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Radiopharmaceuticals for Diagnosis

PROGRESS REPORT

For the Period 1 January 1988 through 31 December 1990

David E. Kuhl, M.D.
Division of Nuclear Medicine
University of Michigan
Ann Arbor, MI 48109

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DISCLAIMER

During this grant period 1 Jan 1988-31 Dec 1990, we have successfully developed a number of new approaches to fluorine-18 labeled compounds, prepared several new radiotracers for both animal studies and eventual clinical trials, and explored the utility of a high-quality industrial robot in radiopharmaceutical applications. The progress during the last grant period is summarized briefly in the following sections. Publications arising from this research are listed below and can be found in Appendix I.

I. Summary of accomplishments.

Project 1. New Methods of [^{18}F]Fluorination.

(1) Production of [^{18}F]fluoride ion. When this grant period began, high specific activity [^{18}F]fluoride ion was not available at this institution. We have constructed and fully tested a small volume (1 ml), high pressure water target for the routine production of [18F]fluoride ion using the ^{18}O(p,n)^{18}F nuclear reaction on enriched water. This target has proven exceptionally successful, and can be used to prepare large quantities of radionuclide (700 mCi) is short times (20 min). This target design is used in our routine production target, used daily for preparation of [^{18}F]fluoride ion for both research in this grant as well as routine production of [^{18}F]FDG and [^{18}F]GBR 12909 for clinical studies. To maximize reliability and allow for periodic cleaning (without concommitant downtime for activation products to decay) we have actually constructed two identical targets which are rotated on a periodic basis. The target is more fully described in publication 17.

(2) New precursors for nucleophilic aromatic [^{18}F]fluorination. We have prepared and fully examined a series of aryl trimethylammonium trifluoromethanesulfonates as easy to prepare, stable, and highly reactive precursors for the synthesis of [^{18}F]aryl fluorides. The use of the cationic precursors allows both the use of lower reaction temperatures and the simple isolation of nuetral, [^{18}F]-labeled products (easily separated from charged precursor). We also examined aryl dimethylsulfonium trifluoromethanesulfonates as precursors, but these proved unsuitable for high yield synthesis of aryl [^{18}F]fluorides.

This method of fluorination proved important in the development of [^{18}F]GBR 13119 and [^{18}F]GBR 12909, new radiopharmaceuticals we have used in animal and human studies of the dopamine reuptake system. Furthermore, the synthesis and application of these cationic precurss has been adopted and duplicated by at least two other research groups worldwide.

This work is reported in publications 2 and 16.

(3) Synthesis of [^{18}F]fluorobenzyl bromide. We have examined the synthesis of 4-[^{18}FF]fluorobenzyl bromide as a precursor for radiopharmaceutical syntheses. The benzyl groups is an important functional group in a number of drugs, including dexetimide (muscarinic antagonist). We have examined three routes to fluorobenzyl bromide. Route (a) utilized the nitrile precursor, which was then reduced to the corresponding aldehyde and brominated. Route (b) again utilized the nitrile precursor, but in this case the nitrile was reduced all the way to the hydrocarbon, which was then brominated. Finally, route (c) utilized the aldehyde as the precursor, which was in the second step brominated; this was the shortest route to the desired product. All of these synthestic routes have worked in low yield on both the carrier and carrier-free scales.

(4) Ring-opening reactions. The opening of cyclic ethers using dimethylboron bromide has been reported to yield the corresponding bromoalcohols, by the nucleophilic attack of bromide ion on the oxygen-Lewis acid complex. We have examined the possibility of interception of the incipient activated ether by [^{18}F]fluoride ion, to yield the corresponing [^{18}F]fluoroalcohol. Although the reagent was successful in the ring-opening of cyclohexene oxide to the corresponding bromohydrin, in reactions where [^{18}F]fluoride was added we could not detect the presence of the [^{18}F]fluorohydrin. We are continuing our efforts in this novel approach to fluorination of alkanes.

5. [^{18}F]Fluorination of thiophene rings. We have extended the nucleophilic aromatic substitution reactions with [^{18}F]fluoride ion to include preparation of fluorothiophenes. The reaction of [^{18}F]fluoride with three thiophene-2-carboxaldehydes was examined., Substitution of the 5-bromo compound gave reasonable yields, but the 4-bromo derivative proved unreactive, and the nitro substituted thiophene

gave small amounts of the [^{18}F]fluorothiophene but mostly decomposition products. These results are consistent with the literature on nucleophilic substitution in thiophene rings. This work is reported in paper 5.

6. Preparation of [^{18}F]trifluoromethyl groups. A new and potentially powerful method for the synthesis of no-carrier-added [^{18}F]trifluoromethyl groups has been devised, in collaboration with scientists from Parke-Davis Co. Reaction of nca [^{18}F]fluoride ion with bromodifluoromethyl groups yields the [^{18}F]trifluoromethyl groups in good radiochemical yield. This reaction does not require high temperatures or Lewis acid catalysts. This is potentially a method for labeling a wide variety of pharmaceuticals which comtain trifluoromethyl substituents. This work is reported in paper 9.

7. Synthesis of [^{18}F]fluoro ethers. In an unusual reaction, we have examined the isotopic substitution of 1,1-difluoro-2,2-dichloroethyl ethers by [^{18}F]fluoride ion to yield the corresponding carrier-added, fluorine-18 labeled ethers. This reaction is an isotopic substitution; reaction in the ring was ruled out by successful synthesis of the benzene analog, and the product obtained was identical by all chromatographic methods with authentic starting material. This substitution by nucleophiles is precedented in the literature of perfluoroalkyl ethers. This work is reported in papers 10 and 29

8. New approach to fluorocatechols. As part of our interests in the synthesis of fluorine-18 labeled catecholamines, such as dopamine, DOPA, and norepinephrine, we have examined a new synthetic route to no carrier added fluorocatechols. This approach utilizes a substituted o-salicaylaldehyde as the synthetic precusrsor, which is first substituted with [^{18}F]fluoride ion and then oxidized to the catechol. By this route we have prepared NCA [^{18}F]fluorocatechol. This work is reported in paper 28.

9. [^{18}F]Fluorination of pyridazines. We have also examined the reaction of [^{18}F][fluoride ion with chloropyridazine, nitrogen heterocycles which may be substitutes for benzene rings in certain drugs. We have successfully prepared several [^{18}F]fluoropyridazines by this approach, including one which does not have activation by a electron-withdrawing group. This work is reported in publication 30.

10. New radiotracer development. This research grant has supported in part the development of four specific types of radiotracers, dopamine reuptake inhibitors, calcium channel blockers, GABA reuptake inhibitors, and norepinephrine reuptake blockers.

a. Dopamine reuptake blockers. We have prepared , fully characterized, and extensively examined in aninmal models the new radioligands [^{18}F]GBR 13119 and [^{18}F]GBR 12909. These are selective, high affinity ligands for the dopamine reuptake site. These radiotracers could be successfully utilized to image the DA uptake sites in animals, and [^{18}F]GBR 12909 was futher developed and brought into clinical trials. In attempts to improve the kinetic behaviour of this compound, a limited QSAR study was done, showing that some alterations (substitution of a thiophene ring) are allowed but other changes (shortening of alkyl chain) are not. The results in this area are in publications 1,2,3,4,5,7,8,12,13,15,19,20,22,23,25,and 26.

b. Norepinephrine reuptake blockers. We have prepared and examined in mice a specific high affinity ligand for the norepinephrine reuptake site, [^{11}C]nisoxetine. Although this radiotracer showed regional specificity and pharmacological specificity expected of a NE reuptake inhibitor, this compound shows high non-specific binding which may limit its usefulness in vivo using PET. This data is reported in publications 6,14 and 15.

c. GABA reuptake inhibitors. In a collaboration with Parke-Davis company we have prepared several GABA reuptake compounds as potential mapping agents for this site. These compounds utilized the chemical synthesis methods developed for the GBR class of compounds. Animal work with these new compounds is underway. The chemical results are described in publications9,24 and 27.

d. Calcium channel blockers. With an interest in the dialkylpiperazine class of calcium channel blockers, due to thier similarity in structure to the dopamine reuptake inhibitors, we have prepared fluorine-18 labeled cinnarizine and flunarizine. Initial animals studies did not show regional binding to the DA uptake site. This work is reported in publications 11 and 21.

Project 2. Applications of Robotics in Radiopharmaceutical Synthesis.

Our initial goals were to incorporate robotics into the synthesis of PET radiotracers. However, continued successful chemical work (funded through DOE grant DE-FG02-87ER60561, D.E. Kuhl, P.I.) has eliminated the need for a robot in most of our syntheses. We have therefore evaluated a robot in three other applications.

(1) Material handling. We have utilized the PUMA robot for the insertion and sealing of product vials into the "rabbits" used in our pneumatic delivery system. This was a high dose, manual operation which defeated the goals of reduction of radiation dose to personnel. The robot proved quite adept at this operation. This need was eliminated, however, by redesign of the rabbits and the pneumatic tube sending station.

(2) Metabolite analysis. A routine, repetitive task in PET studies is the analysis of blood samples for radiotracer metabolites. This is an excellent applications point for robotics. To this end, we have modified standard laboratory equipment (syringes, centrifuges, vortex mixers) to opertate in tandem with the PUMA robot. The robot is thus capable of performing metabolite analyses using standard SEP-PAK technology for separations.

In this work we have showed the feasibility of a robotic approach to this task. It was clear that a simpler, less expensive robot (i.e., fewer degrees of freedom in the robot arm) would function just as well.

(3) Preparation of Copper-62 generators. Our most recent application of the robot is in the preparation of generators for copper-62. The chemistry behind the production of the parent nuclide, zinc-62, and assembly of a resin column used for the generator have been reproduced from the literature (our cyclotron, at 30 MeV, can prepare zinc-62 readily). We have assembled a compact robot cage (Figure 1) which fits inside a standard-sized chemical hood. The robot operates in the inverted position, as this provides the greatest envelope of working space. Specialized hands for this robot have been constructed in our facility. The robot system is currently undergoing evaluation as a safe, automated method for the production of copper-62 for PET studies in our institution.

Project 3. Review of fluorine-18 chemistry.

This grant has also supported the work by the Project Leader Michael R. Kilbourn in the research for and writing of a comprehensive review of fluorine-18 radiopharmaceutical chemistry, which is in press (to be published by National Academy of Sciences). This is publication 31.

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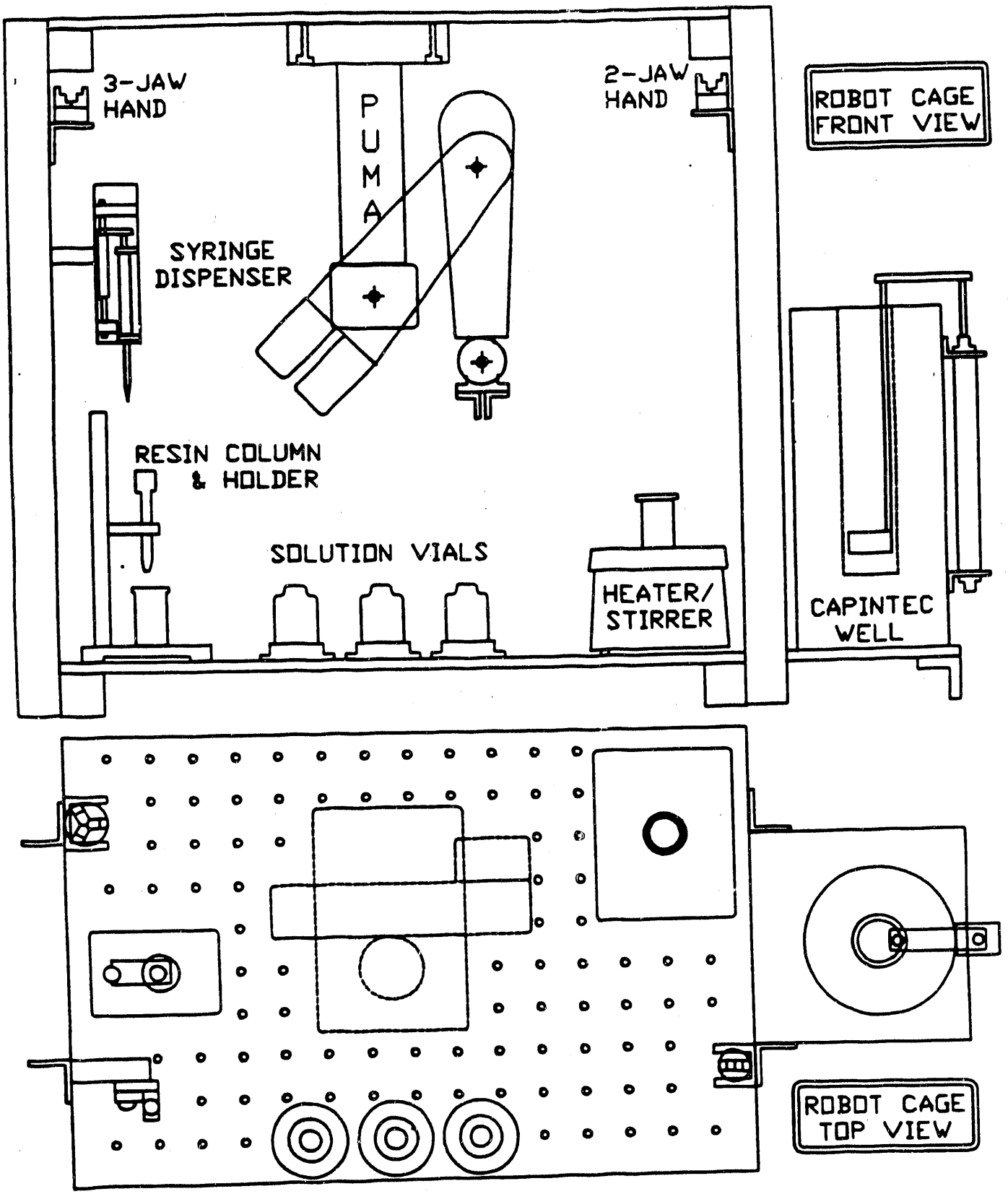
3-JAW
HAND
PUMA
2-JAW
HAND
ROBOT CAGE
FRONT VIEW
SYRINGE
DISPENSER
RESIN COLUMN
& HOLDER
SOLUTION VIALS
HEATER/
STIRRER
CAPINTEC
WELL
ROBOT CAGE
TOP VIEW

FIGURE 1

II. Publications 1 Jan 88 - 31 Dec 90

Manuscripts

1. Kilbourn MR, Haka MS: Synthesis of [^{18}F]GBR 13119, a presynaptic dopamine uptake antagonist. Appl Radiat Isot 1988; 39:279-282.
2. Haka MS, Kilbourn MR, Watkins GL: Aryl trimethylammonium trifluoromethane- sulfonates as precursors to aryl [^{18}F]fluorides: Improved synthesis of [^{18}F]GBR 13119. J Labelled Cmpd Radiopharm 1989; 27: 823-833.
3. Kilbourn MR, Haka MS, Mulholland GK, Sherman PS, Pisani T. Regional brain distribution of [^{18}F]GBR 13119, a dopamine uptake inhibitor, in CD-1 and C57B1/6 mice. Eur J Pharm. 1989; 166:331-334.
4. Kilbourn MR, Carey JE, Koeppe RA, Haka MS, Hutchins GD, Sherman PS, Kuhl DE: Biodistribution, dosimetry, metabolism and monkey PET studies of [^{18}F]GBR 13119. Imaging the dopamine uptake system in vivo. Nucl Med Biol 1989; 16:569-576.
5. Kilbourn MR. Thiophenes as phenyl bio-isosteres: Application in radiopharmaceutical design I. Dopamine uptake antagonists. Nucl. Med. Biol. 1989; 16:681-686.
6. Haka MS, Kilbourn MR. Synthesis and regional brain distribution of [^{11}C]nisoxetine, a norepinephrine uptake inhibitor. Nucl Med Biol. 1989:16;771-774.
7. Kilbourn MR. In vivo binding of [^{18}F]GBR 13119 to the brain dopamine uptake system. Life Sciences 1988; 42:1347-1353.
8. Haka MS and Kilbourn MR; Synthesis of [^{18}F]GBR 12909, a dopamine reuptake inhibitor. J Labeled Compds Radiopharm, in press
9. Kilbourn MR, Pavia MR, and Gregor VE; Synthesis of fluorine-18 labelled GABA uptake inhibitors. Appl Radiat Isot, in press
10. Kilbourn MR and Subramanian R. Synthesis of fluorine-18 labeled 1,1,-difluoro-2,2,-dichloroethyl aryl ethers by ^{18}F-for-^{19}F exchange. J Labeled Compds Radiopharm
11. Kilbourn MR: Synthesis of [^{18}F]flunarizine. Nucl. Med. Biol, submitted
12. Kilbourn MR, Mulholland GK, Sherman PS, and Pisani T: In vivo recovery of dopamine uptake sites after MPTP treatment of C57BL/6 mice. Biogenic Amines, submitted

Abstracts

13. Kilbourn MR, Haka MS, Ciliax GH, Kuhl DE: Synthesis and regional brain uptake of [F-18]GBR 13119, a dopamine uptake blocker. J Nucl Med 1988; 29:767.
14. Kilbourn MR, Jewett DM: Syntheses of carbon-11 labeled nisoxetine and fluoxetine, monoamine reuptake inhibitors. J Nucl Med 1988; 29:932.
15. Kilbourn MR, Haka MS, Mulholland GK, Jewett DM, Kuhl DE: Synthesis of radiolabeled inhibitors of presynaptic monoamine uptake systems: [^{18}F]GBR 13119 (DA), [^{11}C]nisoxetine (NE) and [^{11}C]fluoxetine (5-HT). J Labeled Cmpds radiopharm 1989; 26:412-414.
16. Haka MS, Kilbourn MR, Watkins GL: Aryl trimethylammonium trifluoromethane- sulfonates as precursors to aryl [^{18}F]fluorides. J Labeled Cmpds Radiopharm 1989; 26:17-19.
17. Mulholland GK, Hichwa RD, Kilbourn MR, Moskwa J: A reliable pressurized water target for F-18 production as high beam currents. J Labeled Cmpds Radiopharm 1989; 26:192-193.
18. Mulholland GK, Mangner TJ, Jewett DM, Kilbourn MR: Polymer-supported nucleophilic radiolabeling reactions with [^{18}F]fluoride and [^{11}C]cyanide ion or quaternary ammonium resins. J Labeled Cmpds Radiopharm 1989; 26:378-380.
19. Kilbourn MR, Haka MS, Ciliax BJ, Penney JB, Young AB: In vivo autoradiography of [^{18}F]GBR 13119 binding in rat brain. Society for Neuroscience, 18th Annual Meeting, Toronto, 1988, Abstract 376.11.
20. Kilbourn MR, Haka MS, Koeppe RA, Ciliax BJ, Penney JB, Kuhl DE: Development of [^{18}F]GBR 13119 for PET studies of the dopamine reuptake system. J. Cerebral Blood Flow and Metabolism. 1989; 9:S15.
21. Kilbourn MR: Synthesis of [F-18]fluorocinnarizine, a calcium channel blocker. J. Nuclear Medicine 1989; 30:753.

22. Mulholland GK, Kilbourn MR, Sherman PS, Pisani TL: In vivo changes in striatal binding of dopamine uptake inhibitor [^{18}F]GBR 13119 and muscarinic receptor ligand [^{11}C]-2a-tropanyl benzilate in MPTP lesioned C57BL/6 mice: dual tracer injection studies. Society for Neuroscience Annual Meeting, 1989, abstract 22.1.
23. Koeppe RA, Kilbourn MR, Frey KA, Penney JB, Haka MS, Kuhl DE: Imaging and kinetic modeling of [F-18]GBR 12909, a dopamine uptake inhibitor. Society of Nuclear Medicine 37th Annual Meeting, Washington, D.C., June 1990.
24. Kilbourn MR, Pavia MS, Gregor V. Synthesis of fluorine-18 labeled inhibitors of GABA reuptake. Society of Nuclear Medicine 37th Annual Meeting, Washington, D.C., June 1990.
25. Haka MS, Kilbourn MR. Synthesis of [^{18}F]GBR 12909 for human studies of dopamine reuptake sites. Society of Nuclear Medicine 37th Annual Meeting, Washington, D.C., June 1990.
26. Van Dort ME, Chakraborty PK, Wieland DM, Kilbourn M. Dopamine uptake inhibitors: Comparison of ^{125}I and ^{18}F-labeled ligands. Society of Nuclear Medicine 37th Annual Meeting, Washington, D.C., June 1990.
27. Kilbourn MR, Pavia MS, and Gregor VE, Syntheses of fluorine-18 labeled inhibitors of GABA (g-aminobutyric acid) reuptake. Eighth Interanational Symposium on Radiopharmaceutical chemistry, Princeton N.J., June 1990
28. Chakraborty PK and Kilbourn MR, A new approach to the synthesis of no-carrier-added fluorine-18 labeled fluorocatechols. Eighth Interanational Symposium on Radiopharmaceutical chemistry, Princeton N.J., June 1990
29. Kilbourn MR and Subramanian R, Synthesis of fluorine-18 labeled 1,1-difluoro-2,2-dichloroethyl ary ethers by isotopic exchange. Eighth Interanational Symposium on Radiopharmaceutical chemistry, Princeton N.J., June 1990
30. Mourad A and Kilbourn MR, [18F]Fluorination of heterocyclic rings: syntheses of [18F]fluoropyridazines by nucleophilic substitution. Eighth Interanational Symposium on Radiopharmaceutical chemistry, Princeton N.J., June 1990

Books

31. Kilbourn MR. Fluorine-18 Labeling of Radiopharmaceuticals. National Academy Press, Washington, D.C., 1990, 149 pp, National Technical Information Service, US Department of Energy, in press

III. Postdoctoral scholars trained 1988-1990

Although this DOE grant has not provided funds for the training of postdoctoral chemists, the grant has been used to support the laboratory work of a number of postdoctoral fellows. The following list of individuals were involved in this work during the grant period.

Dr. G. Leonard Watkins
Director of PET Chemistry
University of Iowa

Dr. Michael S. Haka
Research Investigator
Division of Nuclear Medicine
University of Michigan
Ann Arbor, MI

Dr. Pulak Chakraborty
Research Fellow
Division of Nuclear Medicine
University of Michigan
Ann Arbor, MI

Dr. Alaa Mourad
Research Fellow
Division of Nuclear Medicine
University of Michigan
Ann Arbor, MI

Dr. Raghu Subramanian
Midwest Research Institute
Kansas City, MO

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