EFFICIENT STRATEGY FOR THE PARALLEL DEVELOPMENT OF MULTIPLE MODALITY IMAGING PROBES USING "CLICK CHEMISTRY"

T.L. MINDT, T. ROSS and R. SCHIBLI

Department of Chemistry and Applied Biosciences, ETH Zurich, Zurich, Switzerland

Introduction: The visualization of specific targets or processes by different imaging modalities (e.g. MRI, SPECT, PET or NIR) is becoming key for understanding drug-target interactions in vitro and in vivo. Efficient synthetic strategies are needed in order to expedite the development of new and tailor-made probes applicable for different imaging techniques. We demonstrate herein that "click chemistry" (the Cu(I)-catalyzed cycloadditon of alkynes and azides) provides a powerful tool for the parallel development of SPECT (Tc-99m), PET (F-18) and NIR (chromophore Cy5.5) tracers for tumor targeting.

Experimental: Azido folate **1** was synthesized in 4 steps and 78% yield. Subsequent "click reactions" with different alkyne-functionalized imaging probes or precursor thereof were performed in aqueous media at rt in the presence of Cu(I). In all cases, the corresponding 1,4-disubstituted 1,2,3-triazole products were obtained as single products in high yields.

Results and Discussion: The versatility of the "click-approach" for multiple imaging applications was successfully demonstrated for the first time using γ-derivatized azido folate **1** (Figure 1). Compound **1** was employed as a common precursor for the parallel development of folate SPECT tracer **2**, PET tracer **3** and NIR probe **4**. While the triazole moiety of compounds **3** and **4** serves solely as a stable linkage between the targeting molecule and the probe, it forms an integral part of the chelating system in SPECT tracer **2**. Thus, "click chemistry" enabled the concise synthesis of an efficient metal chelator without the use of protective groups while *simultaneously* attaching it to the targeting molecule. Formation of the chelator and labeling with "$[M(CO)_3]^+$" (M= ^{99m}Tc, Re) could be achieved by one-pot procedures, a remarkable efficient entry to metal-labeled conjugates. SPECT tracer **2** was assessed in vitro and in vivo with xenografted mice (data of PET tracer **3** will be presented by T. Ross et al). These preliminary experiments showed specific uptake in folate receptor positive tumors and organs. Thus, 1,2,3-triazole containing SPECT probes represent promising alternatives to histidine-based tracers while offering the benefits and efficiency of "click chemistry".

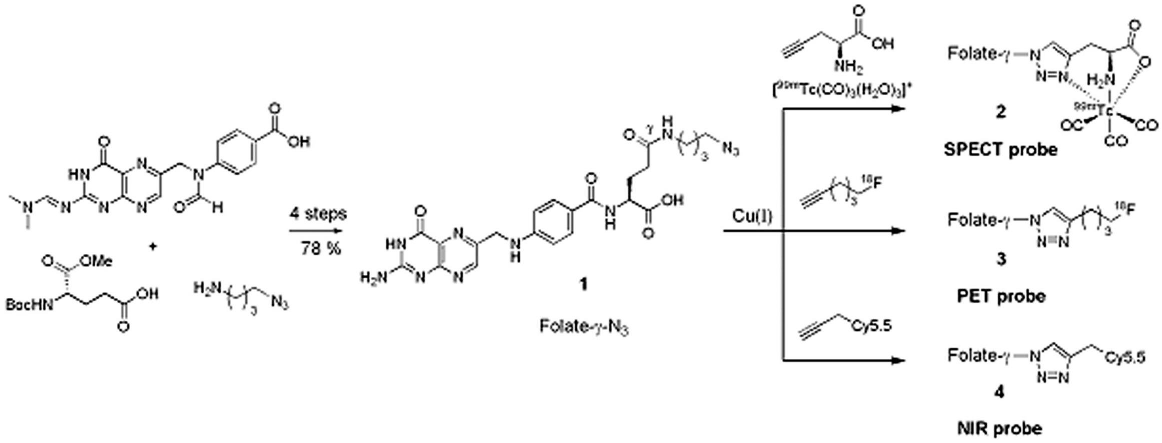


Fig. 1

Conclusion: "Click chemistry" provides a simple, reliable and efficient tool for the preparation of a variety of probes. Employment of the same precursor and functionalization strategy will facilitate a direct comparison of different imaging modalities.

Keywords: SPECT, PET, NIR, Folate Derivatives

RADIOSYNTHESIS OF ^{18}F-LABELLED FOLIC ACID DERIVATIVES USING A DIRECT METHOD AND “CLICK CHEMISTRY”

T.L. ROSS [1], T. MINDT [2], S. BAUMANN [1], A. WEBER [1], V. GROEHN [3], M. HONER [1], R. SCHIBLI [2], P.A. SCHUBIGER [1] and S.M. AMETAMEY [1]

[1] Center of Radiopharmaceutical Science of ETH, PSI and USZ, ETH Zurich, Zurich, Switzerland; [2] Department of Chemistry and Applied Biosciences, ETH Zurich, Zurich, Switzerland; [3] Merck Eprova AG, Schaffhausen, Switzerland

Introduction: The folate receptor (FR) is known to be over-expressed on most epithelial cancer cells while it is scarcely expressed in normal tissues. Folic acid is a high affinity ligand ($K_D \sim 10^{-9}$) for the FR. Based on the folic acid structure, a number of ^{99m}Tc-radiopharmaceuticals have been synthesised and successfully evaluated [1]. However, to date ^{18}F-labelled folates for the routine PET imaging of FR-positive tumours are lacking. Our group developed and evaluated, *in-vitro* and *in-vivo*, the first ^{18}F-labelled folate [2], but the radiosynthesis was time-consuming and gave low radiochemical yields (RCY) of 4%. In order to improve RCY and avoid a multi-step synthesis, we developed two new approaches to ^{18}F-folates.

Experimental: The direct ^{18}F-labelling method is based on a fluoro-for-nitro exchange (Figure 1).

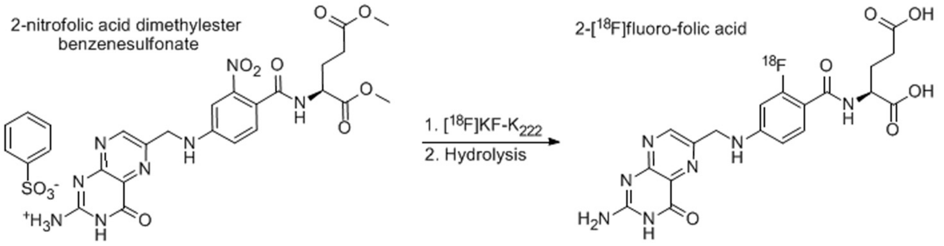


Fig. 1. Direct ^{18}F-labelling method. Synthesis time is ~50 min with a RCY of 35 - 50% (before hydrolysis). Conditions: DMF, TEA, 140°C, 20 min.

In a second approach, “click chemistry” [3] is employed, a Cu(I) catalyzed 1,3-dipolar cycloaddition (Figure 2).

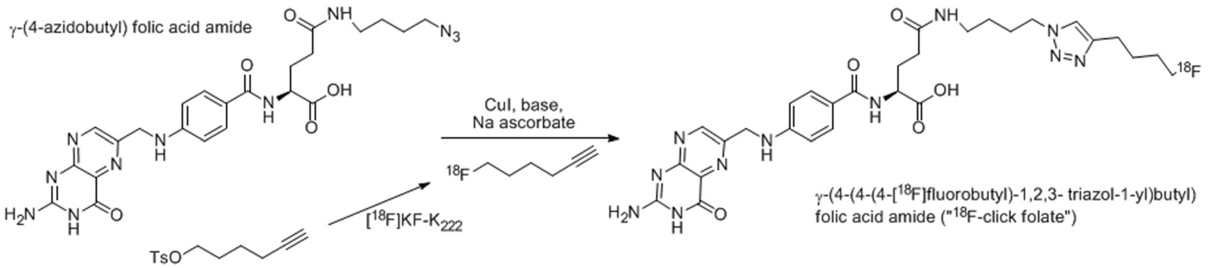


Fig. 2. Click ^{18}F-labelling approach. Synthesis time is ~60 min with RCY of 25–35%. ^{18}F-labelling of 6-[^{18}F]fluoro-1-hexyne: ACN, 100°C, 10 min. ^{18}F-click: DMF, H_2O, TEA, CuI, Na ascorbate, rt, 15 min.

Results and Discussion: We successfully ^{18}F-labelled two folic acid derivatives in good yields. Both approaches are convenient to accomplish and avoid time-consuming multi-step syntheses. Preliminary *in-vitro* studies using the reference compound “^{19}F-click folate” show nanomolar K_i-values similar to those of folic acid.

Conclusion: Both ^{18}F-labelled folates are potential agents for FR-positive tumours and will be validated *in-vivo*. First *in-vivo* studies are in progress.

References: [1] C. Müller et al., Bioconjugate Chem. **17** (2006), 797-806. [2] A. Bettio et al., J. Nucl. Med. **47** (2006), 1153-1160. [3] J. Marik et al., Tetrahedron Lett. **47** (2006), 6681-6684.

Keywords: ^{18}F-Labeling, Folic Acid, Folate Receptor, Click Chemistry, Tumour Imaging

CLICK LABELLING WITH 2-(^{18}F)FLUOROETHYLAZIDE FOR PET

M. GLASER and E. ÅRSTAD

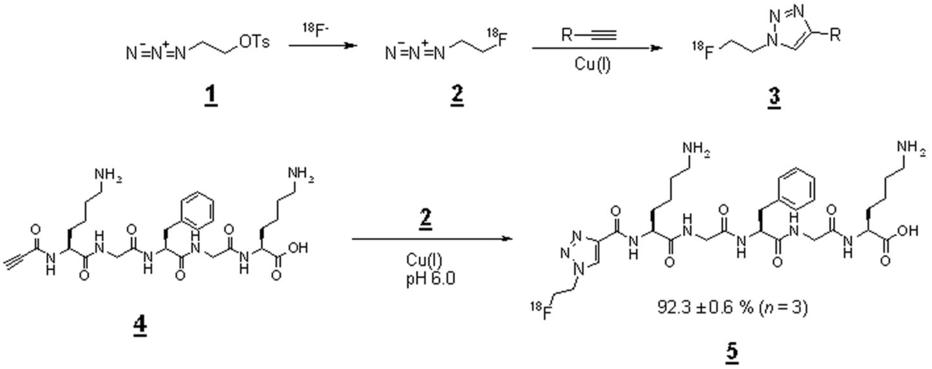
Hammersmith Imanet Ltd (part of GE Healthcare), Hammersmith Hospital, London, United Kingdom

Introduction: ^{18}F-labelled PET tracers are often produced in a sequence of two or more reactions, involving the formation of a small functionalized labelling agent that can be coupled to a vector molecule. With the exception of fluoroalkyl halides, most labelling agents available for ^{18}F are confined to conjugation of residual amino functionalities. The lack of alternative labelling positions, and the high lipophilicity of the coupling groups that are formed, make optimization of peptide based biomarkers difficult.

The recent discovery that Cu(I) catalyses the Huisgen 1.3-dipolar cycloaddition of terminal alkynes and organo azides to form 1,2,3-triazoles,[1, 2] often referred to as 'Click Chemistry',[2] has proven particularly valuable in combinatorial chemistry.[3] The reaction provides a versatile tool for coupling drug like fragments in high yields and under mild conditions, and the 1,2,3-triazole formed is biologically stable, with a polarity and size similar to amide bonds.[4] Herein we report a different approach involving 2-[^{18}F]fluoroethylazide (**2**) as the labelling agent, which allow a wide selection of commercially available alkynes to be used as building blocks for library synthesis of PET tracers.

The aim of the study was to evaluate the scope of Click Chemistry for creating libraries of new ^{18}F tracers using the novel labelling agent 2-[^{18}F]fluoroethylazide (**2**).

Results and Discussion: 2-[^{18}F]fluoroethylazide **2** was prepared from tosylate **1** and isolated in radiochemical yields of 60-70%. Subsequent coupling of **2** to a small library of alkynes was investigated using two catalytic systems, Cu^{2+}/ascorbate and copper granules. Most substrates provided excellent yields after 15 min reaction time at 80°C with both catalytic systems, and the most reactive substrates provided quantitative incorporation of **2** after 15 min at ambient temperature. The method was extended to prepare model peptide **5** by incorporating commercially available propargyl glycine into the peptide sequence to form precursor **4**. Using the Cu^{2+}/ascorbate catalytic system, quantitative incorporation of **2** into the peptide was achieved after 15 min at ambient temperature, affording 92.3 ± 0.6% ($n = 3$) decay corrected isolated yield of **5** following purification by HPLC.



1 **2** **3**

4 **5**

References: [1] Tornøe, C.W. et al., *J. Org. Chem.* 67, 3057 (2002), [2] Rostovtsev, V.V. et al., *Angew. Chem. Int. Ed.* 41, 2596 (2002), [3] Pagliai, F. et al., *J. Med. Chem.* 49, 467 (2006), [4] Bock, V.D. et al., *Eur. J. Org. Chem.*, 51 (2006).

Keywords: Click Chemistry, Dipolar Cycloaddition, Fluorine-18, Peptide Labelling, PET

AN EFFICIENT ^{18}F-LABELING METHOD FOR PET STUDY: HUISGEN 1,3-DIPOLAR CYCLOADDITION OF BIOACTIVE SUBSTANCES AND ^{18}F-LABELED COMPOUNDS

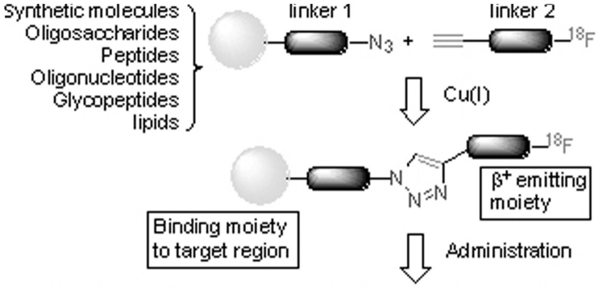
U. SIRION[1], **H.J. KIM**[1], **B.S. LEE**[1], **S.J. LEE**[1,2], **S.J. OH**[2] **and D.Y. CHI**[1]

[1]Department of Chemistry, Inha University, Inchon, Korea; [2]Department of Nuclear Medicine, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea

Introduction: F-18 can be readily incorporated into small organic compounds by simple nucleophilic substitution reaction. In contrast, F-18 labeling reaction of biomolecules such as peptides, oligosaccharides, and oligonucleotides definitely requires very mild reaction conditions. Typically, both [^{18}F]fluorobenzaldehyde and *N*-succinimidyl-4-[^{18}F]fluorobenzoate ([^{18}F]SFB) have been utilized as F-18 labeled prosthetic groups for the preparation of bioconjugates by reductive amination and acylation with amine functionality of biomolecules, respectively. However, such reaction conditions might be harmful to many biomolecules. Besides, [^{18}F]SFB needs multi-step synthesis as well as extended reaction time, which is critically disadvantageous for F-18 because of its short half-life.

Recently, Sharpless and co-workers have developed a room temperature 1,3-dipolar cycloaddition of azides and alkynes using copper(I) salts, which is now well-known representative 'click reaction'. After then, this reaction has been widely applied to many bioorganic and medicinal research fields, and is expected to be an alternative or superior method for the preparation of F-18 labeled biomolecules in light of following advantages. 1) relatively mild reaction conditions even in aqueous media, 2) no protected groups necessary, 3) no need to neutralize reaction media before and after reaction.

Results and Discussion: [^{18}F]Fluorination was performed in *t*-BuOH solvent at 100°C for 20 min with 85-95% radioTLC yield. After cooling to room temperature, acetylene or azide compound and aq. Na-ascorbate were added and then, aq. $CuSO_4$ was added into the mixture to initiate the second reaction. 1,2,3-Triazole formations were completed within 30 min for non-polar molecules or 10 min for polar molecules, being monitored by radio-TLC. Herein, we report the application of click reaction catalyzed by Cu(I) salt to F-18 labeling, providing an optimal cold and hot reaction conditions (one-pot two-step no interim purification), ten examples (overall 66-93% radioTLC yield), and simple purification.



Real-time molecular imaging of living substance with PET.

Conclusion: We expect that the Cu(I)-catalyzed click reaction would be an efficient and potential conjugation method to make a number of F-18 labeled biomolecules, which will be reliable radiotracers for PET image of human body.

Keywords: Cu(I)-Catalyzed 1,2,3-Triazole Synthesis, One-Pot Two-Step [^{18}F]Fluorination

SYNTHESIS OF ^{18}F-LABELED NEUROTENSIN(8-13) VIA COPPER-MEDIATED 1,3-DIPOLAR (3+2)CYCLOADDITION REACTION

F. WÜST, T. RAMENDA and R. BERGMANN

Institut für Radiopharmazie, Forschungszentrum Dresden-Rossendorf, Dresden, Germany

Introduction: Click chemistry is a generic term for a set of reactions, which make use of several selective and modular building blocks to create heteroatom C-X-C links. The copper(I)-catalyzed 1,2,3-triazole formation from azides and terminal acetylenes according to a 1,3-dipolar [3+2]cycloaddition is a particularly powerful linking reaction due to its high degree of specificity and the biocompatibility of the reactants. Recently the first application of click chemistry in 18F chemistry has been reported [1]. Herein we report an alternative click chemistry strategy suitable for 18F labeling reactions.

Experimental: The labeling strategy was exemplified by the synthesis of 18F-labeled neuropeptide neurotensin NT(8-13) [^{18}F]3 (Fig. 1).

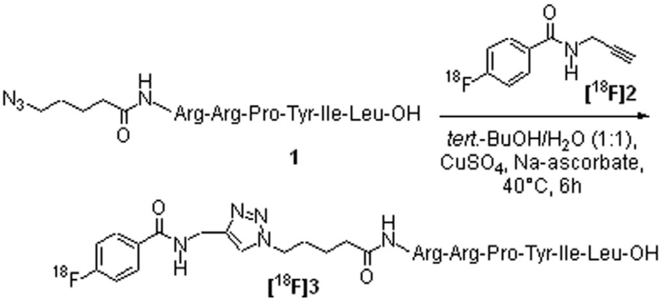


Fig. 1. Synthesis of ^{18}F-labeled NT(8-13) via click chemistry.

Results and Discussion: 4-[^{18}F]Fluoro-N-(prop-2-ynyl)benzamide [^{18}F]2 as novel terminal alkyne building block was prepared in 90% radiochemical yield by the reaction of readily available N-succinimidyl-4-[^{18}F]fluorobenzoate [^{18}F]SFB with propargyl amine. Purification of [^{18}F]2 was performed by solid phase extraction. Coupling of 18F-labeled terminal alkyne with azide-functionalized NT(8-13) 1 gave the corresponding 18F-labeled NT(8-13) derivative [^{18}F]3 in 66% yield as determined by radio-HPLC. Receptor binding of NT(8-13) derivative [19F]3 was investigated using the human colon adenocarcinoma cell line HT-29. The peptides inhibited the binding of [^{3}H]-NT, showing a typical sigmoid curve. The in vitro binding affinity of NT(8-13) derivative [19F]3 was determined to be 66 nM.

Conclusion: In summary, the use of [^{18}F]fluoro-N-(prop-2-ynyl)benzamide [^{18}F]2 as suitable click chemistry building block allows the convenient radiolabeling of 18F-labeled small molecular weight compounds as well as peptides as exemplified for the synthesis of 18F-labeled NT(8-13) [^{18}F]3. This offers the application of click chemistry to the synthesis of a broad array of novel 18F-labeled compounds.

References: [1] J. Marik, J.L. Sutcliffe, Tetrahedron Lett. 47 (2006) 6681-6684.

Keywords: Click Chemistry, 18F-Labeling, PET

COMPARISON OF THREE DIFFERENT PROSTHETICALLY RADIOLABELED VERSIONS OF A20FMDV2: PEPTIDE RADIOLABELING, BIODISTRIBUTION AND *IN VIVO* IMAGING

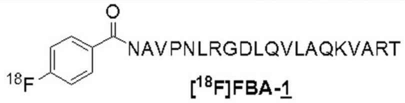
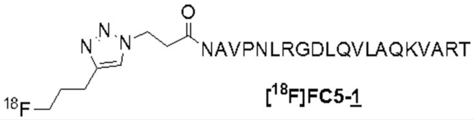
J. MARIK, S.H. HAUSNER, M.K.J. GAGNON and J.L. SUTCLIFFE

Biomedical Engineering, University of California Davis, Davis, CA, USA

Introduction: Fluorine-18 labeled peptides are a rapidly emerging field of targeted PET imaging probes. Several approaches to incorporate ^{18}F-bearing prosthetic groups have been developed in the past decade. The prosthetic groups are first radiolabeled with ^{18}F and subsequently attached to the peptide of interest. A20FMDV2 (1) is a peptide derived from the foot and mouth disease virus envelope protein responsible for interaction with the αvβ6 receptor on the host cell. Previously, we prepared the 4-[^{18}F]fluorobenzoic acid ([^{18}F]FBA) labeled analogue [^{18}F]FBA-1 and successfully imaged αvβ6 in a mouse model using PET.

Experimental: In this study we compared 1 radiolabeled with three different [^{18}F]-bearing prosthetic groups. The peptide 1 was radiolabeled with [^{18}F]FBA or 2-[^{18}F]fluoropropionic acid ([^{18}F]FPA) using a solid phase approach [1,2], and with 5-[^{18}F]fluoropentyne ([^{18}F]FC5) conjugated to 3-azidopropionyl modified peptide using click chemistry [3]. ($n \geq 3$; Table)

Results and Discussion: Comparative microPET imaging and biodistribution studies were carried out. Comparison of the three compounds *in vivo* revealed overall similar biodistribution patterns (n = 3, 1h p.i.). Best positive tumor-to-control and positive tumor-to-blood ratios were seen for [^{18}F]FBA-1 (3.1:1, 2.5:1) and [^{18}F]FC5-1 (3.3:1, 2.4:1); values for [^{18}F]FPA-1 were both 1.9:1. All compounds were rapidly metabolized and cleared renally. None of the urine samples taken at 1h p.i. showed any unmetabolized radiotracer. Despite identical peptide sequence and comparable biodistribution, radio-RP-HPLC analysis of urine samples revealed differences: while three radioactive metabolites were seen for [^{18}F]FBA-1, both [^{18}F]FPA-1 and [^{18}F]FC5-1 yielded two metabolites.

Compound	Reaction steps	Reaction type	Synthesis time (since EOB)	RC yield (decay corrected)
 **[^{18}F]FBA-1**	4	Solid phase	137 min	7.8%
 **[^{18}F]FPA-1**	4	Solid phase	171 min	4.6%
 **[^{18}F]FC5-1**	2	Solution	66 min	7.2%

Conclusion: In conclusion, we compared three ^{18}F-prosthetic groups for peptide radiolabeling and *in vivo* imaging. We observed that small differences caused by the prosthetic groups can have a noticeable effect in pharmacokinetics. Thus, besides other factors such as radiolabeling position and radioisotope, the correct choice of prosthetic group should also be carefully considered and tested for each individual peptide tracer.

References: [1] Sutcliffe-Goulden JL, O'Doherty MJ, Bansal SS, *Bioorg. Med. Chem. Lett.*, **10**, 1501-1503 (2000). [2] Marik J, Hausner SH, Fix LA, Gagnon MKJ, Sutcliffe JL, *Bioconj. Chem.*, **17**, 1017-1021 (2006). [3] Marik J, Sutcliffe JL, *Tet. Lett.*, **47**, 6681-6684 (2006).

Keywords: Solid Phase Peptide Radiolabeling, Biodistribution, Click Chemistry, Integrin, Positron Emission Tomography Imaging PET

A ^{18}F-LABELED GLUCOSE ANALOG: SYNTHESIS VIA CLICK CHEMISTRY AND EVALUATION

D.H. KIM, Y.S. CHOE, I. LEE, J.Y. CHOI, Y. CHOI, K.H. LEE and B.T. KIM

Nuclear Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea

Introduction: Radiolabeled glucose analogs are attractive probes for metabolic imaging. However, there are no suitable glucose radiotracers, except [F-18]FDG. Since click chemistry was introduced by Sharpless and coworkers, it has been known as a useful reaction to generate novel pharmacophores. In the present study, we synthesized a novel F-18 labeled glucose analog via click chemistry and evaluated it in *in vitro* assay using hexokinase.

Experimental: 4-[(2-[F-18]Fluoroethyl)-1-(β-D-glucopyranosyl)]-1*H*-1,2,3-triazole ([F-18]**1**) was prepared using two methods. 3-Butyn-1-ol was reacted with tetraacetylated 1-azidoglucose in the presence of sodium ascorbate and $CuSO_4$. The resulting triazole was treated with toluenesulfonyl chloride in the presence of triethylamine. The tosyl precursor was reacted with $n$$Bu_4N$[F-18]F in a mixture of acetonitrile and *t*-butanol at 100°C for 25 min. Subsequent deprotection of the acetyl groups in 0.05 N NaOH was carried out at room temperature for 3 min. In a separate experiment, 3-butyn-1-ol was reacted with toluenesulfonyl chloride in the presence of triethylamine, and the tosyl precursor was reacted with $n$$Bu_4N$[F-18]F at 100°C for 25 min. The resulting radiofluorinated mixture was reacted with 1-azidoglucose in the presence of sodium ascorbate and $CuSO_4$. The product was purified by reverse phase HPLC using a mixture of water and methanol as solvents (100:0 to 70:30 for 20 min). [F-18]**1** was added to hexokinase solution containing ATP and $MgCl_2$, stirred at room temperature for 10 min, and analyzed by radio-TLC. [F-18]FDG was also assayed for a comparison.

Results and Discussion: Cycloaddition between terminal alkyne and tetraacetylated 1-azidoglucose generated the triazole in high yield (85-90%). Of synthetic methods of [F-18]**1**, triazole formation followed by radiofluorination gave the product in overall 20-25% yield and with high effective specific activity (39-42 GBq/μmol), whereas the synthesis in the reversed order gave the product in 9-12% yield. [F-18]**1** was co-eluted with **1** on HPLC, confirming its identity. In enzymatic assays, [F-18]FDG was completely converted to [F-18]FDG-6-phosphate by hexokinase, whereas [F-18]**1** was not phosphorylated under the same condition.

Conclusion: This result demonstrated that [F-18]**1** was readily prepared via click chemistry. Enzymatic assay of [F-18]**1** suggests that glucose analogs substituted at C1 may not be recognized as a substrate of hexokinase.

Keywords: F-18 Labeled Glucose Analog, Click Chemistry, Triazole, Hexokinase