Supporting Information for:

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

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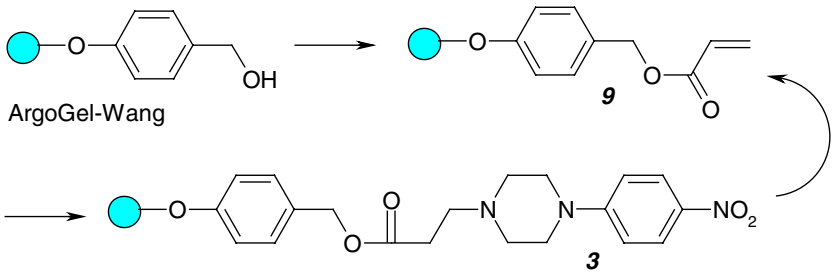
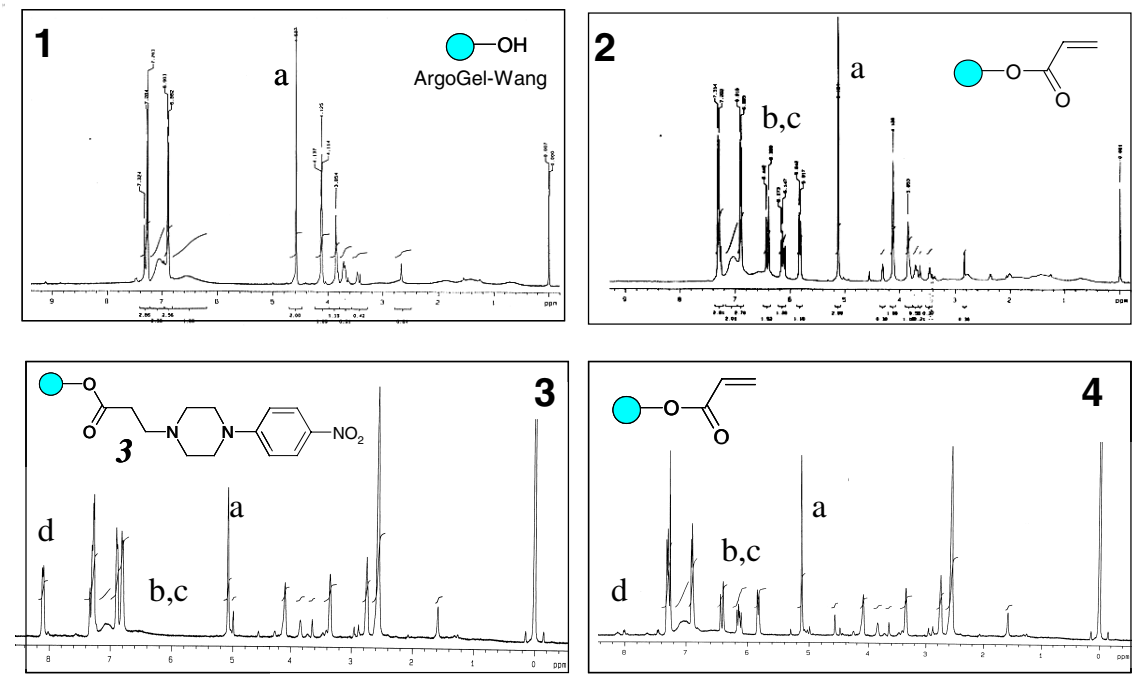
Preparation of p-nitrophenyl piperazine resin *3*;
Monitoring of resin-bound species by solid-phase NMR.

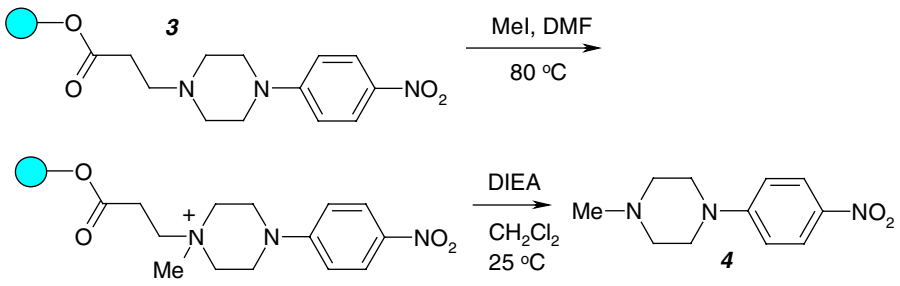


Resin ***3*** was prepared from ArgoGel-Wang resin (Argonaut Technologies, San Carlos, CA) by the method of Morphy *et al* (*Tetrahedron Lett.* **1996**, *37*, 3209-3212). Solid-phase magic angle spinning (SP-MAS) NMR (Fitch et al, *J. Org. Chem.* **1994**, *59*, 7955-7956) was used for reaction monitoring. The formation of the resin-bound acryloyl ester ***9*** (spectrum **2**) was characterized by a shift of the benzylic protons (**a**) from 4.6 ppm (ArgoGel Wang spectrum **1**) to 5.1 ppm, and the appearance of olefinic protons (**b**,**c**) between 5.8 and 6.4 ppm. Michael addition of 4-nitrophenyl piperazine to give resin ***3*** (spectrum **3**) was indicated by the disappearance of the olefinic protons (**b**,**c**) and the appearance of the aromatic protons (**d**) *ortho* to nitro at 8.1 ppm.

Alkylation and release was also carried out by the method of Morphy *et al* using methyl iodide. Spectrum **4** shows the regeneration of acryloyl ester ***9*** by the disappearance of nitrophenyl peaks (**d**) and reappearance of the olefin peaks (**b**,**c**).



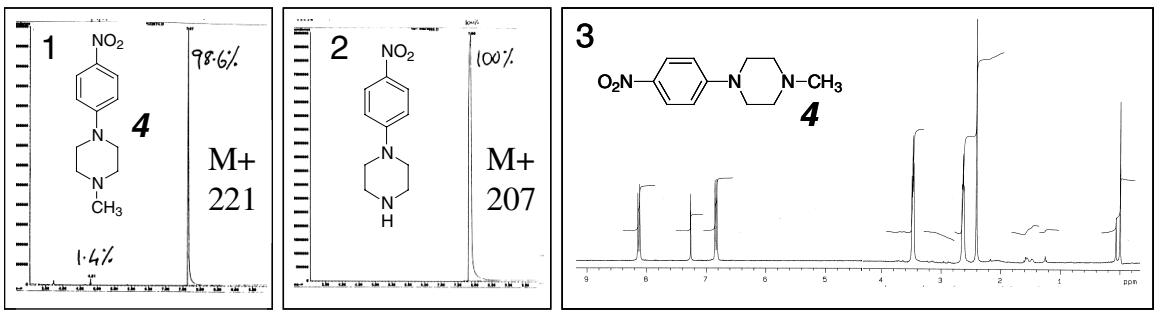
Production of *4* via safety-catch linker using sub-stoichiometric amounts of alkylating agent.



Resin ***3*** (5.0 g, 0.39 mmol/g, 1.95 mmol) was treated with DMF (20 mL) and methyl iodide (25 microL; 0.39 mmol, 0.2 eq). The mixture was heated at 80 C for 20 min then drained and washed well with DMF (3x), CH_2Cl_2 (3x). CH_2Cl_2 (25 mL) was added, followed by DIEA (3.3 mL, 19.5 mmol, 10 eq). The mixture was shaken at 20 C for 20 min then drained and washed well with CH_2Cl_2. The collected filtrates were evaporated under reduced pressure then taken up in CH_2Cl_2 / 5% $NaHCO_3$ (10 mL each). Aqueous phase was further extracted with CH_2Cl_2 (2 x 10 mL). Combined organic extracts were dried over K_2CO_3 and evaporated under reduced pressure to give N-methyl, N'-(4-nitro-phenyl)-piperazine ***4*** as a bright yellow solid (39.0 mg, 9.1 % based on resin loading, 45.6 % based on MeI; spectrum **3**, below). GC-MS chromatographic analysis of the crude product was performed (trace 1, below), as well as the corresponding 4-nitrophenyl-piperazine starting material (Aldrich; trace 2). NMR of the released material ***4*** is shown in trace 3. High resolution mass spectroscopy (EI) calculated for $C_{11}H_{16}N_3O_2^+$ 222.1237; observed 222.1238.

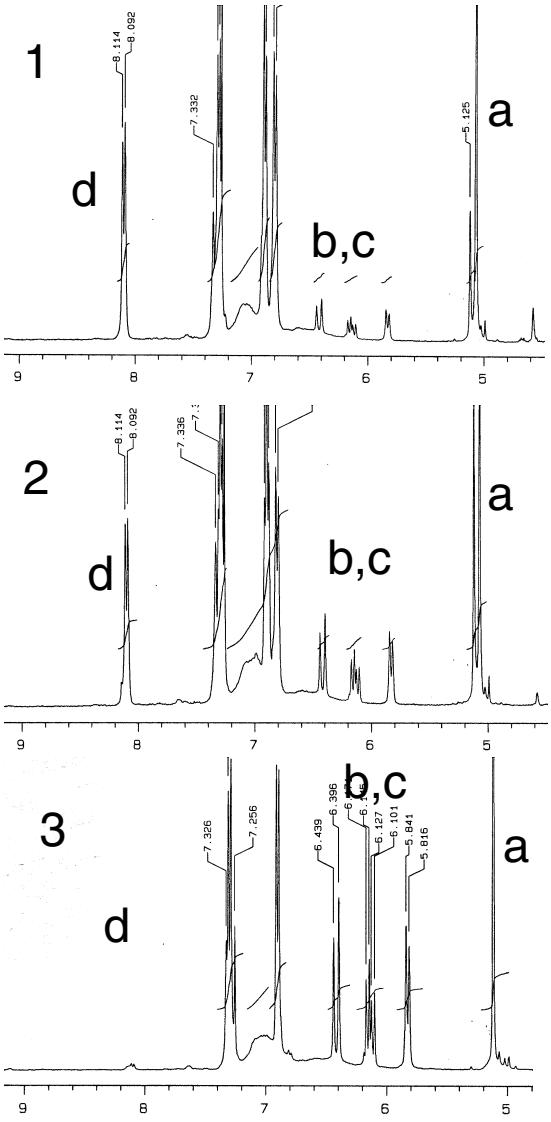
As expected, no unmethylated material was detected in the cleavage from the safety-catch resin. As further confirmation, a sample of resin ***3*** was subjected to the DIEA cleavage conditions without prior methylation. No material was released under these conditions.

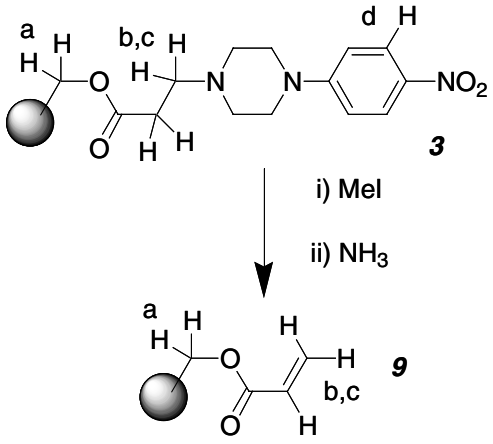
The methylation/release cycle was repeated three further times with 0.2, 0.2, and 5 eq of MeI respectively, giving 44.4, 43.0, and 158 mg of ***4*** (yield of 10.2, 10.0, and 36.7 % based on resin loading).



Production of *4* via safety-catch linker using sub-stoichiometric amounts of alkylating agent: Monitoring by solid-phase NMR.

For the experiment described on the previous page, resin samples were analyzed by solid-phase, magic-angle spinning proton NMR (Fitch *et al*, *J. Org. Chem.* **1994**, *59*, 7955-6). Spectra 1 – 3 below show the gradual conversion of resin ***3*** to ***9*** through several methylate/cleave cycles. Spectrum 1: single treatment with 0.2 eq MeI; Spectrum 2: 2x treatment with 0.2 eq MeI; Spectrum 3: treatment with excess (10 eq) MeI. The relevant resonances are labeled on the spectra and on the structures of starting resin ***3*** and product resin ***9***.





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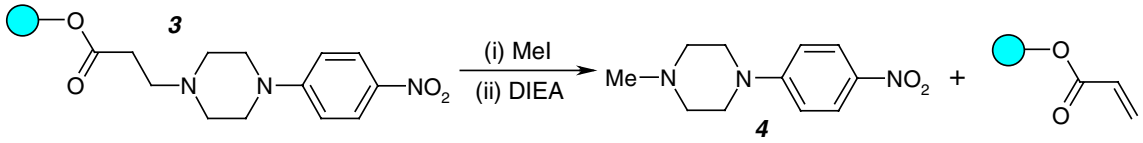
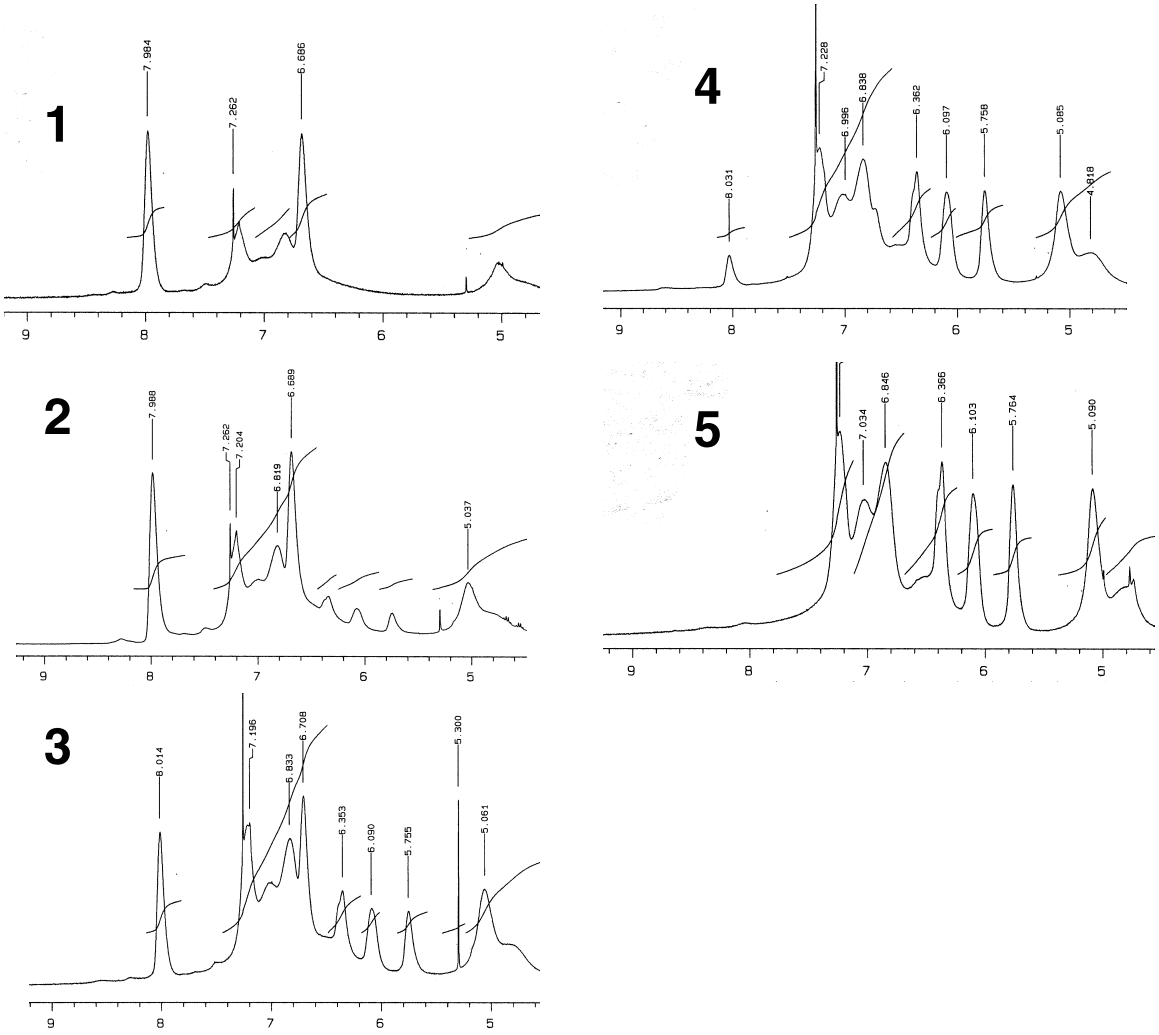
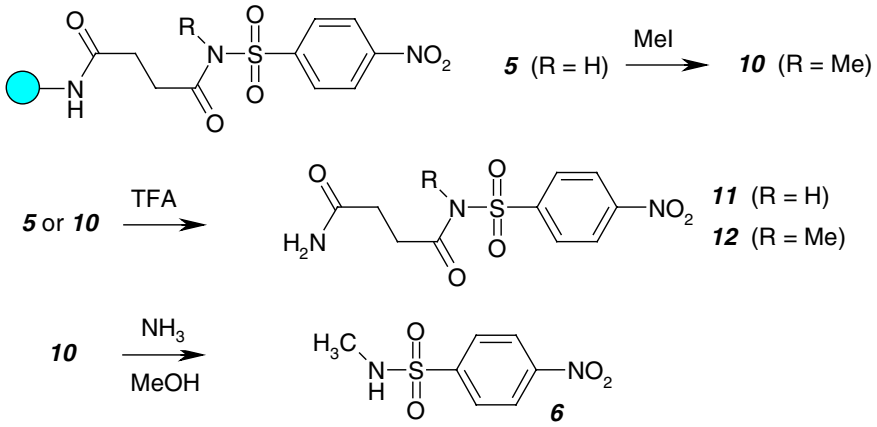
Alkylation and product release using sub-stoichiometric amounts of methyl iodide (polystyrene resin).



A series of methylate/release cycles were carried out on high-loaded polystyrene resin (2.0 g, 2.5 mmol/g, 5.0 mmol) similar to that described for ArgoGel resin ***3*** (above), and the SP-MAS NMR spectra collected for the resin after each cycle. Although significant line-broadening is observed for polystyrene resin in comparison to the gel-form ArgoGel resin, useful information is still obtained regarding reaction progress. Spectrum **1** is for polystyrene resin ***3***. Spectra **2**-**5** show the sequential release of p-nitrophenyl piperazine ***4*** on each cycle of methylation (with 0.2 eq MeI)/cleavage, as shown by the increase in peaks due to acrylate resin (e.g. 5.8 ppm) vs. resin-bound nitrophenyl (8.0 ppm).



Methylation of sulfonamide resin *5* and release of N-methyl sulfonamide *6*.

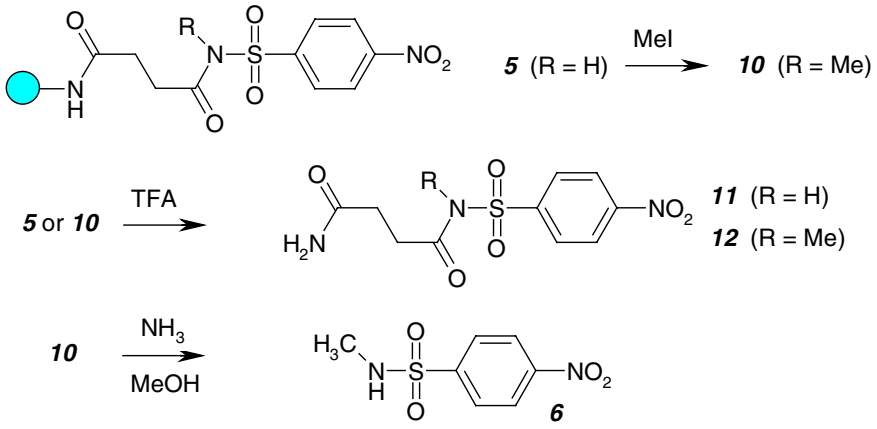


Resin ***5*** (1.0g, 0.36 mmol/g, 0.36 mmol) prepared as described previously (Maclean *et al., Org. Lett.* **2001**, *3*, 2977-2980) was washed with N-methyl pyrrolidinone (NMP) then treated with a solution of methyl iodide in NMP (1.0 M, 72 L, 0.072 mmol, 0.2 eq). Further NMP was added sufficient to slurry the resin (ca. 2 mL) and the mixture heated to 50 C for 15 min, then drained and washed with NMP (2x) and CH_2Cl_2 (3x).

A small sample (ca. 5 mg) of methylated resin was removed and treated with TFA (100 L), shaken for 10 min, then the supernatant was evaporated, taken up in 1:1 CH_3CN/H_2O (150 L), and analyzed by LC-MS indicating 7-10 % of ***12*** by UV, the remainder being unmethylated ***11***.

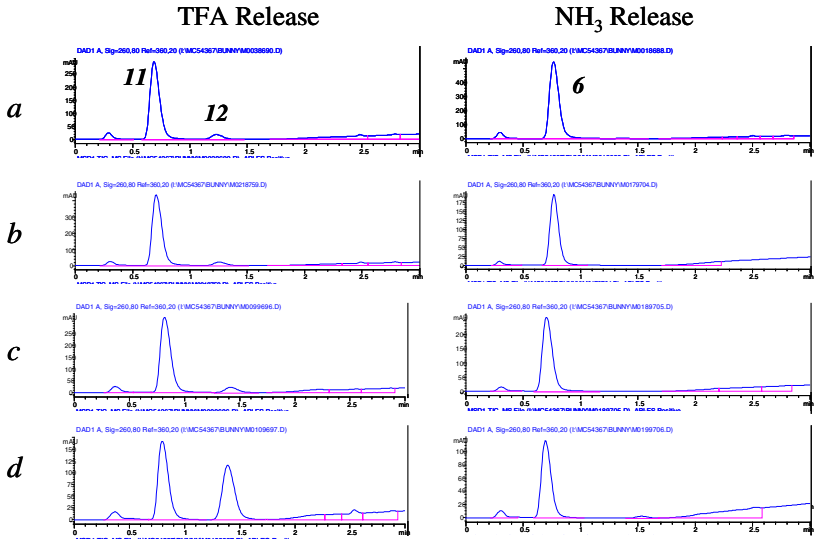
The remaining resin was treated with methanolic ammonia (2M; 4 mL), shaken at room temp. for 5 min, then drained and washed with methanol (x2) and CH_2Cl_2 (x3). The combined filtrates were evaporated to dryness to give ***6*** as a pale yellow film (5.9 mg; 7.5%). The resin was further washed with NMP (3x) and the methylation/release procedure repeated two more times with 0.2 eq MeI (8.0, 8.0% yield of ***6***) then with 10 eq MeI (45.5% yield). LC-MS analysis of crude products ***12*** and ***6*** after each cycle are shown on the next page. Further analytical data for ***6*** and related products can be found in the supporting information for Maclean *et al*.

Methylation of sulfonamide resin *5* and release of N-methyl sulfonamide *6*. LC-MS Analysis



See previous page for experimental description. Resin ***5*** was subjected to four successive cycles of methylation (labeled *a-d* in the figure below) using respectively 0.2, 0.2, 0.2, and 1.0 eq of methyl iodide. At each stage, two small samples of resin were removed and treated with (i) TFA (to release a mixture of ***11*** and ***12*** ; traces in left column of the figure) and (ii) methanolic ammonia (to release ***6*** ; right column in figure). Compounds ***6***, ***11***, and ***12*** were identified by the mass spectral analysis of the appropriate peaks in the chromatograms shown below (detection is UV, 220 nm).

The TFA-released samples show methylation level (ratio of ***12*** to ***11***) of 7-10 % in each of the first three cycles, in good agreement with the observed mass yield of released product ***6*** (see previous page). The ammonia-released products at each cycle are of high purity. In particular, there is no evidence of the release of any unalkylated material. To further demonstrate the selective retention of unalkylated material, resin ***5*** was treated with methanolic ammonia without prior treatment with methyl iodide. No material was released.



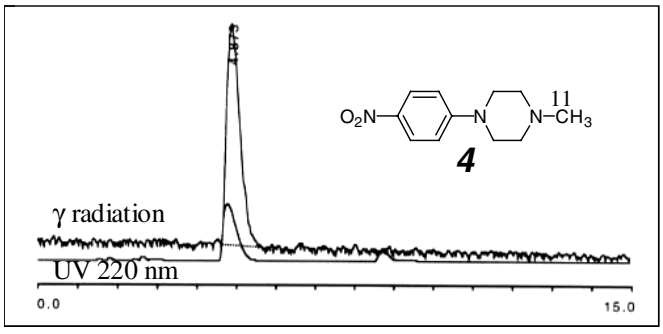
Production of [^{11}C]CH_3I

No carrier added [^{11}C]CO_2 was produced via ^{14}N(p,)^{11}C nuclear reaction. Irradiation of 200 psi of normal nitrogen gas containing 1.5% of oxygen in an aluminum-target body (volume 14.6 mL) with 11 MeV protons (40 A for 60 min) typically produced 1700 mCi of [^{11}C]CO_2 which upon reaction with $LiAlH_4$ followed by HI, yielded 300-500 mCi of [^{11}C]CH_3I (specific activity: 5 Ci/ mol).

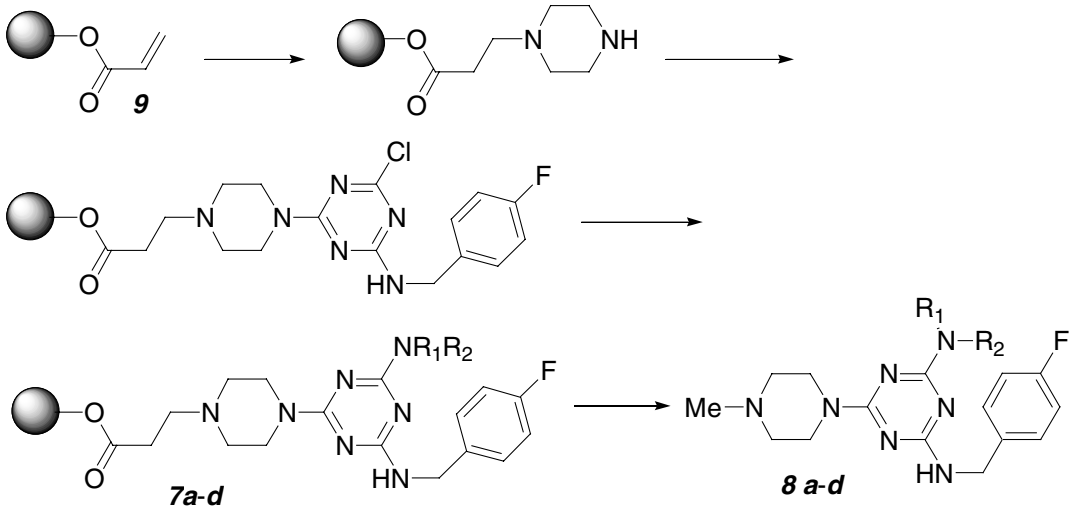
Quaternization of resin-bound tertiary amine with [^{11}C]CH_3I and isolation of final radiolabeled product

Quaternization of resin-bound tertiary amine was carried out using a specially designed, remote, semi-automated reaction apparatus. [^{11}C]CH_3I was trapped in 0.5 mL of a dipolar aprotic solvent (e.g. acetonitrile, dimethylformamide, dimethylsulfoxide, or tetrahydrofuran) and added to a glass column filled with 50-60 mg of resin-bound tertiary amine. The glass column was heated with stirring to 50-100°C for 10 min. The liquid phase was then drained from the column and the resin washed with 2 x 1 mL of the same solvent used for the reaction (to remove unreacted [^{11}C]CH_3I). The ^{11}C-labeled quaternary ammonium resin was then reacted with 0.1 mL of the solvent used for the reaction and heated to 50-70°C for 5 min. The liquid was drained from the column, diluted with 20 mL of water and passed through an activated C-18 SepPak. The SepPak was washed with 2 x 10 mL of sterile water to remove the organic solvent and then the ^{11}C-labeled tertiary amine was eluted with 1 mL of 100% ethanol. The ethanolic solution was diluted with normal saline (9 mL) and passed through a 0.22 M sterilizing filter into a multidose sterile injection vial. Production took 30-60 min to complete after end of bombardment.

Decay-corrected yields of ***4*** from tertiary amine resin ***3*** were 21.4 mCi to 30.2 mCi in four consecutive experiments starting from 300-370 mCi of ^{11}C-CH_3I, with radiochemical purity of at least 95 % in each case. HPLC trace shown below (Knauer HPLC system with a Phenomenex Luna C18 reversed phase column (250 x 4.6 mm), isocratic elution with 3:1 methanol/water at a flow of 1.5 mL/min).



Preparation of Triazine Precursor Resins *7* and Triazines *8*



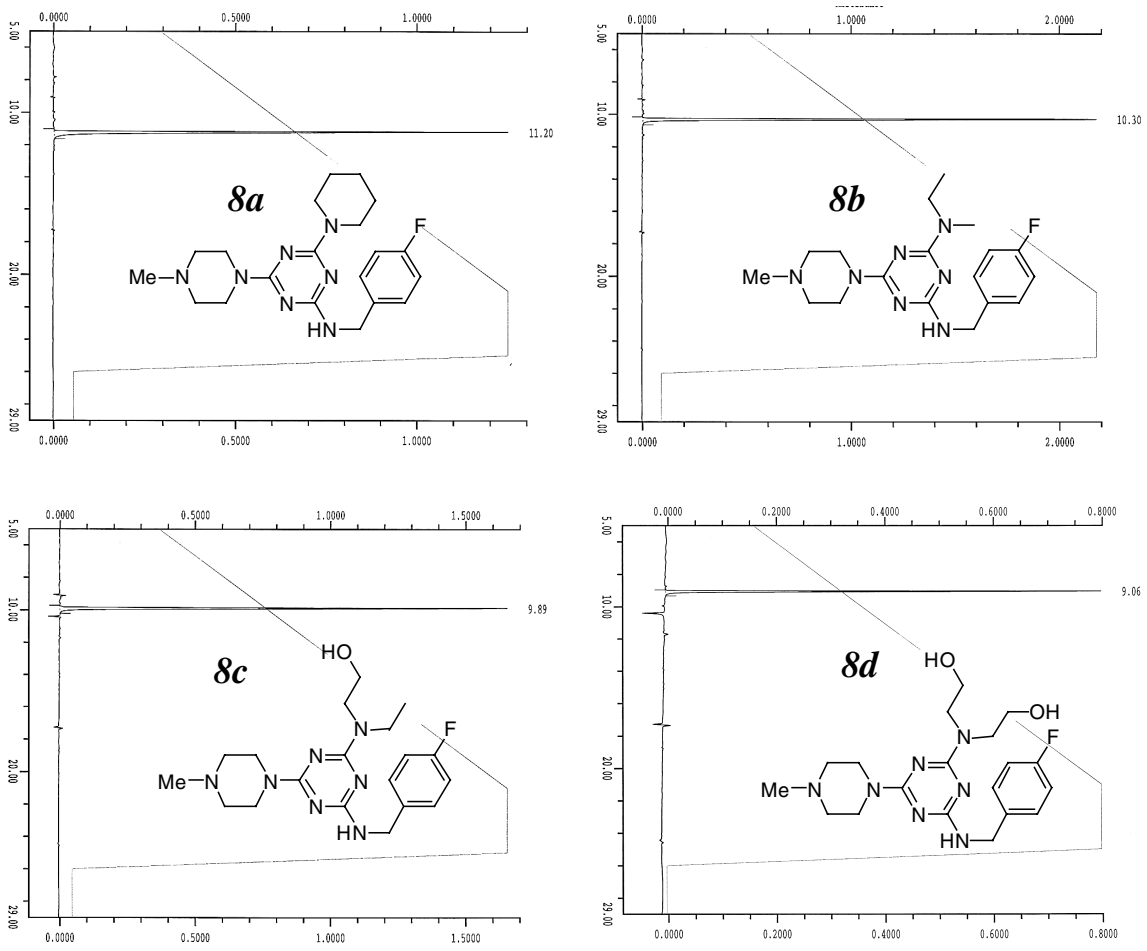
Acryloyl resin ***9*** (ArgoGel Wang, 5.0 g, 0.39 mmol/g, 1.95 mmol) was treated with piperazine (1.56 g, 19.5 mmol, 10 eq) and DMF (25 mL). After shaking overnight the resin was washed well with DMF (25 mL x 3) and CH_2Cl_2 (25 mL x 3) to give piperazine resin ***13***. Resins ***7a-d*** were prepared by the method of Moon *et al.* (*J. Am. Chem. Soc.* **2002**, *124*, 11608-9) by treating resin ***13*** with 1-(4-fluorobenzylamino)-3,5-dichlorotriazine (4 eq, 60 C), then splitting into four portions and reacting each portion with one of the following amines (4 eq, 120 C): (a) piperidine; (b) N-methyl ethylamine; (c) N-methyl ethanolamine; (d) N-(3-hydroxyethyl) ethanolamine to give precursor resins ***7a-d***.

Cold triazines ***8a-d*** were prepared as described for compound ***4*** above. 5 eq of MeI were used in the alkylation step. Analyses of each of the four crude products ***8a-d*** by HPLC using two different mobile phases (acidic and basic conditions) are shown on the next two pages, followed by high-resolution mass spectra for each sample on the third page.

[^{11}C]-methyl products ***8a-d*** were prepared as described above. RadioHPLC analyses are shown on the fourth page.

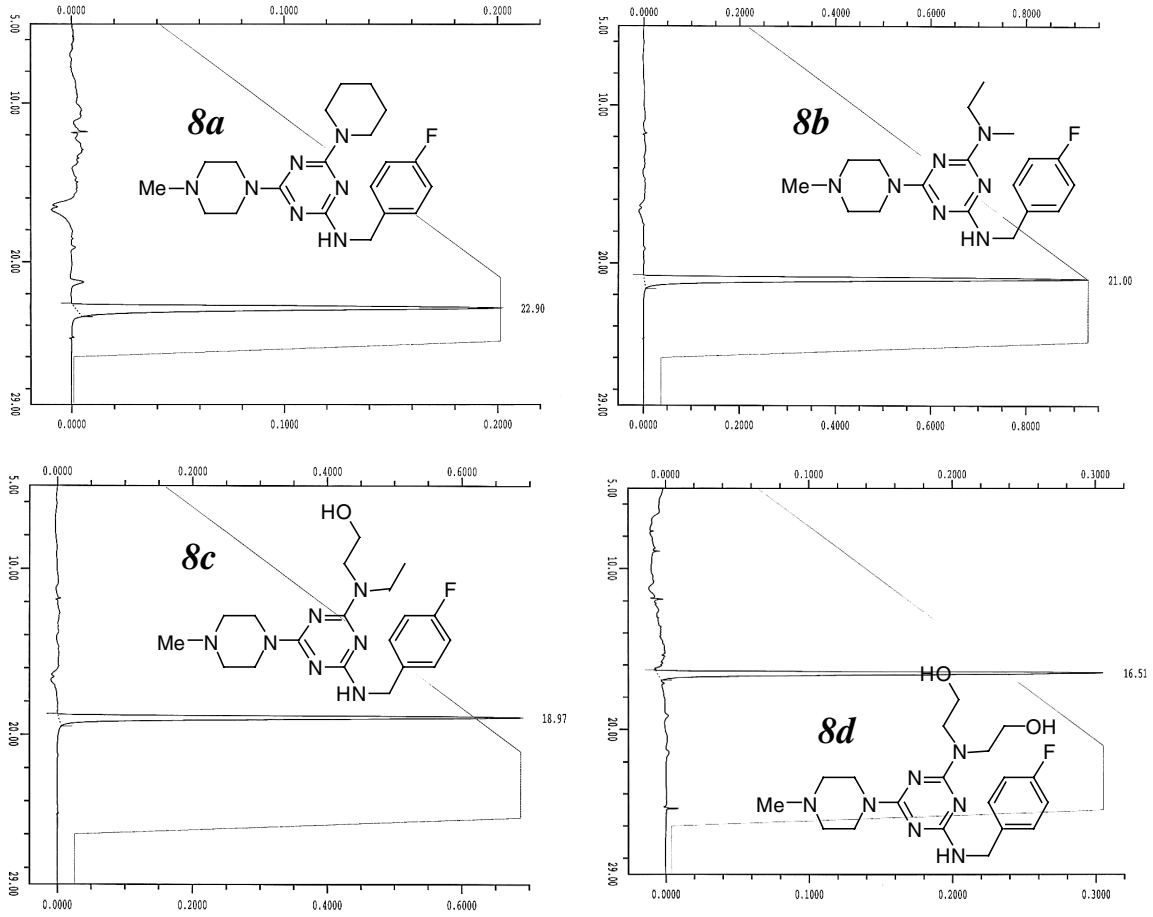
Triazines *8a-d* : HPLC analysis, solvent system A

HPLC data for crude compounds ***8a-d*** after release from REM resin. Samples were analyzed using Beckman System Gold HPLC equipment with UV detection at 220 nm. Column was a 250 x 4.6 mm C18 reversed phase Phenomenex column. Mobile phase for data on this page was: A: 0.1% TFA in water; B: 0.1% TFA in acetonitrile. The indicated gradient shows the percent of B at each time.

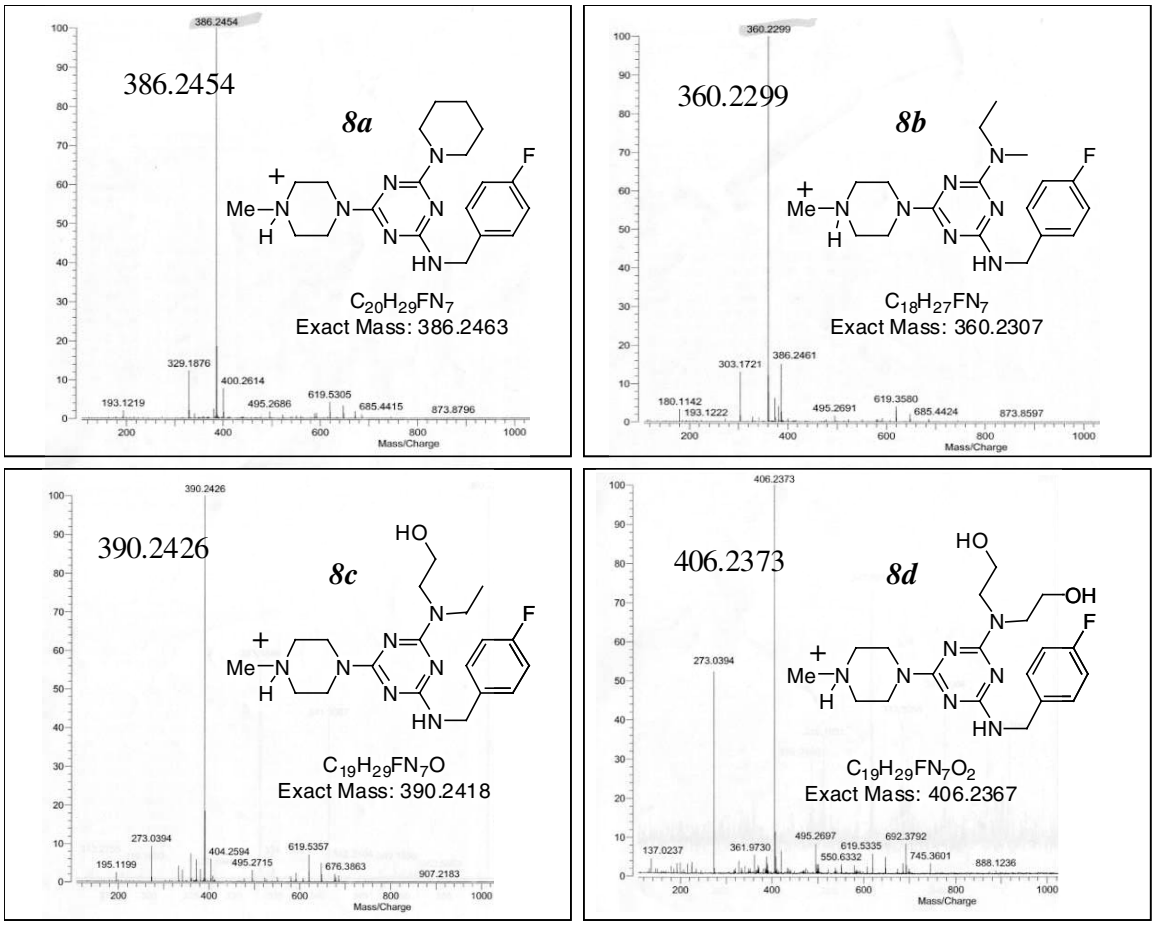


Triazines *8a-d* : HPLC analysis, solvent system B

HPLC data for crude compounds after release from REM resin. Samples were run using Beckman System Gold HPLC equipment with UV detection at 220 nm. Column was a 30 cm x 4.5 mm diameter C18. Mobile phase for data on this page was: A: 50 mM aq ammonium acetate; B: 2% A in acetonitrile. The indicated gradient shows the percent of B throughout the run.



Triazines *8a-d* : High resolution MALDI mass spectral analysis



Triazines *8a-d* : Radiochemical preparation

Radio-HPLC data for crude ^{11}C-labeled compounds ***8a-d*** after release from REM resins ***7a-d*** with [^{11}C]-methyl iodide by the method described earlier for ***4***. Samples were analyzed on Knauer HPLC system with a Phenomenex Luna C18 reversed phase column (250 x 4.6 mm), isocratic elution with 3:1 methanol/water at a flow of 1.5 mL/min. Upper trace on each chromatogram is detection of gamma radiation; lower trace is UV detection at 220 nm. r.t. = retention time; purity refers to the radiochemical trace. The major radiochemical peak co-eluted with the authentic, cold material in all cases.