Organic & Biomolecular Chemistry

Cite this: *Org. Biomol. Chem.*, 2012, **10**, 3871

www.rsc.org/obc

PAPER

Single-step radiofluorination of peptides using continuous flow microreactor†

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Received 3rd January 2012, Accepted 23rd March 2012
DOI: 10.1039/c2ob00015f

^{18}F radiolabelling of peptides bearing two different prosthetic groups was successfully conducted in a continuous flow microfluidic device for the first time. Radiochemical yields were dependent on precursor concentration, reaction temperature and flow rate. The choice of leaving group had a dramatic influence on the reaction outcome. Rapid reaction optimization was possible.

Introduction

^{18}F-labelled peptides are used as radioactive imaging agents for positron emission tomography (PET), an *in vivo* imaging technique that has gained broad application in nuclear and molecular medicine and in drug development during the last decade.[1–4] The conventional synthesis of ^{18}F-peptides by direct radiofluorination requires heating in basic conditions at elevated temperatures resulting often in decomposition of the starting material (precursor) and/or the product.[5] Another disadvantage is that a large excess of the peptidic precursor (milligrams) is required to obtain the radiolabelled product (micrograms) in high radiochemical yields (RCY). Therefore, purification of the final product is necessary by time-consuming high performance liquid chromatography (HPLC) in order to remove the excess precursor. The short half-life of ^{18}F radioisotope (109.7 minutes) poses a constraint on the acceptable synthesis time. It has been suggested, that microfluidic devices could bring multiple advantages to radiopharmaceutical tracer development.[6] Several groups reported the radiolabelling of a variety of small molecules with short-lived radioisotopes, such as ^{18}F and ^{11}C, in a microreactor.[7–11] Herein we investigated if a microfluidic device could be used for the radiolabelling of peptides with ^{18}F using small amounts of precursor and preferably under mild conditions.

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†Electronic supplementary information (ESI) available: Representative UPLC and TLC chromatograms of a reaction mixture, comparison chart of selected analytical data obtained using UPLC and TLC, and an example of product purification procedure using a cartridge. See DOI: 10.1039/c2ob00015f

Experimental†

Peptidic precursors **1–3** (Fig. 1) and their corresponding non-radioactive fluorinated compounds (reference compounds) were generously provided by Bayer HealthCare (Berlin, Germany).

No-carrier-added ^{18}F-fluoride was produced *via* the ^{18}O(p,n)^{18}F nuclear reaction by irradiation of isotopically enriched ^{18}O-water in a fixed-energy Cyclone 18/9 cyclotron (IBA).[12] Dried ^{18}F-fluoride–cryptate complex was prepared using standard separation and azeotropic drying procedure in the presence of Kryptofix 2.2.2 and potassium carbonate.[10]

The radiolabelling reactions were performed using the NanoTek continuous flow system (Advion BioSciences). The reactions were conducted in DMSO and were optimized by varying reaction temperature, precursor concentration, reagent ratio, and flow rate (residence time). Each reaction condition was tested at least three times. On exit from the microreactor the reaction mixtures were quenched with aqueous trifluoroacetic acid (0.1%) and analysed using ultra performance liquid chromatography (UPLC) and thin layer chromatography (TLC) to determine radiolabelling efficiency.‡ Product identity was confirmed by co-elution with the nonradioactive reference compound.

Results and discussion

Three peptides, bombesin derivatives of 7–8 amino acids, conjugated to a prosthetic group containing trimethylammonium

‡All commercially available reagents and solvents were used as received. For liquid chromatography Waters Acquity UPLC system connected in line with a FlowStar LB 513 radiodetector (Berthold Technologies) was used. The analytical column was a UPLC dedicated reversed-phase Acquity BEH C18, particle size 1.7 μm, 100 × 2.1 mm (Waters). The mobile phase consisted of a gradient of water or aqueous buffer and acetonitrile. The gradient was developed for each peptide. Reversed-phase TLC plates were Alugram RP-18W (Macherey-Nagel). The mobile phase was a mixture of 50 mM phosphate buffer (pH 7.4) with acetonitrile 3 : 7. Developed TLCs were visualized with InstantImager (Canberra Packard).

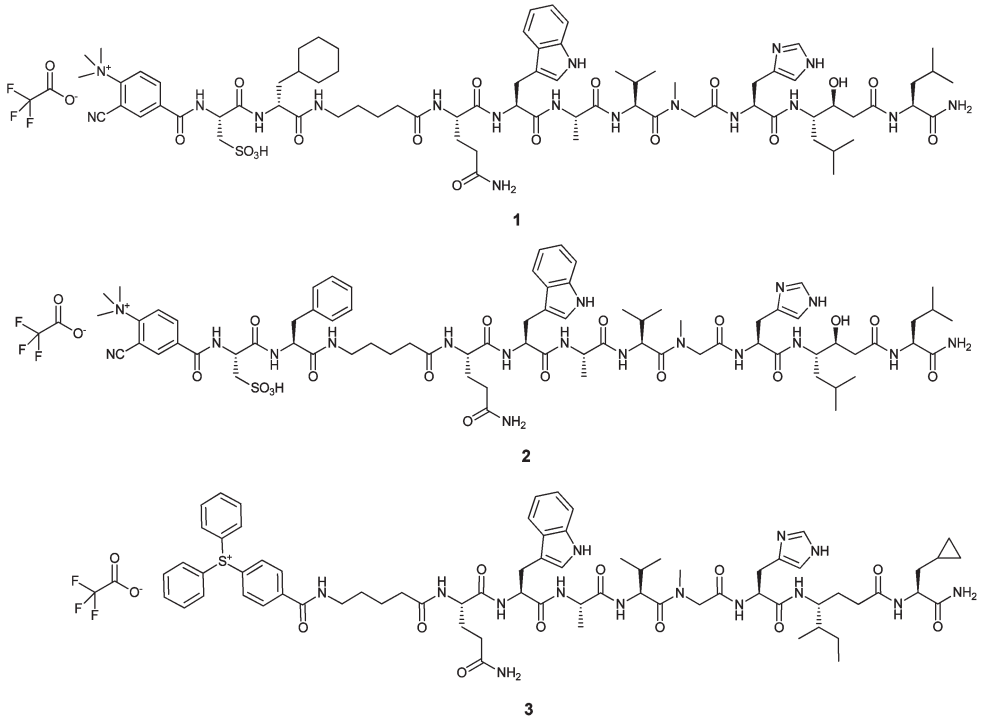
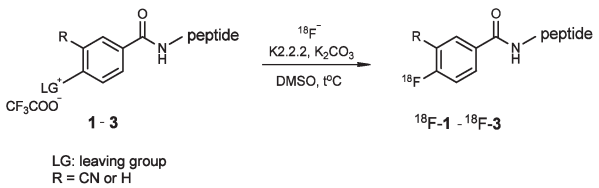


Fig. 1 Chemical structures of precursors **1–3**.



Scheme 1 Radiolabelling of peptides with nucleophilic ^{18}F.

leaving group or triarylsulfonium moiety (Fig. 1) were radiolabelled with ^{18}F in the microreactor (Scheme 1). No side products were detected in the reaction mixture for all described peptides and reaction conditions.

As reported earlier, the trimethylammonium leaving group can be replaced by ^{18}F under conventional heating in a vial in DMSO at 70–95 °C for 10–15 minutes and results in ~5–20% isolated decay corrected (d.c.) RCY after HPLC purification.[13] The optimal temperature in the microreactor was 70–80 °C yielding labelling efficiency up to 90% (estimated by UPLC§)

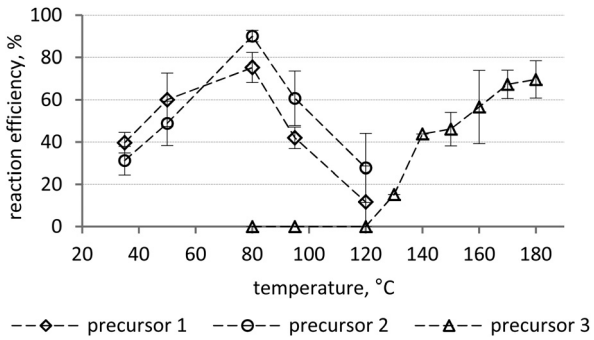


Fig. 2 Influence of the reaction temperature on the labelling efficiency, estimated by UPLC. All reactions were performed at a flow rate of 20 µl min^{-1}. Precursor concentrations were 0.5 mg ml^{-1} for precursor **1**, 1.0 mg ml^{-1} for precursor **2**, and 4 mg ml^{-1} for precursor **3**. The standard deviation range is inside the symbol area if it is not visible.

(Fig. 2). More reproducible labelling results were obtained in this temperature range as well. The reaction progressed even at 35 °C resulting in 30–40% labelling yield. This is an important finding since it opens an opportunity to label thermally labile peptide molecules with ^{18}F radioisotope under very mild conditions.

Moreover, at such low temperatures it is very unlikely for the radiolabelled peptide to lose its biological activity due to racemization when heated in a basic environment. The reaction efficiency dropped significantly when the reaction temperature was raised over 90 °C, probably due to peptide degradation. The reaction efficiency increased with higher concentration of the starting material and reached a plateau. Accounting for the fact that such precursors are often in limited availability,

§ UPLC was used for the purpose of estimation of the reaction efficiency. For this, an aliquot of each quenched reaction mixture solution was injected into UPLC. The obtained chromatograms were processed using dedicated UPLC Empower software. For each chromatogram all radioactive peaks were integrated and the percentage of the corresponding product peak was plotted on graphs as a function of reaction conditions (Fig. 2–4). Normally, these estimated values are higher than the radiochemical yield of the isolated product. Trends in radiolabelling reaction efficiency estimated by TLC were in accordance with the UPLC data, although the values (reaction efficiency, %) were somewhat lower (see ESI;† Comparison of analytical data obtained using UPLC and TLC for peptide **2**). The percentage yields obtained (radiolabelling reaction efficiency) are relative and were used to compare and select the best reaction conditions. These are not absolute isolated RCYs.

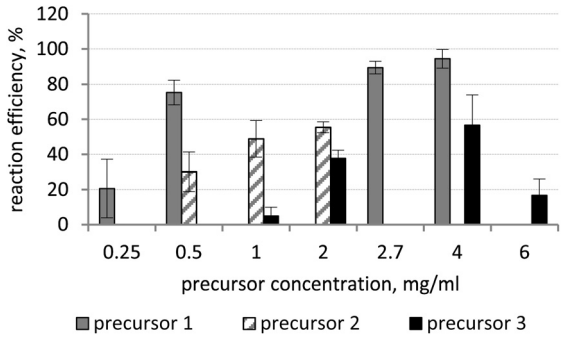


Fig. 3 Influence of the precursor concentration on the labelling efficiency, estimated by UPLC. Reactions were performed at 80 °C for precursor **1**, at 50 °C for precursor **2**, and at 160 °C for precursor **3**. Flow rate was 20 µl min^{-1}.

reactions could be performed even at a concentration of 0.5 mg ml^{-1} with reasonable yields (Fig. 3). For example, we radiolabelled precursor **1** at a concentration of 0.5 mg ml^{-1}, 80 °C, and 50 µl min^{-1} flow rate with 61% radiolabelling efficiency as estimated by UPLC. Purification of the reaction mixture on a SepPak C18 Light cartridge (Waters) resulted in 43% isolated d.c. RCY. Flow rates between 10 and 50 µl min^{-1} had no influence on reaction efficiency for precursor **1** (Fig. 4). For precursor **2**, an increase in radiolabelling yield with slower flow rates was more noticeable at lower reaction temperatures (Fig. 4). Slower flow rate results in longer reaction time. It should be noted that potassium carbonate was used as a base during reactions in the microreactor. The use of potassium carbonate gave negligible yields in conventional radiosyntheses; therefore caesium carbonate was employed instead. A difference in solubility may play a role: caesium carbonate is more soluble in organic solvents than potassium carbonate. Usually dry water-free solvents are required for nucleophilic radiofluorination reactions. It may be that potassium carbonate remains mostly undissolved during conventional heating while microreactor conditions, usually characterized by high surface area to volume ratio of reactants, may improve its chance for interaction, thus giving better yields than in a vial-based radiosynthesis.

It was found that harsh conditions were required for the direct radiolabelling of precursor **3**, containing the diarylsulfonium leaving group, if potassium carbonate is used as a base. This is also the case in conventional radiosynthesis (unpublished data). The radiolabelling was negligible at temperatures below 130 °C (Fig. 2). The optimal temperature was established at 170–180 °C, giving about 70% labelling efficiency at 20 µl min^{-1} flow rate and 4 mg ml^{-1} precursor concentration. The precursor concentration has a much more pronounced influence on the reaction outcome for the diarylsulfonium than for the trimethylammonium leaving group (Fig. 3). The reaction efficiency increased more than 7-fold when the concentration of the precursor was doubled from 1 to 2 mg ml^{-1}. At 4 mg ml^{-1} the radiolabelling yield still rose significantly but further increase of the precursor concentration resulted in a reduction in labelling efficiency. So far, we have no explanation for this phenomenon. The influence of the flow rate was similar to the other two precursors and was more noticeable at 160 °C and below (Fig. 4). Radiolabelled compound ^{18}F-**3** could be reliably produced in

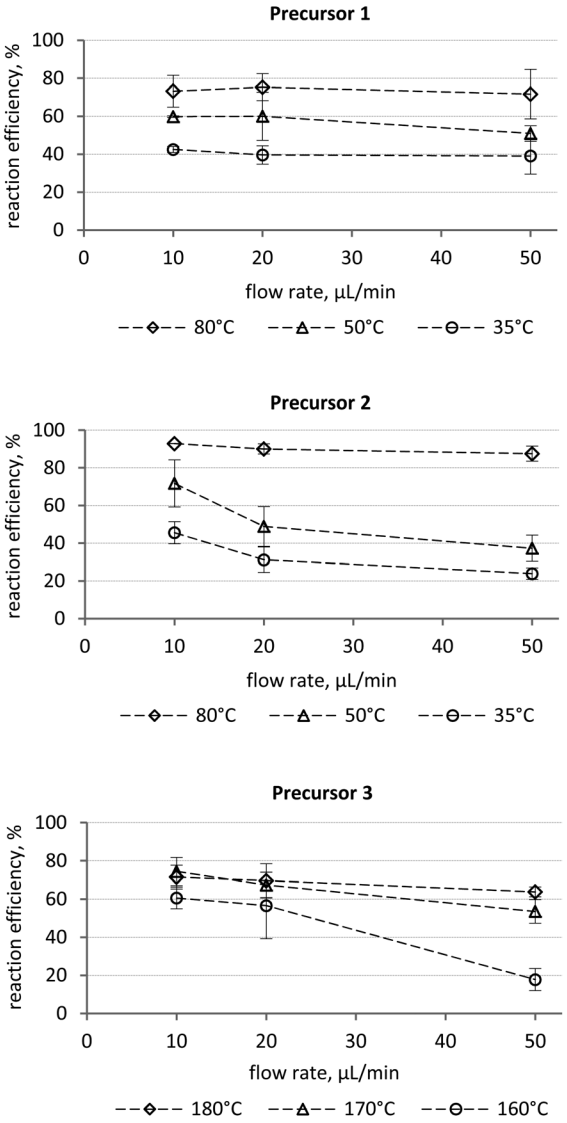


Fig. 4 Influence of the flow rate on the labelling efficiency, estimated by UPLC. Precursor concentrations were 0.5 mg ml^{-1} for precursor **1**, 1.0 mg ml^{-1} for precursor **2**, and 4 mg ml^{-1} for precursor **3**.

22–28% (n = 5) isolated d.c. RCY at optimized conditions (170 °C, 20 µl min^{-1} flow rate, 4 mg ml^{-1} precursor concentration).

Conclusions

Direct radiolabelling of peptides with ^{18}F-fluoride can be successfully achieved in a reproducible manner using the Advion NanoTek continuous flow microreactor. Fast synthesis time and general module setup allows for rapid optimisation of the reaction conditions and multiple on-demand productions of a given radiolabelled peptide. The amount of precursor required for the radiolabelling can be substantially decreased, in particular for peptides having trimethylammonium leaving group, which facilitates purification and helps to save precious starting material. Incorporation of a cartridge purification system and analytical HPLC into the module design would be beneficial.

Acknowledgements

We thank Dr Lee Collier from Advion Biosciences Inc. (Ithaca, NY, USA) for continuous support during set-up and testing of the NanoTek synthesizer.

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