A fluorine-18 radiolabelling method enabled by rhenium(I) complexation circumvents the requirement of anhydrous conditions

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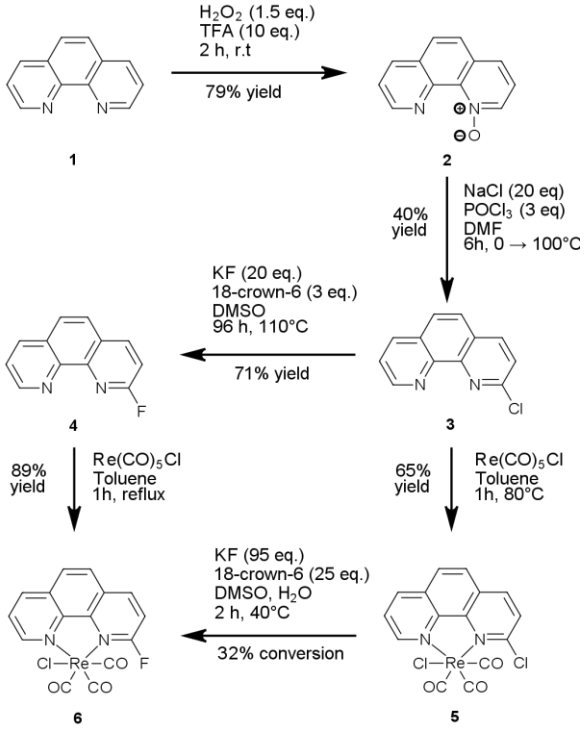
Abstract: Azeotropic distillation is typically required to achieve fluorine-18 radiolabelling during the production of positron emission tomography (PET) imaging agents. However, this time consuming process also limits fluorine-18 incorporation, due to radioactive decay of the isotope and its adsorption to the drying vessel. In addressing these limitations, we herein report the fluorine-18 radiolabelling of one model rhenium(I) complex, which is significantly improved under conditions that do not require azeotropic drying. This work could open towards the investigation of a simplified metal-mediated late stage radiofluorination method, which would expand upon the accessibility of new PET and PET-optical probes.

Main Text:

Positron emission tomography (PET) plays an important clinical role towards unveiling the underlying biochemistry of diseases *via* the administration of positron (β^+) emitting radiopharmaceuticals. Radiosynthesis of most PET tracers needs to be performed quickly, in order to obtain a large enough dose for a successful PET imaging experiment, due to the radioactive decay of the β^+ emitting isotope. Therefore the production of PET radiotracers is performed using automated synthesis modules, and many research groups have worked towards integrating microfluidic technologies to reduce radiation dose, miniaturise the workspace and improve production.[1] Fluorine-18 is the most commonly employed PET radioisotope, owing to its 97% β^+ decay profile, ideal 109.7 min half-life and low 0.64 MeV β^+ energy.[2] However, one drawback is that fluorine-18 is generally produced by the cyclotron mediated $^{18}O(p,n)^{18}F$ reaction on oxygen-18 enriched water, which affords [^{18}F]fluoride in an aqueous environment, that is typically unreactive towards nucleophilic substitution; nonetheless, the interest in employing this unfavorable aqueous environment has inspired some innovative approaches.[3] Traditionally, in order to provide an anhydrous environment, the [^{18}F]fluoride in water is trapped on an anion exchange resin (thus also removing radioactive cationic impurities), eluted with a complexing agent in aqueous/organic solvent mixture, and azeotropically dried in a distillation vial. This azeotropic drying is an inefficient and time consuming process (>15 mins[4]) during which period, radioactivity (≥10%) is lost due to the radioactive decay of the fluorine-18 isotope, as well as non-specific adsorption of [^{18}F]fluoride to the surface of the distillation vial, leading to lower radiochemical yield (RCY) of the PET radiotracer.[3,5]



Scheme 1. Synthesis of radiolabelling precursors **3** and **5**, and non-radioactive standards **4** and **6**. Refer to SI sections 1.1 - 1.5 for detailed procedures.

Having been inspired by recent efforts to couple PET imaging with an optical tracer to complement disease diagnosis[6], and given our experience in the use of phosphorescent rhenium(I) tricarbonyl diimine systems as optical markers for live cell imaging and diagnosis of pathologies[7], we sought to investigate

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the fluorine-18 radiolabelling of this class of rhenium complexes as potential precursors for the future development of PET-optical multimodal markers. Scheme 1 shows the synthetic route used to synthesise the ligand (**3**) and complex (**5**) precursors, as well as the non-radioactive standards **4** and **6**. *N*-Oxidation of 1,10-phenanthroline allowed for selective chlorination in the 2-position by the Vilsmeier reagent to afford **3**. Nucleophilic aromatic substitution with azeotropically dried fluoride, using 18-crown-6 as a phase transfer catalyst, then afforded the novel 2-fluoro-1,10-phenanthroline ligand (**4**). Ligands **3** and **4** were then chelated to a Re(I) metal centre to attain complexes **5** and **6**, respectively (SI 1.1 - 1.5).

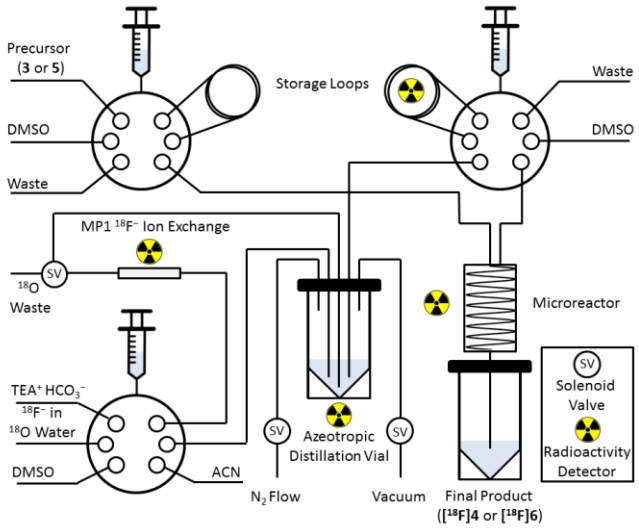

Figure 1**.** Simplified schematic of the flow chemistry configuration assembled for the automated syntheses of compounds [^{18}F]**4** and [^{18}F]**6**.

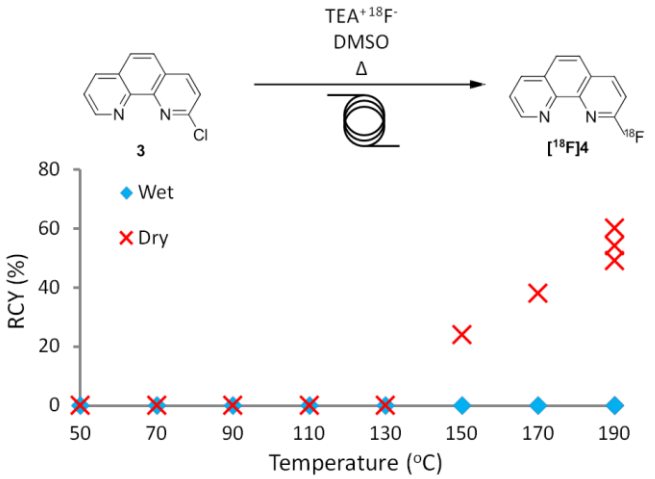

Figure 2**.** Temperature dependence of nucleophilic aromatic substitution of ligand **3** with [^{18}F]fluoride under azeotropically dried (red crosses) and non-azeotropically dried (blue squares) conditions.

The radiosyntheses of [^{18}F]**4** and [^{18}F]**6**, from **3** and **5** precursors respectively, were automated *via* a flow chemistry set-up adapted from our previous work[8] as shown in Figure 1. Within this set-up, aqueous [^{18}F]fluoride was first passed through an MP1 cartridge and eluted with tetraethylammonium bicarbonate ($TEA^+HCO_3^-$) in 90% CH_3CN:H_2O solution. The resulting [^{18}F]TEA^+F^- complex was then either azeotropically dried (*dry* conditions) or not azeotropically dried (*wet* conditions), before being loaded into the microreactor alongside the desired precursor solution (**3** or **5**).

The RCYs obtained for the radiosynthesis of [^{18}F]**4** from **3** under varying temperature conditions are illustrated in Figure 2. These conditions employed the same residence times (47 s) and precursor amounts (0.08 - 0.12 µmol) within the microreactor. Under traditional *dry* conditions, the RCY of ligand [^{18}F]**4** followed the expected trend, whereupon the yield increases as a function of temperature.[8] In this instance, [^{18}F]**4** only formed at temperatures greater than 130°C, providing a maximum RCY of 61% at 190°C. The radiosynthesis of complex [^{18}F]**6** from **5** was then attempted under analogous dry conditions, and the dependence of RCY from temperature is illustrated in Figure 3.

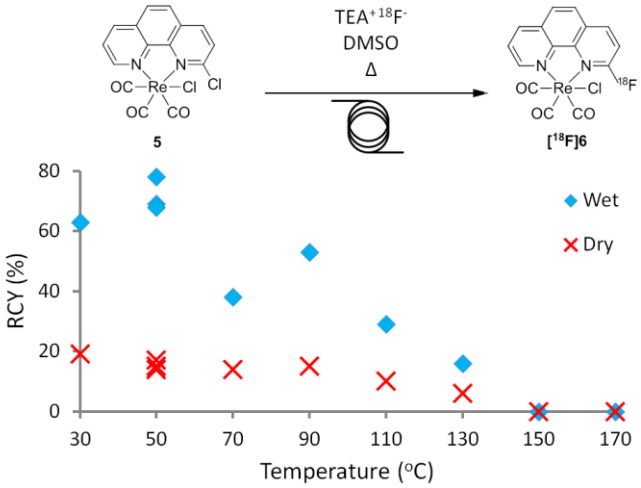

Figure 3. Temperature dependence of nucleophilic aromatic substitution of complex **5** with [^{18}F]fluoride under azeotropically dried (red crosses, *dry*) and not azeotropically dried (blue squares, *wet*) conditions.

Intriguingly, in this case we observed an opposite trend to the ligand, whereupon the complex [^{18}F]**6** only formed at temperatures below 150°C (in contrast to the ligand [^{18}F]**4** which required higher temperatures to afford a radioproduct). The RCYs for the complex [^{18}F]**6** were also noticeably lower than that of the ligand [^{18}F]**4**, with a maximum of 19% RCY obtained at 30°C; in addition, it was clearly noticeable the appearance of a UV-absorbing degradation product (Figure 4). We suspected that this by-product could be due to base-mediated degradation of **5**, and we indeed verified this by analyzing in HPLC a DMSO solution of **5** spiked with 5 µL of 5N NaOH. Therefore, we sought to suppress this undesired process by modifying the basicity of the reaction media. Thus, the radiosynthesis of [^{18}F]**6** was performed under *wet* conditions, whereupon azeotropic drying was excluded while preparing the radiofluorinating solution,

which realized a 10% v/v content of H_2O. Such *wet* conditions have been shown previously to be influential in the formation of different reaction products[9] or improved RCYs; to our delight, the radiosynthesis of [^{18}F]**6** under *wet* conditions was successful and we achieved as large as 78% RCY at a temperature as low as 50°C. Residence times and precursor amounts were also optimized in order to maximize the RCY (refer to SI 3.1), yielding [^{18}F]**6** in as high as 87% RCY when using 0.38 µmol of precursor within a residence time of 47 s. Significantly less of the degradation product from **5** was observed under these *wet* and low temperature conditions, confirming the desired switch from degradation to improved nucleophilic displacement by [^{18}F]fluoride in these aqueous conditions. To verify that the Re(I) centre was influential in facilitating nucleophilic substitution in the 2- position in such extremely mild conditions, we also performed the radiosynthesis of the ligand [^{18}F]**4** under identical *wet* conditions which, as expected, afforded no radioproduct at all as shown in Figure 2. It is suspected that the electron withdrawing nature of the Re(I) metal centre, due to the *π*-acceptance of the CO ligands, may activate the 2-position of phenanthroline for improved nucleophilic substitution at low temperatures. Mechanistic studies are currently underway in our labs to explain why the presence of water favors the radiofluorination reaction so greatly.

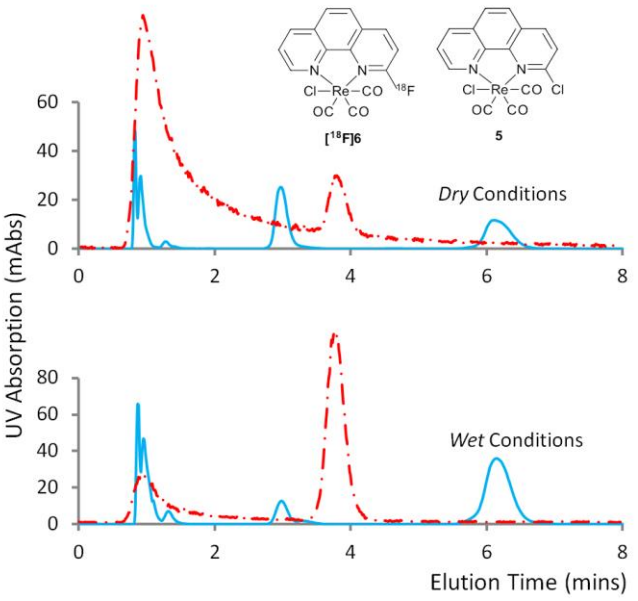

Figure 4**.** UV absorbance (254nm, solid blue) and superimposed radioactive (dashed red) profiles, for *dry* and *wet* analogous conditions (top and bottom, respectively). UV active degradation by-product (R_t: 3 min) is reduced under wet conditions. R_t: [^{18}F]F^- (RAD, 1 min), [^{18}F]**6** (RAD, 3.7 min) **5** (UV, 6.2 min).

The direct radiolabelling of the Re(I) tricarbonyl diimine complex was also compared to a two-step automated radiosynthetic process.[10] Using microfluidic technology, we assembled a second microreactor in series, alongside a fourth pump to supply the $Re(CO)_5Cl$ precursor, as shown in Figure 5. RCYs as great as 35% were able to be achieved using ≥ 5× stoichiometric equivalents of $Re(CO)_5Cl$ compared to **3**, as shown in Figure 6, despite the residual basic conditions from the reaction in the first microreactor. Therefore, this unprecedented two-step microfluidic approach indeed led to the desired final product by marginally increasing total process time (4 *vs.* 10 min), but provided a reduced RCY compared to the direct milder radiofluorination of **5**. A process involving the purification of [^{18}F]**4** and performance of a separate 2nd step reaction was not attempted, as it would have involved a much longer and more complex system resulting in further radioactive decay.

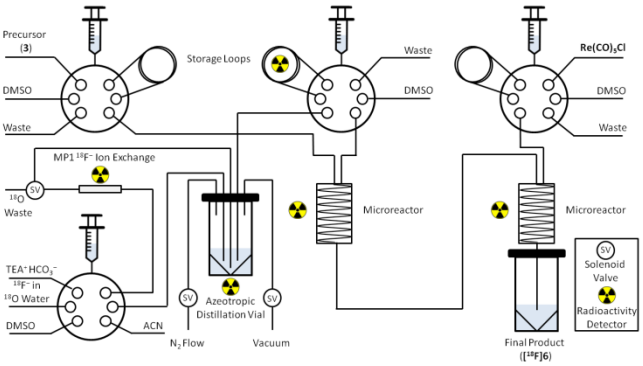

Figure 5**.** Simplified schematic of the flow chemistry configuration assembled for the automated two-step radiosynthesis of [^{18}F]**6**.

Before performing the radiolabelling using microfluidic systems, we were unable to synthesize **6** from **5** using the usual heated and anhydrous conditions which afforded **4** from **3**. Given the resulting formation of [^{18}F]**6** from **5** under *wet*, low temperature conditions, we revisited the bulk non-radioactive synthesis and attempted the reaction of **5** to **6** under analogous conditions. UV reaction monitoring revealed that complex **6** did indeed form under these *wet* and low temperature conditions (32% HPLC conversion) thus demonstrating how fast and efficient microfluidic optimization can be also used to identify the best reaction conditions for bulk syntheses.

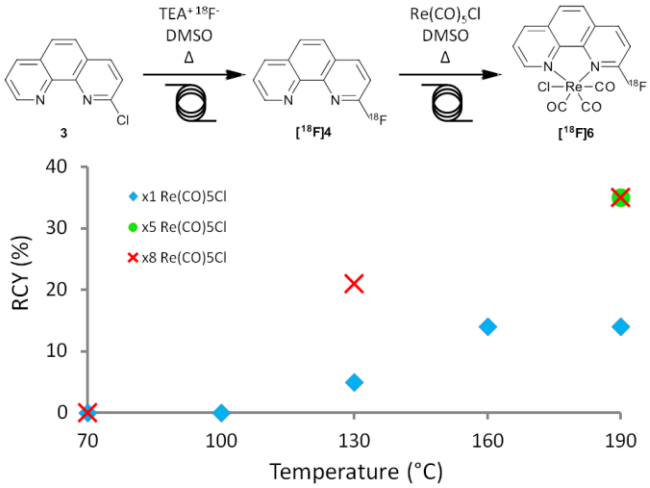

Figure 6. Temperature dependence (2nd step) of the two-step radiofluorination to form complex [^{18}F]**6** under azeotropically dried conditions using one (blue squares), five (green circle) and eight (red crosses) stoichiometric equivalents of the $Re(CO)_5Cl$ precursor.

Photophysical properties of **6** were also assessed, in order to investigate the effect of replacing a chloro for a fluoro substituent in the 2- position of phenanthroline, given our initial research incentives to develop PET-optical probes. We therefore verified that these Re(I) complexes afforded similar quantum yields (Φ = 0.3%), lifetimes (τ = 27 - 41 ns), emission maxima (λex = 410 nm, λem = 653 - 654 nm) and monoexponential decay profiles in DMSO (SI 4.1 - 4.3).

In summary, the fluorine-18 radiolabelling of a ligand complexed to Re(I) was found to afford optimal RCYs under remarkably mild conditions, without the need for azeotropic distillation and with a reduction of >100°C for the optimal temperature. Further investigations will clarify the involvement of the Re(I) centre in the radiofluorination mechanism, and will seek to apply this radiolabelling strategy toward improving the RCY of existing PET tracers, as well as toward accessing new PET and PET-optical probes.

Experimental Section

Aqueous [^{18}F]fluoride was produced on an IBA Cyclone 18 Twin cyclotron (ANSTO, Camperdown, Australia) using the ^{18}O(p,n)^{18}F nuclear reaction. Microfluidic radiosyntheses were performed in Discovery Mode using a NanoTek LF Microfluidic Synthesis System (Advion, Ithaca, NY) connected to a standard laptop using the NanoTek software, V1.4.0 GMP Lite. Microreactors were made of fused silica tubing (100 µm × 2 m), coiled tightly into a brass ring containing a thermoresistent polymer to hold the tubing in place. RadioHPLC analyses were carried out using a Shimadzu system comprised of a CBM-20 controller, LC-20AD pump, SIL-20AHT autoinjector, SPD-M20A PDA detector (for UV analyses) and a Lablogic Posi-RAM gamma detector. Analyses were performed using isocratic conditions consisting of CH_3CN/H_2O/TFA mobile phases (7.95:91.95:0.10% v/v for [^{18}F]**4** and 64.95:34.95:0.10% v/v for [^{18}F]**6**), on a Chromolith RP column (monolith system, Merck, 50 × 4.6 mm). RCYs were calculated using the radio-HPLC profiles, integrating the radioproduct peak against all the other radioactive peaks (inclusive of the unreacted [^{18}F]fluoride), using Laura V4.1.70 SP2 HPLC data analysis software.

The microfluidic experiments were carried out on the scale of 29±10 MBq, and afforded [^{18}F]**6** with a molar activity of 71±12 MBq.nmol^{-1}.

Experimental details concerning the general syntheses, structure elucidation, radiochemistry and photophysical characterisations of the reported compounds can be found within the SI sections 5.1 – 5.3.

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Keywords: fluorine-18 radiolabelling • microfluidics • PET chemistry • rhenium complexes • late stage fluorination

[1] Representative examples: a) G. Pascali, P. Watts and P. A. Salvadori, *Nucl. Med. Biol.* **2013**, *40*, 776–787; b) A. Lebedev, R. Miraghaie, K. Kotta, C. E. Ball, J. Zhang, M. S. Buchsbaum, H. C. Kolb and A. Elizarov, *Lab Chip* **2013**, *13*, 136–145; c) C.-C. Lee, G. Sui, A. Elizarov, C. J. Shu, Y.-S. Shin, A. N. Dooley, J. Huang, A. Daridon, P. Wyatt, D. Stout, H. C. Kolb, O. N. Witte, N. Satyamurthy, J. R. Heath, M. E. Phelps, S. R. Quake and H.-R. Tseng, *Science* **2005**, *310*, 1793–1796; d) C. J. Steel, A. T. O'Brien, S. K. Luthra and F. Brady, *J. Label. Compd. Radiopharm.* **2007**, *50*, 308–311; e) P. W. Miller, H. Audrain, D. Bender, A. J. deMello, A. D. Gee, N. J. Long and R. Vilar, *Chem. Eur. J.* **2011**, *17*, 460–463.

[2] S. M. Ametamey, M. Honer and P. A. Schubiger, *Chem. Rev.* **2008**, *108*, 1501–1516.

[3] Representative examples: a) C. F. Lemaire, J. J. Aerts, S. Voccia, L. C. Libert, F. Mercier, D. Goblet, A. R. Plenevaux and A. J. Luxen, *Angew. Chem. Int. Ed.* **2010**, *49*, 3161–3164; b) R. Richarz, P. Krapf, F. Zarrad, E. A. Urusova, B. Neumaier and B. D. Zlatopolskiy, *Org. Biomol. Chem.* **2014**, *12*, 8094-8099; c) J. Aerts, S. Voccia, C. Lemaire, F. Giacomelli, D. Goblet, D. Thonon, A. Plenevaux, G. Warnock and A. Luxen. *Tetrahedron Lett.* **2010**. *51*, 64-66; d) S.H. Wessmann, G. Henriksen and H. J. Wester. *NuklearMedizin.* **2012**. *51*, 1-31; e) F. Basuli, X. Zhang, E. M. Jagoda, P. L. Choyke and R. E. Swenson. *Nucl. Med. Biol.* **2016**. *43*, 770-772; f) J-H. Chun, S. Telu, S. Lu and V. W. Pike. *Org. Biomol. Chem.* **2013**. *11*, 5094-5099.

[4] L. Matesic, A. Kallinen, N. Wyatt, T. Q. Pham, I. Greguric and G. Pascali, *Aust. J. Chem.* **2014**, *68*, 69–71.

[5] S. Lindner, C. Rensch, S. Neubaur, M. Neumeier, R. Salvamoser, V. Samper and P. Bartenstein, *Chem. Commun.* **2016**, *52*, 729–732.

[6] Representative examples: a) Z. Li, T. P. Lin, S. Liu, C. W. Huang, T. W. Hudnall, F. P. Gabbai and P. S. Conti, *Chem. Commun.* **2011**, *47*, 9324–9326; b) S. Liu, D. Li, Z. Zhang, S. G. K. Prakash, P. S. Conti and Z. Li, *Chem. Commun.* **2014**, *50*, 7371–7373; c) T. Temma, N. Kondo, M. Ono and H. Saji, *J. Nucl. Med.* **2015**, *56*, 1127; d) J. A. Hendricks, E. J. Keliher, D. Wan, S. A. Hilderbrand, R. Weissleder and R. Mazitschek, *Angew. Chem.* **2012**, *51*, 4603–4606; e) K. Chen, Z.-B. Li, H. Wang, W. Cai and X. Chen, *Eur. J. Nucl. Med. Mol. Imaging*, **2008**, *35*, 2235–2244; f) F. Ducongé, T. Pons, C. Pestourie, L. Hérin, B. Thézé, K. Gombert, B. Mahler, F. Hinnen, B. Kühnast, F. Dollé, B. Dubertret and B. Tavitian, *Bioconjugate Chem.* **2008**, *19*, 1921–1926; g) W. Guo, X. Sun, O. Jacobson, X. Yan, K. Min, A. Srivatsan, G. Niu, D. O. Kiesewetter, J. Chang and X. Chen, *ACS Nano* **2015**, *9*, 488–495.

[7] Representative examples: a) M. V. Werrett, P. J. Wright, P. V. Simpson, P. Raiteri, B. W. Skelton, S. Stagni, A. G. Buckley, P. J. Rigby and M. Massi, *Dalton. Trans.* **2015**, 44, 20636–20647; b) C. A. Bader, R. D. Brooks, Y. S. Ng, A. Sorvina, M. V. Werrett, P. J. Wright, A. G. Anwer, D. A. Brooks, S. Stagni, S. Muzzioli, M. Silberstein, B. W. Skelton, E. M. Goldys, S. E. Plush, T. Shandala and M. Massi, *RSC Adv.* **2014**, *4*, 16345–16351.

[8] G. Pascali, L. Matesic, T. L. Collier, N. Wyatt, B. H. Fraser, T. Q. Pham, P. A. Salvadori and I. Greguric, *Nat. Protoc.*, **2014**, *9*, 2017–29.

[9] Representative examples: a) G. Pascali, M. De Simone, L. Matesic, I. Greguric and P. A. Salvadori, *J. Flow Chem.* **2014**, *4*, 86–91; b) T. R. Neal, S. Apana and M. S. Berridge, *J. Label. Compd. Radiopharm.*, **2005**, *48*, 557–568.

[10] G. Pascali, G. Nannavecchia, S. Pitzianti and P. A. Salvadori, *Nucl. Med. Biol.* **2011**, *38*, 637 – 644.

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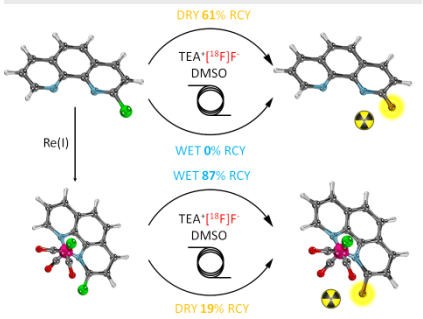
A novel fluorine-18 radiolabelling method has been found to circumvent the need for preliminary azeotropic distillation. The method involves complexation to a rhenium(I) centre, and is of particular importance in the expanded scope of positron emission tomography (PET) imaging agent design. Results were verified through the monitoring of multiple reaction conditions by employing microfluidic technologies in flow.



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