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Highly efficient click labeling using 2-[¹⁸F]fluoroethyl azide and synthesis of an ¹⁸F *N*-hydroxysuccinimide ester as conjugation agent

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Abstract

Introduction—Click labeling using 2-[¹⁸F]fluoroethyl azide has been proven to be promising methods of radiolabeling small molecules and peptides, some of which are undergoing clinical evaluations. However, the previously reported method afforded low yield, poor purities and under desirable reproducibility.

Methods—A vacuum distillation method was used to isolate 2-[¹⁸F]fluoroethyl azide, and the solvent effect of acetonitrile (ACN) and dimethylformamide (DMF) on the click labeling using Cu(I) from copper sulfate/sodium ascorbate was studied. The labeling conditions were optimized to radiosynthesize a hydroxysuccinimide ester (NHS).

Results—2-[¹⁸F]fluoroethyl azide was isolated by the vacuum distillation method with > 80% yield within 10 min in a “pure” and click-ready form. It was found that the amount of DMF was critical for maintaining high levels of Cu(I) from copper sulfate/sodium ascorbate in order to rapidly complete the click labeling reaction. The addition of bathophenanthrolinedisulfonic acid disodium salt (BPDS) to the mixture of copper sulfate/sodium ascorbate also greatly improved the click labeling efficiency. Through exploiting these optimizations, a base-labile *N*-hydroxysuccinimide (NHS) ester was rapidly radiosynthesized in 90% isolated yield with good chemical and radiochemical purities.

Conclusions—We have developed a general method to click-label small molecules efficiently using [¹⁸F]2 for research and clinical use. This NHS ester can be used for conjugation chemistry to label antibodies, peptides and small molecules as PET tracers.

Keywords

PET; Fluorine-18; fluoroethyl azide; click chemistry; hydroxysuccinimide ester

1. Introduction

The identification of new small-molecule ligands for numerous biological targets has led to the growth of the non-invasive imaging technique positron emission tomography (PET) in

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both clinical and research fields [1, 2]. PET imaging studies require the radiolabeling of a compound with ideal biological properties in high yield with good chemical and radiochemical purity. Fluorine-18, which is readily available in high specific activity from most medical cyclotrons, is widely used for PET radiolabeling of small molecules because of its small size and favorable decay characteristics (100% positron, $t_{1/2} = 109.7$ min). Typical fluorine-18 radiolabeling employs nucleophilic substitution of a good leaving group with [^{18}F]fluoride, which is produced in [^{18}O]water and azeotropically dried in the presence of Kryptofix 222 and potassium carbonate or another base at elevated temperatures [3, 4]. Because this reaction proceeds under harsh and basic conditions, attempts to label temperature- or base-sensitive products may result in a lot of radiolabeled and non-radioactive by-products, poor yields, and high variability. Furthermore, optimization of labeling conditions and the necessary HPLC purification for removal of by-products is costly, time-consuming and requires highly-trained personnel. The widespread dissemination of PET radiopharmaceuticals demands a facile method to label compounds with fluorine-18.

Since the term “click chemistry” was introduced in 2001[5], Cu(I)-catalyzed azide-alkyne cycloaddition (CuAAC) [6, 7] has been extensively utilized in many fields of chemistry [8], including PET radiolabeling [9]. This Cu(I)-catalyzed Huisgen 1,3-dipolar cycloaddition of terminal alkynes and organic azides affords 1,4-disubstituted 1,2,3-triazoles exclusively, in high yield and under mild conditions. The 1,2,3-triazole group is stable to acid and basic hydrolysis, as well as reductive and oxidative conditions due to its aromaticity. Furthermore, this triazole group is biologically stable [10] and possesses a polarity and size similar to that of an amide bond [11], thereby improving water solubility [12] and allowing the synthesis of a wide-range of compounds with biological potential [13]. Because of these features of the click reaction and the readily accessible building blocks with azide and alkyne, click labeling has become a valuable and powerful tool in PET chemistry [9].

Many fluorine-18 labeled alkynes and azides have been synthesized for labeling of small molecules and peptides as PET tracers using click chemistry [9]. Among the reported compounds used for click labeling of small molecules and conjugation with peptides, 2- [^{18}F]fluoroethyl azide ([^{18}F]**2**) appeared to be the most promising fluorine-18 labeled azide [14]. Its advantages include: 1) it can be used with a wide variety of commercial available or easily synthesized small molecules and pharmaceuticals that contain an alkyne as a building block; 2) **2** is the lowest molecular weight azide that is suitable for labeling with F-18, and facilitates the introduction of a triazole group with the least steric effect to a labeled molecule; 3) the incorporation yield of [^{18}F]**2** has been reported to be >95% and it can be distilled/purified from the reaction mixture [14]. [^{18}F]**2** has been previously used to successfully label small molecules and peptides [14–20]. However, [^{18}F]**2** was isolated only in ~50% yield with impurities that are difficult to separate, resulting in low specific activity of final products [14, 16, 17], and the click labeling conditions in the literature sometimes required elevated temperatures or long reaction times [14].

During our labeling of a variety of small molecule PET tracers with [^{18}F]**2**, a highly efficient method for the isolation by vacuum distillation of [^{18}F]**2** was developed, and the subsequent click labeling conditions were optimized in order to afford final products at room temperature within 10 minutes, and with high purity and specific activity after facile HPLC purification. Here we describe this distillation procedure and our exploration of the solvent effects on the click chemistry which facilitated optimization of the reaction. This improved method was demonstrated by the radiosynthesis of a base-labile NHS ester in high yield using the click labeling with [^{18}F]**2**. Our method will provide an efficient and general procedure for the click labeling of small molecules using [^{18}F]**2** as a synthon for PET labeling.

2. Materials and methods

2.1. General

All chemicals were obtained from standard commercial sources and used without further purification. All reactions were carried out by standard air-free and moisture-free techniques under an inert nitrogen atmosphere with dry solvents unless otherwise stated. Flash column chromatography was conducted using silica gel. Melting points were uncorrected.

Routine ^1H and ^{13}C NMR spectra were recorded at 300 or 400 MHz. All chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane (TMS). All coupling constants (J) are given in Hertz (Hz). Splitting patterns are typically described as follows: s, singlet; d, doublet; t, triplet; m, multiplet. ESI/MS was performed on a Waters ZQ 4000 single quadrupole mass spectrometer equipped with an electrospray ionization (ESI) LC-MS interface. High performance liquid chromatography (HPLC) was performed with an ultraviolet detector and a well-scintillation NaI (TI) detector and associated electronics for radioactivity detection. An Agilent SB-C18 250×9.4 mm 5μ semi-preparative column (A) and an Agilent SB-C18 250×4.6 mm 5μ analytical column (B) were used for preparation and analysis respectively. [^{18}F]Fluoride was produced at Washington University by the $^{18}\text{O}(\text{p},\text{n})^{18}\text{F}$ reaction through proton irradiation of enriched (95%) [^{18}O] water in the RDS111 cyclotron. Radio-TLC was accomplished using a Bioscan AR-2000 imaging scanner (Bioscan, Inc., Washington, DC). Published methods were used for the synthesis of compound **1** [21], **2** [14], **3** [22], and **5** [23] according to literature.

2.2. 1. Synthesis of 4-((1-(2-fluoroethyl)-1H-1,2,3-triazol-4-yl)methoxy)benzaldehyde (**4**)

—To a solution of **3** (0.2 g, 1.25 mmol) in DMF (5 mL) was added a solution of **2** (0.2 g, 2.25 mmol) in DMF (5 mL), followed by addition of a mixture of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (70 mg in 0.9 mL water) and sodium ascorbate (500 mg in 1.8 mL water). The reaction mixture was stirred at room temperature for 1 h, and then diluted with water (100 mL). Ethyl acetate (3×50 mL) was used to extract the product from the aqueous phase. The combined organic phase was washed by saturated NaHCO_3 solution (3×20 mL), dried over Na_2SO_4 . After the solvent was removed under reduced pressure, the crude product was purified by silica gel flash chromatography using ethyl acetate (EtOAc) to afford **4** as a white solid (247 mg, 79 %). mp, $76\text{--}77^\circ\text{C}$; ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 9.84 (s, 1H), 8.28 (s, 1H), 7.84 (d, $J = 8.8$, 2H), 7.21 (d, $J = 8.8$, 2H), 5.26 (s, 2H), 4.65–4.88 (m, 4H); ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) δ 191.77, 163.38, 142.65, 132.22, 130.31, 125.72, 115.62, 83.16, 81.49, 61.83, 50.61, 50.42.

2.2.2. Synthesis of 2,5-dioxopyrrolidin-1-yl 4-((1-(2-fluoroethyl)-1H-1,2,3-triazol-4-yl)methoxy)benzoate (**7**)

—To a solution of **6** (50 mg, 0.18 mmol) in ACN (0.8 mL) and EtOAc (1 mL) was added a solution of **2** (40 mg, 0.45 mmol) in DMF (1 mL), followed by addition of a mixture of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (2.5 mg in 25 μL water), sodium ascorbate (7.5 mg in 25 μL water) and BPDS (6 mg in 100 μL 4:1 water/DMF). The mixture was stirred at room temperature and the progress monitored by TLC. When silica TLC indicated that the starting material **6** was consumed, the reaction was quenched with water (30 mL) and TFA (100 μL). Ethyl acetate (3×20 mL) was used to extract the product, and the organic phase was dried over Na_2SO_4 . After ethyl acetate was removed under reduced pressure, the crude product was purified by silica gel chromatography using ethyl acetate to afford **7** as a white solid (56.4 mg, 85 %). mp, $156.0\text{--}157.5^\circ\text{C}$; ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 8.28 (s, 1H), 8.01 (d, $J = 9.2$, 2H), 7.25 (d, $J = 9.2$, 2H), 5.29 (s, 2H), 4.65–4.88 (m, 4H), 2.84 (s, 4H); ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) δ 170.88, 163.96, 161.68, 142.48, 132.80, 125.79, 117.04, 116.03, 83.15, 81.48, 61.96, 50.62, 50.43, 25.95; ESI/MS: 363.5 ($\text{M} + \text{H}^+$).

2.3.1. Radiosynthesis of 2-[¹⁸F]fluoroethyl azide (2) and vacuum distillation— [¹⁸F]fluoride (up to 50 mCi in 100–500 μ L [¹⁸O]water) was transferred to a BD vacutainer (13 $\times$ 75 mm, 5 mL, glass, no additives) containing K₂₂₂ (5.6 mg, 14.9 μ mol) and K₂CO₃ (1 mg, 7.2 μ mol), then the mixture was dried by azeotropic distillation at 105 °C using ACN (3 $\times$ 1 mL) under a gentle flow of N₂ gas. When the drying was close to finish, the vacutainer was removed from the oil bath and the solvent residue (< 100 μ L) was removed by a flow of N₂ at room temperature. The vacutainer was capped and connected to a dry ice trap (10 mL Pyrex tube with screw-cap) via Teflon tubing (I.D. 1.0 mm) (see Figure 1). After a solution of **1** (2 mg, 8.3 μ mol) in ACN (200 μ L) was added to the vacutainer, it was shaken and heated at 88 °C for 5 min, directly followed by vacuum distillation which was achieved by a 50 mL syringe. The distillation lasted for 1 min, after which N₂ gas (10 mL) was released to the vacutainer. After this distillation procedure was repeated, the Pyrex tube was removed from the dry ice bath and warmed to room temperature in a water bath for the subsequent click labeling. The processing time is about 10 min from the beginning of the labeling reaction, and the isolated yield is >80 % (n > 30), determined by measuring the distilled activity (decay corrected).

Warning: [¹⁸F]**2** is extremely volatile and should be handled only in a fume hood. Low boiling point solvents (such as dichloromethane and diethyl ether) are not recommended as TLC solvents. A charcoal trap is sufficient to trap [¹⁸F]**2** and should be attached to any vents of the system.

2.3.2. General method of click labeling using [¹⁸F]2** and CuSO₄ and sodium ascorbate as Cu(I) source: radiosynthesis of [¹⁸F]**4**—**A solution of CuSO₄·5H₂O (5 mg, 20.0 μ mol) in water (50 μ L) and a solution of sodium ascorbate (15 mg, 75.7 μ mol) in water (50 μ L) were mixed, and when the color of the mixture changed from black to yellow in minutes, the above mixture was added, along with a solution of **3** (1.0 mg, 6.2 μ mol) in DMF (200 μ L), to the distilled [¹⁸F]**2** in ACN (200 μ L) at room temperature. The reaction mixture was shaken occasionally during the reaction. For analysis, an aliquot of the reaction mixture was diluted in ACN or HPLC mobile phase for analysis by silica gel TLC (developed in ethyl acetate; R_f: 0.0 (unknown), 0.48 ([¹⁸F]**4**), 0.88([¹⁸F]**2**)) or by reversed phase HPLC (column B, 40 % ACN, 60 % water, 0.1 % TFA, 1 mL/min, 272 nm; Retention time (min): 2.2 (unknown), 5.6 ([¹⁸F]**4**), 6.2([¹⁸F]**2**).

2.3.3. Radiosynthesis of 7 using CuSO₄, sodium ascorbate and BPDS as Cu(I) source—A solution of CuSO₄·5H₂O (1 mg, 4.0 μ mol) in water (10 μ L) and a solution of sodium ascorbate (3 mg, 15.1 μ mol) in water (10 μ L) were mixed; when the color of the mixture changed from black to yellow, the copper salt solution was mixed with a solution of BPDS (1.2 mg, 2.0 μ mol) in 1 : 4 DMF/H₂O (20 μ L). A portion of above mixture (25 μ L), along with **6** (1 mg, 3.66 μ mol) in DMF (200 μ L), was added to the distilled [¹⁸F]**2** in ACN (200 μ L). After 5 min at room temperature, the reaction mixture was diluted with a solution of 10% ACN, 90% water, 0.1% TFA(3 mL) for reversed-phase HPLC purification using column A and mobile phase (32% ACN, 68% water, 0.1% TFA) at 4 mL/min, and UV at 240 nm. [¹⁸F]**7** was collected at 15min in 90% yield (decay corrected). The HPLC fraction containing [¹⁸F]**7** was diluted in water (50 mL), then [¹⁸F]**7** was isolated by solid-phase extraction using a C18 SepPak by passing the diluted sample through the SepPak. The SepPak was rinsed with water (10 mL), and dried under a stream of N₂ gas. [¹⁸F]**7** can be eluted from the SepPak in DMF for immediate conjugation, or in either dichloromethane or diethyl ether, to afford a dried form of [¹⁸F]**7** after drying over Na₂SO₄ and removal of solvents.

3. Results and Discussion

3.1. Radiosynthesis of [^{18}F]2 and vacuum distillation

The radiosynthesis of [^{18}F]2 was carried out as described in the literature under typical labeling conditions (Scheme 1). Up to 95 % analytical yield is expected when carried out at 80 °C for 15 min, and published literature indicated that a flow trap method could be used to isolate [^{18}F]2 from the reaction mixture at 130 °C in 63% distillation efficiency or 54% decay corrected radiochemical yield [14]. Following this protocol for our synthesis of [^{18}F]2, the volatile [^{18}F]2 was unexpectedly difficult to trap, even when employing a liquid nitrogen trap with the recommended flow distillation method. This difficulty led us to seek a more efficient technique. Vacuum distillation is routinely used in organic synthesis for isolation, such as 'bulb to bulb' distillation or Kugelrohr distillation. Vacuum can also be employed in chemistry and radiochemistry for transferring liquid materials between containers. Thus, a vacuum "bulb to bulb" distillation method (see Figure 1) was developed for the efficient isolation of [^{18}F]2 from the reaction mixture. At the end of the incorporation reaction of [^{18}F]2, [^{18}F]2 was transferred under vacuum almost instantly, along with the near- or above-boiling point ACN as the carrier, from the reaction vessel via a Teflon tubing to a dry ice-cooled trap. The vacuum (up to 20 torr) was achieved with a 50 mL syringe. Subsequently, nitrogen was released to the reaction vessel to ensure the transfer of any residual [^{18}F]2 to the trap under vacuum. Finally, the trap was warmed in a water bath to room temperature for click labeling. The entire process (incorporation, distillation, and warm-up) required < 10 min to afford [^{18}F]2 in a click-ready form in a minimal volume of ACN (200 μL), which has shown to have great solvent effect on the click labeling (see the discussion below). Because the distillation was carried out in a closed system, almost no [^{18}F]2 escaped from the trap; this method avoids releasing [^{18}F]2 to the environment. To our knowledge, such an application of vacuum in radiochemistry has not been previously reported, and this technique should be useful for the isolation of other volatile radioactive materials from reaction mixtures.

The nucleophilic substitution reaction of **1** with [^{18}F]fluoride (Scheme 1) appears to be very fast and robust. Under the labeling conditions similar to those reported [14], within 2.5 min of heating at 88 °C, the labeling reaction was completed, reaching a plateau in isolated yield (Table 1). Longer reaction time did not increase the yields. In addition, labeling reactions carried out from 80 °C to 90 °C or using 0.5 mg to 2 mg of **1** afforded similar isolated yields (data not shown). The high efficiency of the nucleophilic substitution reaction of **1** clearly benefits from the activation of the adjacent azide group in **1**. On average, we were able to isolate [^{18}F]2 in > 80% yield (decay corrected, $n > 30$) within 10 min in the click-ready form. The distillation efficiency was not determined; however, the high isolation yields suggest that the efficiency is close to quantitative.

The isolated [^{18}F]2 was analyzed by a reversed phase HPLC with the UV at 220 nm. As shown in Figure 2, [^{18}F]2 (retention time: 7.7 min) was isolated as the only radioactive peak in a very chemically pure form with no major UV peaks around it. This is ideal for the HPLC purification of the click labeling mixture in order to achieve a chemically and radiochemically pure final product with high specific activity. From the standpoint of purity, the vacuum distillation method should be superior to the reported flow distillation in that the flow-trap distillation at 130 °C could, in principal, generate significantly more side-products, including the observed elimination by-product vinyl azide from **1**. It should be noted that, low specific activity due to impurities has been reported when the flow-trap distillation is used [14, 16, 17].

The major UV peak in the distilled reaction mixture of [^{18}F]2 was observed at 12 min (Figure 2). NMR analysis of the reaction mixture in CD_3CN suggests that this peak is vinyl

azide, an elimination by-product of the labeling reaction. Elimination reaction is a common side-reaction in ^{18}F labeling using nucleophilic substitution of ethylene sulfonate precursors under basic conditions, especially at above 100 °C. The facile formation of vinyl azide during the radiolabeling of **2** may also be due to the activation of azide in **1** as that in the nucleophilic substitution. The low-boiling point vinyl azide was isolated efficiently by the vacuum distillation method. It was estimated by HPLC that 25–40 % starting material **1** was distilled as vinyl azide. Therefore, the large amount of vinyl azide became the limiting reagent in the click labeling, in that the stoichiometry requires more than one equivalent of alkyne substrate in order to complete the click-labeling reaction. However, because of the large difference in retention time between ^{18}F **2** and vinyl azide in HPLC, if the alkyne substrates are small molecules, separation of the click-labeled product of ^{18}F **2** from that of vinyl azide should be easy (e.g. Figure 3). Vacuum-distilled ^{18}F **2** is not suitable to label macromolecules, such as proteins and nanoparticles, with high specific activity, due to stoichiometry limitations.

3.2. Click labeling-solvent effect

During the development of click labeling of small molecule PET tracers, we found that small changes in the volume of solvent used (e.g. 200 vs. 300 μL ACN and 200 vs. 250 μL DMF) afforded different results in the completion of the click labeling. This observation led us to explore the solvent effect on click labeling. One advantage of click chemistry is that a variety of solvents can be used. However, we only studied the solvent effect of organic solvents, ACN and DMF, which are commonly used in labeling reactions and are well suited to reversed phase HPLC purification. Copper sulfate and sodium ascorbate, which are the most used source of Cu(I) catalyst for click labeling [9], were used here because they afforded good yield [14] and are easy to work up. The click reaction of ^{18}F **2** and **3** (Scheme 2) was used as the model reaction to investigate the solvent effect, because not only is the propargyl group a good building block for click labeling, but also because benzaldehyde derivatives are common conjugation agents in conjugation chemistry.

The results of solvent effects are shown in Table 2 and 3. The reaction mixture was analyzed by silica gel radio-TLC and confirmed by reversed-phase HPLC. Three radioactive peaks were observed as ^{18}F **2**, ^{18}F **4**, and a small amount of unknown by-product in radio-TLC and HPLC. The nature of the by-product was not identified and it is not known whether it is specific to **3**, but it was determined that this peak was not from the decomposition of ^{18}F **2**, which was stable under the reaction condition (data not shown). When 1 mg of **3** was used in the model reactions (data not shown), all the click labeling reactions were very fast, except for the reaction using the reported condition [14]; as a result, the effect of solvent on the rate of conversion of ^{18}F **2** was not evident. When the amount of **3** was reduced to 0.1 mg, which in principle will reduce the reaction rate by 100-fold due to the second order kinetics with respect to an alkyne substrate **3** [24], the solvent effect could be observed. As shown in Table 2, increases in the amount of DMF will greatly increase the rate of conversion of ^{18}F **2**, while increases in the amount of ACN will decrease the rate. The most important factor in click chemistry appears to be the need to maintain Cu(I) at a high level at all times during reaction [8]. In the model reactions where more DMF was used, the Cu(I) mixture remained in the form of a yellowish suspension of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ and sodium ascorbate in the click labeling mixture, and the labeling reaction was always very fast and retaining. When more ACN was added, white solids precipitated out very quickly, and the labeling reaction slowed or even stopped completely. The effect of water on the reaction rate was also studied (Table 3). When more water was used (e.g. 200 μL instead of 100 μL), the reaction mixture became homogenous and colorless, but the reaction slowed down greatly and not retaining. Only when a large amount of DMF was used, the reactions with more water were retaining. All the above observations are apparently related to the form of Cu(I),

the mixture of copper sulfate and sodium ascorbate, in the reaction mixture, however, the influence of solvents on the form of Cu(I) is beyond the scope of this paper. Kinetic effects on the rate of click labeling reactions were also observed when the reaction volume varied. This was because of the second order kinetics with respect to alkyne substrate **3** and Cu(I) catalyst [24]. A change in the volume of the reaction or in the concentrations of reactants will result in a tremendous change in the rate of click labeling. Therefore, a minimal amount of solvent is required to complete the click labeling within a short time at room temperature. Obviously, solvents have great effects on the rate of click labeling reactions using [^{18}F]**2**. It appears that a sufficient amount of DMF is critical for maintaining the level of Cu(I) in the solution, affording fast and retaining click labeling.

Bathophenanthrolinedisulfonic acid disodium salt (BPDS) has been used to promote the Cu(I) catalyzed click reaction by stabilizing the Cu(I) catalyst in organic synthesis and bioconjugations. As shown in Table 4, the addition of BPDS to the mixture of copper sulfate and sodium ascorbate greatly increases the rate of click labeling, resulting in complete conversion of [^{18}F]**2** within 5 min. However, one potential disadvantage in using BPDS is that it may complicate the HPLC purification of very polar compounds.

3.3. Radiosynthesis of an NHS ester using click chemistry

An *N*-hydroxysuccinimide (NHS) ester is perhaps the most common acylating agent in conjugation chemistry. *N*-Succinimidyl-4- ^{18}F fluorobenzoate ([^{18}F]SFB), as an ^{18}F labeled NHS ester, is the most used prosthetic group for ^{18}F -labeling of peptides and proteins [25, 26]. The synthesis of [^{18}F]SFB requires several steps and affords only 30–50 % yields [24]. Recently, a PEGylated NHS ester was synthesized using Cu(I) catalyzed click labeling, but only in 34 % radiochemical yield from the ^{18}F -PEG-alkyne [27]. To demonstrate the ease of click labeling of base-labile compounds using [^{18}F]**2**, we synthesized an ^{18}F -labeled NHS ester ([^{18}F]**7**) using Cu(I) catalyzed click chemistry. Non-radioactive **7** was synthesized as shown in Scheme 3. [^{18}F]**7** was synthesized by a similar procedure. When a standard $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ and sodium ascorbate mixture was used as the Cu(I) catalyst source, the basic reaction mixture resulted in some decomposition of [^{18}F]**7**. When the more efficient $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, sodium ascorbate, and DBPS combination was explored as the Cu(I) catalyst source, click labeling of **6** with [^{18}F]**2** in 5 min afforded [^{18}F]**7** in 90 % HPLC isolated yield with good chemical and radiochemical purity (see Figure 3 for HPLCs). The total synthesis time of [^{18}F]**7** is short: < 10 min for the synthesis of click-ready [^{18}F]**2**, 5 min for click labeling, and < 20 min for HPLC purification of [^{18}F]**7**. [^{18}F]**7** is stable in the HPLC mobile phase, and can be isolated using standard solid phase extraction techniques either in a dry form (eluted with ether or dichloromethane and followed by evaporation of solvents) or in DMF (< 300 μL). [^{18}F]**7** has been used successfully to label small molecules and dendrimers (results will be published elsewhere), and may be superior to [^{18}F]SFB in several aspects, such as facile synthesis, labeling yields, *in vivo* stability, and the introduction of a triazole group.

3.4. General considerations of click labeling using [^{18}F]**2**

In the development of PET radiotracers, we must consider the reaction time, the yield, the easy workup, and facile purification of a labeled compound; a radiolabeled compound with good chemical and radiochemical purities is required to serve as a true tracer for PET. The current method was developed based on these considerations. One-pot reactions have been used to synthesize ^{18}F labeled amino acids with very good results [28], but this method requires much more of the alkyne substrate because of the stoichiometry, and may result in low specific activity and has an increased potential for side reactions, due to basic reaction conditions. The vacuum distillation method described here afforded [^{18}F]**2** in only 200 μL ACN; this is critical, because in order to take advantage of the second-order kinetics with

respect to alkyne substrates and Cu(I) catalyst, the total reaction volume must be minimized. A sufficient amount of DMF is required to maintain a high level of Cu(I) during the reaction and more than one equivalent of the alkyne substrate is needed to complete the reaction because vinyl azide is the limiting reagent. The addition of water to the reaction mixture may slow down the reaction, and may result in low solubility of lipophilic compounds; a mixture of copper sulfate and sodium ascorbate is the choice of Cu(I) catalyst because of the easy workup and facile HPLC purification. We have employed these procedures with great success when using [^{18}F]**2** to label a variety of compounds, including chemically-labile ones. Next to direct nucleophilic substitution using [^{18}F]fluoride, [^{18}F]**2** appears to be the most useful ^{18}F labeled synthon to label small molecules both in terms of labeling yields and due to the rapid and efficient synthesis and purification using the procedure that we have developed.

4. Conclusions

A vacuum distillation method was developed to isolate [^{18}F]**2** in more than 80% yield within 10 min in a “pure” and click-ready form. It was found that sufficient amount of DMF is critical to maintain the high level of Cu(I) in the reaction mixture to ensure the click labeling is rapidly completed. A base-labile NHS ester (**7**) was synthesized as an example of this new method in short time in up to 90% isolated yield using [^{18}F]**2** and copper sulfate, sodium ascorbate and DPBS as the Cu(I) source. Not only can this method be used to label small molecules in high yield and with easy purification, but also the [^{18}F] labeled click-NHS ester can be used for conjugation chemistry with primary amines. We have developed a general method to label small molecules with fluorine-18 with good radiochemical yield, easy purification and high chemical and radiochemical purities. We believe that this novel method could also be of significance for the automated production of [^{18}F]**2**.

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Abbreviations

BPDS	bathophenanthrolinedisulfonic acid disodium salt
DCC	dicyclohexylcarbodiimide
DCM	dichloromethane
DMF	dimethylformamide
EtOAc	ethyl acetate
K₂₂₂	Kryptofix 222
ACN	acetonitrile
NHS	N-hydroxysuccinimide
PET	positron emission tomography
TFA	trifluoroacetic acid

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Scheme 1.

a. [¹⁸F]fluoride, K₂₂₂, K₂CO₃, ACN, 80–90 °C

Scheme 2.

Scheme 3.

a, *N*-hydroxysuccinimide, DCC, DCM/Dioxane;

b, **2**, CuSO₄·5H₂O, sodium ascorbate, BPDS, DMF/ethyl acetate

Figure 1.
Vacuum distillation of [^{18}F]2

Figure 2.HPLC chromatograph of distilled $[^{18}\text{F}]\mathbf{2}$ **Note:** Agilent SB-C18 C18 250×4.6 mm 5 μ , 30% ACN, 70% water, 0.1% TFA, 1 mL/min, 220 nm.

Figure 3.
HPLC chromatograph of [^{18}F]7 purification.

Note: Agilent SB-C18 C18 250 $\times$ 9.4 mm 5 μ , 32% ACN, 68% water, 0.1% TFA, 4 mL/min, 240 nm.

Table 1 Isolated yields of [^{18}F]**2** vs reaction time

Time (min)	2.5	5	10	15
Yield (%)	87.3	86.9	81.5	84

Note. Yields were determined by the activity in the reaction vessel before and after distillation (n = 1).

Table 2

Solvent effect on the click reaction of [¹⁸F]**2** and **3** using Cu(I) from copper sulfate and sodium ascorbate

Entry	1	2	3	4	5	6
A	200	200	200	100	300	200
B	150	200	250	200	200	100
C	100	100	100	100	100	200
2	82	31	5	5	37	84
4	14	56	77	89	55	12
By-product	4	12	18	6	8	4
2	64	16	0	0	21	46
4	28	71	83	94	73	44
By-product	7	13	17	6	6	10
2	58	11	0	0	14	34
4	37	77	83	93	78	62
By-product	5	12	17	7	8	4
Note	Reaction mixture	White precipitate	White precipitate	Yellowish suspension	White precipitate	Colorless solution

^a n = 1;

^b A: [F-18]**2** in ACN, one tenth of distilled; B: **3** (0.1 mg, 0.62 μmol) in DMF; C: CuSO₄·5H₂O (5 mg, 20.0 μmol) and sodium ascorbate (15 mg, 75.7 μmol) in water;

^c Radiochemical yield was determined by radio-TLC (silica TLC/ethyl acetate).

Table 3

Solvent effect (water) on the click reaction of **2** and **3** using Cu(I) from copper sulfate and sodium ascorbate

Entry	1	2	3	4	5	6
A	200	100	100	100	100	100
B	200	200	200	200	400	800
C	200	200	400	800	800	1600
2	77	13	29	74	41	34
4	21	83	69	25	57	62
By-product	2	4	2	1	2	4
2	40	3	16	66	23	18
4	37	93	82	33	74	80
By-product	3	4	2	1	3	2
2	38	0	3	28	10	4
4	59	96	95	38	88	95
By-product	3	4	2	4	2	1

^a n = 1;

^b A: [F-18]**2** in ACN, one tenth of distilled; B: **3** (0.1 mg, 0.62 μmol) in DMF; C: CuSO₄·5H₂O (5 mg, 20.0 μmol) and sodium ascorbate (15 mg, 75.7 μmol) in water;

^c Radiochemical yield was determined by radio-TLC (silica TLC/ethyl acetate).

Table 4 Click reaction of [^{18}F]**2** and **3** using Cu(I)/DPBS as catalyst

Entry		1	2	3
Solvent ^a (μL)	A	100	100	100
	B	100	100	100
	C	10	30	50
5 min ^b (%)	2	12	0	0
	4	75	91	92
	By-product	12	9	8
15 min ^b (%)	2	10	0	0
	4	79	91	92
	By-product	11	9	8
Note	Reaction mixture	clear	clear	clear

^an = 1;^bA: [^{18}F]**2** in ACN; B: **3** (0.05 mg, 0.31 μmol) in DMF; C: CuSO₄·5H₂O (0.17 mg, 0.67 μmol), sodium ascorbate (0.5 mg, 2.52 μmol) per 10 μL water/DMF;^cRadiochemical yield was determined by radio-TLC (silica TLC/ethyl acetate).