Preclinical Evaluation of a Novel \(^{18}\)F-Labeled dTCO-Amide Derivative for Bioorthogonal Pretargeted Positron Emission Tomography Imaging

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ABSTRACT: Pretargeted positron emission tomography (PET) imaging based on the bioorthogonal inverse-electron-demand Diels–Alder reaction between tetrazines (Tz) and trans-cyclooctenes (TCO) has emerged as a promising tool for solid tumor imaging, allowing the use of short-lived radionuclides in immune-PET applications. With this strategy, it became possible to achieve desirable target-to-background ratios and at the same time to decrease the radiation burden to nontargeted tissues because of the fast clearance of small PET probes. Here, we show the synthesis of novel \(^{18}\)F-labeled dTCO-amide probes for pretargeted immuno-PET imaging. The PET probes were evaluated regarding their stability, reactivity toward tetrazine, and pharmacokinetic profile. \(^{18}\)F|MICA-213 showed an extremely fast kinetic rate (10,553 M\(^{-1}\) s\(^{-1}\) in 50:50 MeOH/water), good stability in saline and plasma up to 4 h at 37 °C with no isomerization observed, and the biodistribution in healthy mice revealed a mixed hepatobiliary and renal clearance with no defluorination and low background in other tissues. \(^{18}\)F|MICA-213 was further used for in vivo pretargeted immune-PET imaging carried out in nude mice bearing LS174T colorectal tumors that were previously treated with a tetrazine-modified anti-TAG-72 monoclonal antibody (CC49). Pretargeted PET imaging results showed clear visualization of the tumor tissue with a significantly higher uptake when compared to the control.

INTRODUCTION

The advances in bioorthogonal chemistry have provided new possibilities for noninvasive molecular imaging.\(^1\)–\(^3\) For example, the inverse-electron-demand Diels–Alder cycloaddition (IEDDA) between trans-cyclooctenes (TCOs) and tetrazines can rapidly generate short-lived radioisotope probes enabling in vitro and in vivo pretargeting imaging studies.\(^4\)–\(^7\)

The remarkable specificity and affinity of antibodies make them extremely attractive vectors for the delivery of diagnostic tools to biological targets, and represent an interesting platform for in vitro and in vivo application of bioorthogonal chemistry.\(^5\)–\(^10\) For radio-immun imaging of solid tumors high tumor-to-nontumor (T/NT) ratios of radiolabeled monoclonal antibodies are desired as well as a low radiation burden to the patient. With the bioorthogonal pretargeting technologies this can be achieved by using a modified mAb (monoclonal antibody) for a specific target that will later bind to a fast-clearing radiolabeled probe. This two-step approach will overcome the slow clearance of antibodies and enhance tumor visualization by clearing all the nonbound antibodies before injection of the radiotracer.\(^11\)–\(^13\) Using a pretargeting approach will also allow the use of shorter-lived positron emission tomography (PET) radionuclides such as fluoride-18 (\(t_{1/2} = 110\) min), that have favorable properties including its decay mode (97% \(\beta^+\) emission), low positron energy (634 keV maximum), and \(\beta^+\) trajectory in tissues (<2.3 mm).\(^14\)

Considerable research has been devoted to the development of mAb–TCO conjugates, and their application for pretargeted tumor imaging has been reported using diverse \(^{18}\)F-labeled tetrazines.\(^15\),\(^16\) However, it has been shown that TCO has a tendency to isomerize to cis-cyclooctene (CCO), which is several orders of magnitude less reactive with tetrazine after prolonged exposure to physiological conditions.\(^17\),\(^18\) Until now, the inverse approach, using mAb–tetrazine and radiolabeled...
TCO has been less extensively explored. In 2016, our group reported for the first time an approach, where tetrazines were used as tags for antibody modification and TCOs as imaging probes in order to avoid the prolonged plasma exposure of TCO bound to Abs leading to its deactivation. In this study, a methyl-tetrazine showed a favorable profile and was selected for the development of a novel mAb−tetrazine conjugate, which displayed good stability and reactivity. Furthermore, this immunoconjugate was used in a pretargeted live-cell fluorescent imaging using a fluorescent TCO probe resulting in the selective and rapid labeling of cells. These promising results suggested the viability of this approach and its translation for in vivo pretargeted PET imaging applications.19

Our group also described the 18F-labeling of a TCO and its biodistribution. Despite the TCO being able to enter in the brain, its fast metabolization with nonspecific accumulation in the bone made its utility limited for pretargeted imaging.20 In 2016, Fox et al. developed a new 18F-labeled sTCO displaying excellent reactivity in the tetrazine ligation and showed good stability in phosphate-buffered saline (PBS) and fetal bovine. The 18F-sTCO conjugated with RGD-tetrazine demonstrated a prominent tumor uptake in vivo with a good-tumor-to-background contrast.21 In 2017, Bormans et al. described an 18F-labeled version of a dTCO showing good kinetic rates with good pharmacokinetic properties when compared with the first generation of TCOs. They have shown a promising starting point using this small dTCO probe in pretargeted PET imaging, using SKOV-3 tumor-bearing mice, allowing the visualization of the tumor uptake. However, a slow isomerization into the corresponding cis-derivative was observed when incubating this tracer in rat plasma, and also the tumor-to-background ratio could be improved with further
optimization. More recently, in 2019, Wang, Li, and co-workers reported a new 18F-labeled trans-5-oxocene (oxoT-CO) also used to construct a PET probe for neurotensin receptor imaging, which presented an improvement in the tumor-to-background ratio while retaining good tumor uptake.

Despite the recent interest directed toward the development of new 18F-labeled small TCO probes, there is a need for improved TCO structures with higher stability and faster reactivity to be used in pretargeted PET imaging. Here, we report the synthesis of novel dTCO-amide PET probes for pretargeted imaging based on a new dTCO-amine building block (Figure 1). Modifications with fluoropyridine and polyethylene glycol (PEG) were made to evaluate stability, reaction with tetrazine, and pharmacokinetic properties. The best probe was selected and an in vivo pretargeted immuno-PET imaging experiment was performed using an LS174T human colorectal cancer model previously treated with a tetrazine-modified anti-TAG-72 monoclonal antibody (CC49).

RESULTS AND DISCUSSION

Synthesis. In order to obtain MICA-212 and MICA-213 cold references (11 and 12, respectively), the main scaffold of dTCO-amine 7 was synthesized, as shown in Scheme 1. Compound 1 (sym-diastereomer) was obtained following a previous reported procedure. Compound 1 was selected as a starting point because, to the best of our knowledge, dTCO derivatives have shown an improved stability when compared to other TCO structures and can be easily stored in the fridge for several months. To obtain the main dTCO-amine scaffold 7, mesylation of 1 was performed followed by the formation of the azide 3 using sodium azide under reflux. The azide product was then reduced to amine 4 using LiAlH₄ following protection of the amine group with trifluoroacetic anhydride to afford 5. Photoisomerization of 5 was performed, under 2.5 h, using a closed-loop flow reactor to afford trans isomer 6 in 53% yield. Compound 6 was then deprotected with sodium hydroxide under reflux to achieve the dTCO-amine scaffold 7 that showed to be resistant to degradation even at high temperatures (reflux). To afford MICA-212 cold reference (11), dTCO-amine (7) was first reacted with 4-(chloromethyl)benzoyl chloride to yield 8 and this was treated with triethylenglycol to afford the pegylated compound 9. Compound 10 was obtained by tosylation and then reacted with tetra-n-butylammonium fluoride to afford the desired reference 11. To achieve MICA-213 cold reference (12), a simple nucleophilic substitution with 6-fluoronicotinoyl chloride was performed.

Radiochemistry. [18F]MICA-212 was synthesized by reaction of 10 with K[18F]F in acetonitrile at 100 °C for 5 min to afford the radiolabeled product (Scheme 2) in 8.7 ± 0.4% isolated radiochemical yield (RCY) decay corrected to EOB (end of bombardment) with 98% radiochemical purity after high-performance liquid chromatography (HPLC) purification. [18F]-MICA-213 was produced by first performing a nucleophilic substitution on compound 7 with 6-chloronicotinoyl chloride to afford the pyridine-chloride precursor 13 that was then reacted with K[18F]F in dimethylformamide (DMF) at 130 °C for 5 min to afford the radiolabeled product (Scheme 2) in 5.5 ± 0.5% isolated RCY (decay corrected to EOB), 98% radiochemical purity after HPLC purification. The identity of both tracers was confirmed by co-injection with the nonradiolabeled references 11 and 12. Both radiotracers showed a moderate lipophilicity with a log D value of 1.30 ± 0.03 for [18F]MICA-212 and 1.73 ± 0.01 for [18F]MICA-213. In order to assess the in vitro stability of the novel dTCO PET probes, we first incubated the radiotracers in PBS at 37 °C for 2 h. The radiochemical purity

**Scheme 2. Radiosynthesis of [18F]MICA-212 and [18F]MICA-213 by 18F-Labeling**

\[ \text{[18F]} \text{MICA-212} \]

\[ \text{[18F]} \text{MICA-213} \]

**Scheme 3. Example of the Kinetic Reaction between MICA-213 Cold Reference (12) and Methyl-Tetrazine-NHS (Scheme S2)**
was 94% for [18F]MICA-212 and 95% for [18F]MICA-213. The plasma stability of the tracers was also evaluated, and after 2 h incubation at 37 °C, 86 ± 1.61% (n = 3) of the intact tracer remained for [18F]MICA-212 and 79 ± 0.07% (n = 3) for [18F]MICA-213. These results were very encouraging because the novel PET probes showed improved in vitro stability when compared with other TCO derivatives reported in the literature (1% after 30 min;20 74% after 1 h;21 79% after 1 h4).

**Stopped-Flow Kinetic Analysis.** The success of the pretargeted approach relies on the speed of the reaction between both components of the IEDDA reaction, therefore the kinetic rate constants between the novel dTCO non-radiolabeled tracers and a methyl-tetrazine-NHS19 (Scheme 3) were evaluated by UV-spectrophotometry at 290 nm under pseudo-first order conditions (Scheme S2). dTCO 11 and 12 were reacted with methyl-tetrazine-NHS at 37 °C in 50:50 MeOH/H2O with a rate constant of 1064 and 10553 M⁻¹ s⁻¹, respectively (Table S1). Based on the observed rate constants, 11 is significantly slower (10-fold less reactive) than the rate of the dTCO 12. This difference may be related to the different solubility of compounds because improved aqueous solubility will have a positive effect on the kinetic rate. In order to compare our results with the literature, dTCO 12 was reacted with the commercial 3,6-di-2-pyridyl-1,2,4,5-tetrazine showing a rate constant of 320812 M⁻¹ s⁻¹ at 37 °C in 50:50 MeOH/H2O (Table S1), which is in the same range as the dTCO (366,000 M⁻¹ s⁻¹) reported by Fox et al.,25 and an improvement when comparing to the recently reported dTCO derivative (1620 M⁻¹ s⁻¹) from Bormans et al.14

**In Vivo μPET Imaging and Biodistribution Studies in Nontumor Bearing Mice.** In order to evaluate the pharmacokinetic profile of the new dTCO PET probes, in vivo dynamic whole-body μPET imaging in parallel with ex vivo biodistribution was performed. Female nude BALB/c healthy mice were injected intravenously (iv) with a radiotracer and organs were collected in different timepoints up to 120 min post tracer injection, as shown in Figure 2 (Tables S2 and S3). Both PET tracers showed a similar mixed hepatobiliary and renal clearance followed by a washout of activity from the kidneys ([18F]MICA-212 11.9 ± 0.7% ID/g at 15 min and 5.0 ± 1.7% ID/g at 60 min; [18F]MICA-213 14.5 ± 3.2% ID/g at 10 min and 4.5 ± 0.7% ID/g at 60 min) and liver ([18F]MICA-212 6.3 ± 0.2% ID/g at 15 min and 3.9 ± 0.9% ID/g at 60 min; [18F]MICA-213 17.1 ± 6.9% ID/g at 10 min and 2.9 ± 0.4% ID/g at 60 min), which together with the lack of significant nonspecific accumulation in other organs, resulted in a general low image background at 60 min p.i. The tracers showed the absence of in vivo defluorination, with no significant bone uptake after 60 min p.i. (2.05 ± 0.12% ID/g for [18F]MICA-212, 0.9 ± 0.2% ID/g for [18F]MICA-213). When comparing both tracers, [18F]MICA-212 showed a higher intestinal uptake than [18F]MICA-213, which together with a low kidney uptake suggests that it is predominantly excreted via the gut with the highest accumulation in the large intestines. Nevertheless, these findings are in accordance with other TCO tracer, modified with a PEG linker, reported in the literature.14 It was also interesting to observe that [18F]MICA-212 (2.6 ± 0.1% ID/g at 15 min p.i.) and [18F]MICA-213 (3.8 ± 1.7% ID/g at 10 min p.i.) revealed a brain uptake, and that in the case of [18F]MICA-212 it persists at longer time points (2.6 ± 0.5% ID/g at 1 h p.i.), which may be due to the presence of a PEG linker. This observation might encourage the potential use of these probes for future applications in PET brain imaging.

**In Vivo Pretargeted μPET Imaging in Tumor Bearing Mice.** Encouraged by the remarkable kinetic rate of MICA-213 toward tetrazine and the favorable pharmacokinetic profile, [18F]MICA-213 was selected for further in vivo evaluation. In vivo pretargeted μPET imaging together with ex vivo biodistribution was performed in LS174T tumor-bearing mice pre-treated with anti-TAG72 mAb CC49 conjugated with a previously described methyl-tetrazine19 24 h prior to the injection of [18F]MICA-213. The TAG72 antigen was selected because of its limited internalization and shedding as well as its overexpression in a wide range of solid tumors, including colorectal cancer.27 The timepoint tracer injection was selected based on previously reported studies,10−12 where low levels of CC49 conjugate are detected in blood after 24 h p.i. The PET imaging time point was selected based on the [18F]MICA-213 biodistribution results that showed a low image background at 60 min p.i., as shown in Figure 3.

The μPET images demonstrated a clear visualization of the tumor, as shown in Figure 4B. In accordance with the biodistribution in healthy mice, the radioactivity in other tissues remained low (<2.5% ID/g), with the kidneys (2.3 ± 0.6% ID/g) and liver (1.7 ± 0.3% ID/g) having the highest uptake because of the mixed hepatobiliary and renal clearance of the radiotracer. The remaining radioactivity in the blood is low, suggesting that the [18F]MICA-213 is primarily reacting with CC49-Tz at the tumor site rather than with mAb that can still be circulating in the blood. This result suggests that there is no need to introduce another step where a clearing agent is injected prior to administration of the PET probe. To check the specificity of [18F]MICA-213, a control experiment was performed by first treating the mice with nonmodified CC49 antibody (200 μg in 200 μL of saline, 4 nmol) and after 24 h were injected with the same dose of the radiotracer (Figure 4).
strategy, where a tetrazine molecule is coupled to a specific antibody with the aim of developing new small TCO-probes for bioorthogonal pretargeted applications. $[^{18}F]$MICA-213 was obtained in a total radiosynthesis time of 50 min with 5% RCY, and >98% radiochemical purity. It demonstrated a promising stability and high reactivity against tetrazine moieties and showed a favorable pharmacokinetic profile in healthy mice with a fast clearance and no defluorination observed. In a pretargeted PET imaging setting, using a colorectal adenocarcinoma model treated with a tetrazine-modified CC49 antibody 24 h prior to radiotracer injection, $[^{18}F]$MICA-213 allowed visualization of the tumor with a significantly higher uptake compared to the control.

Additional optimization can be done based on the dTCO-amide scaffold to reduce background radiation and enhance tumor uptake in pretargeted imaging applications. Furthermore, the brain uptake, demonstrated within the first minutes of PET biodistribution, suggest potential application of small hydrophobic $^{18}$F-labeled PET probes for imaging of brain targets or internalizing targets.

**MATERIALS AND METHODS**

**General.** All chemicals and reagents were purchased from commercial suppliers (Sigma-Aldrich, Acros, TCI-Europe, Tractus or BioRad) and used without further purification. All solvents were reagent-grade or higher and used without further purification. PBS (0.01 M, pH 7.4) solutions were obtained from diluting of a 0.5 M stock purchased from Gentest Life technology.

NMR spectra were recorded on a Bruker AVANCE DRX 400 MHz spectrometer. $^1$H and $^{13}$C spectra are referenced to residual solvent peaks, coupling constants are given in Hz. HRMS analysis was performed using a Q-TOF II instrument (Waters, Manchester, UK). Ultra performance liquid chromatography–mass spectrometry analyses were performed on a Waters acuity UPLC system coupled to a Waters TQD ESP mass spectrometer and TUV detector. Kinetics, UV–vis measurements were performed on a stopped-flow system. All data obtained from kinetic and UV–vis measurements were analyzed with a GraphPad Prism 6 (San Diego, CA).

Chromatographic purifications were performed with a Biotage Isolera One flash system equipped with an internal variable dual-wavelength diode array detector (200–400 nm). Preparative HPLC used in the synthesis was performed on a Waters 2545 HPLC equipped with a Waters 2998 diode array detector, a Micromass Quattro microTM model, a Waters 2545 HPLC equipped with a Waters TQD ESP mass spectrometer and TUV detector. Kinetics, UV–vis measurements were performed on a stopped-flow system. All data obtained from kinetic and UV–vis measurements were analyzed with a GraphPad Prism 6 (San Diego, CA).

**Radiochemistry.** The radiosynthesis was carried out in an automated synthesis module (FluorSynthon III, Comecer Netherlands). No-carrier added aqueous $[^{18}F]$ fluoride was produced in an Eclipse HP cyclotron (Siemens) using the $^{18}$O(p,n)$^{18}$F reaction by proton bombardment of $^{18}$O$_2$(Rotem Industries), and passed through an ion exchange resin (Sep-Pak Accell Plus QMA Light cartridge (Waters)). $[^{18}F]$ was eluted from the resin in the reactor vial with 1 mL of a mixture of 0.03 M K$_2$CO$_3$/0.07 M Kryptofix2.2.2 in CH$_3$CN/H$_2$O (95:5 (v/v)), and evaporated to dryness. The remaining traces of water were removed by azotropic distillation using CH$_3$CN. For $[^{18}F]$MICA-212, tosylate precursor solution (5 mg) in dry ACN was added and reacted for 5 min at 90 °C and for $[^{18}F]$MICA-213, the chloro-pyridine precursor solution (5 mg) in dry DMF was added, and reacted for 5 min at 130 °C. After cooling down to 80 °C, 1 mL of buffer was added and the

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**CONCLUSIONS**

Pretargeted PET imaging is a promising approach allowing tumor visualization with a high target-to-background ratio while sparing normal tissues from radiation burden. Recently, we have implemented a new in vitro bioorthogonal IEDDA strategy, where a tetrazine molecule is coupled to a specific antibody with the aim of developing new small TCO-probes for pre-targeted imaging. Using this approach, one could avoid the prolonged plasma exposure of TCO bound to Abs which often leads to its deactivation, and also, having a more hydrophobic-labeled TCO, can bring some interesting advantages when cell membranes or even the blood–brain-barrier need to be crossed.

In this work, we developed novel dTCO amide PET probes for bioorthogonal pretargeted applications. $[^{18}F]$MICA-213 allowed visualization of the tumor with a significantly higher uptake compared to the control.
mixture was passed through a Sep-Pak Alumina N Light cartridge (Waters) (preconditioned with 10 mL of water), before injection on the HPLC. $^{[18F]}$MICA-212 was purified using a Phenomenex Luna C18 250 × 10 mm (5 μm) HPLC column using a mobile phase of NaOAc 0.05 M pH 5.5/EtOH (55:45 v/v) at a flow rate of 4 mL/min. $^{[18F]}$MICA-212 Rf = 17.5 min. $^{[18F]}$MICA-213 was purified using a Grace Platinum EPS C18 150 × 10 mm (5 μm) HPLC column with a mobile phase NaOAc 0.05 M pH 5.5/EtOH (65:35 v/v) at a flow rate of 4 mL/min. $^{[18F]}$MICA-213 Rf = 12.5 min. Radiotracers were sterile-filtered and diluted with 0.9% NaCl to reduce the ethanol concentration <10% in the final formulation. Radiochemical purity was determined by analytical reverse-phase HPLC using a Waters XBridge C18 formulation. Radiochemical purity was determined by nonlinear regression analysis of the data points using GraphPad Prism software (v. 6.00, GraphPad Software Inc.).

**Animal Experiments.** Animals were kept under a temperature-controlled environment (12 h light/dark cycle, 20–24 °C and 40–70% relative humidity) in IVC cages with food and water ad libitum. The human colon cancer cell line LS174T (ATCC CL-188) was obtained from the ATCC and maintained in Eagle’s Minimal Essential Medium (Sigma) supplemented with 10% fetal bovine serum, 2 mM l-glutamine, 1 mM sodium pyruvate, and 1% penicillin–streptomycin (Invitrogen) at 37 °C and 5% CO2. Tumor-free or tumor-bearing female nude BALB/c mice (20–25 g, Charles River Laboratories) were inoculated subcutaneously with 5 × 10⁶ LS174T cells in 100 μL of sterile PBS and were used 7–10 days after tumor inoculation. The tumors reached a size of approximately 70–200 mm³. At the end of each experiment, the mice were euthanized by cervical dislocation. Experimental procedures and protocols were performed in accordance with European Directive 86/609/EEC Welfare and Treatment of Animals and were approved by the local ethical commission (2017-10, University of Antwerp, Belgium).

**In Vivo μPET Imaging Studies.** Pretargeted PET imaging studies in healthy mice (n = 4) and LS174T tumor-bearing mice (n = 5) were performed by iv injection via the lateral tail vein with 7.4 MBq of $^{[18F]}$MICA-213. Acquisition data of the dynamic whole-body PET scans were acquired during 60 min (12 × 10, 3 × 20, 3 × 30, 3 × 60, 3 × 150, and 9 × 300 s frames) for healthy mice and static whole-body PET images 60 min p.i. for LS174T tumor-bearing mice, using an Inveon small animal PET-CT scanner (Siemens). After each PET acquisition, a whole-body CT scan was performed to obtain anatomic information. During the PET-CT scanning, mice body temperature was maintained using a heating pad. For quantitative analysis, PET data were reconstructed using 3-dimensional ordered subset expectation maximization (OSEM3D, 16 subsets and 2 iterations) and 18 maximum a posteriori iterations including scatter and attenuation correction. PET images were additionally reconstructed on a 128 × 128 × 159 matrix with a voxel size of 0.776 × 0.776 × 0.776 mm. Corrections to the PET data were applied using a CT-based attenuation and single scatter simulation scatter. For the dynamic scans, time activity curves for the liver, kidney, urinary bladder, muscle, brain, and bone were generated using PMOD (version 3.6; PMOD Technologies). An absolute measure of the tracer uptake in the tissue, normalized images were scaled according to the percent-injected dose (% ID/mL = tissue uptake from the scanner [kBq/mL]/injected dose [kBq] × 100).

**Ex Vivo Biodistribution Studies.** Healthy mice and LS174T tumor-bearing mice were injected with $^{[18F]}$MICA-212 (7.4 MBq per mouse, n = 3/time point) and $^{[18F]}$MICA-213 (7.4 MBq per mouse, n = 3/time point) via the lateral tail vein. At selected time points (15, 30, 60, or 120 min) blood was collected and mice were euthanized by cervical dislocation. Organs and tissues of interest were harvested, weighed, and blotted dry. The sample radioactivity was counted in a γ-counter (Wizard² 2480, PerkinElmer) and the uptake of the radiotracer are presented as the injected dose per gram (% ID/ g).

**In Vitro Stability Evaluation.** The in vitro stability was assessed by incubation of $^{[18F]}$MICA-212 and $^{[18F]}$MICA-213 in PBS (0.01 M, pH 7.4) or mouse plasma at 37 °C for up to 2 h. Both radiotracers (185–370 kBq) were incubated in...
100 μL of PBS or mouse plasma and, at selected time points, quenched by adding ACN (100 μL), followed by vortexing and centrifugation (5 min, 4000g) to remove the proteins. For each sample and time point, the radioactive supernatant (100 μL) was analyzed by analytical radio-HPLC. The HPLC eluate was collected in fractions of 30 s, and the radioactivity was counted in an automated γ-counter.

Data Analysis. The data are expressed as a mean ± SD. Statistical analysis was performed using Prism (version 6.01; GraphPad Software). Statistical significance between different groups were analyzed by the one-way analysis of variance followed by Bonferroni correction. Statistical significance between two data sets was evaluated by the unpaired two-tailed Student t-test. The difference between groups was considered statistically significant when p < 0.05.

ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.9b03584.

Experimental details; equipment information; chemical structures and additional data of all precursor molecules; radio-HPLC chromatograms, second order reactions rates data, and ex vivo biodistribution data from the pretargeted PET imaging experiment (PDF)

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