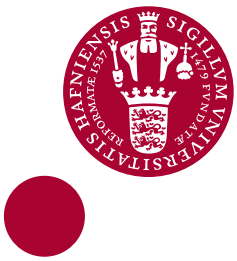

Convenient Entry to ^{18}F-Labeled Amines through the Staudinger Reduction

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Communication

Radiolabeled Synthons

Convenient Entry to ^{18}F-Labeled Amines through the Staudinger Reduction

E. Johanna L. Stéen,[a,b][‡] Vladimir Shalgunov,[a][‡] Christoph Denk,[c] Hannes Mikula,[c] Andreas Kjær,[b,d] Jesper L. Kristensen,[a] and Matthias M. Herth*[a,b]

Abstract: Fluorine-18 possesses outstanding decay characteristics for positron emission tomography (PET) imaging. Therefore, it is ideally suited for clinical applications. As such, improved strategies to incorporate fluorine-18 into bioactive molecules are of utmost importance. Indirect ^{18}F-labeling with amino-functionalized synthons is a convenient and versatile approach to synthesize a broad variety of PET tracers. Herein, we report a method to convert ^{18}F-labeled azides to primary amines by means of the Staudinger reduction. Aliphatic and aromatic ^{18}F-labeled azides were converted into the corresponding amines with high conversion yields. The method was easily automated. From a broader perspective, the applied strategy results in two useful synthons from a single precursor and thus increases the flexibility to label diverse chemical scaffolds with minimal synthetic effort.

Introduction

Positron emission tomography (PET) is a powerful and non-invasive nuclear imaging technique, which makes use of radiolabeled molecules (PET tracers) at tracer levels to study biochemical processes.[1] PET is routinely used in the clinic for diagnosis and staging of diseases, as well as for treatment monitoring.[2] The technique has also found widespread use in drug development to study pharmacokinetics and pharmacodynamics.[1a–b] Fluorine-18 (^{18}F) is the most frequently applied radionuclide for PET in the clinic. Its decay characteristics result in excellent image resolution and acceptable radiation burden, while its half-life of 110 min is convenient for clinical investigations and commercial distributions.[3] Consequently, several strategies have been developed to efficiently incorporate fluorine-18 into bioactive molecules.[3] Indirect labeling strategies applying small ^{18}F-labeled synthons, which can easily be attached to target molecules, are of special relevance for structures that are not suitable for direct ^{18}F-nucleophilic substitution approaches.[3]

Over the years, a number of synthons have been developed and among these structures, ^{18}F-labeled amines represent an interesting platform for indirect labeling.[3] For example, they can be applied in the formation of amide, sulfonamide, urea and carbamate motifs, as well as in reductive alkylations and Michael additions. Up to date, various methods have been published to synthesize ^{18}F-labeled aliphatic and aromatic amines.[3,4] Synthons such as 4-[^{18}F]fluoroaniline and 4-[^{18}F]-fluorobenzylamine can be obtained via aromatic fluorination of an electron-deficient precursor and subsequent reduction.[4] The syntheses of aliphatic amines like 2-[^{18}F]fluoroethylamine ([^{18}F]**2**) are usually based on a two-step fluorination-and-deprotection strategy.[5] The first ^{18}F-labeled intermediate in such strategies cannot be directly used for an indirect labeling approach. In 2012, Glaser et al. reported a novel synthetic approach, which was based on a Cu-mediated reduction of 2-[^{18}F]fluoroethylazide ([^{18}F]**1**, Figure 1).[6] This method has the advantage that two radiolabeled synthons, an azide and an amine, are synthesized within one reaction sequence (Figure 2). Multiple pathways for indirect ^{18}F-labeling can be tested while starting from a single precursor. This reduces synthetic effort and adds flexibility to the tracer development process, especially when a library of structurally related ^{18}F-labeled compounds has to be evaluated.

Our aim with the present study was to explore methods beyond the Cu-mediated reduction of azides to access amines. In particular, we aimed to substitute Cu with an organic reducing agent that would allow for performing the reduction in homogeneous solution, which in turn would facilitate automation of the procedure (Figure 1). The Staudinger reduction is reported

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Supporting information and ORCID(s) from the author(s) for this article are available on the WWW under https://doi.org/10.1002/ejoc.201801457.

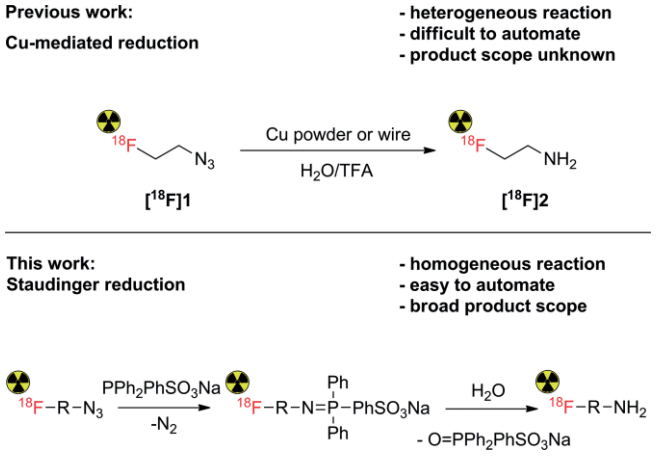


Figure 1. Comparison of radiosynthetic methods to access ^{18}F-labeled amines from azides. PPh_2PhSO_3Na: sodium diphenylphosphinobenzene-3-sulfonate.

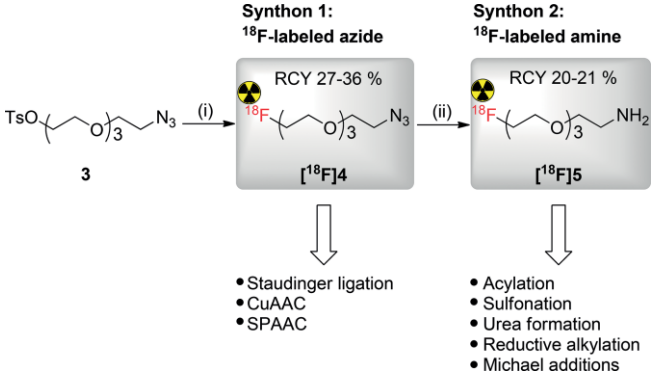


Figure 2. Two different ^{18}F-labeled synthons produced from one precursor: (i) [^{18}F]KF, K_2CO_3, K_{222}, DMSO, 120 °C, 10 min; (ii) 1. PPh_3, MeCN, 100. °C, 10 min; 2. NaOH, 100 °C, 10 min. RCYs are decay corrected.

to rapidly reduce azides to amines in conventional organic chemistry.[7] Therefore, we explored if this type of reduction could also be used for radiochemical applications.

Results and Discussion

The feasibility of the approach was tested on an ^{18}F-fluorinated aliphatic azide, [^{18}F]**4**, which has previously been used for indirect labeling of both peptides and small molecules (Figure 2).[8] Therefore, we envisioned that the corresponding amine [^{18}F]**5** could also have widespread application. In fact, we intended to use [^{18}F]**5** to radiolabel a tetrazine (**6**, Supporting Information). Tetrazines are highly interesting compounds due to their participation in bioorthogonal ligations with dienophiles, in particular *trans*-cyclooctenes.[9]

Initial studies of the Staudinger reduction of [^{18}F]**4** were carried out using 50 mg triphenylphosphine in tetrahydrofuran (THF) (0.5–1.0 mL). The mixture was heated in a sealed vial at 100 °C for 10 min. Thereafter, water was added to hydrolyze the formed iminophosphorane and afford the corresponding amine [^{18}F]**5**. The radiochemical conversion (RCC) varied significantly (20–95 %) under these conditions, which was suspected to be a result of inconsistent hydrolysis (see Figure S1 in the Supporting Information). Changing from water to aqueous NaOH solution (20 mM) for the iminophosphorane hydrolysis stabilized the detected RCCs at ≥80 %. In a next step, we modified the reaction conditions by reducing the amount of triphenylphosphine from 50 to 5 mg and changing the solvent from THF to acetonitrile (MeCN). These modifications were carried out in order to facilitate future automation. THF is not compatible with tubes, seals and fittings of our automated synthesis modules. Moreover, large amounts of triphenylphosphine make separation and purification tedious. Regardless of these adjustments, RCCs of the reaction remained at the same high and reproducible level (≥80 %).

In order to investigate the utility of [^{18}F]**5** as a synthon for indirect ^{18}F-labeling, one tetrazine derivative and three other model compounds were prepared from [^{18}F]**5** via acylation, thiourea formation and reductive alkylation (Figure 3). To minimize total radiosynthesis time, we carried out the Staudinger reduction and the following modifications as a one-pot procedure. Acylations and thiourea formation were successfully performed using this procedure. Amine [^{18}F]**5** reacted readily with benzoyl chloride (PhCOCl) and phenyl isothiocyanate (PhNCS), respectively forming the corresponding benzamide [^{18}F]**8** (75±13 % RCC over 2 steps, n = 4) and thiourea [^{18}F]**9** (73±6 % RCC over 2 steps, n = 3). Coupling of [^{18}F]**5** to tetrazine **6** gave [^{18}F]**7** (75±1 % RCC over 2 steps, n = 2). One-pot reductive alkylation with benzaldehyde (PhCHO) in the presence of $NaBH_3CN$ and acetic acid (AcOH) was unsuccessful. However, after the removal of precipitated triphenylphosphine/triphenylphosphine oxide by solid-phase extraction, [^{18}F]**5** could be converted into the secondary amine [^{18}F]**10** in a RCC of 40±4 % (over 2 steps, n = 2).

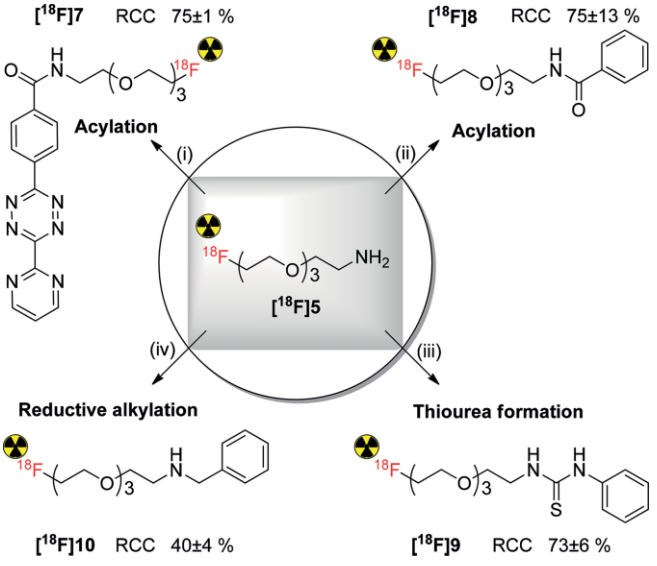
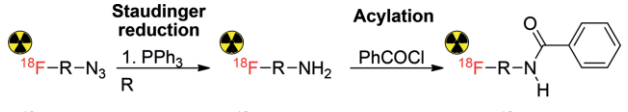


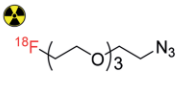
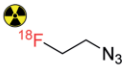
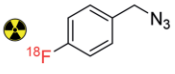
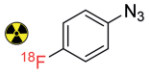
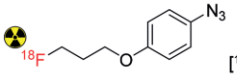
Figure 3. Versatile indirect labeling through acylation, thiourea formation and reductive alkylation using amine [^{18}F]**5** as a synthon. (i) Tetrazine **6** (Supporting Information), r.t. 5 min; (ii) PhCOCl, r.t. 5 min; (iii) PhNCS, 100 °C, 5 min; (iv) PhCHO, $NaBH_3CN$, AcOH, 120 °C, 5 min.

Inspired by the successful preparation of [^{18}F]**5**, the Staudinger reduction was applied to a series of different ^{18}F-labeled azides to investigate the substrate scope of the reaction

(Table 1). The selected azides were based on aliphatic and aromatic substrates that have been frequently used for indirect ^{18}F-labeling approaches.[3,4] All investigated azides were converted into corresponding amines applying the same conditions as used for [^{18}F]**4**. High RCCs were observed for all structures except for [^{18}F]**1**, which was only converted into [^{18}F]**2** in moderate yields (Table 1). Subsequent coupling of the respective amines to benzoyl chloride produced the corresponding ^{18}F-labeled benzamides in good yields (Table 1). The RCC of the acylation was independent of the concentrations of benzoyl chloride in the investigated range of 10 and 100 mM.

Table 1. Substrate scope of the Staudinger reduction.



Azide substrates		Amine RCC (Staudinger reduction)[a] (%)	Amide RCC (over 2 steps)[b] (%)	
			Aliquot[c]	Batch[c]
	[^{18}F]**4**	**83±6** (n = 14)	**75±13** (n = 5)	**65±3** (n = 3)
	[^{18}F]**1**	**35±3** (n = 4)	**22±5** (n = 6)	**24±4** (n = 4)
	[^{18}F]**11**	**64±1** (n = 3)	**55±3** (n = 3)	**55±3** (n = 3)
	[^{18}F]**12**	**84±4** (n = 3)	**89±4** (n = 3)	**86±3** (n = 3)
	[^{18}F]**13**	**70±6** (n = 3)	**70±5** (n = 4)	**69±8** (n = 2)

[a] All Staudinger reductions were performed at 100 °C for 10 min for step 1 and 2, respectively. [b] All acylations were performed at r.t. for 5 min. [c] See Experimental Section. All RCCs have been determined by radio-TLC.

Finally, we demonstrated the amenability of the Staudinger procedure to automation. This was possible by using a water-soluble triphenylphosphine derivative. Sodium diphenylphosphinobenzene-3-sulfonate (PPh_2PhSO_3Na) showed to be as effective in reducing [^{18}F]**4** to [^{18}F]**5** as triphenylphosphine (RCC in the range of 93±3 % (n = 2)). No precipitate was formed and subsequent coupling of [^{18}F]**5** to [^{18}F]**8** could be carried out in a one-pot automated procedure. The whole synthesis procedure including HPLC purification took no longer than 150 min and benzamide [^{18}F]**8** was isolated in 16–18 % RCY from [^{18}F]fluoride; RCY of [^{18}F]**8** calculated from the azide [^{18}F]**4** was 57 % (all RCYs are decay corrected). A molar radioactivity of 60 GBq/μmol could be determined for [^{18}F]**8** at the end of the synthesis.

Conclusions

In the present work, we have investigated the applicability of the Staudinger reduction as an approach to access ^{18}F-labeled amino-functionalized synthons from ^{18}F-labeled azides. The reduction of ^{18}F-labeled aliphatic and aromatic azides using triphenylphosphine or sodium diphenylphosphinobenzene-3-sulfonate proceeded in high RCCs. The amines could successfully be used for indirect ^{18}F-labeling of a small set of model compounds. Moreover, the procedure was easily automated. We believe that the Staudinger reduction is a valuable tool that can be used to access amino-functionalized synthons for radiosynthesis of a wide scope of PET tracers.

Experimental Section

General information: For all reactions, RCCs were determined by radio-TLC and RCYs were decay corrected to the amount of radioactivity at the start of the synthesis. Identity of all radiofluorinated products was confirmed by co-elution with ^{19}F-reference compounds on HPLC and TLC. The syntheses of the ^{19}F-reference compounds and radiosyntheses of ^{18}F-labeled azides are described in the Supporting Information, along with characterization data (NMR spectra and HPLC chromatograms).

General procedure for the Staudinger reduction yielding [^{18}F]2,5,14–16: The corresponding ^{18}F-labeled azide (approximately 120 MBq) dissolved in dry MeCN (1 mL) was added to a 4 mL glass vial containing triphenylphosphine (5 mg). The vial was sealed and heated at 100 °C for 10 min. Thereafter, 20 mM aqueous NaOH solution (0.6–0.8 mL) was added and the mixture was heated for an additional 10 min, before it was cooled to room temperature. The reaction was analyzed by radio-TLC.

Acylation of [^{18}F]5 yielding [^{18}F]7: Crude [^{18}F]**5** was neutralized with 1 M aqueous HCl and afterward filtered through a 0.22 μm nylon filter. An aliquot (40–50 μL, 5–10 MBq) of this solution was mixed with 0.1 M aqueous $KHCO_3$ (30 μL) and tetrazine **6** (0.1 mg) dissolved in dry MeCN (30 μL). The mixture was stirred for 5 min at room temperature and analyzed by radio-TLC.

General procedure for the acylation of ^{18}F-labeled amines [^{18}F]2,5,14–16 with benzoyl chloride yielding [^{18}F]8,17–20: *"Aliquot method"*: An aliquot (50–100 μL, 0.5–10 MBq) of the crude mixture of the corresponding ^{18}F-labeled amine was diluted with an equal volume of MeCN. Benzoyl chloride (1 μL, 8.5 μmol) was added. This mixture was stirred for 5 min at room temperature. *"Batch method":* Benzoyl chloride (1–2 μL, 8.5–17 μmol) was added to the crude mixture of the corresponding ^{18}F-labeled amine (20–100 MBq) dissolved in a MeCN/water mixture (4:3, 1–1.5 mL). This mixture was stirred at room temperature for 5 min. The aliquot and the batch method were analyzed by radio-TLC.

Thiourea formation yielding [^{18}F]9: An aliquot (50 μL, 2–3 MBq) of crude [^{18}F]**5** was added to phenyl isothiocyanate (1 μL) dissolved in dry MeCN (25 μL). The mixture was heated at 100 °C for 5 min and afterward analyzed via radio-TLC.

Reductive alkylation yielding [^{18}F]10: A solution of crude [^{18}F]**5** (700 MBq) was diluted with 0.05 M aqueous HCl (5 mL). This solution was first filtered through a 0.22 μm nylon filter and thereafter passed through a Sep-Pak C18 cartridge. The eluate was basified with 2 M aqueous NaOH to pH > 11, before it was trapped on a second Sep-Pak C18 cartridge. The second cartridge was dried with a nitrogen flow for 2 min and eluted with dry MeOH (1 mL). To an aliquot (0.3 mL, 18–20 MBq) of this solution, benzaldehyde (0.6 mg), $NaBH_3CN$ (2 mg) and glacial AcOH (5 μL) dissolved in dry MeOH (0.2 mL) were added. The vial was sealed and heated at 120 °C for 10 min. Afterward, the reaction was analyzed by radio-TLC.

Automated Staudinger reduction and acylation yielding [^{18}F]8: Azide [^{18}F]**4** was trapped on a Sep-Pak C18 cartridge. This cartridge was dried with a helium flow for 2 min. Afterward [^{18}F]**4** was eluted from the cartridge with dry MeCN (1 mL) into a 7 mL glass vial. Sodium diphenyl-phosphinobenzene-3-sulfonate (3 mg) dissolved in aqueous 20 mM NaOH (0.6 mL) was added. The mixture was heated at 100 °C for 17 min and then cooled to 80 °C. Benzoyl chloride (2 µL) dissolved in MeCN (0.1 mL) was subsequently added, and the mixture was stirred for 5 min without heating. Afterward, the solution was diluted with water (2 mL) and [^{18}F]**8** purified by semi-preparative HPLC using a Luna 5µ C18(2) 100 Å (250 × 10 mm) column. The flow rate was set to 4 mL/min and the eluent was water/MeCN/trifluoroacetic acid (70:30:0.1 v/v/v). [^{18}F]**8** had a retention time of 12.5 min. The radiochemical purity (RCP) was >95 % and was determined by radio-HPLC (Supporting Information). RCY was decay corrected to the start of the synthesis.

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Keywords: Fluorine · Radiochemistry · Azides · Amines · Reduction

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