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Synthesis of 5'-N-(2-[^{18}F]Fluoroethyl)-carboxamidoadenosine: a Promising Tracer for Investigation of Adenosine Receptor System by PET Technique

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SUMMARY

5'-N-(2-[^{18}F]Fluoroethyl)-carboxamidoadenosine ([^{18}F]FNECA), a promising ^{18}F-labelled adenosine agonist has been prepared by two different synthetic routes. In the first, [^{18}F]fluoride was reacted with 5'-N,N-ethylene-2',3'-O-isopropylidenecarboxamido-adenosine and after removing the protective group [^{18}F]FNECA was obtained in a low radiochemical yield (1±1%, mean±sd, n=7, decay corrected). In the second, 2-[^{18}F]fluoro-ethylamine was synthesised according to the literature and reacted with 2',3'-O-isopropylideneadenosine-5'-uronic acid in the presence of a coupling agent. The following hydrolysis step provided the [^{18}F]FNECA with a modest radiochemical yield (24±9%, n=17, based on [^{18}F]fluoride-activity). After purification by preparative reverse phase HPLC 18.9-166.5 MBq (0.51-4.5 mCi) [^{18}F]FNECA was obtained with a specific activity of 2.35±1.14 TBq/mmol (63.5±30.9 Ci/mmol, n=3). The total synthesis took 200 min and the decay corrected radiochemical yield based on [^{18}F]F$^-$ activity was 17±9% (n=5) with more than 99.9 % radiochemical purity. This second route provides sufficient [^{18}F]FNECA for the subsequent biological evaluation using PET-technique.

Key-words: Adenosine receptors, NECA, FNECA, radiofluorination, PET

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INTRODUCTION

Adenosine appears to mediate a wide variety of physiological functions. Many of its effects can be attributed to the action at receptors located on the cell surface. There are three sub-types of adenosine receptors: A_1, A_2 and A_3 with A_2 further sub-divided into A_{2A} and A_{2B} (1). As the number of receptor ligands labelled with positron emitting radionuclides for PET investigation is limited (2,3), and in addition, each of them is antagonist (labelled either with ^{11}C or ^{18}F) (4), synthesis of labelled agonists is needed for a deeper understanding of biochemical phenomena related to adenosine receptor system.

5'-Carboxamidoadenosines are known as non-selective adenosine receptor agonists, among which 5'-N-ethylcarboxamidoadenosine (NECA) binds to A_1, A_2 and A_3 receptors with K_i values in the nanomolar range (5). The C2 modification of 5'-carboxamidoadenosines, however, has led to the potent A_{2A}- (6), while the N6 substitution resulted in A_3 selective receptor agonists (7), respectively. Moreover, 5'-carboxamidoadenosines without further modification are the most active A_{2B} agonists (5).

Fluorine-18 is a convenient labelling agent due to its relatively long half-life (109.6 min), which permits multistep syntheses requiring relatively long reaction times and study of biochemical processes which are relatively slow. In addition, its low β^+ energy (635 keV) is advantageous from the imaging point of view. 5'-N-(2-[^{18}F]Fluoroethyl)-carboxamidoadenosine ([^{18}F]FNECA), as a fluorine-18 labelled analogue of NECA, can possibly serve as a PET isotope labelled agonist with adenosine receptor specificity. According to preliminary pharmacological tests it seems to be equipotent to NECA (results will be published separately). Furthermore, the modification of the presented synthesis route by labelling adenosine derivatives substituted at C2 or N6 position can result in ^{18}F-labelled receptor ligands of A_{2A} and A_3 selectivity, respectively.

EXPERIMENTAL

Materials and methods

Solvents and chemicals (p.a. grade) were obtained from Merck (Darmstadt, Germany) or Sigma-Aldrich (Gillingham, England). Reaction mixtures were purified by column chromatography on silica gel (Kieselgel 60, 230-400 mesh, Merck). Nuclear magnetic resonance spectra were obtained on a Bruker WP 200 spectrometer (200 MHz) using Me_4Si as internal standard. TLC was performed on silica gel (Kieselgel $60F_{254}$ Merck) using solvent systems specified in the text.

HPLC analyses were carried out using μBondapack™ C18 125A 10 μm 3.9X300 mm (Waters) reverse phase column (system A), whilst the preparative reverse phase HPLC separations were completed on a LiChrospher® 100RP-18 (10 μm) 10X250 mm column (Merck, Darmstadt) (system B). The retention times and the solvent systems (gradient mixture of ethanol and water) are summarised in Table 1.

A calibration curve gained by UV absorption data of inactive FNECA at λ=261 nm was used to determine the specific activity of the synthesised [^{18}F]FNECA. All the yields of the radiochemical syntheses given in the text are decay corrected.

Table 1. RP-HPLC retention times (min)

Compound	System A (analytical)	System B (preparative)
Fluoride-ion	1.8	-
5'-N-(2-Fluoroethyl)-2',3'-O-isopropylidene-carboxamidoadenosine (FIPNECA)	10.9	15.2-16.1
5'-N-(2-Fluoroethyl)-carboxamidoadenosine (FNECA)	8.5	12.1-13.4

System A: flow rate 2 ml/min; t=0 min 0% ethanol, linearly increased to 25% at t=8 min; linearly increased to 40% at t=12 min
System B: flow rate 5 ml/min; t=0 min 0% ethanol, linearly increased to 60% at t=15 min then 60% ethanol to t=17 min

^{18}F nuclide was produced in [^{18}F]fluoride-ion form directly in target by the MGC20E cyclotron at the Institute of Nuclear Research, Debrecen from ^{18}O(p,n)^{18}F nuclear reaction, with 14.5 MeV proton beam (yield was 500-700 GBq/C). The [^{18}O]-enriched water used as target material was purchased from CAMPRO Scientific, The Netherlands.

SYNTHESIS OF INTERMEDIATES AND FINAL PRODUCT

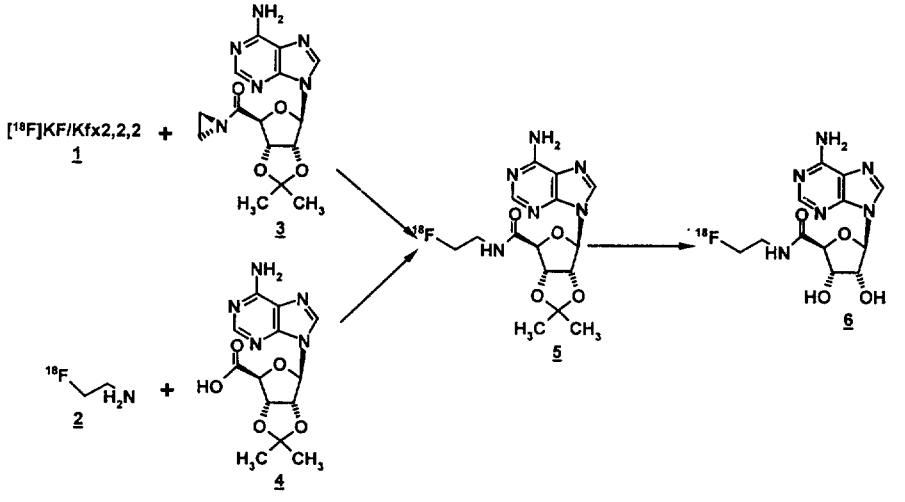

Figure 1. Reaction scheme for the synthesis of [^{18}F]FNECA

2',3'-O-Isopropylideneadenosine-5'-uronic acid(4)

9.0 g (57 mmol) $KMnO_4$ was dissolved in 250 ml water and added to the stirred solution of 5.8 g (19 mmol) of 2',3'-O-isopropylidene-adenosine and 3.2 g (57 mmol) KOH in 1250 ml water. The oxidation reaction took 3 days at room temperature to come to completion. The excess of oxidant was destroyed by adding 65 ml of 6.5% H_2O_2. The mixture was filtered through Celite, the filtrate concentrated to about 100 ml and acidified to pH 3 with 2 M HCl solution. The resulting precipitate was filtered and recrystallized from water to give 4.6 g (77%) of acid **4** as a white solid (mp 273-276 °C)

^{1}H-NMR (in DMSO-d_6) δ: 8.24(s, 1H), 8.10(s, 1H), 7.32(s, 2H), 6.36(s, 1H), 5.54(dd, 1H), 5.44(d, 1H), 4.72(d, 1H), 1.55(s, 3H), 1.35(s, 3H)

5'-N,N-Ethylene-2',3'-O-isopropylidene-carboxamidoadenosine (3)

0.5 g (1.56 mmol) of acid **4** was suspended in 5 ml of anhydrous pyridine containing 0.25 ml (1.56 mmol) of N,N'-diisopropylcarbodiimide (DICI) as the coupling agent. The suspension was stirred and 88 µl (1.56 mmol) of ethyleneimine (aziridine) was added dropwise. The stirring was continued until the solution became clear and TLC analysis (MeCN/Water=95:5) showed the reaction had been completed. The solution was evaporated to dryness and the solid was re-dissolved in cold water. After removing the formed N,N'-diisopropylurea, the solution was extracted with methylenechloride (3 X 10 ml). The combined CH_2Cl_2 extracts were washed with water, dried over $MgSO_4$ and evaporated to dryness. The residue was taken up in a small amount of methylenedichloride and used for column chromatography (eluted with MeCN/Water=95:5). Yield: 180 mg (35%) of imide **3** as a white foam.

^{1}H-NMR (in $CDCl_3$) δ: 8.35(s, 1H), 8.05(s, 1H), 6.37(s, 1H), 5.64(s, 2H), 5.45(m, 2H), 5.02(s, 1H), 4.24(m, 2H), 3.98(m, 2H), 1.45(s, 3H), 1.29(s, 3H)

5'-N-(2-Fluoroethyl)-2',3'-O-isopropylidene-carboxamidoadenosine (FIPNECA)(5)

0.32 g (1.0 mmol) of **4** was suspended in 5 ml anhydrous pyridine containing 0.16 ml (1.0 mmol) DICI and 0.1 g (1.0 mmol) 2-fluoroethylamine hydrochloride. The clear yellowish solution obtained after 2 hours mixing was evaporated to dryness and the partially solid residue was re-dissolved in water. The precipitated urea was removed and the filtrate was extracted with chloroform (3 X 10 ml). The combined chloroform extracts were washed successively with 2M HCl and distilled water, dried over $MgSO_4$ and evaporated to dryness. The residue was dissolved in chloroform and purified on a column of Silica gel eluted with MeCN/water=95:5, yielding 0.328 g (85.0%) of the title compound (mp 189-190.5°C).

^{1}H-NMR (in $CDCl_3$): δ 8.31(s, 1H), 7.87(s, 1H), 7.66(m, 1H), 6.23(s, 2H), 6.10(d, 1H), 5.40(m, 2H), 4.71(s, 1H), 4.28(m, 4H), 1.65(s, 3H), 1.38(s, 3H)

5'-N-(2-Fluoroethyl)-carboxamidoadenosine (FNECA)(6)

A solution of 0.2 g (0.55 mmol) **5** in 20 ml of 90% formic acid was kept at room temperature for 24 h, then evaporated to dryness. The partially crystalline residue was purified by column chromatography using CH_2Cl_2/MeOH=6:1 as eluent. Yield: 77 mg (48%), (mp 218-221°C, crystal rearrangement at 136-140°C)

^{1}H-NMR (in DMSO-d_6): δ 8.44(s, 1H), 8.24(s, 1H), 7.52(s, 2H), 6.00(d, 1H), 5.83(d, 1H), 5.60(d, 1H), 4.64(m, 2H), 4.37(dd, 1H), 4.17(m, 1H), 3.47(m, 4H)

Potassium-[^{18}F]fluoride/Kryptofix2,2,2 adduct(1)

Irradiated target water (0.1 ml) containing [^{18}F]fluoride ion (0.45-8.33 GBq, 12.1-225 mCi) was passed through an anion exchange column (DOWEX 1X8, 0.1g) and eluted with 1.0 ml 3.5 mg/ml potassium-carbonate solution to a reaction vessel previously loaded with Kryptofix2.2.2 (14 mg). After removing water by consecutive azeotropic distillation with acetonitrile under nitrogen flow at 80 °C the title adduct was obtained, and served as a powerful fluorinating agent in the following radiofluorination reactions.

2-[^{18}F]Fluoroethylamine(2)

2 was synthesised according to the modified procedure of Gilissen *et al* (8): 10 mg of N-[2-(p-toluenesulfonyloxy)ethyl]-phthalimide in 0.5 ml of anhydrous acetonitrile was added to the dry potassium-[^{18}F]fluoride/Kryptofix2,2,2 adduct (0.42-5.41 GBq, 11.3-146.2 mCi). The reaction mixture was incubated at 78 °C for 30 min and then evaporated to dryness. Finally 100 µl of hydrazine monohydrate was added to the residue to cleave the phthalyl protecting group (yield: 27±11%, n=23).

5'-N-(2-[^{18}F]Fluoroethyl)-2',3'-O-isopropylidene-carboxamidoadenosine ([^{18}F]FIPNECA)(5)

Method A

100 mg (0.29 mmol) of **3** in 2 ml of anhydrous DMF was loaded into a

vessel containing 291-858 MBq (7.86-23.2 mCi) of dry potassium-[^{18}F]fluoride/Kryptofix2,2,2 adduct. The reaction was allowed to proceed at 120 °C for 30 min with a successive evaporation of the reaction mixture to dryness under a stream of nitrogen (yield: 1±1%, n=7).

Method B

37-1924 MBq (1-52 mCi) 2-[^{18}F]fluoroethylamine was distilled into the suspension of 20 mg acid **4** in 1.0 ml of pyridine containing 25 µl of DICI, and the solution was kept at 60 °C for 40 min, then evaporated to dryness under a stream of nitrogen (yield: 94±13 %, n=17).

For the reliable identification of the radioactive product, it was co-eluted in each synthesis with inactive FIPNECA as reference material using an analytical RP-column with UV- and a radioactivity detector coupled in series using eluent system A as shown in Table 1.

5'-N-(2-[^{18}F]Fluoroethyl)-carboxamidoadenosine ([^{18}F]FNECA)(6)

To the former residue 1.0 ml of formic acid (90%) was added and incubated at 100°C for 15 min. The solution was evaporated under a stream of nitrogen at the same temperature and re-dissolved in 1.0 ml of water. The mixture was filtered by syringe filter (13 mm, Whatman) and the filtrate was purified by preparative reverse phase column (system A) and after evaporation under vacuum, the product was redissolved in 0.5 ml of saline (4.45-166.5 MBq, 0.12-4.5 mCi, n=5).

As in the case of compound **5**, the identification of the radioactive product was assured by co-eluting it with the inactive FNECA on RP-analytical column (system A).

RESULTS AND DISCUSSION

5'-N-(2-[^{18}F]Fluoroethyl)-carboxamidoadenosine ([^{18}F]FNECA), a promising ^{18}F-labelled adenosine agonist, has been prepared by two different

synthetic routes. The radiolabelling with [^{18}F]fluoride-ion was accomplished either in a direct reaction of the radioactive ion with a precursor containing an appropriate activated group to accept the fluoride or in a reaction with a small molecule capable of subsequently forming a covalent bond with the desired molecule or its precursor.

In the first approach the aziridine moiety of compound **3** reacted with [^{18}F]F$^-$ to form, after ring opening, the desired labelled compound **5** containing the corresponding 2-[^{18}F]fluoroethylamino group. According to our experimental data, this reaction, however, took place to only a very small extent: labelled compound **5** was obtained with a yield of 1±1% in terms of [^{18}F]fluoride activity. According to the TLC analysis of the radiofluorination reaction mixture, the precursor carrying the aziridine moiety remained mainly unreacted under the conditions applied: besides a large spot corresponding to the precursor, only some unidentified smaller spots could be detected on the TLC sheet in UV-light.

In the second route, 2-[^{18}F]fluoroethylamine was synthesised according to ref. (8) with a yield of 27±11 % based on [^{18}F]fluoride activity and distilled to a vessel containing the suitable precursor **4**, capable of accepting the 2-[^{18}F]fluoroethylamine moiety through an amide bond in the presence of the carbodiimide coupling agent. The yield of this coupling reaction was as high as 94±13 % based on the activity of the distilled 2-[^{18}F]fluoroethylamine. The acyl chloride derivative of **4** in pyridine had been also tested in the coupling reaction, however, the extent of the conversion had been lower, and not as reproducible as with the acid **4**.

The isopropylidene ring was removed by 90% formic acid almost quantitatively (97±5 %) to give **6**, our target molecule. The formic acid was removed under a stream of nitrogen, and the residue was redissolved in water and filtered off. As the filtrate contained a number of inactive side-products, it was purified by preparative reverse phase HPLC. The loss of radioactivity during filtration and preparative chromatography proved to be 30±9 % (n=5).

The overall decay corrected yields based on [^{18}F]fluoride activity were 1±1% (method A) and 17±9 % (method B), respectively, and the specific activity was determined to be from 1.04-3.11 TBq/mmol (28-84 Ci/mmol) at the end of synthesis. The second route provides sufficient [^{18}F]FNECA for the subsequent biological evaluation in PET studies.

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