AN EFFICIENT, ONE-POT SYNTHESIS OF 2-DEOXY-2-[^{18}F]FLUORO-ACETAMIDO-D-GLUCOPYRANOSE (N-[^{18}F]FLUOROACETYL-D-GLUCOSAMINE), POTENTIAL DIAGNOSTIC IMAGING AGENT

Masao Tada,[1*] Atsushi Oikawa,[1] Ren Iwata,[2] Takehiko Fujiwara,[1] Kazuo Kubota,[1] Taiju Matsuzawa,[1] Hiroshi Sugiyama,[3] Tatsuo Ido,[2] Kiichi Ishiwata.[2] and Tachio Sato[1]

[1]Research Institute for Tuberculosis and Cancer, Tohoku University, 4-1 Seiryomachi, Sendai 980, Japan

[2]Cyclotron and Radioisotope Center, Tohoku University, Aramaki-aza-aoba, Sendai 980, Japan

[3]Chemical Research Institute of Non-Aqueous Solutions, Tohoku University, 2-1-1 Katahira, Sendai 980, Japan

SUMMARY

A rapid, one-pot synthesis of 2-deoxy-2-[^{18}F]fluoroacetamido-D-glucopyranose (N-[^{18}F]fluoroacetyl-D-glucosamine) (1) starting from [^{18}F]fluoride and ethyl bromoacetate is described. The synthesis was accomplished by a combination of halogen exchange, alkaline hydrolysis, and condensation. [^{18}F]Fluoride was produced by the ^{18}O (p, n) ^{18}F nuclear reaction using the cyclotron. The total synthesis time, the radiochemical yield, and purity of (1) are ca. 90 min, ca. 9.1%, and >98%, respectively. Sugar (1) showed the diagnostic tumor-imaging activity.

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*To whom correspondence should be addressed.

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INTRODUCTION

In previous papers (1-3), we have reported on the syntheses of some 2-deoxy-2-[^{18}F]fluorohexopyranoses and [1-^{11}C]-hexopyranoses. 2-Amino-2-deoxyhexopyranoses are of wide spread occurence in nature existing as their N-acetylated derivatives in various polysaccharides and mucopolysaccharides of microbial and animal origin, where they play an important role in physiological process, and also in a number of antibiotics (4). The simultaneous incorporation of 2-acetamido-2-deoxy- and 2-deoxy-2-fluoroacetamido-D-glucopyranose into hyaluronic acid (one of mucopolysaccharides) by mammalian cells has been reported (5). Toole et al. reported that hyaluronate concentrations were most elevated in the connective tissue interface between the tumor mass and the neighboring host tissue (6).

As part of the synthetic study of hexopyranoses labelled with positron emitting radionuclides, the synthesis of 2-deoxy-2-[^{18}F]fluoroacetamido-D-glucopyranose (1), potential diagnostic imaging agent, will be reported here in detail.

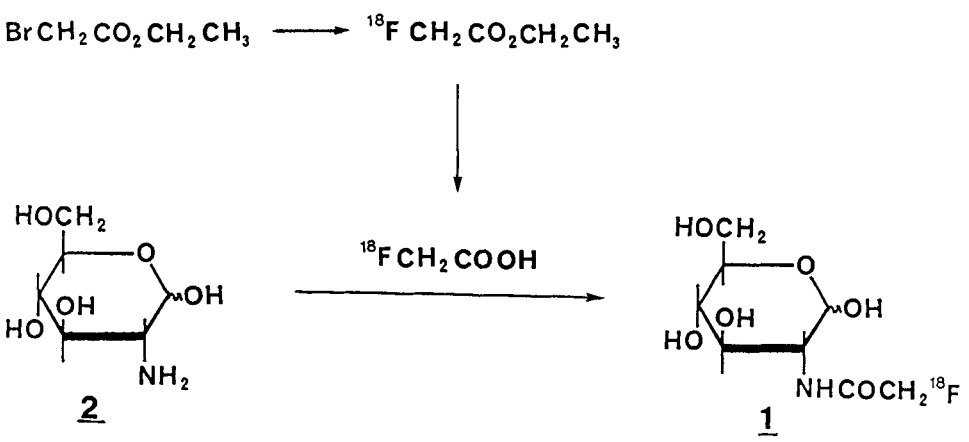
RESULTS AND DISCUSSION

Nonradioactive 2-deoxy-2-fluoroacetamido-D-glucopyranose has been synthesized from tetra-O-acetyl-2-amino-2-deoxy-D-glucopyranose (7-10) or unprotected 2-amino-2-deoxy-D-glucopyranose (2)(8) with fluoroacetic acid. The small scale preparation of fluoro[1-^{14}C]acetic acid from chloro[1-^{14}C]acetic acid via methyl ester has been reported (11). Recently, Block et al. have reported the nucleophilic ^{18}F-exchange

reaction in α-substituted acid esters and the no-carrier-added [^{18}F]fluoroacylation via fluorocarboxylic acid esters (12). We have adapted these methods to prepare 2-deoxy-2-[^{18}F]-fluoroacetamido-D-glucopyranose (1) with some modifications.

The unprotected amino sugar condenses smoothly with acylamino acid (13) or fluoroacetic acid (8) in the presence of dicyclohexylcarbodiimide for a day at room temperature. To shorten the reaction time is prerequisite for the synthesis of compounds labelled with a short-lived radionuclide (^{18}F: β^+ decay, $t_{1/2}$=110 min). We found that heating (2) with fluoroacetic acid at 82°C for 20 min in the presence of the carbodiimide gave the desired sugar in ca. 50% yield. Analyses with high performance liquid chromatography (HPLC) and thin-layer chromatography (TLC) confirmed that the coupling time of 20 min was adequate. This change in reaction temperature brought about a considerable reduction of time required.

Ethyl bromoacetate and potassium fluoride were heated for 10 min in the presence of 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosan (Kryptofix 222). After addition of aqueous alkali, the resulting mixture was further heated for 2 min and a mixture of hydrochloride of (2) and dicyclohexylcarbodiimide was then added to the mixture, which was heated for 20 min. The temperature of the reactor was maintained at ca. 82°C during a period of all reactions. After removal of an excess of the carbodiimide , 2-deoxy-2-fluoroacetamido-D-glucopyranose was obtained by HPLC technique in an 18% yield. Methyl bromoacetate was also treated with the aboved manner to afford the sugar in a 10% yield, and the same influence of ester components in bromoacetic acid esters was reported (12). An efficient, one-pot synthesis of 2-deoxy-2-fluoroacetamido-D-glucopyranose has been established in this way and the radiolabelled run has been carried out.



[^{18}F]Fluoride was produced via the ^{18}O (p, n) ^{18}F nuclear reaction with a circulating 20% enriched [^{18}O]water target using the Tohoku University Cyclotron (14). The ^{18}F nuclide thereby formed was converted into potassium [^{18}F]fluoride with potassium carbonate. After addition of Kryptofix 222, the resulting mixture was submitted to the one-pot synthesis to afford the desired sugar (1) in ca. 9.1% radiochemical yield.

Compound (1) appeared to have good potential as a clinical tumor seeking agent (15), and the medical uses of (1) are being further investigated.

EXPERIMENTAL

Kryptofix 222 and TLC plates were purchased from E. Merck AG. Bromoacetic acid esters were from Wako Chemical Ltd. and distilled under a reduced pressure. The other reagents were obtained commercially (Wako) and used without further purification. The purity of each compound was always checked by TLC. HPLC analyses were carried out either with a Waters Assoc. model 6000 equipped with a refractive index detector or with a Waters Assoc. model 4500 equipped with a radioactivity monitor. Melting point was determined using a hot-stage apparatus and is uncorrected. Optical rotation was recorded with a JASCO model DIP-181 instrument.

2-Deoxy-2-fluoroacetamido-D-glucopyranose. A 0.2 mole scale preparation of this sugar was carried out by a procedure similar to that reported (8). Recrystallization from methanol gave the sugar as crystallites, m.p. 180-181.5°C, $[\alpha]_D^{18}$ +26.5 (2 h, c 1, water); lit.(7). m.p. 189-192°C, $[\alpha]_D^{20}$ +31 (2 h, c 1, water); lit.(8). m.p. 161-163°C, $[\alpha]_D^{25}$ +22.8 (3 h, c 0.3, water); lit.(9). m.p. 154°C, $[\alpha]_D^{25}$ +23.6 (1 h, c 1, water); lit.(10). m.p. 171-173°C. The retention times of this sugar in various HPLC systems are shown in Table 1.

2-Deoxy-2-[^{18}F]fluoroacetamido-D-glucopyranose (1). [^{18}F]Fluoride was produced from the proton bombardment of 20% enriched [^{18}O]water by the ^{18}O (p, n) ^{18}F nuclear reaction at the Cyclotron (14). To the aqueous solution of [^{18}F]fluoride, a mixture of aqueous potassium carbonate (33 μmol/0.2 ml) and Kryptofix 222 (72 μmol, 27 mg) was added. The resulting solution was dried at 90°C in stream of dry nitrogen gas. To the residue, a solution of ethyl bromoacetate (0.2 mmol, 33.4 mg) in acetonitrile (1 ml) was added.
The mixture was heated at 82°C for 10 min with stirring and cooled. After addition of 0.5 N aqueous potassium hydroxide (0.4 ml), the reaction mixture was further heated at 82 C for 2 min. To the resulting mixture, a mixture of hydrochloride of (2) (0.2 mmol, 43.2 mg) and dicyclohexylcarbodiimide (0.5 mmol, 103 mg) in pyridine (0.5 ml) was then added. The mixture was heated at 82°C for 20 min with stirring, diluted with water (2 ml) to decompose an excess of the carbodiimide, and filtered. The filtrate was washed with ethyl ether (10-ml x 2) using a Mixxor (liquid-liquid extraction system; Lidex Co. Ltd., Israel), and evaporated to dryness under a reduced pressure. The residue was dissolved in water (0.5 ml) and the solution was chromatographed over an ion retardation resin (AG 11-A8, 2 ml) column using water as elution solvent. The

Table 1: Retention Times of 2-Deoxy-2-fluoroacetamido-D-glucopyranose in Various HPLC Systems

Run	Column Size (mm)	Mobile Phase[a] Ratio(v:v)	Flow Rate (ml/min)	Retention Time (min)
1	μ-Bondapak-CH[b] (3.9 x 300)	90:10	1.5	7.3
2		85:15	1.5	5.1
3		75:25	1.5	3.5
4	YMC-Pack PA-03[c] (4.6 x 250)	75:25	1.5	4.6
5	YMC-Pack PA-23[c] (10.0 x 250)	85:15	5.0	13.4
6		75:25	5.0	6.5

a) Mobile phase: Acetonitrile:Water.
b) Waters Associate, USA.
c) Yamamura Chem. Lab. Co., Jpn.

eluent was then mixed with an approximately equal portion of acetonitrile, passed through a Sep-Pak C_{18} cartridge (Waters), and eluted with aqueous acetonitrile (1:1, v/v). The effluent was concentrated to 1/10 of its original volume and then subjected to preparative HPLC under the similar conditions of Run 6 shown in Table 1. A radioactivity peak corresponding to (1) was then collected and the identity of the peak was confirmed by analytical HPLC (Runs 2 and 4 shown in Table 1). The total synthesis time, the radiochemical yield, and purity of (1) are ca. 90 min, ca. 9.1%, and >98%, respectively.

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