019**, *30*, 1210.
(63) Boltjes, A.; Shrinidhi, A.; van de Kolk, K.; Herdtweck, E.; Dömling, A. *Chem. Eur. J.* **2016**, *22*, 7352.
(64) Wang, Y.; Shaabani, S.; Ahmadianmoghaddam, M.; Gao, L.; Xu, R.; Kurpiewska, K.; Kalinowska-Tluscik, J.; Olechno, J.; Ellson, R.; Kossenjans, M. *ACS Cent. Sci.* **2019**, *5*, 451.