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Safety-Catch Linker Strategies for the Production of Radiopharmaceuticals Labeled with Positron-Emitting Isotopes

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Positron emission tomography (PET) is a powerful technique for determining noninvasively the biodistribution of radiolabeled materials. PET has found broad utility as a clinical modality for the diagnosis and staging of cancer and other indications,[1] and is an emerging tool in drug discovery.[2] The existence of positron-emitting isotopes of carbon, nitrogen, oxygen, and fluorine permits PET in vivo analysis of radiolabeled drug molecules, but their short half-life[3] poses severe synthetic limitations.[4]

Instead of optimizing the preparation of individual radiolabeled compounds, we reasoned that it may be useful to be able to screen a multitude of compounds whose synthesis had been developed with PET radiolabeling in mind. Thus, analogous to the role of combinatorial methods in drug discovery, screening such a "PET-ready" library could allow rapid selection and optimization of tracer molecules.

We sought to produce labeled material by a route that could also allow preparation of combinatorial libraries. However, finding chemistry to bridge these fields is not straightforward. In PET chemistry, only trace levels of labeled reagents are available, resulting in low conversions of precursor and chromatographic isolation of desired product. In contrast, combinatorial methods normally force reactions to completion using excess reagents, and chromatography is avoided if at all possible.

We envisioned attaching precursor compounds to a solid support via a suitable safety-catch linker,[5] set up so that treatment with a reagent available in radiolabeled form would permit release of a radiolabeled product in a second step. Thus, in library preparation, an excess of "cold" reagent would ensure a high yield of the desired product. In contrast, in the PET lab, only a trace of radiolabeled material would be obtained, but the same desired material would still be the only released product, because unlabeled precursor remains attached to the resin, and unreacted labeling agent can be washed away prior to product release.

[^{11}C]-Methyl iodide is widely used in PET for alkylating amines, alcohols, and thiol groups.[6] In addition, alkylation is the priming step for safety-catch linkers such as the REM linker[7] and related strategies[8] for tertiary amines (**1**, Scheme 1), and the amide-releasing Kenner linker.[5a,9] The alkyl residue in Kenner's strategy remains attached to the resin upon cleavage, so we designed the "reverse Kenner" linker to release alkyl sulfonamides (**2**, Scheme 1).[10] We thus evaluated the behavior of the REM and reverse Kenner linkers for their PET-ready capability.

Our initial studies were carried out using nonradioactive reagents. Resins **3** and **5** (Scheme 2; prepared as described[7a,10]) could each be completely alkylated by methyl iodide in less than one half-life of carbon-11 (Figure 1). Cleavage of alkylated material with base was even more rapid, being complete in less than 1 min for each resin with methanolic ammonia. Triethylamine or Hunig's base were equally effective in releasing **4**, but solid amines[11] were unacceptably slow. No unalkylated material was released in any case, and pure products **4** and **6** were obtained without chromatography.

To more closely match the conditions of the radiochemistry lab, resins **3** and **5** were alkylated with substoichiometric quantities of methyl iodide (Figure 2). No decline in yield or purity occurred over at least three methylate/cleave cycles. Yields of 30−50% based on methyl iodide offered promise for PET studies.

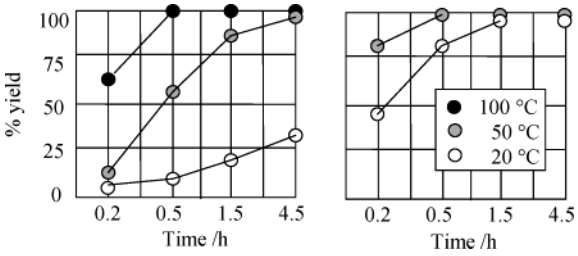
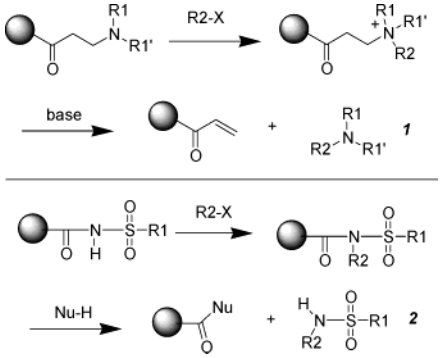


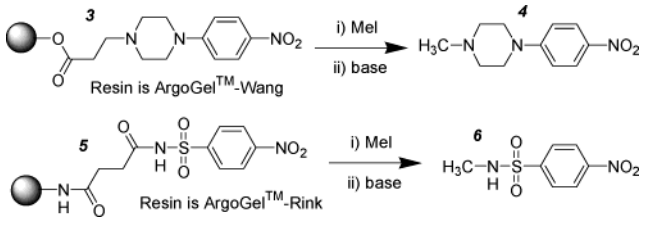
Figure 1. Alkylation of (left) resin **3**, (right) resin **5** with 1 M MeI in NMP (5 equiv) at the indicated temperature. Yields are by weight of product released on subsequent treatment with NH_3/MeOH.

Scheme 1. Alkylation/Release Using REM Resin (Top) and Reverse Kenner Resin (Bottom)[a]



[a] If reagent R2-X is radiolabeled (such as [^{11}C]-MeI), then a radiolabeled product (**1**, **2**) is released.

Scheme 2



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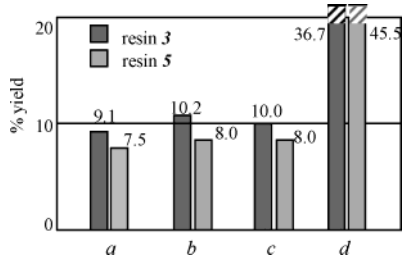
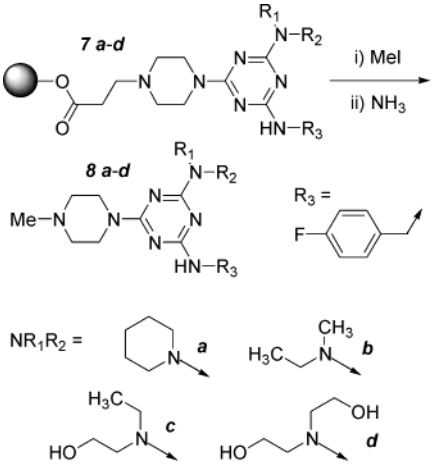


Figure 2. Yields of **4**, **6** in successive rounds of alkylation/release. Cycles a−c, 0.2 equiv of MeI; cycle d, 10 equiv. Values are based on weight of pure material. Resin **3** was alkylated at 80 °C for 20 min; **5** was treated at 50 °C for 15 min in each cycle. Total yields are 66% and 69%, respectively, based on stated loading of resin.

Scheme 3. A Small Library of PET-Ready Triazines



We thus applied the safety-catch linker approach to the generation of radiolabeled materials. Cyclotron-derived [^{11}C]-CO_2 was converted to [^{11}C]-methyl iodide by $LiAlH_4$ and HI as reported,[12] and then it was heated with REM resin **3** in a specially designed remote synthesis apparatus. Decay-corrected yields of 5−10% (based on [^{11}C]-MeI) were consistently obtained, giving up to 7.5 mCi of [^{11}C]-labeled material with radiochemical purities of at least 95% without chromatography. The "spent" resin was reusable, although yields were lower than with fresh resin (2−5%). Although the radiochemical yields were lower than in cold experiments, the amount of material was more than sufficient for use in in vivo studies using the microPET small animal scanner.[13] Sulfonamide resin **5** gave lower yields (2−3%; 0.75−1.7 mCi) and purity in initial tests. However, no unalkylated material was released, which would facilitate purification if desired.

On the basis of these initial data, the REM resin was chosen to prepare a small library of resin-bound precursors (**7a**−**d**, Scheme 3). A series of secondary amines were placed around a common triazine core, linked to the resin via a piperazine residue. The amines were chosen to provide products **8a**−**d** of varied log *P* for subsequent in vivo analysis. The availability of several hundred amines and other reagents which can be substituted on the triazine ring allows this chemistry to generate many thousands of possible PET precursors.[14]

Resin-bound precursors **7a**−**d** were treated as above, with cold and [^{11}C]-MeI to give **8a**−**d**. High-quality material was obtained in all cases. Radiochemical purity was 94−98% for ^{11}C-labeled materials with yields ranging from 3.8% to 9.8% (mean 6.8%, n = 9).

In conclusion, we have demonstrated the generation of compounds labeled with carbon-11 using two different safety-catch linkers, and the preliminary application of the REM linker for combinatorial production of radiolabeled materials. While potentially avoiding chromatography in the isolation of PET-labeled materials is a desirable development, the major advance of the safety-catch strategy may be that it enables the production of large numbers of "PET-ready" compounds using the techniques of combinatorial chemistry. In addition, the solid-supported strategy enables a generic approach to the synthesis of PET agents which should expand the application of PET in the development of new tracers and drugs.

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Supporting Information Available: Experimental details for the preparation of resin-bound precursors, and the release of products under radioactive and nonradioactive conditions (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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