SYNTHESIS AND QUALITY CONTROL OF LONG-CHAIN ^{18}F-FATTY ACIDS

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SUMMARY

Introduction of fluorine-18 into various positions of long-chain fatty acids is described. The potential heart and liver radiopharmaceuticals 16-^{18}F-hexadecanoic acid, 17-^{18}F-heptadecanoic acid, 2-^{18}F-, and (9,10)-^{18}F-stearic acid have been prepared by nucleophilic F-for-Br exchange in the melt of the corresponding bromo-fatty acid methylesters in acetamide, followed by alkaline or acid hydrolysis. In the case of 17-^{18}F-heptadecanoic acid saturation yields of about 30% have been obtained at an optimum reaction temperature of 150 ^{o}C and about 1 mg of KF-carrier.

Key Words: Long-Chain ^{18}F-Fatty Acids, Exchange in Melt, High Pressure Liquid Chromatography

INTRODUCTION

The chemistry of fluorine has become a field of increasing interest, and new fluorination methods have been introduced (1,2). Moreover, it was demonstrated that many fluorine-containing compounds are useful for medical applications (e.g. as tumor inhibitors), exhibiting an enhancement of the pharmacological activity due to the unique properties of the fluorine atom. Consequently, the development of radiopharmaceuticals using the positron emitting radionuclide ^{18}F ($T_{1/2}$ = 110 min) is also a rapidly growing field. Fluorine-18 offers

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some unique properties for nuclear medicine: It can be produced both in a nuclear reactor and in a cyclotron by various reactions. In contrast to the other organic positron emitters, such as ^{11}C ($T_{1/2}$ = 20 min), ^{13}N ($T_{1/2}$ = 10 min), and ^{15}O ($T_{1/2}$ = 2 min), which can also be used in conjunction with positron emission tomography, the relative long half-life of fluorine-18 allows a distribution within a radius of a few hundred kilometers from the site of production. In addition, it has the lowest β^+-energy (0.6 MeV) with a range of only 2.5 mm in H_2O, thus giving rise to a higher resolution in positron emission tomography. Synthesis and purification of the products can often be performed in an appropriate time of about two half-lives and last not least, the high energy of the C-F bond can lead to an enhanced in-vivo stability when compared with other halogens (for a recent review, cf. 3).

Problems may arise, however, when classical fluorination methods are applied, either because the reaction times are too long and/or when large amounts of fluorine-carrier are required. Thus, classical methods have to be changed or modified in order to obtain reasonable radiochemical yields and high specific activities.

Our method of synthesis differs from those previously described in the literature (4-11) by the chain length of the fatty acids (C_{16}-C_{18}), and/or the use of lower concentrations of fluorine-carrier. Furthermore higher radiochemical yields could be obtained by systematically optimizing the reaction conditions.

In previous studies in our laboratory (12) various α- and ω-halogenated fatty acids labelled with ^{34m}Cl, ^{77}Br, and ^{123}I were prepared for comparative measurements of myocardial extraction and elimination rates. An extension of these pharmacokinetic and biochemical investigations which has recently been performed by us using the corresponding ^{18}F-labelled fatty acids, described in this work, will be reported elsewhere (13).

EXPERIMENTAL

Materials: KF and acetamide were purchased from Merck (Darmstadt) with a purity of > 99 %. The acetamide was further recrystallyzed from benzene and dried over P_2O_5. The methylesters of 16-Br-hexadecanoic acid and 17-Br-heptadecanoic acid were obtained by Emka-Chemie (Markgröningen). 2-Br-stearic methylester and (9,10)-Br-stearic methylester were prepared in our laboratory by bromination of stearic acid with P/Br_2 and addition of HBr to oleic acid,respectively, followed by esterification in methanolic BF_3-solutions.

Irradiations: Irradiations were performed at the Jülich compact cyclotron CV 28 with a water target. The water for the irradiations was of ultra high quality (AMPUWA, E. Fresenius KG, Bad Homberg). According to the reaction $^{16}O(^3He,p)^{18}F$, 10 ml of water were irradiated with a ^{3}He-beam (36 MeV, 10 μA) for about 20 minutes, giving rise to about 20 to 30 mCi of carrier-free ^{18}F in water.

Synthesis: KF-carrier was added to the ^{18}F-containing water, which was evaporated to about 200 μl and transferred into a

quartz break-seal ampoule of 3 ml. The water was pumped off and evaporated to dryness by heating with a flame until a pressure of 10^{-3} torr was reached. After cooling, 20 mg of bromofatty acid methylester and 100 mg acetamide were added, the ampoule was sealed under vacuum and heated in an oil bath while stirring. After a given reaction time saponification of the ester was carried out by refluxing the solution in 2 ml of 5N methanolic KOH or, in the case of 2-^{18}F-stearic acid, in 5N H_2SO_4 for 30 minutes. After transferring the contents into a 60 ml extraction vessel, washing the ampoule with 10 ml of water and adding 3 ml H_2SO_4 (1:6) to the combined solutions, the products were extracted 3 times with n-heptane at 80 ^{o}C (1x10 ml, 2x5 ml each). The organic fractions, after centrifugation and phase separation, were evaporated to dryness, the residue was dissolved in 2 ml of warm n-heptane, and the ^{18}F-labelled fatty acid purified by high pressure liquid chromatography (hplc).

The experimental conditions for the hplc-separations were as follows: Chromatograph: WATERS 6000 A; column: WATERS μ-Bondapak NH_2 30x0.4 cm. For 16-^{18}F-hexadecanoic acid, 17-^{18}F-heptadecanoic acid, and (9,10)-^{18}F-stearic acid, n-heptane/acetic acid (998/2) at a flow of 3 ml/min, and for 2-^{18}F-stearic acid n-heptane/diethylether/acetic acid (58/40/2) at a flow of 3 ml/min was used. A summary of hplc-conditions used is given in Table I. Simultaneous radioactivity measurements of the ^{18}F-labelled organic products with a well-type NaI(Tl) scintillation crystal indicated the presence of the desired ^{18}F-labelled fatty acid as main product and a small amount of the corresponding methylester.

TABLE I : Preparation and purification of ^{18}F-labelled fatty acids by F-for-Br exchange in the corresponding Br-methylester systems with subsequent hydrolysis and separation by hplc.

Product	Labelling Procedure	Reaction Time [min]	Hydrolysis	Radiochemical Yield [%]*	High Pressure Liquid Chromatography	Flow [ml/min]	Net Retention Time [min]
2-^{18}F-stearic acid	F-for-Br exchange in acetamide (180 °C)	60	30 min reflux in 5N H_2SO_4	10	Waters μ-Bondapak NH_2: 30 cm long, 0.4 cm i.d.; n-heptane/diethylether/acetic acid=58/40/2	3	21
16-^{18}F-hexadecanoic acid, 17-^{18}F-heptadecanoic acid	F-for-Br exchange in acetamide (120°-180°C)	1-60	30 min reflux in 5N methanolic KOH	4-30	Waters μ-Bondapak NH_2: 30 cm long, 0.4 cm i.d.; n-heptane/acetic acid=998/2	3	11-12
(9,10)-^{18}F-stearic acid	F-for-Br exchange in acetamide (150° and 180°C)	60	30 min reflux in 5N methanolic KOH	4	Waters μ-Bondapak NH_2: 30 cm long, 0.4 cm i.d.; n-heptane/acetic acid=998/2	3	11-12

*Average of at least 2 individual runs. Experimental error ± 10-15 %.

RESULTS AND DISCUSSION

Radiochemical yields, conditions of reaction and of hplc-separations are summarized in Table I. In a more detailed study, the 17-^{18}F-heptadecanoic acid was chosen to study the time dependence of radiochemical yield from 1 to 60 min at a temperature of 150 ^{o}C. In each case the subsequent alkaline saponification of the 17-^{18}F-heptadecanoic methylester was carried out under identical conditions by refluxing the solution in 5N methanolic KOH for 30 minutes. The results are shown in Fig. 1.

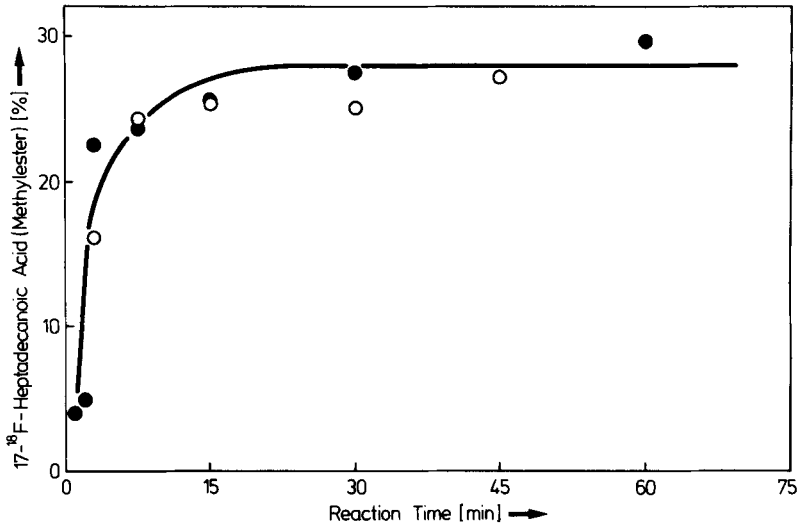

Fig. 1 Time dependence of F-for-Br exchange in the 17-Br-heptadecanoic acid methylester system. (Reaction conditions: 1 mg KF, 100 mg acetamide, 20 mg 17-Br-heptadecanoic acid methylester, 150 ^{o}C).

- o Radiochemical yields of 17-^{18}F-heptadecanoic acid methylester.
- ● Radiochemical yields of 17-^{18}F-heptadecanoic acid after saponification of 17-^{18}F-heptadecanoic acid methylester by refluxing in 5N methanolic KOH solution for 30 min.

Average deviations are within ± 10-15 %.

It can be seen that within the first minutes a fast increase of the yields of 17-^{18}F-heptadecanoic acid takes place and reaches a saturation value of about 30 %. These findings are in agreement with the kinetic investigations of Robinson who, although with lower yields, found similar curves for ^{18}F-fatty acid esters with shorter chain-lengths (5). The yields given in Fig. 1 are the sum from two reaction steps, i.e. the F-for-Br exchange in the melt and the following hydrolysis. Although the C-F bond in the primary position of an aliphatic hydrocarbon chain is known to be very stable against hydrolysis, the experiments were repeated without the step of saponification. It could be shown (see Fig. 1) that within the experimental error (± 10-15 %) the radiochemical yields of 17-^{18}F-heptadecanoic acid methylester were identical with those of the free 17-^{18}F-heptadecanoic acid found in the "one-pot" synthesis described in the experimental part. Obviously, hydrolysis occurs quantitatively.

The rapid increase of the F-for-Br exchange yields within the first minutes is an interesting effect which is in contrast to other nucleophilic fluorination procedures, where long reaction times and large amounts of KF are necessary to obtain reasonable yields. Obviously, the nucleophilic power of F^- in very dry solutions of potassium fluoride in molten acetamide reaches its maximum already within the time necessary to obtain a homogeneous solution of the ^{18}F-KF. One of the factors limiting the radiochemical yields is based upon the fact that besides its nucleophilicity the fluoride ion in aprotic solvents exhibits considerable basic properties, thus causing elimination as competing reaction.

The results of the temperature dependence on the radiochemical yields of 17-^{18}F-heptadecanoic acid is shown in Fig. 2.

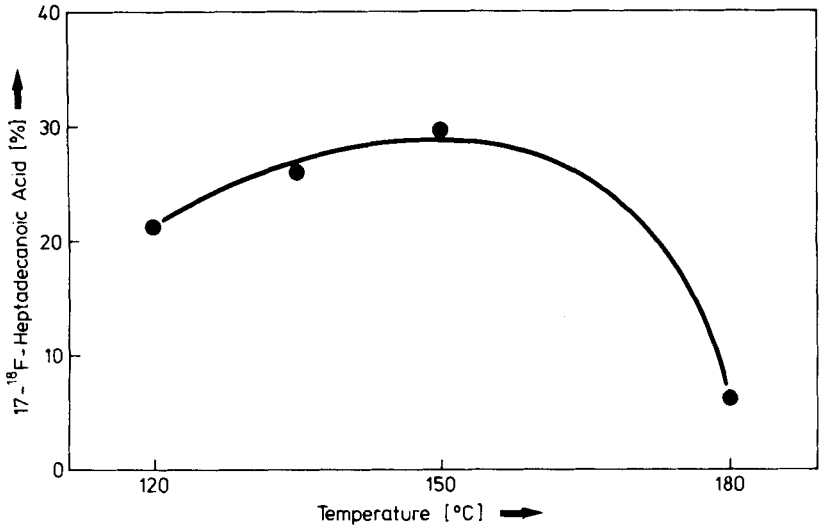

Fig. 2 Temperature dependence of F-for-Br exchange in the 17-Br-heptadecanoic methylester system. (Reaction conditions: 1 mg KF, 100 mg acetamide, 20 mg 17-Br-heptadecanoic acid methylester. Reaction time t_1 = 60 min, followed by saponification in refluxing 5N methanolic KOH solution, t_2 = 30 min). Average deviations are within ± 10-15 %.

The times for the exchange reaction t_1 and for the saponification t_2 were kept constant (t_1 = 60 minutes, t_2 = 30 minutes). In the low temperature range an increase of the yields is observed reaching a maximum at about 150 °C, followed by a decrease at elevated temperatures. These results can be interpreted in terms of a temperature dependent F-for-Br exchange rate in the acetamide melt and a possible decomposition of the product above 150 °C. In addition, elimination reactions as mentioned above may play a predominant role with increasing temperature.

The dependence of the radiochemical yields of 17-^{18}F-heptadecanoic methylester on the addition of KF-carrier is shown in Fig. 3. It can be seen that after an initial increase in yield

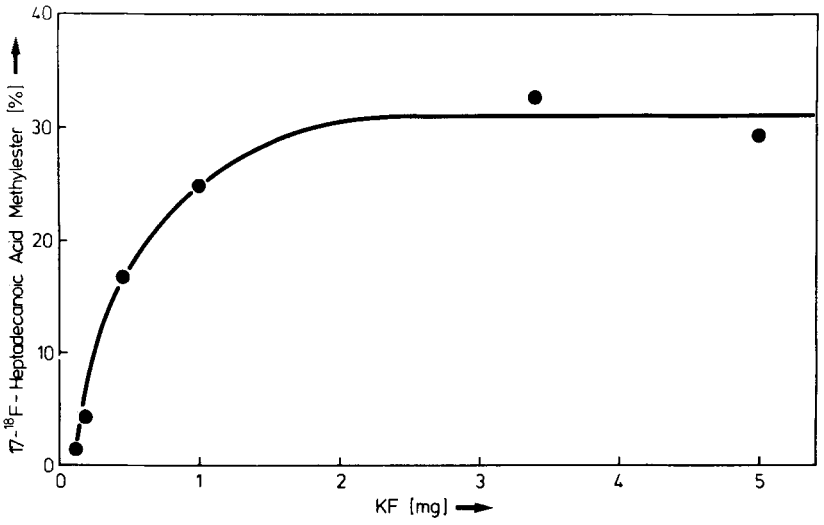

Fig. 3 Carrier-dependence of F-for-Br exchange in the 17-Br-heptadecanoic acid methylester system. (Reaction conditions: KF, 100 mg acetamide, 20 mg 17-Br-heptadecanoic acid methylester, 150 °C, 30 min). Average deviations are within ± 10-15 %.

a saturation is reached at about 30 %. Two conclusions can be drawn from the shape of the curve: i) addition of more than about 1 mg KF-carrier does not lead to a considerable increase of yields, indicating that the losses of ^{18}F are due to competing reactions. As mentioned above the basic properties of F^- in dry acetamide may cause elimination reactions in the starting material,
ii) at low carrier-concentrations the yields decrease drastically, demonstrating that an absolutely carrier-free synthesis cannot be carried out.

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