SYNTHETIC STUDIES WITH [^{18}F]p-FLUOROBENZENEDIAZONIUM CHLORIDE
APPLICATION TO THE SYNTHESIS OF A RADIOLABELLED GLUCOCORTICOID: [^{18}F]WIN 44577

Anthony L. Feliu
Department of Neurology
Memorial Sloan-Kettering Cancer Center
1275 York Avenue
New York, NY 10021 USA

Summary

A route was devised to synthesize no-carrier-added or carrier-added [^{18}F]p-fluorophenylhydrazine from aqueous [^{18}F]fluoride. Key reaction in this three-step sequence is the reduction of [^{18}F]p-fluorobenzenediazonium chloride with sodium cyanoborohydride. The utility of this method is demonstrated by the synthesis of [^{18}F]WIN 44577, an N-(p-fluorophenyl)pyrazole-containing corticosteroid, which exhibits exceptionally high in vitro binding affinity for type-II glucocorticoid receptors, and which is currently under evaluation as a possible radioligand for positron emission tomographic studies.

Keywords: corticosteroids, diazonium salts, fluorine-18, p-fluoroaniline, p-fluorophenylhydrazine, sodium cyanoborohydride

Introduction

[^{18}F]Fluoride is often regarded as the favored radioprecursor for synthesizing labelled drugs to study receptor-ligand interactions using positron emission tomography (PET), because the half-life of fluorine-18 (110 min) is compatible with typical in vivo ligand distribution and binding kinetics, and because [^{18}F]-fluoride can be cyclotron-produced in high specific activity using the ^{18}O(p,n) reaction. Accordingly, developing methods to incorporate no-carrier-added (NCA) [^{18}F]fluoride into organic molecules continues to be an area of active research (1).

Early synthetic efforts to prepare fluorine-18-labelled aromatics used either the Balz-Schiemann reaction (thermolytic decomposition of diazonium tetrafluoroborates) or the Wallach reaction (thermolytic decomposition of aryl triazenes), but the resulting products invariably suffered from low radiochemical yield and/or low specific activity. More recently, a nucleophilic aromatic substitution method to introduce fluorine-18 has been demonstrated by Brookhaven chemists, whereby no-carrier-added [^{18}F]fluoride displaces a leaving group on electron deficient benzene rings (2). The yields of [^{18}F]fluorobenzenes are highest for systems "activated" with non-enolizable electron-withdrawing groups (para-cyano and para-nitro), although at least one example has been reported

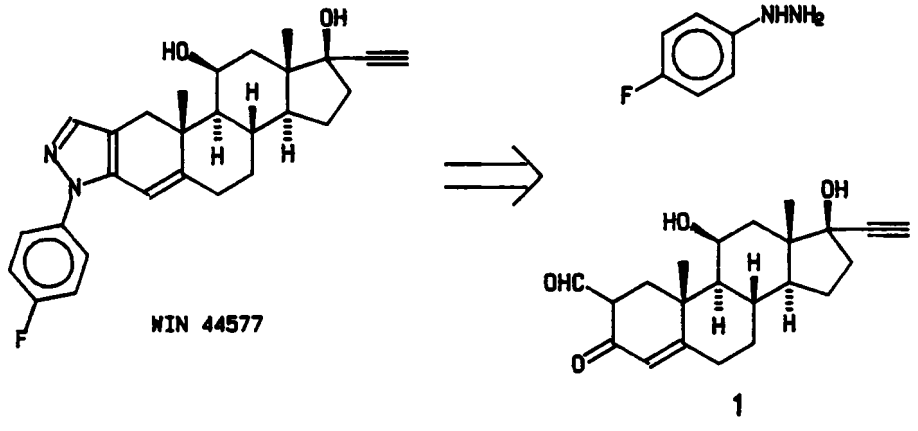
0362–4803/88/111245–10$05.00

Received March 9, 1988
Revised April 11, 1988

using an enolizable group (para-cyclopropylcarbonyl) (3). The nucleophilic aromatic substitution method has been exploited in the synthesis of [^{18}F]butyrophenone neuroleptics, but has yet to be extended to other classes of drugs.

In support of our plans to study cerebral glucocorticoid receptor (GR)-ligand interactions in vivo with PET, we targeted for radiolabelling WIN 44577, a unique, fluorine-containing steroid which exhibits high in vitro GR binding affinity (4,5). Retrosynthetic analysis of WIN 44577 (scheme 1) by cleavage of the pyrazole ring affords steroid component **1** and p-fluorophenylhydrazine. Interest in this drug prompted development of the radiochemical route to [^{18}F]p-fluorophenylhydrazine described in this report.

Scheme 1

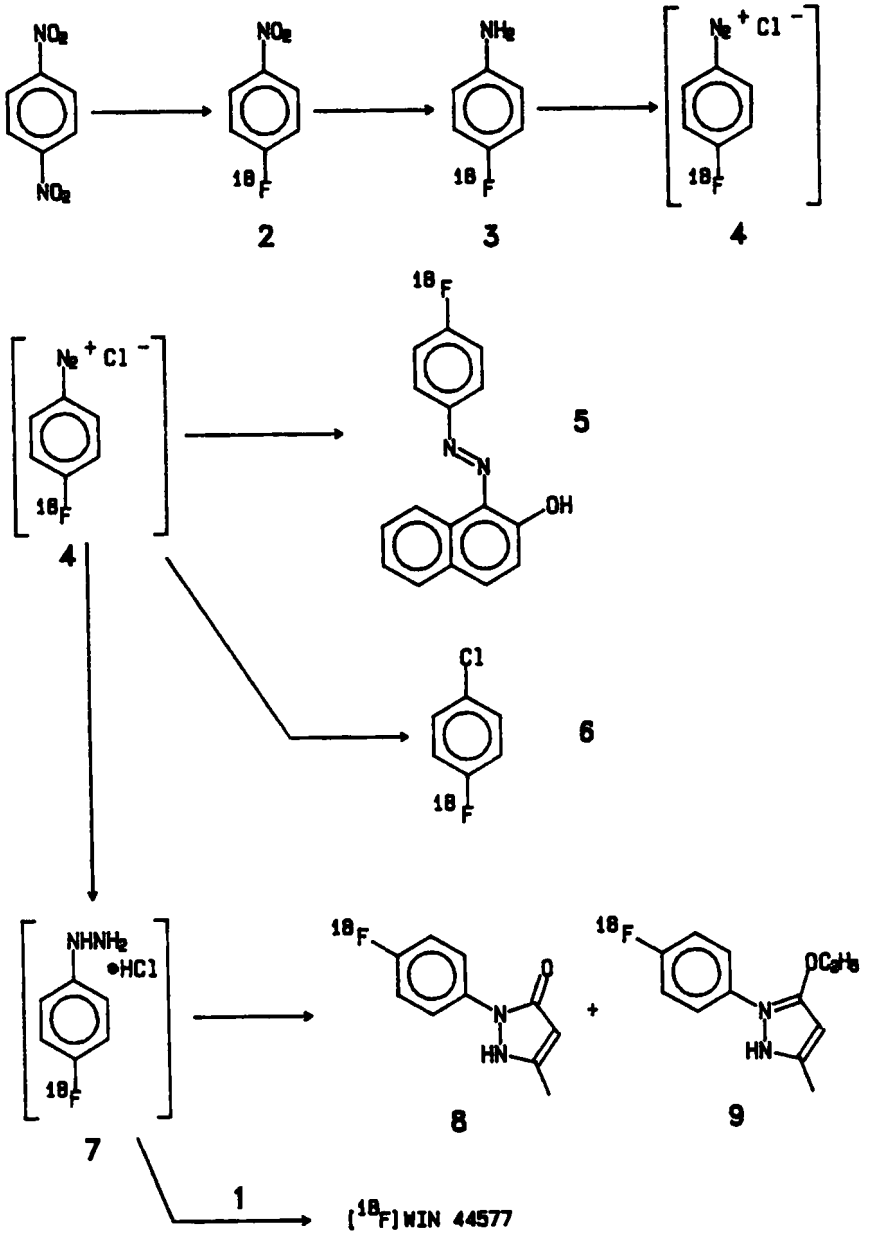


Results

Starting point for the synthetic studies (scheme 2) was NCA [^{18}F]p-fluoronitrobenzene (2), available in high yield from p-dinitrobenzene and NCA [^{18}F]-fluoride via nucleophilic aromatic substitution (2). An aqueous workup for this reaction, employing a Sep-pak C-18 cartridge, was used to wash away the dimethylsulfoxide (DMSO) solvent, which would otherwise consume reducing agent in the next step, since DMSO can readily undergo reduction to dimethylsulfide under mildly reducing conditions.

Conversion of aromatic nitro compounds to anilines is most often accomplished with classical reagents, such as tin and hydrochloric acid. Although this combination successfully reduced the nitro group of **2** at tracer concentrations (Sn, conc. HCl, reflux, 15 min; then alkaline C-18 Sep-pak workup, 85% radiochemical yield), complete separation of the tin salts, which seemed to interfere with the diazotisation, proved to be problematic, because [^{18}F]p-fluoroaniline (3) is poorly retained on a Sep-pak cartridge. Exposing **2** to methanolic sodium boro-

Scheme 2


NO2
NO2
18F
2
NH2
18F
3
N2+ Cl-
18F
4
N2+ Cl-
18F
4
18F
N
N
OH
5
Cl
18F
6
NHNH2
•HCl
18F
7
18F
N
HN
O
8
+
18F
OC2H5
N
HN
9
1
[18F]WIN 44577

hydride and palladised charcoal (6), on the other hand, not only gave **3** in excellent radiochemical yield (>90%), but permitted a simple workup (HCl quench, catalyst filtration, solvent evaporation) and produced only inert byproducts (sodium chloride and boric acid).

Initial experiments tested the feasibility of diazotising **3**, and then compared the reactivity of diazonium salt **4** with macroscale chemistry.

Primary anilines are traditionally detected in organic unknowns by diazotisation with sodium nitrite in hydrochloric acid, followed by quenching with alkaline 2-naphthol, to afford characteristic orange-red azo dyes. Experiments with this sequence readily confirmed the susceptibility of **3** to undergo diazotisation. The radiochemical yield of NCA coupling product **5** (27% from **3**, after preparative radio-HPLC) was modest compared to the same reaction performed cold on a multigram scale (88% crystallized yield), but it illustrated the feasibility of generating diazonium salt **4**. Since coupling product **5** is chemically very distinct from other [^{18}F]labelled intermediates and by-products in the synthesis, it served as a convenient derivative for determining the specific activity of NCA [^{18}F]p-fluoroaniline **3** (and thus established the specific activity of products obtained in subsequent steps). The mass of **5** derived from two individual runs was quantified by HPLC/UV, demonstrating specific activities of 1.3 and 3.0 Curies per micromole at end-of-synthesis.

A second reaction used to evaluate the reactivity of **4** confirmed the earlier results. Thus, exposing diazonium salt **4** to Sandmeyer conditions (CuCl, conc. HCl, 75°, 15 min; extractive workup) afforded [^{18}F]p-chlorofluorobenzene **6**. At NCA level, **6** was produced in 20% radiochemical yield (after flash chromatography), and with carrier (10 µmol) the radiochemical yield of **6** was 47%. By comparison, on a gram scale, a Sandmeyer reaction of p-fluoroaniline afforded p-chlorofluorobenzene in 66% yield after distillation (7).

Having demonstrated the intermediacy of fluorine-18-labelled diazonium salt **4** with two familiar reactions, attention was turned to the synthesis of [^{18}F]p-fluorophenylhydrazine **7**.

Arylhydrazines are usually prepared by reducing diazonium salts with excess sodium sulfite, a method discovered by Emil Fischer. On a gram scale, this method gave a good yield of p-fluorophenylhydrazine hydrochloride from p-fluoroaniline (72%; literature (8) 74%). Unfortunately, at tracer concentrations, classical Fischer-style conditions proved unworkable. Difficulties encountered when scaling down the reaction included facile air oxidation of the hydrazine free base during workup, pH dependence of the reduction, and a relatively long reaction time. Thus, after considerable experimentation, this method had to be abandoned.

Hydrazines are formally derived from diazonium salts by hydride reduction, so this possibility was considered next as an alternative to the Fischer method. Even though such a notion proposed the juxtaposition of apparently incompatible

chemical species-- a diazonium salt stable only in strongly acidic media, and a hydride reagent quenched by acid-- it was reasoned that, under appropriate conditions, the powerful electrophile Ar-N≡N$^+$ would compete successfully with hydronium ions for hydride. Indeed, when powdered sodium cyanoborohydride (9) was added to an ice-cold solution of p-fluorobenzenediazonium chloride, a tan solid separated as gas evolution occurred. Residual cyanoborohydride was decomposed by gently heating the mixture until gas evolution ceased. Finally, the solid was filtered and then reprecipitated from hydrochloric acid to afford the hydrazine hydrochloride in 42% yield.

The diazotisation/cyanoborohydride sequence proved ideal for radiotracer synthesis (**3** → **4** → **7**) because excess reducing agent rapidly decomposed in the acidic reaction medium. In this way, [^{18}F]p-fluorophenylhydrazine **7** was made available for subsequent chemistry without the risk of air oxidation during workup. To quantify the yield of this novel method, **7** was derivatised in situ with ethyl acetoacetate, affording a good yield of air-stable heterocycles **8** and **9** (3:1 product ratio, combined radiochemical yield from **3** after workup with Sep-pak and preparative radio-HPLC purification: 22% NCA, 53% CA). The observed product ratio for **8** to **9** was pH dependent, but, as this was only a model system, the yield of **7** was simply regarded as the sum of the individual yields of **8** and **9**.

Finally, the practical utility of the diazotisation/cyanoborohydride reduction route to [^{18}F]p-fluorophenylhydrazine **7** was illustrated by synthesis of NCA [^{18}F]WIN 44577. To effect the pyrazole-forming condensation (**7** + **1** → WIN, Scheme 2), the reaction mixture containing **7** was first buffered to pH 4-4.5 with sodium acetate, and then β-ketoaldehyde precursor **1** was added as an ethanolic solution. It is apparent that the desired WIN compound is one of two possible regioisomeric [3,2-c]pyrazoles which could form during this condensation reaction, and in fact, production of both such regioisomers was noted in a related steroid system (10). Radio-HPLC analysis of the (**7** + **1**)-condensation reaction mixtures consistently showed two steroid-derived species in a 2:1 ratio. The major species corresponded to WIN, while the minor one (completely separable from WIN, but not characterized) was presumed to be the undesired regioisomer. After the usual Sep-pak workup and preparative radio-HPLC purification, [^{18}F]WIN 44577 was isolated from the condensation reaction in 11% radiochemical yield (based on [^{18}F]p-fluoroaniline **3**). Overall, from [^{18}F]fluoride, NCA [^{18}F]WIN 44577 could be synthesized in approximately 6% radiochemical yield in 100 min from start-of-synthesis.

Metabolic studies to evaluate the potential utility of this radiotracer for PET are underway, and the results will be reported in due course.

Experimental

General: Analytical HPLC was conducted on a 4.6x250 mm 5μ Spherisorb ODS-2 column with various acetonitrile-buffer (0.2 M acetic acid, 0.05 M sodium acetate) or acetonitrile-water mixtures flowing at 1 mL/min. Peaks were detected

with UV (254 nm) and/or radioactivity (solid scintillator) flow detectors. Preparative radio-HPLC purifications of **5**, **8**, **9**, and WIN were performed on a 10x250 mm 5μ Spherisorb ODS-2 column as specified in the individual writeups.

Representative analytical HPLC data expressed as capacity factors (k') are as follows: with 65:35 acetonitrile-water, **2**, 2.4; **3**, 2.6; **5**, 13.3; **6**, 3.5; WIN, 6.7; with 55:45 acetonitrile-buffer, **2**, 4.0; **3**, 2.4; **6**, 6.4; **8**, 2.6; **9**, 7.5.

Disposable Sep-pak C-18 cartridges (Waters Associates, Milford, MA) were activated with ethanol (3-5 mL) and water (5 mL) prior to use. All radiochemical yields are decay corrected. The identity of radioactive compounds was confirmed by coelution of UV-absorbing and radioactive peaks with cold standards. All non-radioactive compounds synthesized in preparative-scale reactions were characterized by IR and proton-NMR spectroscopy.

11β,17β-Dihydroxy-17α-ethynyl-2-formyl-androst-4-en-3-one (**1**): This steroidal precursor was synthesized in six steps from cortisone 21-acetate using standard chemistry as outlined in scheme 3, and the reader is referred to the caption for experimental details and references.

[^{18}F]p-Fluoroaniline (F-18 → **2** → **3**): [^{18}F]Fluoride (15-35 mCi, generated by the ^{18}O(p,n) reaction on 95⁺ atom-% [^{18}O]water) was delivered into a borosilicate glass vessel containing tetramethylammonium hydroxide (1 μmol). The solution was evaporated to dryness at 120° under a stream of nitrogen, and further dried by coevaporation with acetonitrile (3x0.3 mL). p-Dinitrobenzene (1.5 mg) in dry DMSO (0.3 mL) was added to the dried [^{18}F]tetramethylammonium fluoride residue. The vessel was covered, and heating was continued for 10 min. After cooling, the reaction mixture was diluted with water (3 mL), passed through a Sep-pak C-18 cartridge, and the cartridge was rinsed with water (2x2 mL). [^{18}F]p-Fluoronitrobenzene [**2**, radiochemical yield from [^{18}F]fluoride: 63-85% (n>10) in 20 min] was eluted from the cartridge with methanol (2x0.75 mL), collected in a test tube containing 10% palladium-on-carbon (1 mg), and treated with sodium borohydride (7 mg). After 10 min, the mixture was quenched with conc. hydrochloric acid (0.2 mL), filtered through cotton, and evaporated in vacuo, leaving a solid residue containing [^{18}F]p-fluoroanilinium hydrochloride **3** [radiochemical yield from **2**: 90%⁺ (n>10) in 25 min], plus inorganic salts, ready for subsequent chemistry.

[^{18}F]1-(p-Fluorophenylazo)-2-naphthol (**3** → **4** → **5**): The residue containing **3** was dissolved in 3 M hydrochloric acid (0.4 mL), cooled to 0°, and treated with cold sodium nitrite solution (1.5 mg/0.1 mL water). After 30 sec, the diazonium solution was transferred rapidly dropwise to an ice-cold solution of 2-naphthol (5 mg) in 3 M sodium hydroxide (1 mL). Next, the reaction mixture was diluted with water (1 mL), passed through a Sep-pak C-18 cartridge, and the cartridge was rinsed with 0.25 M sodium hydroxide (1 mL). The crude coupling product was eluted from the cartridge with dichloromethane (2x1.5 mL), dried with potassium carbonate, and evaporated. The dark residue was subjected to prep-HPLC (75:25 acetonitrile-water @ 4 mL/min, retention time 12 min), affording pure [^{18}F]coupl-

ing product **5** [average radiochemical yield from **3**: 27% (n=5, range 26-29%), 15 min reaction/workup plus 20 min for prep-HPLC].

1-(p-Fluorophenylazo)-2-naphthol: A mixture of p-fluoroaniline (3.0 g, 27.1 mmol) in 6 N hydrochloric acid (12 mL) cooled to -5° was treated dropwise over 20 min with ice-cold sodium nitrite solution (2.11 g, 30.6 mmol, in 6 mL water). The resulting diazonium salt solution was next transferred dropwise to an ice-cold solution of 2-naphthol (4.25 g, 29.5 mmol) in 1 N NaOH (40 mL). A voluminous red-orange precipitate formed at once. The reaction mixture was filtered; the filter cake was washed with dilute sodium hydroxide and water, and then dried in vacuo. The solid was recrystallized from absolute ethanol (≈250 mL) to afford the coupling product as fine red needles [6.37 g, 88%].

[^{18}F]p-Chlorofluorobenzene (**3** → **4** → **6**): The residue containing **3** (plus 1 µL cold p-fluoroaniline in carrier-added experiment) was dissolved in 4 M hydrochloric acid (0.4 mL), cooled to 0°, and treated with cold sodium nitrite solution (1.5 mg/0.1 mL water). After 30 sec, the diazonium solution was transferred into a hot (75°) solution of freshly-prepared (11) copper(I) chloride (10 mg) in conc. hydrochloric acid (0.5 mL). The reaction was continued at 75° for 15 min. Next, the reaction mixture was diluted with water (3 mL), passed through a Sep-pak C-18 cartridge, and the cartridge was rinsed with water (2 mL). Crude **6** was eluted from the cartridge with dichloromethane, dried with potassium carbonate, and flash chromatographed (silica gel, 11x100 mm, dichloromethane eluant) to give [^{18}F]p-chlorofluorobenzene **6** [from **3**: 20% NCA, 47% CA radiochemical yield, in 40 min].

p-Chlorofluorobenzene: A solution of p-fluorobenzenediazonium chloride (18.1 mmol, prepared under standard conditions at 0°) in 6 M HCl (12 mL) was added rapidly dropwise to hot (75°), freshly-prepared (10) copper(I) chloride (27 mmol, in 8 mL 6 M HCl). Heating was continued for 5 min after the end of addition. Next, after cooling, the dark mixture was partially neutralized with saturated sodium bicarbonate (40 mL), and extracted with dichloromethane (40 mL). The extract was dried with potassium carbonate, evaporated in vacuo, and bulb-to-bulb distilled in vacuo to give pure p-chlorofluorobenzene [1.54 g, 66% yield].

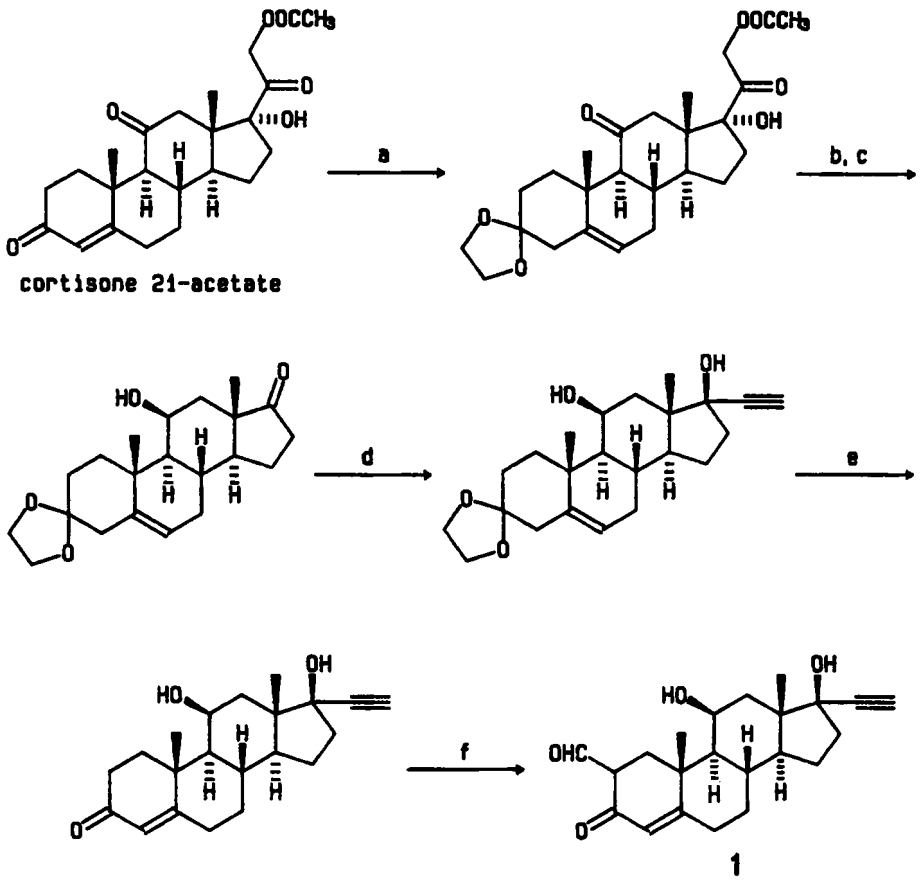
[^{18}F]p-Fluorophenylhydrazine, [^{18}F]1-(p-fluorophenyl)-3-methyl-(2H)pyrazol-5-one, and [^{18}F]1-(p-fluorophenyl)-3-methyl-5-ethoxy-(2H)pyrazole (**3** → **4** → **7** → **8** + **9**): The residue containing **3** (plus 1 µL cold p-fluoroaniline in carrier-added experiments) was dissolved in 4 M hydrochloric acid (0.4 mL), cooled to 0°, and treated with cold sodium nitrite solution (1.5 mg/0.1 mL water). After 30 sec, sodium cyanoborohydride (7 mg powder) was added. The flask was removed from the ice bath. Gas evolution could be observed as the excess cyanoborohydride underwent protonolysis. After about 1 min, the reaction was immersed in a 75° bath (gas evolution quickly ceased). To derivatise **7** in situ, the reaction mixture was brought to room temperature, ethyl acetoacetate (2-3 drops) was added, and heating was resumed for 10 min. After cooling, the reaction mixture was neutral-

ized (pH=7.0±0.5) with disodium phosphate (200mg/2 mL water) plus 1 M sodium hydroxide (1-1.5 mL), passed through a C-18 Sep-pak, and the cartridge was further rinsed with water (2 mL). The crude product mixture, **8** + **9**, was eluted from the cartridge with dichloromethane, dried with sodium sulfate, and evaporated. This residue was subjected to prep radio-HPLC (55:45 acetonitrile-buffer @ 3 mL/min, retention times for **8**, 6 min and **9**, 16 min), giving fractions containing pure **8** and **9** in 3:1 ratio [average radiochemical yield of **8+9** from **3**: 21% NCA (n=3, range 20-23%), 53% CA (n=2, range 48-57%), 40 min reaction/workup plus 20 min for prep-HPLC].

p-Fluorophenylhydrazine hydrochloride: A well-stirred solution of p-fluorobenzenediazonium chloride (23.2 mmol, prepared under standard conditions at 0°) in 4 M HCl (20 mL) was treated portionwise with powdered sodium cyanoborohydride (1.50 g, 23.9 mmol) over ten min. Considerable gas evolution occurred and a dark flock formed during the addition. The reaction was allowed to continue 1 hr at room temperature, and then 1.25 hr at 70°, by which time gas evolution ceased. After cooling to room temperature, the reaction mixture was diluted with 6 M HCl (10 mL) and further cooled in ice. The solid was filtered, washed with 6 M HCl (3 x 7 mL), and air dried, leaving crude p-fluorophenylhydrazine hydrochloride (2.68g) as a light brown powder. This crude material was purified by dissolution in 30 mL warm water, reprecipitation with conc HCl (4 mL), cooling to 0°, filtration, and drying (off-white powder, 1.60g, 42%, homogeneous by HPLC).

[^{18}F]WIN 44577 (**3** → **4** → **7** → WIN): The residue containing **3** was dissolved in 4 M hydrochloric acid (0.4 mL), cooled to 0°, and treated with cold sodium nitrite solution (1.5 mg/0.1 mL water). After 30 sec, sodium cyanoborohydride (7 mg powder) was added. The flask was removed from the ice bath. Gas evolution could be observed as the excess cyanoborohydride underwent protonolysis. After about 1 min, the reaction was immersed in a 75° bath and heating was continued until gas evolution ceased (1-2 min). The reddish brown solution containing [^{18}F]p-fluorophenylhydrazine **7** was buffered to pH 4-4.5 with sodium acetate (150 mg/0.3 mL water) [Note: pH control at this step was essential for a successful condensation reaction], ß-ketoaldehyde **1** (4 mg/0.5 mL EtOH) was added, and the mixture was heated at 75° for 10 min. After cooling, the reaction was diluted with water (3 mL), passed through a Sep-pak C-18 cartridge, and the cartridge was further rinsed with water (2 mL). The crude NCA product was eluted from the cartridge with dichloromethane, dried with sodium sulfate, and evaporated. This residue was subjected to prep radio-HPLC (65:35 acetonitrile-water @ 4 mL/min, retention time 11 min) to afford radiochemically pure [^{18}F]WIN 44577 [average radiochemical yield from **3**: 11% (n=3, range 9-14%), 40 min reaction/workup plus 20 min for prep-HPLC].

Scheme 3



a: ethylene glycol, tosic acid, 80°, distill glycol/water azeotrope in vacuo, 97%

b: sodium borohydride, sodium hydroxide, ethanol, reflux, 4.5 hr

c: sodium periodate, aqueous ethanol, room temp, 4.5 hr, steps b+c 65%

d: lithium acetylide/ethylenediamine complex, DMSO, room temp, 1 hr, 91%

e: 1 part 1 M HCl to 5 parts acetone, room temp, 18 hr, 85%

f: ethyl formate, sodium methoxide, pyridine, room temp, 3 hr, 85%

REFERENCES: steps a/b/c: 12; step d: compare 13; step f: 14

Acknowledgements

Financial support of this work was provided by the NINCDS under grant NS-23473.

The author is indebted to Dr. Albert E. Soria, Sterling-Winthrop Research Institute, Rensselaer, NY, for providing a sample of WIN 44577.

This work was presented in part at the Seventh International Symposium on Radiopharmaceutical Chemistry, Groningen, the Netherlands, July 1988.

References

1. Berridge, M.S.; Tewson, T.J. - Appl. Radiat. Isot. 37: 685-693 (1986).

2. Attina, M.; Cacace, F.; Wolf, A.P. - J. Label. Comp. Radiopharm. XX: 501-514 (1983).

3. Shiue, C.Y.; Fowler, J.S.; Wolf, A.P.; McPherson, D.W.; Arnett, C.D.; Zecca, L. - J. Nucl. Med. 27: 226-234 (1986).

4. Winneker, R.C.; Russell, M.M.; Might, C.K.; Schane, H.P. - Steroids, 44: 447-457 (1984).

5. McEwen, B.S. - unpublished results (Rockefeller University).

6. Neilson, T.; Wood, H.C.S.; Wylie, A.G. - J. Chem. Soc. 371, (1962).

7. Wallach, O.; Heusler, Fr. - Liebigs Ann. 243: 219-244 (1888).

8. Schiemann, G.; Winkelmüller, W. - Ber. dtsch. chem. Gesel. 66B: 727-732 (1933).

9. Lane, C.F. - Synthesis, 135-146 (1975).

10. Hirschmann, R.; Buchschacher, P.; Steinberg, N.G.; Fried, J.H.; Ellis, R.; Kent, G.J.; Tishler, M. - J. Amer. Chem. Soc., 86: 1520-1527 (1964).

11. Furniss, B.S.; Hannaford, A.J.; Rogers, V.; Smith, P.W.G; Tatchell, A.R., eds. - Vogel's Textbook for Practical Organic Chemistry, Longman Group, New York 1978, p. 287.

12. Liston, A.J.; Howarth, M. - J. Org. Chem., 32: 1034-1041 (1967).

13. Baran, J.S. - J. Med. Chem., 10: 1188-1190 (1967).

14. Stonner, F.W. - U.S. Patent 3,539,556 (1970).