Synthesis of Positron Labeled Photoactive Compounds: ^{18}F Labeled Aryl Azides for Positron Labeling of Biochemical Molecules

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We have prepared various [^{18}F]fluorine labeled aryl azides as novel photoactive compounds suitable for positron labeling of biochemical molecules.

The introduction of fluorine substituents to aryl azides can be expected to have dramatic effects on their nature and reactivity toward photolysis.[1-3] Positron labeled reagents for labeling proteins or peptides have recently attracted considerable attention due to their wide applicability in biochemistry and positron emission tomography (PET).[4-7]

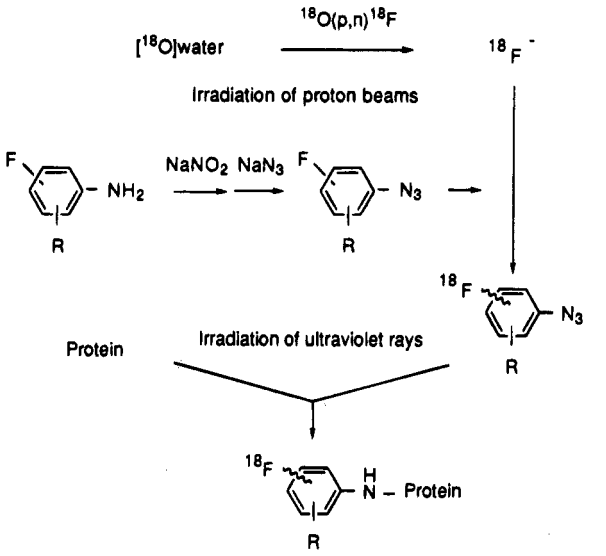
Various radiolabeled azide compounds are often used in biochemistry for radiolabeling biological molecules by photolysis,[8-12] but there have been no reports on the preparation or use of fluorine-18 labeled azides. We now report a novel synthesis of ^{18}F-labeled aryl azides which will have wide application in biochemistry and nuclear medicine as a means for ^{18}F-fluorine labeling for proteins, peptides, and nucleic acids.[13]

[^{18}F]Fluorine has one of the longest half-lives (110 min) among all positron emitters and it is eagerly desired to label biochemical target molecules with it for studies of human brain and cardiovascular functions by PET. However, the target molecules generally cannot be labeled directly by [^{18}F]fluoride due to their instability at the high temperatures required for the reaction. Several methods are known for radiolabeling proteins.[9-12] For example, some radiolabeled active esters, such as *N*-hydroxysuccinimide esters, have been prepared by multistep preparation procedures.[11] However, these synthesis procedures are too complicated to use routinely.

One-step synthesis of ^{18}F labeled active esters, using exchange reactions with [^{18}F]fluoride, seems to be rather difficult due to the thermoinstability of the active esters, with the result that nobody has reported a synthesis with practically useful yields.

To overcome these problems we investigated the use of fluoro aromatic azide compounds that are unstable to light but quite thermostable even at high temperatures.

Scheme 1



We anticipated that fluoroaryl azides could be labeled by a one-step ^{18}F-for-^{19}F isotopic exchange reaction combined with a single-column HPLC purification.

Various single photon radiolabeled phenyl azide derivatives have been known and used for labeling biochemical molecules such as proteins, peptides, and nucleic acids. However, no positron labeled azide compounds have been reported. We therefore, pursued a novel one-step positron labeling of several azide compounds with [^{18}F]fluoride. A precursor for such ^{18}F labeled azides has to have at least one leaving group and one electron-withdrawing group for activation as well as the azide group. Furthermore, we confirmed that for the [^{18}F]fluorination of *o*-fluoronitrobenzene the F-for-F (F–F) isotopic exchange reaction predominates by far over the F-for-nitro (F–NO_2) exchange.[14] Thus the fluoro group is the leaving group of choice in the presence of a nitro group for activation. We synthesized several precursor molecules with an azide group for binding to biochemical molecules, and fluorine or nitro groups to take the roles of the leaving group and electron-withdrawing group, respectively [e.g., 3,4-difluorophenyl azide (3,4-DFA, 53%), 3,5-difluorophenyl azide (3,5-DFA, 53%), 2,4,6-trifluorophenyl azide (2,4,6-TFA, 55%), and pentafluorophenyl azide (PFA, 59%)].

4-Fluorophenyl azide (4-FA), 4-fluoro-3-nitrophenyl azide (FNA), 3,4-DFA, and 3,5-DFA were prepared from the corresponding aniline derivatives,[1,2] while 2,4,6-TFA and PFA were prepared by a modification of Leyva's method.[3]

FNA was used as a precursor possessing a nitro group, and 4-FA (prepared from 4-fluoroaniline, yield: 65%) was used as a precursor without an activating group.

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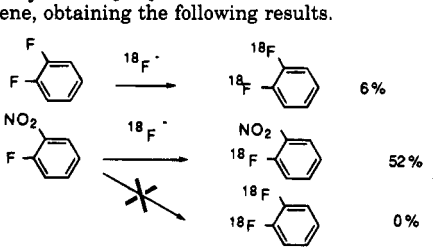
(14) An azide group is neutral electronically and does not activate a leaving group. We investigated preliminarily the reactivity of several substituted precursors which have no azide group in the exchange reaction with fluoride. We compared both the products and their radiochemical yields of [^{18}F]fluorination of difluorobenzene and fluoronitrobenzene, obtaining the following results.



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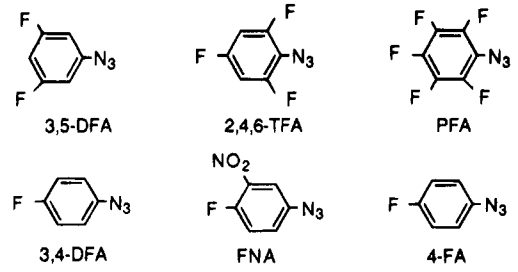
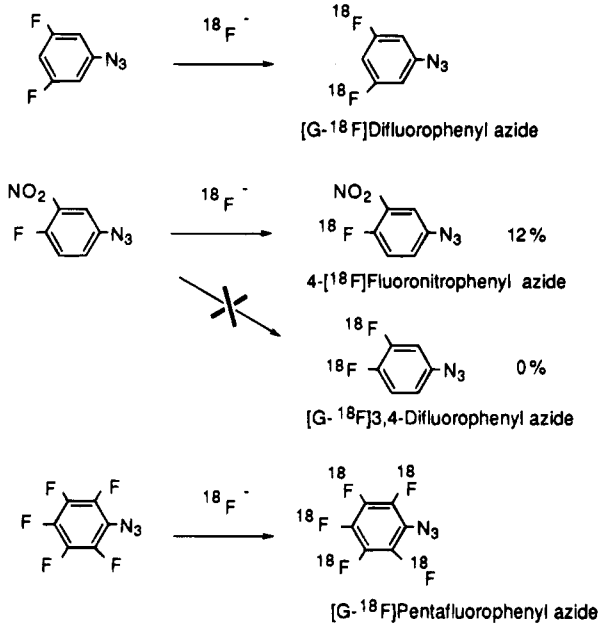


The stability of aryl azides at high temperature was confirmed by measuring the decrease in purity of 3,5-DFA and FNA at 170 °C in DMSO as a function of time (Table 1). These results show that the phenyl azides are quite thermostable.



Then, the precursor phenyl azides were used for F-for-F isotopic exchange (Table 2). The synthetic apparatus was composed of two operating units; one for the F-for-F isotopic exchange reaction and the other for HPLC purification.[15] The reactor vessel, which was shielded from light, was made of glassy-carbon and tightly sealed with a molded polyether ether ketone (PEEK) block. The results in Table 2 show that the desired ^{18}F-labeled phenyl azides were obtained in high purity using the one-step reaction which does not require skilled hands. The precursor 4-FA did not undergo F-for-F exchange due to the lack of any activating group. A fluorine atom at positions ortho to a nitro group is known to be reactive with an amino group. Therefore, [^{18}F]FNA may be less suitable as the ^{18}F-labeling reagent.

To assess the ability of the radiolabeled azides to bind with protein in aqueous media, an aliquot (3 mL) of a 2×10^{-4} M solution of cytochrome C (horse heart) was mixed in a Pyrex tube with DMSO (0.5 mL) containing [G-^{18}F]3,5-DFA, and photolysis was carried out with UV light (8 W, 252 nm, 2 h). Protein assay was conducted by size exclusion chromatography [detection: 409 nm (cytochrome C λ_{max}), flow rate: 1 mL/min, TSKgel G3000SW (Tosoh Corporation, Tokyo)] with 0.05 M phosphate buffer (pH 7.5) as the eluent. The macromolecule fraction was obtained (retention time: 5.8 min) with radioactivity. The radiochemical yield was 6.7% (0.6 μCi of the macromolecule fraction was obtained from a part (9 μCi) of the reaction solution). The macromolecule fraction, separated from a similar reaction mixture of DFA and cytochrome C by size exclusion chromatography (Sephadex G-25 with 0.05 M phosphate buffer at pH 7.5) showed a strong ^{19}F-NMR signal (−118.3 ppm) that proved attachment of the photoprobe to cytochrome C. Total reaction time was 175 min (^{18}F-azide synthesis, 55 min; protein coupling, 120 min).

We are now labeling several other proteins, and peptides, using our fluorine-18 labeling system, in ordre to verify that our present method, using [^{18}F]fluoro aromatic azides, can be used for the study of such molecules in vivo either with biochemical or PET techniques.

Table 1. Stability of Phenyl Azides in DMSO at 170 °C with Base[a]

	purity (%)			purity (%)	
time (min)	FNA	3,5-DFA	time (min)	FNA	3,5-DFA
0	100	100	20	99.3	99.2
10	100	99.7	60	97.2	95.9

[a] Each solution of FNA (18 mg) and 3,5-DFA (15 mg) in DMSO (0.5 mL) containing potassium carbonate (2 mg) and potassium oxalate (0.1 mg) was heated at 170 °C for 10−60 min. Aliquots were withdrawn at each time intervals and analyzed with HPLC for the remaining starting material. [Eluent: acetonitrile:water = 55:45 (v/v), column: Asahipak ODP-5, 4.6 mm × 150 mm (Shoko Co., Ltd, Tokyo), detection: UV 250 nm (DFA λ_{max})].

Table 2. Synthesis of Fluorophenyl Azides Using [^{18}F]Fluoride[a]

entry	precursor	radiochem yield (%)	radiochem purity (%)[b]	chem purity (%)[b]
1	3,5-DFA	11	>99	>99
2	2,4,6-TFA	6	>99	>99
3	2,3,4,5,6-PFA	2	>99	>99
4	3,4-DFA	1	>99	>99
5	FNA	12	>99	>99
6	4-FA	0	–	–

[a] Reagents and conditions: [^{18}F]Fluoride was obtained by irradiating (5-30 min, 15 μA) [^{18}O]water (10−99%) with 18 MeV protons [nuclear reaction: ^{18}O(p, n)^{18}F] generated with an azimuthaly-varying-field cyclotron (Cypris HM-18, Sumitomo Heavy Industries Ltd., Tokyo). The whole irradiated water (2.4 g) was recovered in a glassy carbon-vessel containing cryptand[2.2.2] (Merck) (15 mg), potassium oxalate (2 mg), potassium carbonate (0.03 mg), and dry acetonitrile (2 mL) and then evaporated to dryness at 105 °C under a low pressure (<2 kPa) in 10 min. A DMSO (0.5 mL; distilled before use) solution containing the precursor (0.1 mmol) was added to the residue and the mixture heated at 160 °C for 20 min. Total synthesis time was 70−80 min. No carrier [^{19}F]fluoride ion was added. The whole [^{18}F]labeling reaction mixture was injected onto the preparative HPLC column [Eluent : MeCN:10 mM NaOH = 50:50 (v/v), Asahipak ODP-50 (Shoko Co. Ltd., Tokyo), 21.5 mm × 250 mm + guard column 21.5 mm × 100 mm, flow rate 10 mL/min, positron monitor : TCS-R81 (Aloka Co. Ltd., Tokyo)] to isolate the labeled compound. [b] Analytical HPLC [Eluent: acetonitrile:water = 50:50 (v/v), Asahipak ODP-5, 4.6 mm × 150 mm, flow rate 0.8 mL/min] was used to check the radiochemical and chemical purities of the purified hot products, using both UV (241 nm) and radioisotope detectors [RLC-700 (Aloka Co. Ltd., Tokyo)].

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