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Ultrafast Photoclick Reaction for Selective $^{18}$F-Positron Emission Tomography Tracer Synthesis in Flow

Youxin Fu,‡ Hugo Helbert,‡ Nadja A. Simeth, Stefano Crespi, Gerbren B. Spoelstra, Jan Maarten van Dijl, Marleen van Oosten, Luiza Reali Nazario, Dion van der Born, Gert Luurtsema, Wiktor Szymanski,* Philip H. Elsinga,* and Ben L. Feringa*

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ABSTRACT: The development of very fast, clean, and selective methods for indirect labeling in PET tracer synthesis is an ongoing challenge. Here we present the development of an ultrafast photoclick method for the synthesis of short-lived $^{18}$F-PET tracers based on the photocycloaddition reaction of 9,10-phenanthrenequinones with electron-rich alkenes. The respective precursors are synthetically easily accessible and can be functionalized with various target groups. Using a flow photo-microreactor, the photoclick reaction can be performed in 60 s, and clinically relevant tracers for prostate cancer and bacterial infection imaging were prepared to demonstrate practicability of the method.

Positron emission tomography (PET) is a key molecular imaging technique, characterized by unparalleled sensitivity. It targets the tissue of interest with tracers functionalized with radioactive, short half-life positron-emitting nuclides for detection by gamma cameras. Therefore, the development of radiopharmaceuticals for PET is highly dependent on our ability to introduce radionuclides efficiently and rapidly into the target chemical structures. The workhorse radionuclide for PET is fluorine-18, which is characterized by a half-life suitable for radiosynthesis and biodistribution ($t_{1/2} = 109.8$ min). Emission of low-energy positrons ($E_{\text{mean}} = 0.64$ MeV) accompanies the decay of $^{18}$F, allowing for relatively high image resolution. Consequently, $^{18}$F is the most widely used radionuclide for the clinical labeling of PET radiopharmaceuticals. Labeling methods to introduce $^{18}$F can be divided into direct and indirect techniques. Most direct labeling strategies rely on the use of $^{18}$F to functionalize a wide range of substrates, introducing $^{18}$F-aryl, $^{18}$F-alkyl, $^{18}$F-CF$_3$ or, very recently, $^{18}$F-SO$_2$ groups. These new direct labeling strategies greatly expanded the applicability of the method in the past years; however, the need for elevated reaction temperatures or the low functional group tolerance stimulated recent efforts toward milder direct labeling methods involving chelation of $^{18}$F or $^{18}$F-exchange reactions on heteroatoms like Si and B.

Hence, indirect labeling is often the preferred option for particularly sensitive substrates. This approach is based on the fluorination of a prosthetic group that is subsequently attached to a tracer in a bioorthogonal reaction.

Due to the limited half-life of $^{18}$F, this final coupling step has to be very efficient and fast. Hence, the copper-catalyzed azide−alkyne cycloaddition (CuAAC) reaction became an attractive labeling method. Its metal-free labeling variants, such as strain-promoted click chemistry (SPAAC) and tetrazine trans-cyclooctadiene cycloadditions (IEDDA), were introduced in an attempt to address the issues related to slow reaction rate and copper toxicity. Considering the challenge to develop very fast, clean, and selective methods for indirect labeling, photochemical click reactions would provide a particularly appealing alternative.

Highly beneficial is that photoclick reactions can combine important requirements to provide a practical indirect labeling protocol, such as high functional group tolerance, ambient reaction conditions, and easy operation in a photoflow reactor, and, importantly, one might achieve extremely high reaction rates without the need for additional reagents or catalysts. The outstanding possibilities offered by photochemical reactions have recently been recognized in several radiochemical applications, i.e., a methylation protocol for $^{18}$F-PET ligand synthesis, photo-redox catalysis for $^{18}$F−C bond formation, photoactivatable aryl azides, and photo-triggered reaction of tetrazoles for radiosynthesis of $^{89}$Zr-labeled proteins.

However, so far a very limited number of photochemical transformations has been utilized as a key step in the indirect labeling of $^{18}$F-PET tracers and none of them provides the modularity and selectivity typical of click reactions.

The photoclick reactions showed for PET tracer synthesis often lack in functional group tolerance or are performed with short irradiation wavelength that can be absorbed by, or damage, common biomolecules. With these challenges in mind, we were aiming to identify and evaluate a fast photoclick reaction that can be conveniently used for the versatile preparation of $^{18}$F-PET tracers under visible light irradiation.
An interesting candidate for such a process is provided by photocycloaddition between 9,10-phenanthrenequinones (PQs) and suitably substituted alkenes (see Figure 1B). This “photoclick” [4+2] cycloaddition was already discovered in the 1940s. However, the need for long reaction times hampered its application in synthesis. Recently, using a suitable high-power LED light source able to excite PQ (λ<sub>max</sub> at 395 nm), the transformation was performed in the minute range in a biological environment. We envisioned that this fast and clean photoclick reaction holds tremendous potential for indirect labeling of tracers with short-lived radioisotopes to produce 18F-PET radiopharmaceuticals (Figure 1B). Here, we present the development of the PQ photoclick reaction into highly efficient batch and flow methodology for ultrafast radiosynthesis and its application for the preparation of 18F-labeled compounds, including a prostate cancer biomarker and a bacterial infection imaging tracer.

By irradiation of PQ with 395 nm light in the presence of electron-rich vinyl ethers (VEs, see Figure 2), the photocycloaddition, proceeding via the triplet state of PQ, furnishes PQ-VE adducts. Establishing the reaction conditions between PQ and VE1 (Figure 2A) allowed the synthesis of the corresponding photocycloadduct PQ-VE1 (see SI, Figure S59) after only 180 s of irradiation (see SI, sections 2 and 4, for photoproduct PQ-VE1 synthesis and characterization by 1H and 13C NMR and HRMS). We also discovered ultra-fast reactivity of cyclic vinyl ethers (Figure 2A). Moreover, control experiments using light−dark cycles showed that changes in the absorption spectrum and product formation follow exclusively a photochemical pathway (see SI, Figure S43).

To explore the scope of the photocycloaddition and enable even higher reaction rates for PET labeling, we investigated the reactivity with PQ of a series of hitherto unexplored cyclic VEs: 3,4-dihydro-2H-pyran-2-methanol (VE2), 2,3-dihydrofur-an (DF), and 2,3-dihydropyran (DP, Figure 2A) were reacted under 395 nm light irradiation with the diketone (for detailed information, see SI, section 4). All substrates showed high reactivity toward PQ and formed adducts exhibiting strong blue fluorescence, even visible by naked eye. The photoclick reactions were monitored by fluorescence spectroscopy (Figure 2A) and reached completion in less than 5 min. Formation of the cycloaddition products was confirmed by NMR and high-resolution mass spectroscopy (see SI, sections 2−4). Gratifyingly, the cyclic vinyl ethers VE2, DF, and DP exhibited significantly higher reaction rates compared to the linear vinyl ether (VE1). The full conversion could be achieved in around 90 s for the cyclic vinyl ethers and 180 s for VE1, respectively. Indeed, the electron properties of the vinyl ether greatly influence the reaction rate (see Figure 2A). The computed energies (SMD(MeCN)-ωB97X-D/def2-SVP level) of the HOMO of the various traps match the observed rates found in the experiments (see Figure 2B and SI, section 5).

The cyclic vinyl ethers are more nucleophilic than the linear analogs and, consequently, more prone to react with the lowest unoccupied β-spin orbital of the triplet PQ.

We then proceeded to extend the scope of this fast photoclick reaction to the fluorinated vinyl ethers F-VE1 (linear) and F-VE2 (cyclic; see Figure 2A). The conversion of both substrates with PQ was monitored by fluorescence spectroscopy (Figure 2A), showing a similar trend as observed for the VE compounds, with the cyclic compound reacting faster (full conversion in 1 min for F-VE2 and 3 min for F-VE1). Formation of the cycloaddition products was confirmed.
These results indicate that F-VEs show excellent reactivity toward PQ, quickly and selectively generating the desired fluorogenic photocycloadducts. With the “cold” reaction conditions in hand, we synthesized the 18F-radiolabeled analogs of the two F-VEs. The fluorination of the corresponding tosylates was performed by rapid (3 min) nucleophilic substitution with azeotropically dried 18F-\(\text{F}^-\)/K222, and the products were purified by distillation, affording 18F-VE1 and 18F-VE2 in moderate to good radiochemical yield (58% and 37%, respectively; for experimental details see SI, section 6). Both compounds could be directly used for the subsequent photoclick reaction. Irradiation of PQ in the presence of both 18F-VE linkers (cf. Figure 3A) showed full conversion of the radioactive starting material; however, the expected 18F-VE-PQ was not formed. (For experimental details of optimization, see also SI, section 4.)

\(^1\)H NMR analysis of the reaction between VE2 and PQ revealed that, without an excess of the trap, namely the VE, photooxidation degraded the product. Consequently, the photochemical reactions at equimolar ratios or with excess of PQ (such as in the radiolabeling experiments) were performed with deoxygenated solvents. To our delight, degradation of the product was prevented, and F-VE2-PQ remained unaffected by NMR and high-resolution mass spectroscopy (see SI, section 2).

Figure 2. (A) Comparison of the conversion of the photocycloaddition of PQ with different VE over time followed by fluorescence spectroscopy (1 cm cuvette, 2 mL sample volume, 25 °C, sample interval 10 s. Concentration: 2.5 μM (PQ), 25 μM (VE), \(\lambda_{ex} = 365\) nm, \(\lambda_{obs} = 403\) nm). (B) Frontier orbitals of the species involved in the reaction (HOMO of VE and lowest unoccupied \(\beta\)-spin orbital of the triplet PQ) at the SMD(MeCN)-\(\omega\)B97X-D/def2-SVP level.
even after 10 min of irradiation (see SI, Figure S51, F-VE2-PQ). Applying oxygen-free conditions to the radiolabeling procedure with $^{18}$F-VE1 resulted in $^{18}$F-labeled product $^{18}$F-VE1-PQ in high radiochemical conversion (RCC, 69%) in 5 min. The use of cyclic $^{18}$F-VE2 resulted in lower conversion to the product (RCC 20% to $^{18}$F-VE2-PQ) compared to the linear VE.

To improve the efficiency of the photoclick reaction and to further reduce the reaction time, the reaction was optimized in a microfluidic flow photoreactor (for details regarding the optimization condition, see SI, section 6). This allowed us to have highly efficient irradiation (effective light penetration) and to automate the protocol. Toward this goal, a commercially available FlowSafe synthesis module$^{20}$ was equipped with two 395 nm LEDs (see Figure 3B). Indeed, performing the reaction this way enhanced the irradiation efficiency significantly and allowed us to achieve high conversions even at residence times as short as 60 s. The high conversion observed in batch for the synthesis of $^{18}$F-VE1-PQ could be improved even further in flow, affording the desired product in 77% RCC (Figure 3C).

We subsequently explored if the substituted PQ derivatives perform equally well in this developed ultrafast click methodology, envisioning the embedding of PQ derivatives into targeting moieties of future tracers for reaction with the vinyl prosthetic groups. A carboxylic acid on the PQ moiety as a handle for further synthetic functionalization and linear $^{18}$F-VE1 was selected as the reaction partner in the photo-induced cyclization reaction. First, the effect of the amide substitution on PQ was assessed by performing the labeling of compound $^{18}$F-VE1-PQ-Amide. The expected product was formed in satisfactory radiochemical conversion (RCC 49%, Figure 3C) after only 60 s of irradiation in flow. In order to test the labeling of potential probes via a direct attachment using an amide linkage, a well-established prostate-specific membrane antigen (PSMA) binding motif was connected to the PQ core structure as a model substrate. PSMA is a biological marker of prostate cancer that is often targeted for diagnosis in PET imaging.$^{20,49-51}$ (Figure 3C). Most of the PET probes developed to target PSMA share the same lysine-urea-glutamate binding motif.$^{20}$

Finally, driven by a clinical need to detect bacterial infections, non-invasively and with high sensitivity,$^{22-34}$ the labeling of the antibacterial agent vancomycin was undertaken. A suitable PQ derivative, PQ-Vanco, was synthesized (for details see SI) and isolated as a mixture of functional isomeric products, as reported before for the vancomycin system (see SI for the fragmentation pattern assignment).$^{35,36}$ Gratifyingly, application of our labeling strategy in vivo resulted in lower conversion to the crude final product, to be performed in less than 10 min. From a practical point of view, the method holds tremendous potential as a novel radiolabeling procedure for $^{18}$F-tracers. Moreover, exploiting the fluorochromophore properties of the photocycloadduct offers prospects toward the development of other (multimodal) imaging protocols.

**ASSOCIATED CONTENT**

**Supporting Information**

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/jacs.1c02229.

Experimental procedures and characterization data for all new compounds, photophysical and chemical studies,
details regarding the computational calculation, and detailed protocols of radiochemistry (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

Witkor Szymanski — Centre for Systems Chemistry, Stratingh Institute for Chemistry, Faculty for Science and Engineering, University of Groningen, 9747 AG Groningen, The Netherlands; Department of Radiology, Medical Imaging Center, University of Groningen, University Medical Centre Groningen, 9713 GZ Groningen, The Netherlands; † orcid.org/0000-0002-9754-9248; Email: w.szymanski@umcg.nl

Philip H. Elsinga — Department of Nuclear Medicine and Molecular Imaging, University of Groningen, University Medical Centre Groningen, 9713 GZ Groningen, The Netherlands; ‡ orcid.org/0000-0003-3635-4305; Email: p.h.elsinga@umcg.nl

Ben L. Feringa — Centre for Systems Chemistry, Stratingh Institute for Chemistry, Faculty for Science and Engineering, University of Groningen, 9747 AG Groningen, The Netherlands; † orcid.org/0000-0003-0588-8435; Email: b.l.feringa@rug.nl

Authors

Youxin Fu — Centre for Systems Chemistry, Stratingh Institute for Chemistry, Faculty for Science and Engineering, University of Groningen, 9747 AG Groningen, The Netherlands; † orcid.org/0000-0001-6942-6534

Hugo Helbert — Centre for Systems Chemistry, Stratingh Institute for Chemistry, Faculty for Science and Engineering, University of Groningen, 9747 AG Groningen, The Netherlands; † orcid.org/0000-0001-8130-883X

Stefano Crespi — Centre for Systems Chemistry, Stratingh Institute for Chemistry, Faculty for Science and Engineering, University of Groningen, 9747 AG Groningen, The Netherlands; † orcid.org/0000-0002-0279-4903

Gerbrin B. Spoelstra — Department of Nuclear Medicine and Molecular Imaging, University of Groningen, University Medical Centre Groningen, 9713 GZ Groningen, The Netherlands; † orcid.org/0000-0002-7063-2834

Jan Maarten van Dijl — Department of Medical Microbiology and Infection Prevention, University of Groningen, University Medical Center Groningen, 9713 GZ Groningen, The Netherlands; † orcid.org/0000-0002-5688-8438

Marleen van Oosten — Department of Medical Microbiology and Infection Prevention, University of Groningen, University Medical Center Groningen, 9713 GZ Groningen, The Netherlands; † orcid.org/0000-0002-7061-6324

Luiza Reali Nazario — Department of Nuclear Medicine and Molecular Imaging, University of Groningen, University Medical Centre Groningen, 9713 GZ Groningen, The Netherlands; † orcid.org/0000-0002-4983-5047

Dion van der Born — FutureChemistry, 6708 PW Wageningen, The Netherlands; † orcid.org/0000-0001-7381-1160

Complete contact information is available at: https://pubs.acs.org/10.1021/jacs.1c02229

Author Contributions

Y.F. and H.H. contributed equally.

Notes

The authors declare the following competing financial interest(s): Dion van der Born is an employee of Future Chemistry which produces the FlowSafe equipment used in this work.

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