A $^{18}$F-labeled dibenzocyclooctyne (DBCO) derivative for copper-free click labeling of biomolecules

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For non-invasive in vivo imaging of processes and pharmacokinetics of radiolabeled biomolecules, the positron emission tomography (PET) is one of the most powerful methods. For PET applications, fluorine-18 has ideal nuclear characteristics and is the most commonly applied radionuclide in PET. The relatively long half-life of 110 minutes enables multi-step radiosyntheses, and the rather low $\beta^{+}$-energy ensures a very high spatial resolution in tomography. The challenge for nuclear chemists consists in finding appropriate $^{18}$F-labeling strategies, especially for sensitive biomolecules. Most of them are sensitive to the commonly used harsh conditions in direct $^{18}$F-labeling reactions such as high temperatures and strong basic conditions. As a result, the development of indirect labeling strategies via $^{18}$F-prosthetic groups, which can subsequently be attached to biomolecules under mild reaction conditions, is needed. Besides, the radiolabeling reaction should allow bioorthogonal $^{18}$F-labeling to treat the multitude of functional groups in bioactive compounds with respect. The most prominent example of such reactions, which fulfills all the mentioned criteria, is the copper(1)-catalyzed azide–alkyne cycloaddition (CuAAC) first published by Sharpless et al. in 2001. This variant of the Huisgen 1,3-dipolar cycloaddition of terminal alkynes and azides enables $^{18}$F-labeling with high specificity and excellent yields under mild conditions. In the past decade, a widespread spectrum of PET tracers has been synthesized using the CuAAC method for $^{18}$F-labeling of bioactive compounds. One of the latest developments is based on an amino acid, which is thought to minimize the influence on the pharmacokinetic properties of the intended radiotracer. As an amino acid derived $^{18}$F-prosthetic group, it is particularly suitable for peptides and proteins. However, with all the advantages of the copper(1)-catalyzed cycloaddition, there is one major disadvantage. The need for cytotoxic copper species as a catalyst in the click reaction causes an extensive work-up guaranteeing the complete removal of copper for in vivo applications. This fact led to the necessity of alternative fast and copper-free click reaction strategies. By using strained alkynes instead of terminal alkynes, copper is no longer needed to catalyze the click reaction. These so-called strain-promoted click reactions were first reported by Baskin et al. and can be carried out between cyclooctyne derivatives and azides or tetrazines as $3 + 2$ cycloaddition. The use of azadibenzocyclooctynes for copper-free click reactions was first reported by Kuzmin et al. in 2010. Recently, Arumugam et al. have published the development of a $^{18}$F-labeled azadibenzocyclooctyne for $^{18}$F-labeling of peptides via a strain-promoted click reaction without the use of a copper species, showing the high potential of this concept for $^{18}$F-labeling of biomolecules. Our aim was to develop a new $^{18}$F-prosthetic group based on (aza)dibenzocyclooctyne (DBCO) for radiolabeling of biomolecules such as peptides and microproteins. For reduced lipophilicity, we introduced a triethylglycol spacer to the (aza)dibenzocyclooctyne. Two different leaving groups, different bases, base concentrations and precursor amounts during radiolabeling were evaluated for optimized $^{18}$F-labeling. Consequently, two DBCO-based precursors and the non-radioactive reference compound were synthesized, and the $^{18}$F-labeling reaction was optimized. Finally, we performed a proof-of-principle click reaction with the new $^{18}$F-labeled prosthetic group and an azido-functionalized cyclic Arg-Gly-Asp (cRGD) peptide as a model system. This peptide is used as the gold-standard vector in targeting the $\alpha_v\beta_3$ integrin. Furthermore, we carried out further copper-free click reactions using a folate-azide for targeting the folate receptor and two $\alpha$-MSH analogue azido-
concentrations, reaction times and different amounts of pre-
mixed using different parameters such as various bases, base
Scheme 2. The radiolabeling of precursors
to yield

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Scheme 1 Synthesis of alkyne-functionalized reference compound 11 and labeling precursors 9 and 10. Regents and conditions: a) sodium, THF, 24 h, rt; b) TEA, p-toluenesulfonyl chloride, DCM, 1 h, 0 °C, rt; c) TEA, methanesulfonyl chloride, 1 h, 0 °C, rt; d) TFA, DCM, 4 h, rt; e) TFA, DCM, 4 h, rt; f) TEG-carboxylic acid, N,N,N′,N′-tetramethyl-O-(1H-benzotriazol-1-yl)uranium-hexafluorophosphate (HBTU), N,N-
disopropylethylamine (DIPEA), DMF, 24 h, rt; g) tetrabutylammonium fluoride, THF, 2 h, 80 °C.

functionalized peptides with high specificities to the melanocortin receptor 1 (MC1R).

The syntheses of reference compound 11 and the 18F-la-
beling precursors 9 and 10 are depicted in Scheme 1. The synthesis started from commercially available triethylene gly-
col 2. In the first step, 2 was reacted with tert-butylacrylate 1 to create a carboxylic acid function enabling the desired am-
ide coupling.18 Compound 3 was then reacted with either p-toluenesulfonyl chloride19 or methanesulfonyl chloride20 to
transfer the hydroxyl function into suitable leaving groups for
the nucleophilic radiofluorination reaction. Subsequently,
protected intermediates 4 and 6 were deprotected by trifluoroacetic acid in dichloromethane at room temperature
to yield 5 and 7.21 Both linker groups were coupled via an
amido bond to the dibenzoacylooctyne (DBCO)-amine 8,
using HBTU N,N,N′,N′-tetramethyl-O-(1H-benzotriazol-1-yl)
uranium-hexafluorophosphate (HBTU) as coupling reagent
and N,N′-disopropylethylamine (DIPEA) as base. The coupling
was performed at room temperature for 12 h to yield the de-
sired precursors for the 18F-fluorination reaction in overall
yields of 28% (for precursor 9) and 56% (for precursor 10)
over four steps. Due to the quite high costs for DBCO-amine
8, we aimed to insert this component in the last synthesis
step. In relation to the amounts of 8, the yields were good to
very high, leading to 56% and 87%, respectively. The refer-
ence compound was synthesized through 19F-fluorination of
9 using tetrabutylammonium fluoride (TBAF) at 120 °C for 2
h to yield 11 in excellent yields of 82%.

The radiofluorination of precursor 9 is depicted in
Scheme 2. The radiolabeling of precursors 9 and 10 was opti-
mized using different parameters such as various bases, base
concentrations, reaction times and different amounts of pre-
cursors. Initially, the use of two different bases,
tetrabutylammonium hydroxide (TBA-OH) and tetra-
ethylammonium bicarbonate (Et4N-HCO3), in acetonitrile was
screened. The use of TBA-OH caused decomposition of the
precursors, and a RCY of only 30% was achievable. The use
of precursor 9 (7.5 mg, 12 μmol) in acetonitrile and teta-
ethylammonium bicarbonate gave the highest RCY of ≥90%
within 10 minutes. For further evaluation of precursors 9 and
10, tetrathymethylene bicarbonate was used as base. With
a base amount below 17 μmol, no 18F-labeling was observed,
while increasing the base amount to higher than 17 μmol
resulted in reduced yields. Besides, the amount of precursor
played an important role. Reaction kinetics were monitored
for 2.5, 5.0 and 7.5 mg (4, 8 and 12 μmol) of precursor 9. By
increasing the amount of precursor (12 μmol), RCYs of ≥90%
after 10 min were observed. Between the two leaving groups
we did not observe significant differences in RCYs.

Isolation of the final 18F-labeled prosthetic group was per-
formed by fixation of the product fraction obtained from
semi-preparative HPLC on a C18 reversed phase cartridge,
followed by elution of the 18F-prosthetic group from the resin
with acetonitrile (1 ml). An exemplary radio-HPLC chromato-
gram of the crude mixture after radiolabeling of [18F]11 is
shown in the ESI.† The solvent was removed under reduced
pressure, and the 18F-prosthetic group was resolved in the de-
sired solvent to perform the subsequent click reaction. The
new 18F-prosthetic group was synthesized and isolated within
only 60 min in an excellent overall yield (n.d.c.) of 34 ± 5%,
ready for copper-free click reactions with azido-functionalized
biomolecules. For the lipophilicity of the 18F-prosthetic
group, a logD value of 1.20 ± 0.07 was calculated using the
octanol–water distribution coefficient.

To test the viability of [18F]11, it was used in the copper-
free cycloaddition with azido-functionalized cRGD peptide
(1 mg, 1.1 μmol), as shown in Scheme 3, as a model system.

Scheme 3 SPAAC of protected azido-functionalized cRGD 18 and the
new prosthetic group [18F]11. Click reaction conditions: PBS buffer/aceto-
nitrile (1:1), 25 °C or 40 °C, 5 min, RCY 93%.
The copper free $^{18}$F-click reaction gave the desired peptide $[^{18}F]_{13}$ in excellent RCY of 93% within 5 min, which shows the particularly high potential of the new prosthetic group for $^{18}$F-labeling of sensitive biomolecules under very mild conditions (25 °C, phosphate-buffered saline (PBS, pH 7.4), 5 min). An exemplary radio-HPLC chromatogram of $[^{18}F]_{13}$ in comparison to the $^{18}$F-prosthetic group $[^{18}F]_{11}$ is shown in the ESI.‡

Furthermore, an azido-functionalized folate derivative as a well-known tumor targeting vector was $^{18}$F-labeled in a copper free click reaction using the new $^{18}$F-prosthetic group. Remarkably, quantitative $^{18}$F-click labeling was observed after a few minutes at room temperature and a good (low) log $D$ value of 0.6 ± 0.07 was determined for the final $^{18}$F-folate. The $^{18}$F-labeled folate can be separated from unreacted $^{18}$F-prosthetic group via radio-HPLC from the unreacted $^{18}$F-prosthetic group. For the $^{18}$F-labeled folate derivates, a low log $D$ value of 0.6 ± 0.07 was determined. High stability was observed in human serum at 37 °C over a period of 1.5 h. Further in vitro and in vivo evaluation of the new $^{18}$F-folate using human KB cells and PET imaging is ongoing. Similarly, investigations and evaluation using the other new $^{18}$F-tracers derived from copper-free $^{18}$F-click labeling in in vivo PET imaging are planned.

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