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The traceless Staudinger ligation with fluorine-18: a novel and versatile labeling technique for the synthesis of PET-radiotracers

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ABSTRACT

The development of rapid radiolabeling techniques under mild reaction conditions involving the short-lived positron emitter fluorine-18 remains a special challenge in organic PET chemistry. This work describes a novel and facile application of the traceless Staudinger ligation as a mild and versatile labeling method for preparation of various radiotracers labeled with fluorine-18.

1. Introduction

Positron emission tomography (PET) and single photon emission computer tomography (SPECT) have extensively been used for non-invasive diagnosis, staging, and therapy control of diseases at the molecular and cellular level and have found numerous applications in the field of clinical oncology, cardiology, and neurology.[1]

The success of PET as the most sensitive molecular imaging technique depends on the availability of suitable radiotracers, which is limited by the speed and efficiency of synthetic methods. In particular, the introduction of the short-lived positron emitter ^{18}F ($t_{1/2}$ = 109.8 min) into biopolymers like peptides, proteins, oligonucleotides, and antibodies remains a special challenge. These compounds usually cannot be labeled directly with ^{18}F at high specific activity due to the required harsh reaction conditions. To circumvent this problem various prosthetic groups, also referred to as bifunctional labeling agents, have been developed for ^{18}F labeling of peptides and proteins under mild conditions. Linkage of ^{18}F-labeled prosthetic groups to proteins can be accomplished via various bioconjugation techniques,[2] including acylation, imidation, photochemical conjugation, and thioether formation. However, every method has advantages and limitations and further work on the development of rapid, clean, and mild synthesis techniques for ^{18}F-labeled proteins is still needed.

In recent years click chemistry has entered into many fields of chemical sciences, including radiopharmaceutical chemistry.[3] The copper(I)-catalyzed 1,2,3-triazole formation involving azides and terminal acetylenes according to a 1,3-dipolar [3+2]cyclo-addition has proved to be a particularly valuable tool for efficient ^{18}F labeling of peptides. Moreover, copper salts as typically required for the [3+2]cycloaddition between azides and alkynes proved to be cytotoxic[4] and in contact with living systems the Cu catalyst must be removed. Alongside other direct and indirect peptide or protein radiolabeling methods with aluminum chelates[5] or like the SiFa technology[6], the Staudinger ligation[7] is another example of a powerful bioconjugation approach to label molecules under mild conditions and proceeds without Cu salts.

The bioorthogonal character[8] of organic azides and phosphanes as the reaction partners, and the mild reaction conditions have made Staudinger ligations a valuable synthesis tool for the interconnection of molecular entities like carbohydrates, amino acids, and proteins to give various hybrid-bioconjugates.[9] Moreover, incorporation of various labels into biomolecules, as frequently exemplified by the use of fluorescence labels, has gained great interest over the last years to study their behavior in complex biological systems.[10]

Recently, a radiolabeling method was pointed out using the traceless Staudinger ligation where the bioactive part of the later radiotracer is located on the phosphane moiety and the labeling was done with 1-azido-2-[^{18}F]fluoroethane.[11] Our approach presents a facile and versatile labeling technique with a novel developed radiofluorinated phosphane which acts as labeling tool and offers the possibility to label a wide variety of azide-functionalized bioactive molecules as exemplified in Figure 1. Therefore, acyl-functionalized phosphanes[12] were prepared and tested as versatile

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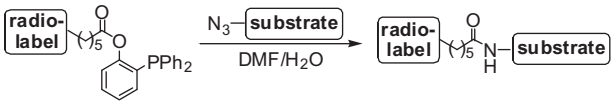

Figure 1. Labeling concept using the traceless Staudinger ligation.

[^{18}F]fluoride containing building blocks for radiolabeling purposes with the traceless Staudinger ligation using various organic model azides.

2. Results and discussion

Phosphane–borane adducts[13] are frequently utilized in organic chemistry and catalysis. Advantageous, BH_3 as 'protecting group' stabilizes phosphorus in the oxidation state +3 and prevents side reactions like the formation of phosphonium salts within the preparation of functionalized phosphanes as starting material for Staudinger ligations. The borane-protected phosphanol **1**[14] functions as key compound for the preparation of various ω-haloacyl functionalized phosphane–borane derivatives. For this purpose, compound **1** was treated with corresponding ω-haloacyl chlorides **2a–c** in the presence of *t*-BuOK as base to obtain **3a–c** in yields between 59% and 96% (Fig. 2).

Conversion of bromo and chloro derivatives **3a–c** into the corresponding iodo compounds **4a–c** as suitable derivatives for the introduction of [^{19}F]fluoride and/or good leaving groups was achieved via a Finkelstein substitution with NaI. Fluorination of the iodo derivatives **4a–c** was accomplished with AgF as fluoride source in acetonitrile for 16 h at ambient temperature to afford the fluoro compounds **6a–c** in good to high yields of 54–73%, whereas reaction of iodides **4a–c** with AgOTs resulted in the formation of tosylates **5a–c** (23–79% yield) with good leaving group capacity as suitable labeling precursors for nucleophilic radiofluorination with [^{18}F]fluoride (Fig. 2).

NMR investigations of compounds **3a–c**, **4a–c**, **5a–c**, and **6a–c** confirm the presence of the BH_3 group while a broad multiplet indicative of the borane protons was observed between $\delta = 1$–2 ppm in the ^{1}H NMR spectra. Formation of the phosphane–borane adducts was further confirmed by the multiplet at $\delta = +20$ ppm in the ^{31}P NMR spectra and a signal at $\delta = -36$ ppm in the ^{11}B NMR spectra. In addition, single crystals from **4c** and **6a** were obtained and X-ray structures determined.[15] Figures 3 and 4 depict the molecular structures of compounds **4c** and **6a**.

Fluorination with AgF as well as tosylation with AgOTs gave the best yields using **4c** as starting material. Thus, we decided to apply tosylate **5c** as labeling precursor for subsequent radiolabeling experiments with no-carrier-added (n.c.a.) [^{18}F]fluoride and **6c** as respective reference compound. However, several attempts to incorporate [^{18}F]fluoride into the borane-containing tosylate **5c** failed due to the formation of stable [^{18}F]fluoroborate adducts. The formation of B-^{18}F bonds by the reaction of n.c.a. [^{18}F]fluoride with organoboron compounds is well documented in radiopharmaceutical chemistry.[16] To avoid undesired B-^{18}F bond formation,

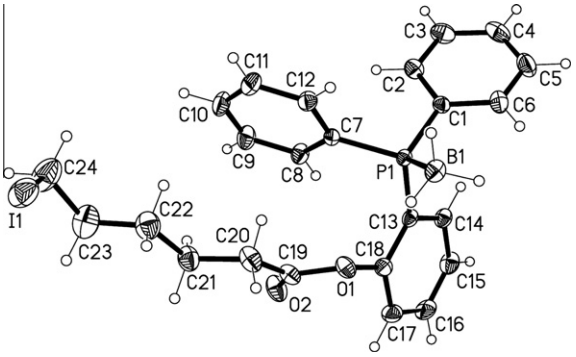

Figure 3. Molecular structure of **4c** in the crystalline state (thermal ellipsoids at the 50% probability level).

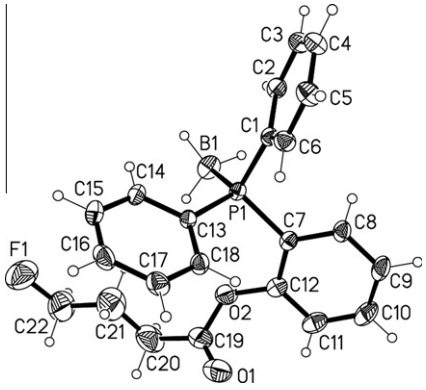

Figure 4. Molecular structure of **6a** in the crystalline state (thermal ellipsoids at the 50% probability level).

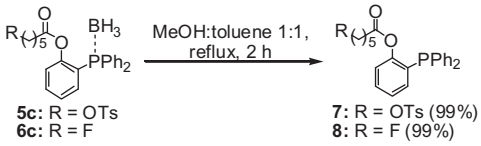

Figure 5. Borane deprotection via methanolysis.

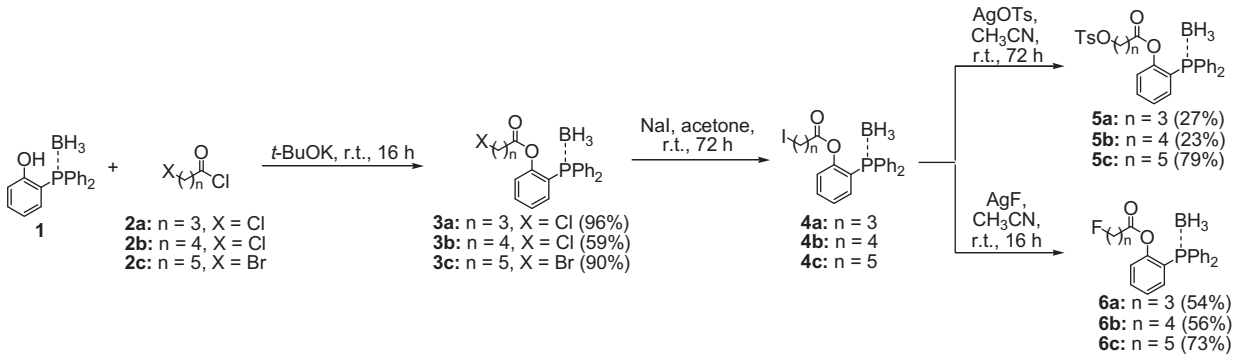

Figure 2. Preparation of the labeling precursors **5a–c** and the non-radioactive fluorinated reference compounds **6a–c**.

the borane in compound **5c** had to be removed prior to the radiofluorination with [^{18}F]fluoride and subsequent traceless Staudinger ligation. Borane deprotection succeeded in nearly quantitative yields without side reactions through treatment of compounds **5c** and **6c** in a methanol/toluene mixture (v/v = 1:1) at 60 °C for 2 h (Fig. 5).[17] ^{31}P NMR spectra showed a singlet at approx. $\delta = -15$ ppm which indicates successful removal of the BH_3 group in compounds **7** and **8**. Commonly used amines for borane deprotection like piperazine or morpholine led to aminolysis of the ester residue in phosphane **5c** and **6c**.

Non-radioactive (fluorine-19) compounds as references for corresponding ^{18}F radiotracers were prepared via the traceless Staudinger ligation and could be obtained by the reaction of the 6-fluorohexanoyl-containing phosphane **8** with several organic model azides like benzyl azide (**9**), azidoacetic acid (**10**) and a 6-azido-galactose derivative **11**. The ligation reaction in a DMF/water mixture (v/v = 10:1) at 90 °C afforded the desired amides **12**, **13,** and **14** in excellent chemical yields of 97–99% after chromatographic purification (Fig. 6).

Radiofluorination of tosylate **7** gave the ^{18}F-labeled phosphane [^{18}F]**8** which acts as fluorine-18 containing building block for the subsequent traceless Staudinger ligation as depicted in Figure 7. The radiochemical yield of [^{18}F]**8** strongly depends on the choice

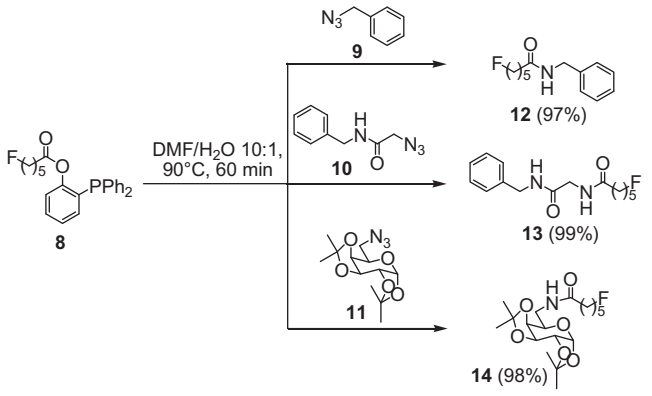

Figure 6. Preparation of reference compounds **12**, **13**, and **14** via traceless Staudinger ligation.

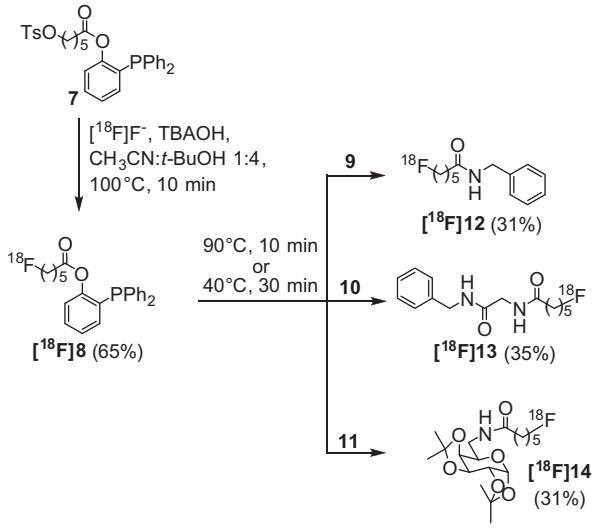

Figure 7. Radiofluorination of [^{18}F]**10** and one pot radiolabeling of [^{18}F]**14**, [^{18}F]**15,** and [^{18}F]**16** using the traceless Staudinger ligation.

of the reaction conditions. An influence of the solvent and used base was investigated. No radiolabeled product was found when DMF or DMSO was used. Furthermore, the amount of labeling precursor **7** was evaluated. Best results for the preparation of [^{18}F]**8** were obtained using [^{18}F]fluoride in the presence of TBAOH as base in a mixture of 500 μL acetonitrile/*t*-BuOH (v/v = 1:4) for 10 min at 100 °C.[18] The results are summarized in Table 1. Due to different retention times of tosylate **7** (t_R = 11.3 min) and fluoro compound [^{18}F]**8** (t_R = 8.5 min) a separation via HPLC proved to be successful (Fig. 9).

Based on these results, the subsequent traceless Staudinger ligations of ^{18}F-labeled phosphane [^{18}F]**8** with several model azides **9**–**11** were evaluated. In a first approach we used the reaction conditions (except of the solvent: acetonitrile/*t*-BuOH, v/v = 1:4) from the preparation of the non-radioactive reference compounds. Therefore, water was added to the reaction vial to obtain a 10:1 (v/v) solvent/water mixture. After the addition of the respective azide the following reaction was carried out at 90 °C for 10 min to achieve complete conversion of [^{18}F]**8**. To test milder conditions it was further shown that at 40 °C also a complete conversion proceeded but with an elongation of 30 min for the ligation process. The overall synthesis time was found to be 50 min at 90 °C and 70 min at 40 °C (end of synthesis). The resulting products [^{18}F]**12**, [^{18}F]**13,** and [^{18}F]**14** were obtained in radiochemical yields ranging from 31% to 35% under both labeling conditions (overall yield after a two step procedure starting from [^{18}F]F^-, decay corrected) and the formation was verified by radio-HPLC and radio-TLC analyses showing the same retention times and R_f values as determined for corresponding non-radioactive reference compounds **12**, **13,** and **14**. An application of other solvents like DMF or DMSO delivered no ligation product. Presumably, the radiolabeled phosphane [^{18}F]**8** was oxidized or destroyed during the heating. Radiosyntheses of phosphane [^{18}F]**8** with subsequent traceless Staudinger ligation are depicted in Figure 7.

Furthermore, a strategy for the radiolabeling of biotin as an example for bioactive molecules was adopted. Therefore, (+)-biotin

Table 1
Selected reaction conditions for the preparation of [^{18}F]**8** from **7**

Entry	Amount of **7** (mg)	Solvent[a]	Base	RCY[b] of [^{18}F]**8** (%)
1	3	CH_3CN	K_2CO_3	2
2	12	CH_3CN	$K_2C_2O_4$	14
3	10	CH_3CN:*t*-BuOH 3:7	TBAOH	18
4	10	CH_3CN:*t*-BuOH 1:9	TBAOH	19
5	10	CH_3CN:*t*-BuOH 1:4	TBAOH	32
6	21	CH_3CN:*t*-BuOH 1:4	TBAOH	65[c]

[a] ^{18}F-labeling was carried out in 500 μL of the respective solvent (mixture) for 10 min.
[b] Radiochemical yield (RCY) of [^{18}F]**8**, determined by radio-TLC, decay corrected.
[c] Mean value from 15 runs (range: ±5%).

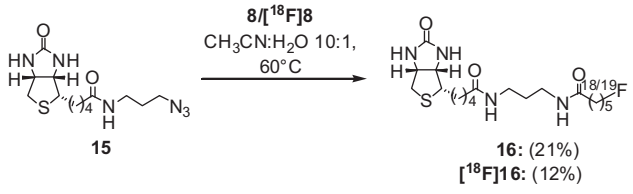

Figure 8. Functionalized biotin compound **15** and (radio)-fluorinated biotin compound **16**/[^{18}F]**16**.

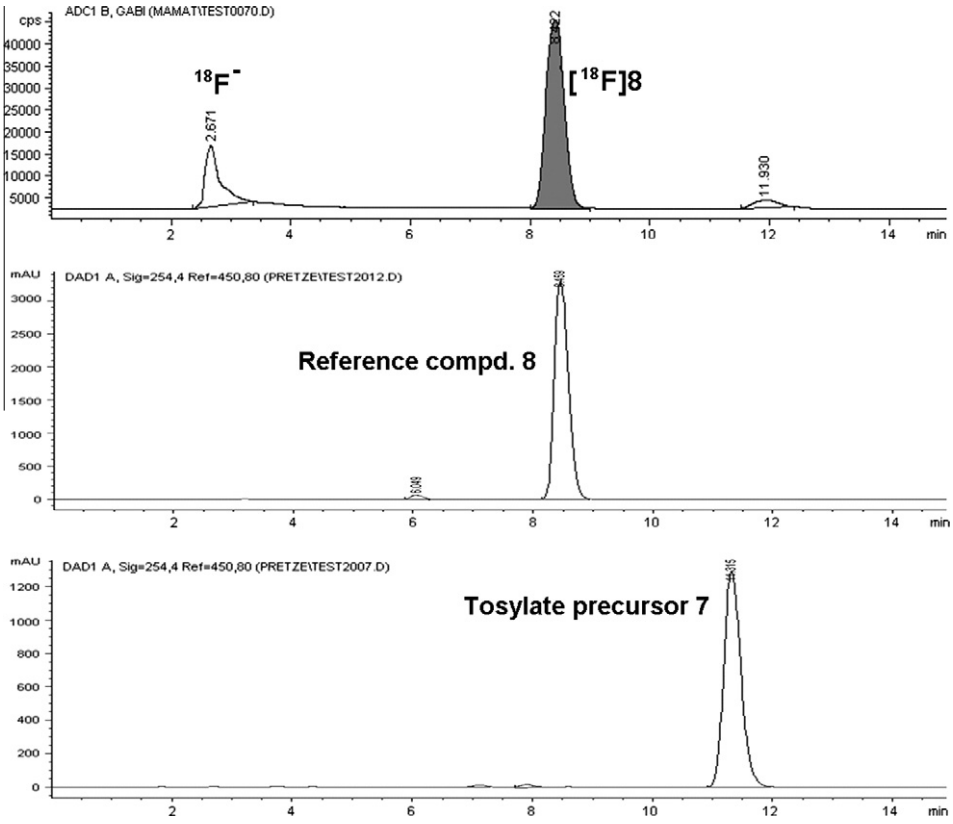

Figure 9. Radio-HPLC diagram (top) of [^{18}F]**8** (γ-trace) and HPLC diagrams of compounds **7** and **8** (both UV trace).

was converted into an amide under Steglich conditions with 3-azidopropanol that leads to **15**. The subsequent traceless Staudinger ligation delivered the non-radioactive fluoro compound **16** in 21% yield. Radiolabeling of **15** with [^{18}F]**8** was carried out at 60 °C for 1 h in a mixture of acetonitrile/water (v/v = 10:1) and gave [^{18}F]**16** in 12% (decay corrected) conversion over a two step procedure (Fig. 8). Verification was done using radio-HPLC and radio-TLC analyses.

3. Conclusion

We developed a straightforward and convenient synthesis route for the introduction of fluorine-18 into small organic and bioactive molecules. Therefore, functionalized phosphanes were synthesized and labeled with [^{18}F]fluoride which act as starting material for the traceless Staudinger ligation. Due to their functionalization with good leaving groups like tosylate these phosphanes still offer the opportunity to introduce chemical as well as radioactive labels into various azide-functionalized (bioactive) compounds. A protection of the central phosphorus with the BH_3 group is essential for a prevention of side reactions like the generation of phosphonium salts or oxidation processes. To test the practical utility, ligation reactions were carried out with good yields to introduce the 6-fluorohexanoyl moiety into several model azides. In addition, a radiolabeling via the Staudinger ligation with the radiofluorinated phosphane was proved successfully in a two-step/one-pot synthesis procedure with the same model compounds under mild reaction conditions and in a short time frame with moderate to good yields compared to other known bio-conjugation approaches. In conclusion, the traceless Staudinger ligation was used as a fast and facile method for the radiofluorination of various small organics as well as bioactive molecules in good yields and mild reaction conditions using this two step/one pot approach.

Acknowledgments

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Supplementary data

Supplementary data (general experimental details of (radio-) chemistry and characterization data) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.09.134.

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