ChemComm

Cite this: *Chem. Commun.*, 2012, **48**, 991–993

www.rsc.org/chemcomm

COMMUNICATION

Copper-free click—a promising tool for pre-targeted PET imaging†

Helen L. Evans,[a] Rozanna L. Slade,[a] Laurence Carroll,*[a] Graham Smith,[a] Quang-De Nguyen,[a] Lisa Iddon,[a] Nazila Kamaly,[a] Henning Stöckmann,[b] Finian J. Leeper,[b] Eric O. Aboagye[a] and Alan C. Spivey[c]

Received 6th October 2011, Accepted 17th November 2011
DOI: 10.1039/c1cc16220a

The copper-free click (CFC) reaction has been evaluated for its potential application to *in vivo* pre-targeting for PET imaging. A promising biodistribution profile is demonstrated when employing [^{18}F]2-fluoroethylazide ([^{18}F]1) and optimisation of the CFC reaction with a series of cyclooctynes shows that reactions proceed efficiently with tantalizing opportunities for application-specific tuning.

Since the discovery of fluorine-18 labelled 2-deoxy-2-fluoroglucose ([^{18}F]FDG) approximately forty years ago, positron emission tomography (PET) has expanded to become a crucial technique in the fight against diseases such as Parkinson's disease and cancer.[1,2] Throughout this period, there has been great interest in developing new radiolabelling methods to expand the range of possible tracers that can be synthesised. One of the most exciting recent developments has been the deployment of the copper(I)-catalysed Huisgen [2, 3]-cycloaddition between alkynes and azides, which is widely referred to as the 'click reaction', following independent development work by Sharpless and Meldal in 2001.[3,4] The use of click chemistry for the synthesis of a number of novel radiotracers has been documented, but to date, none have reached the clinic, in part due to the difficulties associated with removing traces of copper, which has been shown to be toxic to humans even at low levels.[5]

The copper-free click (CFC) reaction, using strained cyclooctynes, was pioneered by Bertozzi, and eliminates any issues of copper toxicity (Scheme 1).[6]

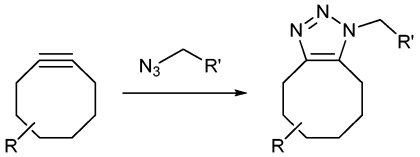
The reaction was developed specifically to enable *in vivo* pre-targeting for fluorescent imaging, albeit for *ex vivo* tumour analysis, due to the limitations of fluorescence detection technology. Pre-targeting refers to a strategy in which a reactive tag (*e.g.* the cyclooctyne) is attached to a specific biomolecule (*i.e.* drug, peptide or antibody) and allowed to localise in the tissue to be imaged (*e.g.* tumour) before systemic administration of a 'pull-down' reagent (*e.g.* an azide having a fluorescent label), which, after wash-out of the excess pull-down reagent, allows the 'pre-targeted' tissue to be imaged. This is in contrast to traditional 'targeted' radiotracers, which accumulate in the desired area of interest with the radiolabelled tag already attached to the targeting group—a protocol which is more severely constrained by the limited half-life of PET radionuclides. The CFC reaction was shown to have superior reaction kinetics relative to other bio-orthogonal reactions such as the Staudinger ligation, in this context.[7,8] We therefore envisaged that the CFC reaction had great potential for analogous *in vivo* pre-targeting for PET imaging. Prompted by a recent preliminary disclosure by Wuest *et al.* of an approach to this in which a radiolabelled cyclooctyne is used in conjunction with a series of functionalised azides,[9] we describe here a complementary approach[10] in which a radioabelled azide is used in conjunction with a series of functionalised cyclooctynes.

Specifically, we report the biodistribution profile of [^{18}F]2-fluoroethylazide (**[^{18}F]1**) in BALB/c nude mice and the reactivity of this radiotracer with a series of five structurally diverse cyclooctynes as a function of temperature and solvent.

We chose [^{18}F]2-fluoroethylazide (**[^{18}F]1**) as our labelled azide, due to its synthesis being well-described in the literature, its small size and its expected rapid clearance from major organs *in vivo* (Scheme 2).[10–13]

Using the method of Glaser *et al.*, tosylate **2** was reacted with a mixture of [^{18}F]fluoride and Kryptofix[2.2.2] in acetonitrile, giving the desired labelled azide **[^{18}F]1** in 30% RCY n.d.c, after distillation.[11]

In order to determine the level of background radioactivity that could be expected when employing the CFC reaction for *in vivo* pre-targeting using this compound, biodistribution data was collected for **[^{18}F]1** in BALB/c nude mice (n = 3) (Fig. 1).



Scheme 1 The copper-free 'click' reaction.

[a] *Comprehensive Cancer Imaging Centre, Department of Surgery & Cancer, Hammersmith Campus, Imperial College, London, United Kingdom. E-mail: l.carroll@imperial.ac.uk; Tel: +44 (0)20 8383 3759*
[b] *Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge, CB2 1EW, UK*
[c] *Department of Chemistry, Imperial College London, South Kensington Campus, London, SW7 2AZ, UK*

† Electronic supplementary information (ESI) available: Synthetic procedures for **3–7** and selected HPLC traces for click reactions. See DOI: 10.1039/c1cc16220a

Scheme 2 Radiosynthesis of [^{18}F]2-fluoroethylazide (**[^{18}F]1**).

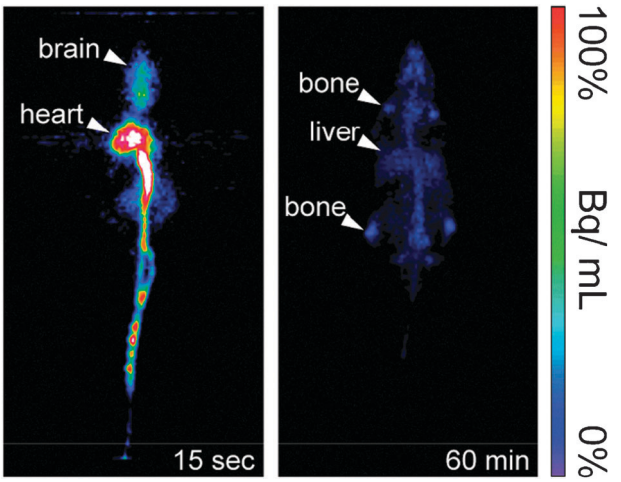


Fig. 1 Representative small animal PET images of a BALB/c nude mouse at t = 15 s and t = 60 min after intravenous injection in the tail of 3.7 MBq of **[^{18}F]1**.

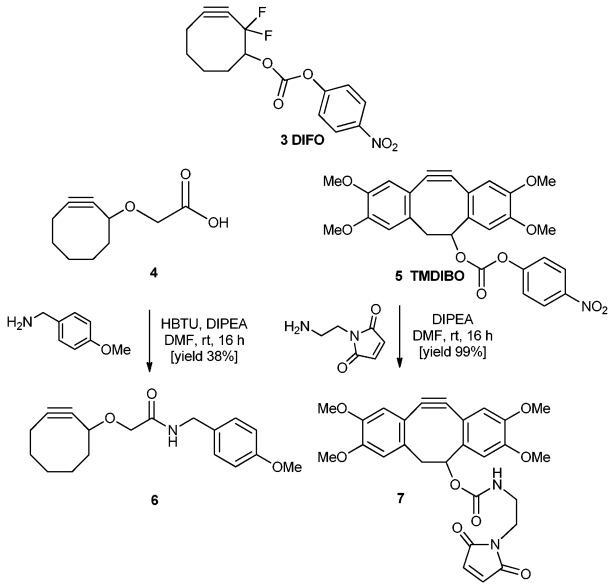


Fig. 2 Structures of cyclooctynes used in the study.

Over the course of the experiment, approximately 9 % of the total radioactivity was eliminated either *via* excretion or from the lungs *via* exhalation. By the end of the scan a small amount of activity was found in the bone, suggesting a minor amount of [^{18}F]fluoride anion generation, but this is not anticipated to present a problem for future imaging, due to its low level relative to the initial injection. The remaining radioactivity was evenly spread throughout the other tissue (see ESI for full biodistribution data†). Azide **[^{18}F]1** is also completely stable in phosphate buffer (pH = 7.4), acetonitrile and dimethylsulfoxide for up to six hours at room temperature. This intrinsic stability, combined with the even distribution within the body within 60 min, suggests that azide **[^{18}F]1** could prove to be an advantageous labelled azide for use following pre-targeting with a CFC reagent.

The synthesis of a wide range of cyclooctynes have been reported for use in CFC reactions, each presenting a unique blend of attributes relevant to this specialist application vis-à-vis synthetic accessibility, stability/reactivity and potential for ligation to biomolecular targets.[14,15] We selected three of these cyclooctynes (**3–5**), as well as further-functionalised derivatives **6** and **7**, for our investigation (Fig. 2).

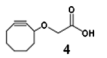
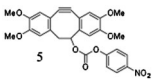
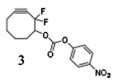
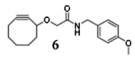
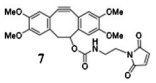
Cyclooctynes **3–5** were synthesised using previously described methods and derivative **6** was obtained by subsequent HBTU coupling of cyclooctyne **4** with 4-methoxybenzylamine.[14,15] Derivative **7** was synthesised by direct coupling of cyclooctyne **6** with *N*-(2-aminoethyl)maleimide. The literature suggests that DIFO (**3**) has a faster reaction rate for the CFC reaction than cyclooctyne **4**, and similarly that TMDIBO (**5**) has a faster reaction rate than DIFO (**3**).[14]

Non-labelled reference samples of the four triazole products derived from CFC reaction of cyclooctynes **3–7** with azide **1** (*cf*. Scheme 1) were prepared by addition of a five-fold excess of azide **1** to each cyclooctyne. After stirring until the reactions reached completion, excess azide **1** was evaporated at reduced pressure to afford the desired triazole products for characterisation and HPLC analysis; for cyclooctynes **3–7**, a mixture of regioisomeric 1, 4, 5-trisubstituted-1, 2, 3-triazoles could be identified by ^{19}F NMR and HPLC in ratios varying from 2 : 1 to 7 : 1.

Next, we examined the corresponding CFC reactions using radiolabelled **[^{18}F]1** under different conditions of temperature, solvent and time (Table 1).

Under our conditions in MeCN at 90 °C, the apparent reactivities of cyclooctynes **3**, **4** and **5**, based on radiochemical yield (RCY) after 15 min, follow the order **3** > **5** > **4** (*cf*. entries 3, 7 and 11) rather than the **5** > **3** > **4** that would be expected based on published literature (*vide supra*). However, TMDIBO (**5**) appears to be unstable at higher temperatures (see ESI file for example HPLC UV traces for reactions at 90 °C†) and so the lower amount of labelled product in this case is probably due to its decomposition being faster than its reaction with **[^{18}F]1**. Interestingly, the amide derivative **6** of cyclooctyne **4** and the carbamate derivative **7** of TMDIBO (**5**) show significantly altered reactivities relative to their acid/alcohol parents: **6** > **4** and **7** < **5** (*cf*. entries 12 and 3 *vs*. 14 and 7). Importantly, both TMDIBO (**5**) and DIFO (**3**) react efficiently with **[^{18}F]1** in water at physiological temperature within the timeframe of an *in vivo* PET scan (*i.e*. ~15 min, entries 5 and 9). This, coupled with azide **[^{18}F]1** having an even distribution profile within the body (*vide supra*), suggests that these cyclooctynes could be suitable for pre-targeting studies by PET. It is evident however that after 60 min in water the RCY of product when using TMDIBO (**5**) is approximately double that when using DIFO (**3**); this contrasts strongly with the situation after 15 min in MeCN where the RCY of product when using TMDIBO (**5**) is over eight-fold lower than when using DIFO (**3**) (*cf*. entries 6 and 10 *vs*. 4 and 8). In view of this inversion of relative reactivity in water as compared to MeCN, TMDIBO (**5**) appears to be the most promising cyclooctyne of those that we have investigated for use in biological studies.

Table 1 Summary of CFC reactions carried out using **[^{18}F]1**

	Substrate[a]	*T*/°C	Time/Mins	Solvent	RCY/%[b,c]
1	 4	40	15	MeCN	4.4
2		40	15	H_2O	0.3
3		90	15	MeCN	24.9
4	 5	40	15	MeCN	7.1
5		40	15	H_2O	33.3
6		40	60	H_2O	36.2
7		90	15	MeCN	31.5
8	 3	40	15	MeCN	62.1
9		40	15	H_2O	26.1
10		40	60	H_2O	17.8
11		90	15	MeCN	97.1
12	 6	90	15	MeCN	60.4
13	 7	40	15	MeCN	3.2
14		90	15	MeCN	9.6

[a] Each reaction carried out using 50 μL of **[^{18}F]1** (typical activity of ~5 MBq); $n = 3$ in all cases. [b] Radiochemical yield as determined *via* radio-HPLC showing conversion of **[^{18}F]1** into the summation of the regioisomers. [c] For further details relating to these data see the ESI file.†

In addition, a cell viability assay was carried out in HCT116 human colon cancer cells, and no toxicity of TMDIBO (**5**) was observed at concentrations up to 100 μM (see ESI for further details†).

We have demonstrated that CFC reactions can be carried out between a short-lived ^{18}F-labelled azide and various cyclooctynes for the first time and that these reactions can be achieved under conditions which appear suitable for *in vivo* pre-targeting for PET imaging. [^{18}F]2-Fluoroethylazide (**[^{18}F]1**) is shown to be a promising initial choice for systemic administration as the pull-down reagent, partnered with a pre-localised cyclooctyne(s)-tagged biomolecule. Both the ease of radiosynthesis of **[^{18}F]1** and its favourable mouse biodistribution profile indicate that this mode of pre-targeting may constitute a more promising strategy than one in which the systemic radiolabelled pull-down reagent is the cyclooctyne.[9] The cyclooctyne partner of choice for biomolecule tagging using this strategy appears to be TMDIBO (**5**), although further studies will be required to confirm this for specific applications. A delicate balance needs to be struck between reactivity of the tag towards azide **[^{18}F]1**, its *in vivo* stability and its ease of synthesis and biomolecule attachment. DIFO (**3**) shows greater reactivity towards azide **[^{18}F]1** in MeCN than TMDIBO (**5**), but in water over longer time periods TMDIBO (**5**) displays more robust reactivity and benefits from a shorter synthesis route for preparation. We are currently exploring the combination of azide **[^{18}F]1** and TMDIBO (**5**) for *in vivo* PET imaging of certain cancers and these studies will be published in due course.

This work was supported by a studentship from the Imperial Cancer Research UK Centre grant (C37990/A12196), and by CR-UK&EPSRC Cancer Imaging Centre at Imperial College London, in association with the MRC and Department of Health (England) grant C2536/A10337 and UK Medical Research Council core funding grant U.1200.02.005.00001.01.

Notes and references

1 B. Beuthien-Baumann, K. Hamacher, F. Oberdorfer and J. Steinbach, *Carbohydr. Res.*, 2000, **327**, 107–118.
2 S. M. Ametamey, M. Honer and P. A. Schubiger, *Chem. Rev.*, 2008, **108**, 1501–1516.
3 H. C. Kolb, M. G. Finn and K. B. Sharpless, *Angew. Chem., Int. Ed.*, 2001, **40**, 2004–2021.
4 C. W. Tornøe, C. Christensen and M. Meldal, *J. Org. Chem.*, 2002, **67**, 3057–3064.
5 G. J. Brewer, *Clin. Neurophysiol.*, 2010, **121**, 459–460; A. Ala, A. P. Walker, K. Ashkan, J. S. Dooley and M. L. Schilsky, *Lancet*, 2007, **369**, 397–408.
6 J. A. Codelli, J. M. Baskin, N. J. Agard and C. R. Bertozzi, *J. Am. Chem. Soc.*, 2008, **130**, 11486–11493.
7 L. Carroll, S. Boldon, R. Bejot, J. E. Moore, J. Declerck and V. Gouverneur, *Org. Biomol. Chem.*, 2011, **9**, 136–140.
8 J. M. Baskin, J. A. Prescher, S. T. Laughlin, N. J. Agard, P. V. Chang, I. A. Miller, A. Lo, J. A. Codelli and C. R. Bertozzi, *Proc. Natl. Acad. Sci. U. S. A.*, 2007, **104**, 16793–16797.
9 V. Bouvet, M. Wuest and F. Wuest, *Org. Biomol. Chem.*, 2011, **9**, 7393.
10 While this manuscript was in preparation Feringa *et al.* published an approach to labelling a cyclooctyne-tagged Lys [3]-bombesin *via* a strain-promoted click reaction using three [^{18}F]-labelled azides: L. S. Campbell-Verduyn, L. Mirfeizi, A. K. Schoonen, R. A. Dierckx, P. H. Elsinga and B. L. Feringa, *Angew. Chem., Int. Ed.*, 2011, **50**, 11117.
11 M. Glaser and E. Årstad, *Bioconjugate Chem.*, 2007, **18**, 989–993.
12 R. Bejot, T. Fowler, L. Carroll, S. Boldon, J. E. Moore, J. Declerck and V. Gouverneur, *Angew. Chem., Int. Ed.*, 2009, **48**, 586–589.
13 G. Smith, M. Glaser, M. Perumal, Q.-D. Nguyen, B. Shan, E. Årstad and E. O. Aboagye, *J. Med. Chem.*, 2008, **51**, 8057–8067.
14 H. Stockmann, A. A. Neves, S. Stairs, H. Ireland-Zecchini, K. M. Brindle and F. J. Leeper, *Chem. Sci.*, 2011, **2**, 932–936.
15 A. Bernardin, A. Cazet, L. Guyon, P. Delannoy, F. Vinet, D. Bonnaffe and I. Texier, *Bioconj. Chem.*, 2010, **21**, 583–588.