Communications to the Editor

Synthesis of High Specific Activity 6-[^{18}F]Fluorodopamine for Positron Emission Tomography Studies of Sympathetic Nervous Tissue

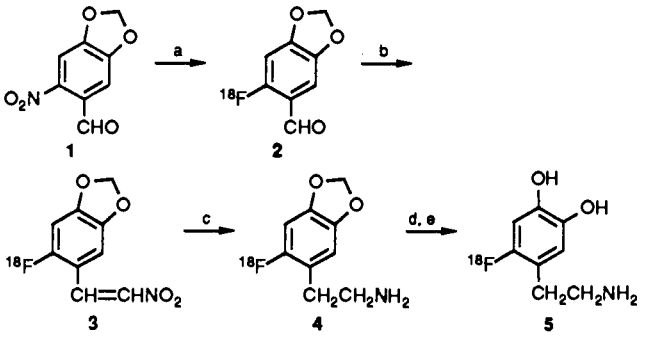
We recently reported the synthesis of high specific activity (+)- and (−)-6-[^{18}F]fluoronorepinephrine (6-[^{18}F]-FNE) via the nucleophilic aromatic substitution reaction.[1] This represented the first synthesis of a no-carrier-added F-18 labeled catecholamine, making it possible to examine these molecules as potential tracers for the activity of the sympathetic nervous system at true tracer levels, well below the mass which would lead to vasoactive reactions or other pharmacological effects. PET (positron emission tomography) studies of racemic 6-[^{18}F]FNE showed rapid, appreciable uptake and long retention in the baboon myocardium which is consistent with previous studies of unlabeled 6-FNE and of tritium-labeled norepinephrine itself.[2,3]

Previous studies have shown that intravenously administered 6-fluorodopamine (6-FDA) is converted to (−)-6-FNE in sympathetic nerve terminals.[4] This led to the suggestion that fluorine-18 labeled 6-FDA might be a candidate for cardiac neuronal imaging with PET. Support for this was demonstrated recently by the application of low specific activity 6-[^{18}F]FDA in PET imaging of cardiac sympathetic innervation and function.[5] This elegant study demonstrated, in vivo, that 6-[^{18}F]FDA serves as a substrate for neuronal uptake and vesicular storage. However, using electrophilic fluorination in the preparation of 6-[^{18}F]FDA resulted in a specific activity of only 0.17 mCi/μmol (1 mCi/mg). Because of the high mass associated with such a specific activity, its administration to anesthetized dog was accompanied by an increase in mean arterial pressure and a decrease in heart rate. Although these effects were short lived (about 3 min), the need to be administer large amounts of physiologically potent molecules seriously limits their application in humans because of toxicity and the possibility that the process being traced may be obscured by pharmacological effects.

For these reasons, and for the purpose of objectively comparing 6-[^{18}F]FNE and 6-[^{18}F]FDA, we report the no-carrier-added synthesis of high specific activity 6-[^{18}F]FDA via the nucleophilic aromatic substitution reaction using [^{18}F]fluoride ion. The kinetics of 6-[^{18}F]FNE and 6-[^{18}F]FDA were compared in the heart of the same baboon.

Chemistry. The synthesis of 6-[^{18}F]FDA is shown in Scheme I. Table I shows the time taken and relative yield (corrected for decay) for each step in the synthesis. Since

Scheme I. Synthesis of NCA 6-[^{18}F]Fluorodopamine[a]



[a] (a) K^{18}F, Kryptofix 222; (b) CH_3NO_2, NH_4OAc, HOAc; (c) $LiAlH_4$; (d) HI, H_3PO_2; (e) semi-prep HPLC.

Table I. Time Elapsed and Relative Yield at Various Stages in the Radiosynthesis of 6-[^{18}F]FDA

step completed	relative yield, mCi (EOB)	total time, min
[^{18}F]fluoride dried at 120 °C azeotropically with CH_3CN	100	0
fluorination at 120 °C for 10 min, crude product extracted with CH_2Cl_2	68	18
nitropropene formation at 100 °C for 5 min, solvent evaporated	55	40
reduction, room temperature, 5 min and 50 °C, 5 min, crude product extracted with CH_2Cl_2	27	65
hydrolysis at 170 °C for 10 min, 6-[^{18}F]FDA collected from semi-prep HPLC	20	105

6-[^{18}F]FDA is more stable than 6-[^{18}F]FNE to the harsh conditions required for hydrolyzing the protected catechol moiety during the synthesis, a commercially available substrate, 6-nitropiperonal (1), was chosen as the precursor of 6-[^{18}F]FDA rather than 3,4-*O*-isopropylidene-6-nitrobenzaldehyde[1] (precursor of 6-[^{18}F]FNE). A higher radiochemical yield for the nucleophilic aromatic substitution step (67–70% when a silicone-coated test tube was used as reaction vessel) was obtained as opposed to 45% when the isopropylidene-protected dihydroxy aromatic substrate was used. Condensation of the isolated crude fluorinated aldehyde **2** with nitromethane,[6] followed by one-step reduction of the resulting nitropropene **3** with $LiAlH_4$, yielded ethylamine **4**. Subsequent hydrolysis with hydriodic acid and purification by HPLC afforded 6-[^{18}F]FDA in a synthesis time of 105 min with a radiochemical yield 20% (EOB) and a specific activity of 2–5 Ci/μmol (EOB).

PET Studies of 6-[^{18}F]FDA in Baboons. PET studies of 6-[^{18}F]FDA in baboons were conducted as previously described for 6-[^{18}F]FNE.[1] The relative myocardial distribution of [^{13}N]ammonia was measured to determine the homogeneity of blood flow. Rapid uptake and concentration of the tracer in myocardial tissue and rapid clearance from blood were observed after intravenous injection of 6-[^{18}F]FDA. There was no change in vital signs at any time during the study. A peak concentration of

(1) Ding, Y.-S.; Fowler, J. S.; Gatley, S. J.; Dewey, S. L.; Wolf, A. P. submitted to *J. Med. Chem.*, in press.
(2) Nimit, Y.; Cantacuzene, D.; Kirk, K. L.; Creveling, C. R.; Daly, J. W. *Life Sci.* **1980**, *27*, 1577.
(3) Chiueh, C. C.; Zukowska-Grojec, Z.; Kirk, K. L.; Kopin, I. J. *J. Pharmacol. Exp. Ther.* **1983**, *225*, 529.
(4) Eisenhofer, G.; Hovevey-Sion, D.; Kopin, I. J.; Miletich, R.; Kirk, K. L.; Finn, R.; Goldstein, D. S. *J. Pharmacol. Exp. Ther.* **1988**, *248*, 419.
(5) Goldstein, D. S.; Chang, P. C.; Eisenhofer, G.; Miletich, R.; Finn, R.; Bacher, J.; Kirk, K. L.; Bacharach, S.; Kopin, I. J. *Circulation* **1990**, *81*, 1606.
(6) Kirk, K. L. *J. Org. Chem.* **1976**, *41*, 2373.

0022-2623/91/1834-0861$02.50/0

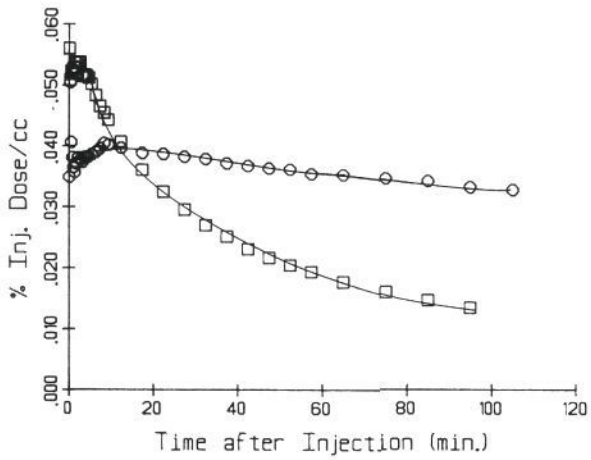


Figure 1. Time course of radiaoctivity in the baboon myocardium (left ventricular wall and septum) after injection of 6-[^{18}F]FNE (○) and 6-[^{18}F]FDA (□).

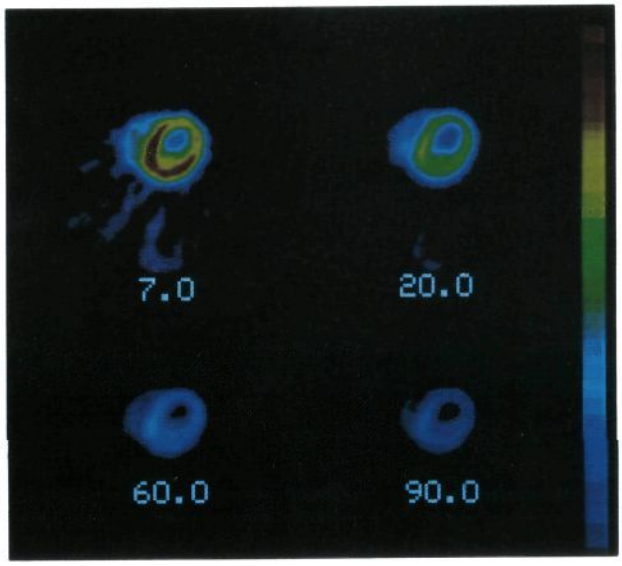


Figure 2. PET images of baboon heart at 7.0, 20.0, 60.0, and 90.0 min after injection of 6-[^{18}F]FDA.

0.053% of the injected dose/cc was observed at 2.2 min postinjection and decreased to half of the peak value by 32 min. The biexponential clearance of radioactivity at times greater than 5 min showed average half-lives of 13 and 117 min for the early and late phases, respectively, in reasonable agreement with previous PET studies.[5] These half-lives were dependent on the starting time chosen for the decay curve analysis demonstrating that the process is not a simple washout but requires a kinetic model with at least two compartments for a more accurate representation. The ratio of myocardium to plasma was 4.5 at 5 min and 4.8 at 100 min. The uptake and clearance of fluorine-18 after the injection of 6-[^{18}F]FDA and 6-[^{18}F]-FNE[1] were compared in the same baboon (Figure 1). 6-[^{18}F]FNE shows more prolonged retention than 6-[^{18}F]FDA with 86% of the peak value remaining in the heart at 60 min postinjection compared to 37% for 6-[^{18}F]FDA. The more rapid clearance of 6-[^{18}F]FDA with respect to 6-[^{18}F]FNE may be due to a number of factors including inefficient β-hydroxylation of 6-FDA in comparison to dopamine, thus leading to efflux out of storage vesicles[4] or different reactivity of the two tracers toward the metabolizing enzymes such as MAO and COMT. 6-[^{18}F]FDA-derived PET images of the baboon heart are shown on Figure 2. The myocardial distribution was similar to that obtained with $^{13}NH_3$, and 6-[^{18}F]FNE (data not shown).

To date, several radiotracers have been developed to study the neuronal activity of heart. These include [^{123}I]-*m*-iodobenzylguanidine (MIBG),[7-9] 6-[^{18}F]fluorometaraminol (6-[^{18}F]FMR),[10-12] and [^{11}C]-*m*-hydroxyephedrine [*m*-[^{11}C]HED],[13] all of which are false transmitters which are relatively stable metabolically. As a result, tracers are irreversibly bound during the entire scanning time scale, potentially limiting their capacity to reflect the turnover rate of endogenous NE.[14,15] Thus it has been recently suggested that tracers more closely resembling the catecholamine neurotransmitters and participating in their metabolic and storage functions may be more useful in diagnosing the range of neuronal impairments.[15]

In summary, this study expands the utility of nucleophilic aromatic substitution by [^{18}F]fluoride ion in the synthesis of high specific activity F-18 labeled catecholamines.[16] The synthesis of 6-[^{18}F]FDA uses a commercially available substrate (6-nitropiperonal) and proceeds in practical yield for PET studies. For example, starting with 100 mCi of [^{18}F]fluoride, 9 mCi of 6-[^{18}F]FDA is obtained in a 2-h synthesis. This would correspond to a total mass of 0.17 μg for 1 mCi, about 6000 times less than for 1 mCi of electrophilically produced 6-[^{18}F]FDA. Thus with NCA 6-[^{18}F]FDA and 6-[^{18}F]FNE reported previously[1] it is now possible to compare the kinetic behavior of these molecules at true trace levels. Even though 6-FDA is known to be converted to 6-FNE in vivo,[4] the present studies have shown the kinetic behavior of the tracers to be strikingly different. Mechanistic studies which are underway with 6-[^{18}F]FDA and with (+)- and (−)-6-[^{18}F]FNE should allow the association of biochemical processes with the kinetic properties of these molecules and allow the choice of the tracer with optimal kinetic and biochemical properties to probe a range of sympathetic neuronal activities. Such a study will not be hampered by the lack of availability of synthetic methods for high specific activity F-18 labeled tracers.

(7) Sisson, J. C.; Wieland, D. M. *Am. J. Physiol. Imaging* **1986**, *1*, 96.
(8) Glowniak, J. V.; Turner, F. E.; Gray, L. L.; Palac, R. T. *J. Nucl. Med.* **1989**, *30*, 1182.
(9) Henderson, E. B.; Kahn, J. K.; Corbett, J. R.; Jansen, D. E.; Pippin, J. J.; Kulkarni, P.; Ugolini, V.; Akers, M. S.; Hansen, C.; Buja, L. M.; Parkey, R. W.; Willerson, J. T. *Circulation* **1988**, *78*, 1192.
(10) Wieland, D. M.; Rosenspire, K. C.; Hutchins, G. D.; Dort, M. V.; Rothley, J. M.; Mislankar, S. G.; Lee, H. T.; Massion, C. C.; Gildersleeve, D. L.; Sherman, P. S.; Schwaiger, M. *J. Med. Chem.* **1990**, *33*, 956.
(11) Rosenspire, K. C.; Gildersleeve, D. L.; Massin, C. C.; Mislankar, S. G.; Wieland, D. M. *Nucl. Med. Biol.* **1989**, *16*, 735.
(12) Schwaiger, M.; Guibourg, H.; Rosenspire, K.; McClanahan, T.; Gallagher, K.; Hutchins, G.; Wieland, D. M. *J. Nucl. Med.* **1990**, *31*, 1352.
(13) Schwaiger, M.; Kalff, V.; Rosenspire, K.; Haka, M. S.; Molina, E.; Hutchins, G. D.; Deeb, M.; Wolfe, E., Jr.; Wieland, D. M. *Circulation* **1990**, *82*, 457.
(14) Kopin, I. J. *Circulation* **1990**, *82*, 646.
(15) Wieland, D. M. Abstract. Heart: In Vivo PET Results. In *Afrontiers in Nuclear Medicine Joint Symposium on In Vivo Imaging of Neurotransmitter Functions in Brain, Heart and Tumors*; American College of Nuclear Physicians, Washington, D.C., Publication No. 90-3, 1990, p 9.
(16) Ding, Y.-S.; Shiue, C.-Y.; Fowler, J. S.; Wolf, A. P.; Plenevaux, A. *J. Fluorine Chem.* **1990**, *48*, 189.

Acknowledgment. This research was carried out at Brookhaven National Laboratories under contract DE-AC02-76CH00016 and the U.S. Department of Energy and supported by its Office of Health and Environmental Research and also supported by the National Institutes of Health, Grant NS-15380. The authors wish to thank Dr. Kenneth Kirk for a sample of 6-fluorodopamine. They are also grateful for the advice and assistance of Bernard Bendriem, Robert MacGregor, Payton King, Karin Karlstrom, Elizabeth Jellett, Colleen Shea, Robert Carciello, Babe Barrett.

Yu-Shin Ding,* Joanna S. Fowler, S. John Gatley Stephen L. Dewey, Alfred P. Wolf, David J. Schlyer
Chemistry Department
Brookhaven National Laboratory
Upton, New York 11973
Received October 22, 1990

Hydrophobicity Parameters for Platinum Complexes

Since the discovery of the antitumor activity of *cis*-$[Pt(NH_3)_2Cl_2]$, many analogues of the type *cis*-$[PtA_2X_2]$ have been synthesized. In vivo the labile ligands, X, are replaced during substitution reactions with nucleophiles such as DNA while the nonleaving groups, A, remain attached to the metal.[1] Structure–activity studies have concluded that charged complexes are inactive because they are not sufficiently hydrophobic.[2] However, hydrophobicities of charged platinum complexes have not been measured.

The partition coefficient (log *P*) is a useful parameter for finding the optimal hydrophobicity of a series of molecules. It is usually calculated from the sum of partition coefficients of the chemical fragments composing the molecule and values permitting such calculations have been tabulated.[3-5] Since partition coefficients for Pt fragments were not available, we wished to determine hydrophobicity parameters for $[>PtCl_2]$ and $[>Pt(H_2O_2)]^{2+}$.

In order to measure hydrophobicity of these groups, we synthesized the molecules in Table I[6] and measured their partition coefficients in an octanol/water emulsion. The nitrato derivatives may undergo hydrolysis in aqueous solution to form aqua complexes such as $\{cis\text{-}[PtA_2(OH)(H_2O)]^{1+}; NO_3^-\}$, $\{cis\text{-}[PtA_2(H_2O)_2]^{2+}; 2NO_3^-\}$, and various dimers and trimers.[7,8] Several pieces of evidence indicate that the nitrato complexes used in our experiments formed uniquely diaqua complexes of the type $\{cis\text{-}[PtA_2(H_2O)_2]^{2+}; 2NO_3^-\}$. Molar conductivities of 10^{-3} to 10^{-4} M aqueous solutions of these compounds were between 230 and 280 (ohm cm^2 mol)$^{-1}$. Onsager plots of the conductivity had identical slopes for these molecules and for the doubly ionized model compounds $\{[Pt(ethylenediamine)(NH_3)_2]^{2+}; 2Cl^-\}$ and $\{[Pt(1,2\text{-diaminocyclohexane})(NH_3)_2]^{2+}; 2Cl^-\}$. After dissolving 3×10^{-2} M compound in water for 2 h at 37 °C, ^{195}Pt NMR spectra[6] contained a single peak corresponding to the monomer diaqua complex.[8]

Table I. Partition Coefficients of the Complexes *cis*-$[PtA_2X_2]$[a]

A	X	log P[b]	optimal dose, μmol/kg	T/C,[c] %
NH_3	Cl	−2.19 ± 0.06	27	200
CH_3NH_2	Cl	−1.68 ± 0.04	76	175
$(CH_3)_2CHNH_2$	Cl	−0.32 ± 0.02	ND	ND
Bu^cNH_2	Cl	0.36 ± 0.04	147	199
Pe^cNH_2	Cl	0.81 ± 0.04	572	172
NH_3	NO_3	−3.36 ± 0.11	3.5	123
CH_3NH_2	NO_3	−3.28 ± 0.08	26	161
Bu^cNH_2	NO_3	−1.71 ± 0.12	43	177
Pe^cNH_2	NO_3	−1.14 ± 0.06	51	193
Hx^cNH_2	NO_3	−0.91 ± 0.14	97	173
Hp^cNH_2	NO_3	−0.35 ± 0.05	92	133
4-$HOCH_2$Py	NO_3	−2.13 ± 0.06	ND	ND
4-CH_3COOPy	NO_3	−1.41 ± 0.13	ND	ND
Py	NO_3	−1.59 ± 0.12	105	125
4-$(CH_3)_2N$Py	NO_3	−0.83 ± 0.08	495	110
4-ClPy	NO_3	−1.06 ± 0.24	183	130

[a] Abbreviations are as follows: Bu^c, cyclobutylamine; Pe^c, cyclopentylamine; Hx^c, cyclohexylamine; Hp^c, cycloheptylamine; and Py, pyridine. [b] Mean ± range of 6–10 independent experiments. [c] Antitumor activity was measured against P388 murine leukemia.[13] Female DBA/2 mice were injected with 10^6 cells on day 0 and treated on day 1 with platinum compounds freshly dissolved in 0.4% Klucel. T/C is the median survival time of treated mice with respect to untreated controls.

Solutions of nitrato complexes were freshly prepared in triply distilled water and chloro compounds in 0.15 M NaCl. In some experiments complexes were dissolved in octanol. Equal volumes of the two phases were shaken at 300 agitations/min at 37 °C. At various times the two phases were separated, aliquots removed, and platinum concentrations determined in the octanol and the water phase with use of a Perkin-Elmer atomic absorption spectrometer Model 603 equipped with a graphite furnace.[9] Results are reported as log *P* where *P* is the concentration of platinum compound in the octanol phase divided by the aqueous phase.

After 3 min the chloro compounds reached an equilibrium between octanol and water. log *P* was independent of concentration and of the phase in which the compound was initially dissolved.

For the nitrato complexes, Pt passed from the aqueous to the organic phase during the first hour of agitation. In the extreme case, $\{cis\text{-}[Pt(Hp^c)_2(H_2O)_2]^{2+}; 2NO_3^-\}$, log *P* varied from −0.38 ± 0.05 to −0.008 ± 0.006. The kinetics were identical whether the emulsion was continuously agitated or not. Hence this phenomena appears to be independent of the partition between the two phases which occurs rapidly. log *P* did not change if complexes were kept in aqueous solution for 2 h at 37 °C prior to mea-

(1) Johnson, N. P.; Butour, J. L.; Villani, G.; Wimmer, F. L.; Defais, M.; Pierson, V.; Brabec, V. In *Progress in Clinical Biochemistry and Medicine*; Clarke, M. J., Ed.; Springer-Verlag: Berlin, 1989; p 1.
(2) Cleare, M. J.; Hoeschele, J. D. *Bioinorg. Chem.* **1973**, *2*, 187.
(3) Hansch, C.; Fujita, T. *J. Am. Chem. Soc.* **1963**, *86*, 1616.
(4) Hansch, C.; Leo, A.; Unger, S. H.; Kim, K. H.; Nikaitani, D.; Lien, E. J. *J. Med. Chem.* **1973**, *16*, 1207.
(5) Nys, G. G.; Rekker, R. F. *Chim. Ther.* **1973**, *5*, 521.
(6) Souchard, J. P.; Wimmer, F. L.; Ha, T. T. B.; Johnson, N. P. *J. Chem. Soc., Dalton Trans.* **1990**, 307.
(7) Lim, M. C.; Martin, R. B. *J. Inorg. Nucl. Chem.* **1976**, *38*, 1911.
(8) Gill, D. S.; Rosenberg, B. *J. Am. Chem. Soc.* **1982**, *104*, 4598.
(9) In the case of the most hydrophilic compounds, the value of log *P* was determined from the platinum concentration in the octanol phase. The signal/noise ratio was >20, and the uncertainty in Table I reflects primarily the precision of the atomic absorption measurements.
(10) Rekker, R. F.; Kort, H. M. *Eur. J. Med. Chem.* **1979**, *14*, 479.
(11) Hansch, C.; Leo, A. *Substituent Constants for Correlation Analysis in Chemistry and Biology*; John Wiley and Sons: New York, 1979.
(12) Farrell, N.; Ha, T. T. B.; Souchard, J. P.; Wimmer, F. L.; Cros, S.; Johnson, N. P. *J. Med. Chem.* **1989**, *32*, 2240.
(13) Auclair, C.; Pierre, A.; Voisin, E.; Pepin, O.; Cros, S.; Colas, C.; Saucier, J. M.; Verschuere, B.; Gros, P.; Paoletti, C. *Cancer Res.* **1987**, *47*, 6254.

0022-2623/91/1834-0863$02.50/0