PREPARATION OF N.C.A. [17-^{18}F]-FLUOROHEPTADECANOIC ACID IN HIGH YIELDS VIA AMINOPOLYETHER SUPPORTED, NUCLEOPHILIC FLUORINATION

Heinrich Hubert Coenen[1], Bernd Klatte[2], Arndt Knöchel[2], Manfred Schüller[1], Gerhard Stöcklin[1]

[1]Institut für Chemie 1, Kernforschungsanlage Jülich GmbH, Postfach 1913, D-5170 Jülich, FRG

[2]Institut für Anorganische und Angewandte Chemie, Universität Hamburg, Martin-Luther-King-Platz 6, D-2000 Hamburg 13, FRG

SUMMARY

A macrocyclic aminopolyether (Kryptofix®2.2.2.) supported labelling method for the preparation of n.c.a. [17-^{18}F]-fluoroheptadecanoic acid is described. The equimolar complex of potassium carbonate and the aminopolyether ($[2.2.2./K]^{+}_{2}CO^{2-}_{3}$) is used to provide ^{18}F-fluoride of high nucleophilicity in a homogeneous solution of acetonitrile. Nucleophilic ^{18}F-for-Br substitution in the methylester of 17-bromoheptadecanoic acid is accomplished with radiochemical yields of 94 + 3%. Subsequent quantitative ester hydrolysis with KOH leads to a simple "one pot" procedure. Minimization of reagent concentrations allows a direct isolation of the product from the reaction mixture by means of reverse phase HPLC. The corrected radiochemical yield of high activity level routine production is 82 + 2% after 90 minutes of synthesis time. The specific activity is > 10,000 Ci/mmol.

Key words: nucleophilic radiofluorination, n.c.a. $^{18}F^{-}$, [17-^{18}F]-fluorofatty acid, aminopolyether 2.2.2.

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INTRODUCTION

A great variety of radiolabelled long-chain fatty acids have been prepared and evaluated as tracers for studying metabolic turnover in the myocardium (for reviews cf. 1-3). Carbon-11 ($T_{1/2}$ = 20 min) labelled fatty acids are used in conjunction with positron emission tomography (PET) for regional dynamic studies (1). Analogue tracers, in particular iodine-123 ($T_{1/2}$ = 13.2 h) labelled fatty acids, have been clinically evaluated using a conventional γ-camera or single photon emission computed tomography (SPECT) (2).

The carbon-11 label has the inherent advantage that it does not induce any biochemical changes. On the other hand, its short half-life makes longer lasting studies difficult. Since the elimination kinetics of the radioactivity from the myocardium is biphasic with a slow second component, a longer lived positron emitter such as fluorine-18 seems to be more suitable. In addition, fatty acids labelled in the ω-position with fluorine-18 lose their label only in the last step of ß-oxidation in contrast to [1-^{11}C]-palmitic acid where the label is lost in the first step of degradation.

Among the fluorinated fatty acids labelled in different positions, the odd-numbered heptadecanoic acid was shown to be most suitable (4). Its final catabolite is the free fluoride ion which is eliminated from the cell, unlike the even-numbered fatty acids which is degraded to the toxic 2-fluoroacetic acid.

Several nucleophilic methods have been used to introduce fluorine into carboxylic acids such as heterogeneous exchange on ion exchange resins (5,6). However, these are inconvenient for the routine production of no-carrier-added (n.c.a.) long-chain fatty acids. N.c.a. 6- and 7-fluoropalmitic acids were prepared by substitution on the corresponding mesylate

derivatives in DMSO with very low yields (7). The non-isotopic bromine-for-fluorine exchange in ω-bromoheptadecanoic acid in an acetamide melt required a relatively high fluoride carrier concentration to obtain reasonable yields of about 30% (8). Only recently higher yields could be obtained with n.c.a. fluoride by adding defined cations to the reaction medium. Tetrabutylammonium hydroxide in a solution of 16-iodohexadecanoic acid methylester in DMSO in the presence of silver oxide led to a 30% yield of the n.c.a. product (9). Recently the halogen exchange in 17-bromoheptadecanoic acid methylester in acetamide melt was optimized by adding K_2CO_3, and n.c.a. yields of 50% were obtained in a synthesis time of about 2 hours (10).

Another approach to facilitate nucleophilic substitution especially with n.c.a. [^{18}F]-fluoride is the use of neutral phase transfer catalysts. While the classical 18-crown-6 did not allow n.c.a. fluorination with useful yields (11,12), a systematic study using aminopolyether (APE) led to high n.c.a. yields in non-isotopic exchange reactions on halogenated alkanes (13). The complex of the APE 2.2.2. and K_2CO_3 was found optimal to provide n.c.a. $^{18}F^-$ of high nucleophilicity in dipolar aprotic solvents. Consequently, this method was applied for n.c.a. labelling of 2-fluoro-2-desoxyglucose (14), aromatic compounds (15) and ω-fluorocarboxylic acids (15) in high yields. The application of this method to the routine production of [17-^{18}F]-fluoroheptadecanoic acid which is presently clinically evaluated (16) is described in this paper.

EXPERIMENTAL

Materials The methylester of 17-bromoheptadecanoic acid and of 17-fluoroheptadecanoic acid and the corresponding free acids were purchased from EMKA-Chemie (Markgröningen, FRG). The aminopolyether Kryptofix®2.2.2. (4,7,13,16,21,24

hexaoxa-1,10-diazabicyclo[8.8.8]hexacosan) and K_2CO_3 as well as the other reagents and solvents used were from Merck (Darmstadt, FRG). The acetonitrile used as reaction solvent was Uvasol grade, and was additionally dried.

Radionuclide production and preparation of reactive fluoride

For the production of fluorine-18 via the $^{18}Ne(d,\alpha)^{18}F$-process a cylindrical gas target (Inconel 600) filled with Ne gas containing 15% H_2 (total pressure 18 bar) is bombarded with 14 MeV deuterons at the Jülich CV 28 compact cyclotron (17). Irradiations are carried out at 25 µA and the $^{18}F^-(H^{18}F)$ is collected by rinsing the target wall with 2-4 ml bidistilled water. Typically 4.6 mg (0.03 mmol) K_2CO_3 and 25.1 mg (0.06 mmol) APE 2.2.2. from an aqueous stock solution are added to the ^{18}F-water which had been transferred to a glassy carbon vessel (Sigradur®-G, 18x70 mm, from Sigri, Meitingen, FRG). While purging with a flow of about 50 ml/min of helium the aminopolyether - potassium fluoride (carbonate) solution is evaporated to dryness at a temperature of about 95 °C in the reaction vessel. Afterwards the glassy carbon vessel is equipped with a reflux condenser and drying tube. The loss of fluorine activity during evaporation is < 1%.

Synthesis of [17-^{18}F]-fluoroheptadecanoic acid

Procedure 1. For optimization of the labelling procedure on an analytical scale the following scheme is followed. Typically 20 mg (0.06 mmol) of 17-bromoheptadecanoic acid methylester are dissolved in 0.5 ml of dry acetonitrile and added to the glassy carbon vessel containing the dry $[2.2.2./K]^{+18}F^-$ and refluxed for 10 minutes at a bath temperature of 110 °C. Subsequently 1 ml of 0.5 N methanolic KOH is added and the solution refluxed for another 15 minutes at the same temperature,

The reaction mixture is diluted with about 14 ml of water and transferred to a 50 ml glass vessel for extraction. 1 ml of H_2SO_4 (1:6) is added after cooling and the solution is extracted with four portions of 5 ml n-heptane at 80 °C. After complete evaporation of the heptane the dry residue is dissolved in the eluant used for HPLC separation (see below). The total time of synthesis is about 120 minutes. The radiochemical yield in the analytical scale procedure is 94 ± 3% (corrected for decay) with < 0.2% losses to the reaction vessel.

Procedure 2. For routine production on a high activity level the synthesis was optimized to avoid the time consuming extraction step and to minimize volumes and reagents for direct injection onto the HPLC column. 20 mg (0.06 mmol) 17-bromoheptadecanoic acid methylester and 0.4 ml anhydrous acetonitrile are added to the glassy carbon vessel. After refluxing for 10 minutes at a bath temperature of 110 °C and cooling, 80 µl 5 N aqueous KOH are directly added to the CH_3CN solution for a 15 minute hydrolysis at the same temperature. Additional 2.5 ml CH_3CN, 0.25 ml H_2O and 150 µl H_2SO_4 (1:6) are added under cooling. Warming of the reaction mixture to about 50 °C results in a clear solution and a white precipitate of salts. The solution is transferred to the heated (50 °C) injection loop of the HPLC system together with 0.5 ml of eluant used for rinsing the reaction vessel and residue. Direct HPLC of the reaction solution is performed on a reverse phase column. The [17-^{18}F]-fluoroheptadecanoic acid fraction is collected in a sterilized flask and evaporated to dryness. The radiochemical yield of the product is 82 ± 3% (corrected), the solid residue in the reaction vessel containes 7 ± 2% of total activity. Batches of about

Table 1: High performance liquid chromatography of heptadecanoic acid derivatives (eluants see text)

Column	Lichrosorb Si-60 (10 µm) (250x4 mm)	Nucleosil RP-18 (7.5 µm) (250x16 mm)
	k'	k'
17-fluoroheptadecanoic acid	5.20	2.64
17-bromoheptadecanoic acid	4.46	3.73
17-fluoroheptadecanoic acid methylester	1.17	5.08
17-bromoheptadecanoic acid methylester	1.04	7.23

10-15 mCi were prepared in 90-95 minutes.

An injectable solution was obtained by adding 2 ml of the patient's serum to the sterile flask and dissolving the n.c.a. [^{18}F]-fluoroheptadecanoic acid under gentle shaking in a 35 ^{o}C water bath for 10 minutes. The recovery of the acid after sterile filtration over a millipore filter (Sephadex®GS, 0.25 µm) was 90% (corrected).

Chromatography and quality control In contrast to previous separations via amine bound polar phases (8), silicagel or reverse phase columns are found to be more reliable and convenient. A normal phase column with LiChrosorb Si-60 (10 µm) as stationary phase is only used for analytical purposes (250 x 4 mm). The eluant used is n-heptane saturated with CH_3CO_2H at a flow rate of 1 ml/min.

For the high activity level routine separation of the fluorofatty acid the complete synthesis solution is injected on a reverse phase column, Nucleosil RP-18 (7.5 µm) of 16 mm

i.d. and 25 cm length. Elution is performed with methanol : water : acetic acid (896:100:4) at a flow rate of 7.4 ml/min. The k'-values of the ester and acids are summarized in Table 1.

A continuous radiochromatographic procedure using a RI-detector in series with a radioactivity detector is applied in the high activity runs. In the low activity analytical separation the fractions are collected and measured in a well-type scintillation counter.

RESULTS AND DISCUSSION

The study of metabolic turnover of radiohalogenated fatty acid analogues does not require no-carrier-added labelling since there is about a 1 mmolar concentration of free fatty acids normally occuring in the blood. Isotopic or non-isotopic carrier does not change the uptake and biokinetics if present in the range of some 10-100 µg (4,18). However, possible toxic effects of fluorinated analogues (cf. 4) and the low solubility of long-chain fatty acids in injectable solutions (18) make high specific activity products very desirable. A reliable high yield for preparations of n.c.a. long-chain ^{18}F-labelled fatty acids has only recently been developed (10), using potassium carbonate as a non-isotopic carrier in the acetamide melt.

A newer approach of anion (fluoride) activation for nucleophilic substitution using neutral aminopolyether in combination with alkali carbonates demonstrated excellent results in aliphatic compounds even on a n.c.a. level (13). Especially the bicyclic aminopolyether 2.2.2. - potassium carbonate complex ($[2.2.2./K]_2^+ CO_3^{2-}$) in dipolar aprotic solvents, preferentially in acetonitrile, allowed exchange with n.c.a. ^{18}F-fluoride under mild conditions. The strong activation of fluoride in this system is based on the very

low interaction of the anion with the cationic complex $[2.2.2./K]^+$ which leads to "naked" fluoride ions in acetonitrile as solvent. The high nucleophilicity and hence reactivity of n.c.a. $^{18}F^-$ is expressed in good exchange rates being almost independent of the leaving group (13). Carbonate as counter ion can be regarded as a non-isotopic carrier with very low nucleophilicity which also provides a basic medium prohibiting the formation of HF.

The preceding systematic study on aminopolyether supported fluorination demonstrated the necessity of using a molar ratio of 2.2.2.:K_2CO_3 (2:1), an almost equimolar ratio of the $[2.2.2./K]^+$ complex to the substrate, and a relatively high absolute concentration of the substrate in order to obtain maximal labelling yields. Unlike the experiments on an analytical scale difficulties were encountered in high activity routine production such as fast evaporation of water without the APE 2.2.2. decomposition, losses of activity to the reaction vessel, and the high amounts of reagents necessary. These problems, however, were overcome as described in the experimental part.

In Table 2 the reaction conditions and radiochemical yields are listed which were performed to evaluate minimal reagent concentrations necessary to obtain optimal yields. Besides cost saving with respect to the relatively expensive substrates, purification and isolation are facilitated using smaller amounts. The direct HPLC-separation of the total reaction mixture was only possible after minimization of the reagents (cf. procedure 2). It can be seen from Table 2 that compared to the previous standard conditions the amounts of solvent and reagents can be reduced by a factor of two to three without significantly changing the radiochemical yield. Further decrease of concentrations leads to a loss of labelling efficiency.

Table 2: Effect of reagent and aminopolyether 2.2.2. concentration on the radiochemical yield of [17-^{18}F]-fluoroheptadecanoic acid

K_2CO_3 [mg]	13.8	4.6	2.3	1.73
2.2.2. [mg]	75.3	25.1	12.6	9.41
CH_3CN [ml]	1.5	0.5	0.5	0.4
17-Br$(CH_2)_{16}CO_2CH_3$ [mg]	50	20	10	7.5
0.5 N meth. KOH [mg]	2	1	0.5	0.4
H_2SO_4(1:6) [ml]	3	2	0.4	0.3
wall activity [%]	< 0.2	< 0.1	< 0.1	< 0.3
17-^{18}F$(CH_2)_{16}CO_2H$ [%]	91±2	94±3	88±2	80±2

reaction temperature for exchange 110 °C, 10 minutes
hydrolysis temperature 110 °C, 15 minutes

Direct chromatography of the total reaction mixture reduces the manipulation steps and losses of activity, and facilitates automation of the labelling process. In consequence, the overall labelling yield is higher than that using the extraction process although the decay corrected yield is only 83 ± 3% compared to the almost quantitative extraction. An advantage of the extraction method is that the aminopolyether is protonated and thus quantitatively removed from the organic phase. In the direct work-up process via the reverse phase HPLC it is eluted in protonated form with the void volume and thus separated from the fluorinated fatty acid. An alternative would be to use reverse phase Sep-Pak cartridges for rapid separation of the fatty acids from the other reagents and salts by elution with acid. But HPLC is needed in any case in order to separate the bromo- from the fluoroheptadecanoic acid.

Partition chromatography on Si-60 is advantageous with respect to the much higher separation capacity. Therefore silica gel has been used routinely as the stationary phase for the preparation of [17-^{123}I]-iodoheptadecanoic acid (19). In the case of fluorofatty acid, however, it is eluted shortly after the mass of the starting material, 17-bromo-heptadecanoic acid (cf. Table 1). Avoiding non-isotopic carrier is therefore critical. In contrast, reverse phase chromatography used in the present study allows elution of the carrier-free product in front of the starting material.

It should be noted that the hydrolysis is separately optimized. The conditions given in procedure 2 are sufficient for quantitative saponification of the ester as checked by both chromatographic systems. Methanolic KOH should not be used in procedure 2 because in this concentrated solution addition of H_2SO_4 leads to reformation of the methylester. This does not occur in the extraction procedure where the reaction solution is strongly diluted with H_2O.

The specific activity is tested under the same reaction conditions (procedure 1) using the Br-for-F exchange in 2-bromoacetic acid ethylester, which can easily be assayed by radio gaschromatography (cf. 6,10). On the basis of the GC-sensitivity the lower limit of mass corresponds to a specific activity of > 10,000 Ci/mmol.

CONCLUSION

The advantages of the aminopolyether 2.2.2. supported reaction are manifold and exceed even those of the acetamide melt method using carbonate as non-isotopic carrier (10), which until now was the best method to prepare n.c.a. ^{18}F-fatty acids in high yields. The yields of the [17-^{18}F]-fluoro-heptadecanoic acid are almost quantitative in a shorter

reaction time using the phase transfer catalyst supported method. The considerably lower temperature does not give rise to side products such as olefines from dehydrohalogenation or ω-hydroxy derivatives. The homogeneous reaction in acetonitrile is not very sensitive to traces of water (< 0.1%) allowing easy handling and high reproducibility. The APE 2.2.2. supported reaction is therefore highly promising for high yield nucleophilic aliphatic and aromatic fluorination for temperature sensitive molecules.

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