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An efficient F-18 labeling method for PET study: Huisgen 1,3-dipolar cycloaddition of bioactive substances and F-18-labeled compounds

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Abstract—The Cu(I)-catalyzed, 1,3-dipolar cycloaddition reaction was applied successfully to the synthesis of small, F-18-labeled biomolecules, and an optimal condition was developed for one-pot, two-step reaction without any interim purifications. This technique was employed in various F-18-labeled, 1,2,3-triazole syntheses with high radiochemical yield.

F-18-labeled compounds have been developed as the most popular radiotracers for use in PET (positron emission tomography) study, an emerging molecular imaging technology for early-stage diagnosis of a variety of human diseases.[1] Among numerous radionuclides, F-18 has not only superior properties such as an appropriate half-life ($t_{1/2} = 110$ min) and a small atomic size similar to hydrogen, but it can also be readily produced and incorporated into small organic compounds by simple nucleophilic substitution reaction. However, in contrast to small organic compounds, very mild reaction conditions are essential for the F-18 labeling reaction of biomolecules such as peptides, oligosaccharides, and oligonucleotides to avoid undesired possible side reactions.[2] Typically, both [^{18}F]fluorobenzaldehyde ([^{18}F]FB-CHO)[3] and *N*-succinimidyl-4-[^{18}F]fluorobenzoate ([^{18}F]SFB)[4] have been utilized as prosthetic groups for the preparation of bioconjugates by the reductive amination or acylation with the amine functionality of biomolecules, respectively. However, such reaction conditions are susceptible to most biomolecules, affording undesired hydrolytic bond-breaking and epimerization. Furthermore, [^{18}F]SFB requires multi-step synthesis and an extended reaction time,[2] which is critically disadvantageous for the F-18 labeling process because of its short half-life of 110 min. Therefore, strong research interest has focused on the development of more biologically friendly reaction conditions for the mild and rapid preparation of F-18-incorporated biomolecules and biomimetic molecules.

Recently, Sharpless et al. developed at room temperature, Huisgen 1,3-dipolar cycloaddition of azides and alkynes using copper(I) salts,[5] which is now recognized as a representative 'click reaction'. Since then, this reaction has been widely applied to many bioorganic and medicinal research fields,[6] because it proceeds under mild and tolerable conditions, in aqueous media, at neutral pH, and at room temperature, all within a reasonable reaction time.

Most recently, Marik and Sutcliffe adopted this reaction for the preparation of F-18-labeled, short peptide fragments, thereby demonstrating its potential use in PET study, in which the conjugation reaction of unprotected peptides was also tested to afford good yield and purity based on the reaction tolerance of the click reaction to other functional groups such as amines, alcohols, and acids.[7] As a Cu(I) catalyst, they used a CuI salt dissolved only in acetonitrile, and not in other organic solvents or water.[8] However, considering both various substance-dependent reaction conditions and the requirement of the biomolecule friendly aqueous media reaction, an

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alternative reaction system is required. Furthermore, previous study was restricted to the use in peptides.

Herein, we describe an alternative synthetic approach for the preparation of F-18-labeled biomolecules using 'click reaction' with $CuSO_4$/Na-ascorbate and several model compounds such as small organic molecules, sugar, amino acid, and nucleotide (Fig. 1).

Preliminary studies to find an optimal condition of 1,3-dipolar cycloaddition for the two-step F-18 labeling procedure were performed using two Cu^I species in four water-containing organic solvents, acetonitrile, DMF, DMSO, and *t*-BuOH, in which 1,3-dipolar cycloaddition of 4-methoxybenzyl azide and phenyl acetylene was employed as a model reaction (Fig. 2).

Between two catalytic systems, $CuSO_4$/Na-ascorbate and CuI, the former showed better performance while the latter yielded a 1,5-disubstituted, 1,2,3-triazole as a by-product and the reaction proceeded slowly (for detail, see Supplementary data). In the $CuSO_4$/Na-ascorbate system, aqueous DMSO was the best

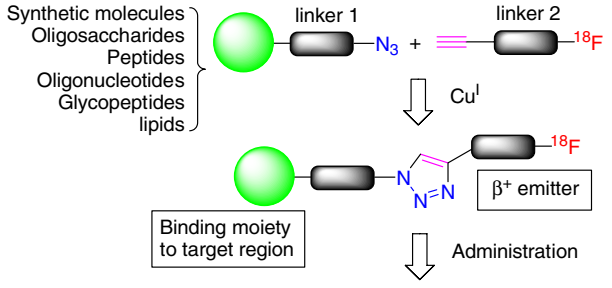


Figure 1. Schematic click conjugation strategy.

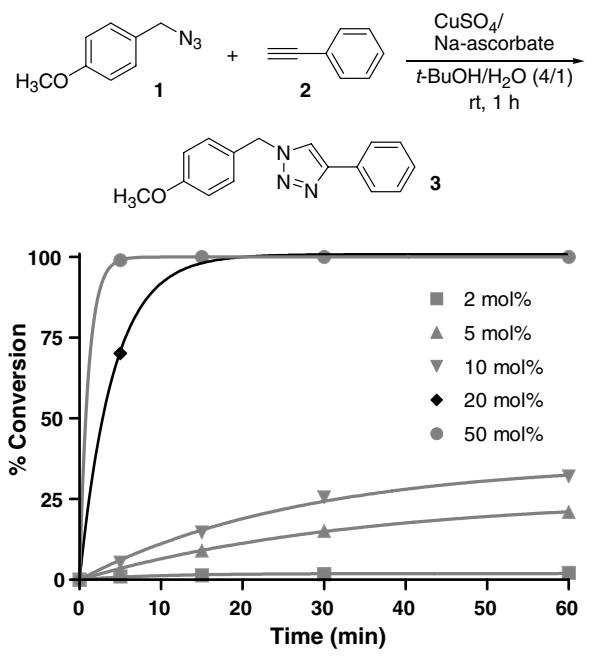


Figure 2. Time versus % conversion curve at different $CuSO_4$/Na-ascorbate concentrations.

reaction media regardless of the water content. However, we hoped to achieve both [^{18}F]fluorination and 1,2,3-triazole formation via a one-pot reaction without the need for solvent change or any interim purifications. Recently, Kim et al. reported that *t*-BuOH solvent shows good reactivity and high selectivity in the [^{18}F]fluorination reaction, by reducing the competitive decomposition reactions of mesylate precursors through the formation of a weak F–H hydrogen bond.[9] Therefore, we eventually chose aqueous *t*-BuOH as the reaction media for [^{18}F]fluorination, and *t*-BuOH/H_2O (4/1) for the second reaction.

Further optimization reaction was performed by varying the amount of $CuSO_4$ from 2 to 50 mol % (Fig. 2) to find the optimal concentration of catalyst in *t*-BuOH/H_2O (4/1) media. With regard to F-18 decay with a half-life of 110 min, 20 mol % of $CuSO_4$ showed a sufficient catalysis effect within a short time.

We designed and synthesized four mesylates, two acetylenes[10] and two azides,[11] as [^{18}F]fluorination precursors (Fig. 3). In addition, 10 counterpart acetylene or azide compounds, including two glucoses,[12] two prolines, and two thymidines, were also prepared (for their synthesis, see Supplementary data). These corresponding fluorinated acetylene and azide compounds are thermally stable and nearly nonvolatile, at the reaction temperature under ambient pressure, and are therefore suitable for one-pot, two-step synthesis.

Under optimal condition and with the acetylene/azide precursors, the F-18-labeled, 1,2,3-triazole formation reaction was examined to validate our reaction condition using acetylene mesylate **4a** and *O*-trityl thymidinyl azide **6** (Scheme 1).

Aqueous [^{18}F]fluoride ion was azeotropically heated with $TBAHCO_3$ (tetrabutylammonium bicarbonate) and CH_3CN to remove any water. Nucleophilic [^{18}F]fluorination of mesylate acetylene **4a** was performed in 0.4 mL of *t*-BuOH at 100 °C for 20 min in the presence of $TBAHCO_3$. The corresponding [^{18}F]fluorinated acetylene [^{18}F]**5** was obtained with an yield of 90%, as determined by radio-TLC integration. The reaction mixture was cooled to room temperature in a water bath. Compound **6** (1.5 equiv), aqueous Na-ascorbate (0.6 M, 50 μL), and aqueous $CuSO_4$ (0.4 M, 50 μL) were added to the reaction mixture in sequence to match the optimal condition (total solvent volume and ratio, and concentration of catalysts) previously determined for the second reaction. 1,3-Dipolar cycloaddition was monitored by radio-TLC every minute until 5 min had elapsed. From the experimental results, the cycloaddition reaction was almost completed

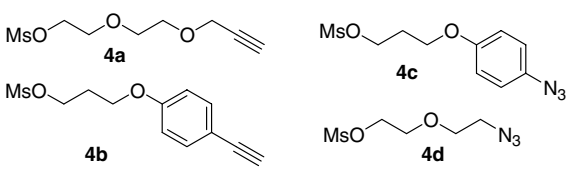
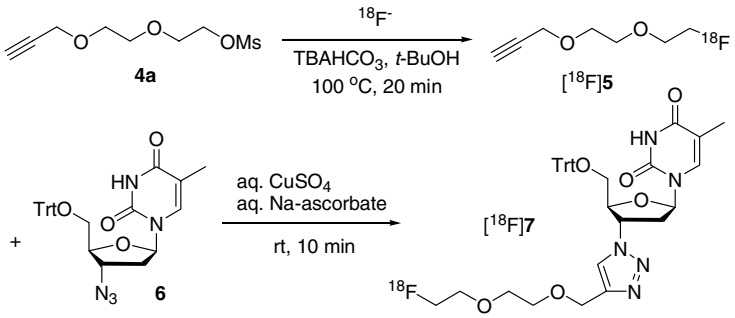


Figure 3. Mesylate precursors.



Scheme 1. Synthesis of F-18-labeled, 1,2,3-triazole compound [^{18}F]**7**.

at 5 min and was entirely so at 10 min (pseudo first-order reaction, $k_{obs} = 0.607\ s^{-1}$, $t_{1/2} = 1.14$ min). In terms of radio-TLC determination, the overall conversion yield was 90%, and the two-step reaction was completed within 40 min from the end of bombardment. As shown in Figure 4, the resulting F-18-labeled, *O*-trityl thymidinyl 1,2,3-triazole was very clearly isolated by HPLC separation. More interestingly, no significant unlabeled thymidinyl by-products were observed on UV detector (254 nm) except unreacted mesylate triazole compound (retention time = 23.0 min). This indicated that other side reactions during the [^{18}F]fluorination of acetylene mesylate **4a** were suppressed by the remarkable reduction of the basicity of the [^{18}F]fluoride and HCO_3^- anion through a hydrogen bond with the *t*-BuOH solvent. This result is consistent with our own recent report.[9] In addition, one of the goals of this research was to perform two-step, F-18 labeling reaction in one-pot without the need for interim work-up and purification. Our procedure enabled both [^{18}F]fluorination and Huisgen 1,3-dipolar cycloaddition to be conducted in the same reaction vessel and solvent system.

Also noteworthy were the remains of the insoluble substances in the reaction mixture after the reaction completion. These remains presumably consisted of poorly soluble, 1,2,3-triazole compounds and copper catalyst formed soon after the mixing of the aqueous $CuSO_4$ and Na-ascorbate. These resulting copper species were mostly removed by filtration with a membrane filter. The product residue was dissolved by washing twice with CH_3CN solvent.

Following the determination of these optimal reaction and purification conditions, we further applied this procedure to various other compounds in both protected and unprotected forms. F-19-incorporated, 1,2,3-triazole compounds were prepared in advance (for detail, see Supplementary data) under the same reaction condition, at a yield of 66–91%. The obtained cold authentic molecules were used to identify the F-18-labeled, 1,2,3-triazole compounds by HPLC coinjection. As shown in Table 1, [^{18}F]fluorinated azides and acetylenes were prepared from the corresponding mesylate precursors **4a–d** in good radio-TLC yields (85–95%) under the same reaction conditions. However, in the second step, small, non-polar, organic compounds [^{18}F]**8–11** needed longer reaction times (up to 30 min) than did the other simple, polar biomolecules [^{18}F]**7**,**12–16**, probably due to their low solubility in the polar solvent system. Conversion yields were limited to 71%, even with the longer reaction times.

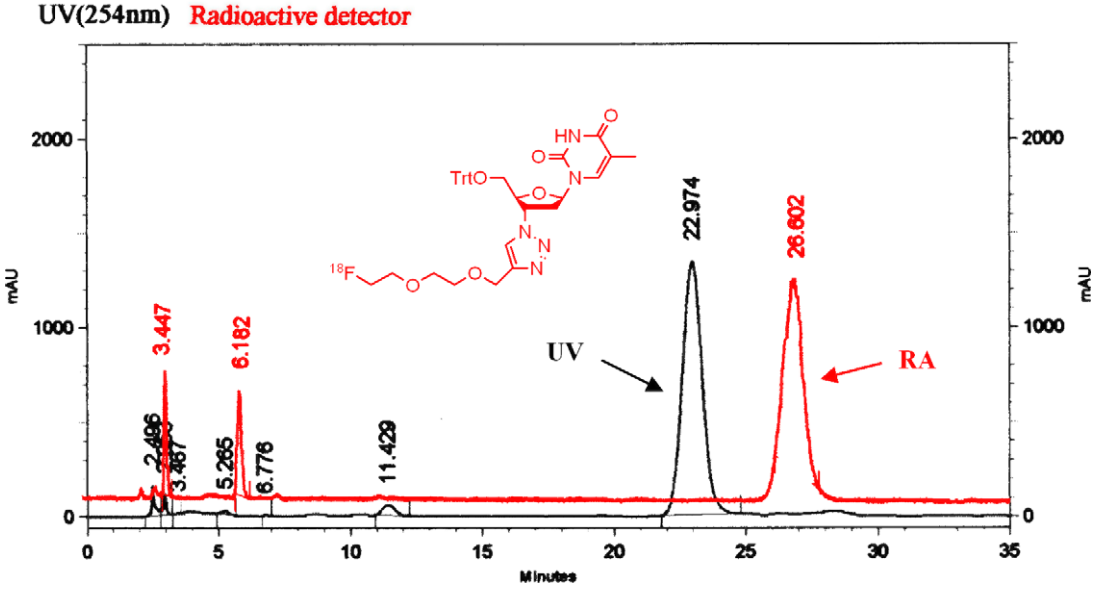
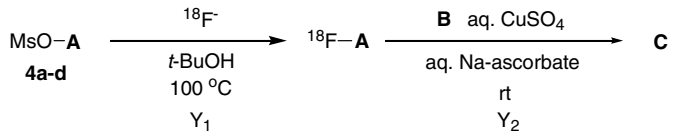
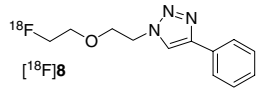
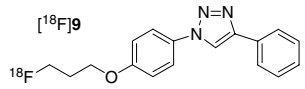
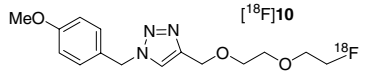
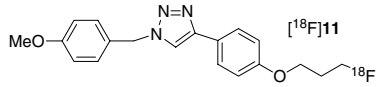
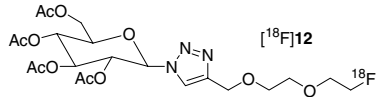
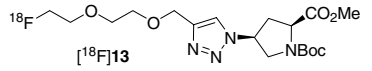
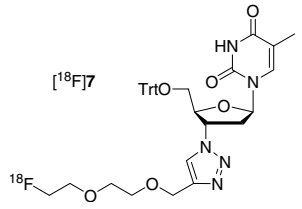
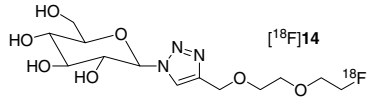
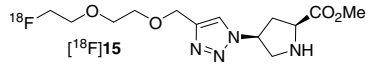
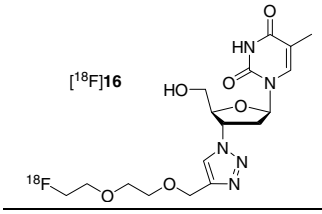


Figure 4. HPLC profile of [^{18}F]**7**.

Table 1. Various one-pot, two-step, F-18 incorporations



Compounds	Time (min) (Y_1/Y_2)	Radio-TLC (%) (Y_1/Y_2)
 [^{18}F]**8**	20/30	92/71
 [^{18}F]**9**	20/30	94/73
 [^{18}F]**10**	20/30	89/100
 [^{18}F]**11**	20/30	95/72
 [^{18}F]**12**	20/10	89/100
 [^{18}F]**13**	20/10	88/100
 [^{18}F]**7**	20/10	85/97
 [^{18}F]**14**	20/10	87/100
 [^{18}F]**15**	20/10	93/100
 [^{18}F]**16**	20/10	92/100

In contrast, 1,2,3-triazole syntheses of [^{18}F]fluorinated acetylene [^{18}F]**5** and polar azide compounds were quantitatively completed within 10 min. [^{18}F]Fluorinated tetraacetyl glucosyl 1,2,3-triazole [^{18}F]**12**, *N*-Boc-protected prolinyl 1,2,3-triazole [^{18}F]**13**, and *O*-trityl thymidinyl 1,2,3-triazole [^{18}F]**7** were synthesized in excellent yields of 97–100%. The corresponding unprotected 1,2,3-triazole compounds [^{18}F]**14**–**16** were also quantitatively prepared under the same condition. This simple preparation of [^{18}F]**14**–**16** confirmed the absence of any need to protect the heteroatom functionality in this conjugation reaction. Consequently, small biomolecules can be covalently bonded to F-18-labeled, prosthetic molecules in the naked form, thereby eliminating the protection step and reducing the reaction time. This result is consistent with a previous report.[7]

In conclusion, the Cu^{I}-catalyzed, 1,3-dipolar cycloaddition reaction was applied successfully to the synthesis of small, F-18-labeled, biomolecule-like compounds, and an optimal reaction condition was developed for one-pot, two-step reaction without interim purification. This condition was employed in various 1,2,3-triazole syntheses of F-18-labeled azides or acetylenes and their corresponding azide or acetylene compounds, including biomolecules. We expect that the reaction condition presented here will widen the application of the click reaction for the preparation of F-18-labeled peptides to various types of biomolecules.

Acknowledgments

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Supplementary data

Experimental procedures and spectral data for all compounds are given in the Supplementary data. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2007.04.048.

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