

THESIS FOR PH.D. DEGREE

**SYNTHESIS OF FLUORINE-18 LABELLED
ADENOSINE-RECEPTOR LIGANDS**

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Debrecen, 2002**

INTRODUCTION

Adenosine appears to mediate quite a number of physiological functions. Many of its effects can be attributed to the action at receptors located on the cell surface. These *adenosine-receptors* are widely distributed throughout the body and are involved in complex regulatory systems, inflammatory and immunological responses, the cardiovascular system and pain. For a deeper understanding of its specific interactions in different physiological processes the use of adenosine receptor ligands may prove to be very instrumental.

Positron emission tomography has become a valuable tool for non-invasive investigation of basic biological and physiological processes. Biological structures, which exist in even as low as pico- or femtomolar concentration range can be easily studied *in vivo* in laboratory animals by PET technique.

Imaging the adenosine-receptors by PET requires selective ligands of high specific activity labelled with a positron-emitting isotope. The small number of the available radiolabelled molecules of the required specificity limits experimentation supported by such ligands. Another drawback is that all PET isotope conjugated adenosine-receptor ligands belong to the group of antagonists.

Being aware of the significance of appropriate adenosine-receptor ligands in clearing up the detailed mechanisms of physiological role of adenosine, we decided to develop adenosine-receptor agonists labelled with a positron-emitting isotope.

Of the most frequently used PET isotopes, carbon-11, nitrogen-13, oxygen-15 and fluorine-18, we chose the latter one as its relatively long half life (110 min) offers a wide scale of experimental protocols as compared to those of the other three short-living (2-20 min) radionuclides. Fluorine-18 permits multistep syntheses and study of moderately slow biochemical processes.

This thesis presents a study of introduction of fluorine-18 isotope to the designed position of molecules of adenosine type. The thesis also describes different strategies to synthesise precursors serving as suitable starting material in the radiosynthesis. As a contribution to the biological evaluation of the radioligands synthesised with sufficient yield, results of biodistribution studies are also summarised.

BACKGROUND AND SUMMARY OF PRECEEDING RESEARCH

Positron Emission Tomography

Positron emission tomography (PET) is a non-invasive biomedical imaging technique, whereby the distribution of biological tracer molecules, labelled by positron emitting isotopes, in the living body can be studied quantitatively. Nowadays it is widely used in the clinical practice, and has proven to be a unique advantageous diagnostic technique in neurology, psychiatry, cardiology and oncology.

Hungary's and the Central European region's first PET centre was established at the University of Medical School of Debrecen in 1994.

PET isotopes

Isotopes used in PET technique should comply with the requirements listed bellow:

- short half-life
- clear β^+ -decay (no α - or β^- -radiation is permitted)
- quantity of γ -photons having energy of other than 511 keV should not exceed 0.1 %

The four medically relevant PET isotopes are carbon-11, nitrogen-13, oxygen-15 and fluorine-18. Among them fluorine is the most favourable isotope of PET, and it is also the isotope used in this study.

Receptor studies with PET

Imaging of receptors, in general, requires selective ligands of high affinity for the receptor to be able to distinguish specific binding to the receptor and unspecific binding to background structures. Moreover, the investigation of different receptor systems by PET requires ligands labelled with β^+ -emitting isotope. By means of this type of radioligands, biological structures existing in even as low as pico- or femtomolar concentration range can be easily investigated *in vivo* in laboratory animals by PET technique. As each practically important PET isotope is cyclotron-isotope of relatively short half life, radiochemical synthesis with incorpo-

ration of the radionuclide into molecule of interest will result in compounds with several orders of magnitude higher specific activity than that of molecules labelled with ^{14}C or ^3H .

Adenosine-receptors

Adenosine receptors are members of a receptor family called purinoseptors (P) with endogenous ligands of adenosine, AMP, ADP and ATP. Purinoseptors can be subdivided into two types, P_1 and P_2 on the base of the preferred agonist: P_1 for adenosine and P_2 for adenine nucleotides. Thus, P_1 receptors are identical with *adenosine receptors*. Adenosine receptors (or P_1 purinoseptors) are divided into four subtypes, A_1 , A_{2A} , A_{2B} and A_3 . Each of them is coupled via a G-protein to the cyclic AMP-producing enzyme *adenylate cyclase*. All receptor subtypes have been recently cloned from different species (including humans), thus they can be solely investigated by using artificially constructed systems.

From the point of view of ligands: for A_1 and A_{2A} and A_3 both selective agonists and antagonists are available, whereas for A_{2B} receptor, to the author's knowledge, no selective ligands have been reported so far.

Agonists

The only natural ligand so far identified for adenosine receptors is the adenosine itself; modification of adenosine at N^6 , 2- and 5'-positions has led to ligands by which adenosine receptors could be classified. N^6 -substituted adenosines have generally proven to be A_1 selective (for example N^6 -cyclopentyl adenosine, CPA). Removal of either 2'-OH or the 3'-OH, however, leads to partial agonists with reduced affinity. The 5' substituted analogues, *e.g.* NECA is a non-selective adenosine receptor ligand: it is approximately equipotent at all subtypes. The 2-modified (*e.g.* alkoxy-) adenosines, conversely, are reported to be potent and selective A_2 agonists. A_3 selective agonists can be synthesised by combining the N^6 , 2- and 5'-modifications.

Antagonists

The prototypic adenosine receptor antagonists were xanthines, theophylline and caffeine. Since then numerous xanthine derivatives have been synthesised and evaluated as adenosine receptor antagonist. Among them some 8-styryl caffeine derivatives were proven to be high affinity antagonists specific to A_{2A} type adenosine receptors.

Fluorine and its isotopes

Fluorine, the most electronegative of all chemical elements stands at the head of the halogen family in group VIIA of the Periodic Table. Fluorine atoms possess the electronic configuration $1s^2 2s^2 2p^5$ and are strictly univalent.

The naturally occurring fluorine is mononuclidic, having isotope of mass number 19 and six artificial fluorine isotopes have additionally been produced, each of them is radioactive.

Among the radioisotopes of fluorine, fluorine-18 is the most favourable isotope of PET, partially of its relatively long half-life (109.7 min) that permits multistep syntheses and study of biochemical processes, which are moderately slow. Its low β^+ -energy is advantageous from imaging point of view. It is also the radionuclide used in this study.

Fluorine-18 can solely be produced in accelerators, mainly in cyclotrons. Among the nuclear reactions have been used, the following two reactions are of major practical importance, in view of their requirements for moderate beam energy and current:

- (1) Carrier added (*c.a.*), low specific activity ^{18}F -fluorine gas is usually produced in $^{20}\text{Ne}(d,\alpha)^{18}\text{F}$ nuclear reaction using Ne/F_2 (v/v 99.9/0.1) mixture as target material.
- (2) Non carrier added (*n.c.a.*), high specific activity ^{18}F -fluoride is generally produced by $^{18}\text{O}(\text{p},\text{n})^{18}\text{F}$ reaction. The target material is ^{18}O -enriched water.

Radiofluorination

As fluorinated biochemicals are not present in biological systems, introduction of fluorine-18 frequently results in labelled molecules of altered biochemical behavior. Thus, the labelling strategy should be directed toward the position that will have as little effect as possible on the characteristics of the parent molecule.

Two most common substitutions employed for making ^{18}F -labelling are F for -H and F for -OH. In view of the original electron density for the molecule to be labelled the substitution F for -OH, whilst considering the size of the fluorine, the F for H seem to be better approach.

Nucleophilic radiofluorination

Incorporation of fluorine into organic molecules by nucleophilic substitution with the fluoride ion has still remained a difficult area. The small size of fluoride-ion and its low polarizability determines F^- to behave as a base rather than nucleophile. This is the reason why under usual substitution conditions the most common reaction of the fluoride ion is elimination.

Fluorine is strongly solvated in aqueous or protic solvents due to its tendency to form hydrogen bonds. This is the reason why nucleophilic radiofluorination reactions are conducted in dipolar aprotic solvents, such as CH_3CN , DMF or DMSO. As the solubility of $[\text{}^{18}\text{F}]\text{F}^-$ is rather low in these aprotic solvents, appropriate phase transfer catalyst is needed. A widely used phase transfer catalyst in radiofluorination is the Kryptofix 2.2.2 of kryptand type.

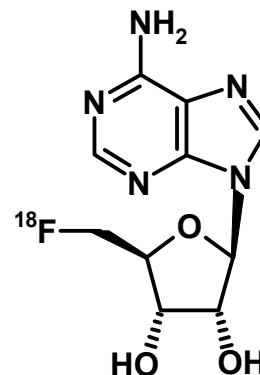
GOAL OF THIS WORK

The number of adenosine-receptor ligands labelled with positron-emitting radionuclides for PET investigation is limited; in addition, each of them is antagonist. Similarly, at our institute, merely some $[\text{}^{11}\text{C}]$ -labelled caffeine derivatives, as antagonists, have been at disposal to investigate adenosine receptor so far. Thus, synthesis of labelled agonists is needed for a deeper understanding of biochemical phenomena related to the adenosine receptor-system.

Fluorine-18 was chosen as labelling isotope, partially because of its relatively long half-life.

Fluorine-18 is available at our institute in [^{18}F]fluoride ion form, which restricts our tools of fluorination, thus we had to plan our reaction to introduce fluorine-18 in designated position merely in nucleophilic radiofluorination. The nucleophilic agent is the [^{18}F]fluoride itself promoted by KryptofixTM2.2.2 as phase transfer catalyst.

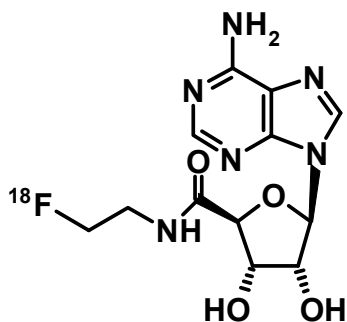
Finally, two molecules to be synthesised were chosen; both of them can be regarded as a model compound. Working out their synthesis is the key to producing series of fluorine-18 labelled specific adenosine receptor ligands by modified labelling procedures.



Our aim was to synthesise 5'-deoxy-5'-[^{18}F]fluoroadenosine and 5'-N-(2-[^{18}F]Fluoroethyl)-carboxamidoadenosine, and the preliminary investigation of biological behaviour of the latter one.

5'-deoxy-5'-[^{18}F]fluoroadenosine can possibly act as a suitable model-compound to synthesise fluorine-18 labelled ligands for investigating the adenosine receptor system by PET technique; the modification of the adenine moiety leads to increased selectivity towards receptor subtypes, for example N⁶-alkyl and cycloalkyl derivatives are the most selective A₁ receptor agonists.

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 some A_{2B}



receptor specificity. Furthermore, the modification of the synthesis route by labelling adenosine derivatives substituted at C2 or N⁶ position can result in ^{18}F -labelled receptor ligands of A_{2A} and A₃ selectivity, respectively. Affinity of NECA to the A₁, A_{2A} and A₃ receptor subtypes is almost equipotent, and can be characterised by dissociation

constants in nM range, thus all three subtypes can be investigated with [^{18}F]FNECA labelled ligand *in vivo*. The given receptor subtype can be independently studied, if the binding sites of other subtypes are blocked by highly selective non-radioactive antagonists.

EXPERIMENTAL

Cyclotron and targetry

In all the experiments during this study the MGC-20-E isochronous cyclotron of ATOMKI (Institute of Nuclear Research of the Hungarian Academy of Sciences) was used for producing fluorine-18 radionuclide. The cyclotron and the beam transport system were manufactured by D. Efremov Scientific Institute of Electrophysical Apparatus (NIEFA) in St. Petersburg.

Automated radiofluorination system

All the radiofluorination experiments were performed on a computer-assisted automated synthesis panel. The most important parts of the panel used during the experiments of this thesis are as follows:

- (1) Irradiated water delivery system
- (2) Module for the rapid separation of [^{18}F]fluoride from ^{18}O -enriched water
- (3) Reaction vessel equipped with thermometers and radioactivity detector

Analytical methods

Analytical methods used in this study are mainly spectroscopic and chromatographic methods. Taking the short half-life of fluorine-18 into consideration, practical and fast analysis methods are required. Furthermore, nuclides, such as fluorine-18, exist in carrier-free form, *i.e.* with a concentration of only around 10^{-9} - 10^{-11} M. This means that their compounds can only be

determined by gamma radiation. This detection was also used in case of radioactive materials in this thesis during HPLC and TLC analysis, as well as monitoring the radioactive materials during transportation and distillation.

Chemistry

^{18}F nuclide was produced directly as [^{18}F]fluoride-ion in target by the MGC20E cyclotron at the Institute of Nuclear Research, Debrecen from $^{18}\text{O}(\text{p},\text{n})^{18}\text{F}$ nuclear reaction, with 14.5 MeV proton beam.

Solvents and chemicals (p.a. grade) were obtained from Merck (Darmstadt, Germany) or Sigma-Aldrich (Gillingham, England) and Reanal (Hungary). [^{18}O]-enriched water was used as target material purchased from CAMPRO Scientific, The Netherlands. All the adenosine related compounds were synthesized at Debrecen PET Centre starting from adenosine.

Biology

The investigations were carried out in compliance with the relevant national laws relating to the conduct of animal experimentation.

Autoradiography (ARG): for in vitro ARG investigation *mouse* brain and heart sections were prepared with V-3000-R/A Vibrotome microtom (Energy Beam Sciences Inc.) and was stored at $-20\text{ }^{\circ}\text{C}$ till use. The specific binding was proven in competition experiments. In case of control labelling, the brain- and heart sections were incubated in physiological salt solution ($\text{pH}=7.2$) of 100 nM ligand-concentration in presence of 20 $\mu\text{Ci}/\text{ml}$ [^{18}F]FNECA. In the competition experiments conducted in parallel, the incubation solution contained 100 μM unlabelled (*cold*) FNECA in addition to 20 μCi [^{18}F]FNECA. The incubation time was 20 min in both cases. Subsequently, sections were washed three times with cool physiological salt solution. The sections were then placed on the plates of Phosphor Imager (Molecular Dynamics), and the ^{18}F activity concentration distribution was determined after 2 hours incubation time.

PET scanning: Average mass of domestic rabbits used for the experiments was 2.1 ± 0.2 kg. The animals were anaesthetised by i.p. injection of urethane (0.5 g/kg body weight) and alfa-chloralose (50 mg/kg body weight) i.p., and one of femoral artery and femoral vein were cannulated. For PET-investigation 0.5-1 mCi/30-60nmol [^{18}F]FNECA radiopharmakon was injected intravenously in 2 ml physiological saline as a 20-30 s bolus. Dynamic PET scans were carried out using a GE 4096 plus PET camera. Fifteen saggital sections were recorded which includes the hole body of the rabbit. Simultaneously, with the PET scan *ca.* 20 arterial blood-samples were taken by syringes treated with heparin previously. Blood taken was centrifuged for 3 minutes with 2000 g, then the radioactivity-concentration was measured by calibrated gamma counter (Canberra-Packard) in plasma, as well as in sediment (cells). Transmission scans for correcting the tissue attenuation were carried out by an inner ^{68}Ge source. For the determination of accumulation kinetics of chosen organs, radioactivity indicating the [^{18}F]FNECA quantity was determined in the same volume on the subsequent PET images.

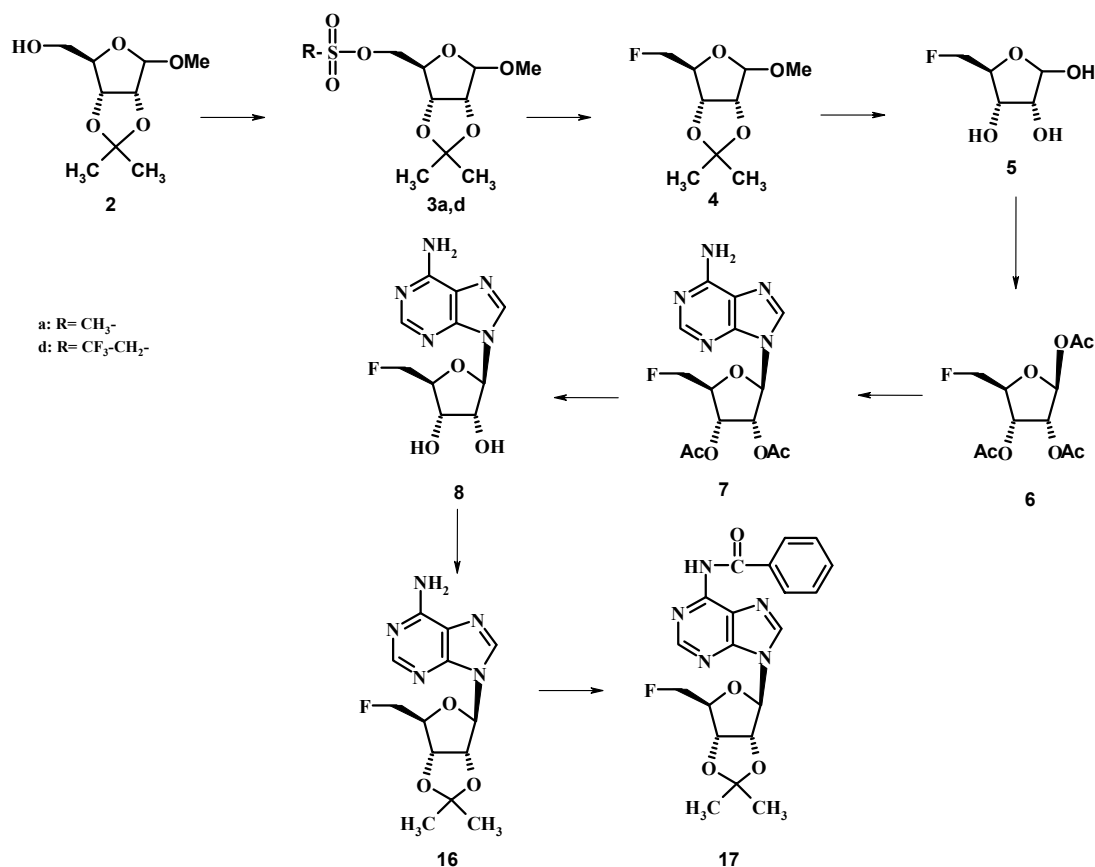
RESULTS AND DISCUSSION

5'-deoxy-5'-fluoro-adenosine

Inactive 5'-deoxy-5'-fluoro-adenosine **8**, the reference material, was synthesised in a long and tedious route, as shown in Scheme 1.

The synthesis started with a suitably protected ribose derivative **2**, which was mesylated in usual manner to obtain **3a**, as crystalline solid. **3a** was then treated with potassium fluoride in methanol at 160°C for 24 hours to obtain fluoride-riboside **4** as analytically pure oil. Alternatively, **4** was obtained through trezylate **3d**. Acid hydrolysis of **4** gave the free sugar **5** as pure, light oil, which was characterized by $^1\text{H-NMR}$. Finally, acetylation of the free sugar **5** with acetic anhydride in pyridine afforded **6** as a crystalline solid. Triacetate **6** is a very important intermediate; it served as a starting material of the key-step of the synthesis for 5'-deoxy-5'-fluoro-adenosine **8**, namely the nucleoside condensation reaction, in which the adenosine struc-

ture formed. Although these condensation reactions are well characterised and thoroughly reviewed, merely the modification of the usual TMSTf-method could give the desired diacetyl-adenosine derivative **7** in a moderate yield; the essence of the modified method was the use of *unprotected* silylated adenine. Lastly, deprotection of **7** in Zemplen's reaction afforded **8**, as highly hygroscopic white crystalline solid, which was characterised by $^1\text{H-NMR}$.



Scheme 1. Synthesis of inactive 5'-deoxy-5'-fluoro-adenosine (and *N*⁶-benzoyl-5'-deoxy-5'-fluoro-2',3'-isopropylideneadenosine)

5'-Deoxy-5'-[^{18}F]fluoro-adenosine, this ^{18}F -labelled adenosine analogue has been prepared in nucleophilic substitution reaction starting from two different precursor families; the nucleophilic agent is, in both cases, the [^{18}F]fluoride itself promoted by KryptofixTM2.2.2 (aminopolyether 2.2.2) as phase transfer catalyst.

Synthesis starting from 5'-deoxy-5'-haloadenosines

An easy and promising route for the production of these compounds seemed to be the nucleophilic substitution starting from 5'-halogenated adenosines. In particular bromine and iodine, have been widely used as leaving groups in aliphatic substitutions. For the brief description of this compound family, 5'-chloride and 5'-fluoride (homogeneous isotope exchange) were also investigated.

We found that 5'-deoxy-5'-haloadenosines can be transformed into 5'-deoxy-5'-[¹⁸F]fluoro-adenosine in radiofluorination reaction at 120 °C using [¹⁸F]KF/Kryptofix2.2.2 as nucleophilic agent in dimethylformamide (DMF). The synthesis time for radiofluorination reaction was 30 min. Under these conditions no reaction was observed, but the desired halogen exchange. The components of the radiofluorination reaction mixture were identified by their R_f values.

The table below lists the radiochemical yields of the 5'-deoxy-5'-[¹⁸F]fluoro-adenosine production in the end of synthesis. Each data point is the average value of three independent measurements.

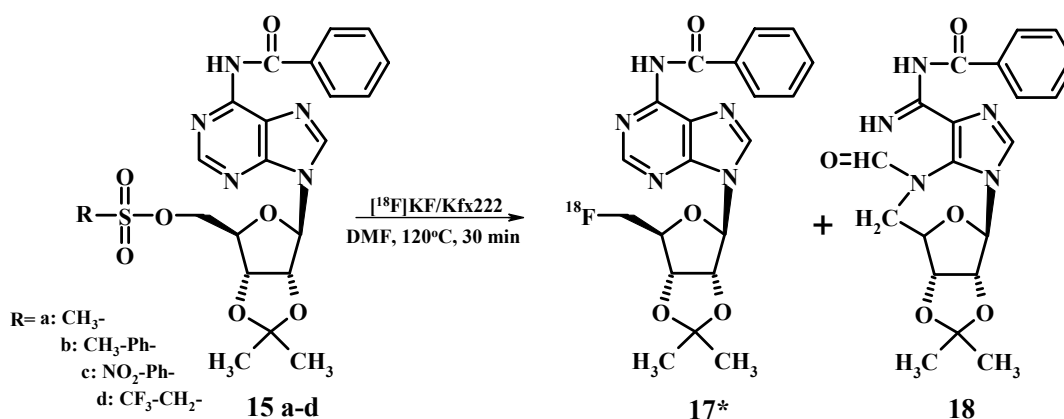
Precursor	Precursor mmol/ml DMF	Precursor mg	Yield %
FAdo	0.13/2	35	0
ClAdo	0.13/2	38	0.25
BrAdo	0.13/2	44	0.49
IAdo	0.13/2	50	1.07

In the homogeneous fluorine isotope exchange reaction, no radioactive peak was detected apart from the starting [¹⁸F]KF/Kryptofix2.2.2 adduct at R_f 0.00. In other cases, fluorine-18 uptake was found as indicated by another radioactive peak at constant R_f value (depending, of course, on the solvent system applied). The reactivity of the precursors corresponds to the I>Br>Cl order; however, the extent of the halogen-fluorine-18 exchange was found to be un-

expectedly low in all cases. This may be attributed to the solvating effect of proton for amino function that considerably reduces the nucleophilicity of the naked ^{18}F -anion.

Synthesis starting from N^6 -benzoyl 2',3'-isopropylideneadenosine-5'-sulfonates

As our first attempt to synthesise 5'-deoxy-5'-[^{18}F]fluoro adenosine starting from unprotected 5'-haloadenosines was proved to be of insufficient yield, we were interested in the radiosynthesis of our goal-molecule in another way, namely starting from the suitably protected 5'-sulfonate derivatives **15a-d**. These compounds *i.e.* N^6 -benzoyl 2',3'-isopropylideneadenosine-5'-sulfonates, including methane-(mesylate), *p*-toluene-(tosylate), *p*-nitro-benzene-(nosylate)-and 2,2,2-trifluoro-ethanesulfonate (tresylate) derivatives, seemed to be suitable precursors to react with naked [^{18}F]fluoride ion to form the corresponding 5'-[^{18}F]fluoro compound **17***. Hydrolysis of this intermediate molecule would give 5'-deoxy-5'-[^{18}F]fluoro adenosine **8***, our target-molecule.



Scheme 2. Reaction of [^{18}F]KF/Kryptofix 2.2.2 toward N^6 -benzoyl 2',3'-isopropylideneadenosine -5'-sulfonates

The isopropylidene group leaves the 5'-OH group free, where the sulfonyl group can be introduced. The acylation of N^6 amino functional group of the adenine moiety, however, drastically decreases the nucleophilicity of the N^3 nitrogen atom.

Moreover, the reactivity of the sulfonyl group has, in general, a profound effect on the product of the reaction: the more reactive the sulfonyl group, the quicker the substitution, and

the formation of common side product *i.e.* elimination is restricted. For this reason we tried to synthesize 5'-trifluoromethane-sulfonate (triflate), one of the most reactive sulfonate. However, despite of all effort we obtained directly **18** from the reaction mixture. This observation referred to the fact that the N³ is still nucleophilic enough to form the N³,5'-cyclonucleoside. Thus, we synthesized sulfonates **15.a-d**, among which only mesylate (**15.a**) and tosylate (**15.b**) proved to be stable enough and could be stored for considerable time under ambient conditions. These four sulfonates served as precursors in the radiofluorination reactions.

The radiofluorination reactions were carried out under usual conditions. The common radioactive product was identified by comparing its R_F-value with that of the authentic inactive material on TLC. The TLC analysis showed that the same radioactive compounds appeared in each reaction. In addition, TLC analysis indicated the appearance of the same inactive product in each reaction, which identified to be compound **18**.

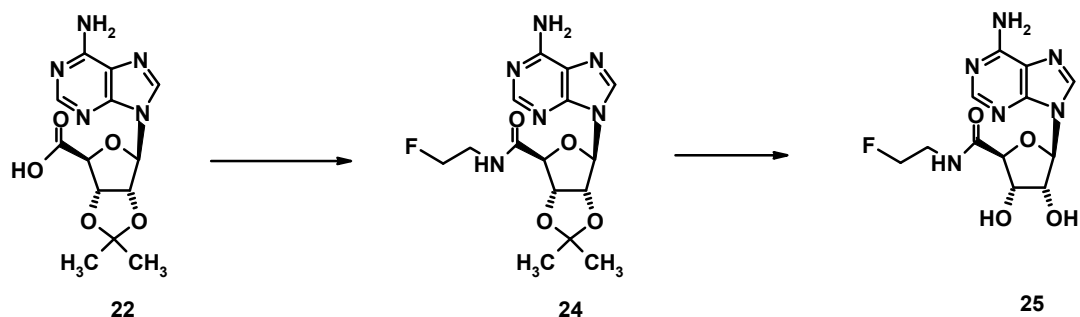
The table below lists the radiochemical yields for the 5'-deoxy-5'-[¹⁸F]fluoro-adenosine product at the end of synthesis. Displayed data represent average values of three independent measurements.

Precursor	Precursor mmol/2ml DMF	Precursor mg	Yield %
Mesylate	9.2	45.0	1.17
Tosylate	9.2	52.1	1.46
Nosylate	9.2	56.2	0.99
Tresylate	9.2	51.3	0.40

These results clearly show that the radiofluorination took place only to some extent; the fluorine-18 uptake was found to be rather low; the main product was proven to be **18**, the cyclized compound.

FNECA

Inactive 5'-N-(2-fluoroethyl)-carboxamidoadenosine **25**, the reference material, was synthesised starting from acid **22** and hydrochloride salt of 2-fluoroethylamin in pyridine, and the hydrolysis of **24** gave **25**, as shown in scheme 3. Its structure was confirmed by ^1H -NMR.

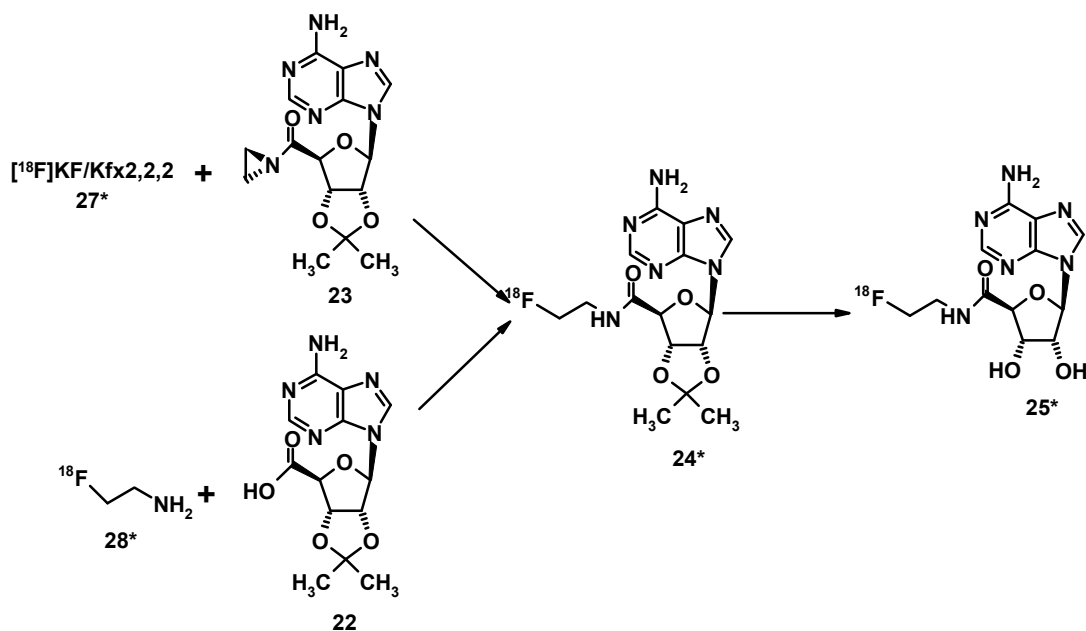


Scheme 3. Synthesis of non-radioactive FNECA

5'-N-(2-[^{18}F]Fluoroethyl)-carboxamidoadenosine ([^{18}F]FNECA), this promising ^{18}F -labelled adenosine agonist has been prepared in two different synthesis routes. The radio-labelling 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 forming subsequently a covalent bond with the desired molecule or its precursor. The routes of this reaction are summarised in Scheme 4.

Synthesis starting from precursor of aziridine type

In first approach, the aziridine moiety of compound **23** was reacted with [^{18}F] F^- to form, after ring opening, the desired labelled compound **24**^{*} containing the corresponding 2-[^{18}F]fluoroethylamino group. This reaction, however, took place to only a very small extent: labelled compound **24**^{*} was obtained with a yield of $1\pm 1\%$ in terms of [^{18}F]fluoride activity.



Scheme 4. Synthesis of 5'-N-(2-[¹⁸F]fluoroethyl)-carboxamidoadenosine in two different ways

Synthesis through 2-[¹⁸F]fluoroethylamine

In the second route, 2-[¹⁸F]fluoroethylamine **28*** was synthesized according to the literature with a yield of 27±11 % based on [¹⁸F]fluoride activity, and distilled to a vessel containing the suitable precursor **22**, capable of accepting the 2-[¹⁸F]fluoroethylamine moiety through an amide bond in the presence of the DICI, a carbodiimide coupling agent.

The yield of this coupling reaction was as high as 94±13 % based on the activity of the distilled 2-[¹⁸F]fluoroethylamine.

The isopropylidene ring was removed by 90 % formic acid almost quantitatively (97±5 %) to give **25***, our target molecule. The formic acid was removed under a stream of nitrogen, and the residue was re-dissolved in water and filtered off. As the filtrate contained lots 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).

This second route provides sufficient amount of [¹⁸F]FNECA for the subsequent biological evaluation in PET studies.

Biological studies

In vitro autoradiographic investigations were carried out to map the A₁ receptor distribution in the mouse brain and heart. High accumulation of the radiolabelled ligand was observed both on brain slices and heart slices. Particularly high [¹⁸F]FNECA uptake was detected in cortex, in hippocampus and in cerebellum. The pattern of brain slices mainly shows the A₁ receptor-distribution of the brain, because, among P₁ receptors expressed in brain, the A₁ receptor possesses the highest density. Receptor-ligand specific binding is supported by the competition experiments conducted, in which, in the presence of predominate non-radioactive “cold” FNECA the [¹⁸F]FNECA accumulation equivalent to a fraction of the control value was observed.

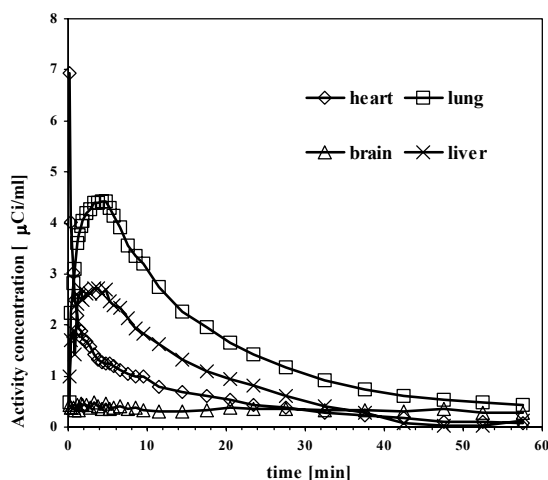


Figure 1. Dynamic ¹⁸F-NECA-accumulation in the organs of rabbit

On the basis of analysis of the artery blood samples taken during the dynamic PET investigation it was concluded that the quantity of the radiopharmaceutical in the blood serum and the [¹⁸F]FNECA concentration reach a maximum at approx. 2 minutes, then tend to decrease with a half-life of approx. 10 minutes.

Agonist [^{18}F]FNECA labelled with positron emitting isotope due to its specific feature discussed briefly above is capable of mapping the distribution for adenosine-receptors of P_1 type, as well as investigating of receptor-regulation processes *in vivo*.

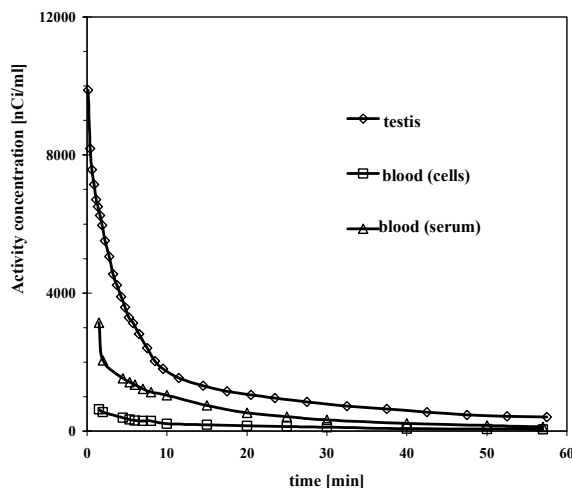


Figure 2. Dynamic PET scan of rabbit – ^{18}F -NECA-accumulation in the blood

CONCLUSIONS

Synthesis of labelled adenosine-receptor agonists is essentially needed for the deeper understanding of biochemical phenomena related to adenosine receptor system. Two molecules to be synthesised were chosen; both of them can be regarded as a model compound: working out their synthesis is the key to producing series of fluorine-18 labelled specific agonist adenosine-receptor ligands by modified labelling procedures.

The first molecule is 5'-deoxy-5'-[^{18}F]fluoro adenosine, which has been prepared in two synthetic routs.

The first attempt to synthesise this labelled molecule was starting from 5'-deoxy-5'-haloadenosines in the halogen exchange reactions. The conversion (*that is* fluorine-18 uptake), however, proved to be rather low, and depended on the strength of the halogen-carbon bond:

0.248 % for the chloride-, 0.488 % for the bromide- and 1.070 % for the iodide-derivative; there was no reaction observed in the case of the fluoro-compound.

In the second route we start from N⁶-benzoyl 2',3'-isopropylideneadenosine-5'-sulfonates. The results obtained showed that fluorine-18 could be introduced into this type of compounds, although also with very low yield: the fluorine-18 uptake was found to be 1.17 % for mesylate, 1.46 % for tosylate, 0.99 % for nosylate and 0.40 % for tresylate.

On the basis of results obtained we concluded that 5'-deoxy-5'-[¹⁸F]fluoro-adenosine can be synthesised merely in small amount starting from a precursor of adenosine structure. Either a new and tedious synthetic route should be worked out including labelling the sugar moiety with fluorine-18 first, and the subsequent coupling reaction with the adenine moiety would give the 5'-[¹⁸F]fluoride derivative, or we should resign to label adenosine at that (5') position and the radioisotope should be introduced into the adenine moiety of a suitable precursor of adenosine-type.

The second goal-molecule, namely 5'-N-(2-[¹⁸F]fluoroethyl)-carboxamidoadenosine ([¹⁸F]FNECA) has also been prepared in two different synthesis routes.

In the first way, [¹⁸F]fluoride ion was reacted with 5'-N,N-ethylene-2',3'-O-isopropylidene-carboxamido-adenosine and after removing the protective group, [¹⁸F]FNECA was obtained in a low radiochemical yield (1±1 %).

In the second route, 2-[¹⁸F]fluoroethylamine was reacted with 2',3'-O-isopropylideneadenosine-5'-uronic acid in the presence of a carbodiimide coupling agent, and the subsequent hydrolysis step provided the [¹⁸F]FNECA with a modest radiochemical yield. After purification by preparative RP-HPLC, 18.9-166.5 MBq (0.51-4.5 mCi) [¹⁸F]FNECA was obtained with a specific activity of 2.35±1.14 TBq/mmol (63.5±30.9 Ci/mmol). The total synthesis took 200 min and the decay-corrected radiochemical yield based on [¹⁸F]F⁻ activity was 17±9 % with more than 99.9 % radiochemical purity. This second route provides sufficient amount of [¹⁸F]FNECA for the subsequent biological evaluation in PET studies.

According to the primary biological studies [^{18}F]FNECA seems to be a very promising radiopharmaceutical for mapping adenosine receptors of the P_1 type in various tissues. Studies both with non-radioactive FNECA and labelled [^{18}F]FNECA have been in progress at Debrecen PET Centre.

PUBLICATIONS

Papers which the thesis based on

1. **Sz. Lehel**, G. Horváth, I. Boros, P. Mikecz, T. Márián, L. Trón: *Synthesis of 5'-deoxy-5'-[¹⁸F]fluoro-adenosine by radiofluorination of 5'-deoxy-5'-haloadenosine derivatives*, **Journal of Radioanalytical and Nuclear Chemistry**, Vol. **245**, No. **2**(2000) **399-401** (Impact factor: 0.605)
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