solvent was added, and the final volume was adjusted to 1 mL with acetone-d_6. All spectra were recorded on a GE QE-300 superconducting spectrometer operating at 300 MHz in the fourier transform mode. One pulse with presaturation mode for the solvent peak was used. For each measurement, the sweep width was 3 kH_z with 200 scans and 16K data size collected. Chemical shifts were reported in hertz downfield from the internal tetramethylsilane standard. Spectra were collected at ambient temperature (298 K).

Calculations. A modification of the Benesi–Hildebrand equation[36] for determining equilibrium constants for complex equilibria has been derived for NMR applications. By considering the chemical shift of protons on molecules undergoing rapid exchange between complexed and uncomplexed states and by following treatments used in NMR studies of hydrogen-bonding equilibria, the equation shown below may be derived[37,38] where

$$1/\Delta_{obsd}{}^{A} = 1/K\Delta_{AD}{}^{A}c_D + 1/\Delta_{AD}{}^{A} \quad (2)$$

$\Delta_{obsd}{}^{A}$ is the observed shift of acceptor protons in the complexing medium, $\Delta_{AD}{}^{A}$ is the shift of acceptor protons in the pure complex, c_D is the concentration of donor in moles per kilogram of solvent; K is the equilibrium constant. This equation requires that $c_D K(\Delta_{AD}{}^{A}/\Delta_{A}{}^{A}) \gg 1$. When $c_D \gg c_A$ (as used in this work), the shift of pure acceptor protons ($\Delta_{A}{}^{A}$) is very small relative to $\Delta_{AD}{}^{A}$; thus, for reasonable K values (≥ 0.1), this condition is usually met. It also assumes that solutions are ideal or that the quotient $\gamma_{AD}/(\gamma_A)(\gamma_D)$ remains constant over the range of solutions studied.

Equilibrium constants (K) were calculated from the NMR data by least-squares regression analysis. The correlation coefficients were analyzed for statistical significance as a test for the linearity of the plot. All coefficients were significantly different from zero, $P < 0.001$. The standard free energy (ΔG) was calculated from K by the equation

$$\Delta G = -RT(\ln K) \quad (3)$$

where R is the gas constant and T the absolute temperature.

The molecular mechanics program MODEL 1.3 kindly provided to the University of North Carolina, Chapel Hill, (UNC-CH) by W. Clark Still (Columbia University) was used to perform approximate force field calculations on representative complexes with a VAX780 computer. This program was modified in our laboratory to utilize a Tektronix 4107 color display terminal with graphics tablet input and the MM2p force field.[39] Parameters were estimated from Meyer et al.[40] A program for matching local coordinates was used for approximately locating the molecular planes of interest before MM2p optimization of the complex. The structure of p-xylene is based on standard geometries and that for the thyroid hormone is based on X-ray studies.[8]

Acknowledgment. We thank Drs. L. G. Pedersen (UNC-CH) and C. T. Burt (this laboratory) for helpful comments and suggestions and Dr. T. Darden (NIEHS) for assistance with the molecular modeling and calculations.

Registry No. TCDD, 1746-01-6.

(36) Benesi, H. A.; Hildebrand, J. H. *J. Am. Chem. Soc.* **1949**, *71*, 2703.
(37) Wilson, N. K. *J. Am. Chem. Soc.* **1972**, *94*(7) 2431.
(38) Berkeley, P. J., Jr.; Hanna, M. W. *J. Phys. Chem.* **1963**, *67*, 846.
(39) Darden, T.; McKinney, J. D.; Gottschalk, K.; Maynard, A. T.; Pedersen, L. G. *J. Am. Chem. Soc.* **1986**, *108*, 207.
(40) Meyer, A. Y.; Allinger, N. L.; Yuk, Y. *Isr. J. Chem.* **1980**, *20*, 57.

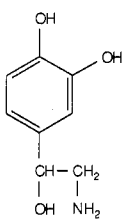
6-[^{18}F]Fluorometaraminol: A Radiotracer for in Vivo Mapping of Adrenergic Nerves of the Heart[1]

Suresh G. Mislankar, David L. Gildersleeve, Donald M. Wieland,* Christopher C. Massin, G. Keith Mulholland, and Steven A. Toorongian

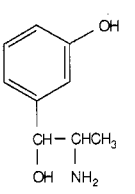
Division of Nuclear Medicine, Department of Internal Medicine, University of Michigan Medical Center, Ann Arbor, Michigan 48109. *Received July 17, 1987*

The false neurotransmitter metaraminol has been ^{18}F labeled and evaluated as a possible heart imaging agent on the basis of its selective accumulation in adrenergic nerves. Reaction of 6-(acetoxymercurio)-*N*-*t*-BOC-metaraminol with acetyl hypofluorite followed by removal of the BOC group provides a regiospecific synthesis of 6-fluorometaraminol (4). Use of acetyl hypo[^{18}F]fluorite gives [^{18}F]-4 in 60 min in 20–42% radiochemical yield. Systemic blockade of the neuronal uptake-1 carrier with desmethylimipramine or systemic destruction of the adrenergic nerves with 6-hydroxydopamine lowers [^{18}F]-4 accumulation ≥85% in all four regions of the rat heart. These preliminary findings suggest that [^{18}F]-4 could be used to assess neuronal damage in various heart diseases by positron emission tomography.

Metaraminol is a substitute adrenergic transmitter that stoichiometrically displaces norepinephrine (NE) from its storage sites within the neuron.[2] Like NE, metaraminol is transported into the adrenergic neuron by the uptake-1 carrier protein, sequestered within storage vesicles, and released by nerve impulse.[3] Metaraminol is less potent than NE in activating postsynaptic adrenergic receptors.[4] Although structurally similar to NE, metaraminol is metabolized by neither catechol-*O*-methyltransferase nor monoamine oxidase.[5] The metabolic stability of metar-



NOREPINEPHRINE



METARAMINOL

(1) A preliminary report of this work has been presented: Mislankar, S. G.; Gildersleeve, D. G.; Mulholland, G. K.; Massin, C. C.; Toorongian, S. A.; Wieland, D. M. 34th Annual Meeting of the Society of Nuclear Medicine, Toronto, Canada, June 2–5, 1987 (Abstract in *J. Nucl. Med.* **1987**, *28*, 625).
(2) (a) Anden, N. E. *Acta Pharmacol. Toxicol.* **1964**, *21*, 260. (b) Shore, P. A.; Busfield, D.; Alpers, H. S. *J. Pharmacol. Exp. Therap.* **1964**, *146*, 194.
(3) (a) Crout, J. R.; Alpers, H. S.; Tatum, E. L.; Shore, P. A. *Science (Washington, D.C.)* **1964**, *145*, 828. (b) Kopin, I. J. *Annu. Rev. Pharmacol.* **1968**, *8*, 377.
(4) Trendelenburg, V.; Muskus, A.; Fleming, W. W.; Alonso de La Sierra, B. G. *J. Pharmacol. Exp. Ther.* **1962**, *138*, 170.

0022-2623/88/1831-0362$01.50/0

Scheme I

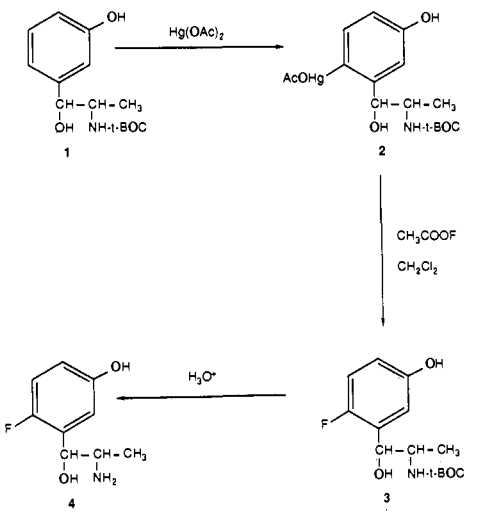


Table I. ^{19}F NMR Chemical Shift Values of Fluorinated Phenylephrines and 6-Fluorometaraminol[a]

fluorophenylephrines[b]		fluorometaraminol	
isomer	chemical shift	isomer	chemical shift
2-fluoro	−66.2		
4-fluoro	−63.7		
6-fluoro	−53.0	6-fluoro	−52.5

[a] Spectra were obtained of the bitartrate or oxalate salts in D_2O; chemical shifts are in ppm relative to CF_3COOH. [b] Compounds supplied by K. L. Kirk.[10]

aminol has prompted us to investigate this false transmitter as a possible radiotracer for probing the integrity of adrenergic neurons of the heart. There is compelling evidence in the literature that [^{3}H]metaraminol can be used in vitro as an index of adrenergic nerve density in various tissues.[6] Initial studies in our own laboratory[7] with [^{3}H]metaraminol suggested that a ^{18}F-labeled metaraminol ($T_{1/2}$ of ^{18}F = 110 min) might be used to quantitatively assess peripheral adrenergic nerve density in vivo by the technique of positron emission tomography (PET).[8] We report here a regiospecific synthesis of 6-[^{18}F]fluorometaraminol (6-FMR) via fluorodemercuration using acetyl hypo[^{18}F]fluorite. Preliminary biological studies demonstrate the high selectivity of this new agent for adrenergic nerves and suggest its possible use in diagnosing neuronal damage in human heart disease.

Chemistry. Scheme I illustrates the procedure used for the synthesis of 6-FMR. The *t*-BOC derivative 1, prepared from metaraminol and di-*tert*-butyl dicarbonate in DMF, was treated with mercuric acetate in 5% acetic acid in 50% aqueous ethanol to give 6-HgOAc derivative **2** as the major product (Figure 1). Flash chromatography on silica gel provided pure **2**. Fluorodemercuration of **2** was achieved with gaseous acetyl hypofluorite in CH_2Cl_2 at ice temperature.[9] Removal of the *t*-BOC group with dilute hydrochloric acid in acetonitrile gave compound **4**, 6-FMR, in 30% isolated yield (based on **2**) following flash chromatography.

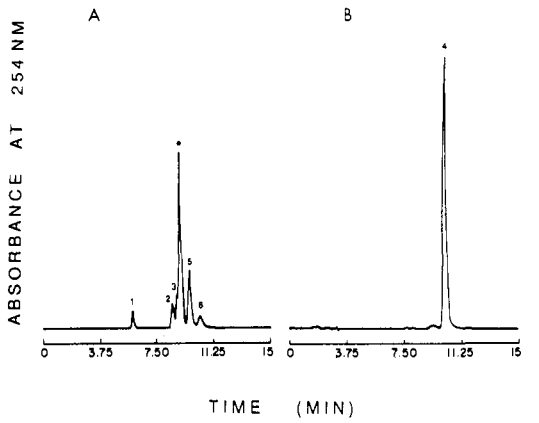


Figure 1. HPLC chromatograms of the reaction mixture of 6-(acetoxymercurio-*N*-*t*-BOC-metaraminol before (A) and after (B) purification by flash chromatography. Peaks 1, 2, and 6 are unknown contaminants while peaks 3, 4, and 5 are, respectively, 4-(acetoxymercurio)-*N*-*t*-BOC-metaraminol, 6-(acetoxymercurio)-*N*-*t*-BOC-metaraminol, and unreacted *N*-*t*-BOC-metaraminol. After purification, the 6-(acetoxymercurio)-*N*-*t*-BOC-metaraminol (peak 4) was >98% pure. Analysis was performed on an Ultrasphere ODS column with 0.01% 2-mercaptoethanol in water–acetonitrile (1/1) as solvent at a flow rate of 0.5 mL/min.

Table II. Tissue Concentrations of 6-[^{18}F]Fluorometaraminol in Dog[a]

tissue	concentration,[b] % kg dose/g	
	dog 1	dog 2
heart[c]	0.96	0.92
blood	0.03	0.03
lung	0.26	0.30
spleen	0.66	0.51
liver	0.46	0.25
muscle	0.05	0.09
adrenal medulla	12.41	10.75

[a] Animals were 20-kg male, mongrel dogs. [b] Radiotracer injections (2 mCi) were iv bolus; animals were killed 1 h following radiotracer injection; values represent mean of duplicate samples. [c] Left ventricle.

Structural evidence for **4** is based on a comparison of its ^{1}H and ^{19}F NMR spectra to the ring-fluorinated isomers of phenylephrine,[10] a close structural analogue of metaraminol (see Table I). Further support for selective functionalization in the 6-position is based on iododemercuration of **2** and subsequent acid hydrolysis to give exclusively 6-iodometaraminol, a compound previously obtained in our laboratory by a different synthetic route.[7]

Evidence for retention of the 1*R*,2*S* configuration of metaraminol during the reaction sequence is based on ^{1}H NMR and reverse-phase HPLC comparison of **4** with a diastereomeric mixture obtained by partially racemizing **4** in refluxing 6 N HCl for 7 days; the ^{1}H NMR of metaraminol and its acid-induced racemate have been re-

(5) Fuller, R. W.; Snoddy, H. D.; Perry, K. W.; Bernstein, J. R.; Murphy, P. J. *Biochem. Pharmacol.* **1981**, *30*, 2831.
(6) (a) Duckles, S. P. *Eur. J. Pharmacol.* **1980**, *67*, 355. (b) Hermann, W.; Graefe, K.-H. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1977**, *296*, 99. (c) Anton, A. H.; Berk, A. I. *Eur. J. Pharmacol.* **1977**, *44*, 161.
(7) Arjunan, P.; Lee, H.; Mislankar, S. G.; Wieland, D. M.; Lin, T. Y.; Sherman, P. S.; Fisher, S. J. submitted for publication in *J. Nucl. Med. Biol.*
(8) Phelps, M. E., Mazziotta, J. C., Schelbert, H. R., Eds. *Positron Emission Tomography and Autoradiography: Principles and Applications for the Brain and Heart*; Raven: New York, 1986.
(9) (a) Visser, G. W. M.; von Halteren, B. W.; Herscheid, D. M.; Brinkman, G. A.; Hoekstra, A. *J. Chem. Soc. Chem. Commun.* **1984**, 655. (b) Visser, G. W. M.; Bakker, C. N. M.; von Halteren, B. W.; Herscheid, J. D. M.; Brinkman, G. A.; Hoekstra, A. *J. Org. Chem.* **1986**, *51*, 1886.
(10) Kirk, K. L.; Olubajo, O.; Buchold, K.; Lewandowski, G. A.; Gusovsky, F.; McCulloh, D.; Daly, J. W.; Creveling, C. R. *J. Med. Chem.* **1986**, *29*, 1982.

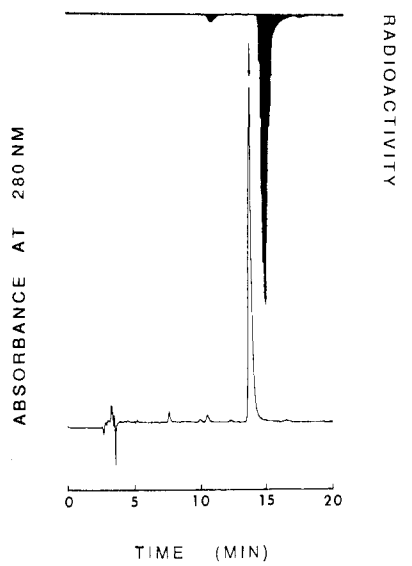


Figure 2. Radio-HPLC chromatogram of 2.4 μCi of 6-[^{18}F]-fluorometaraminol, showing both radiochemical (>95%) and chemical (>93%) purity. Radioactivity trace is shown in black and the ultraviolet trace is in white. Analysis was performed on an Ultrasphere IP column with a solvent system of 25 mM sodium acetate buffer, pH 4.75, containing 10 mM sodium pentanesulfonate and 25% methanol at a flow rate of 1.0 mL/min.

ported by Saari and co-workers.[11]

Radiochemistry. Scheme I was also employed for the radiosynthesis of ^{18}F labeled 4. A SEP-PAK (Waters silica cartridge) was used instead of flash chromatography for the purification of [^{18}F]-3. Gaseous acetyl hypo[^{18}F]fluorite was generated by a previously described method.[12] The radiosynthesis of [^{18}F]-4 from 2 could be accomplished in less than 60 min. With continued optimization of this process, radiosynthetic times of less than 25 min should be attainable. Radiochemical yields based on acetyl hypo[^{18}F]fluorite for 10 runs ranged from 20% to 42%; chemical purity was >93%, and radiochemical purity was >95% as shown by radio-HPLC (Figure 2). Specific activity at end-of-synthesis was 500–1200 mCi/mmol.

Biological Results. The concentration of radioactivity in various tissues following intravenous injection of [^{18}F)-4 was determined in rats and dogs as shown in Table II. [^{18}F]-4 showed preferential accumulation in organs with rich noradrenergic innervation such as heart and spleen. The dog adrenal medulla, a tissue known to actively sequester exogenous NE,[13] shows high concentration levels of radioactivity. The heart-to-blood and heart-to-lung radioactivity concentration ratios for [^{18}F]-4 were 25 and 4, respectively, at 1 h after intravenous injection in the dog. Studies to be reported separately using radio-HPLC analysis of tissue extracts have shown that the sole source of radioactivity in the dog heart is [^{18}F]-4.[14]

Treatment of rats with the selective NE uptake inhibitor desmethylimipramine (DMI) prior to injection of [^{18}F]-4 resulted in a >90% decrease in radioactivity in all regions of the rat heart compared to controls (Table III). Similar results were observed when [^{3}H]NE was used as the radiotracer. Ablation of the sympathetic nerves of the rat heart resulted in an 82% decrease in [^{18}F]-4 concentration in the left ventricle (Table IV). The degree of sympathectomy in the heart was confirmed by parallel studies with [^{3}H]NE.

Table III. Desmethylimipramine (DMI) Blockade of [^{18}F]-4 and [^{3}H]NE Uptake in the Rat

	^{18}F concentration, % dose/g ± SEM[a]			
tissue	control	DMI[b]	% change[c]	% change [^{3}H]NE[d]
left ventricle	2.69 ± 0.31	0.15 ± 0.02	−94	−97
right ventricle	3.68 ± 0.34	0.20 ± 0.03	−95	−96
left atrium	3.41 ± 0.18	0.24 ± 0.04	−93	−95
right atrium	3.06 ± 0.10	0.24 ± 0.03	−92	−95
spleen	0.82 ± 0.05	0.20 ± 0.02	−75	−86
liver	1.04 ± 0.07	0.77 ± 0.05	−26	*e*
blood	0.06 ± 0.01	0.12 ± 0.01	+81	+55

[a] Normalized to 250 g body weight; results are the means of four rats per study group; duplicate samples were taken of each tissue. [b] DMI·HCl (10 mg/kg, ip) or, in the case of controls, an equal volume of physiological saline (ip) was administered 30 min prior to iv tracer injection. All animals were sacrificed 1.5 h after tracer injection (5 μCi). [c] $p \leq 0.001$ for all heart tissues. [d] Comparative results when [^{3}H]NE was used as the radiotracer; five rats were used per study group. [e] Not determined.

Table IV. Effect of 6-Hydroxydopamine (6-OHDA) Sympathectomy on 6-[^{18}F]Fluorometaraminol Concentration in the Rat

	^{18}F concentration, % dose/g ± SEM[a]			
tissue	control	6-OHDA treated[b]	% change	% change [^{3}H]NE[d]
left ventricle	2.67 ± 0.20	0.42 ± 0.06	−84	−85
right ventricle	3.42 ± 0.13	0.46 ± 0.03	−87	−92
left atrium	3.47 ± 0.06	0.41 ± 0.07	−88	−92
right atrium	3.91 ± 0.18	0.49 ± 0.11	−87	−94
spleen	0.88 ± 0.08	0.35 ± 0.04	−60	−70
liver	0.73 ± 0.07	1.20 ± 0.11	+64	*e*
blood	0.07 ± 0.01	0.11 ± 0.01	+57	0

[a] Normalized to 250 g body weight; results are the means of six rats per study group; duplicate samples were taken of each tissue. [b] 6-OHDA·HBr (100 mg/kg, ip) or, in the case of controls, an equal volume of physiological saline (ip) was administered 5 days prior to iv tracer injection (9–10 μCi). All animals were sacrificed 1.5 h after tracer injection. [c] $p \leq 0.001$ for all heart tissue. [d] Comparative results when [^{3}H]NE was used as the radiotracer; five rats per study group. [e] Not determined.

Discussion

The synthesis of 6-FMR described in this paper is based in part on recent reports that have applied the mercuration/[^{18}F]fluorodemercuration technique to a variety of simple aromatic compounds,[9] including the synthesis of 6-[^{18}F]fluoro-DOPA.[15] It is notable in the present synthesis of 6-[^{18}F]FMR that neither the phenol group nor the chiral benzylic hydroxy group need to be protected. The [^{18}F]fluorodemercuration approach represents an improvement in both the radiochemical yield and regioselectivity achieved by other electrophilic fluorination methods.[16]

A potential limiting factor in the human use of [^{18}F]-radiotracers synthesized by this technique is the low specific activity achievable. The carrier amount of 6-[^{19}F]FMR present in our preparation of 6-[^{18}F]FMR is only slightly lower than the expected pharmacological dose range of 6-FMR based on levels of metaraminol known to cause a pressor effect.[17] This potential problem might be

(11) Saari, W. S.; Raab, A. W.; Engelhardt, E. L. *J. Med. Chem.* **1968**, *11*, 1115.
(12) Jewett, D. M.; Potocki, J. F.; Ehrenkaufer, R. E. *Synth. Commun.* **1984**, *14*, 45.
(13) Wieland, D. M.; Mangner, T. J.; Inbasekaran, M. N.; Brown, L. E.; Wu, J. L. *J. Med. Chem.* **1984**, *27*, 149.
(14) Rosenspire, K. C.; Gildersleeve, D. L.; Wieland, D. M., unpublished findings.
(15) Luxen, A.; Barrio, J. R.; Bida, G. T.; Satyamurthy, N. *J. Labelled Cmpd. Radiopharm.* **1986**, *23*, 1066 (Abstract).
(16) Firnau, G.; Garnett, E. S.; Chirakal, R.; Sood, S.; Nahmias, C.; Schrobilgen, G. *Int. J. Appl. Radiat. Isot.* **1986**, *37*, 669.

alleviated by using slow intravenous infusions rather than bolus injections. Efforts are under way in our laboratory to enhance the specific activity of 6-[^{18}F]FMR by using no-carrier-added [^{18}F]fluoride in nucleophilic processes.[18] The ^{18}F tagging of more potent pressor agents such as NE itself may require specific activities higher than presently achievable by electrophilic fluorination techniques.[19,20]

The high concentrations (Table II) of fluorine-18 observed in catecholamine-rich organs such as heart, spleen, and adrenal medulla are consistent with the predicted neuronal localization of 6-[^{18}F]FMR. The large decreases in fluorine-18 activity observed in these tissues following inhibition of the uptake-1 transporter with DMI further support the neuronal sequestration of 6-[^{18}F]FMR. The Michaelis–Menten kinetic parameters for metaraminol transport by the uptake-1 carrier are very similar to those of the endogenous transmitter NE.[21] Neuronal localization in the heart was also confirmed by the decrease observed in 6-[^{18}F]FMR uptake following systemic destruction of the peripheral sympathetic nerves with 6-OHDA; an 85% or greater decrease in tracer concentration was observed in all four heart regions assayed. Use of 6-[^{18}F]FMR as a PET imaging agent should permit a more quantitative assessment of neuronal alterations in various heart diseases than is presently achievable with the neuronal agent (*m*-[^{123}I]iodobenzyl)guanidine and single-photon tomography.[22]

Experimental Section

Melting points were determined on a Mel-Temp apparatus and are uncorrected. Mass spectra were recorded on a Finnigan 4000 spectrometer. ^{1}H NMR spectra were recorded at 60 MHz with a Varian EM-360 spectrometer and ^{19}F NMR spectra were recorded at 282.34 MHz on a Bruker 300 MHz spectrometer. IR spectra were recorded on a Perkin-Elmer 283B infrared spectrometer. Optical rotations were recorded on a Perkin-Elmer EM-241 polarimeter.

Thin-layer chromatography was conducted with Analtech 0.25-mm glass plates precoated with silica gel GF. For flash column chromatography, E. Merck silica gel-60, 230–400 mesh, was used. Metaraminol ((−)-(*m*-hydroxyphenyl)propanolamine) bitartrate salt was obtained as a gift from Merck Sharp & Dohme Research Laboratories or purchased from Sigma Chemical Co. C, H, and N analyses were performed by Spang Microanalytical Laboratory, Eagle Harbor, MI. Radio thin-layer chromatograms were obtained on a Berthold TLC linear analyzer LB 282 equipped with data acquisition system LB 500. Two HPLC systems were used; the first was a Beckman Instruments Model 344 gradient liquid chromatograph with a Beckman Model 155-00 UV/vis detector (254 or 280 nm) and a Waters Associates Model 740 data module. The second HPLC system consisted of a Waters Associates Model 680 automated gradient controller, two 510 pumps, and UGK injector with a Kratos SF773 UV/vis detector (280 nm), a Waters 740 data module, and a Radiomatic Instruments Flo-One DR radioactivity flow detector with Model CU data acquisition system upgrade and 340-μL solid scintillant cell installed.

***N*-*t*-BOC-metaraminol (1).** To a suspension of 6.1 g (19.2 mmol) of metaraminol bitartarate in 15 mL of dimethylformamide (DMF) was added 5.6 mL (40 mmol) of triethylamine. The reaction mixture was stirred under argon for 30 min. To the resulting pale yellow solution was added 4.6 g (21.0 mmol) of di-*tert*-butyl dicarbonate in 5 mL of DMF, and the reaction mixture was stirred overnight at ambient temperature. The DMF was distilled under high vacuum, and the residue was dissolved in 50 mL of ethyl acetate and 15 mL of water. The aqueous layer was extracted twice with 15 mL of ethyl acetate, and the combined organic layers were washed with brine and dried over sodium sulfate. The solvent was removed under reduced pressure to give 4.7 g (92%) of *N*-*t*-BOC-metaraminol as a glassy solid. A small portion of this was purified by flash column chromatography using ethyl acetate–hexane (1:2) to give analytically pure compound: ^{1}H NMR ($CDCl_3$) δ 0.85–1.0 (3 H, d, 9 Hz), 1.45 (9 H, s), 3.45–4.2 (2 H, m), 4.55–5.1 (2 H, m), and 6.5–7.35 (5 H); IR (KBr) 1670 cm^{-1}; EIMS, *m/e* 267 (M^+), 211, 194, 176, 167, 144, 123, 95, 88, 77, 57, 44. Anal. ($C_{14}H_{21}NO_4$) C, H, N.

6-(Acetoxymercurio)-*N*-*t*-BOC-metaraminol (2). To a solution of 400 mg (1.49 mmol) of *N*-*t*-BOC-metaraminol in 10 mL of 50% aqueous ethanol containing 5% acetic acid was added 238 mg (0.75 mmol) of mercuric acetate. The resulting mixture was stirred at 50–55 °C until the solution was free of Hg^{2+} ions; usually, 35–40 min sufficed. The ethanol was removed under reduced pressure. The residue was extracted with 50 mL of chloroform and dried over sodium sulfate. The solvent was removed in vacuo, and the residue was purified by flash chromatography on silica gel (3.0 × 20.0 cm column) using as the mobile phase chloroform–ethanol–acetic acid in a gradient from 94:4:0.01 to 94:6:0.01 to yield 235 mg (60%) of pure 6-(acetoxymercurio)-*N*-*t*-BOC-metaraminol as a white fluffy solid, mp 115–118 °C. The high purity of **2** was confirmed after each synthesis by HPLC (see Figure 1): ^{1}H NMR ($CDCl_3$) δ 0.92 (3 H, d, 9 Hz), 1.45 (9 H, s), 2.00 (3 H, s), 3.6–4.1 (2 H, m), 6.5–7.35 (4 H, m); IR (KBr) 1680 cm^{-1}. Anal. ($C_{16}H_{23}NO_6Hg$) C, H, N.

6-Fluoro-*N*-*t*-BOC-metaraminol (3). Gaseous acetyl hypofluorite (0.10 mmol) was bubbled through a solution of 64 mg (0.12 mmol) of 6-(acetoxymercurio)-*N*-*t*-BOC-metaraminol in 10 mL of acetonitrile for 10 min. The solvent was removed under reduced pressure, and the residue was purified by silica gel Sep-Pak with 99:1 CH_2Cl_2–methanol to yield 16 mg (55%) of fluffy solid, which was recrystallized from petroleum ether and ether: mp 132–133 °C; ^{1}H NMR ($CDCl_3$) δ 1.1 (2 H, d, 9 Hz), 1.5 (9 H, s), 3.45–5.2 (4 H, m), and 6.7–7.5 (4 H, m); ^{19}F NMR ($CDCl_3$ with $CFCl_3$ internal standard) δ −129.02 (br s); IR (KBr) 1670 cm^{-1}; EIMS, *m/e* 286 ($M^+ + 1$), 285, 230, 212, 194, 141, 57, 44. Anal. ($C_{14}H_{20}FNO_4$) C, H, N.

6-Fluorometaraminol Bitartrate (4). To a solution of 70 mg (0.24 mmol) of 6-fluoro-*N*-*t*-BOC-metaraminol in 5 mL of acetonitrile was added 5 mL of 3 N hydrochloric acid. The solution was stirred at room temperature for 30 min. The solvent was removed under reduced pressure to yield 45 mg (99%) of pure 6-fluorometaraminol as an oil. The product was crystallized as a bitartrate salt by treating it with 40 mg of (+)-D-tartaric acid in 2 mL of boiling methanol for 5 min. Recrystallization from methanol–ethyl acetate gave 65 mg of **4** (mp 155–157 °C, $[\alpha]^{25}_D$ −18.2° (*c* 0.13, methanol)] as the free base: ^{1}H NMR of free base in (CD_3COCD_3) δ 1.2 (3 H, d, 9 Hz), 3.3–4.1 (1 H, m), 5.5 (1 H, br d), 6.5–7.3 (3 H, m), 8.1–8.7 (2 H, br s); ^{19}F NMR (D_2O, CF_3CO_2H internal standard) δ −52.5 (ddd *J* = 10.1, 5.8, and 5.8 Hz). Anal. ($C_9H_{12}FNO_2 \cdot C_4H_6O_6 \cdot H_2O$) C, H, N.

[^{18}F]Fluorometaraminol ([^{18}F]-4). [^{18}F]F_2 (1.3–1.5 Ci/mmol) was passed through an acetyl hypofluorite generator consisting of 1 g of a solid 1:1 complex of KOAc–HOAc until 15 mCi of radioactivity was deposited on the generator.[12] The gaseous effluent from the generator, acetyl hypo[^{18}F]fluorite, was bubbled into a solution of 15.0 mg (0.028 mmol) of 6-AcOHg-*N*-*t*-BOC-metaraminol[2] in 4 mL of CH_2Cl_2 at 0 °C over 5–10 min. The solution was transferred to a silica gel SEP-PAK and eluted with 2 mL of CH_2Cl_2–EtOAc (5:1) and 10 mL of CH_2Cl_2–EtOAc (7:3) while 3-mL fractions were collected. Radioactive heart fractions were combined, and the solvent was removed in vacuo. The residue was dissolved in 4 mL of CH_3CN–6 N HCl (15:1), and the solution was stirred at 20 °C for 10 min. The solvent was

(17) American Medical Association *Drug Evaluations*, 6th ed.; W. B. Saunders; Philadelphia, 1986.
(18) Berridge, M. S.; Crouzel, C.; Comar, D. *J. Labelled Cmpd. Radiopharm.* **1985**, *22*, 687.
(19) Fowler, J. S.; Wolf, A. P. *The Synthesis of Carbon-11, Fluorine-18, and Nitrogen-13 Labeled Radiotracers for Biomedical Applications*; Nuclear Science Series (NAS-NS-3201); Technical Information Center, U.S. Department of Energy, 1982; p 56.
(20) Chiueh, C. C.; Zukowska-Grojec, Z., Kirk, K. L.; Kopin, I. J. *J. Pharmacol. Exp. Ther.* **1983**, *225*, 529.
(21) Ross, S. B. In *The Mechanism of Neuronal and Extraneuronal Transport of Catecholamines*; Paton, D. M., Ed.; Raven: New York, 1976, p 67.
(22) Kline, R. C.; Swanson, D. P.; Wieland, D. M.; Thrall, J. H.; Gross, M. D.; Pitt, B.; Beierwaltes, W. H. *J. Nucl. Med.* **1981**, *22*, 129.

removed under reduced pressure, and the residue was dissolved in 0.05 N sodium acetate buffer, pH 4.5, for injection. Aliquots were removed for radio-TLC analysis on silica gel (CH_2Cl_2–EtOH, 90:10) to detect residual [^{18}F]-3 (R_f of 3 was 0.42, R_f of 4 was 0.00); radio-TLC purity was >99%. See Figure 2 for radio-HPLC purity. Specific activity, which was 500–1200 mCi/mmol, was determined by UV/radio-HPLC.

Tissue Distribution Study. This was performed in two male mongrel dogs (20.1 and 20.4 kg). Animals received a bolus intravenous injection (cephalic vein) of 2 mCi of [^{18}F]-4 in 2.0 mL of sterile 0.150 M acetate buffer (pH 4.5). The dogs were sacrificed 1 h later by rapid iv injection of sodium pentobarbital. Duplicate tissue samples (15–80 mg) of organs were excised, washed free of blood with 0.9% saline solution, blotted dry, quickly weighed, and counted on a Packard 5780 autogamma counter for 1 min. The technique for isolating adrenomedullary tissue has been described previously.[13] To normalize for differences in animal weights, tissue concentrations are expressed in terms of percent kilogram dose per gram (% kg dose/g).[23] Radioactive concentrations in Tables II–IV are decay corrected.

Pharmacological Studies. The selective uptake of [^{18}F]-4 and [^{3}H]NE was inhibited in female Sprague–Dawley rats (Charles River Breeding Laboratories, Inc., Wilmington, MA) weighing 230–290 g, by the ip injection of desmethylimipramine hydrochloride (Revlon Care Group, Tuckahoe, NY), 10 mg/kg.[24] The [^{3}H]NE (*levo*-[7-^{3}H]), specific activity 15–20 Ci/mmol, was obtained from Du Pont NEN, Wilmington, DE. Significance levels were determined by the Student's *t* test.

The adrenergic neurons of the rat heart were impaired in rats (Sprague–Dawley) by the ip injection of 6-hydroxydopamine hydrobromide (Aldrich Chemical Co., Milwaukee, WI) freshly dissolved in physiological saline, 100 mg/kg, 5 days prior to the tracer experiments.[25]

Acknowledgment. We gratefully acknowledge support of this research by the National Institutes of Health (Grants HL 27555 and CA 09015). We thank Dr. Kenneth L. Kirk of the National Institutes of Health for graciously providing us with samples of 2-, 4-, and 6-fluorophenylephrine for ^{19}F NMR analysis, Phillip S. Sherman and Susan J. Fisher for conducting the animal studies, and Linder Markham for preparing the manuscript. We are indebted to Dr. William Kerr for the use of the radiochemical laboratories at the Phoenix Memorial Building, University of Michigan and to the staff of the Cyclotron/PET Facility of the University of Michigan Medical Center for providing ^{18}F.

(23) Kirschner, A. S.; Ice, R. D.; Beierwaltes, W. H. *J. Nucl. Med.* **1975**, *16*, 248.

(24) (a) Axelrod, J. G.; Herttling, G.; Potter, L. *Nature (London)* **1962**, *194*, 297. (b) Daly, J. W.; Creveling, C. R.; Witkop, B. *J. Med. Chem.* **1966**, *9*, 280.

(25) DeChamplain, J.; Nadeau, R. Fed. Proc., *Fed. Am. Soc. Exp. Biol.* **1971**, *30*, 877.

Quantitative Structure–Activity Relationships for the Inhibition of *Escherichia coli* Dihydrofolate Reductase by 5-(Substituted benzyl)-2,4-diaminopyrimidines

Ren-Li Li*[†] and Martin Poe[‡]

Department of Medicinal Chemistry, Beijing Medical University, Beijing, China, and Merck Institute for Therapeutic Research, Rahway, New Jersey 07065-0900. *Received May 11, 1987*

Quantitative structure–activity relationships for the inhibition of *Escherichia coli* (MB 1428) dihydrofolate reductase (DHFR) by 61 5-(substituted benzyl)-2,4-diaminopyrimidines are reported and analyzed. The 61 compounds include 17 congeners whose activities have not been previously reported, five of which have a 5′-substituent larger than a methoxy group. The correlation equations indicated that the molar refractivity (MR) values of the 5′-substituent, just as with the 3′- and 4′-substituents, contributed maximally at the value of 0.79 with no increment of binding for compounds with MR larger than 0.79 (which corresponds to a 5′-methoxy substitution). This experimental result is in agreement with the crystal structure of the *Escherichia coli* DHFR–trimethoprim complex, which shows a reasonably large trimethoprim-binding site. The inhibition of *E. coli* (MB 1428) DHFR by nine of the 17 new benzylpyrimidines is at lower concentrations than for trimethoprim. However, all 17 are much less potent than trimethoprim in inhibition of growth of *E. coli* (1515).

Dihydrofolate reductase (DHFR) plays a crucial role in DNA synthesis; its inhibition can be used to control growth in any organism: animal, plant, insect, or microorganism. By studying DHFR from host and from pathogen, one can establish selectivity in growth inhibition (a therapeutic index) before commencing expensive animal testing.

Trimethoprin [5-(3′,4′,5′-trimethoxybenzyl)-2,4-diaminopyrimidine, 44] is a potent dihydrofolate reductase inhibitor and is widely used as bacteriostatic agent in combination with sulfamethaxazole.[1] Trimethoprim is a selective inhibitor of DHFR. Burchall[2] has shown the variability of the inhibitory power of trimethoprim with DHFR from different microorganisms, as well as mammalian sources. The apparent K_i values in nanomolar obtained for trimethoprim are 1.35 (*Escherichia coli* DHFR),[3] 132 (*Lactobacillus casei* DHFR),[4] 170 000 (human lymphoblast DHFR),[5] and 7900 (bovine liver DHFR).[6] Thus, trimethoprim binds from 60 to 100 000 times more tightly to the bacterial enzymes than to the mammalian enzymes.

Because of the clinical success of the antimicrobial agent trimethoprim, the structure–activity relationships for 5-

[†] Beijing Medical University.
[‡] Merck Institute for Therapeutic Research.

(1) Bushby, S. R. M.; Hitchings, G. H. *Br. J. Pharmacol. Chemother.* **1968**, *33*, 72.
(2) Burchall, J. J. *J. Infect. Dis.* **1973**, *128*, S437.
(3) Dietrich, S. W.; Blaney, J. M.; Reynolds, M. A.; Jow, P. Y. C.; Hansch, C. *J. Med. Chem.* **1980**, *23*, 1205.
(4) Hansch, C.; Li, R. L.; Blaney, J. M.; Langridge, R. *J. Med. Chem.* **1982**, *25*, 777.
(5) Li, R. L.; Hansch, C.; Matthews, D.; Blaney, J. M.; Langridge, R.; Delcamp, T. J.; Susten, S. S.; Freisheim, J. H. *Quant. Struct.-Act. Relat. Pharmacol., Chem. Biol.* **1982**, *1*, 1.
(6) Blaney, J. M.; Dietrich, S. W.; Mark, A. R.; Hansch, C. *J. Med. Chem.* **1979**, *22*, 614.

0022-2623/88/1831-0366$01.50/0