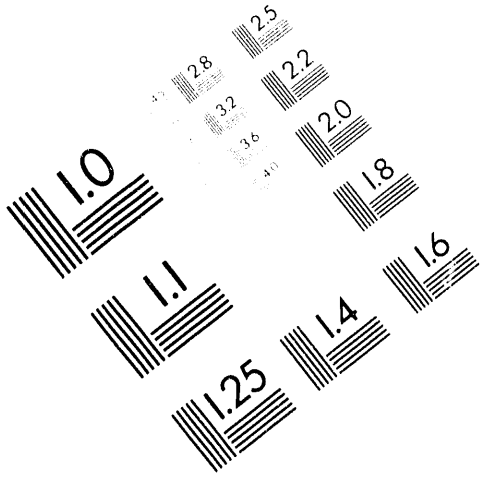

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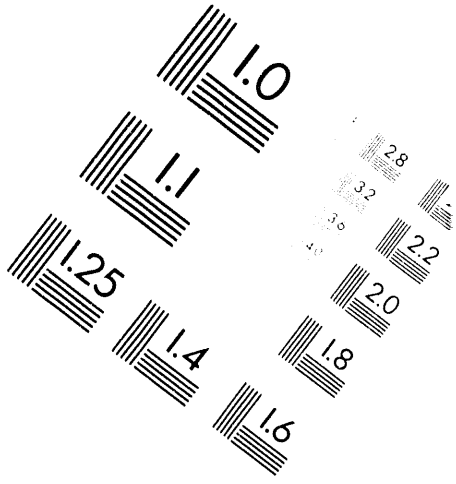

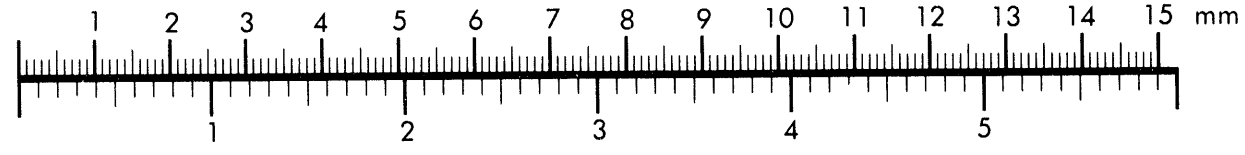

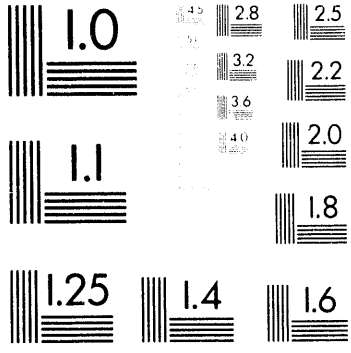

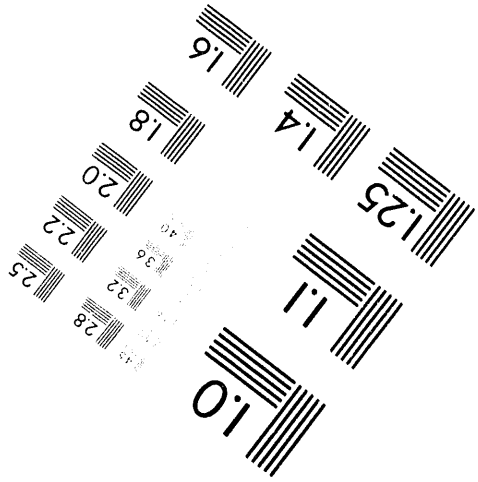

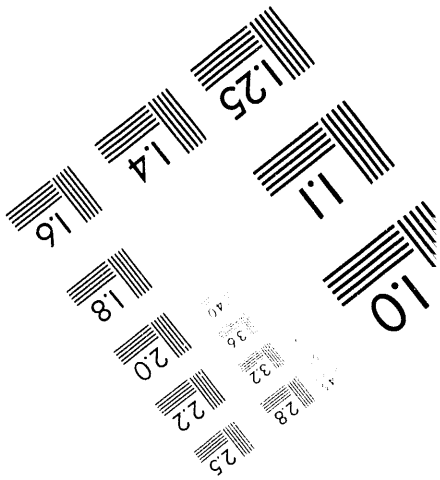

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DOE/ER/61006-3

Synthesis of Tracers Using Automated Radiochemistry and Robotics

Final Progress Report

for Entire Grant Period August 1, 1990 - October 31, 1993

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Proposal Abstract

The synthesis of high specific activity radiotracers labeled with short-lived positron-emitting radionuclides for use in positron emission tomography (PET) often requires handling large initial quantities of radioactivity for a successful tracer preparation and PET study. High specific activities are required when preparing tracers for use in PET studies of neuroreceptors, which are small biomacromolecular recognition sites that are finite in number and easily saturated. With the current demands for production of radiotracers for PET, a fully automated approach for tracer synthesis is highly desirable. This proposal involves the development of a system for the **S**ynthesis of **T**racers using **A**utomated **R**adiochemistry and **R**obotics (STARR) for this purpose.

While the long range objective of the proposed research is the development of a totally automated radiochemistry system for the production of major high specific activity ^{11}C-radiotracers for use in PET, the specific short range objectives of the proposed research are the automation of ^{11}C-methyl iodide ($^{11}CH_3I$) production via an integrated approach using both radiochemistry modular labstations and robotics, and the extension of this automated capability to the production of several radiotracers for PET.

Keywords: radiochemistry, PET, robot, radiotracer, radiopharmaceutical

Summary of Progress

The results presented here are consistent with the overall goal of the project to develop a system for the synthesis of tracers using automated radiochemistry and robotics. The research supported by this grant supports the DOE mission through its development of remote methods of handling radioactive materials, with special emphasis on robotics and automated radiochemistry technology development, for the radiochemical synthesis of positron emitting tracers for application to problems of public health. The research directly impacts on the DOE mission in that the development of positron emission tomography and related technology currently supported by DOE research is necessary for continued progress in labeled compound development, biomedical research, and clinical application of PET.

To accomplish the objectives of this project required that common features involved in radiochemical syntheses with carbon-11 be identified and that labstations be acquired or built to address the radiochemical handling during each step of the synthesis (see Figure 1).

Common Features of C-11 Tracer Syntheses

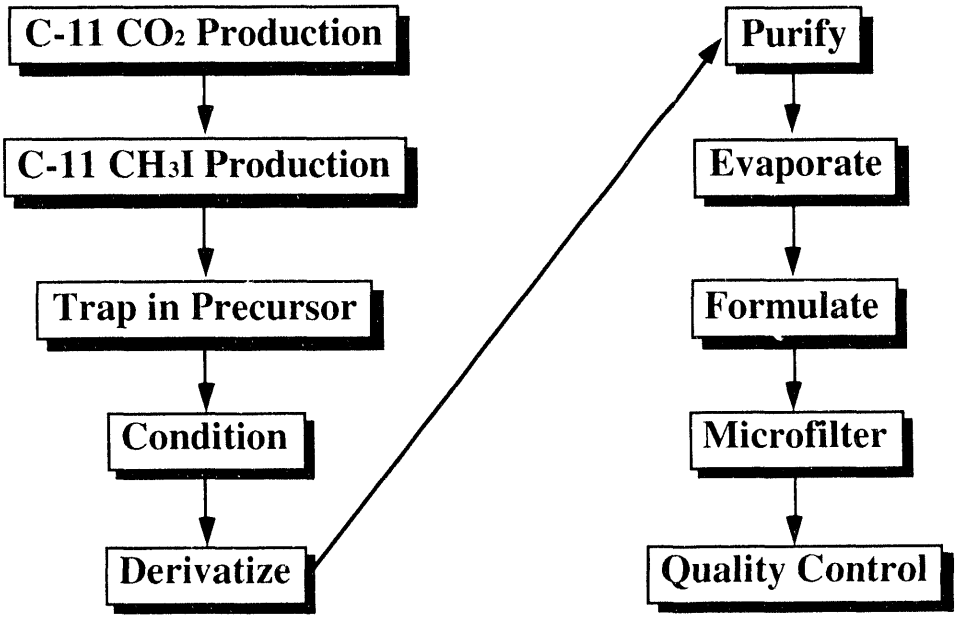


Figure 1.

As described below, the initial robotic arm was a Zymark Zymate II. The system was upgraded during this project to a Zymate XP (see Figure 2). Several of the labstations (e.g., the microfiltration station and the methyl iodide station) were custom built on-site.

Equipment

- **Commercially available laboratory robot**
  Zymark Zymate II, Zymate XP (installed 11/91)
- **Commercially available workstations**
  Zymark capping station, master lab station (syringes)
- **Custom built workstations**
  methyl iodide, microfiltration
- **High Performance Liquid Chromatography**
  Waters Associates, Rheodyne, ORTEC

Figure 2.

The labstations were organized around the Zymark Zymate robotic arm (see Figure 3).

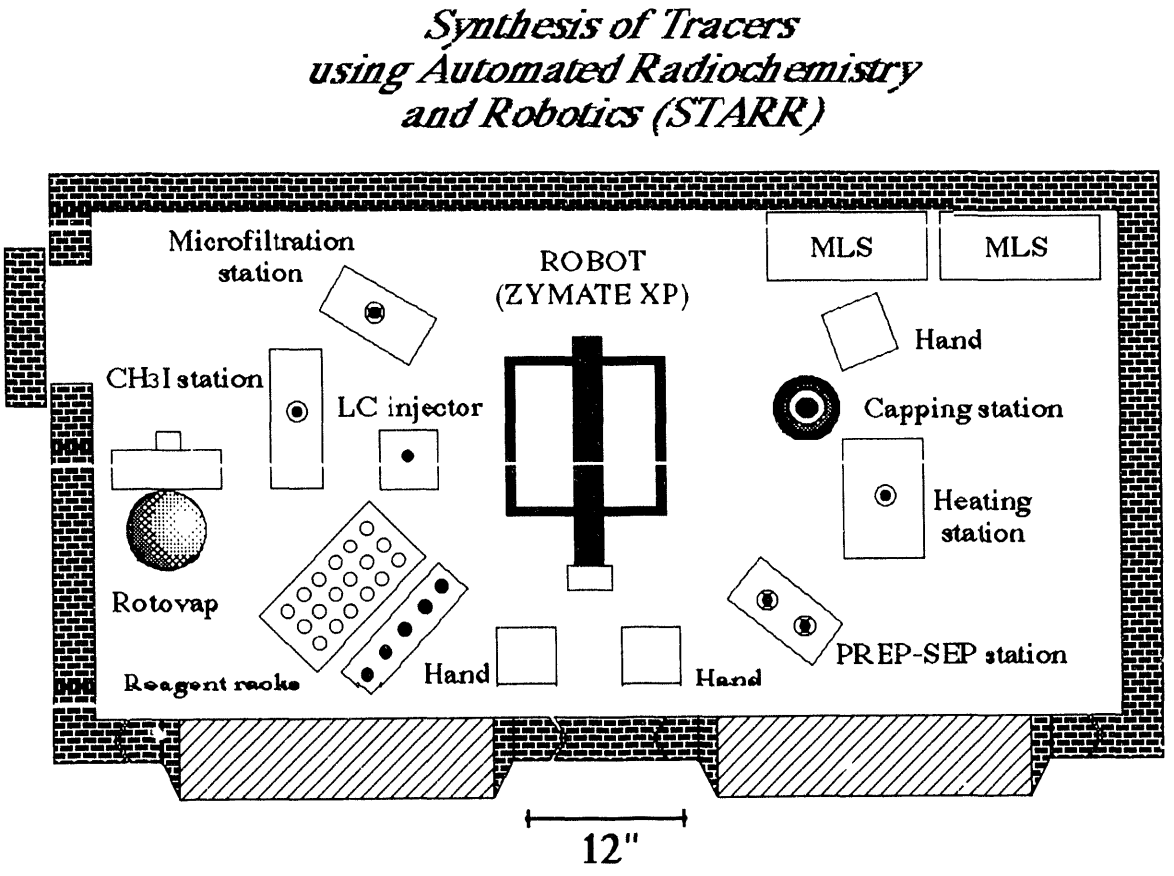


Figure 3.

When funding of this research began in August 1990, a prototype labstation for the production of ^{11}C-methyl iodide was tested using manual operation to determine baseline values for comparison with the fully automated version. Average times of synthesis, percent radio-chemical yields, and specific activities were determined (see Table I). The prototype labstation was interfaced to a Zymark Z830 power and event controller and the initial version of an EASY-LAB ^{11}C-methyl iodide production program was written and debugged. The preparation of ^{11}C-methyl iodide is the initial part of the synthesis of more complex radiotracers (see Figure 4). The "top-down" program for a more complex tracer synthesis (e.g., ^{11}C-methyl spiperone) is shown in Figure 5.

Breakdown of Synthesis

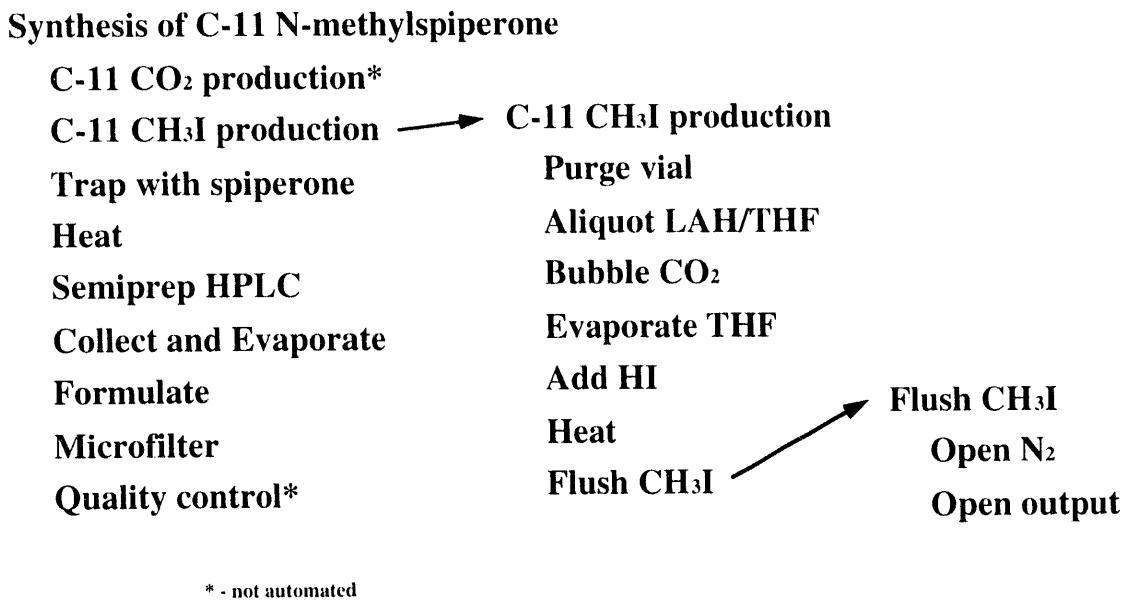


Figure 4.

Top-Down Approach

TOP LEVEL PROGRAM

SYNTHESIS.OF.C11.METHYLSPIPERONE
- **PRODUCE.C11.CH3I**
- **TRAP.C11.CH3I.IN.PRECURSOR**
- **HEAT.PRECURSOR**
- **PREP.HPLC**
- **WAIT.FOR.PRODUCT.TO.ELUTE**
- **COLLECT.IN.ROTOVAP.N.EVAP**
- **ADD.SALINE.N.BICARB**
- **MICROFILTER**
- **SIGNAL.END.OF.SYNTHESIS**
- **CLEAN.UP**

Figure 5.

The initial version of the program utilized simple event timers based on previous production experience with the manual version. Based on our experience, the average time for trapping of ^{11}C-carbon dioxide and conversion to ^{11}C-methanol via lithium aluminum hydride reduction in tetrahydrofuran solution was 60 seconds and the average time for evaporation of the tetrahydrofuran solution was 45 seconds. The average time for release of ^{11}C-methanol and conversion to ^{11}C-methyl iodide was 75 seconds. In the initial version of the automated program, individual timers were set at 150% of average manual times. The results of the first automated ^{11}C-methyl iodide productions are shown in Table I.

Table I. Labstation ^{11}C-methyl iodide production

	Time of synthesis	Radiochemical yield[a]	Specific activity[b]
Manual operation	180 ± 14 seconds	65 ± 8 %	2576 ± 182 mCi/μmole
Automated operation	270 seconds	38 ± 11 %	871 ± 98 mCi/μmole

a - based on average ^{11}C-carbon dioxide production
b - calculated at end-of-production

Based on the results obtained, the use of simple timers was determined to be inadequate for the intended production of high specific activity ^{11}C-methyl iodide. Feedback from a radioactivity detector in close proximity to the reaction vessels used in the ^{11}C-methyl iodide production was necessary and was incorporated into the next version of the EASY-LAB program. This modification indicated the location and quantity of ^{11}C during the various stages of the production. It was also determined that the transfer of the lithium aluminum hydride-tetrahydrofuran solution required modification to limit the introduction of carrier carbon dioxide during the transfer.

Using feedback from a small semiconductor radioactivity detector to determine when the next step in the process should proceed (the plateau of radioactivity), the labstation performance (with respect to the average time-of-synthesis) was comparable to the manual operation (see above table). The average specific activity showed a modest increase (1010 ± 58 mCi/μmole). This specific activity was lower than that observed when a

fully manual synthesis was done under comparable beam currents and cyclotron target conditioning.

There were several potential sources of carrier in the system including dissolved carbon dioxide in the LAH/THF solution. From our previous experience with manual syntheses of high specific activity tracers, the storage and transfer of the LAH/THF solution were considered most important. A special LAH/THF reagent chamber was constructed of Lexan®. The chamber, which has a narrow port to allow the syringe needle to enter, was under constant argon overpressure exiting through the port. The LAH/THF solution was prepared manually in a stainless steel inert atmosphere box. The syringe hand was programmed to clean with THF and purge the needle with argon prior to withdrawing the reagent. The syringe was filled with argon which was slowly expelled prior to entering the chamber to minimize contamination from carrier.

During the second year of the project, a new faster robotic arm (Zymark Zymate XP) was installed. The faster arm minimized the delay in moving air sensitive reagents and reaction vessels during the radiochemical synthesis. With the faster robot, a new robot control system was required. This required reprogramming and additional debugging of the automated modular labstation.

As a result of the additional precautions taken to minimize carrier carbon contamination, the average specific activity at the end of methyl iodide production was increased to 3515 ± 279 mCi/μmole (determined by UV standard curves). This specific activity was only slightly lower than that observed in comparable fully manual synthesis under comparable beam currents and cyclotron target conditioning.

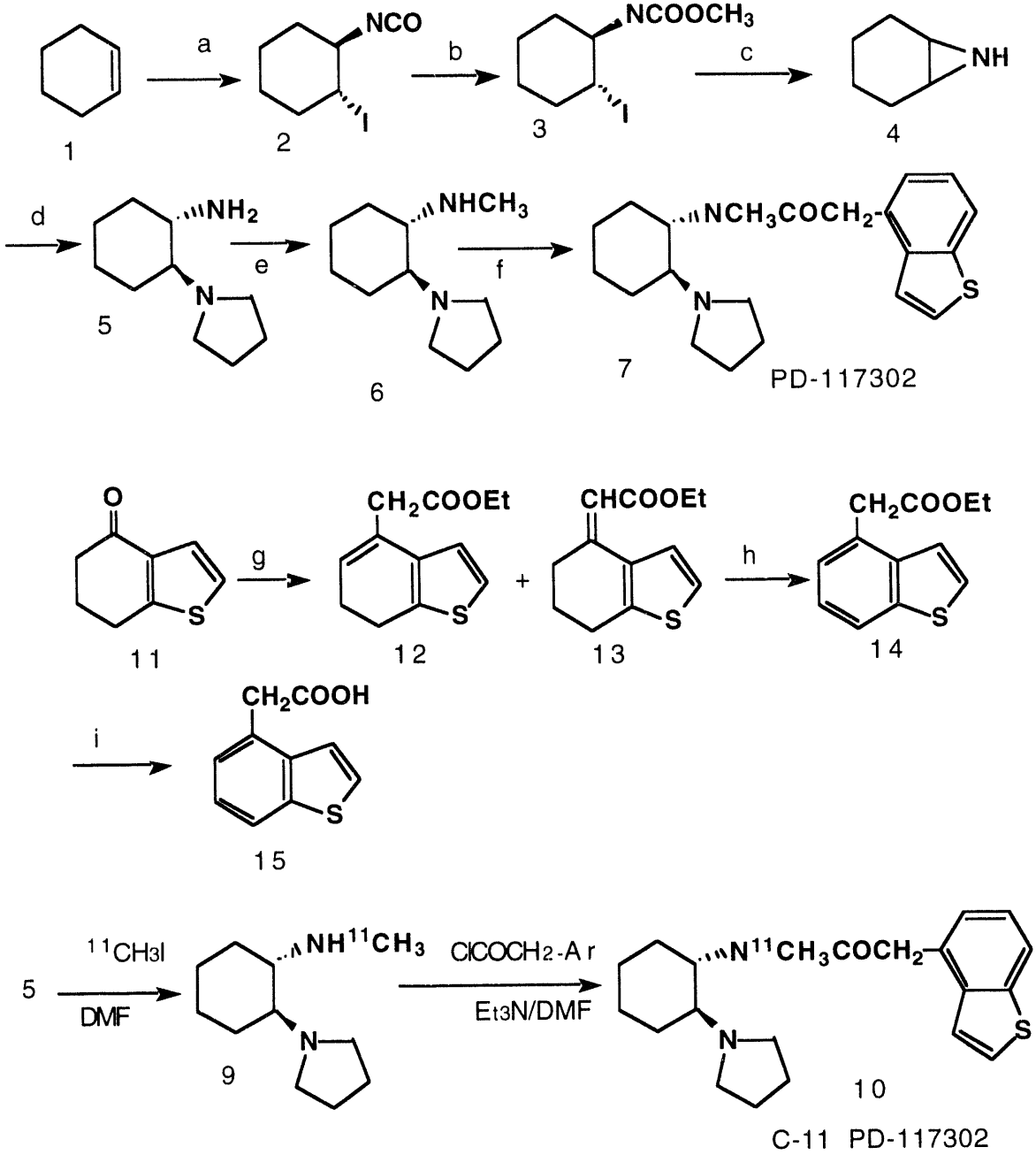
The first radiotracer that was prepared using this system was ^{11}C-methionine. Radiomethylation to produce ^{11}C-methionine using the automated system proceeded as anticipated (high radiochemical yield). ^{11}C-methionine was prepared by S-alkylation of the appropriate nor-methyl precursor, S-homocystiene. The synthesis required minimal modification of the previous automated program due to the facile purification using reduced pressure rotary evaporation and redissolving of the product in aqueous media. Synthesis times were comparable to those achieved with a fully manual synthesis of ^{11}C-methionine was performed.

The synthesis of 3-N-[^{11}C-methyl]spiperone was more challenging in that it required the use of high performance liquid chromatography (HPLC) during the radiochemical purification. 3-N-[^{11}C-methyl]spiperone

was purified using "timed" HPLC separations (i.e., averaging elution times from several manual syntheses and collecting the eluant based solely on the averaged times). From high specific activity ^{11}C-methyl iodide produced as described above, the robotic synthesis of 3-N-[^{11}C-methyl]spiperone using STARR was completed in average synthesis time is approximately 28 minutes (compared to under 17 minutes when the synthesis is performed manually). The average specific activity at end-of-synthesis from STARR is approximately 1875 mCi/μmole (compared to over 2500 mCi/μmole when the synthesis is performed manually). The increase in synthesis time was attributed in part to the use of slower flow rate on the HPLC semipreparative purification (the slower flow rate appears to increase the reliability of the timed collections of product in the HPLC eluant). The increase in the synthesis time was also attributed to the lack of a suitable means for determining the endpoint in the evaporation of the HPLC solvent from the collected eluant.

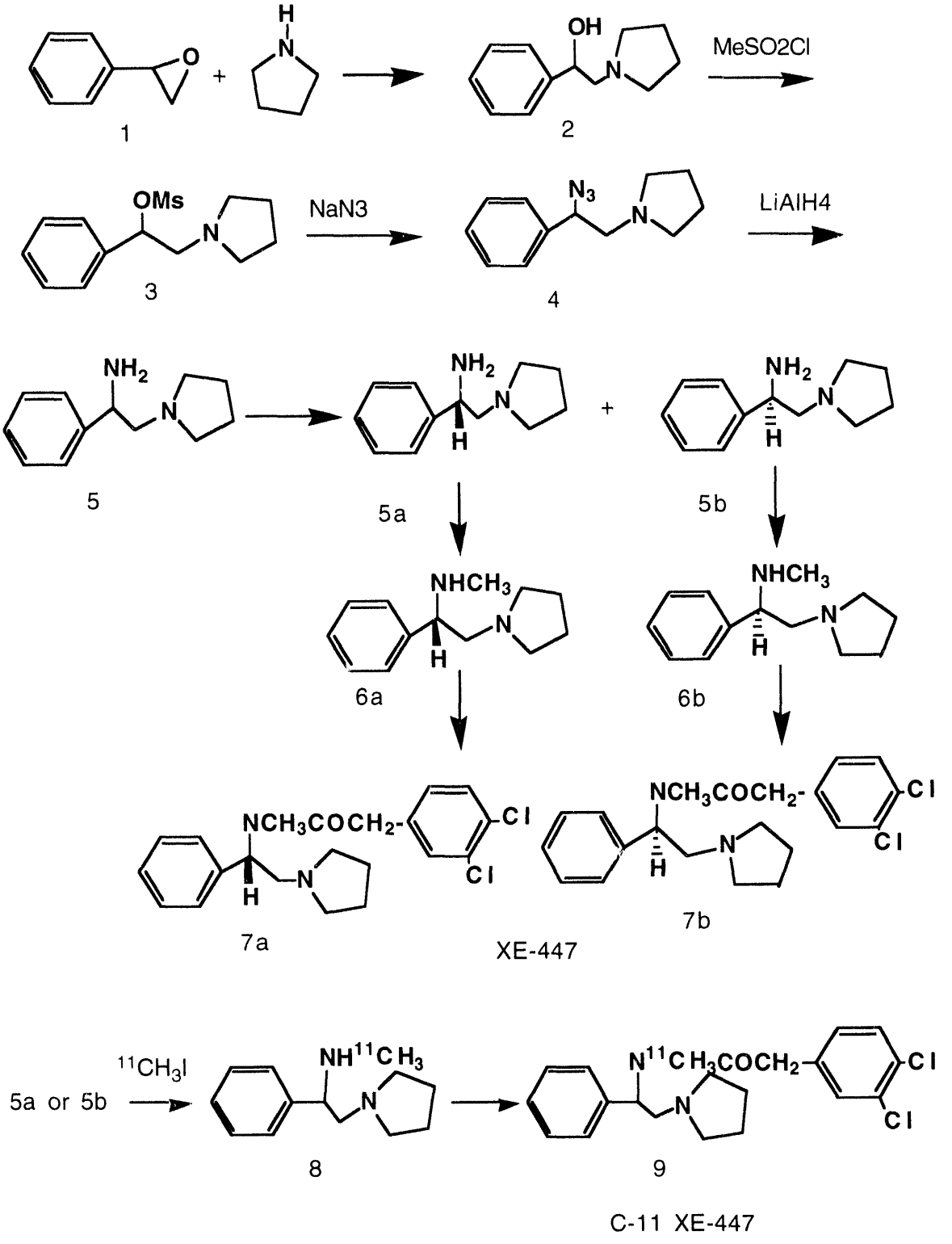
The variability in elution times of high specific activity remains a problem in the totally automated production of these radiotracers. The variability is great enough to limit the widespread use of this method. An "intelligent" on-line HPLC monitoring program which will adjust collection parameters according to observed UV and radiochromatographic signals from detectors is still required. Until such a program is available, the preparative chromatography used in the syntheses of radiotracers like [^{11}C]-carfentanil and 3-N-[^{11}C-methyl]spiperone will require operator intervention to determine when the desired tracer is eluting from the HPLC column.

During the final year of this grant, developmental radiochemistry on several opiate kappa subtype ligands was also performed. Four ligands with subtype specificity for the kappa opiate receptor were studied: CI-977, PD-117304, U-69593, and XE-447. The chemical structures, precursor syntheses, and radiolabeling are schematically shown below (see Figure 6, 7, 8, and 9). Radiomethylation of the appropriate diamine precursor followed by acylation with the appropriate acid chloride was used for the preparation of each tracer. Specific activities and times of syntheses were comparable to other tracers in this series (Noble et al., 1992). Due to problems stemming from the difficulties associated with the HPLC separation of a small quantity of the desired radiochemical from a relatively large quantity of starting material, the syntheses of these compounds with STARR were not attempted.

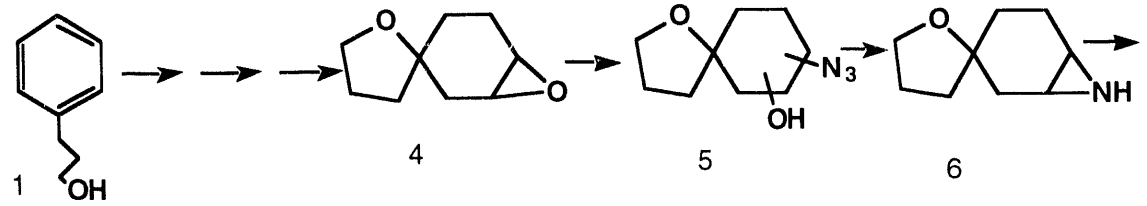


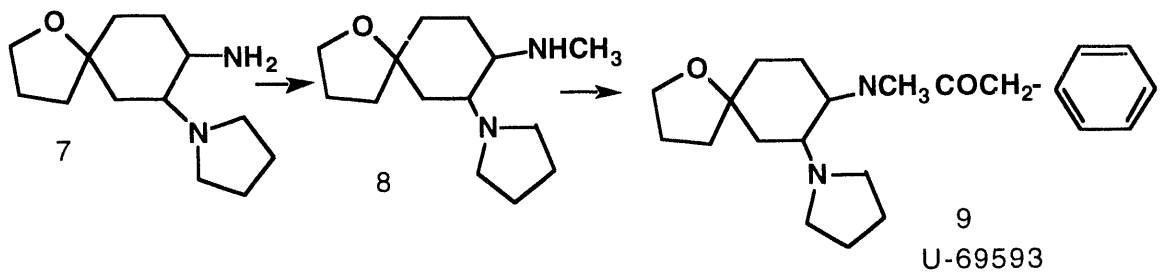
a. $AgOCN/I_2$, ether; b. CH_3OH, CH_3ONa; c. KOH/CH_3OH; d. pyrrolidine/THF/H_2O, NH_4Cl; e. ethyl formate; f. 4-benzo-thiopheneacetyl chloride/Et_3N/CH_2Cl_2; g. $Zn/I_2/BrCH_2COOEt$/Benzene/Ether; h. Chloranil/Xylenes; i. 10% NaOH.

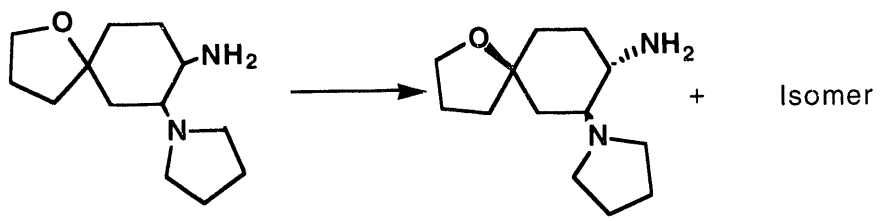
Synthesis of [^{11}C]PD-117304

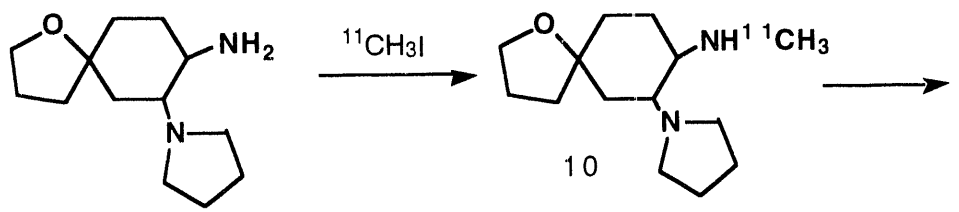


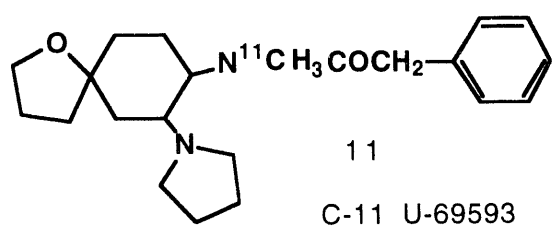
Synthesis of [^{11}C]XE-447



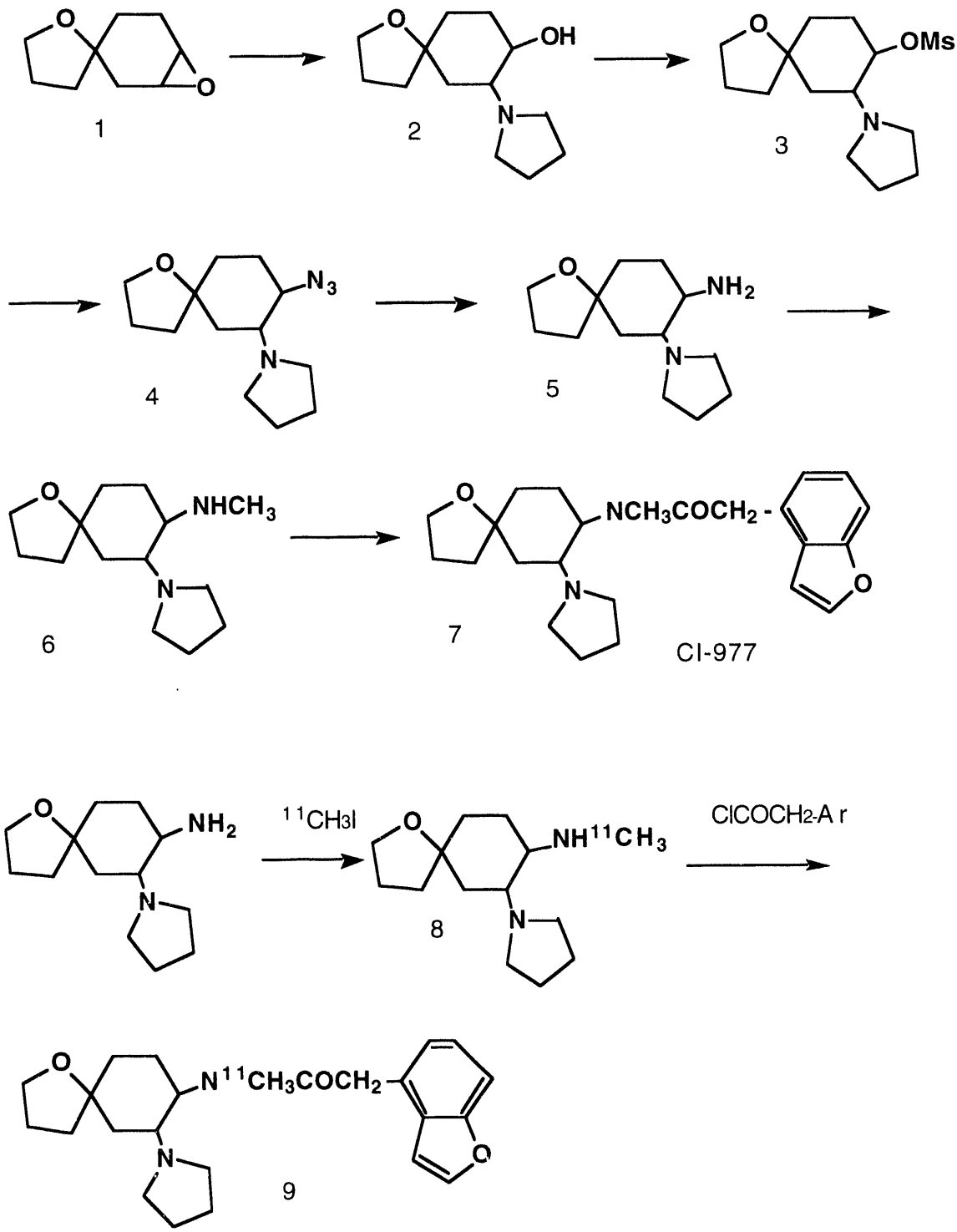








Synthesis of [^{11}C]U-69593



C-11 CI-977

Synthesis of [^{11}C]CI-977

Publications

Several publications are still in the preparation stages. The manuscript titles are listed below and preprints will be forwarded to the Department when the manuscripts are submitted for review. Each manuscript acknowledges the support of the Department of Energy grant number DOE/ER/61006.

Dannals RF: A simple labstation for the routine production of carbon-11 labeled methyl iodide. Appl Radiat Isot, Int J Rad Appl Instrum Part A, in preparation, 1994.

Dannals RF: Synthesis of tracers using automated radiochemistry and robotics. Appl Radiat Isot, Int J Rad Appl Instrum Part A, in preparation, 1994.

Zhang X, Dannals RF, Ravert HT, Musachio JL, Mathews WB: Synthesis of radiotracers for studying opiate κ subtype receptors using positron emission tomography: [^{11}C]XE-447, [^{11}C]CI-977, [^{11}C]PD117302, [^{11}C]U-69593. J Label Compds Radiopharm, in preparation, 1994.

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