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A New Strategy for Preparing Molecular Imaging and Therapy Agents Using Fluorine-Rich (Fluorous) Soluble Supports

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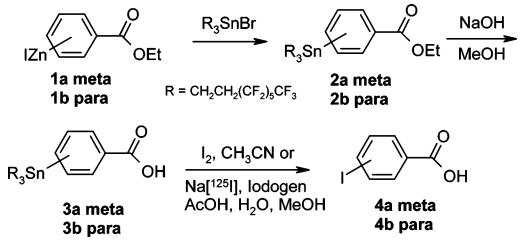
The increasing use of molecular radioimaging for diagnostic medicine, drug development, and biological research[1,2] has created a need for new labeling methods that afford radiotracers in high effective specific activity without having to employ time-consuming purification protocols. In the case of tracers which utilize targeting vectors for delivering the radionuclides to cell surface receptors, the key impurity that must be removed is the excess substrate present during labeling. Unlabeled starting material can compete with the small amount of tracer for the target which typically leads to reduced image quality. It is particularly imperative that residual starting material be removed when tracers are being employed in small animal imaging studies, so that sufficient levels of activity can be administered in order to be able to acquire realistic imaging data without violating the tracer principle or causing unwanted biological effects.[2]

To achieve "precursor-free" formulations without having to resort to HPLC, which is not an attractive option particularly for radionuclides with short half-lives, a number of groups have developed solid-phase labeling strategies.[3−8] In solid-phase labeling, a substrate is bound to an insoluble support in such a manner that it will only be released into solution if it reacts with a radionuclide. Unreacted material remains bound to the support and is removed by simple filtration. One of the disadvantages common to all reactions that are performed on insoluble supports is that preparation of ultrahigh purity resin-bound substrates can be problematic. If reactions used to prepare precursors are not quantitative upon labeling, impurities will be formed, which must then be removed using traditional purification methods.

An alternative approach is to use soluble supports in place of cross-linked resins. This would allow for purification and characterization of precursors using standard solution-phase techniques, while retaining the ability to separate labeled compounds from the corresponding starting materials. The approach described here employed fluorous supports, which have been used successfully as soluble synthetic platforms.[9−14] The first target was a system based on an iodo-demetalation reaction using a fluorine-rich tin support. This is directly analogous to the solid-phase labeling system developed by Hunter and co-workers.[4,6]

To investigate the feasibility of the fluorous labeling strategy, meta and para benzoic derivatives were attached to a "heavy" fluorous support through a tin linker (Scheme 1). In an attempt to prepare the target compounds, a fluorous tin bromide[15] reported by Curran and co-workers was reacted with the dilithio salt of *p*-bromobenzoic acid. Using this approach, **3b** could only be isolated in 40% yield. A superior approach involved reacting the fluorous tin bromide with organozinc derivatives of benzoic acid ethyl ester, which are commercially available.[16] Compounds **2a** and **2b** were isolated by simple extraction into perfluorinated hexanes (FC-72).

Scheme 1



Saponification of the esters using NaOH led to the desired products in excellent yields. Compounds **3a** and **3b** were fully characterized and their purities (>98% in both cases) determined by HPLC. The fact that precursors can be characterized by traditional methods and analyzed (or purified if necessary) by HPLC is a major advantage of the fluorous labeling method over an analogous solid-phase technique.

To verify that the tin−aryl bond was still reactive toward iodo-demetalation and that the fluorine-rich starting material and reaction byproducts could be effectively separated from the desired products, compounds **3a** and **3b** were iodinated using 0.5 equiv of "cold" I_2. Purification of the products was accomplished by passing the reaction mixtures down fluorous solid-phase extraction (FSPE) cartridges following dilution of the reaction mixtures with water. FSPE cartridges contain modified reversed-phase silica that selectively retain fluorine-rich compounds, which in the case presented here includes unreacted **3a**/**3b** and the tin support cleaved during labeling. The cartridges were washed with water to remove residual salts followed by 80% MeOH−H_2O, where the collected fractions showed only one major peak according to HPLC, which matched retention times of authentic standards. ESMS, which is very sensitive to the presence of the fluorous tin compounds, showed that the fractions containing the desired products (**4a**/**4b**) were not contaminated with any residual fluorous tin derivatives.

In light of the success of the cold experiments, compounds **3a** and **3b** were combined with [^{125}I]NaI in the presence of iodogen. After 3 min, the reactions were diluted with water and the mixtures passed down FSPE cartridges.[17] Washing with water eluted residual salts and iodide, while washing with 80% MeOH−H_2O selectively eluted the desired products, which were both obtained in 85% radiochemical yield and 98% radiochemical purity. Compounds **3a** and **3b** could be recovered by elution with THF. FSPE in conjunction with fluorous scavengers has likewise been employed to purify ^{35}S-radioligands.[18]

Benzoic acid derivatives were selected as the model substrates because they are useful starting materials for the preparation of a range of different types of labeled compounds, including benzamides, which are currently being investigated as agents for imaging melanomas.[19−22] To demonstrate their potential utility, compounds **3a** and **3b** were converted to propyl benzamides **6a** and **6b** (R′ =

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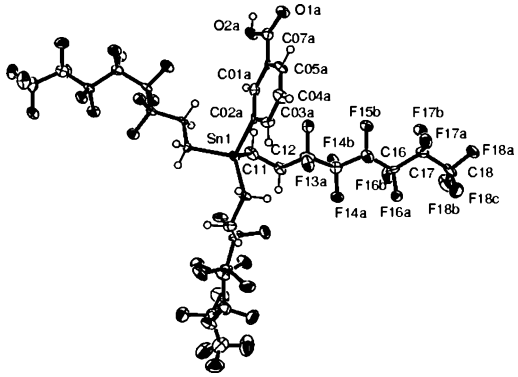
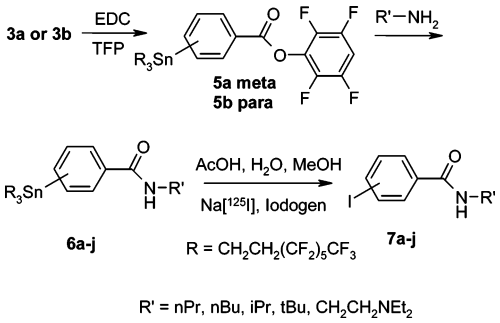


Figure 1. ORTEP drawing of **3a** (30% thermal probability ellipsoids).

Scheme 2. Synthesis and Labeling of Fluorous-Tagged Benzamides



*n*Pr) via the TFP active esters, which were prepared by reacting the acids with tetrafluorophenol and EDC (Scheme 2). Addition of excess amine followed by extraction into a fluorous solvent (FC-72) and chromatographic purification produced the desired products in good yield. Both compounds were radiolabeled under the conditions described above and the products (**7a**, **7b**) isolated in greater than 85% yield and 99% purity.

In addition to producing compounds in high effective specific activity, one of the other advantages of employing fluorous supports is that libraries of radiopharmaceuticals can be readily synthesized. This feature can be used to improve the rate by which promising lead candidates are discovered particularly when compared to conventional one molecule at a time discovery strategies. To demonstrate that the fluorous supports reported here can be used to create libraries of both precursors and labeled compounds, a small collection of benzamides (10) (Scheme 2) were prepared from compounds **5a** and **5b**. An excess of a series of simple amines were added to reaction wells containing one of the two fluorous active esters and the products purified by taking advantage of the nonfluorous nature of the reagents and reaction byproducts. All products, which were fully characterized and their purities verified by HPLC, were subsequently labeled with ^{125}I in high radiochemical yield (>85%), and the products were obtained in greater than 98% purity. It is noteworthy that the active esters **5a** and **5b** can also be labeled and the products isolated in high yield. Radiolabeled active esters are particularly useful for radiolabeling macromolecular targeting agents, such as proteins and antibodies.

From these results, it is clear that the fluorous labeling strategy (FLS) allows for facile purification of labeled compounds using a simple solid-phase extraction procedure. In addition to purifying labeled compounds, as mentioned previously, FLS also enables precursors to be purified and characterized by techniques used for small molecule pharmaceuticals. This point is further illustrated by the fact that we were able to obtain an X-ray structure of **3a** (Figure 1). For compounds which are destined for clinical use, the ability to unequivocally establish the purity and structure of precursors is a key aspect of getting regulatory approval.

In conclusion, a convenient new strategy for radiolabeling and purifying radiopharmaceuticals was developed. We are currently expanding the reported technique to include developing methods for labeling libraries of fluorous-tagged substrates with a range of nuclides beyond solely isotopes of iodine. We are also developing an automated labeling and purification system based on the FLS that is suited to routine production using high levels of activity.

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Supporting Information Available: X-ray data along with synthetic procedures and characterization data for **2a**, **2b**, **3a**, **3b**, **4a**, **4b**, **5a**, **5b**, **6a**−**j**, and **7a**−**j**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) *Handbook of Radiopharmaceuticals*; Welch, M. J., Redvanly, C. S., Eds.; John Wiley & Sons Ltd.: Etobicoke, ON, 2003.
(2) (a) Eckelman, W. C. *Nucl. Med. Biol.* **2002**, *29*, 777−782. (b) Jagoda, E. M.; Vaquero, J. J.; Seidel, J.; Green, M. V.; Eckelman, W. C. *Nucl. Med. Biol.* **2004**, *31*, 771−779.
(3) Flanagan, R. J. U.S. Patent 4,874,601, 1989.
(4) Culbert, P. A.; Hunter, D. H. *React. Polym.* **1993**, *19*, 247−253.
(5) Kawai, K.; Ohta, H.; Channing, M. A.; Kubodera, A.; Eckelman, W. C. *Appl. Radiat. Isot.* **1996**, *47*, 37−44.
(6) Hunter, D. H.; Xizhen, Z. *J. Label. Compd. Radiopharm.* **1999**, *42*, 653−661.
(7) (a) Dunn-Dufault, R.; Pollak, A.; Fitzgerald, J.; Thornback, J. R.; Ballinger, J. R. *Nucl. Med. Biol.* **2000**, *27*, 803−807. (b) Pollak, A.; Roe, D. G.; Pollock, C. M.; Lu, L. F. L.; Thornback, J. R. *J. Am. Chem. Soc.* **1999**, *121*, 11593−11594.
(8) Mundwiler, S.; Candreia, L.; Häfliger, P.; Ortner, K.; Alberto, R. *Bioconjugate Chem.* **2004**, *15*, 195−202.
(9) Curran, D. P. *Synlett* **2001**, *9*, 1488−1496.
(10) *Handbook of Fluorous Chemistry*; Gladysz, J. A., Curran, D. P., Horvath, I. T., Eds.; Wiley-VCH Verlag GmbH & Co. KGaA: Weinheim, Germany, 2004.
(11) Studer, A.; Hadida, S.; Ferritto, R.; Kim, S.-Y.; Jeger, P.; Wipf, P.; Curran, D. P. *Science* **1997**, *275*, 823−826.
(12) Zhang, W. *Tetrahedron* **2003**, *59*, 4475−4489.
(13) Zhang, W. *Chem. Rev.* **2004**, *104*, 2531−2556.
(14) Cavazzini, M.; Montanari, F.; Pozzi, G.; Quici, S. *J. Fluorine Chem.* **1999**, *94*, 183−193.
(15) Hoshino, M.; Degenkolb, P.; Curran, D. P. *J. Org. Chem.* **1997**, *62*, 8341−8349.
(16) The organozinc compounds are available from Aldrich Inc. In some instances, the product comes as a mixture of meta- and para-substituted isomers. Prior to each reaction, check the purity by ^{1}H NMR.
(17) Fluorous solid-phase extraction (FSPE) cartridges are available from Fluorous Technologies Inc.
(18) Zhang, A. S.; Elmore, C. S.; Egan, M. A.; Melillo, D. G.; Dean, D. C. *J. Label. Compd. Radiopharm.* **2005**, *48*, 203−208.
(19) Auzeloux, P.; Papon, J.; Azim, E. M.; Borel, M.; Pasqualini, R.; Veyre, A.; Madelmont, J.-C. *J. Med. Chem.* **2000**, *43*, 190−198.
(20) Eisenhut, M.; Mohammed, A.; Mier, W.; Schönsiegel, F.; Friebe, M.; Mahmood, A.; Jones, A., G.; Haberkorn, U. *J. Med. Chem.* **2002**, *45*, 5802−5805.
(21) Moins, N.; D'Incan, M.; Bonafous, J.; Bacin, F.; Labarre, P.; Moreau, M.-F.; Mestas, D.; Noirault, E.; Chossat, F.; Berthommier, E.; Papon, J.; Bayle, M.; Souteyrand, P.; Madelmont, J.-C.; Veyre, A. *Eur. J. Nucl. Med.* **2002**, *29*, 1478−1484.
(22) Moins, N.; Papon, J.; Seguin, H.; Gardette, D.; Moreau, M.-F.; Labarre, P.; Bayle, M.; Michelot, J.; Gramain, J.-C.; Madelmont, J.-C.; Veyre, A. *Nucl. Med. Biol* **2001**, *28*, 799−808.

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