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Click for PET: rapid preparation of [^{18}F]fluoropeptides using Cu^{I} catalyzed 1,3-dipolar cycloaddition

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Abstract—Cu^{I} catalyzed 1,3-dipolar cycloaddition 'click chemistry' was used to prepare ^{18}F-radiolabeled peptides. Three ω-[^{18}F]-fluoroalkynes were prepared in yields ranging from 36% to 81%. Conjugation of ω-[^{18}F]fluoroalkynes to various peptides decorated with 3-azidopropionic acid via Cu^{I} mediated 1,3-dipolar cycloaddition yielded the desired ^{18}F-labeled products in 10 min with yields of 54–99% and excellent radiochemical purity (81–99%). The total synthesis time was 30 min from the end of bombardment.

Fluorine-18 labeled targeting peptides are becoming more widely used as in vivo imaging agents of various physiological and pathological processes using positron emission tomography (PET).[1–3] The short half-life isotope ^{18}F (β^{+}, $t_{1/2} = 110$ min) is attached to the target molecule via a prosthetic group. Although a variety of ^{18}F-labeled prosthetic groups have been developed, only a limited number of chemical reactions have been utilized to incorporate the prosthetic groups into peptides including, acylation,[4–10] alkylation,[11] and oxime formation.[12,13] The most commonly used acylation approach requires protection of other acylation prone groups within the peptide sequence. We previously developed a solid phase radiolabeling approach to avoid multiple acylation reactions by using a fully protected peptide sequence.[14,15] Using the alkylation approach, ^{18}F-fluorothiols were chemoselectively alkylated with *N*-chloroacetylated peptides.[11] The labeling of aminooxy-modified peptides has been achieved by conjugation with 4-[^{18}F]fluorobenzaldehyde.[12,13] In both the alkylation and oxime formation reactions, the number of synthetic steps is reduced but unfortunately the reagents used may potentially react with other functional groups within the peptides and the products are often species that are susceptible to hydrolysis or oxidation. Therefore a rapid, selective, generic method for radiolabeling peptides is required.

'Click' chemistry makes use of a few chemical reactions selectively providing high yields of products from a wide variety of easily accessible building blocks.[16,17] Undoubtedly, the most explored reaction is the Cu^{I} catalyzed formation of 1,2,3-triazole using Huisgen 1,3-dipolar cycloaddition of terminal alkynes with azides.[18,19] This reaction is highly regioselective leading to 1,4-disubstituted 1,2,3-triazoles (*anti*-isomer).[18,19] This transformation can be performed in an aqueous media using readily accessible reagents and without exclusion of atmospheric oxygen. Since this reaction between alkyne and azide is orthogonal to any functional group[20–24] it can be performed without the protection of other functional groups within the peptide sequence.[25] The 1,4 disubstituted 1,2,3-triazole product is relatively stable, posses a large dipole moment and the nitrogen atoms in positions two and three serve as weak hydrogen bond acceptors improving the solubility of the product in water.[16] Although the Cu^{I} catalyzed 1,3-dipolar cycloaddition of terminal alkynes with azides provides the product in excellent yield and purity the transformation is slow and requires hours for completion. The microwave-assisted three-component copper catalyzed cycloaddition of alkynes and in situ formed azides was previously reported to provide the products at 75–125 °C in 10–15 min.[26] In order to avoid epimerization we explored the two-component Cu^{I}

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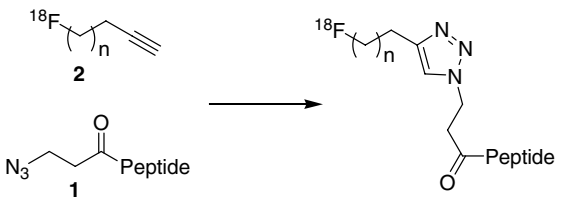


Scheme 1. Labeling of peptides using 1,3-dipolar cycloaddition.

catalyzed 1,3-dipolar cycloaddition of ω-[^{18}F]fluoroalkynes **2** to *N*-(3-azidopropionyl) peptides **1** (Scheme 1).

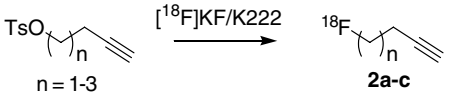
Peptides **1** were assembled on solid phase using standard Fmoc chemistry and an automatic synthesizer. 3-Azidopropionic acid (Azpr) was synthesized as previously described from β-propiolactone[27] and coupled to the N-terminus of the peptide or the N$^{\varepsilon}$ of a lysine residue. In order to increase the water solubility and separate the prosthetic group from the biologically active peptide the hydrophilic linker (Ebes) was inserted between the *N*$^{\varepsilon}$-(3-azidopropionyl)lysine and the peptide sequence.[28] The *N*-(3-azidopropionyl) peptides **1** were cleaved from the solid support and purified using semi-preparative RP-HPLC.[30]

Terminal ω-fluoroalkyne **2** was prepared by nucleophilic substitution of the corresponding tosylate with [^{18}F]fluoride.[31] The [^{18}F]fluoride anion was produced by the ^{18}O(p,n)^{18}F nuclear reaction on isotopically enriched [^{18}O]H_2O and converted to the [^{18}F]KF/K222 complex using 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]-hexacosane (Kryptofix, K222). The nucleophilic substitution of toluenesulfonyl moiety with [^{18}F]fluoride was facilitated by heating and the volatile product distilled. 4-[^{18}F]fluoro-1-butyne **2a** (bp 45 °C), 5-[^{18}F]fluoro-1-pentyne **2b** (bp 76 °C), and 6-[^{18}F]fluoro-1-hexyne **2c** (bp 106 °C) were prepared and purified by co-distillation with acetonitrile in 10 min with decay corrected yields and purity as illustrated in Table 1.

The kinetically driven Cu^{I} catalyzed 1,3-dipolar cycloaddition of azides and alkynes requires relatively long periods of time to obtain quantitative yields,[16] thus it might not appear to be suitable for the preparation of compounds labeled with short half-life radioisotopes. However, in the case of no-carrier added (n.c.a) radiochemical synthesis the ratio of reactants and catalysts differs from traditional chemistry. Particularly, the azide component **1** and catalyst are in a huge excess to ω-[^{18}F]fluoroalkyne **2**. The initial experiments with Cu^{I} generated in situ using $CuSO_4$/sodium ascorbate catalytic system provided **2b**-YGGFL in 10% yield at room temperature in 30 min. Further optimization of the catalytic system showed that the quantitative incorporation of ω-[^{18}F]fluoroalkyne in 10 min could be achieved when $CuSO_4$ was replaced with CuI (1 equiv) and a nitrogen base (10 equiv). Sodium ascorbate (10 equiv) was required to prevent oxidation of Cu^{I} to Cu^{II} by atmospheric oxygen.[32] We used 0.025 equiv of *N*-(3-azidopropionyl)peptide **1** for each reaction. It was previously demonstrated that the presence of the nitrogen base is necessary when CuI is used as a source of Cu^{I}. We tested several bases, and the best yields were obtained with *N*,*N*-diisopropylethylamine (DIEA) (Table 2). We found that the use of piperidine provided the product in a short period of time; however, formation of some un-identified byproducts was observed. The RP-HPLC analysis of all ^{18}F-labeled peptides showed only a single product indicating that the reaction proceeded regioselectively to yield 1,4-disubstituted 1,2,3-triazoles as previously reported.[18,19] In the case of **2b**-AGDLHVLR (Table 2, entry 5), pyridine (5 equiv) was added to improve the purity of the product. The effect of pyridine or 2,6-lutidine to suppress undesired byproduct formation associated with the use of CuI has been demonstrated previously.[18] The reaction provided the products in moderate to good yields and the use of bipyridine or phenanthroline based ligands[29] could further improve the yields and purity of the presented radiolabeling procedure, thus requiring a smaller amount of copper catalyst.

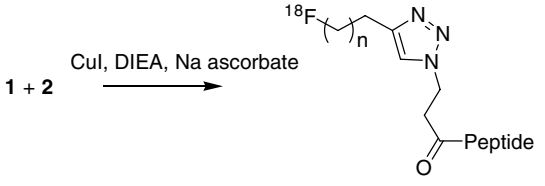
The radiolabeled peptides were isolated from the reaction mixture using a C18 Sep-Pak extraction. Un-reacted ω-[^{18}F]fluoroalkyne was evaporated together with the eluant to yield the ^{18}F-labeled peptides in excellent radiochemical purity. The radiochemical purity of the products was determined by RP-HPLC analysis. The n.c.a. radiolabeled peptides were obtained with specific activity greater than 35 GBq μmol^{-1}. The peptides radiolabeled using **2b** (Table 2, entries 2, 4, 5) were compared to the corresponding ^{19}F-labeled peptides (Fig. 1). For the synthesis of ^{19}F-labeled peptides, the desired 5-[^{19}F]fluoro-1-pentyne was prepared from the corresponding alcohol using (diethylamino)sulfur trifluoride (DAST).[33] The ^{19}F-labeled peptides were obtained

Table 1. Synthesis of [^{18}F]fluoroalkynes; radiochemical yields and radiochemical purity



[^{18}F]fluoroalkyne	*n*	Yield [%]	Purity [%]
2a	1	36	98
2b	2	81	98
2c	3	61	99

Table 2. Synthesis of [^{18}F]fluropeptides using Cu^{I} catalyzed 1,3-dipolar cycloaddition; radiochemical yields and radiochemical purity



Entry	[^{18}F]fluoropeptide	Yield [%]	Purity [%]
1	**2a**-YGGFL	54	95
2	**2b**-YGGFL	97	98
3	**2c**-YGGFL	62	99
4	AGDLHVLR-Ebes-Lys(**2b**)	97	81
5	**2b**-AGDLHVLR	99	87

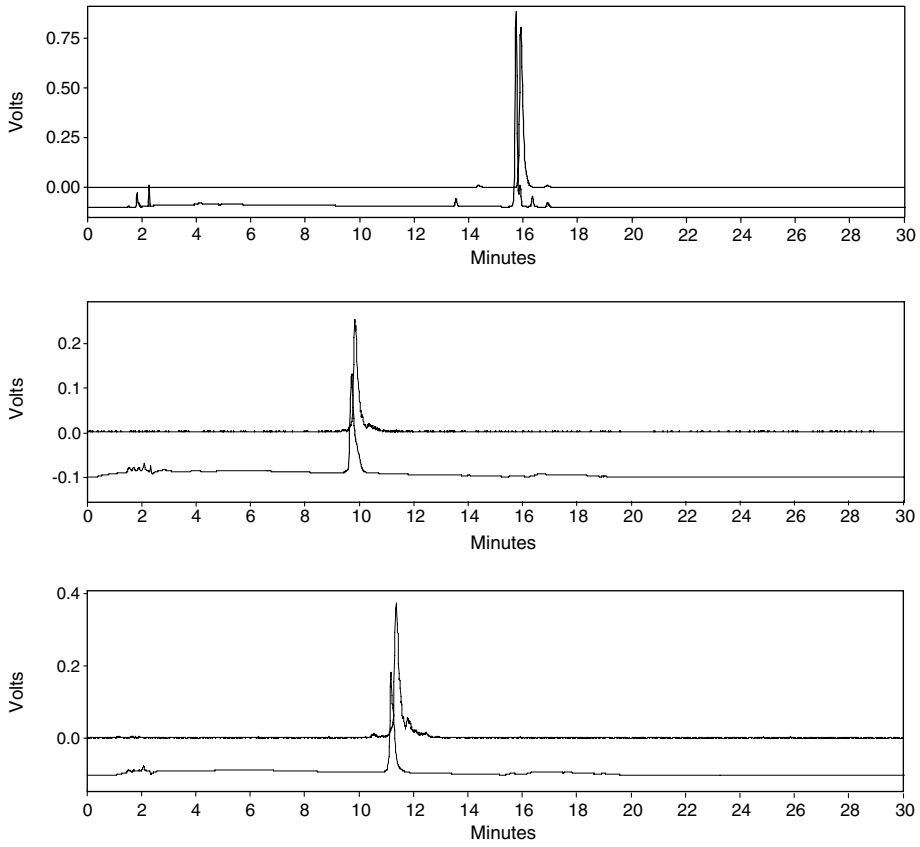


Figure 1. Reversed phase HPLC analysis (UV 220 nm bottom trace, radioactivity top trace) of [^{18}F]fluoropeptides co-injected with corresponding ^{19}F-standards: **2b**-YGGFL (top), AGDLHVLR-Ebes-Lys(**2b**) (center), **2b**-AGDLHVLR (bottom). The Radio-HPLC detector was connected in series after the UV detector accounting for the slight difference between retention times of ^{19}F and ^{18}F labeled peptides.

by the reaction of 5-[^{19}F]fluoro-1-pentyne with the corresponding *N*-(3-azidopropionyl)peptides **1** under the identical conditions as used for radiolabeling, and subsequently purified and characterized.[34] The product of the 1,3-cycloaddition was, in all cases, the only observed and isolated product of this transformation.

In conclusion, we have developed a very fast and efficient method for radiolabeling peptides with ^{18}F for targeted imaging using PET. Three ω-[^{18}F]fluoroalkynes were prepared in yields ranging from 36% to 81% by nucleophilic substitution of *p*-toluenesulfonyl moiety and purified by distillation. Conjugation of ω-[^{18}F]fluoroalkynes to the various peptides decorated with 3-azidopropionic acid, via Cu^{I} mediated 1,3-dipolar cycloaddition provided the desired ^{18}F-labeled products in 10 min with yields of 54–99% and excellent radiochemical purity (81–99%). The total synthesis time was 30 min from the end of bombardment. We successfully demonstrated that click chemistry can be used for rapid preparation of ^{18}F-labeled compounds.

Acknowledgements

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30. *N-(3-azidopropionyl)peptides*. Compound **1a** Azpr-YGGFL: MS (MALDI) *m/z* 674.289 ($M+Na^+$); calcd ($M+Na^+ = C_{31}H_{41}N_9O_7Na$) 674.303 Da. Compound **1b** AGDLHVLR-Ebes-Lys(Azpr): MS (MALDI) *m/z* 1334.720 ($M+H^+$); calcd ($M+H^+ = C_{57}H_{100}N_{21}O_{16}$) 1334.766 Da. Compound **1c** Azpr-AGD- LHVLR: MS (MALDI) *m/z* 976.526 ($M+H^+$); calcd ($M+H^+ = C_{41}H_{70}N_{17}O_{11}$) 976.544 Da.
31. *General procedure for the radiochemical synthesis of ω-[^{18}F] fluoroalkynes* (**2**). The [^{18}F]fluoride was captured on an ion exchange ^{18}F Trap and Release Column and eluted with 1.5 mL of K222/K_2CO_3 solution (15 mg K222, 3 mg K_2CO_3 in 6% water/ACN). The acetonitrile was evaporated under a gentle stream of nitrogen at 100 °C. The remaining water was removed from K222/K[^{18}F]F complex by azeotropic distillation with three times 1 mL of acetonitrile. The solution of ω-alkynyl *p*-toluenesulfonate (50 μL) in anhydrous acetonitrile (1.5 mL) was added and the sealed vial 1 was connected to vial 2 via a 20 cm of 1/16 in ID silicon tubing; vial 2 was vented using a short drying tube. Vial 1 was heated to 100 °C and the second vial was placed to −78 °C bath. ω-[^{18}F]fluoroalkyne was distilled together with acetonitrile into the second vial within 10–15 min.
32. *General procedure for the radiochemical synthesis of [^{18}F] fluoropeptides*. The ω-[^{18}F]fluoroalkyne **2** in acetonitrile was added to the solution of sodium ascorbate (25 mg, 0.13 mmol), CuI (2.5 mg, 0.013 mmol), *N*-(3-azidopropionyl)peptide **1** (0.3 mg, 0.0003 mmol), DIEA (25 μL, 0.14 mmol) in the mixture of DMF (0.2 mL), water (0.25 mL) and acetonitrile (0.25 mL). The resulting orange solution was stirred vigorously at room temperature for 10 min. The reaction mixture was diluted with water (10 mL) and passed through C-18 Sep-Pak cartridge. The product was trapped on the cartridge, washed with water (5 mL) and eluted with 1% AcOH in ethanol. The solvent and remaining ω-[^{18}F]fluoroalkyne were evaporated by a gentle stream of nitrogen. The products were analyzed using RP-HPLC and the identity was confirmed by co-injection with the appropriate cold ^{19}F-labeled peptide (Fig. 1).
33. *5-fluoro-1-pentyne* ([^{19}F]**2b**). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.77–1.89 (m, 2H), 1.91 (t, $J = 2.5$ Hz, 1H), 2.28 (td, $J = 7.0$, 2.5 Hz, 2H), 4.44 (t, $J = 6.0$ Hz, 1H), 4.53 (t, $J = 6.0$ Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.43 (d, $J = 5.9$ Hz), 29.26 (d, $J = 20.2$ Hz), 68.94, 82.30 (d, $J = 164.9$ Hz), 82.91; ^{19}F NMR (470 MHz, $CDCl_3$) δ −220.3.
34. *[^{19}F]fluoropeptides*. [^{19}F]**2b**-YGGFL: MS (MALDI) *m/z* 760.336 ($M+Na^+$); calcd ($M+H^+ = C_{36}H_{48}N_9O_7F_1Na$) 760.356 Da. [^{19}F]AGDLHVLR-Ebes-Lys(**2b**): MS (MALDI) *m/z* 1420.775 ($M+H^+$); calcd ($M+H^+ = C_{62}H_{107}N_{21}O_{16}F$) 1420.819 Da. [^{19}F]AGDLHVLR-Ebes-Lys(**2b**): MS (MALDI) *m/z* 1420.775 ($M+H^+$); calcd ($M+H^+ = C_{62}H_{107}N_{21}O_{16}F$) 1420.819 Da.