Published in final edited form as:

Org Biomol Chem. 2018 January 17; 16(3): 363–366. doi:10.1039/c7ob02532g.

[¹⁸F]ODIBO: a universal prosthetic group for highly efficient radiolabelling of macromolecules *via* strain-promoted alkyne-azide cycloaddition

Mehdi Boudjemeline^a, Christopher D. McNitt^b, Vladimir V. Popik^b, and Alexey P. Kostikov^a

^aMcConnell Brain Imaging Centre, Montreal Neurological Institute, McGill University, Montreal, Canada

^bDepartment of Chemistry, University of Georgia, Athens, GA, USA

Abstract

A novel prosthetic group for efficient radiolabeling of macromolecules have been developed. [¹⁸F]oxadibenzocyclooctyne ([¹⁸F]ODIBO) is synthesized in high radiochemical yield and applied for nearly quantitative conjugation to azide-tagged peptides and proteins at room temperature and low substrate concentrations. The resulting bioconjugates are chemically and radiochemically pure and free of toxic solvents and catalysts.

Positron Emission Tomography (PET) is a molecular imaging modality which enables visualization of biochemical processes *in vivo* by detecting radioactive decay of the isotope attached to the biologically active molecules¹. Among positron-emitting isotopes, fluorine-18 is considered a gold standard for imaging applications due to its favorable chemical properties and decay characteristics ($T_{1/2} = 109.7$ min, 97% β^+ , $E(\beta^+)_{\max} = 634$ keV). The PET radiotracers based on large bioconjugates, such as peptides and proteins, have lately proved promising for clinical and pre-clinical imaging due to their high affinity and specificity to the target. However, macromolecules are often structurally incompatible with the harsh reaction conditions required to incorporate fluorine-18 and other PET isotopes, necessitating the use of so-called prosthetic groups – small organic molecules that are first radiolabeled and then conjugated to the substrate under biocompatible conditions. Given the radioactive half-life constraints, the latter reactions must be fast, selective, high yielding and preferably biocompatible, proceeding at room temperature and in non-toxic solvent – thus overlapping with the requirements of “click” reactions. Several recently discovered biocompatible conjugations have found applications in PET radiochemistry in the last two decades². Copper (I) catalyzed acetylene-azide cycloaddition (CuAAC)³, considered a prototypical “click” reaction, found somewhat limited utility in biomedical applications, particularly in syntheses of radiopharmaceuticals for clinical nuclear medicine due to the presence of Cu(I) ions⁴ which cause oxidative degradation of DNA⁵ or coordinate peptides and proteins⁶. Strict current Good Manufacturing Practices (cGMP) regulations require extensive quality control protocols to analyze each batch of radiotracer for the

Correspondence to: Alexey P. Kostikov.

There are no conflicts to declare.

absence of a cytotoxic copper contamination. Additional challenges are often posed by the liquid chromatography purification where inorganic copper salts tend to precipitate, clogging the columns and reducing their efficiency in product purification. More recently reported biocompatible copper-free strain-promoted alkyne-acetylene cycloaddition (SPAAC)⁷ has quickly attracted interest of PET radiochemists. We previously reported the radiolabeling of peptides with the [¹⁸F]ADIBO prosthetic group, which was obtained in high radiochemical yield (RCY) in one step from the corresponding tosylate precursor⁸. Several model azide-tagged peptides were efficiently radiolabeled in absence of copper catalyst at room temperature in ethanol with >95% RCYs. However, the major limitation of the [¹⁸F]ADIBO as a prosthetic group for radiolabeling of peptides, proteins and other macromolecules is the moderate rate of cycloaddition ($k = 0.43 \pm 0.05 \text{ M}^{-1}\text{s}^{-1}$)⁹, requiring relatively high concentration of the substrate to achieve high RCYs. Nevertheless, other ¹⁸F-fluorinated ADIBO-based radioactive prosthetic groups have been developed and applied for peptide and protein radiolabeling^{10–12}. Furthermore, in a reverse approach ¹⁸F-fluorinated azides were used for labeling of DIBO-tagged substrates^{13, 14}. More recently, the faster inverse-electron demand Diels–Alder reaction between tetrazines and *trans*-cyclooctenes (IEDDA, k up to $2000 \text{ M}^{-1}\text{s}^{-1}$)¹⁵ has been extensively studied in a context of PET radiochemistry¹⁶, although unpredictable *in vivo* stability¹⁷, substrate-dependent reaction rate variability¹⁸ and complicated chemical synthesis¹⁹ currently limit its application. Our aim in this work was to develop a novel prosthetic group for radiolabeling of macromolecules via SPAAC with improved conjugation kinetics. Bent cyclooctyne triple bond promotes early cycloaddition transition state, lowering activation energy and, consequently, increasing reaction rate of SPAAC. Recently reported oxadibenzocyclooctyne (ODIBO) have shown up to 100-fold increase in the rate of cycloaddition ($k \sim 45 \text{ M}^{-1}\text{s}^{-1}$) presumably due to reduced bond angle at sp-hybridized carbon compared to ADIBO²⁰. Here we wish to report our development of [¹⁸F]ODIBO – a novel universal prosthetic group for radiolabeling of peptides and proteins at unprecedentedly low concentration of the substrate (Fig 1).

Non-radioactive precursor ODIBO-EG₄-OTs (**3**) and standard ODIBO-EG₄-F (**4**) were synthesized according to the previously published general procedure (Fig 2). Briefly, photo-ODIBO-OH (**1**) synthesized as previously reported¹³, was reacted with TBS-EG₄-OTs in DMF in the presence of potassium carbonate. TBS protection was removed using hydrofluoric acid in acetonitrile and the resulting alcohol reacted with tosyl chloride in the presence of trimethylamine in dichloromethane to yield photo-ODIBO-EG₄-OTs (**2**). Photodecarbonylation upon irradiation with 350 nm lamp in methanol resulted in the formation of precursor ODIBO-EG₄-OTs (**3**) in 34 % overall yield starting from photo-ODIBO-OH. Standard ODIBO-EG₄-F (**4**) was synthesized from precursor upon treatment with TBAF in THF at reflux in 73% yield. Importantly, final compounds **3** and **4** showed good chemical stability upon storage at –20°C, as even after 1 year [¹⁸F]ODIBO could be prepared in high RCY as described below.

We have previously optimized radiolabeling conditions for [¹⁸F]ADIBO by the nucleophilic radiofluorination of the corresponding tosylate precursor ADIBO-OTs with [¹⁸F]fluoride. Among three different base/phase catalyst systems, tetraethylammonium bicarbonate (TEAB) provided the best radiochemical yield. Later, similar conditions were found optimal

for the radiolabeling of ^{18}F -TEG-DBCO by independent group¹⁰. We successfully translated those previously developed conditions for the radiolabeling of ODIBO-OTs with ^{18}F fluoride using TEAB as a base. Among three aprotic dipolar solvents commonly used in ^{18}F fluorination, acetonitrile provided the best yield of incorporation. Importantly, ODIBO cyclooctyne core showed stability towards nucleophilic fluorination conditions as only small amounts of radioactive impurities were observed in the reaction mixture in this solvent. Purification of ^{18}F ODIBO (^{18}F 4) from the unreacted precursor was achieved by standard reversed phase preparative high performance liquid chromatography (prep-HPLC) and remained stable in HPLC eluate throughout the day. The radiosynthesis of ^{18}F ODIBO was fully automated using our remotely controlled synthesis module (Scintomics GRP), equipped with an HPLC system and desired compound was regularly obtained in $11.5 \pm 1.8\%$ isolated yield not corrected for decay (Fig 3) within 65–70 min.

Using ^{18}F ODIBO as a prosthetic group we first radiolabeled the previously reported azide-tagged peptides octreotate- N_3 (**5**) and model 6-mer peptide N_3 -YWKTCT (**6**). Native 6-mer peptide YWKTCT (**7**) was used as a negative control (Fig 4).

Non-radioactive conjugates were obtained as a mixture of two regioisomers upon coinubation of **4** with the peptides **5–7** in acetonitrile/water solution and used as cold standards to monitor the progression of the radioconjugation. ^{18}F ODIBO was reacted with the peptides at different concentration in a similar manner and the reaction mixtures were analyzed by analytical HPLC. At 400 μM concentration, octreotate- N_3 was nearly quantitatively (97%) radiolabeled in as short as 5 min reaction time (Table 1, entry 1). This already represents an improvement of bioconjugation efficiency over previously reported ^{18}F ADIBO, which allowed peptide radiolabeling at higher concentrations of 1 mM or above upon 10–30 minutes incubation. Next, we have lowered the concentration of peptide to 40 μM . Amazingly, the conversion of ^{18}F ODIBO remained excellent, as the radiolabeled peptide accounted for 92% of the total radioactivity after 10 min incubation at room temperature in a water/acetonitrile mixture (Table 1, entry 2). Somewhat lower radiolabeling efficiency was observed for the other model 6-mer azide-tagged peptide **6**. The conversion at 500 μM was 95% (Table 1, entry 3), while reducing the concentration to 95 μM led to 90% conjugation efficiency within 10 min coinubation (Table 1, entry 4). Nevertheless, both peptides were efficiently and selectively radiolabeled in clean fashion in high yields within 10 minute at rt. The UV trace on the chromatogram showed the presence of unreacted peptide as the only non-radioactive impurity. Likewise, a small amount of unreacted ^{18}F ODIBO was the only radioactive impurity following the reaction, thus no side-products were observed. As expected, coinubation of ^{18}F ODIBO with unmodified 6-mer peptide **7** lacking azide tag showed no reaction (Table 1, entry 6), confirming selectivity of the prosthetic group towards the azide group.

Next, we applied this technique towards radiolabeling of proteins, the other important biomolecules with favorable characteristics for molecular imaging. We chose albumin from

rat serum (8) and apo-transferrin (9) as the model proteins and successfully tagged them with an azide group (8- N_3 and 9- N_3 , respectively) using the commercially available amine-reactive N_3 -PEG₄-NHS ester as a linker. Crucial for this approach was the purification of the tagged protein from the unreacted linker to avoid [^{18}F]ODIBO-to-linker conjugation. This was accomplished by the size exclusion chromatography using commercially available disposable cartridges (NAP-5). Tagged proteins were then radiolabeled by coincubation with [^{18}F]ODIBO in aqueous buffers at different concentration of the substrate. Remarkably, at the concentration of 1 mg/mL (~15 μ M) both proteins were radiolabeled with very high yields (Table 2, entries 1 and 4). These results highlight the efficiency of the SPAAC conjugation using [^{18}F]ODIBO prosthetic group, as most previously developed groups showed lower conjugation efficiency at such low concentration of the substrate. Furthermore, decreasing the concentration to 0.1 mg/mL (~1.5 μ M) still provides moderate radiochemical yields (Table 2, entries 2 and 5). At 0.01 mg/mL (~0.15 μ M) concentration, lower than 20% yields of labeled proteins were observed in the reaction mixture even after 30 min of coincubation with [^{18}F]ODIBO (Table 2, entries 3 and 6). Negative control experiment using unmodified albumin showed no presence of radiolabeled protein (Table 2, entry 7), thus again confirming the selectivity of the [^{18}F]ODIBO towards the azide group.

Conclusions

In summary, a novel universal synthon for radiolabelling of various macromolecules has been successfully developed. [^{18}F]ODIBO can be reliably synthesized from the corresponding tosylate precursor in high isolated radiochemical yields. The prosthetic group was then reacted with peptides modified with azide group at the *N*-terminus for nearly quantitative bioconjugation at low micromolar concentrations. Next, the prosthetic group was applied for the site-specific radiolabelling of azide-tagged proteins at even lower molar concentration of 15 μ M and below. Results of our work bring the rate of SPAAC closer to that of IEDDA and could therefore further promote application of this technique in PET radiochemistry. Currently, we are attempting to radiolabel nanoparticles with azide-modified surfaces using [^{18}F]ODIBO.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We would like to thank the staff at the radiochemistry facility of the Montreal Neurological Institute (MNI), in particular Mr. Dean Jolly and Dr. Gassan Massarweh for access to the radiochemistry facility, Mr. Robert Hopewell for proof-reading the manuscript and Mr. Pierre-Luc Rochon for help with protein modification. This work was financially supported by the McGill University start-up grant (243617) awarded to A.P.K and NSF grant (CHE-1565646) awarded to V.V.P.

Notes and references

1. Khalil, M. Basic science of PET imaging. 1. Springer Berlin Heidelberg; New York, NY: 2016.
2. Meyer JP, Adumeau P, Lewis JS, Zeglis BM. Bioconjug Chem. 2016; 27:2791–2807. [PubMed: 27787983]

3. Kolb HC, Finn MG, Sharpless KB. *Angew Chem Int Ed Engl.* 2001; 40:2004–2021. [PubMed: 11433435]
4. Gaetke LM, Chow CK. *Toxicology.* 2003; 189:147–163. [PubMed: 12821289]
5. Chikira M. *J Inorg Biochem.* 2008; 102:1016–1024. [PubMed: 18314194]
6. Li S, Cai H, He J, Chen H, Lam S, Cai T, Zhu Z, Bark SJ, Cai C. *Bioconjug Chem.* 2016; 27:2315–2322. [PubMed: 27583984]
7. Baskin JM, Prescher JA, Laughlin ST, Agard NJ, Chang PV, Miller IA, Lo A, Codelli JA, Bertozzi CR. *Proc Natl Acad Sci U S A.* 2007; 104:16793–16797. [PubMed: 17942682]
8. Arumugam S, Chin J, Schirmacher R, Popik VV, Kostikov AP. *Bioorg Med Chem Lett.* 2011; 21:6987–6991. [PubMed: 22024032]
9. Orski SV, Sheppard GR, Arumugam S, Arnold RM, Popik VV, Locklin J. *Langmuir.* 2012; 28:14693–14702. [PubMed: 23009188]
10. Kettenbach K, Ross TL. *MedChemComm.* 2016; 7:654–657.
11. Carpenter RD, Hausner SH, Sutcliffe JL. *Acs Med Chem Lett.* 2011; 2:885–889. [PubMed: 24900276]
12. Bouvet V, Wuest M, Wuest F. *Org Biomol Chem.* 2011; 9:7393–7399. [PubMed: 21938294]
13. Campbell-Verduyn LS, Mirfeizi L, Schoonen AK, Dierckx RA, Elsinga PH, Feringa BL. *Angew Chem Int Ed Engl.* 2011; 50:11117–11120. [PubMed: 21956935]
14. Evans HL, Slade RL, Carroll L, Smith G, Nguyen QD, Iddon L, Kamaly N, Stockmann H, Leeper FJ, Aboagye EO, Spivey AC. *Chem Commun (Camb).* 2012; 48:991–993. [PubMed: 22158912]
15. Blackman ML, Royzen M, Fox JM. *J Am Chem Soc.* 2008; 130:13518–13519. [PubMed: 18798613]
16. Reiner T, Zeglis BM. *J Labelled Comp Radiopharm.* 2014; 57:285–290. [PubMed: 24347429]
17. Rossin R, van den Bosch SM, Ten Hoeve W, Carvelli M, Versteegen RM, Lub J, Robillard MS. *Bioconjug Chem.* 2013; 24:1210–1217. [PubMed: 23725393]
18. Karver MR, Weissleder R, Hilderbrand SA. *Bioconjug Chem.* 2011; 22:2263–2270. [PubMed: 21950520]
19. Carroll L, Evans HL, Aboagye EO, Spivey AC. *Org Biomol Chem.* 2013; 11:5772–5781. [PubMed: 23907155]
20. McNitt CD, Popik VV. *Org Biomol Chem.* 2012; 10:8200–8202. [PubMed: 22987146]

Figure 1. Radiolabeling of various macromolecules via SPAAC with $[^{18}\text{F}]\text{ADIBO}$ (ref 8) and $[^{18}\text{F}]\text{ODIBO}$ (this work).

a) TBS-EG₄-OTs, K₂CO₃, DMF, 53 %; b) HF, CH₃CN, 98%; c) TsCl, Et₃N, DCM, 85%;
d) hv, 350 nm, MeOH, 78%; e) TBAF, 66°C, THF, 73%

Figure 2.
Syntheses of ODIBO-OTs and ODIBO-F.

Figure 3.
Radiosynthesis of $[^{18}\text{F}]\text{ODIBO}$ from ODIBO-OTs

Figure 4.

Model azide-tagged peptides octreotate-N₃ (**5**) and N₃-YWKTCT (**6**), and a negative control peptide YWKTCT (**7**).

Table 1Azide-tagged peptides radiolabeling with [^{18}F]ODIBO.

Entry	Peptide	C, μM	Time, min	RCY, % ^a
1	5	400	5	97
2	5	40	10	92
3	6	500	10	95
4	6	95	10	90
5	6	30	10	70
6	7	500	10	0

^adetermined by radioHPLC

Table 2Azide-tagged proteins radiolabeling with [^{18}F]ODIBO.

Entry	Protein	C, μM	Time, min	RCY, % ^a
1	8-N ₃	15	10	96
2	8-N ₃	1.5	10	80
3	8-N ₃	0.15	25	5
4	9-N ₃	15	10	91
5	9-N ₃	1.5	10	60
6	9-N ₃	0.15	25	17
7	8	15	30	0

^a determined by radioTLC