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

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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 practicality of the method.

Positron emission tomography (PET) is a key molecular imaging technique, characterized by unparalleled sensitivity.[1] 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_{mean} = 0.64 MeV) accompanies the decay of ^{18}F, allowing for relatively high image resolution.[1] 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,[2,3] or, very recently, ^{18}F-SO_3 groups.[4] These new direct labeling strategies greatly expanded the applicability of the method in the past years;[5,6] however, the need for elevated reaction temperatures or the low functional group tolerance[7−10] stimulated recent efforts toward milder direct labeling methods involving chelation of Al^{18}F and ^{18}F-^{19}F-exchange reactions on heteroatoms like Si and B.[11−14] Hence, indirect labeling is often the preferred option for particularly sensitive substrates.[15,16] 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.[16,17] Hence, the copper-catalyzed azide−alkyne cycloaddition (CuAAC) reaction became an attractive labeling method.[18−20] Its metal-free labeling variants, such as strain-promoted click chemistry (SPAAC)[21−25] and tetrazine *trans*-cyclooctene cycloadditions (IEDDA),[26−30] were introduced in an attempt to address the issues related to slow reaction rate and copper toxicity (Figure 1A).[31,32] Considering the challenge to develop very fast, clean, and selective methods for indirect labeling, photochemical click reactions would provide a particularly appealing alternative (Figure 1B).

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[33] have recently been recognized in several radiochemical applications, i.e., a methylation protocol for ^{11}C-PET ligand synthesis,[34] photo-redox catalysis for ^{18}F−C bond formation,[35] photoactivatable aryl azides, and photo-triggered reaction of tetrazoles for radiosynthesis of ^{89}Zr-labeled proteins.[36−39] 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,[40−42] and none of them provides the modularity and selectivity typical of click reactions.

The photoreactions showcased for PET tracer synthesis often lack in functional group tolerance[41,42] or are performed with short irradiation wavelength that can be absorbed by, or damage, common biomolecules.[43] 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.

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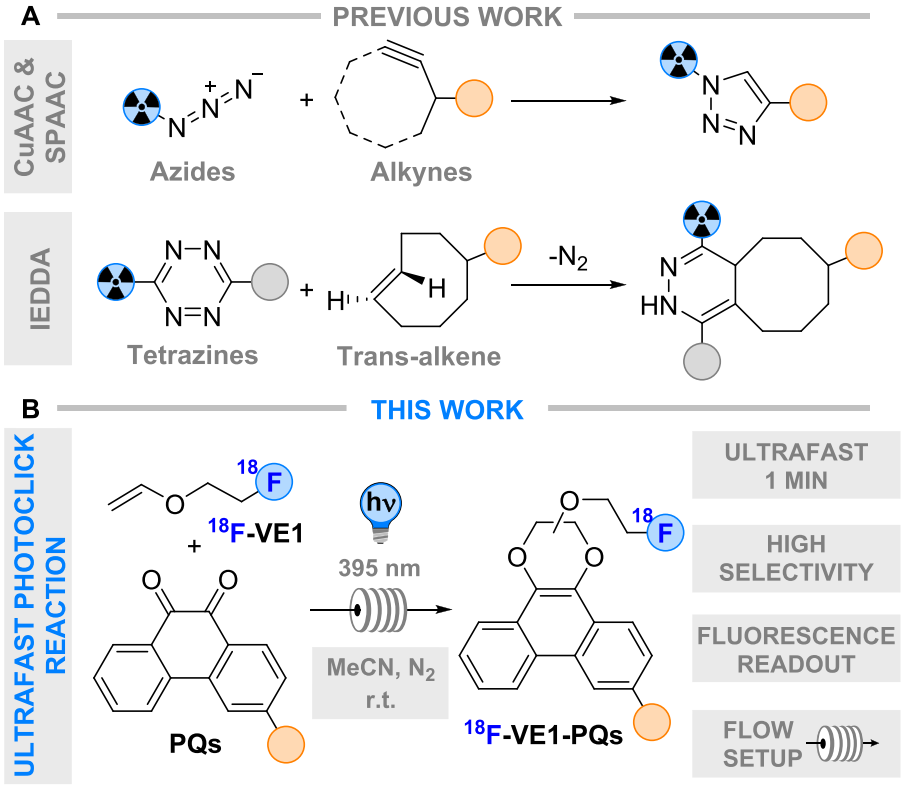

Figure 1. Overview of different "click" reactions used in PET tracer synthesis: (A) the established CuAAC, SPAAC, and IEDDA techniques and (B) the novel ultrafast photoclick reaction presented here.

An interesting candidate for such a process is provided by photocycloaddition between 9,10-phenanthrenequinones (**PQs**) and suitably substituted alkenes (see Figure 1B).[44] This "photoclick" [4+2] cycloaddition[44−47] was already discovered in the 1940s. However, the need for long reaction times hampered its application in synthesis.[47] Recently, using a suitable high-power LED light source able to excite **PQ** (λ_{max} at 395 nm), the transformation was performed in the minute range in a biological environment.[48] We envisioned that this fast and clean photoclick reaction holds tremendous potential for indirect labeling of tracers with short-lived radioisotopes to produce ^{18}F-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 ^{18}F-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 ^{1}H and ^{13}C 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-2*H*-pyran-2-methanol (**VE2**), 2,3-dihydrofuran (**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

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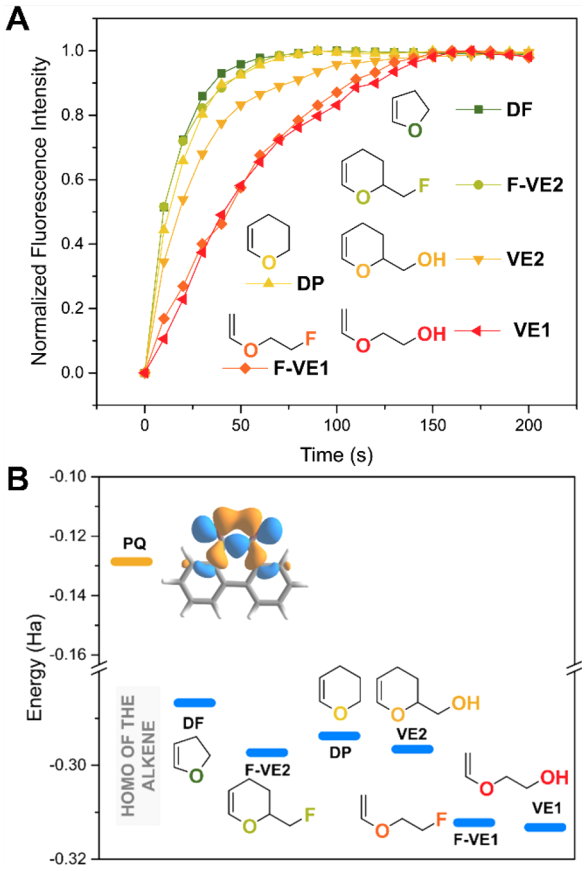

Figure 2. (A) Comparison of the conversion of the photocycloaddition of **PQ** with different **VE**s over time followed by fluorescence spectroscopy (1 cm cuvette, 2 mL sample volume, 25 °C, sample interval 10 s. Concentration: 2.5 μM (**PQ**), 25 μM (**VE**), λ_{ex} = 365 nm, λ_{obs} = 403 nm). (B) Frontier orbitals of the species involved in the reaction (HOMO of **VE**s and lowest unoccupied β-spin orbital of the triplet **PQ**) at the SMD(MeCN)-ωB97X-D/def2-SVP level.

by NMR and high-resolution mass spectroscopy (see SI, section 2).

These results indicate that **F-VE**s show excellent reactivity toward **PQ**, quickly and selectively generating the desired fluorogenic photocycloadducts. With the "cold" reaction conditions in hand, we synthesized the ^{18}F-radiolabeled analogs of the two **F-VE**s. The fluorination of the corresponding tosylates was performed by rapid (3 min) nucleophilic substitution with azeotropically dried $^{18}F^-/K_{222}$, and the products were purified by distillation, affording **^{18}F-VE1** and **^{18}F-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 **^{18}F-VE** linkers (cf. Figure 3A) showed full conversion of the radioactive starting material; however, the expected **^{18}F-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**s, 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

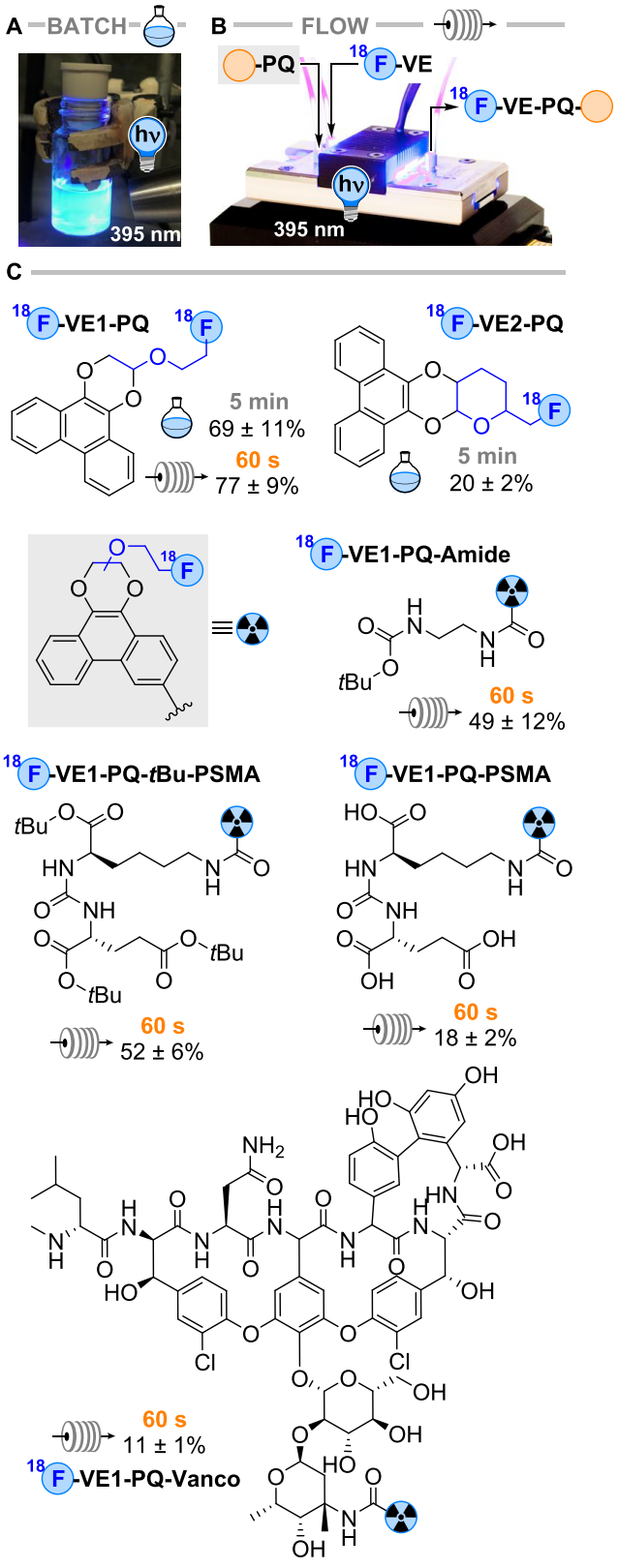

Figure 3. (A) Batch reaction setup. (B) Flow reaction setup. (C) Scope of "hot" **F-VE-PQ**s including the potential prostate cancer tracer **^{18}F-VE1-PQ-PSMA** and the bacterial infection imaging agent **^{18}F-VE1-PQ-Vanco** (only one isomer displayed; see also SI). The RCC values over three repetitions are reported with the corresponding error associated with the measurements.

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J. Am. Chem. Soc. 2021, 143, 10041−10047

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,[52−54] 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).[55,56] Gratifyingly, application of our labeling strategy in flow led to successful radiolabeling of this highly functionalized drug in 60 s, under irradiation with 395 nm light (RCC 11 ± 1%), without the need of protecting groups. This is the first report of the synthesis of a ^{18}F-labeled vancomycin derivative, which highlights the versatility and utility of the synthetic approach for a large and complex multifunctional molecule of high biomedical relevance.

To assess the potential of this novel vancomycin derivative, the *in vitro* binding properties of **^{18}F-VE1-PQ-Vanco** were determined. Lipophilicity (LogP) of **^{18}F-VE1-PQ-Vanco** was found to be −0.68 ± 0.10 (mean, standard deviation). Little tracer degradation was observed in human plasma, with >90% of tracer remaining intact after 2 h incubation at 37 °C. A fraction of 22% of **^{18}F-VE1-PQ-Vanco** was found to be bound to plasma protein after 3 h incubation. An *in vitro* assay was performed using the Gram-positive bacterium *Staphylococcus aureus* and the Gram-negative bacterium *Escherichia coli*. We observed significant binding of the **^{18}F-VE1-PQ-Vanco** by *S. aureus*, but not by *E. coli*, which is consistent with the mode of action of the parent compound vancomycin. Specifically, after 30 min incubation, a 17-fold higher accumulated activity was detected for *S. aureus* compared to *E. coli* (Figure 4).

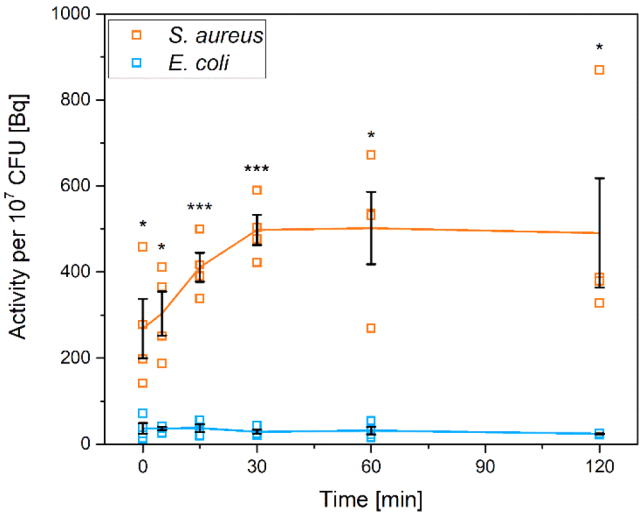

Figure 4. Accumulation of radioactivity over time (0, 5, 15, 30, 60, and 120 min) in *S. aureus* (orange) and *E. coli* (blue) grown in liquid culture, corrected for colony forming units (CFUs). The fitting line represents the mean ± standard error of the mean (in black); Student's *t* test: *P < 0.05, ***P < 0.001.

Altogether, these results suggest that **^{18}F-VE1-PQ-Vanco** can be of great value for the selective detection of infections caused by *S. aureus* and other Gram-positive bacteria through PET imaging.

In summary, we successfully developed a novel, extremely fast photoclick reaction for the synthesis of short-lived ^{18}F-PET tracers. The respective **^{18}F-VE** and **PQ** precursors are synthetically easily accessible. By functionalizing the **PQ** moiety with a carboxylic acid handle, various target-specific agents, such as the clinically relevant PSMA ligand and the antibacterial agent vancomycin, can be readily attached via an amide bond. By using a commercially available, automated module equipped with a flow photo-microreactor, the reaction time could be optimized to only 1 min under visible (violet) light irradiation, representing a considerable improvement compared to most current methods. Our strategy enables the complete process, from the stage of dried $^{18}F^{-}/K_{222}$ 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 fluorescence 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,

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J. Am. Chem. Soc. 2021, 143, 10041−10047

details regarding the computational calculation, and detailed protocols of radiochemistry (PDF)

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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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