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PAPER

Copper-free click chemistry with the short-lived positron emitter fluorine-18†

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The copper-free strain-promoted click chemistry between ^{18}F-labeled aza-dibenzocyclooctyne [**^{18}F]FB-DBCO** and various azides is described. [**^{18}F]FB-DBCO** was prepared in 85% isolated radiochemical yield (decay-corrected) through acylation of amino aza-dibenzocyclooctyne **1** with *N*-succinimidyl 4-[^{18}F]fluorobenzoate ([^{18}F]SFB). [**^{18}F]FB-DBCO** showed promising radiopharmacological profil with fast blood clearance as assessed with dynamic small animal PET studies. Metabolic stability of [**^{18}F]FB-DBCO** was 60% of intact compound after 60 min post injection in normal Balb/C mice and blood clearance half-life was determined to be 53 s based on the time-activity-curve (TAC). Copper-free click chemistry was performed with various azides at low concentrations (1–2 μM) which differed in their structural complexity in different solvents (methanol, water, phosphate buffer and in bovine serum albumin (BSA) solution). Reaction proceeded best in methanol (>95% yield after 15 min at room temperature), whereas reaction in BSA required longer reaction times of 60 min and 40 °C upon completion.

Introduction

The development of radiolabeled molecular probes for molecular imaging with positron emission tomography (PET) and single-photon emission computed tomography (SPECT) has become a complex chemical science. Especially PET as the most sophisticated imaging methodology has evolved into a powerful non-invasive molecular imaging technique which provides functional information on physiological, biochemical and pharmacological processes in living subjects.[1] Among the available PET radionuclides, short-lived positron emitter fluorine-18 (^{18}F, $t_{1/2}$ = 109.8 min) is particularly useful due to its favorable nuclear and chemical properties.[2] ^{18}F has found numerous applications for the labeling of both, small molecules and compounds of high molecular weight like peptides, proteins, and oligonucleotides. However, radiochemistry with ^{18}F differs significantly from conventional chemistry. Radiosyntheses involving ^{18}F require fast and efficient reactions while considering the extraordinary stoichiometry resulting from the typically produced submicromolar amounts of radiolabeled compounds.

In recent years bioorthogonal chemistry has entered into many fields of chemical, biological, and material sciences.[3] Bioorthogonal chemical reporter strategy has emerged as a versatile method for the labeling of biomolecules like nucleic acids, carbohydrates, peptides, and proteins.

An especially powerful set of bioorthogonal chemistry reactions is summerized under the term "click chemistry", which has also strongly influenced current radiopharmaceutical chemistry.[4] Click chemistry describes a class of reactions using several selective and modular building blocks to create heteroatom C–X–C links enabling chemoselective ligation reactions to label biologically relevant compounds. Bioorthogonal click reactions include Staudinger ligation, Cu(I)-catalyzed [3 + 2] azide-alkyne cycloaddition, copper-free strain-promoted [3 + 2] azide-alkyne cycloaddition, and various reactions based on inverse-electron demand Diels–Alder reactions. These click chemistry concepts have been applied to the labeling of molecular probes, covalent labeling of active enzymes, site- and residue-specific labeling of proteins, labeling of cell surface glycans, and, more recently, to *in vivo* chemistry for pretargeted imaging and therapy of cancer.[5]

Cu(I)-catalyzed [3 + 2] azide-alkyne cycloaddition and Staudinger ligation have successfully introduced into organic PET chemistry with the short-lived positron emitter ^{18}F.[6,7] As a result, numerous ^{18}F-labeled PET radiotracers have been prepared *via* Cu(I)-assisted cycloaddition between azides and terminal alkynes and to a lesser extent through Staudinger ligation. Advances of click chemistry for radiolabeling reactions with ^{18}F and other radionuclides for molecular imaging purposed have been recently summarized in excellent reviews.[4]

However, slow reaction kinetics as encountered for the Staudinger ligation and the use of cytotoxic copper have led to the search of alternative fast and copper-free click chemistry concepts applicable to radiochemisty with the short-lived positron emitter ^{18}F for molecular probe development and pretargeted molecular imaging *in vivo*.

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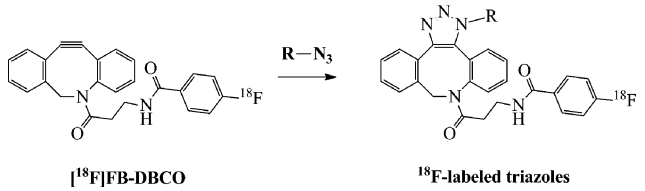
In this work we report the first example of radiolabeling reactions with ^{18}F employing copper-free click reaction based on strain-promoted cycloaddition reactions using an ^{18}F-labeled aza-dibenzocyclooctyne derivative (**[^{18}F]FB-DBCO**). The concept was tested through the reaction of **[^{18}F]FB-DBCO** with various azides to evaluate versatility of the reaction for rapid incorporation of short-lived positron emitter ^{18}F under mild conditions (Scheme 1).



Scheme 1 Copper-free click chemistry between ring-strained [^{18}F]FB-DBCO and various azides to give ^{18}F-labeled triazoles. Only one regioisomer is shown.

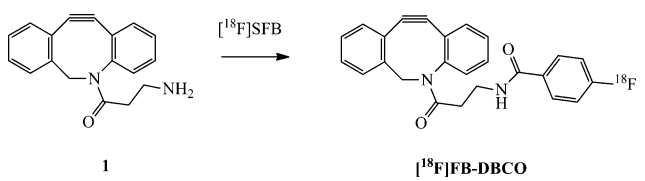
Moreover, we also studied metabolic stability and radiopharmacological profile of **[^{18}F]FB-DBCO** by means of small animal PET in mice. Copper-free click chemistry reaction in buffer and bovine serum albumin (BSA) was studied to determine potential of the reaction for future pretargeted click chemistry *in vivo*.

Results and discussion

Synthesis and radiopharmacological evaluation of ^{18}F-labeled aza-dibenzocyclooctyne [^{18}F]FB-DBCO

N-(3-Aminopropionyl)-5,6-dihydro-11,12-didehydrodibenzo-[*b*,*f*]azocine **1** is commercially available (Click Chemistry Tools), and a large variety of analogues can easily be synthesized through acylation reaction using primary amine group in compound **1**. For radiolabeling of compound **1** with short-lived positron emitter ^{18}F we decided to apply acylation reaction with prominent bifunctional labeling agent 4-succinimidyl-[^{18}F]fluorobenzoate ([^{18}F]SFB).

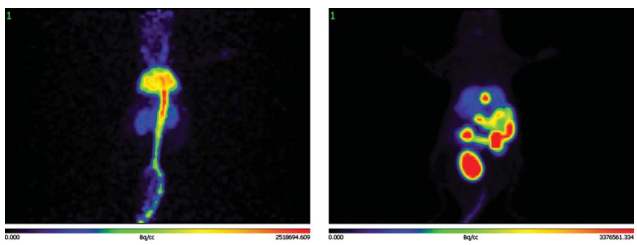
We and others showed that [^{18}F]SFB is a highly suitable prosthetic group for fast and convenient radiolabeling of a broad variety of different compounds under mild reaction conditions. [^{18}F]SFB can easily be prepared according to various automated synthesis procedures recently published in literature making prosthetic group [^{18}F]SFB readily available for acylation reactions with ^{18}F.[8] The preparation of **[^{18}F]FB-DBCO** *via* acylation of primary amine in compound **1** with [^{18}F]SFB is depicted in Scheme 2.



Scheme 2 Radiosynthesis of [^{18}F]FB-DBCO employing acylation with bifunctional labeling agent [^{18}F]SFB.

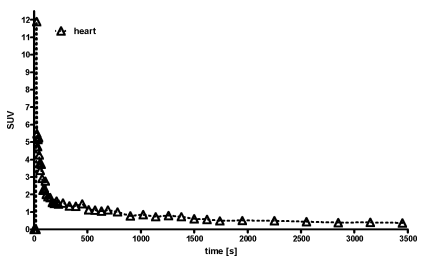
Primary amine **1** and [^{18}F]SFB were dissolved in acetonitrile and incubated for 30 min at 40 °C. Monitoring the progress of the reaction with radio-TLC indicated complete conversion (>95%) of [^{18}F]SFB into **[^{18}F]FB-DBCO** after 30 min. After HPLC purification, [^{18}F]FB-DBCO was obtained in excellent radiochemical yields of 85% (decay-corrected) within 60 min starting from [^{18}F]SFB. The radiochemical purity exceeded 95%.

Radiopharmacological profile of **[^{18}F]FB-DBCO** was evaluated in normal Balb/C mice using small animal PET and radiometabolite analysis. **[^{18}F]FB-DBCO** was completely stable for 60 min in methanol and phosphate buffer (pH 7.4). Metabolite analysis of arterial blood samples confirmed that compound **[^{18}F]FB-DBCO** displayed reasonable metabolic stability *in vivo*, reaching 60% of intact compound after 60 min post injection in normal Balb/C mice as determined with radio-TLC. Dynamic small animal PET studies showed rapid clearance of **[^{18}F]FB-DBCO** from the blood and from most other tissues and organs. Most activity was found in the bladder, gall bladder and intestines (Scheme 3).



Scheme 3 Representative small animal PET images (maximum intensity projection) of a normal Balb/C mouse 5 s (left) and 60 min (right) after single intravenous administration of 6 MBq of **[^{18}F]FB-DBCO**.

The blood clearance half-life of **[^{18}F]FB-DBCO** was determined to be 53 s based on the time-activity-curve (TAC) over the heart derived from dynamic small animal PET study (Scheme 4).



Scheme 4 Representative time activity curve (TAC) of the blood after single administration of [^{18}F]FB-DBCO.

The rapid blood clearance and the reasonable metabolic stability are important requirements for using ^{18}F-labeled aza-dibenzo-cyclooctyne **[^{18}F]FB-DBCO** as radiolabeled low molecular weight compound within the proposed pretargeted strategy *in vivo* based on copper-free click chemistry with corresponding azides.

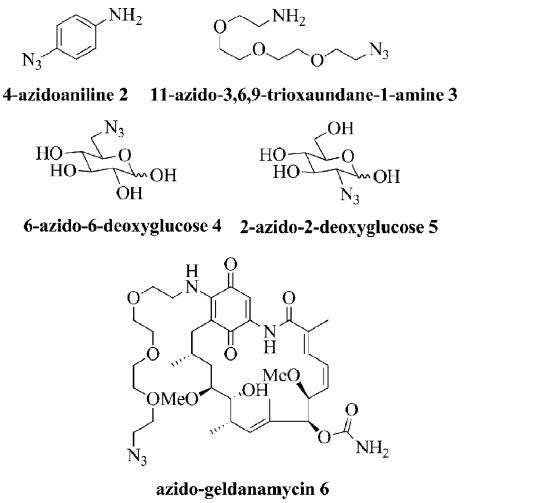
Copper-free strain-promoted click chemistry of [^{18}F]FB-DBCO with various azides

^{18}F-Labeled aza-dibenzocyclooctyne **[^{18}F]FB-DBCO** was further studied in several strain-promoted click chemistry reactions with different azides. Recently, aza-dibenzo-cyclooctynes have been used for fast and efficient enzyme PEGylation[5b] and the preparation of metabolically labeled glycoconjugates of living cells

through copper-free [3 + 2] cycloaddition with various azides.[5f] This approach was further expanded through the use of nitrones in strain-promoted alkyne-nitrone cycloadditions to form the corresponding *N*-alkylated isoxazolines with very fast reaction kinetics.[9]

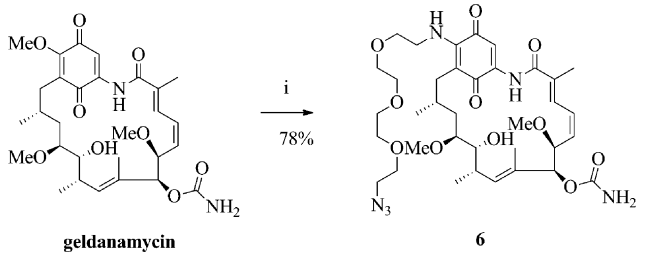
We treated [^{18}F]**FB-DBCO** with various azides possessing different structural complexities. [^{18}F]**FB-DBCO** was reacted with simple aromatic azides like 4-azidoaniline **2** and aliphatic azides like 11-azido-3,6,9-trioxaundane-1-amine **3**, carbo-hydrates like 2-azido-2-deoxyglucose **4** and 6-azido-6-deoxy-glucose **5**, and complex natural product geldanamycin **6**, which has been modified with a linker containing an azide group. Geldanamycin is an inhibitor of heat-shock protein 90 (Hsp90), which has become an important drug target in oncology over the last years.[10]

Structures of all azides used in the copper-free click chemistry reaction are given in Scheme 5.



Scheme 5 Structures of azides used for copper-free strain promoted click chemistry.

Azides **2**, **3**, **4**, and **5** are commercially available, whereas azido-functionalized geldanamycin derivative **6** was prepared according Scheme 6.



Scheme 6 *Reaction conditions*: (i) 11-azido-3,6,9-trooxaundane-1-amine **3**, DMF, room temperature, 30 min.

As a methoxy quinone, geldanamycin rapidly undergoes Michael addition and β-elimination with primary amines to furnish the corresponding vinylogous amine product.[10c,d] Treatment of geldanamycin with a five-fold excess of 11-azido-3,6,9-trioxaundane-1-amine **3** afforded azido-functionalized geldanamycin derivative **6** in 78% isolated yield as a purple solid.

All reference compounds for copper-free strain-promoted click chemistry were obtained through treatment of azides displayed in Scheme 5 with FB-DBCO according to Scheme 1. In contrast to the reaction depicted in Scheme 1 employing tracer concentrations of [^{18}F]**FB-DBCO**, 1.5 to 2.0 equivalents of respective azides **2**, **3**, **4**, **5**, and **6** were reacted with one equivalent of FB-DBCO. The reaction resulted in the formation of two distinct regioisomers (1,4-triazole and 1,5-triazole regioisomer), which is a well known phenomenon for this type of reaction when no copper catalyst is used.[11] However, in some cases both regioisomers were not separable by HPLC-purification. All reactions were performed on a μM-scale (15–30 μM), and products were isolated using HPLC purification. Characterization of corresponding alkylated triazole derivatives **7**, **8**, **9**, **10** and **11** was performed with ^{1}H NMR and/or mass spectrometry. Cold FB-DBCO was prepared according to Scheme 2 through reaction of amine **1** with 1.3 equivalents of SFB in CH_2Cl_2 in good chemical yield of 72% after purification with column chromatography.

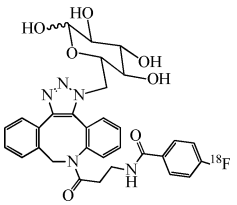
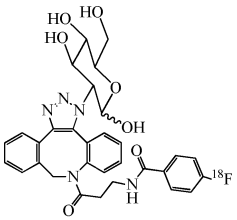
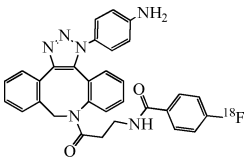
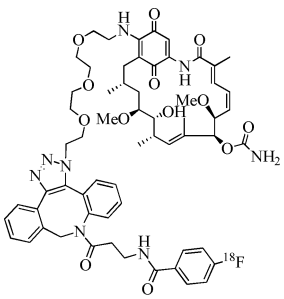
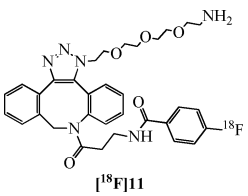
Reaction of [^{18}F]**FB-DBCO** with azides **2–6** at low concentrations (1–2 μM) was performed at room temperature and 40 °C, and in different solvents, including methanol, water, phosphate buffer (pH 7.4), and bovine serum albumin (BSA) solution to evaluate speed of the reaction under different conditions according to Scheme 1. The results are summarized in Table 1.

In the first set of reactions we tested readily water-soluble glucose derivatives 6-azido-6-deoxyglucose **4** and 2-azido-2-deoxyglucose **5** (entries 1 and 2). Reaction of 6-azido-6-deoxyglucose **4** with [^{18}F]**FB-DBCO** in methanol at room temperature gave desired click chemistry product [^{18}F]**7** in almost quantitative yield after a reaction time of 15 min at room temperature. This is consistent with the previously reported high reactivity of aza-dibenzocyclooctynes with various azides.[5b,5f] Reactivity was slightly decreased when reaction was performed in phosphate buffer (pH 7.4) and BSA solution. In the latter case a reaction time of 60 min was necessary to achieve a 90% conversion of [^{18}F]**FB-DBCO** into triazole [^{18}F]**7** as determined by radio-TLC analysis.

Somewhat lower conversion rates were achieved when 2-azido-2-deoxyglucose **5** was used as the azide source in the click chemistry reaction (entry 2). This observation can be explained by the less accessible azide group in 2-azido-2-deoxyglucose **5** in comparison to 6-azido-6-deoxyglucose **4**.

As an aromatic azide, 4-azidoaniline **2** showed comparable reactivity in methanol as the solvent as found for both azido-substituted glucose derivatives **4** and **5** (entry 3). However, solubility problems prevented performance of the click reaction in phosphate buffer and BSA solution. Complex azido-functionalized geldanamycin derivative **6** also posed solubility problems, and reaction with [^{18}F]**FB-DBCO** proceeded in a DMSO/water mixture to afford a 69% conversion of [^{18}F]**FB-DBCO** into click chemistry product [18F]**10** after a reaction time of 60 min at 40 °C (entry 4). Aliphatic azide 11-azido-3,6,9-trioxaundane-1-amine **3** represents an interesting azide-containing linker for potential conjugation and functionalization of specific targeting vectors like antibodies and proteins for subsequent pretargeting. Compound **3** is readily soluble in water, and the reaction with [^{18}F]**FB-DBCO** proceeded in good radiochemical yields of 75% based upon conversion of

Table 1 Copper-free click chemistry with [^{18}F]FB-DBCO

Entry	Reaction conditions[a]	RCY (%)[b]	Product[c]
1	A	98	 [^{18}F]7
	B	97	
	C	90	
2	A	85	 [^{18}F]8
	B	69	
	C	75	
3	A	82	 [^{18}F]9
4	D	69	 [^{18}F]10
5	E	75	 [^{18}F]11

[a] All reactions were carried out with 1–2 μM of the respective azide. The following reaction conditions were used: A) methanol, room temperature, 15 min; B) phosphate buffer, 40 °C, 30 min; C) 3.50% bovine serum albumin (BSA) solution in water, room temperature, 60 min; D) DMSO/water (1/1), 40 °C, 60 min.; E) water, 40 °C, 30 min. [b] Radiochemical yield determined by radio-TLC representing the percentage of the F-labeled triazole product present in the reaction mixture. [c] Only one regioisomer formed during the reaction is shown.

[**^{18}F]FB-DBCO** into triazole [**^{18}F]11** after a reaction time of 30 min at 40 °C (entry 5).

Summary and conclusions

We have developed a convenient and simple method for copper-free click chemistry with the short-lived positron emitter fluorine-18. To the best of our knowledge, this is the first report on the application of copper-free strain-promoted click chemistry involving aza-dibenzocyclooctynes in ^{18}F chemistry. The commercial availability of amine-functionalized aza-dibenzocyclooctyne **1** and the readily availability of prosthetic group [^{18}F]SFB make the proposed ^{18}F-labeled building block [**^{18}F]FB-DBCO** an ideal starting material for subsequent copper-free strain-promoted click chemistry reactions.

The copper-free strain-promoted click chemistry with [**^{18}F]FB-DBCO** was tested with various azides, which differed in their structural complexity ranging from simple aliphatic and aromatic azides, azido-functionalized carbohydrates to complex natural products like azido-functionalized geldanamycin. The presented experiments clearly demonstrate the feasibility to use copper-free strain-promoted click chemistry between aza-dibenzocyclooctyne [**^{18}F]FB-DBCO** and various azides at low concentrations as little as 1–2 μM for the convenient radiolabeling of small molecules under mild conditions. Moreover, this approach has the potential to be extended to other classes of compounds like peptides, proteins and oligonucleotides. The opportunity to perform the reaction in the absence of copper under physiological conditions makes this approach especially attractive for the mild radiolabeling of proteins and antibodies with the short-lived positron emitter fluorine-18. However, compared to alternative bioorthogonal reactions like tetrazine/*trans*-cyclooctene cycloaddition, our approach proceeded with much slower reaction rate which seems not to be feasible for *in vivo* chemistry-based pretargeting.[4h] Alternative strategies which include more reactive aza-dibenzocyclooctynes and the application of nitrones as more reactive coupling partners are currently under investigation.

Experimental

General methods

^{1}H NMR and ^{13}C NMR spectra were recorded on a 500 MHz or 600 MHz (Varian Unity) spectrometers. Chemical shifts are given in ppm referenced to internal standards (s = singlet, bs = broad singlet, d = doublet, dd = doublet of doublet, ddd = doublet of doublet of doublet, t = triplet, m = multiplet). Mass spectra were recorded using a Micromass ZabSpec Hybrid Sector-TOF by positive mode electrospray ionization. Thin layer chromatography (TLC) was monitored using HF_{254} silica gel. All solvents were dried and/or distilled prior to utilization. Crude reaction mixtures were analyzed by TLC and HPLC. HPLC analysis was performed on a semi-preparative Luna C18 column (100 Å, 10 μm, 250 × 10 mm).

The eluting solvent started with an acetonitrile/water gradient from (15/85 to 50/50, v/v) for 8 min at a flow rate of 3 mL min^{-1}, followed by a 8 min gradient from (50/50 to 70/30, v/v) and finally a 14 min at 70/30. UV detection was performed at 220 nm and 254 nm. Radioactivity detection was performed using a well-scintillation NaI (Tl) detector.

Chemical syntheses

5,6-Dihydro-11,12-didehydrodibenzo-[*b*,*f*]azocino-3-oxoprop-yl-4-fluorobenzamide (FB-DBCO). *N*-(3-Aminopropionyl)-5,6-dihydro-11,12-didehydrodibenzo-[*b*,*f*]azocine **1** (34 mg, 123 µmol) was dissolved in 1 mL of dichloromethane and added drop-wise to a solution of SFB (39 mg, 164 µmol) in dichloromethane (4 mL). The reaction mixture was stirred for 20 min at 25 °C and progress of the reaction was monitored by TLC (hexane/ethyl acetate 1/1). Upon completion, the reaction mixture was concentrated under reduced pressure and the residue was then purified by column chromatography (hexane/ethyl acetate 1/1) to afford 35 mg (72%) of the desired product as a pale-yellow oil. TLC analysis, R_f 0.5; ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.08 (ddd, 1H, J = 3.9 Hz, J = 7.7 Hz, J = 16.6 Hz), 2.53(ddd, 1H, J = 3.8 Hz, J = 7.2 Hz, J = 16.7 Hz), 3.42–3.56 (m, 2H), 3.67 (d, 1H, J = 13.9 Hz), 5.16 (d, 1H, J = 13.9 Hz), 6.66 (t, 1H, J = 12.0 Hz), 6.98–7.04 (M, 2H), 7.18 (dd, 2H, J = 0.8 Hz, J = 7.5 Hz), 7.29–7.43 (M, 6H), 7.47–7.52 (M, 2H), 7.71 (d, 1H, 7.5 Hz). ^{13}C NMR (150.8 MHz, $CDCl_3$): 34.86, 35.63, 55.52, 107.75, 114.7, 115.3, 115.4, 122.5, 122.9, 125.6, 127.3, 128.2, 128.5, 128.6, 128.9, 129.1, 129.2, 130.7, 132.2, 147.9, 150.9, 166.0, 172.3. *m/z* (ESI) $C_{25}H_{19}FN_2O_2$ ([M+Na$^+$]) calcd. 421.1322, found 421.1322.

17-[11-Azido-3,6,9-trioxaundane-1-amino]-17-desmethoxy geldanamycin 6. Geldanamycin (100 mg, 178.4 µmol) and 11-azido-3,6,9-trioxaundane-1-amine **3** (177 µL, 892 µmol) were dissolved in DMF (5 mL). The mixture was stirred at room temperature for 30 min. HCl (1 N, 50 mL) was added and the mixture was extracted with dichloromethane. After drying with $MgSO_4$ and evaporation of the solvent, the residue was purified by column chromatography (methanol/CH_2Cl_2 1/9) to give 104 mg (78%) of the desired product as a purple solid. TLC analysis (methanol/CH_2Cl_2 1/9), R_f 0.7. HPLC analysis, t_R = 18.3 min. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.97 (d, J = 6.5 Hz, 3H), 0.99 (d, J = 6.5 Hz, 3H), 1.80 (s, 3H, CH_3), 2.02 (s, 3H, CH_3), 3.27 (s, 3H, OCH_3), 3.36 (s, 3H, OCH_3), 3.39 (t, J = 5.2 Hz, 2H), 3.45 (m, 1H), 3.48–3.57 (m, 2H), 3.69 (m, 6H), 4.30–4.32 (m, 2H), 5.19 (s, 1H), 5.86 (t, J = 10.9 Hz, 1H), 5.90 (d, J = 9.4 Hz, 1H), 6.58 (t, J = 10.9 Hz, 1H), 6.66 (m, 1H), 6.95 (bd, J = 10.9 Hz, 1H), 7.27 (s, 1H), 9.17 (s, 1H). *m/z* (ESI) $C_{36}H_{54}N_6O_{11}$ ([M+H]$^+$) calcd. 747.3929, found 747.3929.

General procedure for copper-free click chemistry of FB-DBCO with azides 2–6. 25 µmol of respective azide compound and 5 mg (12.5 µmol) of FB-DBCO was stirred in methanol (1.5 mL) at room temperature for 30 min. Product was purified by semi-preparative HPLC, and formed regioisomers were collected and analysed with HR-MS.

FB-DBCO-coupled triazole-7. Regioisomer 1 (48%), t_R = 12.0–12.3 min.; regioisomer 2 (37%) 12.5–12.8 min *m/z* (ESI) $C_{31}H_{30}FN_5O_7$ ([M+Na$^+$]) calcd. 626.2021, found 626.2014 (regioisomer 1), 626.2013 (regioisomer 2).

FB-DBCO-coupled triazole-8. Regioisomer 1 (46%), t_R = 12.0–12.3 min.; regioisomer 2 (39%) 12.5–12.8 min *m/z* (ESI) $C_{31}H_{30}FN_5O_7$ ([M+Na$^+$]) calcd. 626.2, found 626.2 (regioisomer 1), 626.2 (regioisomer 2).

FB-DBCO-coupled triazole-9. Yield: 95%. Formation of inseparable regioisomers. t_R = 15.1–15.5 min. *m/z* (ESI) $C_{31}H_{25}FN_6O_2$ ([M+Na$^+$]) 12 calcd. 555.2, found 555.2.

FB-DBCO-coupled triazole-10. Compound **6** (5.1 mg, 6.8 µmol) and FB-DBCO (2 mg, 5 µmol) were stirred in MeOH/CH_2Cl_2 (1 : 1, 1.5 mL) for 10 h at 40 °C. The two formed regioisomers could not be separated by HPLC. Regioisomers eluted between 18.6–18.9 min. Yield: 51%. ^{1}H NMR (500 MHz, $CDCl_3$): 0.98 (d, 3H, J = 6.5 Hz), 1.11 (d, 3H, J = 6.5 Hz), 1.64–1.82 (M, 9H), 2.02 (s, 3H), 2.08 (ddd, 1H, J = 3.9 Hz, J = 7.7 Hz, J = 16.6 Hz), 2.40 (dd, 1H, J = 10.7 Hz, J = 10.3 Hz), 2.53(ddd, 1H, J = 3.8 Hz, J = 7.2 Hz, J = 16.7 Hz), 2.67 (d, 1H, J = 13.0 Hz), 2.74 (m, 1H), 3.26 (s, 3H), 3.35 (s, 3H) 3.42–3.62 (m, 20H), 3.67 (d, 1H, J = 13.9 Hz), 4.30 (d, 1H, J = 10 Hz), 5.16 (d, 1H, J = 13.9 Hz), 5.19 (s, 1H), 5.85 (d, 1H, J = 15.5 Hz), 5.87 (d, 1H, J = 15.7 Hz), 6.09 (bs, 1H), 6.25 (bs, 1H), 6.58 (t, 1H, J = 11.5 Hz), 6.66 (t, 1H, J = 12.0 Hz), 6.95–7.04 (M, 3H), 7.18 (dd, 2H, J = 0.8 Hz, J = 7.5 Hz), 7.22–7.26 (M, 3H), 7.29–7.43 (M, 5H), 7.47–7.52 (M, 2H), 7.71 (d, 1H, 7.5 Hz). *m/z* (ESI) $C_{61}H_{74}FN_8O_{13}$ ([M+Na$^+$]) 12 calcd. 1167.5, found 1167.5.

FB-DBCO-coupled triazole-11. Yield: 49%. Formation of inseparable regioisomers. t_R = 13.5–13.8 min. *m/z* (ESI) $C_{33}H_{37}FN_6O_5$ ([M+Na$^+$]) 13 calcd. 639.3, found 639.2.

Radiosyntheses

No-carrier added aqueous [^{18}F]fluoride was produced using the TR19/9 (Advanced Cyclotron systems Inc.) cyclotron at the Edmonton PET Center (Ep = 17.8 MeV) by irradiation of enriched [^{18}O]water (3.0 mL, Rotem Germany, >98% enrichment) *via* the ^{18}O(p,n)^{18}F nuclear reaction. [^{18}F]SFB was synthesized following the procedure reported by Mäding *et al.*[8a] Overall synthesis was usually carried out in less than 45 min providing [^{18}F]SFB in decay-corrected yield of 60% and in a radiochemical purity greater 95%.

5,6-Dihydro-11,12-didehydrodibenzo-[*b*,*f*]azocino-3-oxoprop-yl-4-[^{18}F]fluorobenzamide ([^{18}F]FB-DBCO). *N*-(3-Aminopropionyl)-5,6-dihydro-11,12-didehydrodibenzo-[*b*,*f*]azocine **1** (1.5 mg, 5.4 µmol) was dissolved in acetonitrile (500 µL) of containing 100 MBq of [^{18}F]SFB. The reaction was stirred for 30 min at 40 °C. Progress of the reaction was monitored by radio-TLC (hexane/ethyl acetate 1/1). Upon completion, the reaction mixture was concentrated and the residue was purified using semi-preparative HPLC. **[^{18}F]FB-DBCO** was isolated in 95% radiochemical yield (decay-corrected). The radiochemical purity exceeded 95%. Radio-TLC (hexane/ethyl acetate 1/1) R_f 0.5, t_R = 18.6 min.

General procedure for copper-free click chemistry of [^{18}F]FB-DBCO with azides 2–6. HPLC-collected peak containing **[^{18}F]FB-DBCO** (20–50 MBq) was evaporated to dryness at 40 °C under reduced pressure. The residue was re-solubilized in methanol, phosphate buffer (pH 7.4), 3.5% BSA solution in water, water, and DMSO/water containing 1–2 µM of the respective azide. All reactions were carried out at 25 °C or 40 °C. Identity of the products was confirmed by radio-HPLC analysis through co-injection of respective reference compounds.

The following reaction conditions were applied:

A) methanol, room temperature, 15 min.

B) phosphate buffer, 40 °C, 30 min.

C) 3.5 BSA solution in water, room temperature, 60 min.

D) DMSO/water (1/1), 40 °C, 60 min.

E) water, 40 °C, 30 min.

[^{18}F]FB-DBCO-coupled triazole-[^{18}F]7. Condition A: Radiochemical yield: 98%; Condition B: Radiochemical yield: 97%; Condition C: Radiochemical yield: 90%, t_R = 11.8–12.5 min for both isomers.

[^{18}F]FB-DBCO-coupled triazole-[^{18}F]8. Condition A: Radiochemical yield: 85%; Condition B: Radiochemical yield: 69%; Condition C: Radiochemical yield: 75%, t_R = 11.9–12.5 min for both isomers.

[^{18}F]FB-DBCO-coupled triazole-[^{18}F]9. Condition A: Radiochemical yield: 82%, t_R = 14.9–15.2 min.

[^{18}F]FB-DBCO-coupled triazole-[^{18}F]10. Condition D: Radiochemical yield: 69%, t_R = 13.1–13.4 min.

[^{18}F]FB-DBCO-coupled triazole-[^{18}F]11. Condition E: Radiochemical yield: 75%, t_R = 18.3–18.6.

Small animal PET in normal mice

All animal experiments were carried out in accordance with guidelines of the Canadian Council on Animal Care (CCAC) and were approved by the local animal care committee of the Cross Cancer Institute. Positron emission tomography (PET) experiments were performed using normal BALB/c mice. The mice were not fasted prior to imaging experiments. The animals were anesthetized through inhalation of isoflurane in 40% oxygen/60% nitrogen (gas flow, 1 L min^{-1}) and body temperature was kept constant at 37 °C for the entire experiment. Mice were positioned and immobilized in the prone position with their medial axis parallel to the axial axis of the scanner and their thorax, abdomen and hind legs (organs of interest: heart, kidneys, bladder,liver) in the centre of the field of view of the microPET® R4 scanner (Siemens Preclinical Solutions, Knoxville, TN, USA). A transmission scan for attenuation correction was not acquired. 4–6 MBq of [^{18}F]**FB-DBCO** in 100–150 µL saline containing 10% of EtOH was injected through a needle catheter into the tail vein. Data acquisition continued for 60 min in 3D list mode. The frames were reconstructed using MAP. The pixel size was 0.085 by 0.085 by 0.12 cm and the resolution in the centre field of view was 1.8 mm. No correction for partial volume effects was performed. The image files were further processed using the ROVER v2.0.21 software (ABX GmbH, Radeberg, Germany). Masks for defining 3D regions of interest (ROI) were set and the ROI's were defined by thresholding. ROI time-activity curves (TAC) were generated for subsequent data analysis. Standardized uptake values (SUV = (activity/mL tissue)/(injected activity/body weight), mL g^{-1}) were calculated for each ROI.

Metabolite analysis

15 MBq [^{18}F]**FB-DBCO** in 100 to 150 µL saline containing 10% of EtOH was injected as a bolus through a catheter into the tail vein of isoflurane anesthetized BALB/c mice. Before radiotracer injection, mice were heparinized by subcutaneous injection of 50 µL heparin (1000 I.U.) and kept under anesthesia during the course of the experiment. At selected time points of 5 and 60 min, the animal was sacrificed and a whole blood sample (approximately 500 µL) was collected. Blood cells were separated by immediate centrifugation (5 min at 13,000 rpm). Proteins within the sample were precipitated by adding ~800 µL methanol to the supernatant following a second centrifugation step (5 min at 13,000 rpm). TLC samples from the plasma fraction were developed and analyzed using radio-TLC as described above.

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References

1 (*a*) M. E. Phelps, *Proc. Natl. Acad. Sci. U. S. A.*, 2000, **97**, 9226; (*b*) M. E. Phelps, *J. Nucl. Med.*, 2000, **41**, 661; (*c*) T. F. Massed and S. S Gambhir, *Genes Dev.*, 2003, **17**, 545; (*d*) A. M. J. Paans, A. van Waarde, P. H. Elsinga, A. T. M. Willemsen and W. Vaalburg, *Methods*, 2002, **27**, 195; (*e*) T. J. McCarthy, S. W. Schwarz and M. J. Welch, *J. Chem. Educ.*, 1994, **71**, 830; (*f*) J. Czernin and M. J. Phelps, *Annu. Rev. Med.*, 2002, **53**, 89; (*g*) J. S. Fowler and A. P. Wolf, *Acc. Chem. Res.*, 1997, **30**, 181.

2 (*a*) P. H. Elsinga;, *Methods*, 2002, **27**, 208; (*b*) V. W. Pike, *Drug Inf. J.*, 1997, **31**, 997; (*c*) B. Langström, T. Kihlberg, M. Bergström, G. Antoni, M. Björkman, B. H. Forngren, P. Hartvig, K. Markides, U. Yngve and M. Ögren, *Acta Chem. Scand.*, 1999, **53**, 651; (*d*) M. C. Lasne, C. Perrio, J. Rouden, L. Barré, D. Roeda, F. Dolle and C. Crouzel, *Top. Curr. Chem.*, 2002, **222**, 201.

3 (*a*) P. Wu, A. K. Feldman, A. K. Nugent, C. J. Hawker, A. Scheel, B. Voit, J. Pyun, J. M. J. Frechet, K. B. Sharpless and V. V. Fokin, *Angew. Chem., Int. Ed.*, 2004, **43**, 3928; (*b*) M. Malkoch, R. J. Thibault, E. Drockenmuller, M. Messerschmidt, B. Voit, T. P. Russell and C. J. Hawker, *J. Am. Chem. Soc.*, 2005, **127**, 14942; (*c*) Q. Wang, T. R. Chan, R. Hilgraf, V. V. Fokin, K. B. Sharpless and M. G. Finn, *J. Am. Chem. Soc.*, 2003, **125**, 3192; (*d*) P. M. Gramlich, C. T. Wirges, A. Manetto and T. Carell, *Angew. Chem., Int. Ed.*, 2008, **47**, 8350; (*e*) S. T. Laughlin and C. R. Bertozzi, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **106**, 12; (*f*) H. C. Kolb, M. G. Finn and K. B. Sharpless, *Angew. Chem., Int. Ed.*, 2001, **40**, 2004; (*g*) M. Kohn and R. Breinbauer, *Angew. Chem., Int. Ed.*, 2004, **43**, 3106; (*h*) I. Chen and A. Y. Ting, *Curr. Opin. Biotechnol.*, 2005, **16**, 35; (*i*) J. M. Antos and M. B. Francis, *Curr. Opin. Chem. Biol.*, 2006, **10**, 253; (*j*) M. E. Hahn and T. W. Muir, *Trends Biochem. Sci.*, 2005, **30**, 26; (*k*) M. B. Soellner, B. L. Nilsson and R. T. Raines, *J. Am. Chem. Soc.*, 2006, **128**, 8820; (*l*) F. L. Lin, H. M. Hoyt, H. van Halbeek, R. G. Bergman and C. R. Bertozzi, *J. Am. Chem. Soc.*, 2005, **127**, 2686; (*m*) R. V. Kolakowski, N. Shangguan, R. R. Sauers and L. J. Williams, *J. Am. Chem. Soc.*, 2006, **128**, 5695; (*n*) J. A. Johnson, M. G. Finn, J. T. Koberstein and N. J. Turro, *Macromol. Rapid Commun.*, 2008, **29**, 1052–1072 .

4 (*a*) C. Wängler, R. Schirrmacher, P. Bartenstein and B. Wängler, *Curr. Med. Chem.*, 2010, **17**, 1092; (*b*) K. Nwe and M. W. Brechbiel, *Cancer Biother. Radiopharm.*, 2009, **24**, 289; (*c*) C. Mamat, T. Ramenda and F. Wuest, *Mini-Rev. Org. Chem.*, 2009, **6**, 21.

5 (*a*) J. A. Prescher and C. R. Bertozzi, *Nat. Chem. Biol.*, 2005, **1**, 13; (*b*) M. F. Debets, S. S. van Berkel, S. Schoffelen, F. P. Rutjes, J. C. van Hest and F. L. van Delft, *Chem. Commun.*, 2010, **46**, 97; (*c*) M. F. Debets, C. W. J. van der Doelen, F. P. J. T. Rutjes and F. L. van Delft, *ChemBioChem*, 2010, **11**, 1168; (*d*) J. M. Baskin and C. R. Bertozzi, *QSAR Comb. Sci.*, 2007, **26**, 1211; (*e*) N. K. Devaraj, R. Upadhyay, J. B. Haun, S. A. Hilderbrand and R. Weissleder, *Angew. Chem., Int. Ed.*, 2009, **48**, 7013; (*f*) X. H. Ning, J. Guo, M. A. Wolfert and G.-J. Boons, *Angew. Chem., Int. Ed.*, 2008, **47**, 2253; (*g*) N. K. Devaraj and R. Weissleder, *Acc. Chem. Res.*, 2011 in press; (*h*) R. Rossin, P. R. Verkerk, S. M. van den Bosch, R. C. Vulders, I. Verel, J. Lub and M. S. Robillard, *Angew. Chem. Int. Ed. Engl.*, 2010, **46**, 3375; (*i*) M. F. Debets, C. W. van der Doelen, F. P. Rutjes and F. L. van Delft, *ChemBioChem*, 2010, **11**, 1168; (*j*) J. M. Baskin, J. A. Prescher, S. T. Laughlin, N. J. Agard, P. V. Chang, L. A. Miller, A. Lo, J. A. Codelli and C. R. Bertozzi, *Proc. Natl. Acad. Sci. U. S. A.*, 2007, **104**, 16793.

6 (*a*) J. Marik and J. L. Sutcliffe, *Tetrahedron Lett.*, 2006, **47**, 6681; (*b*) T. L. Ross, M. Honer, P. Y. Lam PY, T. L. Mindt, V. Groehn, R. Schibli,

P. A. Schubiger and S. M. Ametamey, *Bioconjugate Chem.*, 2008, **19**, 2462; (*c*) T. Ramenda, T. Kniess, R. Bergmann, J. Steinbach and F. Wuest, *Chem. Commun.*, 2009, 7521.
7 (*a*) M. Pretze, F. Wuest, T. Peppel, M. Köckerling and C Mamat, *Tetrahedron Lett.*, 2010, **51**, 6410; (*b*) L. Carroll, S. Boldon, R. Bejot, J. E. Moore, J. Declerck and V. Gouverneur, *Org. Biomol. Chem.*, 2011, **9**, 136; (*c*) A. Gaeta, J. Woodcraft, S. Plant, J. Goggi, P. Jones, M. Battle, W. Trigg, S. K. Luthra and M. Glaser, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 4649.
8 (*a*) P. Mäding, F. Füchtner and F. Wuest, *Appl. Radiat. Isot.*, 2005, **63**, 329; (*b*) G. Vaidyanathan and M. R. Zalutsky, *Nat. Protoc.*, 2006, **1**, 1655; (*c*) F. Wuest, L. Vogler, M. Berndt and J. Pietzsch, *Amino Acids*, 2009, **36**, 283; (*d*) J. Pietzsch, R. Bergmann, K. Rode, C. Hultsch, B. Pawelke, F. Wuest and J. van den Hoff, *Nucl. Med. Biol.*, 2004, **31**, 1043.
9 X. Ning, R. P. Temming, J. Dommerholt, J. Guo, D. B. Ania, M. F. Debets, M. A. Wolfert, G. J. Boons and F. L. van Delft, *Angew. Chem., Int. Ed.*, 2010, **49**, 3065.
10 (*a*) Y. Fukuyo, C. R. Hunt and N. Horikoshi, *Cancer Lett.*, 2010, **290**, 24; (*b*) T. Taldone, W. Sun and G. Chiosis, *Bioorg. Med. Chem.*, 2009, **17**, 2225; (*c*) R. C. Clevenger, J. M. Raibel, A. M. Peck and B. S. Blagg, *J. Org. Chem.*, 2004, **69**, 4375; (*d*) Z. Q. Tian, Y. Liu, D. Zhang, Z. Wang, S. D. Dong, C. W. Carreras, Y. Zhou, G. Rastelli, D. V. Santi and D. C. Myles, *Bioorg. Med. Chem.*, 2004, **12**, 5317.
11 V. V. Rostovtsev, L. G. Green, V. V. Fokin and K. B. Sharpless, *Angew. Chem., Int. Ed.*, 2002, **41**, 2596.