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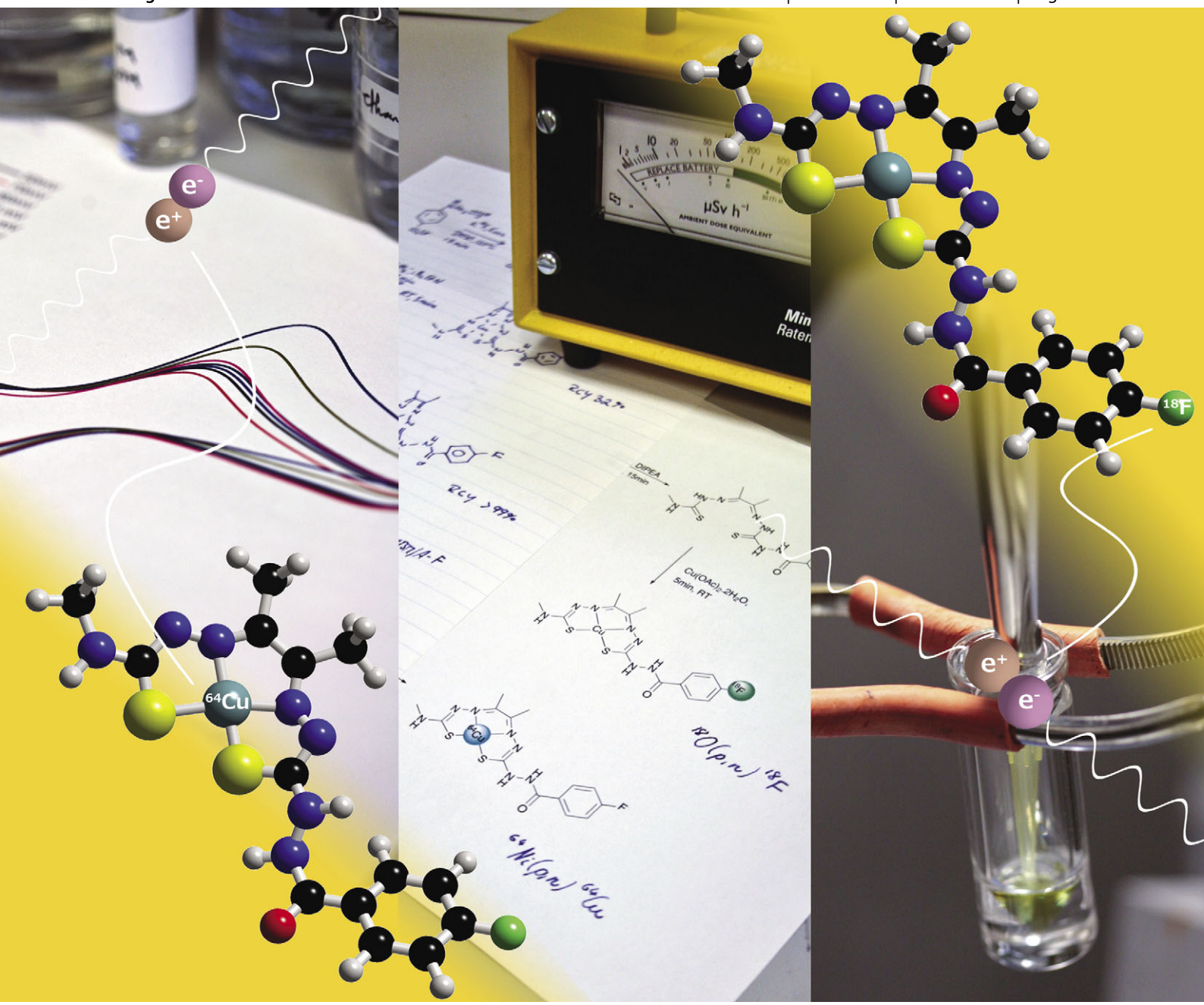

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FEATURE ARTICLE
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Orthogonal ^{18}F and ^{64}Cu labelling of functionalised *bis*(thiosemicarbazonato) complexes†

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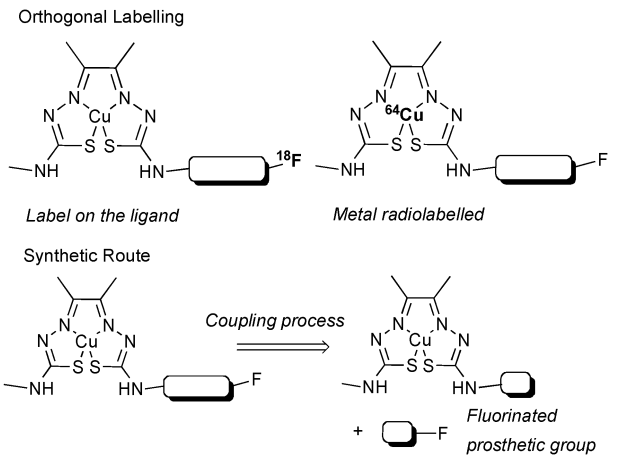
The synthesis of three pairs of orthogonally labelled fluorinated Cu *bis*(thiosemicarbazonato) complexes is presented. These are the first examples of ^{18}F-labelled Cu(II)-complexes designed to serve as new hypoxia selective PET tracers and as mechanistic probes to study the mode of action of this class of markers. *In vitro* evaluation revealed that the fluorinated Cu-complex derived from amide coupling is suitable for *in vivo* work.

Low oxygen level in cells, known as hypoxia, has direct implications for the treatment of various diseases including cancer.[1,2] Hypoxic tumours are typically more difficult to eradicate with a lower survival rate for patients not diagnosed correctly.[3] It is therefore critical to identify hypoxic tumours from the outset, so that treatment can be personalised. A commonly used non-invasive diagnostic method to detect tumours is positron emission tomography (PET).[4–6] Nitroimidazole-based tracers such as [^{18}F]fluoromisonidazole ([^{18}F]FMISO), and metal complexes, for example diacetyl-di-(N^4-methyl-thiosemicarbazonato)-[^{64}Cu]copper(II) ([^{64}Cu]-Cu-ATSM) have been developed to target and image hypoxic tumours. These metal complexes are labelled at *the metal*.[7–10]

Recently, we have questioned whether complexes structurally related to Cu(II)-ATSM but ^{18}F-labelled at *the ligand*, might serve as new hypoxia selective radiotracers for PET. The wide availability of ^{18}F would open the possibility to use these new compounds for hypoxia imaging at a large number of clinical sites worldwide. In addition, these new ^{18}F-labelled metal complexes, structurally related to Cu(II)-ATSM, are unique probes to bring new insight into the mode of action of this class of hypoxia selective radiotracers by examining the metabolic fate of the copper complex *in vivo*. Valuable information on the mechanism of these new complexes would be gained by examining the biodistribution profile of both the ligand and the metal. This can be achieved if these two entities could be radiolabelled independently. This so-called "orthogonal labelling" strategy requires the preparation of a pair of structurally identical Cu-ATSM derivatives labelled either at the metal with ^{64}Cu or at the ligand with ^{18}F (Scheme 1). Careful SAR studies conducted by Lewis *et al.* and Kelly *et al.* indicate that there appears to be strong correlation between the redox potential of the copper complex and its hypoxia selectivity.[11,12] Typically, the redox potential is controlled primarily by the substitution pattern of the carbon backbone, whereas modification of the exocyclic amino group modifies lipophilicity and biodistribution.[13–16] These observations combined with synthetic considerations suggest that manipulation of the exocyclic amino group is more suitable for ^{18}F-labelling. We opted for a synthetic route based on the use of ^{18}F-labelled prosthetic groups as this modular approach should comply with the challenges associated with the handling of short half-life ^{18}F (109.7 min) and allow for easier structural variation.

Three reactions were selected based on the availability of well-established protocols to access the necessary ^{18}F-labelled component: an imine condensation with [^{18}F]4-fluorobenzaldehyde, an amide bond formation with [^{18}F]4-fluorobenzoic acid and a Huisgen 1,3-dipolar cycloaddition with [^{18}F]2-fluoro-1-azidoethane. Both imine and amide bond formation reactions were reported in the literature to access various functionalized *bis*(thiosemicarbazonato)copper(II) complexes metal radiolabelled.[16] In contrast, click chemistry is unprecedented for copper radiopharmaceutical synthesis despite its extensive use in the context of chemical biology and more recently in radiochemistry.[17–20] This is likely due to possible complication



Scheme 1 Orthogonal labelling: concept and retrosynthetic approach.

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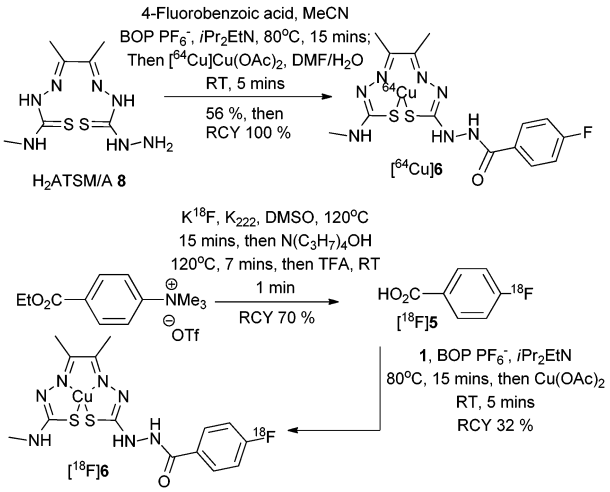
arising from the propensity of proligands (or zinc complexes) to metallate (or transmetallate) in the presence of Cu^{2+} ions, which are typically used to promote 1,3-dipolar cycloadditions of azides with alkynes. This strategy will therefore require careful synthetic planning.

Initial work began by studying the coupling of Zn-ATSM/A **1** with 4-fluorobenzaldehyde **2**.[13] Imine condensation performed in acetonitrile at 80 °C delivered an inseparable *E*/*Z* mixture (1 : 4) of hydrazone **3** in 56% yield. Transmetallation with [^{64}Cu]Cu(OAc)$_2$ was quantitative within 5 min to give the ^{64}Cu-complex **4** radiochemically pure as judged by radio-HPLC. For the preparation of the novel orthogonally labelled [^{18}F]**4**, [^{18}F]4-fluorobenzaldehyde [^{18}F]**2** was firstly prepared in acetonitrile by nucleophilic aromatic substitution of 4-trimethylammoniumbenzene triflate with [^{18}F]$^-$/K$^+$-Kryptofix 222.[21] Condensation of [^{18}F]**2** with Zn-ATSM/A **1** led to the formation of [^{18}F]**3** (*E*/*Z* ratio = 5 : 1) in 94% RCY. Subsequent transmetallation of [^{18}F]**3** with Cu(OAc)$_2$ gave the desired complex [^{18}F]**4**. All radiochemical yields are reported decay-corrected. The identity of [^{18}F]**2**, [^{18}F]**4** and labelled [^{64}Cu]**4** was unambiguously confirmed by comparison of their radio-HPLC trace with the UV-HPLC trace of independently prepared and fully characterized non-radioactive material (Scheme 2).

4-Fluorobenzoic acid **5** was selected to validate the amide coupling strategy. To access the novel amide complex **6** radiolabelled at the metal, H_2ATSM/A **8** was coupled with 4-fluorobenzoic acid in the presence of *O*-benzotriazole-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (BOP PF_6^-) and *N*,*N*-diisopropylethylamine. Addition of [^{64}Cu]-Cu(OAc)$_2$ to the resulting proligand **7** led quantitatively to [^{64}Cu]**6**. Orthogonal ^{18}F-labelling required access to [^{18}F]**5**, which was made available within 30 min upon fluorination of ethyl 4-trimethylammoniumbenzoate triflate with [^{18}F]$^-$/K$^+$-Kryptofix 222 followed by hydrolysis. Amide coupling of [^{18}F]**5** with **8** followed by metallation using Cu(OAc)$_2$ gave [^{18}F]**6** in an overall RCY of 32%. The synthesis of [^{18}F]**6** from [^{18}F]**5** took less than 20 min (Scheme 3).

[^{18}F]-2-Fluoro-1-azidoethane **9** was considered next to investigate the value of Huisgen 1,3-dipolar cycloadditions

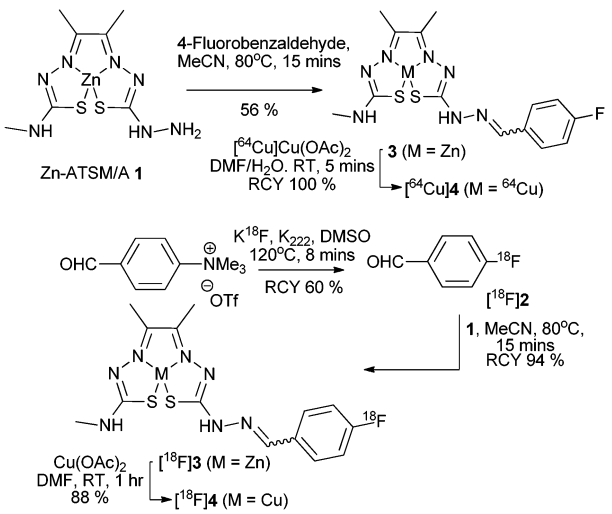


Scheme 3 Synthesis of complex **6**.

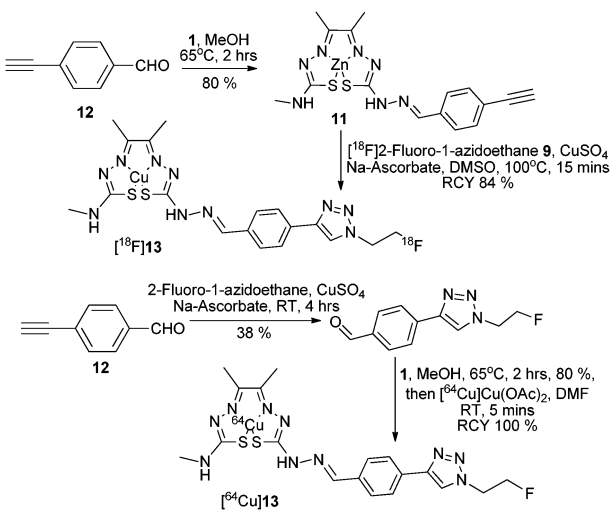
("click reaction") for the synthesis of copper-based tracers (Scheme 4). Following a recent literature procedure,[22] [^{18}F]**9** was prepared by nucleophilic fluorodetagging of the corresponding fluorous sulfonate and subsequent facile purification by fluorous solid phase extraction (FSPE). The Zn-complex **11** was prepared by imine condensation of Zn-ATSM/A **1** with aldehyde **12**.

Pleasingly, upon treatment with $CuSO_4$ and Na-ascorbate, the 1,3-dipolar cycloaddition with [^{18}F]-2-fluoro-1-azido-ethane was successful in DMSO at 100 °C within 15 min. Subsequent addition of Cu(OAc)$_2$ led to [^{18}F]**13** with a RCY of 84%. The synthesis of [^{64}Cu]**13** was uneventful following a strategy reversing the order of the synthetic steps, the click coupling between aldehyde **12** and 2-fluoro-1-azidoethane being carried out prior to imine condensation with Zn-ATSM/A **1**. The resulting Zn-complex was transmetallated under standardised conditions leading to [^{64}Cu]**13** in high radiochemical yield and purity.

To compare the electrochemical properties of the new complexes with the parent compound Cu-ATSM, the Cu(II/I) redox couple was measured for complexes **4**, **13** and **6** giving values of −0.60 V, −0.64 V and −0.62V *vs.* SCE respectively



Scheme 2 Synthesis of complex **4**.



Scheme 4 Synthesis of complex **13**.

Table 1 Cell uptake studies

	21% (normoxic)	0% (hypoxic)
[^{64}Cu]Cu-ATSM[a,24]	10%	65%
[^{64}Cu]Cu-ATSM[b]	21.3 ± 0.3%	47.6 ± 0.9%
[^{64}Cu]Cu-ATSM[c]	30.3 ± 2.4%	41.1 ± 2.2%
[^{64}Cu]**4**[b]	53.1 ± 1.5%	48.8 ± 1.3%
[^{64}Cu]**6**[c]	53.8 ± 2.3%	63.5 ± 0.9%
[^{64}Cu]**13**[a]	82.6 ± 1.1%	91.5 ± 0.1%
[^{64}Cu]**13**[c]	38.6 ± 0.4%	35.6 ± 3.4%

[a] EMT6 suspension assay, 1 h. [b] HT1080 suspension assay, 1 h. [c] HT1080 adherent, 3 h.

(for Cu(II)-ATSM: −0.62 V *vs.* SCE).[23] These data indicate that all new complexes display an electrochemical behavior similar to Cu(II)-ATSM. While the relationship between lipophilicity and cellular uptake is expected to be complex, this property is critically important in the context of bio-distribution. With a log *P* value of 1.50, Cu(II)-ATSM is a suitable tracer despite its relatively high liver uptake.[11] Log *P* values of 1.55, 1.70 and 2.50 were measured for complexes **6**, **13** and **4**, respectively. These data indicate that **6** and **13** emerge as the closest Cu(II)-ATSM analogues since their log *P* values are similar. With a higher log *P* value of 2.50, complex **4** might sequester itself within membranes becoming inaccessible to intracellular reducing agents within the cytosol, and therefore cannot be elected a suitable candidate for *in vivo* experimentation.

Hypoxia selectivity can be measured *in vitro* by oxygen dependent cellular uptake measurements (See SI for details†). Hypoxia selectivity of the fluorinated complexes **4**, **6** and **13** was tested and compared with [^{64}Cu]Cu-ATSM (Table 1). Using the HT1080 suspension assay, [^{64}Cu]**4** showed approximately 10% higher uptake in hypoxic cells compared to normoxic cells after 10 min, however after 1 h, uptake under normoxia had become higher than that under hypoxia. Data for [^{64}Cu]**6** were much more encouraging, and in the HT1080 adherent assay it showed excellent hypoxia selectivity with 64% uptake after 3 h in hypoxic cells compared to 54% in normoxic cells. The 10% difference is equivalent to that observed for [^{64}Cu]Cu-ATSM, although uptake of [^{64}Cu]**6** was higher in comparison at both oxygen concentrations. Although a preliminary EMT6 suspension assay on [^{64}Cu]**13** showed some selectivity for hypoxic cells (but of lower magnitude than [^{64}Cu]Cu-ATSM), in the adherent assay with HT1080 cells no significant difference in uptake of the complex under normoxic and anoxic conditions was observed. From these results [^{64}Cu]**6** was earmarked for future *in vivo* study.

In conclusion, the fluorinated *bis*(thiosemicarbazone) complexes **4**, **6** and **13** were prepared and radiolabelling with ^{18}F or ^{64}Cu led to six new radiotracers. These are the first examples of ^{18}F-labelled diacetyl-di(N^4-methyl-thiosemicarbazonato)-copper(II) derivatives. It has been demonstrated for the first time that 'click' chemistry is a viable transformation to functionalize *bis*(thio-semicarbazonato) complexes. Known methodologies were improved for compatibility with ^{18}F-labelling ($t_{1/2}$ = 109.7 min). *In vitro* data indicate that the fluorinated complex **6** is suitable for *in vivo* investigation. Current work is underway to determine whether ^{18}F-labelled Cu(II) *bis*(thiosemicarbazonato) complexes can be used as PET imaging tracers to measure hypoxia and to enhance our understanding of the mode of action of this family of markers.

Notes and references

1 J. M. Brown, *Cancer Res.*, 1999, **59**(23), 5863–5870.
2 J. M. Brown and W. R. Wilson, *Nat. Rev. Cancer*, 2004, **4**(6), 437–447.
3 W. A. Denny, *Eur. J. Med. Chem.*, 2001, **36**(7–8), 577–595.
4 B. Beuthien-Baumann, K. Hamacher, F. Oberdorfer and J. Steinbach, *Carbohydr. Res.*, 2000, **327**(1–2), 107–118.
5 P. Blower, *Dalton Trans.*, 2006, 1705–1711.
6 G. J. R. Cook, S. Houston, S. F. Barrington and I. Fogelman, *J. Nucl. Med.*, 1998, **39**(1), 99–103.
7 [^{18}F]Fluoromisonidazole. [^{18}F]FMISO, in: Molecular Imaging and Contrast Agent Database (MICAD) [database online]. Bethesda (MD): National Library of Medicine (US), NCBI; 2004–2008. Available from: http://micad.nih.gov.
8 S.-M. Eschmann, F. Paulsen, M. Reimold, H. Dittmann, S. Welz, G. Reischl, H.-J. Machulla and R. Bares, *J. Nucl. Med.*, 2005, **46**(2), 253–260.
9 A. L. Vavere and J. S. Lewis, *Dalton Trans.*, 2007, 4893–4902.
10 K. A. Wood, W. L. Wong and M. I. Saunders, *Nucl. Med. Biol.*, 2008, **35**(4), 393–400.
11 J. Dearling, J. Lewis, G. Mullen, M. Welch and P. Blower, *J. Biol. Inorg. Chem.*, 2002, **7**(3), 249–259.
12 C. Kelly, M. Kelly, R. Bejot, S. Bayly, Q. Guo, J. Dilworth, V. Gouverneur, M. Brady and J. Declerck, *J. Nucl. Med.*, 2008, **49**, 122P.
13 J. P. Holland, F. I. Aigbirhio, H. M. Betts, P. D. Bonnitcha, P. Burke, M. Christlieb, G. C. Churchill, A. R. Cowley, J. R. Dilworth, P. S. Donnelly, J. C. Green, J. M. Peach, S. R. Vasudevan and J. E. Warren, *Inorg. Chem.*, 2007, **46**(2), 465–485.
14 M. Christlieb, H. S. R. Struthers, P. D. Bonnitcha, A. R. Cowley and J. R. Dilworth, *Dalton Trans.*, 2007, 5043–5054.
15 S. R. Bayly, R. C. King, D. J. Honess, P. J. Barnard, H. M. Betts, J. P. Holland, R. Hueting, P. D. Bonnitcha, J. R. Dilworth, F. I. Aigbirhio and M. Christlieb, *J. Nucl. Med.*, 2008, **49**(11), 1862–1868.
16 P. D. Bonnitcha, A. L. Vavere, J. S. Lewis and J. R. Dilworth, *J. Med. Chem.*, 2008, **51**(10), 2985–2991.
17 M. Glaser and E. Arstad, *Bioconjugate Chem.*, 2007, **18**(3), 989–993.
18 H. C. Kolb, M. G. Finn and K. B. Sharpless, *Angew. Chem., Int. Ed.*, 2001, **40**(11), 2004–2021.
19 V. V. Rostovtsev, L. G. Green, V. V. Fokin and K. B. Sharpless, *Angew. Chem., Int. Ed.*, 2002, **41**, 2596–2599.
20 C. W. Tornøe, C. Christensen and M. Meldal, *J. Org. Chem.*, 2002, **67**, 3057–3064.
21 G. Vaidyanathan and M. R. Zalutsky, *Nat. Protoc.*, 2006, **1**(4), 1655–1661.
22 R. Bejot, T. Fowler, L. Carroll, S. Boldon, J. E. Moore, J. Declerck and V. Gouverneur, *Angew. Chem., Int. Ed.*, 2009, **48**(3), 586–589.
23 A. R. Cowley, J. R. Dilworth, P. S. Donnelly, A. D. Gee and J. M. Heslop, *Dalton Trans.*, 2004, 2404–2412.
24 J. L. J. Dearling, J. S. Lewis, D. W. McCarthy, M. J. Welch and P. J. Blower, *Chem. Commun.*, 1998, 2531–2532.

