Recent Progress using the Staudinger Ligation for Radiolabeling Applications

Mamat, C.; Gott, M.; Steinbach, J.;

Originally published:

March 2018

Journal of Labelled Compounds and Radiopharmaceuticals 61(2018), 165-178

DOI: https://doi.org/10.1002/jlcr.3562

Perma-Link to Publication Repository of HZDR:

https://www.hzdr.de/publications/Publ-25545

Recent Progress using the Staudinger Ligation for Radiolabeling Applications

Constantin Mamat[a,b,*], Matthew Gott [a], and Jörg Steinbach [a,b]

a) Institut für Radiopharmazeutische Krebsforschung, Helmholtz-Zentrum Dresden-Rossendorf, Bautzner Landstraße 400, D-01328 Dresden, Germany

b) Fachbereich Chemie und Lebensmittelchemie, Technische Universität Dresden, D 01062 Dresden, Germany

* Correspondence to: Constantin Mamat, Institut für Radiopharmazeutische Krebsforschung, Helmholtz-Zentrum Dresden-Rossendorf, Bautzner Landstraße 400, D 01328 Dresden, Germany; e-mail: c.mamat@hzdr.de

Short title: Radiolabeling via Staudinger Ligation

Key words: fluorine-18, Staudinger Ligation, click chemistry, bioorthogonal

Abstract:

The increasing application of positron emission tomography (PET) and single photon emission computer tomography (SPECT) in radiopharmacy and nuclear medicine has stimulated the development of a multitude of novel and versatile bioorthogonal conjugation techniques. Currently, there is particular interest in radiolabeling biologically active, high molecular weight compounds like peptides, proteins or antibodies, but also for the labeling of small organic compounds. An enormous challenge in radiolabeling these biologically active molecules is that the introduction of radiohalogens like fluorine-18 as well as various radiometals proceeds under harsh conditions, which could destroy the biomolecule.

The Staudinger Ligation is one of the most powerful bioorthogonal conjugation techniques. The reaction proceeds over wide temperature and pH ranges; an amide (peptide) bond is formed as the ligation unit, which minimizes distortion of the structure; no isomers are obtained; and the reaction proceeds without any metal catalyst. Due to this adaptability, this robust ligation type is a perfect candidate with a high potential for various applications in the field of radiopharmacy for the labeling of biomolecules under mild conditions.

This review summarizes recent research concerning the implementation of the Staudinger Ligation for radiolabeling applications.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/jlcr.3562

Introduction

Mild and site-selective labeling reactions are often required for the radiolabeling of biomacromolecules as they have a high number of functional groups.[1] In principle, radiolabeling of such compounds with fluorine-18, especially biologically active high molecular weight compounds like peptides, proteins and antibodies, still represents a considerable challenge and has generated immense interest.[2,3] Well established methods are known for the mild and selective introduction of radiometals using either protecting groups or the pre-labeling concept.[4] However, fluorine-18 is mostly introduced as [^{18}F]fluoride under harsh reaction conditions like organic solvents and high temperatures. Thus, fast labeling strategies are needed to introduce fluorine-18 under mild conditions using radiolabeling building blocks also referred as fluorine-18-containing prosthetic groups with high specific activities. Due to these facts, a labeling strategy has to be developed for almost every one of these high molecular weight compounds.[5] To overcome the above mentioned obstacles, bioorthogonal click-labeling reactions were developed in the past and successfully applied to allow the selective introduction of fluorine-18 under mild conditions, with high radiochemical yields (RCY) and high specific activity (A_S).[6,7,8]

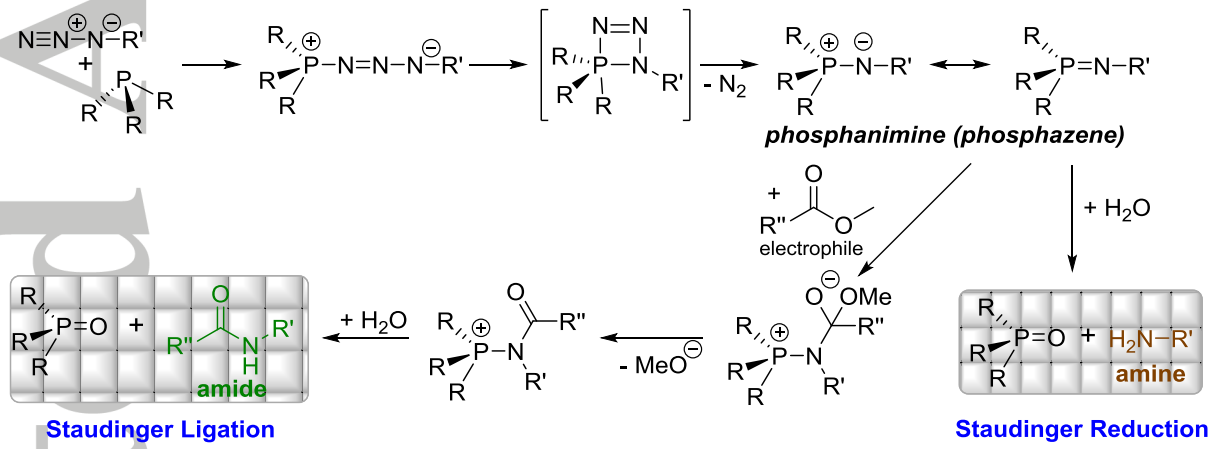
The bioorthogonal concept refers to all reactions which can proceed inside living systems without interfering with native biochemical processes. Several specifications have to be fulfilled for a reaction to be considered as biorthogonal.[9] First and most importantly, selective reactions should be applied to avoid side-reactions with other functional groups found in the biological starting compounds. The functional groups of both reactants should only react with each other and must be inert to the remaining chemical and biological functionalities of the molecules. Secondly, the resulting covalent bond should be chemically inert to biological reactions and should not affect the (native) biological behavior of the desired molecule. Thirdly, the reaction should have fast reaction kinetics. This fact is especially important for working with short-lived positron emitters like fluorine-18 ($t_{1/2}$ = 110 min) or carbon-11 ($t_{1/2}$ = 20 min) and for *in vivo* pre-targeting strategies. Finally, the non-toxicity of the reactants as well as the catalysts are of high importance for *in vivo* applications, and the reaction should proceed under biological conditions taking into account pH, aqueous environments, and temperature.[10]

The terms “click chemistry” and “bioorthogonal reactions” have entered into the field of radiochemistry and radiopharmacy. Both terms describe a set of labeling reactions which make use of several site-selective and modular building blocks as well as facilitate chemoselective ligations to radiolabel biologically relevant compounds. In this context, the Cu(I)-mediated triazole formation from azides and terminal alkynes according to the 1,3-dipolar Huisgen cycloaddition as well as the strain-promited Cu-free variant are particularly powerful ligation reactions, due to their high degree of specificity and the biocompatibility of both starting materials. In the same way, both variants of the Staudinger Ligation were applied. The absence of catalysts makes these reactions tremendously attractive for radiolabeling purposes. Neither azides, phosphanes, alkenes nor tetrazines react with other functional groups commonly present in biopolymers like peptides or proteins. Hence, there is no need for protective group chemistry. Thus, bioorthogonal click chemistry is a very attractive approach for the evaluation of novel potent radiotracers for molecular imaging purposes.

Origin of the Staudinger Ligation

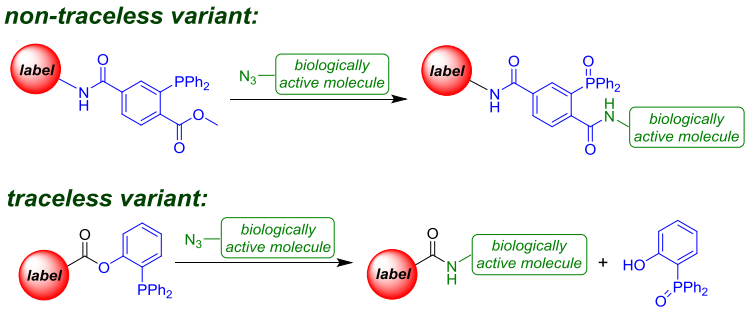
In 1919, Hermann Staudinger and his colleague Jules Meyer were the first to describe the reaction of organic azides with phosphanes.[11] They obtained phosphane imines (phosphazenes or aza-ylides) as products and elemental nitrogen as by-product under absence of water. A hydrolytic cleavage of the phosphane imines occurs immediately by the addition of water to the reaction mixture yielding the respective primary amine and phosphane oxide.[12] The formation of a highly stable oxygen phosphorus double bond is the driving force for this reaction, which is classically known as Staudinger reduction.[13]

Phosphane imines, in general, are able to react with other compounds having an electrophilic center instead of water. The utilization of carboxylic esters leads to compounds containing an acid amide bond between the organic moiety of the former azide and the organic residue of the former ester after hydrolysis of the intermediate. Thus, this intermolecular variant of the Staudinger Ligation (three component reaction) was applied in the past for conjugation purposes.[14,15,16,17] Both reaction mechanisms are illustrated in Scheme 1.[18,19]



Scheme 1. Overview of the mechanism of the Staudinger reduction and the intermolecular Staudinger Ligation.

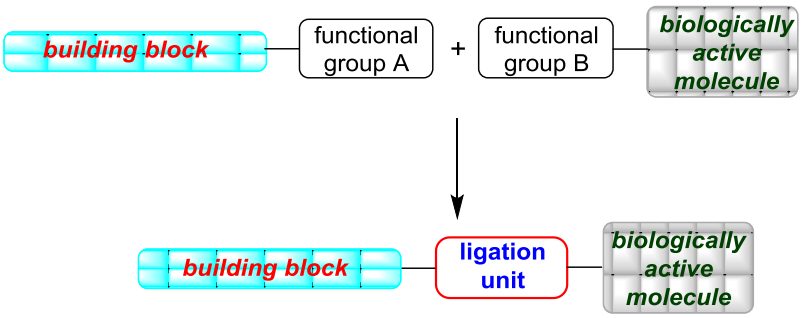
The traceless[20,21] and the non-traceless[22] versions of the bioorthogonal Staudinger Ligation (intramolecular, two component reactions) were discovered in the year 2000 (Scheme 2). Being two of the first truly biorthogonal ligation reactions, both variants of the Staudinger Ligation have found various applications such as: connecting carbohydrates and peptides, labeling of bioactive molecules with fluorescence dyes and radionuclides, chemoselectively modifying of peptides and proteins, modifying polymers, preparing special lactams and other (macrocyclic) ring systems, and synthesizing glycosyl amides. Additionally, the absence of cytotoxic copper salts makes both variants of the Staudinger Ligation highly interesting for potential *in vitro* and *in vivo* applications in contrast to other catalyzed ligation reactions like the 1,3-dipolar Huisgen cycloaddition. Overviews about the general utilization of both Staudinger Ligation variants are given by four excellent reviews[23,24,25,26] and several more general reviews containing bioconjugate techniques based on biorthogonal reactions.[27,28,29,30,31,32]



Scheme 2. Variants of the (intramolecular) Staudinger Ligation.

Role of the ligation unit

The size of the building block plays an essential role in the case of radiolabeling, especially with radiometal complexes. The final labeled compound can lose its pharmacological impact due to the influence of the building block. To minimize this influence, the building block should be reduced to its minimum size without losing the ability to stably connect the radionuclide.

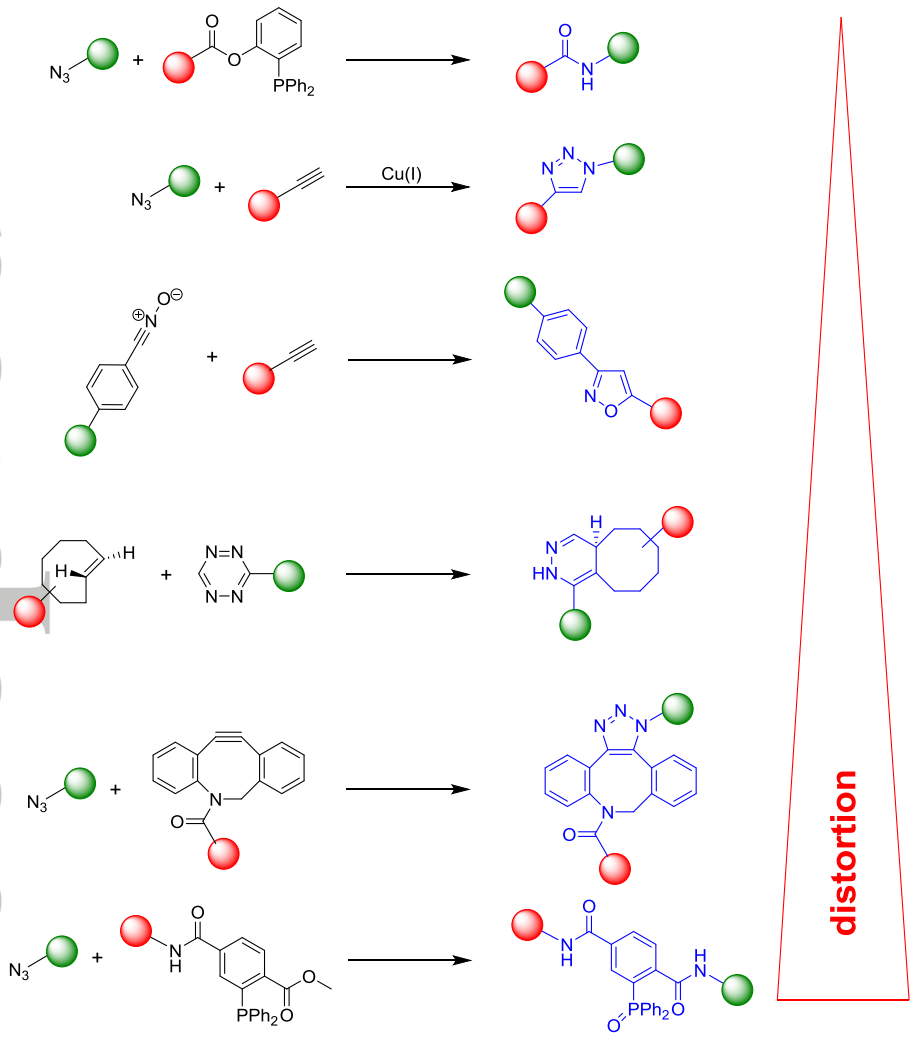


Scheme 3. Influence of the ligation unit for radiolabeling purposes.

In this case, the ligation unit also plays a highly important role. In general, a ligation unit is formed when a functional group A from the building block reacts with a functional group B from the biologically active molecule (Scheme 3). Depending on the applied functional groups, the resulting ligation unit has a different size and/or a distorted structure which can also influence the biological or pharmaceutical behavior of the ligation product beside the size of the actual building block. For instance, a triazole unit (M = 67 g/mol) is formed using the Huisgen-click-reaction whereas a benzotriazole residue (M = 246 g/mol) is yielded when using the strain-promoted click reaction. The lowest size for the ligation unit was found using the traceless Staudinger Ligation (M = 43 g/mol) which only consist of an amide bond. An overview of the influence of the ligation unit regarding their distortion is presented in Scheme 4 and Table 1.

Table 1. Overview of the ligation reactions used for radiolabeling in relation to the (molar) mass (ΔM) of the ligation unit.

ligation reaction	**ligation unit (connection)**	**ΔM** [g/mol]
traceless STAUDINGER Ligation	acid amide	43
HUISGEN-click-reaction	triazole	67
nitrileoxide-based cycloaddition	phenylisoxazole	145
tetrazine-click-reaction	octahydrocycloocta[*d*]pyridazine	164
strain-promoted click-reaction	8,9-dihydro-1*H*-dibenzo[*b*,*f*][1,2,3]triazolo[4,5-*d*]azocine	246
non-traceless STAUDINGER Ligation	triphenylphospane oxide	350

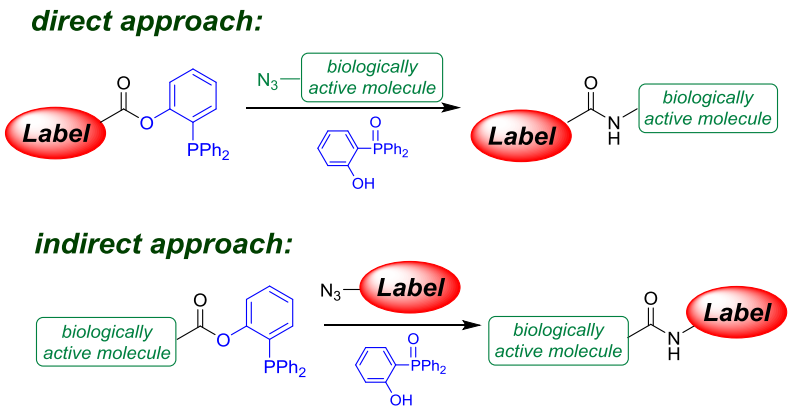


Scheme 4. Overwiew of the ligation reactions used for radiolabeling in relation to the (molar) mass (ΔM) of the ligation unit.

Traceless variant of the Staudinger Ligation

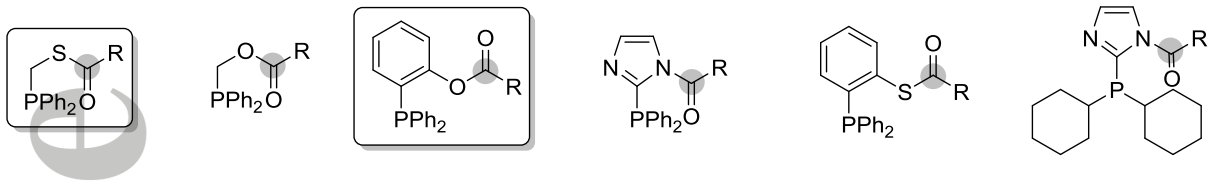
The traceless Staudinger Ligation was independently developed by the groups of Bertozzi[21] and Raines[20] in 2000. Advantageously, the ligation product does not contain the phosphane oxide residue, no metal catalyst is required, and only a carboxylic amide bond (native peptide bond) is formed as the ligation unit, which leads to less distortion. For these reasons, the traceless variant was used for the connection and chemoselective modification of peptides and proteins.[20,33,34,35,36] Other applications consist of the fluorescence labeling of bioactive molecules,[37,38,39] the modification of polymers,[40] the preparation of special lactams,[41,42] or the synthesis of glycosyl amides.[43,44]

In the case of labeling reactions with dyes or with radionuclides, two approaches of the traceless Staudinger ligation are described.[45] First, in the direct ligation approach, the label is connected to the phosphane and the azide function is bound to the biologically active molecule. The opposite connection of the starting materials is found in the indirect approach, with the phosphane bound to the biologically active molecule and the respective label containing the azide function. Both approaches are shown in Scheme 5.



Scheme 5. Approaches of the traceless Staudinger Ligation.

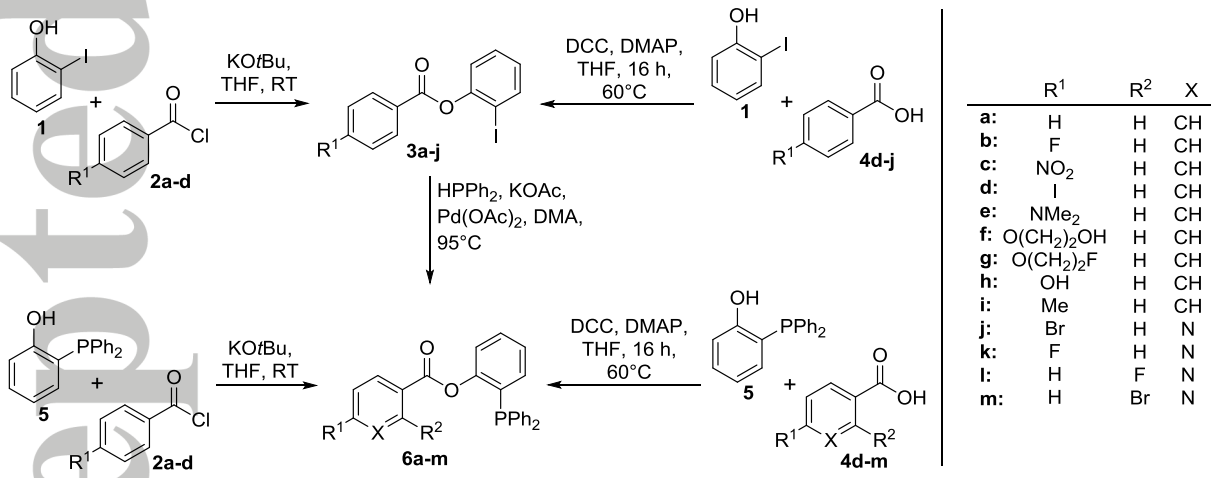
Various functionalized phosphanes based on aliphatic, aromatic and heteroaromatic skeletons containing an electrophilic residue immediately next to the P(III) center were developed in the past. An overview is given in Scheme 6. The electrophilic species is either an ester, thioester or an amide. The nature of this bond has strong influence on the reaction rate of the ligation reaction, the stability against oxidation processes, and the formation of unwanted by-products (due to hydrolysis during the ligation reaction). For radiolabeling purposes, the highlighted structures are mainly in use.



Scheme 6. General composition of phosphane building blocks for the traceless Staudinger Ligation, phosphanes highlighted in the box are in use for radiolabeling purposes (the electrophilic center is highlighted in grey).

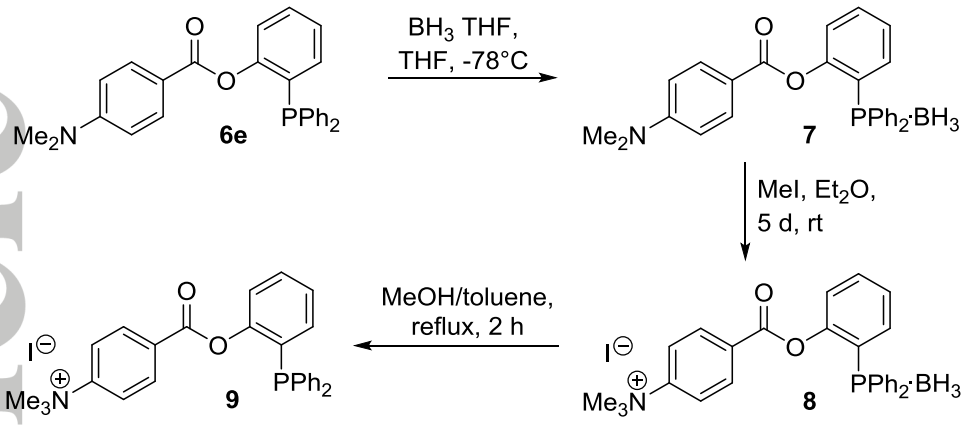
Direct approach

The direct approach of the traceless Staudinger Ligation applied for radiolabeling purposes with fluorine-18 was first published in 2009.[46] Phosphanes containing a benzoate or nicotinate residue functionalized in *ortho*- or in *para*-position were reported. A novel synthesis procedure was developed using 2-iodophenyl esters and diphenylphosphane as starting material to obtain these functionalized phosphanes. Additionally, conventional esterification procedures were used with the respective benzoyl chlorides **2a-d** and phosphanol **5** or the Steglich esterification of **4d-m** with **5**.[47] First, 2-iodophenyl esters **3a-j** were synthesized from 2-iodophenol (**1**) and acid chlorides **2a-d** or the acids **4d-j**. In the second step, phosphanes **6a-h** were obtained in 58-89% yield using the palladium-catalyzed P-C- cross-coupling reaction of **3a-j** and diphenylphosphane with $Pd(OAc)_2$ as catalyst. The syntheses of these phosphanes are summarized in Scheme 7.



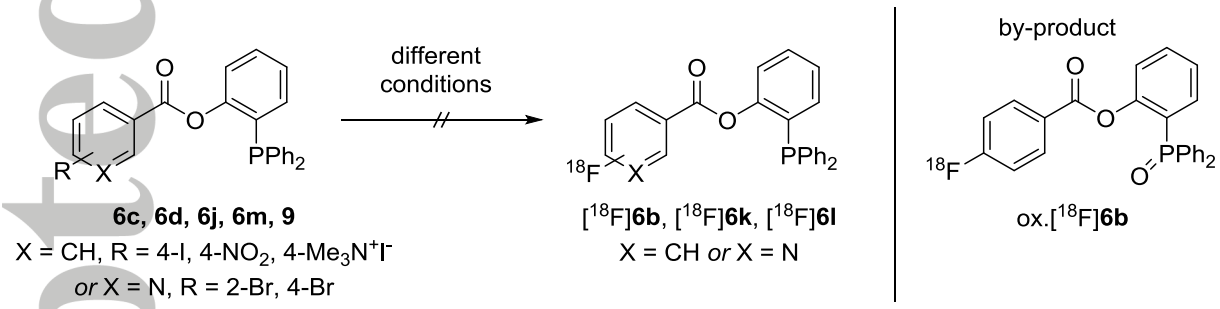
Scheme 7. Preparation of functionalized phosphanes as reference compounds and precursors for fluorine-18 labeling.

This approach was primarily designed for the introduction of the 4-[^{18}F]fluorobenzoate moiety as alternative for the use of succinimidyl 4-[^{18}F]fluorobenzoate ([^{18}F]SFB).[48,49] Thus, 4-nitro- and 4-aminobenzoate **6c** and **6e** were prepared as precursors and 4-fluorobenzoate **6b** as the non-radioactive reference compound. For the introduction of the 4-trimethylammonium precursor, compound **6e** was reacted with methyl iodide. To avoid unwanted formation of phosphonium salts, **6e** was first protected with borane. Then, borane-protected derivative **7** was treated with methyl iodide to give ammonium compound **8**. Finally, the BH_3 group of **8** was removed with methanol to give the final precursor **9**. The reaction pathway is illustrated in Scheme 8.



Scheme 8. Synthesis pathway to prepare ammonium precursor **9**.

Radiofluorination of precursor **6c** was tested under different conditions by varying the solvent, temperature, base, and the time, but the desired ^{18}F-phosphane [^{18}F]**6b** was only obtained in a maximum RCY of 1-2% (DMSO, 120°C, 40 min). Additionally, a by-product was found in 5-6% RCY, which was determined to be the oxidized phosphane ox.[^{18}F]**6b**. This formation could be an indication for a faster kinetics of the Staudinger reduction in comparison to the Staudinger ligation under radiolabeling conditions. Radiofluorinations using precursors **6d**, **6j**, **6m**, and **9** were not successful.



Scheme 9. Radiolabeling using precursors **6c**, **6d**, **6j**, **6m**, and **9.**

To avoid using harsh reaction conditions for a nucleophilic introduction of [^{18}F]fluoride into aromatic compounds, the 4-fluoromethylbenzoate moiety was chosen instead[50] allowing an introduction of [^{18}F]fluoride under milder conditions.[51] Furthermore, the cross-coupling strategy was chosen to overcome problems with the oxidation of the phosphorous during the introduction of fluorine-18. For this purpose, the respective ^{18}F-labeled 2-iodophenylester was prepared and coupled with diphenylphosphane in a Pd-catalyzed cross coupling reaction to yield the resulting phosphane building block. Such cross-coupling reactions are known in fluorine-18 chemistry.[52]

For this approach, three different precursors **10**, **11**, and **12** were prepared and the ^{18}F-labeled 2-iodophenylester [^{18}F]**13** was synthesized. As shown in Table 2, the RCY is dependent on the leaving

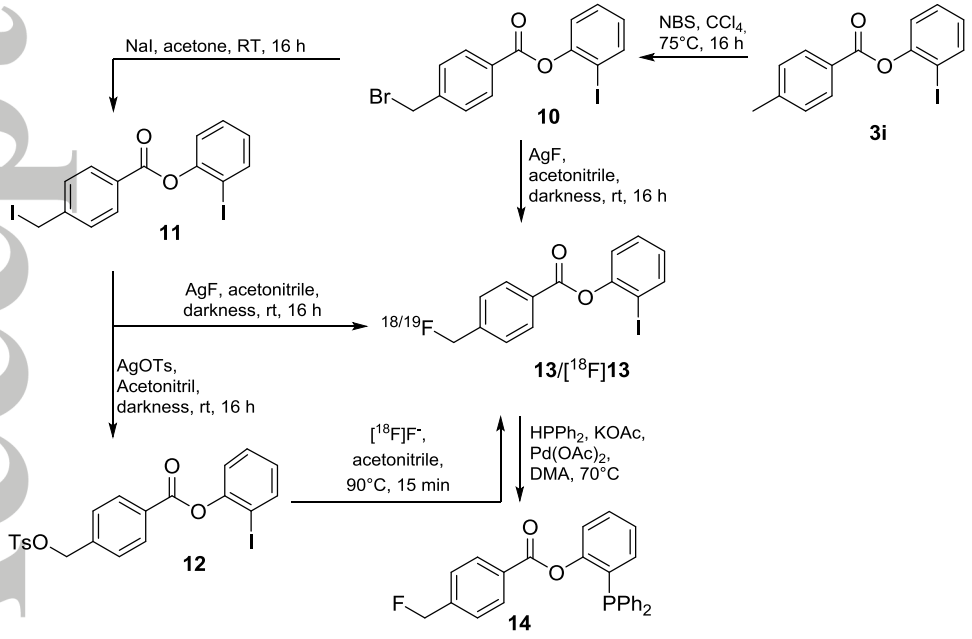
group and amount of the precursor. The highest RCY of 66% was obtained when using a relatively high amount of precursor **12** in acetonitrile at 90°C for 15 min.

Table 2. Radiolabeling conditions for the preparation of 2-iodophenylester [^{18}F]**13**.

	precursor	leaving group	amount	solvent	reaction time	RCY
1	**10**	Br	3.34 mg	acetonitrile	30 min	1%
2	**10**	Br [a)]	4.31 mg	acetonitrile	15 min	7%
3	**10**	Br	3.34 mg	acetonitrile	MW [c)]	1%
4	**11**	I	2.33 mg	acetonitrile	15 min	1%
5	**11**	I [b)]	5.21 mg	acetonitrile	20 min	46%
6	**12**	OTs	3.21 mg	acetonitrile	30 min	25%
7	**12**	OTs	5.20 mg	acetonitrile	30 min	33%
8	**12**	OTs	5.20 mg	acetonitrile	MW [c)]	36%
9	**12**	OTs	9.52 mg	ACN/*t*-BuOH	15 min	2%
10	**12**	OTs	10.30 mg	acetonitrile	15 min	66%

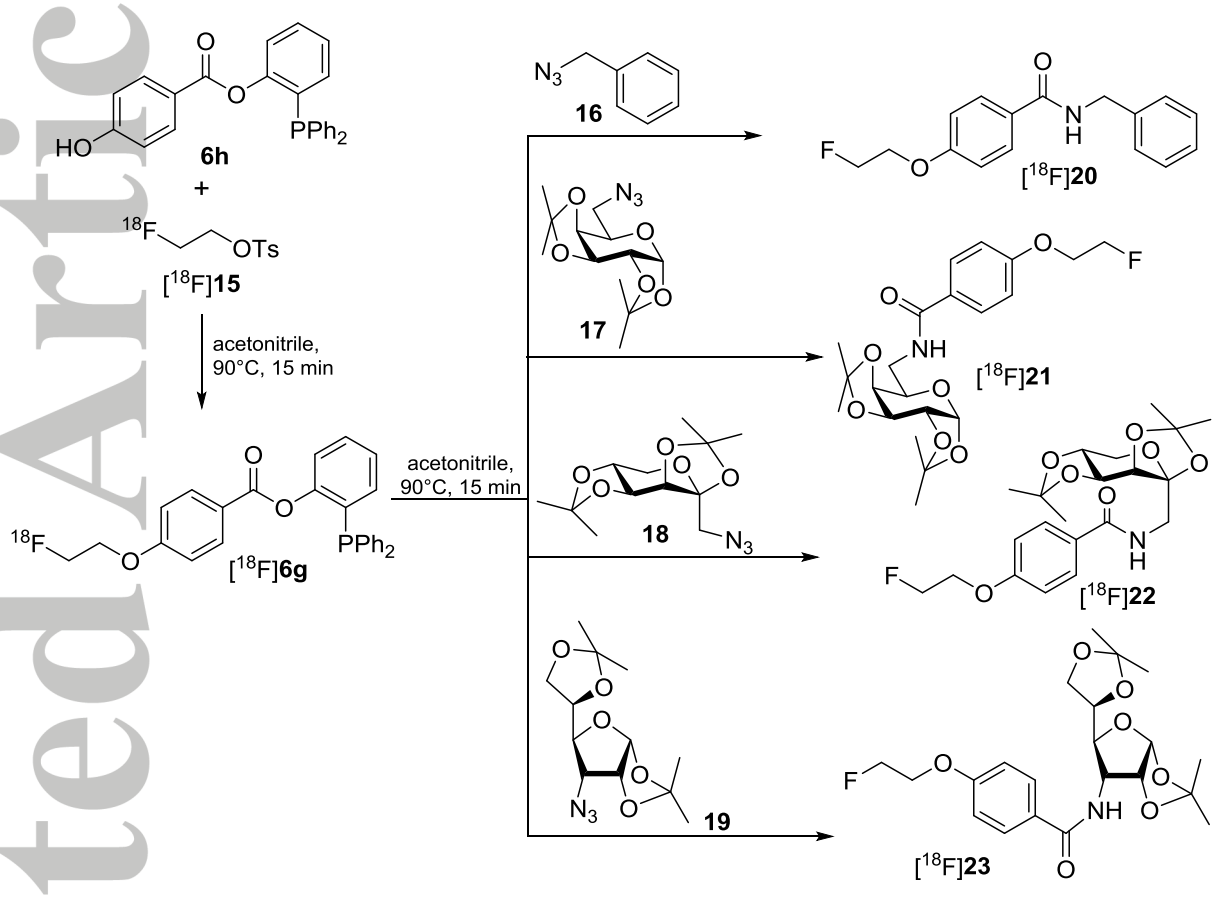
a) $AgNO_3$ was added, b) AgOMs was added, c) MW conditions: 5 min/50 watt.

The final cross coupling step to prepare the non-radioactive reference compound **14** and the labeling building block [^{18}F]**14** was performed using diphenylphosphane, KOAc and $Pd(OAc)_2$ in *N*,*N*-dimethylacetamide under an argon atmosphere. The cross coupling reaction to the appropriate non-radioactive reference compound **14** was successful. Phosphane **14** was synthesized in 41% yield, but the preparation of radiolabeling building block [^{18}F]**14** failed (Scheme 10).



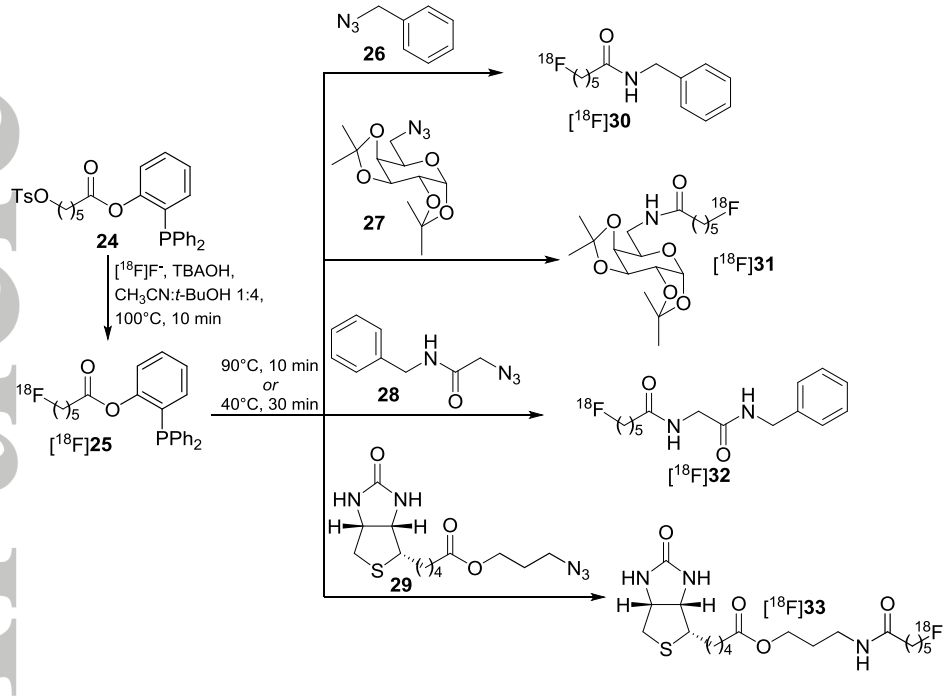
Scheme 10. Preparation of precursors **10**, **11,** and **12**, and the fluoromethyl-containing phosphanes **13**, [^{18}F]**13**, and **14**.

Based on previously described results, a two-step-radiolabeling procedure for the traceless Staudinger Ligation was developed starting with 4-hydroxy derivative **6h**. First, **6h** was reacted with [^{18}F]fluoroethyl tosylate ([^{18}F]**15**) and the appropriate labeling building block [^{18}F]**6g** was obtained. Next, the labeling step was accomplished with four sample azides **16-19** without isolating [^{18}F]**6g** beforehand. The respective radiolabeled Staudinger products [^{18}F]**20**-[^{18}F]**23** were obtained in a one-pot reaction in 17-21% RCY after three steps. The overview is shown in Scheme 11.



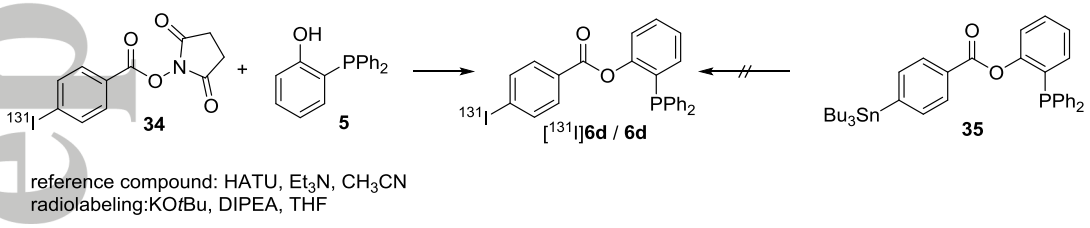
Scheme 11. Preparation of radiolabeling building block [^{18}F]**6g** and radiofluorination of **16**-**19** using the traceless Staudinger Ligation.

To avoid the obstacles of the previously mentioned three-step procedure using [^{18}F]fluoroethyl tosylate ([^{18}F]**15**), a more simple one-step approach for the preparation of the fluorine-18 containing phosphane building block [^{18}F]**25** (RCY = 65%, d.c.) based on aliphatic esters was developed.[53,54] A five carbon atom chain length was chosen for precursor **24**, and different bases, radiofluorination agents and solvents were evaluated for the optimization of the radiolabeling procedure. The highest RCY's for [^{18}F]**25** were obtained using [^{18}F]fluoride in the presence of tetrabutylammonium hydroxide in a mixture of acetonitrile/t-BuOH (v/v = 1:4)[55] for 10 min at 100°C using a relatively high amount of precursor **24**. Based on these conditions, several azide functionalized model compounds **26-29** including a carbohydrate moiety and (+)-biotin were subsequently labeled with [^{18}F]**25** using the traceless Staudinger Ligation with decay corrected RCY's of [^{18}F]**30**-[^{18}F]**33** ranging from 12 to 31% (Scheme 12).



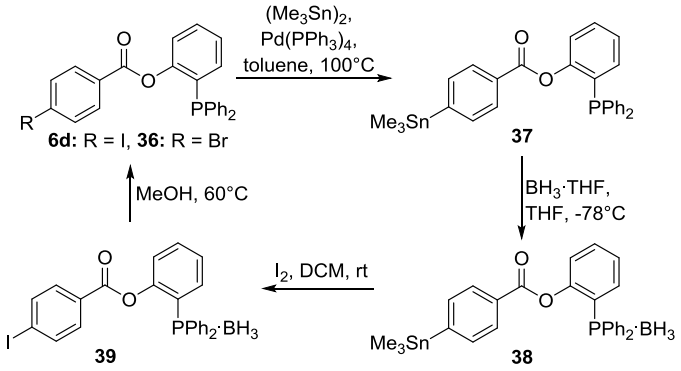
Scheme 12. Preparation of radiolabeling building block [^{18}F]**25** and radiofluorination of [^{18}F]**30**-[^{18}F]**33** using the traceless Staudinger Ligation.

Two publications were found describing the traceless Staudinger ligation and in this case the preparation of a radioiodine-labeled phosphane building block. In 2011, Vaidyanathan and colleagues published a procedure for the synthesis of [^{131}I]**6d** either from stannyl precursor **35** or from succinimidyl 4-[^{131}I]iodobenzoate ([^{131}I]SIB) [^{131}I]**34** (RCY = 68.2±11.6%),[56] however the pathway using the stannyl precursor **35** failed (Scheme 13). A reason could be that iodine was mostly introduced as I^+-species in an electrophilic aromatic substitution reaction. This species or the oxidizing agent, which is required for the formation of the I^+-species, can also lead to unwanted reactions with the phosphorous of compound **35**.



Scheme 13. Synthesis of the ^{131}I-containing building block [^{131}I]**6d**.

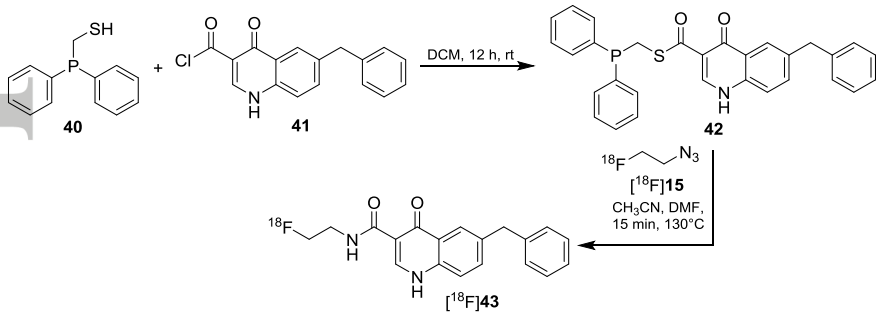
Later in 2015, Mamat *et al.* presented a synthesis procedure starting from trimethylstannyl precursor **38**, which was borane-protected prior to the reaction with elemental iodine.[57] Next, elemental iodine was introduced leading to borane-protected **39** without any side reactions like oxidation or other degradation. Finally, the BH_3-group of **39** was removed to obtain phosphane **6d** using methanol at 60°C.



Scheme 14. Alternative introduction of iodine via electrophilic aromatic substitution into borane-protected, stannylated phosphane **37**.

Indirect approach

Two labeling strategies have been published for the introduction of fluorine-18 using the indirect approach of the traceless Staudinger Ligation. In 2010, Gaeta *et al.* reported the preparation and radiolabeling of $GABA_A$ receptor binding 4-quinolones with an inhibitory constant K_i ranging from 0.7 to 3.7 nM.[58] For labeling purposes, the bioactive site of molecule **41** was modified and reacted with (diphenylphosphino)methanethiol (**40**) to give precursor **42**. Furthermore, 2-[^{18}F]fluoroethyl azide ([^{18}F]**15**) was applied as the azide labeling building block. The following Staudinger Ligation was accomplished in a mixture of acetonitrile and DMF for 15 min at 130 °C and [^{18}F]**43** was obtained after purification using preparative HPLC with a 75% RCY, not d.c. (30–50 MBq) and a molar activity A_m = 0.9 GBq/µmol (radiochemical purity > 95%); see Scheme 15. Biological data showed that only a modest fraction of the compound crossed the blood–brain barrier, with peak uptake of 0.12% of the injected dose (0.11% ID/g). *Ex vivo* autoradiographic analysis of prefrontal cortex, striatum and thalamus slices indicated significant differential binding of [^{18}F]**43** consistent with the distribution of $GABA_A$ receptors.

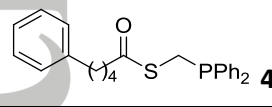
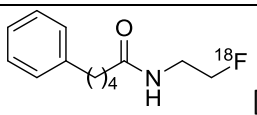
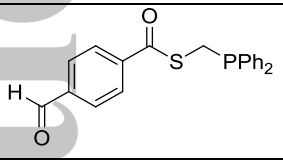
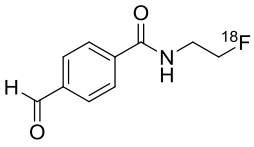
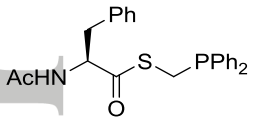
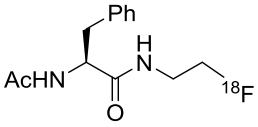
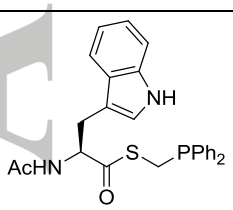
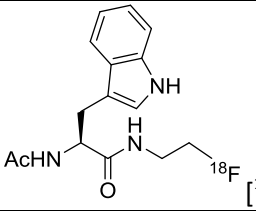


Scheme 15. Preparation of radiotracer [^{18}F]**43** for imaging of $GABA_A$ receptor.

The second work was published by Gouverneur *et al.* in 2011 using a similar strategy.[59] Phosphane functionalized sample molecules **44** and **45** as well as amino acid derivatives **46** and **47** were reacted with the labeling building block, 2-[^{18}F]fluoroethyl azide ([^{18}F]**15**). All of these chosen molecules contain a carboxylic acid function which was mandatory for the connection with the phosphane unit. Thioesters **44-47** were obtained as precursors when using (diphenylphosphino)methanethiol (**40**) as phosphane unit. For radiolabeling, two labeling conditions were applied: (A) THF/H_2O (4/1), 120 °C,

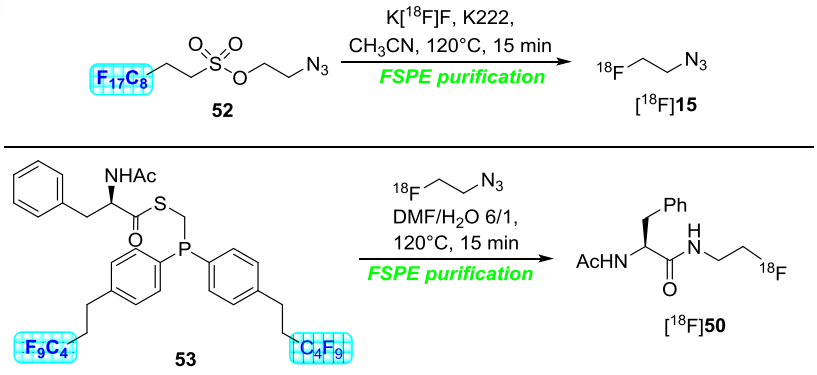
15 min and (B) DMF/H_2O (6/1), 120 °C, 15 min, but no difference in RCY was observed (Table 3). All radiolabeled compounds [^{18}F]**48**-[^{18}F]**51** were obtained with RCY's >95%.

Table 3. Scope of the labeling reaction under Staudinger conditions.

Starting material	Conditions	RCY‡	Product
 **44**	A	> 95%	 [^{18}F]**48**
 **45**	A	> 95%	 [^{18}F]**49**
 **46**	A B	> 95% > 95%	 [^{18}F]**50**
 **47**	A	> 95%	 [^{18}F]**51**

‡ = conversion from 2-[^{18}F]fluoroethyl azide ([^{18}F]**15**).

The goal of this paper was the development of a simplified purification procedure based on fluorous solid phase extraction (FSPE) using highly fluorinated starting materials. In a previous publication, 2-[^{18}F]fluoroethyl azide ([^{18}F]**15**) was successfully synthesized from the perfluorinated sulfonate precursor **52**.[60] [^{18}F]**15** was obtained in a RCY of 76% and a molar activity of 10 GBq/µmol after purification via FSPE. For the radiolabeling within the Staudinger Ligation, phosphane **53** was prepared containing two perfluorobutyl groups. FSPE was used again for the purification of the radiofluorinated Staudinger ligation product. In a sample reaction phenyl alanine [^{18}F]**50** was prepared in a high RCY of >95% using this strategy.

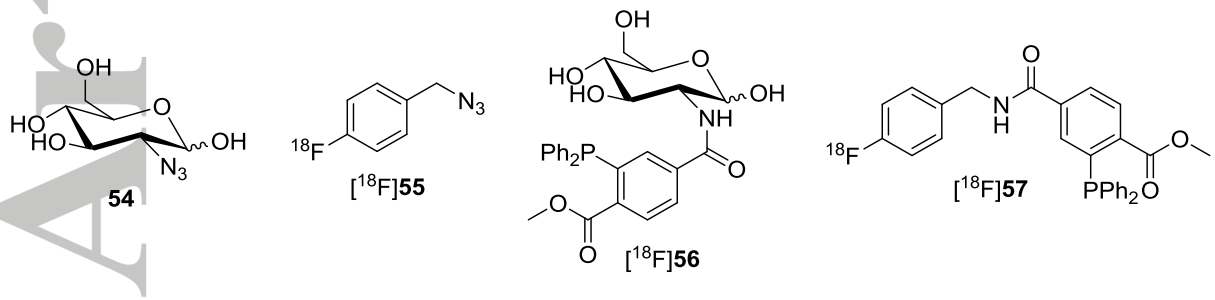


Scheme 16. Sample reaction for the preparation of the azide building block [^{18}F]**15** and sample Staudinger Ligation to label phenylalanine **53**.

Non-traceless Staudinger Ligation

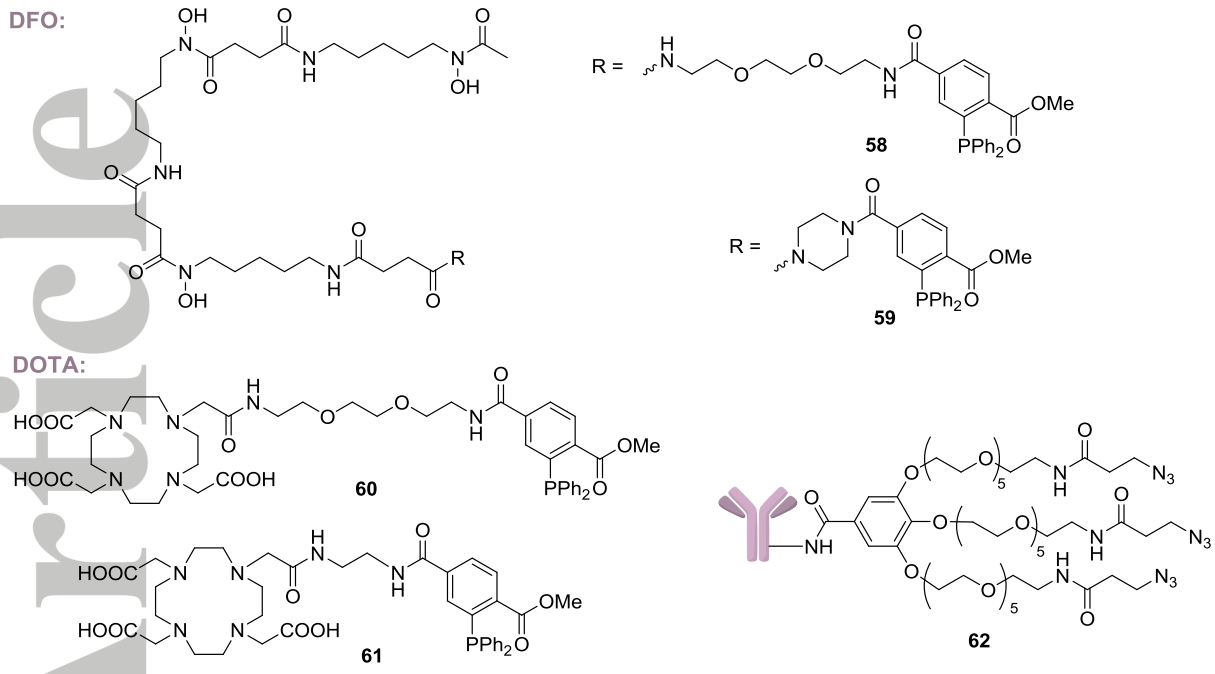
In 2000, Bertozzi and co-workers discovered the non-traceless variant of the Staudinger Ligation for the modification of cell surfaces with biotin.[22] Since then, this variant has also gained access for radiolabeling purposes, but only a few applications are known.

Two patents were published by Robillard and Gruell in 2006 demonstrating the application of the non-traceless ligation for radiolabeling with fluorine-18.[61,62] In one patent, they claimed a procedure to prepare a ^{18}F-labeled glucose derivative from 2-azido-2-deoxy-D-glucose (**54**) and [^{18}F]fluorophosphane [^{18}F]**56** as alternative for [^{18}F]FDG (2-deoxy-2-[^{18}F]fluoroglucose), but unfortunately no experimental procedure was found even for the preparation of ^{18}F-phosphane [^{18}F]**57** shown in Scheme 17.[63]



Scheme 17. Azide- and phosphane-building blocks [^{18}F]**55**-[^{18}F]**57** for radiolabeling purposes using the non-traceless Staudinger Ligation.

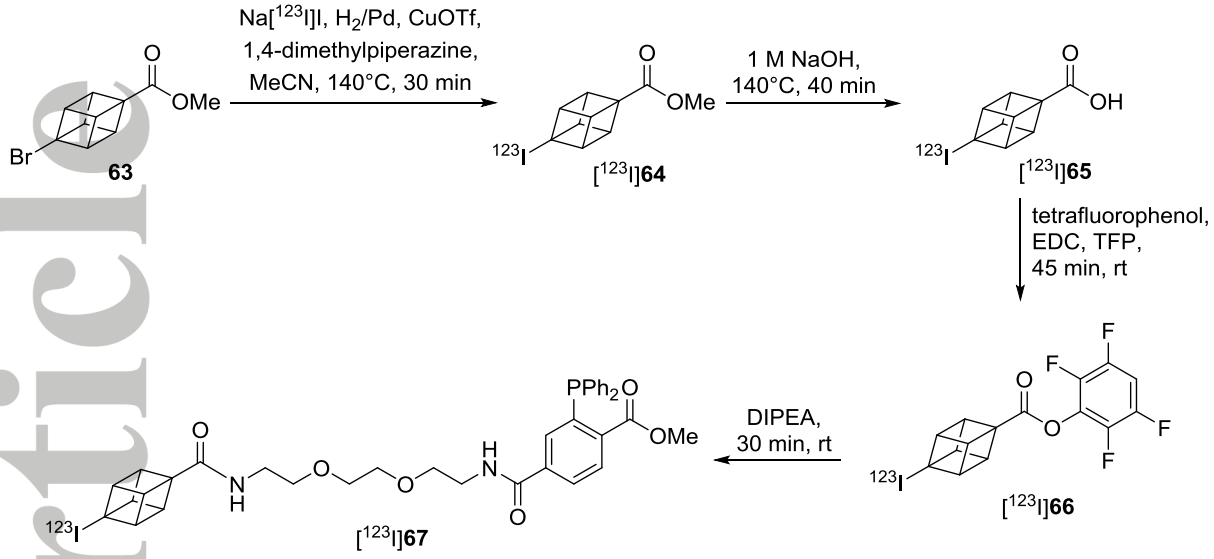
In a later publication, several functionalized phosphanes **58-61** were prepared either with DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) for ^{177}Lu and DFO (deferoxamine) for ^{89}Zr and $^{67/68}$Ga (Scheme 18) or a moiety for the connection of ^{123}I/^{131}I (Scheme 19).[64] For the pretargeting approach, the anti-CD44v6 chimeric mAb U36 was modified with azido functions. The purification of the modified antibody **62** was done via dialysis. The average number of the azide residue was determined via UV measurements to be 4, 6, 8, or 15. Thus, the efficiency of the modification was 37-42%.



Scheme 18. Chelators **58-61** for ^{67}Ga/^{68}Ga, ^{89}Zr, ^{177}Lu and structure of the azide-functionalized mAb **62**.

To avoid oxidation of the phosphorous, an antioxidant (Na_2SO_3) was added during the radiolabeling of the phosphanes with the radiometals. The labeling efficiency of the phosphanes is generally high (>95%), but also the respective oxidation products were found in yields of 9-20%.

A cubanecarboxylic acid derivative was used as basis for the preparation of radiolabeling building blocks bearing iodine-123 (Scheme 19). Thus, 4-bromo-1-cubanecarboxylic acid (**63**) was used as starting material and reacted with CuOTf to give the precursor. [^{123}I]Iodide was then added to the reaction mixture and heated at 140°C for 30 min. Methyl 4-[^{123}I]iodo-1-cubanecarboxylat ([^{123}I]**64**) was obtained after HPLC purification and was then saponified to yield [^{123}I]**65** and converted into its activated ester [^{123}I]**66**. Finally, the 4-[^{123}I]iodo-1-cubanecarboxylat residue was connected to the phosphane to give the radiolabeling building block [^{123}I]**67** containing approx. 5% of the oxidized product.



Scheme 19. ^{123}I/^{131}I-labeling of a phosphane for the non-traceless Staudinger Ligation.

The first *in vitro* Staudinger Ligation was performed with ^{89}Zr- and ^{67}Ga-DFO-containing phosphane **58** as well as with ^{177}Lu-containging DOTA-phosphane **60** and to study the efficiency and stability in Human serum albumin (HSA) of this ligation by SDS-PAGE. For this preparation, 24 equivalents of the respective labeled phosphine were added to 1 equiv. of the N_3-mAB containing approx. 24 azide functionalities in PBS buffer (6 v/v% EtOH) at 37°C for 2 h. As a result, all three phosphines are only slowly oxidized with a chemical half life of about 10 h and a ligation efficiency between 20 and 25% at 37°C. Furthermore, 93% of the formed Staudinger products remained intact after 48 h incubation at 37% in 0.9% NaCl or 80% HSA. In a pretargeting approach, *in vivo* experiments with tumor-free mice were accomplished with sequential administration of the azide-functionalized mAb and ^{67}Ga-containing DFO-phosphane **59**. No difference in biodistribution was found between these mice and mice which did not receive the antibody. Additional HPLC analyses revealed no evidence for a Staudinger Ligation *in vivo* in mice.

Conclusion

This review has summarized the applications and the scope of both variants of the Staudinger Ligation as bioorthogonal reactions for the introduction of radionuclides into small organic compounds as well as biomacromolecules. These reactions have become a metal-catalyst-free tool for radiolabeling high molecular weight pharmacologically relevant molecules, like peptides or proteins, but also for radiolabeling small compounds of interest. For labeling purposes, fast and high yielding reactions are required and strategies for a convenient purification in an acceptable time span. In this case, the kinetics of the Staudinger Ligations is sufficient for labeling applications even with short-lived radionuclides. Oxidation processes, which can occur at the phosphorus of the phosphane unit were prevented using the borane-protected compounds or by adding antioxidants. Nevertheless, an *in vivo* application seems to be difficult, since the Staudinger reduction appears to be faster than the Staudinger Ligation *in vivo* under these conditions.

Various examples of the traceless variant demonstrate the introduction of fluorine-18. Furthermore, a labeling strategy for radioiodine was presented using this approach. The non-traceless variant was used for the introduction of radiometals such as Lu-177, Ga-67, and Zr-87 for pretargeting purposes. Additionally, the synthesis route to a ^{123}I-containing radiolabeling building block was presented.

The use of special resins like FSPE for fast and convenient purification makes this labeling reaction interesting especially but not only for the introduction of short-lived radionuclides under mild labeling conditions. Overall it has been shown that the Staudinger Ligation can function as a valuable tool for radiolabeling purposes.

References

[1] Li Z, Conti PS. Radiopharmaceutical chemistry for positron emission tomography. Adv Drug Delivery Rev 2010;62:1031-1051.

[2] Olberg DE, Hjelstuen OK. Labeling strategies of peptides with ^{18}F for positron emission tomography. Curr Topics Med Chem 2010;10:1669-1679.

[3] Lei M, Tian M, Zhang H. Click labeled peptides with fluorine-18 for PET imaging. Curr Med Imaging Rev 2010;6:33-41.

[4] Wängler C, Schirrmacher R, Bartenstein P, Wängler B. Click-chemistry reactions in radiopharmaceutical chemistry: fast & easy introduction of radiolabels into biomolecules for in vivo imaging. Curr Med Chem 2010;17:1092-1116.

[5] Liu S, Shen B, Chin FT, Cheng Z. Recent progress in radiofluorination of peptides for PET molecular imaging. Curr Org Synth 2011;8:584-592.

[6] Schirrmacher R, Wängler C, Schirrmacher E. Recent developments and trends in ^{18}F-radiochemistry: Syntheses and applications. Mini-Rev Org Chem 2007;4:317-329.

[7] Mamat C, Ramenda T, Wuest FR. Recent applications of click chemistry for the synthesis of radiotracers for molecular imaging. Mini-Rev Org Chem 2009;6:21-34.

[8] Glaser M, Robins EG. 'Click labelling' in PET radiochemistry. J Labelled Compd Radiopharm 2009;52:407-414.

[9] Bertozzi CR. A decade of bioorthogonal chemistry. Acc Chem Res 2011;44:651-653.

[10] Meyer J-P, Adumeau P, Lewis JS, Zeglis BM. Click Chemistry and Radiochemistry: The First 10 Years. Bioconjugate Chem 2016;27:2791-2807.

11 Staudinger H, Meyer J. Über neue organische Phosphorverbindungen III. Phosphinmethylenderivate und Phosphinimine. Helv Chim Acta 1919;2:635-646.

12 Staudinger H, Hauser E. Über neue organische Phosphorverbindungen IV Phosphinimine. Helv Chim Acta 1921;4: 861-886.

13 Gololobov YG, Kasukhin LF. Recent advances in the staudinger reaction. Tetrahedron 1992;48:1353-1406.

14 Garcia J, Urpi F, Vilarrasa J. New synthetic tricks - triphenylphosphinemediated amide formation from carboxylic-acids and azides. Tetrahedron Lett 1984;25:4841-4844.

15 Garcia J, Vilarrasa J, Bordas X, Banaszek A. New synthetic tricks - onepot preparation of N-substituted phthalimides from azides and phthalic-anhydride. Tetrahedron Lett 1986;27:639-640.

16 Bosch I, Romea P, Urpi F, Vilarrasa J. Alternative procedures for the macrolactamization of omega-azido acids. Tetrahedron Lett 1993;34:4671-4674.

17 Kosiova I, Janicova A, Kois P. Synthesis of coumarin or ferrocene labeled nucleosides via Staudinger ligation. Beilstein J Org Chem 2006;2:No. 23. DOI:10.1186/1860-5397-2-23.

18 Tian WQ, Wang YA. Mechanisms of Staudinger Reactions within Density Functional Theory. J Org Chem 2004;69:4299-4308.

19 Lin FL, Hoyt HM, van Halbeek H, Bergman RG, Bertozzi CR. Mechanistic Investigation of the Staudinger Ligation. J Am Chem Soc 2005;127:2686-2695.
20 Nilsson BL, Kiessling LL, Raines RT, Staudinger Ligation: A Peptide from a Thioester and Azide. Org Lett 2000;2:1939-1941.
21 Saxon E, Armstrong JI, Bertozzi CR. A "Traceless" Staudinger Ligation for the Chemoselective Synthesis of Amide Bonds. Org Lett 2000;2:2141-2143.
22 Saxon E, Bertozzi CR. Cell Surface Engineering by a Modified Staudinger Reaction. Science 2000;287:2007-2010.
23 Köhn M, Breinbauer R. Die Staudinger-Ligation – Ein Geschenk für die Chemische Biologie. Angew Chem 2004;116:3168-3178.
24 Schilling CI, Jung N, Biskup M, Schäpers U, Bräse S. Bioconjugation via azide-Staudinger ligation: an overview. Chem Soc Rev 2011;40:4840-4871.
25 van Berkel SS, van Eldijk MB, van Hest JCM, Staudinger-Ligation als Methode zur Biokonjugation. Angew Chem 2011;123:8968-8989.
26 Wang Z-PA, Tian C-L, Zheng J-S. The recent developments and applications of the traceless-Staudinger reaction in chemical biology study. RSC Adv 2015;5:107192-107199.
27 Hermanson GT. Bioconjugate Techniques. London: Academic Press; 2008.
28 van Swieten PF, Leeuwenburgh MA, Kessler BM, Overkleft HS. Bioorthogonal organic chemistry in living cells: novel strategies for labeling biomolecules. Org Biomol Chem 2005;3:20-27.
29 King M, Wagner A. Developments in the Field of Bioorthogonal Bond Forming Reactions – Past and Present Trends. Bioconjugate Chem 2014;25:825-839.
30 Patterson DM, Nazarova LA, Prescher JA. Finding the Right (Bioorthogonal) Chemistry. ACS Chem Biol 2014;9:592-605.
31 McKay CS, Finn MG. Click Chemistry in Complex Mixtures: Bioorthogonal Bioconjugation. Chem Biol 2014;21:1075-1101.
32 Gentilucci L, Tosi P, Bauer A, De Marco R. Modern tools for the chemical ligation and synthesis of modified peptides and proteins. Future Med Chem 2016;8:2287-2304.
33 Nilsson BL, Kiessling LL, Raines RT. High-Yielding Staudinger Ligation of a Phosphinothioester and Azide To Form a Peptide. Org Lett 2001;3:9-12.
34 Kleineweischede R, Hackenberger CPR. Chemoselective Peptide Cyclization by Traceless Staudinger Ligation. Angew Chem Int Ed 2008;47: 5984-5988.
35 Soellner MB, Dickson KA, Nilsson BL, Raines RT. Site-Specific Protein Immobilization by Staudinger Ligation. J Am Chem Soc 2003;125:11790-11791.
36 Grandjean C, Boutonnier A, Guerreiro C, Fournier J-M, Mulard LA. On the Preparation of Carbohydrate−Protein Conjugates Using the Traceless Staudinger Ligation. J Org Chem 2005; 70:7123-7132.
37 Tsao M-L, Tian F, Schultz PG. Selective Staudinger Modification of Proteins Containing *p*-Azidophenylalanine. ChemBioChem 2005;6:2147-2149.
38 Wodtke R, König J, Pigorsch A, Köckerling M, Mamat C. Evaluation of novel fluorescence probes for conjugation purposes using the traceless Staudinger Ligation. Dyes Pigm 2015;113:263-273.
39 Mamat C, Köckerling M. Preparation of 4-Halobenzoate-Containing Phosphane-Based Building Blocks for Labeling Reactions Using the Traceless Staudinger Ligation. Synthesis 2015;47:387-394.
40 Pötzsch R, Fleischmann S, Tock C, Komber H, Voit BI. Combining RAFT and Staudinger Ligation: A Potentially New Synthetic Tool for Bioconjugate Formation. Macromolecules 2011;44:3260-3269.
41 David O, Meester WJN, Bieräugel H, Schoemaker HE, Hiemstra H, van Maarseveen JH. Intramolecular Staudinger Ligation: A Powerful Ring-Closure Method To Form Medium-Sized Lactams. Angew Chem Int Ed 2003;42:4373-4375.
42 Masson G, den Hartog T, Schoemaker HE, Hiemstra H, van Maarseveen JH. Intramolecular Staudinger Ligation towards Biaryl-Containing Lactams. Synlett 2006:865-868.
43 Bianchi A, Bernardi B. Traceless Staudinger Ligation of Glycosyl Azides with Triaryl Phosphines: Stereoselective Synthesis of Glycosyl Amides. J Org Chem 2006;71:4565-4577.
44 Nisic F, Speciale G, Bernardi A. Stereoselective Synthesis of α- and β-Glycofuranosyl Amides by Traceless Ligation of Glycofuranosyl Azides. Chem Eur J 2012;18:6895-6906.
45 Pretze M, Pietzsch D, Mamat C. Recent Trends in Bioorthogonal Click-Radiolabeling Reactions Using Fluorine-18. Molecules 2013;18:8618-8665.
46 Mamat C, Pretze M, Steinbach J, Wüst F, Radiofluorination via Staudinger Ligation. J Labelled Compd Radiopharm 2009;52:S142.

47 Mamat C, Flemming A, Köckerling M, Steinbach J, Wuest FR. Synthesis of Benzoate-Functionalized Phosphanes as Novel Building Blocks for the Traceless Staudinger Ligation. Synthesis 2009:3311-3321.
48 see e.g.: Kuchar M, Pretze M, Kniess T, Steinbach J, Pietzsch J, Löser R. Site-selective radiolabeling of peptides by ^{18}F-fluorobenzoylation with [^{18}F]SFB in solution and on solid phase: a comparative study. Amino Acids 2012;43:1431-1443.
49 Mamat C, Weiß DH, Köckerling M. X-ray Structures of Succinimidyl Halobenzoates. Crystals 2017;7:90.
50 Mamat C, Franke M, Peppel T, Köckerling M, Steinbach J. Synthesis, structure determination, and (radio-)fluorination of novel functionalized phosphanes suitable for the traceless Staudinger ligation. Tetrahedron 2011;67:4521-4529.
51 Wüst F, Müller M, Bergmann R. Synthesis of 4-([^{18}F]fluoromethyl)-2-chlorophenylisothiocyanate: A novel bifunctional ^{18}F-labelling agent. Radiochim Acta 2004;92:349-353.
52 Review: Pretze M, Große-Gehling P, Mamamt C. Cross-Coupling Reactions as Valuable Tool for the Preparation of PET Radiotracers. Molecules 2011;16:1129-1165.
53 Pretze M, Wuest F, Peppel T, Köckerling M, Mamat C. The traceless Staudinger ligation with fluorine-18: A novel and versatile labeling technique for the synthesis of PET-radiotracers. Tetrahedron Lett 2010;51:6410-6414.
54 Pretze M, Flemming A, Köckerling M, Mamat C. Synthesis and radiofluorination of iodophenyl esters as tool for the traceless staudinger ligation. Z Naturforsch B 2010;65b:1128-1136.
55 Kim DW, Jeong H-J, Lim ST, Sohn MH, Katzenellenbogen JA, Chi DYJ. Facile nucleophilic fluorination reactions using tert-alcohols as a reaction medium: Significantly enhanced reactivity of alkali metal fluorides and improved selectivity. Org Chem 2008;73:957-962.
56 Vaidyanathan G, White B, Affleck DJ, McDougald D, Zalutsky MR. Nucl Med Biol 2011;38:77-92.
57 Mamat C, Köckerling M. Preparation of 4-Halobenzoate-Containing Phosphane-Based Building Blocks for Labeling Reactions Using the Traceless Staudinger Ligation. Synthesis 2015;45:387-394.
58 Gaeta A, Woodcraft J, Plant S, Goggi J, Jones P, Battle M, Trigg W, Luthra SK, Glaser M. Use of 2-[^{18}F]fluoroethylazide for the Staudinger ligation-Preparation and characterisation of $GABA_A$ receptor binding 4-quinolones. Bioorg Med Chem Lett 2010;20:4649-4652.
59 Carroll L, Boldon S, Bejot R, Moore JE, Declerck J, Gouverneur V. The traceless Staudinger ligation for indirect ^{18}F-radiolabelling. Org Biomol Chem 2011;9:136-140.
60 Bejot R, Fowler T, Carroll L, Boldon S, Moore JE, Declerck J, Gouverneur V. Fluorous synthesis of ^{18}F radiotracers with the [^{18}F]fluoride ion: Nucleophilic fluorination as the detagging process. Angew Chem 2009;121:594-597.
61 M.S. Robillard, H. Gruell, Patent: WO2006/038184A2; M.S. Robillard, H. Gruell, Compounds, kits and methods for use in medical imaging. (Chem Abstr 2006;144:376466).
62 M.S. Robillard, H. Gruell, Patent: WO 2006/038185A2; M.S. Robillard, H. Gruell, Targeted imaging and/or therapy using the Staudinger ligation. (Chem Abstr 2006;144:398254).
63 SciFinder recherche March 2017.
64 Vugts DJ, Vervoort A, Stigter-van Walsum M, Visser GWM, Robillard MS, Versteegen RM, Vulders RCM, Herscheid JDM, van Dongen GAMS. Synthesis of Phosphine and Antibody–Azide Probes for *in Vivo* Staudinger Ligation in a Pretargeted Imaging and Therapy Approach. Bioconjugate Chem 2011;22:2072-2081.

Recent Progress using the Staudinger Ligation for Radiolabeling Applications

Constantin Mamat[a,b,*], Matthew Gott [a], and Jörg Steinbach [a,b]