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Fluorous ligand capture (FLC): a chemoselective solution-phase strategy for isolating ^{99m}Tc-labelled compounds in high effective specific activity†

Justin W. Hicks, Laura E. Harrington and John F. Valliant*

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A new approach for preparing ^{99m}Tc-labelled compounds in high effective specific activity was developed by utilizing a novel fluorous ligand capture (FLC) agent and a chemoselective filtration strategy. This paradigm eliminates the need to use HPLC to obtain technetium(I) based molecular imaging probes free from residual precursor.

Molecular radioimaging using positron emission tomography (PET) and single photon emission computed tomography (SPECT)[1] make it possible to visualize biochemical changes *in vivo* thereby providing the opportunity for earlier detection and molecular characterization of diseases and injuries. These methods depend upon targeted molecular imaging probes; radiolabeled compounds which can localize medical isotopes to sites in direct proportion to the concentration of a specific target. When developing molecular radioimaging probes for specific receptors, a B_{max}/K_D of 10 or greater is desirable to achieve high target to non-target ratios.[2,3] For systems of modest expression levels it is essential that the probe have high affinity for the target and that the concentration of competing ligands be minimized to prevent non-specific binding which has a detrimental impact on target to non-target ratios and the general utility of a probe.[4]

The traditional process of preparing SPECT radiopharmaceuticals using ^{99m}Tc, the most widely used radionuclide in diagnostic medicine, involves the use of instant kits which contain a reductant, buffers and a large excess of the ligand to be labelled. Upon the addition of technetium, the product mixture contains the desired product and unlabelled vector which is problematic in the case of molecular imaging probes because the excess unlabelled precursor often maintains similar binding affinity as the radiotracer for the target.[3,5] For research, preparative HPLC is a common method for removing the excess unlabelled ligands; however, this approach is not optimal for routine clinical use, primarily because of prolonged operator exposure to radiation and the general length of time required for purification. Staff exposure during the preparation and dispensing of PET and SPECT radiopharmaceuticals has been an issue of concern and attempts are made to minimize sample handling or maximize shielding.[6,7]

The move toward solid-phase extraction and automated synthesis and purification systems will serve to reduce operator exposure by minimizing sample handling and accelerating preparation and purification times as well as facilitating the process of meeting GMP guidelines; the current focus on processes that can be easily automated is a general trend in the field.[8] An alternative strategy that has been developed to address the issue of removal of excess unlabeled precursor without HPLC are solid-phase labelling methods[9,10] which are effective in removing the excess ligand but the labelling yields are not ideal.[9,10] An additional disadvantage is that not all radiopharmaceuticals can be linked to solid-supports such that the desired product is released upon labelling.

An alternative approach is to employ ligand capture following labelling; both solid and liquid-based systems are possible. Reported solid-phase capture reactions, such as those used for mass spectrometric identification of phosphoproteins[11] and those for capture and removal of bacterial pathogens[12] are generally not suitable for working with radioactive compounds. Moreover, solid-phase labelling methods, which are favoured in high throughput screening of combinatorial libraries,[13] have often been shown to result in high non-specific binding of the radionuclide to solid supports leading to decreased radiochemical yields.[10] An alternative approach would be to employ a solution-phase ligand capture system based on fluorous chemistry.[14] Ley and coworkers have used a comparable "phase-switch" method to isolate reagents and scavengers used in synthetic transformations.[15]

Fluorous radiolabelling methods offer a convenient means to purify radiolabelled compounds. The general approach to date involved systems whereby the fluorous group is cleaved upon reaction with the radionuclide. The labelled compound can then be isolated by passing the reaction mixture through a fluorous solid-phase extraction cartridge where the precursor is selectively retained.[16,17] Our approach is to incorporate the fluorous synthon into the scavenging agent which will capture the unreacted ligand after the radiolabelling reaction. This route avoids the step of preparing the fluorous precursor of the desired radiolabelled product.

A fluorous copper chelate complex that can selectively bind unlabelled ligand, rendering it fluorous, without degrading the

Departments of Chemistry and Chemical Biology & Medical Physics and Applied Radiation Sciences, McMaster University, Hamilton, ON, Canada. E-mail: valliant@mcmaster.ca; Fax: +905-522-7776; Tel: +905-525-9140 ext. 20182

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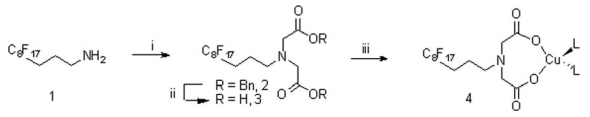


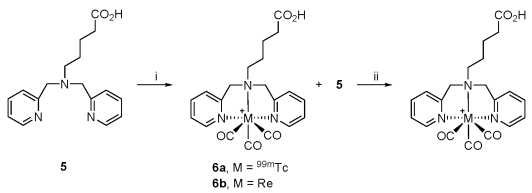
Scheme 1 (i) $BrCH_2CO_2Bn$, DIPEA (ii) H_2, Pd/C, CH_3OH (iii) $CuBr_2$, EtOH.

labelled compound was developed as the FLC reagent. The soluble ligand capture agent was prepared by combining 3-(perfluorooctyl)propyl amine (**1**) with 2-bromobenzylacetate to yield **2** in 98% yield (Scheme 1). Use of unprotected bromoacetic acid led to a large mixture of side products which could not be easily separated from the desired chelate. Following hydrogenation, the copper complex was prepared using a saturated $CuBr_2$ in EtOH solution which produced the product as a blue precipitate in 91% yield.

To test the extraction efficiency, **4** was combined with a simple Tc(I) chelate system, bispyridyl valeric acid (**5**) prior to working with a peptide-targeted agent (Scheme 2). **5** was chosen because it contains a highly effective chelator which forms inert complexes with the $[^{99m}Tc(CO)_3]^+$ core.[18] In addition, the ligand has a strong UV absorption making it possible to determine the extraction efficiency by HPLC.

Compound **5** (3.3 μmol) was combined with $[^{99m}Tc(CO)_3(H_2O)_3]^+$ in a microwave reactor for 2 min at 150 °C (Scheme 2). The γ-HPLC chromatogram displayed the formation of the desired BPV-Tc complex (t_R = 12.9 min.), with a retention time matching the Re standard (t_R = 12.6 min.)[19] while unlabelled BPV ligand could be seen in the corresponding UV trace (t_R = 10.7 min.). Compound **4** (10 mg) was dissolved in 0.5 mL DMF and then added to reaction mixture. After stirring for 5 min, the mixture was loaded onto a F-SPE cartridge and a fluorophobic wash of 20% water in MeOH was used to elute the BPV-Tc complex. Analytical HPLC (Fig. 1) showed that **6a** was obtained as the only radioactive product with greater than 99% of the ligand removed and an average of 8.5 ± 1.6% (n = 4) of the activity remained on the cartridge (Table 1). The compound could then potentially be dried by rapid evaporation on the Biotage V10 and reconstituted as an injectible formulation. Ethanol can be substituted for methanol to give a formulation suitable for injection (following dilution with an isotonic aqueous solution).

For comparison, the solid-phase analogue of **4** was prepared following the method of Ley *et al.*[15,20] and evaluated as a ligand capture reagent. Three Amberlite IRC-784 resins of different particle sizes were used (20–75, 75–150, and 150–300 μm). These resins, which are functionalized with iminodiacetic acid moieties, were loaded with copper by shaking a suspension of the resins in saturated solutions of $CuSO_4$ for 24 h. After thorough washing, the resins were loaded into standard solid-phase extraction tubes (100 mg) and activated prior to use with



Scheme 2 (i) $[M(CO)_3(OH_2)_3]^+$, microwave heating (ii) **4** then FSPE.

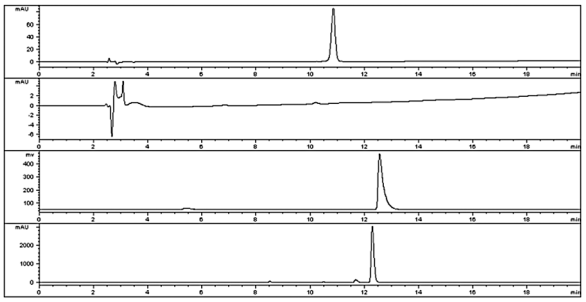


Fig. 1 HPLC chromatograms associated with the FLC of **5**/**6a**. Top: UV trace of the crude reaction mixture showing **5** prior to FLC. Second from top (note scale change): UV trace following FLC. Third: γ trace following FLC showing only **6a**. Note that the UV and radioactivity detectors are connected in series.

Table 1 Comparison of ligand removal methods

Method	Ligand removed	Non-specific binding
Solid-phase (20–75 μm)	86 ± 2%	27 ± 3%
Solid-phase (75–150 μm)	90 ± 3%	22 ± 1%
Solid-phase (150–300 μm)	91 ± 5%	21 ± 1%
FLC (**5**/**6a**)	> 99%	8 ± 2%
FLC (**7**/**8a**)	95 ± 1%	12 ± 1%
Preloaded FLC (**5**/**6a**)	99 ± 1%	2 ± 1%
Preloaded FLC (**7**/**8a**)	95 ± 1%	33 ± 3%

an acidic saline solution (pH = 4). The mixture containing **5** and **6a** was dripped onto the resin slowly, where approximately 1/3 of the activity was eluted during the loading process and another 40–50% was eluted with a 3 mL saline wash. Further washes with saline and then MeOH did not elute the significant amounts (> 20%) of activity that remained on the cartridge.

The solid-phase extraction procedure, which also required a C-18 SPE to eliminate residual copper, was able to remove between 86 and 91% of the excess ligand which is reasonable but less effective than the fluorous system. In addition, the high non-specific binding to the solid-support greatly favours the FLC method where the inherently poor interaction between fluorous compounds and polar organic/inorganic molecules was particularly advantageous.

An alternative strategy, a hybrid of the FLC and solid phase methods, was investigated whereby fluorous silica preloaded with **4** was used to purify the model reaction mixture. As the radiolabelling mixture passed through the silica, the excess ligand was captured by **4** and remained behind while the product was eluted. This approach is more amenable to an instant kit type strategy. As a test, compound **4** was preloaded onto a 2g F-SPE cartridge using a 1 : 1 mixture of MeCN and DMF containing 5 drops of 1N HCl and the reaction mixture containing **5** and **6a** added subsequently. Following a gradient elution, **6a** was selectively eluted with 99% of the ligand removed and 90% of the activity recovered. This represents an improvement over the solid phase extraction procedure however it requires more manipulation.

With the successful extraction of the free dipyridyl amine chelate using the FLC method, the focus shifted to peptide conjugates. Peptides are attractive vectors for targeting radiometals to specific proteins. For this work, a dipyridyl amine

conjugate of a LTVSPWY peptide that is known to target erbB2 receptors was prepared.[21] ErbB2 is a human epidermal growth factor whose over expression is present in approximately 30% of breast cancer cases. In such cases, the prognosis is poor as this receptor is normally a sign of a metastasizing tumor.[22] To incorporate the dipyridyl ligand in this peptide, an analogue derived from Fmoc-protected lysine as opposed to the valeric acid derivative was used (see ESI for structure†). The peptide (**7**) and the Re analogue (**8b**) were prepared using a CEM Liberty synthesizer according to a literature procedure.[22]

Compound **7** was combined with $[^{99m}Tc(CO)_3(OH_2)_3]^+$ in a microwave reactor for 10 min at 60 °C. The γ-HPLC chromatogram showed a peak representing the ^{99m}Tc-peptide complex (**8a**) (t_R = 15.6 min.) which matched the retention time of the Re standard (t_R = 15.2 min.) while the unlabelled peptide could be seen in the UV chromatogram (t_R = 13.0 min.). To remove the unlabelled ligand, **4** was added to the reaction mixture (20 mg). After 10 min of sonication the pale blue solution was loaded onto a FSPE cartridge and **8a** was eluted using a fluorophobic wash of 20% water in CH_3CN. A Biotage V10 rapid evaporation system was used to remove the solvent and the residue was redissolved into 1 mL of 25% CH_3CN in water for HPLC analysis. The UV chromatogram showed that an average of 95 ± 1% of the ligand had been removed demonstrating that the FLC method can be applied to larger ligands as well as small molecule chelates. Unfortunately, but not unexpectedly, the loss of product from non-specific binding increased (average of 12 ± 1%, n = 3). The value is still superior to solid-phase capture. Purification of the labelled peptides using the FSPE cartridge precoated with **4** was also attempted. The approach resulted in 95% of the ligand being removed but higher loss of product (33%). The reason for this is likely interaction with uncapped silica groups on the SPE cartridge. Evaluating different solid-phase materials is a focus of current research.

The results described demonstrate that fluorous ligand capture is a feasible alternative to HPLC for the purification of new Tc(I)-based molecular imaging probes. This system can be readily adapted for use with an automated synthesis platform which is routinely used in manufacturing and purifying radiopharmaceuticals. The general utility of this method for other Tc chelates needs to be assessed including a more detailed measurement of the optimal and relative stability constants. Nonetheless, a change in how and where Tc radiopharmaceuticals are prepared (*i.e.* in large central radiopharmacies equipped with automated synthesis units as opposed to small hospital pharmacies) in combination with the development of new labelling and purification strategies should allow for more effective agents to be developed and formulated.[23]

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