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# 'Click labelling' in PET radiochemistry

## Matthias Glaser* and Edward G. Robins

**The copper(I)-catalysed 1,3-dipolar cylcoaddition of terminal alkynes with substituted azides has seen a rapidly growing popularity in a wide range of applications. This versatile coupling reaction has been also recognised as an invaluable tool to meet the high radiosynthetic demands of tracers for Positron Emission Tomography (PET). Here, we are reviewing publications from 2006 to March 2009.**

**Keywords:** click; PET; review

## Introduction

The reaction of a terminal alkyne with a substituted azide was discovered by Arthur Michael 116 years ago.[1] Later, Rolf Huisgen carried out systematic studies of this particular reaction and other 1,3-dipolar cycloadditions.[2] However, it was only after the publication of the copper(I)-catalysed variant by Tornøe and Meldal in 2001 that the potential of the transformation could be fully exploited.[3] The chemistry was subsequently further developed in parallel by the groups of Meldal and Sharpless and became synonymous with the broader concept of 'Click chemistry', also known as copper(I) catalysed cycloaddition of an alkyne and azide (CuAAC).[4–6] Figure 1 shows the two general pathways of the 1,3-dipolar cycloaddition. The role of copper(I) in the catalytic cycle has been investigated in depth.[7–9]

The merits of that cyclization reaction are in particular high chemoselectivity and regiospecificity, excellent yields as well as mild reaction conditions. These features and numerous applications have been compiled in various outstanding and comprehensive review articles.[7,8,10,11] The importance of the CuAAC for biosciences has been also reviewed recently.[12,13]

This Mini Review article is aiming to provide a brief overview on the CuAAC in terms of applications for Nuclear Medicine, and more specifically for Positron Emission Tomography (PET). PET plays an important role in non-invasively and quantitatively imaging of tissue function on a molecular level. The positron emitting radionuclides are produced by cyclotrons or generator systems.[14–17] There are however some constraints in the radiosynthesis of tracers for PET. These limitations are essentially dictated by the relatively short half-lives of the common positron-emitting radionuclides such as fluorine-18 ($t_{1/2} = 109.7$ min) and carbon-11 ($t_{1/2} = 20.4$ min).[17] In consequence, there arises the need for appropriate shielding measures and process automation. Thus, the challenge for the researcher is to develop radiochemical syntheses that are rapid, high yielding, chemoselective, and do not require deprotection steps.

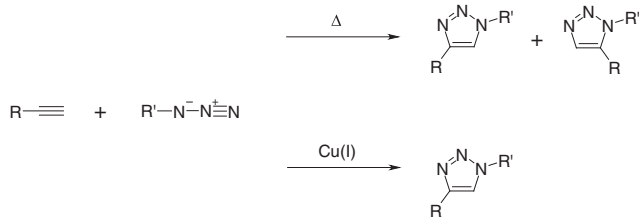


**Figure 1.** The 1,3-dipolar cycloaddition of substituted alkynes and azides.

It has been demonstrated in the recent years that the CuAAC does offer a solution to all of these demands. The heterocyclic moiety of 1,4-disubstituted 1,2,3-triazoles is stable under *in vivo* conditions.[18] The triazole linker also introduces some degree of polarity into the tracer[9] and can be seen as a surrogate for the amide bond.[19] Therefore, adding a 1,2,3-triazole group is expected to be advantageous with regard to the pharmacological properties of the resulting radiotracer moleclule.

## Click labelling with fluorine-18

### $^{18}$F-Reagents for Click labelling

Fluorine-18 is usually only available to the PET radiochemist as a poorly reactive aqueous fluoride. The labelling conditions for [$^{18}$F]fluoride can be incompatible with some substrates. For this reason, the $^{18}$F–C bond formation step needs to be separated from the molecule to be labelled. The common approach is to synthesize $^{18}$F-labelling reagents that bind to the substrate under mild conditions in a chemoselective fashion. Functionalised alkynes and azides both are relatively inert compounds. However, they will couple readily with each other in the presence of a copper(I) catalyst to give exclusively the 1,4-disubstituted 1,2,3-triazole (Figure 1).

Both alkynes and azides have been radiolabelled with fluorine-18. Table 1 gives a chronological list of the currently known $^{18}$F-reagents for Click labelling. Alkyne and azide groups can be easliy introduced into the required labelling precursors.

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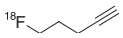
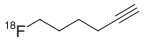
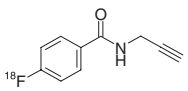
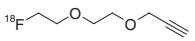
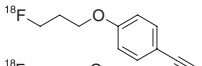
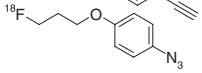
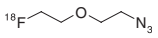
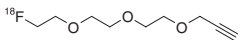
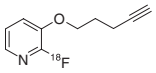
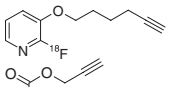
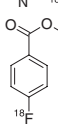
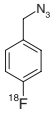
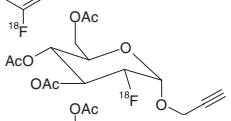
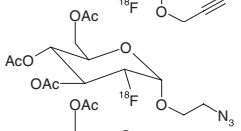
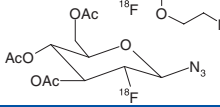
**Table 1.** Preparation of $^{18}$F-labelling reagents for Click Chemistry.

| No | $^{18}$F-labelling reagent | Reaction conditions[a] | RCY (%) | Reference |
|---|---|---|---|---|
| 1 |  | Tosylate precursor, MeCN, 100°C, 10–15 min, distillation | 36[b] | 20 |
| 2 |  | Tosylate precursor, MeCN, 100°C, 10–15 min, distillation | 81[b] | 20 |
| 3 |  | Tosylate precursor, MeCN, 100°C, 10–15 min, distillation | 61[b] | 20 |
| 4 |  | Tosylate precursor, MeCN, 80°C, 15 min, distillation | 55[b] | 21 |
| | | Precursor: $C_8F_{17}(CH_2)SO_3(CH_2)_2N_3$, MeCN, 120°C, 15 min, FSPE | >76[c] | 22 |
| 5 |  | Intermediate: *N*-succinimidyl 4-[$^{18}$F]fluoro benzoate, propargylamine, total synthesis time: 98 min, purification by C18 SPE | 27[b] | 23 |
| 6 |  | Mesylate precursor, $^t$BuNHCO$_3$, $^t$BuOH, 100°C, 20 min, no purification | 88[d] | 24 |
| 7 |  | Mesylate precursor, $^t$BuNHCO$_3$, $^t$BuOH, 100°C, 20 min, no purification | 95[d] | 24 |
| 8 |  | Mesylate precursor, $^t$BuNHCO$_3$, $^t$BuOH, 100°C, 20 min, no purification | 94[d] | 24 |
| 9 |  | Mesylate precursor, $^t$BuNHCO$_3$, $^t$BuOH, 100°C, 20 min, no purification | 92[d] | 24 |
| 10 |  | Tosylate precursor, DMSO, 110°C, 30 min, HPLC purification | 84.3 ± 2.1[b] | 25 |
| 11 |  | 2-Nitro or 2-trimethylammonium precursor, 165°C, 5 min C18 SepPak and HPLC purification | 30–35[e] | 26 |
| 12 |  | 2-Nitro or 2-trimethylammonium precursor, DMSO, 110°C, 15 min, HPLC and $^t$C18-SepPak purification | 50, 42[b] | 27 |
| 13 |  | 2-Trimethylammonium precursor, MeCN, 130°C, 15 min | 58 ± 31[b] | 28 |
| 14 |  | (1) 2-Trimethylammonium precursor, (2) $NaBH_4$ (3) HBr (4) azido resin; total synthesis time: 75 min | 34[b] | 29 |
| 15 |  | Triflate precursor, $KH_2PO_4$, MeCN, 85°C, 5 min | 7[d] | 30 |
| 16 |  | Triflate precursor, $KH_2PO_4$, MeCN, 85°C, 5 min | 8–9[d] | 30 |
| 17 |  | Triflate precursor, $KH_2PO_4$, MeCN, 85°C, 5 min | 71 ± 10[d] | 30 |

[a] [$^{18}$F] KF/Kryptofix 2.2.2/$K_2CO_3$ except for entries 6–8.
[b]Isolated and decay-corrected.
[c]Estimated after coupling with *N*-propargylbenzamide.
[d]Measured by radio TLC.
[e]Measured by analytical HPLC, decay-corrected.

Interestingly, there seems to be, however, a preference for $^{18}$F-modified alkynes. A series of aliphatic $^{18}$F-alkynes **1–3** was published in 2006.[20] These peptide labelling reagents were isolated by distillation. Similarly, the complementary 2-[$^{18}$F]fluoroethylazide **4** could be obtained by distillation using acetonitrile as a carrier.[21,31] Recently, reagent **4** has been prepared by nucleophilic detagging on fluorous phase.[22] A broad range of commerical alkyne building blocks can be used to obtain peptide and other alkyne substrates for the CuAAC. It should be noted that the boiling point of the stable 2-[$^{19}$F]fluoroethylazide has not been measured yet. (The estimated boiling point should be lower than 150°C, based on the b.p. of the related 2-chloroethyl azide with 46°C at 24.8 torr.[32]).

The $^{18}$F Click labelling reagents are typically purified by chromatographic means. It has been also shown that the CuAAC can be performed as a simple one-pot chemistry without the need to purify either the $^{18}$F-alkyne or $^{18}$F-azide intermediates.[24,33]

In addition, a number of more larger labelling reagents have been published – as shown in Table 1. The added building blocks will have a greater impact on the overall properties of the tracer. However, these reagents are also beneficial in controling the polarity of the resulting triazole molecule. Also, an added chromophore might be desired.

Propargylamide **5** was obtained from *N*-succinimidyl 4-[$^{18}$F]fluorobenzoate, an established prosthetic group.[23] The azidomethyl prosthetic group **14** has been prepared in three steps from 4-[$^{18}$F]fluorobenzaldehyde using an automated process.[29] Since reagents **5**, **13**,[28] and **14** are featuring an aromatic $^{18}$F bond, they are expected to warrant superior *in vivo* stabilities.[34] Compounds **6**, **9**, and **10** are more polar $^{18}$F-alkynes with glycol

**Table 2.** $^{18}$F-labelled small molecules obtained by Click Chemistry.

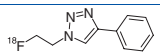
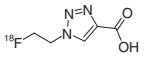
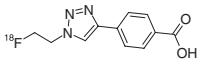
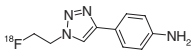
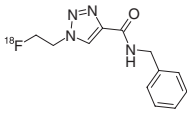
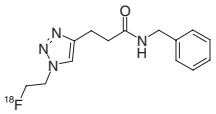
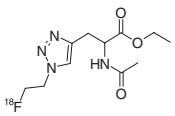
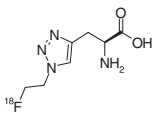
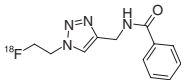
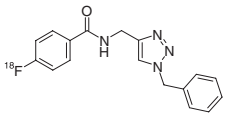
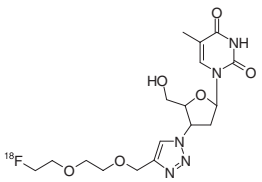
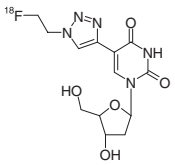
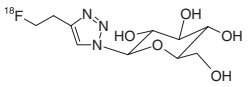
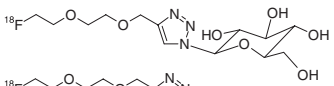
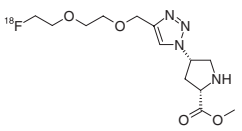
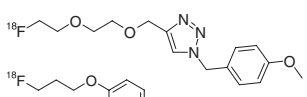
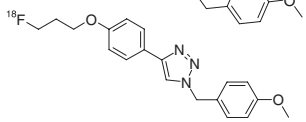
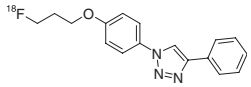
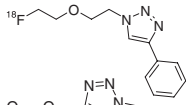
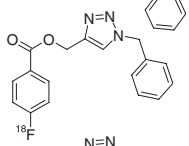
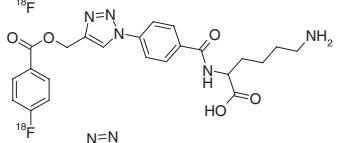
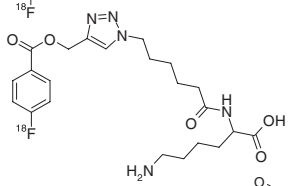
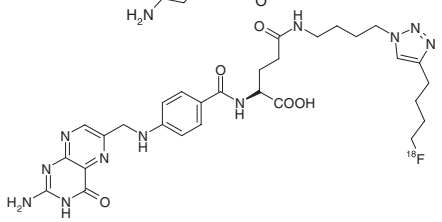
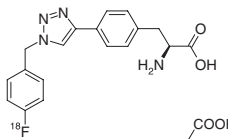
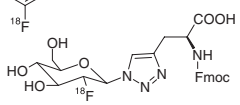
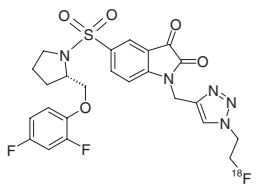
| No | [$^{18}$F]-1,2,3-Triazole | $^{18}$F-Reagent | Reaction conditions | RCY (%) | Reference |
|---|---|---|---|---|---|
| 18 |  | 4 | $CuSO_4$/Na-ascorbate, pH 6.0, MeCN, DMF, 80°C, 15 min | 84[a] | 21 |
| 19 |  | 4 | $CuSO_4$/Na-ascorbate, pH 6.0, MeCN, DMF, 80°C, 15 min | 61[a] | 21 |
| 20 |  | 4 | $CuSO_4$/Na-ascorbate, pH 6.0, MeCN, DMF, 80°C, 15 min | 93[a] | 21 |
| 21 |  | 4 | $CuSO_4$/Na-ascorbate, pH 6.0, MeCN, DMF, 80°C, 15 min | >98[a] | 21 |
| 22 |  | 4 | $CuSO_4$/Na-ascorbate, pH 6.0, MeCN, DMF, 80°C, 15 min | >98[a] | 21 |
| 23 |  | 4 | $CuSO_4$/Na-ascorbate, pH 6.0, MeCN, DMF, 80°C, 15 min | 98[a] | 21 |
| 24 |  | 4 | $CuSO_4$/Na-ascorbate, pH 6.0, MeCN, DMF, 80°C, 15 min | >98[a] | 21 |
| 25 |  | 4 | $CuSO_4$/Na-ascorbate, HPLC purification | 55 | 35 |
| 26 |  | 4 | $CuSO_4$/Na-ascorbate, pH 6.0, MeCN, DMF, 80°C, 15 min | >98[a] | 21 |
| 27 |  | 5 | $CuSO_4$/Na-ascorbate, $H_2O$, MeCN, 40°C, 20 min | 95[b] | 23 |
| 28 |  | 6 | $CuSO_4$/Na-ascorbate, $^t$BuOH, r.t., 10 min | 100[b] | 24 |
| 29 |  | 4 | $CuSO_4$/Na-ascorbate, DMF, pH 6.0, 80°C, 20 min | 70 | 36 |
| 30 |  | 1 | CuI/Na-ascorbate, MeCN, 2,6-lutidine, 90°C, 10 min, HPLC purification | 30[c] | 37 |
| 31 |  | 6 | $CuSO_4$/Na-ascorbate, $^t$BuOH, r.t., 10 min | 100[b] | 24 |
| 32 |  | 6 | $CuSO_4$/Na-ascorbate, $^t$BuOH, r.t., 10 min | 100[b] | 24 |

**Table 2.** *Continued*.

| No | [$^{18}$F]-1,2,3-Triazole | $^{18}$F-Reagent | Reaction conditions | RCY (%) | Reference |
|---|---|---|---|---|---|
| 33 |  | 6 | $CuSO_4$/Na-ascorbate, $^t$BuOH, r.t., 30 min | 100[b] | 24 |
| 34 |  | 7 | $CuSO_4$/Na-ascorbate, $^t$BuOH, r.t., 30 min | 72[b] | 24 |
| 35 |  | 8 | $CuSO_4$/Na-ascorbate, $^t$BuOH, r.t., 30 min | 73[b] | 24 |
| 36 |  | 9 | $CuSO_4$/Na-ascorbate, $^t$BuOH, r.t., 30 min | 71[b] | 24 |
| 37 |  | 13 | $CuSO_4$/Na-ascorbate, $^t$BuOH/DMSO | 88 ± 4 | 28 |
| 38 |  | 13 | $CuSO_4$/Na-ascorbate, $^t$BuOH/DMSO | 79 ± 33 | 28 |
| 39 |  | 13 | $CuSO_4$/Na-ascorbate, $^t$BuOH/DMSO | 75 ± 5 | 28 |
| 40 |  | 3 | CuI/DIPEA, 2,6-lutidine, MeCN, 80–60°C in 20 min, HPLC purification | 65–80[a]<br>25–35 | 38 |
| 41 |  | 14 | CuI/Na-ascorbate, DIEA, DMF, 15 min r.t., tC18-SepPak purification | 90[b] | 29 |
| 42 |  | 17 | (1) NaOH, 60°C, 5 min, HCl<br>(2) $CuSO_4$/Na-ascorbate, *t*-butanol, 60°C, 10 min | 60[a] | 30 |
| 43 |  | 4 | $CuSO_4$/Na-ascorbate, pH 6.0, DMF, MeCN, r.t., 30 min, HPLC purification | 65 ± 6 | 39 |

[a]RCY measured by radio-HPLC.
[b]RCY measured by radio-TLC.
[c]Overall yield with decay correction.

structures that can be of advantage for triazole purification and the pharmacokinetics of the tracer.[24,25] Also, two similar 2-[$^{18}$F]fluoropyridine alkynes **11** and **12** have been reported.[26,27]

Three glucose-based Click labelling reagents **15**, **16**, and **17** have been published recently.[30] These polar prosthetic groups could be also useful for the design of labelled biomacromolecules with better pharmacokinetics.

## Small $^{18}$F-containing molecules by Click labelling

As mentioned above, the short half-life of fluorine-18 imposes certain constraints on the radiochemistry. Fortunately, it was found that under optimised and mild conditions the CuAAC can be completed within 5–30 min. The labelling reaction is driven by a large excess of the coupling partner for the

no-carrier-added $^{18}$F-fluorine component that creates pseudo-first-order conditions. Table 2 shows an overview of small $^{18}$F-triazole molecules and the corresponding reaction conditions.

$^{18}$F-Triazoles **18–24** and **26** have been synthesised as model compounds to study the general labelling chemistry and substituent effects of neighbouring groups in peptides.[21] It was concluded that the alkyne function is tolerated at both peptide terminals and as a propargyl glycine side chain without compromising labelling yields. Propiolic acid was the only alkyne to give low radiochemical yields of **19**. This might be explained by an interference of the carboxylic group with the catalytical copper centre. Compound **25**, the deprotected $^{18}$F-triazole glycine derivative of **24**, has been already evaluated *in vivo* with the intention to develop a new non-natural amino acid marker for PET oncology.[35]

$^{18}$F-Triazole **27** was prepared as a model compound to explore the potential of [$^{18}$F]fluoro-*N*-(prop-2-ynyl)benzamide (**5**) for peptide Click labelling. The radiochemistry benefited from an automated radiosynthesis of the $^{18}$F-SFB precursor.[23]

Thymidine-based PET tracers are of interest as cell proliferation markers for oncology. Here, the thymidine derivative 3′-azido-3′-deoxythymidine (AZT) and an *N*-5 propargyl thymidine precursor have been successfully Click labelled with fluorine-18 to form compounds **28** and **29**, respectively.[24,36,40,41] In these applications the advantage of a labelling chemistry, that does not rely on protecting groups, becomes obvious. Similarly, $^{18}$F Click labelled glucose triazoles such as compounds **30** and **31** can be easily obtained.[24,37]

Click chemistry with $^{18}$F does not always require the isolation of the labelling reagent. For example, compounds **31–36** have been synthesised in good radiochemical yields without prior a purification step of the $^{18}$F-reagent.

The cycloaddition chemistry of propargyl 4-[$^{18}$F]fluorobenzoate has been explored for the three model triazoles **37–39**.[28]

The folate receptor has been recently studied using the Click labelled folate analogue **40**.[38] The tracer was evaluated by PET in nude mice bearing tumors from folate receptor expressing human nasopharyngeal carcinoma cells (KB tumors). The *ex vivo* biodistribution showed 3.13% ID/g specific uptake in the xenografts after 45 min. The authors noted as a limitation the predominantly hepatobiliary excretion of $^{18}$F-species. This was possibly due to an increased lipophilicity of the tracer, caused by the aliphatic carbon chains between both the triazole and $^{18}$F.

Recently, 4-ethynyl-substituted phenylalanine has been labelled with azide **14** in a model reaction.[29] The resulting labelled amino acid **41** has not yet been evaluated as a tracer *in vivo*.

An interesting approach to $^{18}$F Click labelling has been based on the 2-deoxy-2-[$^{18}$F]fluoroglucose derivative **17** leading to the substituted glycine **42**.[30] Although the radiochemistry protocol relies on protective groups, it opens a new avenue in PET peptide labelling.

The isatin probe **43** has been designed as a caspase-3-specific inhibitor for imaging of apoptosis.[39] The tracer demonstrated good *in vivo* stability in mice (i.e. 86.1 ± 3.7% and 61.3 ± 5.9% of parent peak by radio-HPLC after 2 and 15 min p.i., respectively). The biodistribution profile revealed a major excretion route by the liver. Encouraging results have been also obtained from RIF-1 tumor bearing mice. After *cis*-dichlorodiammine platium(II) (CDDP) treatment, a 2.9-fold increase in uptake of **43** compared with the untreated tumor has been observed.

### $^{18}$F-Peptides by Click labelling

The $^{18}$F labelling of peptides has been the area that has benefited the most by Click chemistry. Here, a chemoselective and high-yielding coupling method without the need for protecting groups is a distinctive advantage. So far, at least 12 instances have been reported (Table 3). Different approaches of the $^{18}$F Click method have been explored for some model peptides such as **44–48**, **50**, and **52**.[20–22]

$^{18}$F-Peptide **49** was studied in nude mice bearing $\alpha_v\beta_6$ receptor expressing human xenografts.[42] The authors evaluated **49** in comparison with solid phase labelling by two established $^{18}$F-fluorocarboxylic prosthetic groups (*N*-succinimidyl-4-[$^{18}$F]fluorobenzoate and *p*-nitrophenyl 2-[$^{18}$F]fluoropropionate). Click labelling was the preferred method but it also required more azido peptide precursor. The authors noted an impact of the prosthetic group on the corresponding biodistribution pattern (i.e. an increased kidney uptake for **49**).

The Click modified peptide **51** showed reduced binding affinity for the neurotensin receptor-1 (NTR1).[23] The $^{18}$F Click labelled nanopeptide **53** has been reported as a potential transglutaminase substrate.[28]

The $^{18}$F-triazole labelled dimeric RGD peptide **54** demonstrated a good tumor uptake (2.1 ± 0.4% ID/g) in a murine subcutaneous U87MG glioblastoma xenograft model. The tracer also showed a rapid renal and hepatic clearance.[25]

The Click labelled neuropeptide [Leu$^5$]Enkephalin **55** has been synthesized as a model compound. The tracer was the first instance of a cycloaddition at an aromatic peptide side chain.[29]

The bicyclic $^{18}$F Click RGD peptide **56** was obtained in a simple one-pot protocol without purification of 2-[$^{18}$F]fluoroethylazide. However, the separation from unlabelled alkyne peptide precursor proved to be difficult. This was attributed to the less favourable size difference between labelling reagent and substrate.[33]

## Click labelling with Carbon-11

The Huisgen reaction is often described as the perfect Click chemistry. The reaction is fast, simple to use, and can be carried out under mild conditions. For the same reasons, carbon-11 methylation has been the prevalent labelling strategy adopted for the preparation of short-lived $^{11}$C-radiopharmaceuticals. To translate the Click chemistry to carbon-11 needs to take into account the even more stringent radiosynthesis time constraints. Recently, Schirrmacher and co-workers described the first example of the Click reaction applied to carbon-11 chemistry as shown in Figure 2.[43]

Using a one-pot procedure, the rapid synthesis of [$^{11}$C]methylazide (**58**) from [$^{11}$C]methyliodide (**57**) and $NaN_3$ was conducted in the presence of CuI catalyst and phenyl acetylene in water at 100°C. The desired $^{11}$C-labelled 1,2,3-triazole (**59**) was isolated in 25% decay-corrected preparative yield in 12–14 min. A significant improvement to the preparative yield of **59** was achieved when a two-step protocol was employed. The more efficient trapping of **57** and conversion to **58** in organic solvent at room temperature provided **58** in 70% decay-corrected isolated yield in 12 min. The subsequent aqueous phase 1,3-dipolar cycloaddition with phenylacetylene at 100°C for 10 min afforded **59** in 60% decay-corrected preparative yield. The utility of Click chemistry to provide $^{11}$C-labelled radiopharmaceuticals was further exemplified through synthesis of the $^{11}$C Click labelled cyclic-TATE peptide. Following an optimised procedure, **60** was

**Table 3.** $^{18}$F-labelled peptides obtained by Click Chemistry.

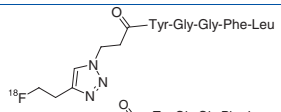
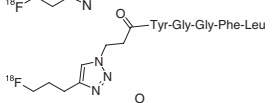
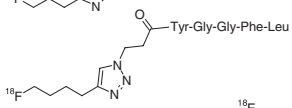
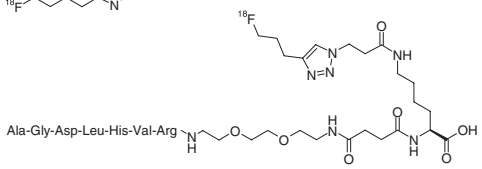
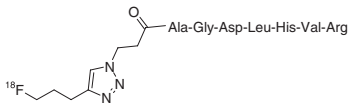
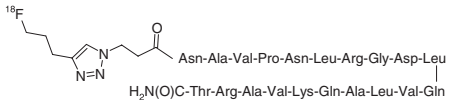
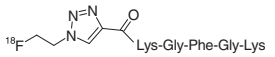
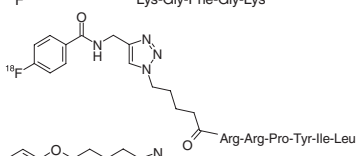
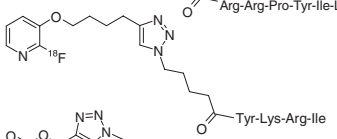
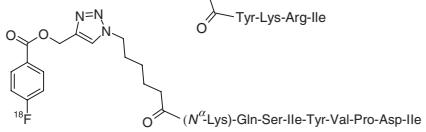
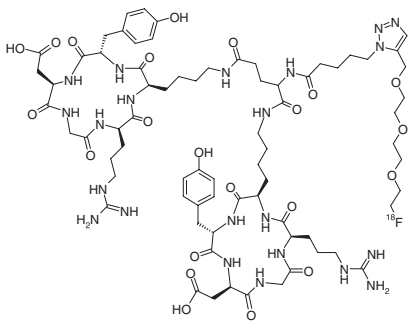
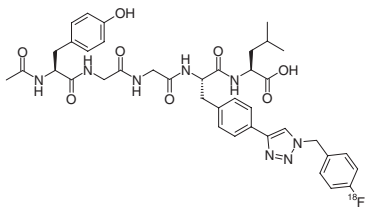
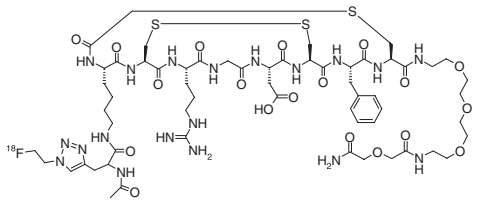
| No | [$^{18}$F]-1,2,3-Triazole | $^{18}$F-Reagent | Reaction conditions | RCY/ (Purity) | Reference |
|---|---|---|---|---|---|
| 44 |   | 1 | CuI, DIEA, Na-ascorbate, DMF, MeCN, water, r.t., 10 min, C18 SepPak purification | 54% (95%) | 20 |
| 45 |   | 2 | CuI, DIEA, Na-ascorbate, DMF, MeCN, water, r.t., 10 min, C18 SepPak purification | 97% (98%) | 20 |
| 46 |   | 3 | CuI, DIEA, Na-ascorbate, DMF, MeCN, water, r.t., 10 min, C18 SepPak purification | 62% (99%) | 20 |
| 47 |   | 2 | CuI, DIEA, Na-ascorbate, DMF, MeCN, water, r.t., 10 min, C18 SepPak purification | 97% (81%) | 20 |
| 48 |   | 2 | CuI, DIEA, Na-ascorbate, DMF, MeCN, water, r.t., 10 min, C18 SepPak purification | 99% (87%) | 20 |
| 49 |   | 2 | CuI, DIEA, Na-ascorbate, DMF, MeCN, water, r.t., 10 min, C18 SepPak and HPLC purification | 8.7 ± 2.3% (> 98%) | 42 |
| 50 |   | 4 | $CuSO_4$/Na-ascorbate, pH 6.0, MeCN, r.t., 15 min | 92.3 ± 0.3% (99%) | 21 |
| 51 |   | 5 | $CuSO_4$/Na-ascorbate, pH 8.4 40°C, 20 min | 66%[a] | 23 |
| 52 |   | 12 | $Cu(MeCN)_4PF_6$/TBTA, DMF, pH 8.5, 37°C, 10 min, semi-preparative HPLC | 18.7%[c] (89 ± 8.6%[a]) | 27 |
| 53 |   | 13 | $CuSO_4$/Na-ascorbate, $^t$BuOH/ DMSO | 37 ± 31% | 28 |
| 54 |  | 10 | $CuSO_4$/Na-ascorbate, 40°C, 20 min, semi-preparative HPLC | 69 ± 11% (> 97%) | 25 |
| 55 |  | 14 | CuI/Na-ascorbate, DIEA, DMF, 15 min r.t., tC18-SepPak purification | 90%[b] | 29 |

**Table 3.** *Continued*.

| No | [$^{18}$F]-1,2,3-Triazole | $^{18}$F-Reagent | Reaction conditions | RCY/ (Purity) | Reference |
|---|---|---|---|---|---|
| 56 |  | 4 | $CuSO_4$/Na-ascorbate, pH 8.0, DMSO, MeCN, 5 min, 80°C [or Cu powder, 15 min r.t., 30 min, HPLC purification] | 70 ± 5% (> 99%) [42 ± 14% (> 99%)] | 33 |

[a]RCY measured by radio-HPLC.
[b]RCY measured by radio-TLC.
[c]Decay-corrected RCY based on $^{18}$F-fluoride.

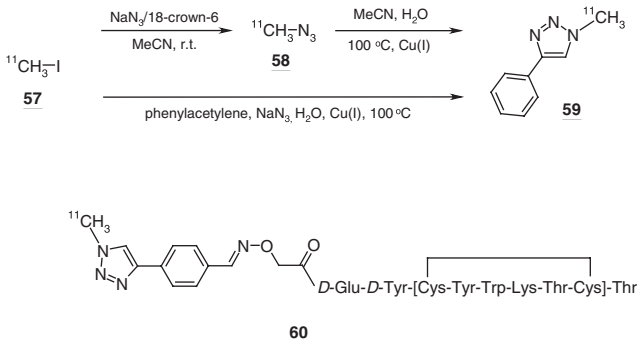



**Figure 2.** Preparation of [$^{11}$C]methyl azide and its use in Click labelling.

prepared in an isolated, decay-corrected yield of 32–35% in a synthesis time of 30 min. One particular difference between the $^{11}$C and $^{18}$F Click chemistries is the higher temperatures required for the $^{11}$C-labelled 1,2,3-triazole formation. It is suggested that more forcing conditions are required due to the lower reactivity of the [$^{11}$C]methylazide, however, this in no way precludes the application of $^{11}$C Click chemistry to the preparation of $^{11}$C-labelled radiopharmaceuticals.

## Conclusions

One of the key challenges facing radiopharmaceutical chemistry is to broaden the spectrum of radiochemical reactions and labelling methods available for the construction of molecular scaffolds. The Cu(I)-catalysed Huisgen reaction has undoubtedly achieved this for fluorine-18 labelling of biomolecules. The application of Click chemistry to carbon-11 has demonstrated the potential of this chemistry for the preparation of radiopharmaceuticals labelled with shorter-lived isotopes.

To date, Click chemistry for PET radiopharmaceuticals has focused on the application of $CuSO_4$/Na-ascorbate or CuI/*N,N'*-diisopropylethylamine/Na-ascorbate catalyst mixtures. However, it is known that for the CuAAC other copper catalytic systems such as nitrogen bases like *tris*(benzyltriazolylmethyl)amine (TBTA) or bathophenanthroline disodium salt (BPDS) can be used.[44,45] In fact, BPDS has been recently applied to label nanoparticles with $^{18}$F-alkyne **10**.[46] Copper carbenes have shown to be extremly effective, exhibiting high turnover numbers at low catalyst loading.[47,48] Reduction in the amount of catalyst used may address potential clinical concerns over metal contamination of radiopharmaceuticals, in particular for Click labelled macromolecules.

The CuAAC of organic azides with alkynes selectively provides 1,4-substituted-1,2,3-triazoles but is limited to the reaction of terminal alkynes only. Zhang *et al.*[49] have already demonstrated that alternative ruthenium-based catalyst systems can provide products of complementary regiochemistry. For example, pentamethylcyclopentadienyl *bis*(triphenylphosphine) ruthenium(II) chloride [$Cp^*RuCl(PPh_3)_2$]-mediated cycloaddition provides 1,5-substituted triazoles and has also been shown to be capable of activating internal alkynes for the synthesis of 1,4,5-substituted triazoles.[50,51] While the regiochemistry of the triazole may be less significant for peptide and macromolecule radiolabelling, the application of ruthenium-based catalysts may provide complementary SAR development of small molecule PET tracers.

The Click labelling method has already become a fashionable tool for PET radiochemistry. As a method for biomolecule labelling it is chemoselective and tolerates a broad range of functional groups. We anticipate that the popularity of Click chemistry in PET will continue to increase and many more examples will follow.[52]

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