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

Ph.D Thesis
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Synthesis of Fluorine-18 Labelled Adenosine-Receptor Ligands

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ABSTRACT

This thesis presents a study of introduction of fluorine-18, a positron-emitting radioisotope to the designed position of molecules of adenosine type. This thesis also includes strategies of synthesis of precursors, into which the fluorine-18 isotope, either in fluoride ion form or as a suitable fluorine-containing small molecule, can be introduced. The synthesized labelled molecules make the investigation of adenosine-receptors by PET technique possible. Preliminary biological evaluation of one of these molecules, namely 5'-N-(2-[¹⁸F]fluoroethyl)-carboxamidoadenosine is also presented.

Two molecules were chosen to synthesise their fluorine-18 labelled forms: *5'-deoxy-5'-fluoro-adenosine* and *5'-N-(2-fluoroethyl)-carboxamidoadenosine*. Both compounds were labelled in two ways, each synthetic route includes nucleophilic fluorination with [¹⁸F]fluoride ion.

5'-Deoxy-5'-[¹⁸F]fluoro-adenosine would serve as a suitable model-compound for the synthesis of fluorine-18 labelled ligands; the appropriate modification of the adenine moiety leads to increased selectivity towards the chosen receptor subtype. As the radiochemical yields proved to be rather low, using either 5'-deoxy-5'-haloadenosines or N⁶-benzoyl 2',3'-isopropylideneadenosine-5'-sulfonates as precursors, we had to resign to label adenosine at that (5') position. Although N⁶-benzoyl 2',3'-isopropylideneadenosine-5'-tosylate, one of these sulfonates was not proven to be a suitable precursor in the radiofluorination reactions, it served as an appropriate starting material in radioiodination reactions.

5'-N-(2-fluoroethyl)-carboxamidoadenosine ([¹⁸F]FNECA), as a fluorine-18 labelled analogue of NECA represents a PET isotope labelled agonist with given adenosine receptor specificity. It was synthesised in the following ways: in the reaction of [¹⁸F]fluoride with 5'-N,N-ethylene-2',3'-O-isopropylidene-carboxamidoadenosine as well as by reacting 2-[¹⁸F]fluoroethylamine with 2',3'-O-isopropylideneadenosine-5'-uronic acid. This latter route provides sufficient [¹⁸F]FNECA for the subsequent preliminary biological evaluation using PET-technique.

According to the preliminary biological evaluation, agonist [¹⁸F]FNECA labelled with positron emitting isotope is capable of mapping the distribution for adenosine-receptors of P₁ type, as well as investigating of receptor-regulation processes *in vivo*.

KEY WORDS: fluorine-18, PET, adenosine receptor ligands, NECA, FNECA, nucleophilic radiofluorination

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THIS THESIS IS BASED ON THE FOLLOWING PAPERS:

Paper I. 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

Paper II. Sz. Lehel, G. Horváth, I. Boros, P. Mikecz, J. Szentmiklósi, T. Márián, L. Trón: *Synthesis of 5'-N-(2-[¹⁸F]Fluoroethyl)-carboxamido-adenosine: a Promising Tracer for Investigation of Adenosine Receptor System by PET Technique*, **Journal of Labelled Compounds and Radiopharmaceuticals**, 43(2000) 807-815

Paper III. Sz. Lehel, G. Horváth, I. Boros, L. Trón: *Investigation for the Nucleophilic Substitution Reaction of [¹⁸F]fluoride ion on the Series of N⁶-Benzoyl 2',3'-Isopropylidene-Adenosine-5'-Sulfonates*, **Journal of Radioanalytical and Nuclear Chemistry**, Vol. 251, No. 3(2002) 413-416

Paper IV. Márián T., Lehel Sz., Lengyel Zs., Balkay L., Horváth G., Mikecz P., Miklovicz T., Fekete I. és Szentmiklósi A. J.: *A [¹⁸F]-FNECA széleskörűen alkalmazható radioligands a purinerg receptorexpresszió PET-vizsgálatához*, **Orvosi Hetilap** 143(21)(2002) Suppl. 3 1319-1322

Publications included partly in this thesis:

Paper V. I. Boros, G. Horváth, Sz. Lehel, T. Márián, Z. Kovács, J. Szentmiklósi, G. Tóth, L. Trón: *[¹¹C]-labelling of some caffeine derivatives for mapping adenosine A_{2a} receptors by PET technique*, **Journal of Radioanalytical and Nuclear Chemistry**, Vol. 242, No. 2(1999) 309-312

Paper VI. Mikecz P., Tóth Gy., Horváth G., Lehel Sz., Kovács Z., Pribóczki É., Boros I., Miklovicz T. és Márián T.: *Radiológyszerek előállítása pozitronemissziós tomográfiás vizsgálatokhoz*, **Orvosi Hetilap** 143(21)(2002) Suppl. 3 1240-12421.

1. 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 existing 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.

2. BACKGROUND AND SUMMARY OF PRECEEDING RESEARCH

2.1. 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 quantitatively¹. 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 1994².

2.1.1. Essence of PET

The main steps of a PET investigation are as follows:

- Labelling the selected compound with positron-emitting radionuclide
- Administering this labelled compound to the subject to be studied
- Imaging the distribution of radioactivity as the function of time
- Eliciting the nature of the biomedical phenomenon³

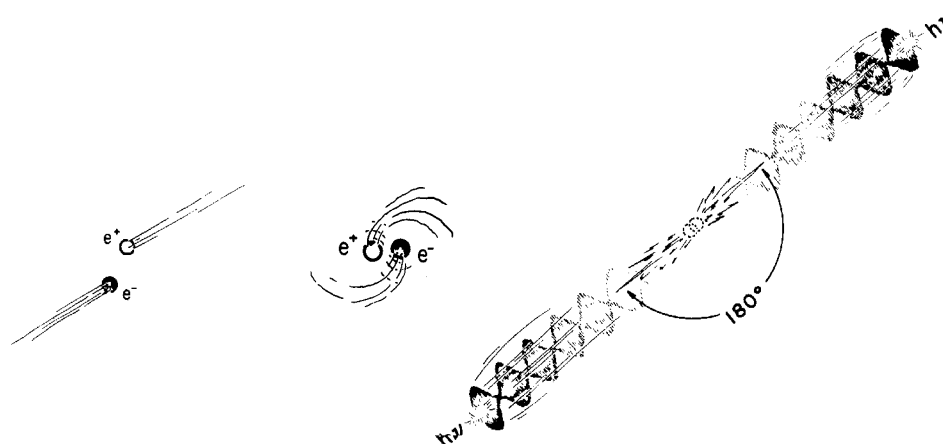


Figure 1. Annihilation of positron in matter (ref. 3)

A positron (β^+) emitted from a decaying nucleus travels a short distance before colliding with an electron of a nearby atom. The positron emitted loses its kinetic energy in matter in a manner similar to that of a *normal* electron: mostly exchanges energy with electrons causing ionisations and excitations. Consequently, the distance that a positron travels depends on its energy⁴. After positron is brought to rest, it combines with an ordinary electron of a nearby atom, forming an atom-like structure called positronium as an intermediate. (Positronium has a half-life of 10^{-7} s order of magnitude⁵.) When a positron interacts with an electron, the two particles annihilate turning the mass of the two particles into the energy of two 511-keV γ -rays that are emitted at 180-degree to each other. These photons easily escape from the human body and can be recorded by external detectors.

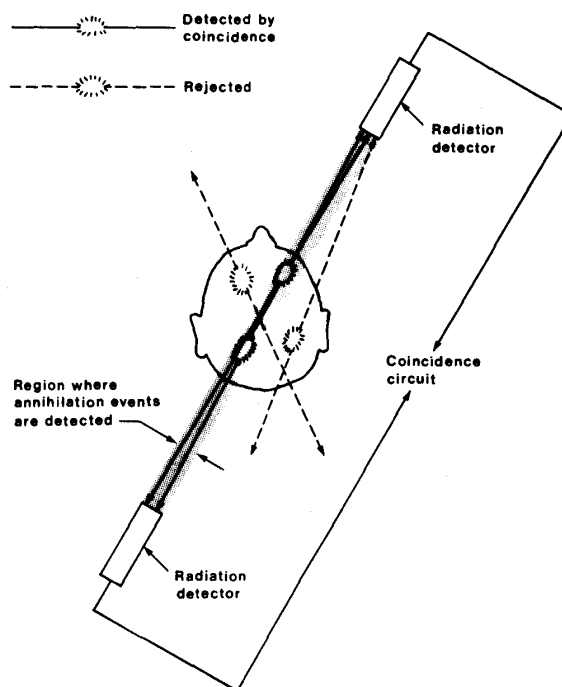


Figure 2. Coincidence detection used in PET technique (ref. 3)

As the annihilation radiation consists of two photons of 511-keV emitted simultaneously and travelling co-linearly in opposite directions, it provides a special detection method called coincidence detection. The basis of detection is that two detectors placed on each side of the annihilation point may register these photons reaching the

detector crystals almost simultaneously (within some nanoseconds). The registered coincidence means that a positron has been emitted somewhere along the line between the two detectors (coincidence line). The PET camera consists of several coincidence detectors arranged in rings and connected to a computer for collection of data. The reconstruction of data by the computer will yield a description of distribution of the radioactivity⁶.

PET is nowadays a routine tool in modern nuclear medicine diagnostics, as well as in research of physiological processes⁷. As PET is capable of providing functional images based on quantitative data of metabolic rate constants and tissue distribution of a certain radiotracer *in vivo*, it might become one of the most important technique of biomedical research.

2.1.2. PET isotopes

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

- 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 %

Although the number of positron emitting radionuclides is large, only a few of them can fulfil the above requirements. The four medically relevant PET isotopes and their decay characteristics are summarised in Table I.⁸

Isotope	Half-life (min)	Maximum positron energy (keV)	Percentage of positron-decay
Carbon-11	20.4	970	99.8
Nitrogen-13	10.0	1190	100
Oxygen-15	2.05	1720	99.9
Fluorine-18	109.7	635	97

Table I. Main physical characteristics of medically important PET isotopes

It can be seen, that the positrons emitted from fluorine-18 possess the lowest energy, thus the distance between the place of positron decay and that of annihilation is the shortest. Consequently, the best resolution can be obtained in images of trace distribution by using this isotope for labelling⁹.

2.1.3. Receptor studies with PET

Positron emission tomography is a valuable technique for quantitative measurement of biochemical functions in living organisms. Among these investigations, the localisation and characterisation of receptor systems are of major importance.

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 incorporation 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 ¹⁰.

2.2. Adenosine-receptors

In a seminal paper, Drury and Szent-Györgyi showed¹¹, that adenosine exerted a large number of biological effects, such as bradycardia and vasodilation. The observation that the actions of adenosine in heart tissue could be blocked by caffeine¹² suggested the existence of an adenosine receptor and by 1970s there were many evidences that receptors exist for adenosine at which methylxantines (such as caffeine) act as antagonists¹³.

Indeed, 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 P₁ purinoseptors) are divided into four subtypes, A₁, A_{2A}, A_{2B} and A₃¹⁴. Each of them is coupled via a G-protein¹⁵ to the cyclic AMP-producing enzyme *adenylate cyclase*. All receptor subtypes have been recently cloned¹⁶ from different species (including humans), thus they can be solely investigated by using artificially constructed systems.

From the point of view of ligands: for A₁ and A_{2A} and A₃ both selective agonists and antagonists are available, whereas for A_{2B} receptor, to the author's knowledge, no selective ligands have been reported so far¹⁷.

Adenosine receptors are widely distributed throughout the body and are involved in complex regulatory systems such as inflammatory and immunological responses, the cardiovascular system and pain. Due to this complexity, it is difficult to separate the therapeutic profit from the side effects, which, up to now, has impeded the develop drugs acting at these receptors¹⁸.

A deeper understanding of its specific interactions in different physiological processes are likely to lead to the use of adenosine receptor ligands, either agonists or antagonists, in wide range of diseases¹⁹.

2.2.1. Agonists

As the only natural ligand so far identified for adenosine receptors is the adenosine itself, it has been extensively used as a probe for the receptor studies. Because of its metabolic lability, more stable analogues have been synthesized. Modification of adenosine at N⁶, 2- and 5'-positions has led to ligands by which adenosine receptors could be classified. Figure 3. shows the structure-activity relationship of modified adenosine. N⁶-substituted adenosines have generally proven to be A₁ selective (for example N⁶-cyclopentyl adenosine, CPA²⁰). Removal of either 2'-OH or the 3'-OH, however, leads to partial agonists with reduced affinity²¹. The 5' substituted analogues, *e.g.* NECA²² 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 A₂ agonists²³. A₃ selective agonists can be synthesised by combining the N⁶, 2- and 5'-modifications²⁴.

As the number of adenosine receptor ligands labelled with positron emitting radionuclide for PET investigation is limited, and in addition, each of them is antagonist (labelled either with ¹¹C or ¹⁸F)²⁵, synthesis of agonists labelled with a positron

emitter is essentially needed for the deeper understanding of biochemical phenomena related to the adenosine-receptor system.

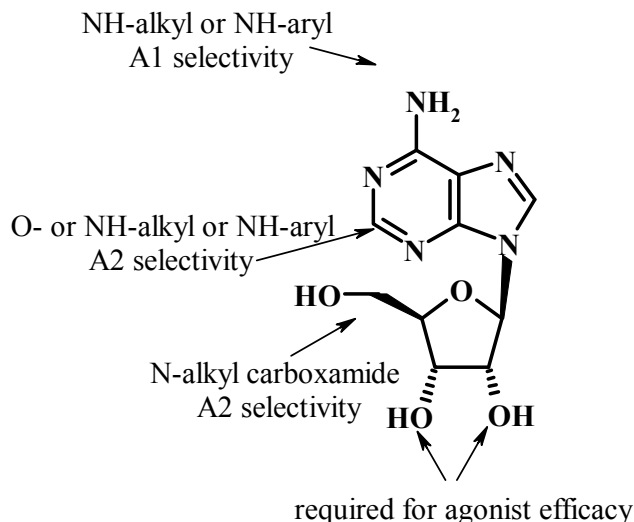


Figure 3. The structure of adenosine showing the effects of structural modifications at various sites on receptor binding²⁶

2.2.2. Antagonists (Paper V.)

The prototypic adenosine receptor antagonists were xanthines, theophylline and caffeine²⁷. 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 receptors²⁸. Perhaps the most common of all biological substitution reactions is methylation. Similarly, the artificial synthesis route of such molecules is the methylation with iodomethane in case of inactive ligands, and with [¹¹C]CH₃I in case of radioactive analogues. These carbon-11 labelled caffeine, as proved selective receptor ligands, are tools for the investigation of A_{2A} adenosine receptors in various tissues using PET technique. This type of pharmacological studies has been in progress at Debrecen PET Centre²⁹.

Although the author has taken part in development of carbon-11 labelled caffeine derivatives, the brief chemical characterization of antagonists, and presentation

of the biological results achieved in Debrecen PET Centre is not the subject of this thesis.

2.3. Fluorine

In this study, fluorine-18 was chosen as the labelling agent. The labelling procedures and strategies cannot be understood without some basic introduction on chemical behaviour of fluorine itself.

2.3.1. Chemical characteristics

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 univalent³⁰. The first member of a periodic group differs quite often from the remaining members of the group³¹. This dissimilarity is clearly demonstrated in this thesis as well: whilst fluoride (or [^{18}F]fluoride ion) failed to react with N⁶-benzoyl 2',3'-isopropylideneadenosine-5'-tosylate, iodide ion (or [^{125}I]iodide ion) readily reacted to form the corresponding radioiodinated product (*cf.* chapter 4.1.2 and 4.1.3).

The fluorine is much too reactive to be found in nature in the elementary form. Thus, it occurs almost entirely as ionic derivatives in minerals such as *fluorspar* (CaF_2), fluorapatite ($\text{Ca}_{10}\text{F}_2(\text{PO}_4)_6$) and cryolite (Na_3AlF_6), the latter being very important in aluminium production point of view.

Of all the single bonds formed by carbon the strongest is with fluorine, which therefore occupies a special place as a substituent in organic chemistry. Thus, organic molecules having fluorine-carbon bonds are very likely to have *in vivo* stability³².

Despite the wide range of its inorganic compounds, fluorine is very rare to find in the organically bound state in nature. It has been identified in only a small number of tropical plants and in two microorganisms. Moreover, merely one fluorinated nucleoside, the type of compounds which is the topic of this dissertation, has been isolated from a living organism: the nucleocidin, 4'-fluoro-5'-O-sulfamoyl adenosine³³.

2.3.2. Fluorinated nucleosides

A fluorine atom at a sugar carbon in nucleotides causes a minor change of the shape of the modified structure³⁴. Some early synthesized 2'-deoxy-2'-fluoro-nucleosides showed promising therapeutic potential, thus, they were synthesized in hope of new drug discovery^{35,36}. In a result, more than 77% of fluorinated nucleosides synthesized today contain fluorine atom at 2'-position³⁷. However, as we saw in chapter 2.3.1., 2' (and 3')-OH groups are required for adenosine receptor agonist efficacy, we had to focus on the 5'-fluorinated adenosine. In addition, the number of nucleosides containing fluorine at C-5' position is restricted; they have been synthesised to date in order to eliminate the possibility of phosphorylation in cells³⁸. As our aim was to synthesize fluorine-18 labelled adenosines, consequently we had to develop new routes to introduce fluorine into 5'-position of the adenosine structure in the ultimate or penultimate step, or, if it is not possible, to elaborate quick reactions with fluorine-containing building blocks. Both strategies are presented in this dissertation.

2.3.3. Isotopes of fluorine

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.

Mass number	Type of decay	Half-life	β -energy (MeV)	γ -energy (keV)
17	β^+	64.8 s	1.7	-
18	β^+ , EX	109,7 min	0.6	-
19	stable	-	-	-
20	$\beta^-(\gamma)$	11.0 s	5.4	1634
21	$\beta^-(\gamma)$	4.16 s	5.3 , 5.7	351, 1395
22	$\beta^-(\gamma)$	4.23 s	5.5	1275 , 2083, 2166
23	$\beta^-(\gamma)$	2.23 s	8.5	1701, 1822 , 3431
24	$\beta^-(\gamma)$	0.34 s		1982
25	$\beta^-(\gamma)$	59 ms		

Table II. Physical data of isotopes for fluorine

Isotopes having a deficiency of neutrons (mass number 17, 18) are positron emitters, those having a surplus of neutrons (mass number 20-23) decay by β^- -emission. The main physical properties of isotopes for fluorine are listed in Table II.³⁹

Among the radioisotopes of fluorine, fluorine-18 is being increasingly used for radiolabelling procedures. Indeed, fluorine-18 is the most favourable isotope of PET, partially of its relatively long half-life 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.

Considering the significant importance of fluorine-18, it is worth getting acquainted with ways of production for this isotope.

2.3.3.1. Production of fluorine-18

Fluorine-18 has no practically important parent nuclide *i.e.* there is no generator system for production of fluorine-18. Therefore, fluorine-18 can solely be produced in accelerators, mainly in cyclotrons.

Nuclear reaction	Target material	Beam energy (MeV)	Chemical form of ^{18}F
$^{20}\text{Ne}(\text{d},\alpha)^{18}\text{F}$	0.1 F_2/Ne	18-23	$[^{18}\text{F}]\text{F}_2$
	15 H_2/Ne	14	$[^{18}\text{F}]\text{HF}$
	6.7 H_2/Ne	14	$[^{18}\text{F}]\text{F}^-$
$^{20}\text{Ne}(^3\text{He},\alpha\text{n})^{18}\text{Ne}-^{18}\text{F}$	2 H_2/Ne	27	$[^{18}\text{F}]\text{HF}$
$^{18}\text{O}(\text{p},\text{n})^{18}\text{F}$	$[^{18}\text{O}]\text{O}_2$	10	$[^{18}\text{F}]\text{F}^-$
	$[^{18}\text{O}]\text{H}_2\text{O}$	15	$[^{18}\text{F}]\text{F}^-$
	$[^{18}\text{O}]\text{CO}_2$	18	$[^{18}\text{F}]\text{F}^-$
$^{16}\text{O}(^3\text{He},\text{p})^{18}\text{F}$	H_2O	40	$[^{18}\text{F}]\text{F}^-$
$^{16}\text{O}(\alpha,\text{d})^{18}\text{F}$	H_2O	30	$[^{18}\text{F}]\text{F}^-$
$^{16}\text{O}(^3\text{He},\text{p})^{18}\text{Ne}-^{18}\text{F}$	H_2O	36	$[^{18}\text{F}]\text{F}^-$
$^{16}\text{O}(\alpha,2\text{n})^{18}\text{Ne}-^{18}\text{F}$	O_2	40	$[^{18}\text{F}]\text{HF}$

Table III. Methods for production of fluorine-18

The numerous methods that have been used for cyclotron production of fluorine-18 are summarized in Table III.⁴⁰ Depending upon the method used, either electrophilic or nucleophilic chemical form of ^{18}F is obtained (see chapter 2.5.).

Among the nuclear reactions listed in Table III. the following two reactions are of major practical importance, in view of their requirements for moderate beam energy and current⁴¹:

- (1) Carrier added (*c.a.*), low specific activity ^{18}F -fluorine gas is usually produced in $^{20}\text{Ne}(\text{d},\alpha)^{18}\text{F}$ 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.

As reaction (2) is used in this study, the next chapter will deal with it in detail.

2.3.3.2. Production of *n.c.a.* [^{18}F] F^- ion in the $^{18}\text{O}(\text{p},\text{n})^{18}\text{F}$ nuclear reaction

The proton irradiation of ^{18}O -enriched water is nowadays the most effective method for the production of *n.c.a.* [^{18}F]fluoride⁴². The main viewpoint in design of target is to reduce the consumption of the expensive [^{18}O]-enriched water. Thus, mainly closed targets are used, and effective target cooling of the front window by helium and the backside with water is mandatory⁴³.

As the targetry used to produce fluorine-18 for the experiments of this thesis differs from the ones used worldwide in the viewpoint of technical realization, it will be thoroughly discussed in chapter 3.1.

2.4. 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 (see ref. 32). These two types of replacements are employed in this study, as well.

2.4.1. Electrophilic radiofluorination

It is strange at first glance that fluorine, being the most electronegative element can react as a partially positive charged species⁴⁴. Indeed, in some fluorine compounds under the conditions of given reactions a partially positive charged fluorine can be formed ($-F^{\delta+}$), thus fluorine (or ^{18}F) can be introduced into electron-rich compounds such as alkenes or aromatic rings.

One of the most important reagents for the synthesis of ^{18}F -labelled products is $[^{18}F]F_2$ ^{45,46}. The production of $[^{18}F]F_2$, to date, has been possible only in the carrier-added form (see nuclear reaction (2) in chapter 2.4.2.1.), meaning that non-radioactive fluorine is utilized in the production procedure.

Sometimes $[^{18}F]F_2$ has proved to be rather reactive and non-selective in labelling processes. For this reason a new electrophilic fluorination agent was developed in early 80's: acetyl hypofluorite⁴⁷. Unfortunately, ^{18}F -labelled CH_3COOF ⁴⁸ can only be obtained from $[^{18}F]F_2$, thus has been accessible only in the carrier-added form.

Labelling with $[^{18}F]F_2$ or $[^{18}F]CH_3COOF$ leads to products of medium specific activity, which is sometimes not high enough for investigation of receptor systems. Care must be taken to avoid the contamination with inactive fluoride isotope during the 18-fluorinating procedures with these agents.

No electrophilic radiofluorination reaction is included in this thesis.

2.4.2. Nucleophilic radiofluorination

Although $[^{18}F]$ fluoride ion can also be introduced to suitable activated aromatic ring having good leaving group, hereinafter we consider merely nucleophilic aliphatic radiofluorination; this type of fluorination is used during this thesis.

Incorporation of fluorine into organic molecules by nucleophilic substitution with the fluoride ion still remains a difficult area. The small size of fluoride-ion (1.36 Å) and its low polarizability determines F^- to behave as a base rather than nucleophile⁴⁹. This is the reason why under usual substitution conditions the most common side reaction is elimination.

As in most nucleophilic substitution reactions, the rate of radiofluorination will be generally affected by the factor, how naked the anion is⁵⁰. Nucleophilic reactivities of anions in gaseous phase are approximately the same as in solution, however, they do not correlate with the gas phase basicities⁵¹. This suggests that solvation of the anions is responsible for the large variations found in the reactivities of the anions in solution⁵². 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 CH₃CN, DMF or DMSO (each of them is used in this study). As the solubility of [¹⁸F]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^{53,54}.

One of the most important variables that can strongly affect the nucleophilic radiofluorination is the nature of the species expelled by the attacking fluoride ion, *i.e.* the nature of the *leaving group*. Halogens have been usually used as leaving groups in aliphatic substitutions, of which iodine and bromide are the most reactive. Sulfonates have also been used with good results. Of these, triflate is the most reactive, however, triflates are sometimes so reactive that they cannot be stored, or cannot even be prepared⁵⁵. Tosylates are more stable and sometimes result in less side-reactions, therefore are often chosen as starting material. These phenomena are also clearly demonstrated also in this thesis. In addition to the reactive groups discussed above, aziridines⁵⁶ and epoxides⁵⁷ also react with [¹⁸F]fluoride to give, after ring opening, the corresponding alkyl fluorides containing a 2-amino and 2-hydroxy groups, respectively. An aziridine type of starting material is also presented in this study.

2.5. Goal of this work

As we saw in chapter 2.2., 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 [¹¹C]-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, the most favourable isotope of PET was chosen as labelling isotope, partially because of its relatively long half-life. Moreover, fluorine-18 is a con-

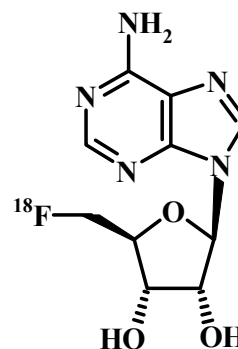
venient labelling agent due to its low β^+ (635 keV) energy, which is advantageous from the imaging point of view (see chapter 2.1.2.).

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 (aminopolyether 2.2.2) as phase transfer catalyst.

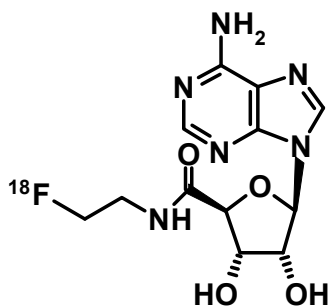
Finally, two molecules to be synthesised were chosen; both of them can be regarded as a model compound. Working out their synthesis is the key to producing series of fluorine-18 labelled specific adenosine receptor ligands by modified labelling procedures. Moreover, one of our target molecules (FNECA) has been expected to possess some selectivity toward A_{2B} receptor subtype.

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 N^6 -alkyl and cycloalkyl derivatives are the most selective A_1 receptor agonists.



5'-N-(2-[^{18}F]fluoroethyl)-carboxamidoadenosine ([^{18}F]FNECA), as a fluo-



rine-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 N^6 position can result in ^{18}F -labelled receptor ligands of A_{2A} and A_3 selectivity, respectively. Affinity of NECA to the A_1 , A_{2A} and A_3 receptor subtypes is almost equipotent, and can be characterised by dissociation con-

stants 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.

Figure 4. shows the sterical arrangement of these two compounds. The coplanar shape of the adenine moiety, which is essential for receptor affinity, can be excellently seen.

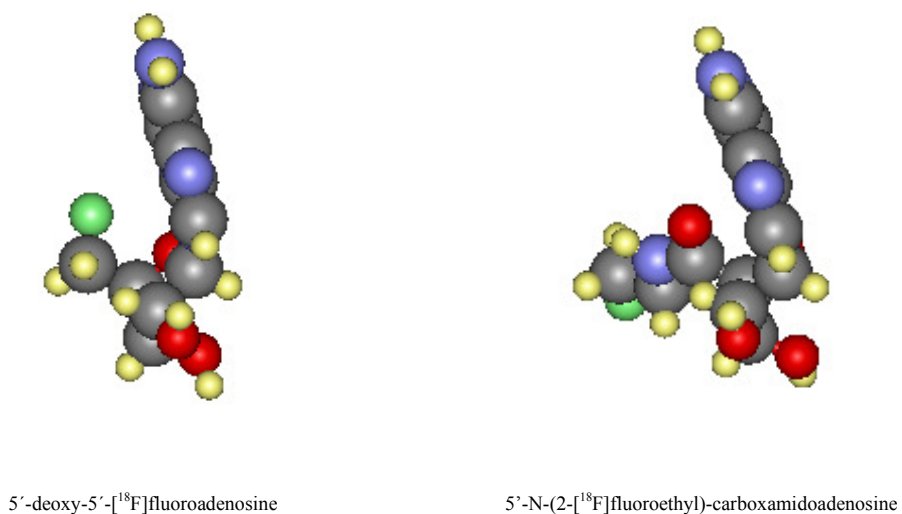


Figure 4. Sterical arrangement of two molecules to be synthesized

3. EXPERIMENTAL

3.1. 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. Petersburg⁵⁸. The picture and parameters table of MGC-20 cyclotron can be seen in Appendix 1. (A.1.).

Taking the special (vertical) orientation of beam-end⁵⁹ into consideration, a new series of the target system had been developed⁶⁰. Gas targets had been easily adopted, however, a completely new liquid (water) target had been planned and developed. The body of this target is made of stainless steel to withstand highly charged particle beams and temperature, moreover, this target material can be regarded as chemically inert. Its main feature is its shape: it consists of two conical cylinders; ¹⁸O-enriched water is placed into the lower cylinder and the upper cylinder serves as gas space. The double window on the top of the target consists of 25 µm thick titanium sheets, which closes the target, and an 11µm *Duraterm* foil, which separates the cyclotron vacuum. Whilst the body of the target is cooled with flowing water, helium stream keeps cold the two window foils. The target can be remotely loaded with [¹⁸O]-enriched water.

The picture of the vertical beam-end and the scheme of the fluorine-18 (water) target can be seen in A.2.

3.2. Automated radiofluorination system

All the radiofluorination experiments were performed on a computer-assisted automated synthesis panel. This panel is also used for production of 2-¹⁸FDG, the most important radiopharmaceutical produced routinely by Debrecen PET Centre⁶¹. The modules of the panel are connected with TeflonTM tubes (Cole-Parmer, USA) and air actuated valves of TeflonTM membrane type (Rheodyne, USA). The most important parts of this 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, which can be warmed by heater coil and cooled with a stream of air, and equipped with thermometers and radioactivity detector

The picture and the graphical interface of the automated nucleophilic radiofluorination panel can be seen in **A.3**.

3.3. 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.

3.3.1. Chromatography

Chromatographic methods used is as follows:

- Column chromatography⁶²
- Thin layer chromatography⁶³
- Paper chromatography⁶⁴
- High performance liquid chromatography⁶⁵

In this study, our target molecules were separated from other components of the reaction mixtures by *column chromatography* on silica gel (Kieselgel-60, 230-400 mesh, Merck); the solvent systems are specified in the text. *Paper chromatography* was used merely for the quick control of radiochemical purity⁶⁶ of produced [^{18}F]fluoride ion⁶⁷, and performed on Cellulose-F chromatographic paper with aqueous 0.015 M sodium acetate as solvent system. *Thin layer chromatography* was performed on silica gel (Kieselgel 60F₂₅₄ Merck) using solvent systems also specified in the text. For *radio-TLC* measurements an MD 100 scanner was used, equipped with

NaI(Tl) scintillation detector (scanning rate: 1.0 cm/min). *High performance liquid chromatography* measurements were performed on Unicam chromatograph equipped with Waters UV-VIS or Unicam DAD detector and/or a NaI(Tl) scintillation detector with rate meter. Column types and solvent systems are characterised in the appropriate chapters.

3.3.2. Spectroscopy

Spectroscopic methods used is as follows:

- ^1H NMR spectroscopy⁶⁸
- UV-VIS spectroscopy⁶⁹

A majority of the elements in the periodic table have at least one isotope that is amenable to NMR studies. Although ^{19}F is an NMR active isotope, each NMR spectrum occurred in this thesis is proton spectrum. The reason why ^1H -NMR was chosen: the author was interested in another part of the fluorine containing molecules. All *^1H -nuclear magnetic resonance spectra* in this thesis were obtained on a Bruker WP 200 spectrometer (200 MHz) using Me_4Si as internal standard. Solvents are specified in the text.

UV-VIS-spectroscopy was used merely for the determination of absorbance maximums of the compounds to be analysed; for this purpose, Unicam DAD detector was solely used.

3.4. Chemistry

3.4.1. General methods

^{18}F nuclide was produced directly as $[\text{}^{18}\text{F}]$ 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.

All the yields of the radiochemical syntheses given in the text are decay-corrected.

3.4.2. Materials

Solvents and chemicals (p.a. grade) were obtained from Merck (Darmstadt, Germany) or Sigma-Aldrich (Gillingham, England) and Reanal (Hungary).

[¹⁸O]-enriched water was used as target material purchased from CAMPRO Scientific, The Netherlands.

3.4.3. Syntheses of non-radioactive materials

Methyl-D-ribofuranoside(1)

8.4 g of D-ribose was suspended in 100 ml of methanol and approx. 5 g of AG-11 ion exchange resin (acidic form) was added. The mixture was refluxed for 20 hours, when the TLC analysis (MeCN/Water=95/5) showed that the starting material had disappeared and the mixture of α - and β -methyl riboside had been formed. The reaction mixture was filtered off and evaporated to dryness to yield 9.2 g of methyl-D-ribofuranoside (quantitative) as crude oil.

Methyl-2',3'-O-Isopropylidene-D-ribofuranoside(2)

9.2 g methyl-D-ribofuranoside was suspended in 50 ml of dimethoxy-propane and 1.4 g of pyrimidinium tosylate (PPTs, 0.1 equ.) was added to this suspension. After the methyl-D-ribofuranoside was dissolved, the TLC analysis (MeCN/Water=95/5) showed that the reaction had been completed. The reaction mixture was then evaporated to dryness and re-dissolved in chloroform. The chloroform solution was washed with water, dried over Na₂SO₄ and evaporated to dryness to form 10.6 g of methyl-2',3'-O-isopropylidene-D-ribofuranoside as a yellowish foam (yield: 92.6 %).

Methyl-2',3'-O-Isopropylidene-5-O-mesyl- β -D-ribose(3a)⁷⁰

To an ice-cold solution of 7.9 g of methyl-2',3'-O-isopropylidene-D-ribofuranoside dissolved in 20 ml of anhydrous pyridine 4.5 ml of mesyl chloride was added in dropwise fashion with vigorous stirring and cooling. The mixture was kept at approx. 5 °C in refrigerator for a day and poured onto 100 ml of ice-water when the mesylate was slowly solidified. The solid material was filtered off, re-suspended in fresh cold water and filtered again to leave a clear crystalline material. After drying over P₂O₅ 5.2 g of methyl-2',3'-O-Isopropylidene-5-O-mesyl- β -D-ribose was obtained (yield: 47 %, mp. 75-76 °C, according to ref. 70, 78-79 °C).

Methyl-2',3'-O-Isopropylidene-5-O-trezy-D-ribose(3b)

The more reactive sulfonate was synthesized as follows: 1.1 g of methyl-2',3'-O-isopropylidene-D-ribofuranoside was dissolved in 25 ml of anhydrous methylene chloride and 2.0 ml of anhydrous pyridine was added to the solution. The mixture was cooled to -20 °C and 0.61 ml of trezyl chloride was added dropwise then let to warm up to room temperature when the TLC analysis showed (Acetone/Hexane=1/1) that the starting material had disappeared. The mixture was poured to ice-water and extracted with chloroform (3x20 ml). The combined chloroform extracts were washed subsequently with water, 1 M H₂SO₄ and saturated NaHCO₃. The dried chloroform solution was then evaporated to dryness to yield 2.1 g of methyl-2',3'-O-isopropylidene-5-O-trezy-D-ribose as crude oil. The TLC analysis (Acetone/Hexane=1/1) showed some impurities; the structure of **3b** was proved by its reaction with fluoride when the appropriate substitution occurred.

Methyl-2',3'-O-Isopropylidene-5-deoxy-5-fluoro- β -D-ribose(4)⁷⁰

1.7 g of methyl-2',3'-O-Isopropylidene-5-O-mesyl- β -D-ribose and 1.7 g of dry potassium fluoride (previously warmed up to 500 °C for 1 hour, then cooled) and 20 ml of methanol were placed into a stainless steel bomb. The bomb was thoroughly sealed and heated at approx. 160 °C for a day. After cooling, the bomb was opened and its content was washed with methanol, and filtered off. The precipitate was washed with methanol and the combined filtrate and washings were evaporated to dryness and the residue was taken up with approx. 50 ml of ether then filtered through Celite. Filtrate was evaporated to dryness to leave 1.1 g (88 %) **4** as off-white oil. As TLC analysis (Acetone/Hexane=1/2)

showed one spot at $R_f=0.8$, no further purification was needed ($n_D^{20}=1.4334$, according to ref. 71 $n_D^{20}=1.4270$).

$^1\text{H-NMR}$ (in CDCl_3) δ : 4.89(d, 1H), 4.55(m, 2H), 4.05(m, 1H), 3.27(s, 3H), 1.40(s, 3H), 1.22(s, 3H), cf. ref. 72

5-Deoxy-5-fluoro- β -D-ribose(**5**)⁷⁰

A mixture of 1.6 g of methyl-2',3'-*O*-isopropylidene-5-deoxy-5-fluoro- β -D-ribose and 50 ml of 0.01 M H_2SO_4 was refluxed for 3 hours, when the TLC analysis (Acetone/Hexane=1/1) showed that the **4** had disappeared. 5 ml of 0.1 M $\text{Ba}(\text{OH})_2$ solution was added to the mixture and the precipitated BaSO_4 was filtered through Celite and the filtrate was extracted with ether then the water phase was evaporated to dryness to yield 1.2 g (quantitative) of **5** as brownish oil.

1,2,3-Tri-*O*-acetyl-5-Deoxy-5-fluoro- β -D-ribose(**6**)

1.2 g purified **5** was dissolved in 6 ml of dry pyridine and, in dropwise fashion, 2.4 ml of acetic anhydride was added to the solution. After keeping at room temperature overnight, the mixture was poured onto ice-water and extracted with chloroform (3x20ml). The combined chloroform extracts were subsequently washed with saturated NaHCO_3 solution (3x10 ml) and water, then dried over MgSO_4 and evaporated to dryness. The oily residue was triturated with a small amount of ether to give 1.5 g (38.5 %) of **6** as white powder (mp.:95-98 °C, according to ref 70, 100-101 °C).

2',3'-*O*-Acetyl-5'-deoxy-5'-fluoroadenosine(**7**)^{Paper I}

A stirred suspension of adenine (100 mg, 0.74 mmol) in 3 ml of 1,1,1,3,3,3-hexamethyldisilazane containing 20 mg ammonium sulphate was heated at reflux for 2 days. The clear solution was evaporated *in vacuo* and the residual oil was co-evaporated with dry toluene and then dissolved in a solution of **6** (150 mg) dissolved in dry 1,2-dichloroethane (10ml). The stirred mixture was cooled to 0°C, and treated dropwise with TMSTf (trimethylsilyl triflate, 325 μl). The mixture was stirred at room temperature for 20 hours then washed with ice-cold saturated sodium hydrogen carbonate solution and filtered. The organic phase was separated, washed with water, dried over MgSO_4 and evaporated to dryness. The residue dissolved in chloroform was applied to a column of silica gel eluted with acetonitrile/water 95/5 to give 31.0 mg (17 %) 2',3'-*O*-acetyl-5'-deoxy-5'-fluoroadenosine as white crystals (mp. 196-198 °C).

$^1\text{H-NMR}$ (in CDCl_3) δ : 8.42(s, 1H), 8.06(s, 1H), 6.33(d, 1H), 5.84(s, 2H), 5.67(m, 1H), 4.86(d, 1H), 4.64(d, 1H), 4.45-4.22(m 2H), 4.22(dd, 1H), 2.19(s, 3H), 2.05(s, 3H)

5'-Deoxy-5'-fluoroadenosine(**Fado**)(**8**)^{Paper I}

31.0 mg of 2',3'-*O*-acetyl-5'-deoxy-5'-fluoroadenosine was then dissolved in dry methanol (4 ml) containing 20 μl 1M sodium methylate in methanol. The reaction mixture was kept at room temperature for 2 hours while the deprotection took place. The mixture was treated with DOWEX 1X8 (H^+ -form) to remove ionic components, filtered and evaporated to dryness. The white solid residue was recrystallized from methanol to give 20 mg (84.7 %) of title compound (mp. 201-203 °C, softening at 160 °C, ref 70, 205-206 °C).

$^1\text{H-NMR}$ (in DMSO-d_6) δ : 8.42(s, 1H), 8.18(s, 1H), 7.31(s, 2H), 5.92(d, 1H), 5.88(d, 1H), 5.12(d, 1H), 4.75(dd, 1H), 4.55(m 2H), 4.22(dd, 1H), 4.08(dd, 2H)

5'-Chloro-5'-deoxyadenosine(**9**)⁷³

A stirred suspension of 1.6 g adenosine in 6 ml of anhydrous acetonitrile was cooled to 0°C, and 1.3 ml of thionyl chloride (SOCl_2) was then added to it. To the yellow solution, 1 ml of anhydrous pyridine was added and the mixture was stirred for 3 hours at 0°C, then let to warm up to room temperature. The colourless precipitate formed was dissolved by adding 20 ml ice cold water and the solvent was neutralised to pH 5-6 with solid NaHCO_3 . This solution was then extracted with ethyl acetate (3x25 ml) and the combined organic extracts were washed with saturated NaHCO_3 solution and water and dried over Na_2SO_4 . Toluene (15 ml) was added to the solution and evaporated to about 15 ml. A new portion of toluene (15 ml) was added and the evaporation was continued to approx. 10 ml. This 10 ml solution was placed to a refrigerator to give 1.6 g mixture of 5'-chloro-5'-deoxy-2',3'-*O*-sulfinyladenosines(**28a/b**).

To a stirred suspension of **28a/28b** in 25 ml methanol 5 ml of water and 2.5 ml concentrated ammonia were added. The stirring was continued for 30 min and the solution was evaporated to dryness to leave a white solid. Crystallising from water yielded 1.4 g (93.5 %) of **9** (mp.:180-190 °C with decomposition, according to ref. 73, 190 °C, decomposition).

¹H-NMR (in CDCl₃) δ: 8.41(s, 1H), 8.20(s, 1H), 7.35(s, 2H), 5.94(d, 1H), 5.67(d, 1H), 5.45(d, 1H), 4.75(dd, 1H), 4.28(dd, 1H), 4.12(dd, 1H), 3.91(m, 2H)

5'-Deoxy-5'-bromoadenosine(**10**)⁷⁴

A solution of 534 mg of adenosine, 500 mg of PPh₃ and 756 mg of CBr₄ in HMPA (5 ml) was stirred at 40 °C for 10 hours. The solution was diluted with approx. 15 ml methanol and evaporated to dryness. The residue was taken up with a small amount of methanol and subjected to column chromatography (Chloroform/Methanol=17/3) to obtain 422 mg (64 %) **10** as yellowish powder (mp.: 140-144 °C).

¹H-NMR (in CDCl₃) δ: 8.32(s, 1H), 8.06(s, 1H), 7.35(s, 2H), 5.91(d, 1H), 5.77(d, 1H), 5.69(d, 1H), 4.67(dd, 1H), 4.16(dd, 1H), 4.09(dd, 1H), 3.79(m, 2H)

5'-Deoxy-5'-iodoadenosine(**11**)⁷⁴

A solution of 534 mg of adenosine, 1.25 g of PPh₃ and 1.9 g of ICl₄ in HMPA (5 ml) was stirred at 60 °C for 1 hour. The solution was extracted with methylene chloride (3x10 ml). The extract was washed with 5 % Na₂S₂O₃ solution and water, evaporated to dryness then taken up with a small amount of methanol and subjected to column chromatography (Chloroform/Methanol=17/3) to obtain 410 mg (55 %) **11** as yellowish powder. As its colour was darkening (showing the iodine liberation) **11** was needed to be stored in a dark glass vessel.

¹H-NMR (in CDCl₃) δ: 8.45(s, 1H), 8.18(s, 1H), 7.35(s, 2H), 6.42(d, 1H), 5.92(d, 1H), 5.84(d, 1H), 4.85(dd, 1H), 4.15(dd, 1H), 3.95(dd, 1H), 3.55(m, 2H)

2',3'-O-Isopropylideneadenosine(**12**)⁷⁵

7.1 g *p*-toluenesulfonic acid was added to a stirred suspension of adenosine (1.0 g) in 300 ml dry, freshly distilled acetone. The suspension cleared quickly and stirring was continued for an hour. To the vigorously stirred solution 6.7 g NaHCO₃ in ice cold water (160 ml) was added and evaporated to dryness. The solid residue was placed in a Soxhlett extractor and extracted with acetone. The acetone solution was then evaporated to dryness and the residue was crystallised from water to give 1.05 g (92 %) of **12** as cream-coloured needles (mp.: 217-218 °C, according to ref. 75, 217.5-218 °C).

2',3'-O-Isopropylideneadenosine-5'-sulfonates(**13a-c**)

2',3'-O-Isopropylideneadenosine-5'-mesylate(**13a**)

1.0 g 2',3'-O-isopropylideneadenosine was dissolved in 6.0 ml dry pyridine. The solution was cooled to -20 °C and 0.41 ml mesyl chloride was added to it dropwise. The mixture was kept at -20 °C for 24 hours then poured onto ice-water and extracted with chloroform. The organic phase was washed subsequently with 5% H₂SO₄ solution, saturated NaHCO₃ and water. The solution was dried over Na₂SO₄ and evaporated to dryness. 0.7 g (60%) **13a** was obtained as white foam. TLC analysis (MeCN/Water=95/5) showed that the product was pure.

2',3'-O-Isopropylideneadenosine-5'-tosylate(**13b**)⁷⁶

1.0 g 2',3'-O-isopropylideneadenosine was dissolved in 6.0 ml dry pyridine. The solution was cooled to -20 °C and 0.68 g tosyl chloride was added to it. The mixture was kept at -20 °C for 24 hours then poured onto ice-water and extracted with ethylene chloride. The organic phase was washed subsequently with 5 % H₂SO₄ solution, saturated NaHCO₃ and water. The solution was dried over Na₂SO₄ and evaporated to dryness and taken up with approx. 3 ml methanol, when **13b** started to crystallize to yield 0.3 g (20 %) white powder (mp.: 190 °C with decomposition).

2',3'-O-Isopropylideneadenosine-5'-nosylate(**13c**)

Nosylate **12c** was synthesised in similar manner starting from 1.0 g 2',3'-O-isopropylideneadenosine and 1.0 g nosyl chloride. After extraction and evaporation the residue was taken up with a small amount of methylene chloride and cooled; 0.4 g (35 %) **13c** formed (mp.: 295 °C with decomposition).

N⁶-Benzoyl 2',3'-O-Isopropylideneadenosine(**14**)⁷⁷

A solution of 10 g 2',3'-O-isopropylideneadenosine in dry pyridine (100 ml) was treated with benzoyl chloride (20 ml) and the reaction mixture was stirred for an hour at room temperature. The solution was poured onto ice-water and extracted with chloroform, the chloroform solution washed with saturated NaHCO₃ solution and water, dried over Na₂SO₄ and evaporated to dryness. The residue was dissolved in pyridine (500 ml), and treated with 500 ml 0.1 M NaOH. After 20 min the mixture

was cooled to approx. 5 °C and 50 ml acetic acid was added. The reaction mixture was then extracted with chloroform; the chloroform layer was washed subsequently with saturated NaHCO₃ solution and water, dried and evaporated to dryness. Re-crystallisation from ethanol gave 8.5 g (65 %) **14** as white crystals (mp.: 130-132 °C, ref. 77, 132-133 °C).

N⁶-Benzoyl 2',3'-O-Isopropylidene-adenosine-5'-sulfonates(15a-d)^{Paper III}

N⁶-Benzoyl 2',3'-O-Isopropylidene-5'-mesylate(15a)

1.5 g N⁶-Benzoyl-2',3'-isopropylideneadenosine **14** was dissolved in 10 ml of dry pyridine (dried over potassium hydroxide pellets), cooled to approx. -20 °C with the subsequent dropwise addition of 0.31 ml of (1.1 equ.) methanesulfonyl chloride. The reaction mixture was allowed to stand at -20 °C for 24 hours, a duration necessary for the reaction to be completed as based on TLC analysis (acetone/hexane=6:4). The mixture was poured onto ice/water, and extracted with chloroform (3X25 ml). The combined chloroform extracts were washed with 10 % sulphuric acid, followed by saturated sodium bicarbonate and water, dried over sodium sulphate and evaporated to dryness leaving **15a** as an off-white oil. As the mesylate proved to be pure by TLC analysis no further purification step was needed (yield 83 %).

¹H-NMR (in DMSO-d₆) δ: 8.74(s, 1H), 8.11(s, 1H), 7.9(m, 2H), 7.4(m, 3H), 6.16(d, 1H), 5.44(dd, 1H), 5.10(dd, 1H), 4.45(m, 3H), 1.60(s, 3H), 1.37(s, 3H)

N⁶-Benzoyl 2',3'-O-Isopropylidene-5'-tosylate(15b)

The tosylate was synthesised in the same manner: to the cooled pyridine solution of **14** 0.85 g *p*-toluenesulfonyl chloride (1.2 equ.) was added portionwise, and was allowed to stand at -20 °C for 48 hours. After extraction, washing, drying and evaporation **15b** was obtained as a crude oil. As **15b** turned out to be impure by TLC analysis, the crude oil was taken up in a small amount of chloroform and applied to column chromatography (acetone/hexane=3:7). Yield 61 %, as a white powder (mp.: 135-137 °C)

¹H-NMR (in DMSO-d₆) δ: 8.79(s, 1H), 8.15(s, 1H), 8.04(m, 3H), 7.60(m, 6H), 6.20(d, 1H), 5.38(dd, 1H), 5.04(dd, 1H), 4.61(m, 2H), 4.18(m, 1H), 2.45(s, 3H), 1.62(s, 3H), 1.36(s, 3H)

N⁶-Benzoyl 2',3'-O-Isopropylidene-5'-nosylate(15c)

0.32 g 4-nitro-benzenesulfonyl chloride was dissolved in 10 ml anhydrous methylene chloride containing 195 µl 2,4,6-trimethyl pyridine, and the mixture was cooled to -30 °C. 0.5 g **14** dissolved in 5 ml dry CH₂Cl₂ was added drop-wise to the cold mixture. The reaction mixture was left to warm up to room temperature within 2 hours and was allowed to stand at approx. 20 °C overnight. The mixture was poured onto ice/water and extracted with chloroform (3X25 ml). The combined chloroform extracts were washed subsequently with 10% sulphuric acid, saturated sodium bicarbonate and water, dried over Na₂SO₄ and evaporated to dryness. The oily residue was taken up in a small amount of chloroform and applied to column chromatography (acetone/hexane=7:3). Yield 64 % of **15c** as yellow foam that contained roughly 15 % of cycled product **18** according to the TLC- and ¹H-NMR analysis.

N⁶-Benzoyl 2',3'-O-Isopropylidene-5'-tresylate(15d)

Tresylate was synthesized in similar way: after the column chromatography **15d** was yielded 52 % as off-white foam that contained approx. 50 % of **18** by TLC.

5'-Deoxy -5'fluoro 2',3'-isopropylideneadenosine(16)^{Paper III}

5'-Deoxy -5'fluoro adenosine (50 mg) was dissolved in the 1:1 mixture of 15 ml anhydrous acetone and dimethoxy propane (DMP) containing 353 mg *p*-toluene sulfonic acid. The reaction mixture was kept at room temperature for 3 hours. Saturated NaHCO₃-solution was then added and extracted with chloroform (3X10 ml). The combined chloroform extracts were dried (over Na₂SO₄) and evaporated to dryness. The partially crystallised raw product was re-crystallised from ethanol to give 57 mg (almost quantitative) of isopropylidene derivative (mp.: 144.5-146 °C).

N⁶-Benzoyl -5'-deoxy -5'fluoro 2',3'-O-Isopropylideneadenosine(17)^{Paper III}

5'-Deoxy -5'fluoro 2',3'-isopropylideneadenosine (57 mg) was dissolved in 850 µl anhydrous pyridine containing 110 µl of benzoyl chloride. After 1 hour, when the TLC analysis (acetone/hexane:4/6) reported on the disappearance of the starting material. The mixture was extracted with chloroform (3X5 ml), dried over Na₂SO₄ and evaporated to dryness. The oily residue was taken up in a

small amount of chloroform and applied to column chromatography (acetone/hexane=4/6). Yield: 54.5 mg (72%) **17** as white foam.

¹H-NMR (in CDCl₃) δ: 8.64(s, 1H), 8.18(s, 1H), 7.82(m, 3H), 7.35(m, 2H), 6.22(d, 1H), 5.15(dd, 1H), 5.00(dd, 1H), 4.71(m, 1H), 4.55(m, 2H), 1.54(s, 3H), 1.32(s, 3H)

5',N⁵-anhydro-4-(N-benzoylcarbamido)-5-formamido-1-(2,3-O-isopropylidene-β-D-ribofuranosyl)-imidazole(18)^{Paper III}

18 was predominantly formed under the radiofluorination conditions applied and during the storage of the more reactive sulfonates (**15c** and **15d**); its structure was proved by NMR measurements (mp. 242-244 °C with gas evolution, according to ref. 78 mp.:236 °C).

¹H-NMR (in DMSO-d₆) δ: 10.32(s, 1H), 8.85(s, 1H), 8.45(s, 1H), 8.07(s, 1H), 8.02(m, 2H), 7.45(m, 3H), 6.25(s, 1H), 5.06(d, 1H), 5.91(dd, 1H), 4.78(s, 1H), 4.56(d, 1H), 3.11(dd, 1H) 1.45(s, 3H), 1.25(s, 3H)

N-(2-Hydroxyethyl)phthalimide(19)⁷⁹

10.0 ml of 2-amino-ethanol and 10.0 g of phthalic anhydride were heated in an oil-bath at approx. 150 °C for 15 min. The melted mixture was poured into a mortar, let to solidify, then the amorphous product was crushed and powdered. N-(2-Hydroxy-ethyl)phthalimide was used in the following reaction without purification.

N-[2-(p-Toluenesulfonyloxy)ethyl]phthalimide(20)

15.7 g N-(2-hydroxyethyl)phthalimide was dissolved in approx. 100 ml dry pyridine and cooled to -18°C. To the cold solution 17.2 g tosyl chloride was added portionwise. The reaction mixture warmed up to 5 °C, and was kept at this temperature for 24 hours and poured onto ice-water and extracted with chloroform. The chloroform extract was washed with dilute H₂SO₄ (5 %), saturated NaHCO₃ solution and water. After drying over Na₂SO₄ the mixture was evaporated to dryness to give yellowish solid, which was re-crystallized from ethyl acetate to yield 23.8 g (83.4 %) **20** as white crystals (mp.: 140-142 °C).

¹H-NMR (in CDCl₃) δ: 7.9-7.6(m, 8H), 7.18(d, 2H), 4.33(t, 2H), 3.94(t, 2H), 2.35(s, 3H)

N-(2-Fluoroethyl)phthalimide(21)⁸⁰

1.0 g N-[2-(p-toluenesulfonyloxy)ethyl]phthalimide was dissolved in 125 ml anhydrous acetonitrile and a mixture of 3.25 ml 0.1 M tetrabutylammonium fluoride (TBAF) in THF and 250 ml dried acetonitrile was added with stirring. The solution was heated at 70 °C for 10 min and evaporated to dryness. The residue was purified by column chromatography (Hexane/Ethyl acetate=6/4) to yield 37 mg (6.6 %) of **21** as yellow powder (mp.:94-97 °C, according to ref. 80, mp.:97-99 °C).

2',3'-O-Isopropylideneadenosine-5'-uronic acid(22)^{Paper II}

9.0 g KMnO₄ was dissolved in 250 ml water and added to the stirred solution of 5.8 g of 2',3'-O-isopropylidene-adenosine (**12**) and 3.2 g KOH in 1250 ml water. The oxidation reaction was completed in 3 days at room temperature. The excess of oxidant was destroyed by adding 65 ml of 6.5 % H₂O₂. 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 form 4.6 g (77 %) of acid **22** as white solid (mp 273-276 °C)

¹H-NMR (in DMSO-d₆) 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-carboxamido-adenosine(23)^{Paper II}

0.5 g of acid **22** was suspended in 5 ml of anhydrous pyridine containing 0.25 ml N,N'-diisopropylcarbodiimide (DICI) as the coupling agent. The suspension was stirred and 88 µl 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 redissolved in cold water. After removing the formed N,N'-diisopropylurea, the solution was extracted with methylene chloride (3 X 10 ml). The combined CH₂Cl₂ extracts were washed with water, dried over MgSO₄ and evaporated to dryness. The residue was taken up in a small amount of methylene chloride and applied for column chromatography (eluted with MeCN/Water=95/5). Yield: 180 mg (35 %) of imide **23** as white foam.

¹H-NMR (in CDCl₃) 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-isopropylidencarboxamido-adenosine (FIPNECA)(24)^{Paper II}

0.32 g of **22** was suspended in 5 ml anhydrous pyridine containing 0.16 ml DICI and 0.1 g 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 precipitating 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 MgSO₄ 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).

¹H-NMR (in CDCl₃): 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)(25)^{Paper II}

A solution of 0.2 g **24** 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 CH₂Cl₂/MeOH=6/1 as eluent. Yield: 77 mg (48 %), (mp 218-221 °C, crystal rearrangement at 136-140 °C)

¹H-NMR (in DMSO-d₆): 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)

3.4.4. Syntheses of radioactive materials**[¹⁸F]F⁻-ion(26^{*})**

¹⁸F nuclide was produced in [¹⁸F]fluoride-ion form directly in the target by the MGC-20E cyclotron at the Institute of Nuclear Research, Debrecen by the ¹⁸O(p,n)¹⁸F nuclear reaction, with 14.5 MeV proton beam (yield was 40-50 mCi/μAh). [¹⁸O] enriched water was used as target material purchased from CAMPRO Scientific (The Netherlands).

Potassium-[¹⁸F]fluoride/Kryptofix2,2,2 adduct(27^{*})

Irradiated target water (0.1 ml) containing [¹⁸F]fluoride ion (0.45-8.33 GBq, 12.1-225 mCi) was passed through an anion exchange column (DOWEX 1X8, ca. 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, that served as a powerful nucleophilic fluorinating agent in the following radiofluorination reactions.

5'-Deoxy-5'-[¹⁸F]fluoro-adenosine(8^{*})^{Paper I}

To the dry potassium-[¹⁸F]fluoride/Kryptofix2,2,2 adduct halo-adenosine (**8-11**) (0.13 mmol in 2 ml anhydrous DMF) was added into the vessel. The reaction was carried out 30 min at 120 °C and quenched by cooling and pouring 0.5 ml of water to the reaction mixture.

N⁶-Benzoyl -5'-deoxy -5'-[¹⁸F]fluoro 2',3'-O-Isopropylideneadenosine(17^{*})^{Paper III}

0.1 ml irradiated target water containing the radionuclide in [¹⁸F]fluoride ion form (12.1-15.5 mCi) was placed into a reaction vessel containing Kryptofix2.2.2 (7 mg) dissolved in 0.5 ml 3.5 mg/ml potassium carbonate solution. After removing water by subsequent azeotropic distillation with acetonitrile under nitrogen flow at 80 °C one of the N⁶-benzoyl 2',3'-isopropylidene-5'-sulfonate (**15a-d**) (9.2 mmol in 2 ml anhydrous DMF) was added into the vessel. The reaction was carried out for 30 min at 120 °C and stopped by cooling and adding 1.0 ml of water to the reaction mixture.

2-[¹⁸F]Fluoroethylamine(28^{*})^{Paper II}

28^{*} was synthesised according to the modified procedure of ref. 80. 10 mg of N-[2-(*p*-toluenesulfonyloxy)ethyl]phthalimide in 0.5 ml anhydrous acetonitrile was added to the dry potassium-[¹⁸F]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 hydrazine monohydrate was added to the residue to cleave the phthalyl protecting group (yield: 27±11%, n=23).

5'-N-(2-[¹⁸F]Fluoroethyl)-2',3'-O-isopropylidencarboxamido-adenosine ([¹⁸F]FIPNECA)(24*) ^{Paper II}*Method A*

20 mg of **23** in 2 ml anhydrous DMF was loaded into a vessel containing 291-858 MBq (7.86-23.2 mCi) of dry potassium-[¹⁸F]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-[¹⁸F]fluoroethylamine was distilled to the solution of 20 mg acid **22** in 1.0 ml pyridine containing 25 µl of DICI, and the solution was incubated at 60 °C for 40 min, then evaporated to dryness under a stream of nitrogen (yield: 94±13 %, n=17).

5'-N-(2-[¹⁸F]Fluoroethyl)-carboxamidoadenosine ([¹⁸F]FNECA)(25*) ^{Paper II}

To the former residue, 1.0 ml 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 redissolved in 1.0 ml 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 *in vacuo*, the product was redissolved in 0.5 ml of saline (4.45-166.5 MBq, 0.12-4.5 mCi, n=5).

3.5. Biology

The investigations were carried out in compliance with the relevant national laws relating to the conduct of animal experimentation. The experimental protocol was approved by the Laboratory Animal Care and Use Committee of University Medical School of Debrecen on the base of Helsinki Declaration.

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 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 (pH=7.2) of 100 nM ligand-concentration in presence of 20 µCi/ml [¹⁸F]FNECA. In the competition experiments conducted in parallel, the incubation solution contained 100 µM unlabelled (*cold*) FNECA in addition to 20 µCi [¹⁸F]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 ¹⁸F activity concentration distribution was determined after 2 hour incubation time.

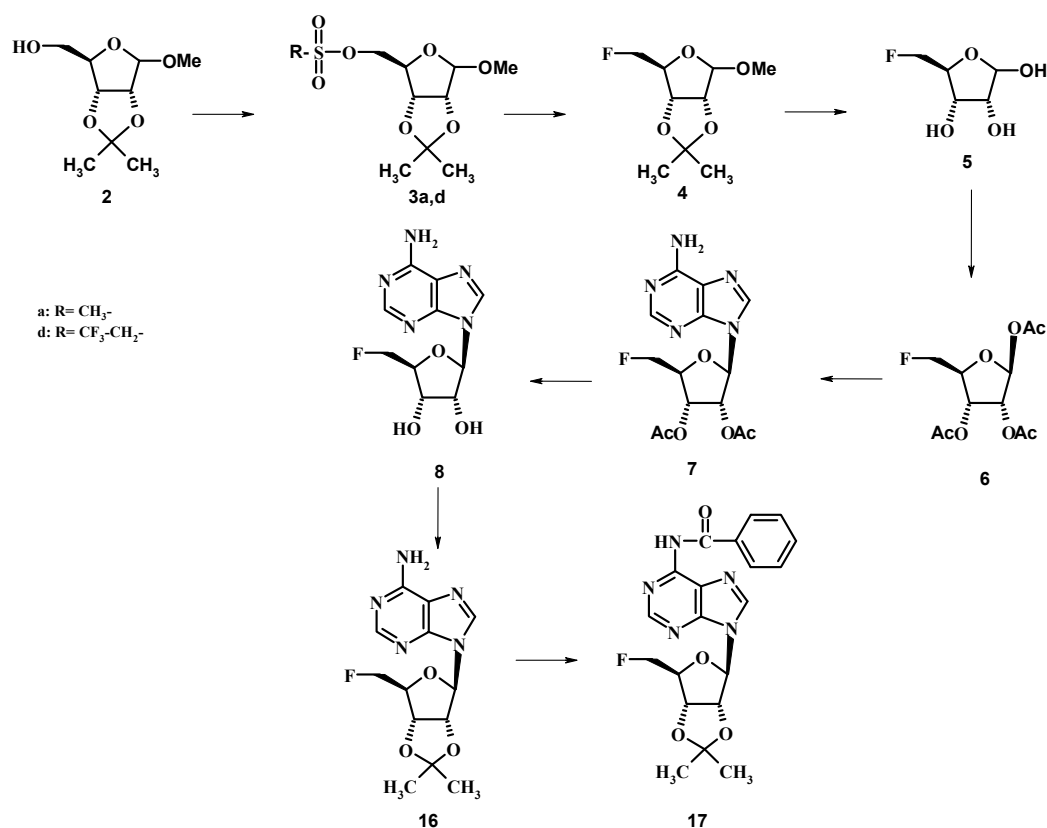
PET scanning. Average mass of domestic rabbits used for the experiments was 2.1±0.2 kg. The animals were anaesthetised by i.p. injection of urethane (0.5 g/kg body weight) and alfa-chloralose (50 mg/kg body weight) i.p., and one of femoral artery and femoral vein were cannulated⁸¹. For PET-investigation 0.5-1 mCi/30-60nmol [¹⁸F]FNECA radiopharmakon was injected intravenously in. 2 ml physiologi-

cal saline as a 20-30 s bolus. Dynamic PET scans were carried out using a GE 4096 plus PET camera. Fifteen sagittal sections were recorded which includes the whole 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.

4. RESULTS AND DISCUSSION

4.1. FAdo

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



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

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 triflate **3d**, although **3d** was proved to be a contaminated oil and was used without further purification. Acid hydrolysis of **4** gave the free sugar **5** as pure, light oil, which was characterized by ¹H-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 structure formed. Although these condensation reactions are well characterised and thoroughly reviewed⁸², merely the modification of the usual TMSTf⁸³-method could give the desired di-acetyl-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 ¹H-NMR.

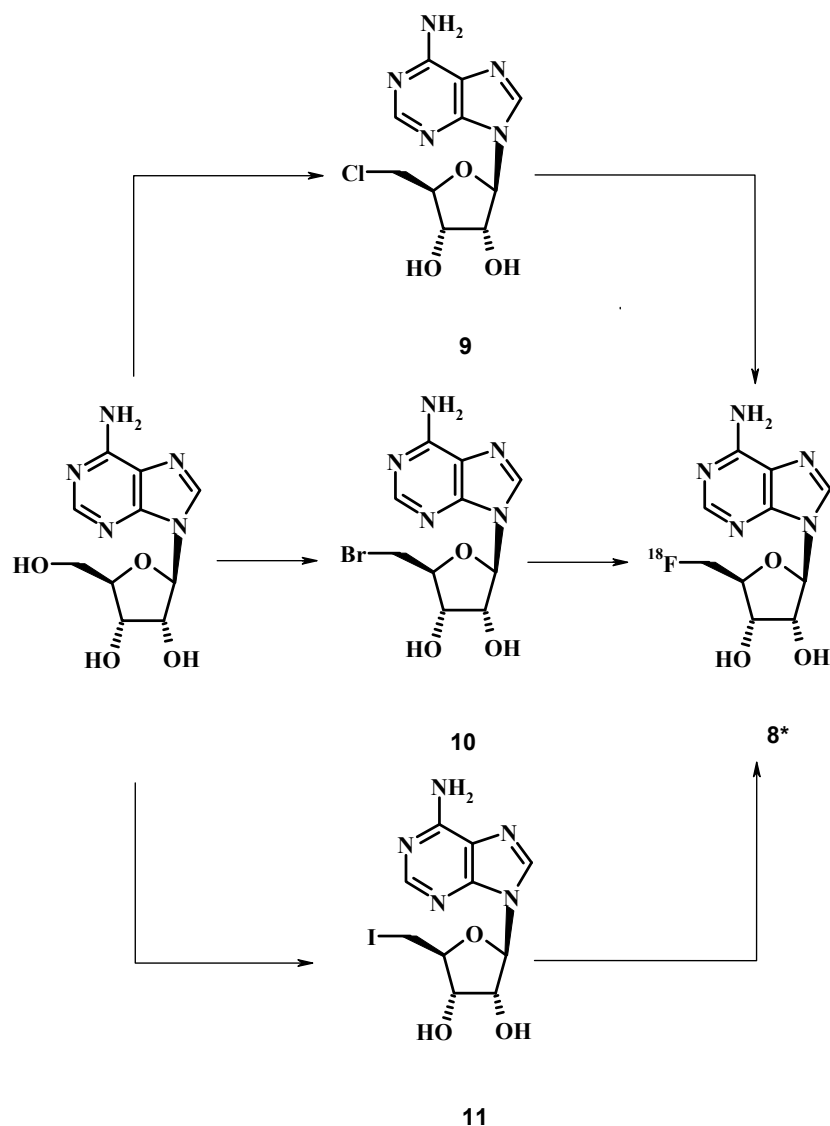
As in paper **III** the fluorination of starting materials gave a protected derivative of **8**, thus we had to synthesise the doubly-protected **17**; isopropylidene and benzoyl groups were introduced under usual conditions.

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

4.1.1. Synthesis starting from 5'-deoxy-5'-haloadenosines (Paper I.)

An easy and promising route for the production of these compounds seemed to be the nucleophilic substitution starting from 5'-halogenated adenosines. As seen in chapter 1.5.2., halogens, 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 (Scheme 2).

In paper **I** 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, which, taking the half-life of ¹⁸F radionuclide into consideration, cannot be lengthened substantially. 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 summarized in Table IV.



Scheme 2. Reaction scheme for the synthesis of 5'-deoxy-5'-[¹⁸F]fluoro-adenosine starting from 5'-halides

	Acetonitrile-Water:95:5	Chloroform-Methanol:5:1
Adenosine	0.09	0.26
5'-Deoxy-5'-fluoroadenosine	0.17	0.31
5'-Chloro-5'-deoxyadenosine	0.21	0.34
5'-Bromo-5'-deoxyadenosine	0.29	0.41
5'-Deoxy-5'-iodoadenosine	0.31	0.45
[¹⁸ F]KF/Kryptofix2.2.2	0.00	0.00
Common radioactive product	0.17	0.30

Table IV. R_f Values of nucleosides in two different solvent systems

Table V. lists the radiochemical yields of the 5'-deoxy-5'-[^{18}F]fluoro-adenosine production 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

Table V. Synthesis of 5'-deoxy-5'-[^{18}F]fluoro-adenosine (120°C for 30 min)

Figure 5. shows the radio thin layer chromatogram of the radiofluorination reaction mixtures. The peak at R_f 0.00 refers to [^{18}F]KF/Kryptofix2.2.2 adduct, and the peak at R_f 0.30 to 5'-deoxy-5'-[^{18}F]fluoro-adenosine, respectively.

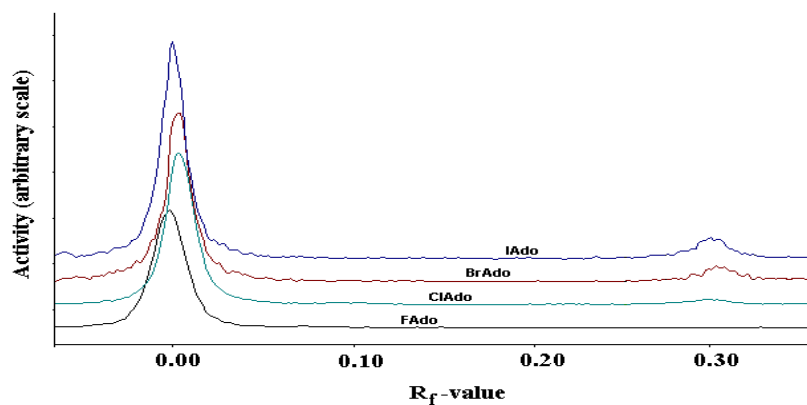


Figure 5. Radio thin layer chromatogram of the radiofluorination reaction mixtures (developed in chloroform/methanol 5/1)

In the homogeneous fluorine isotope exchange reaction, no radioactive peak was detected apart from the starting [^{18}F]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 con-

stant 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 unexpectedly 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.

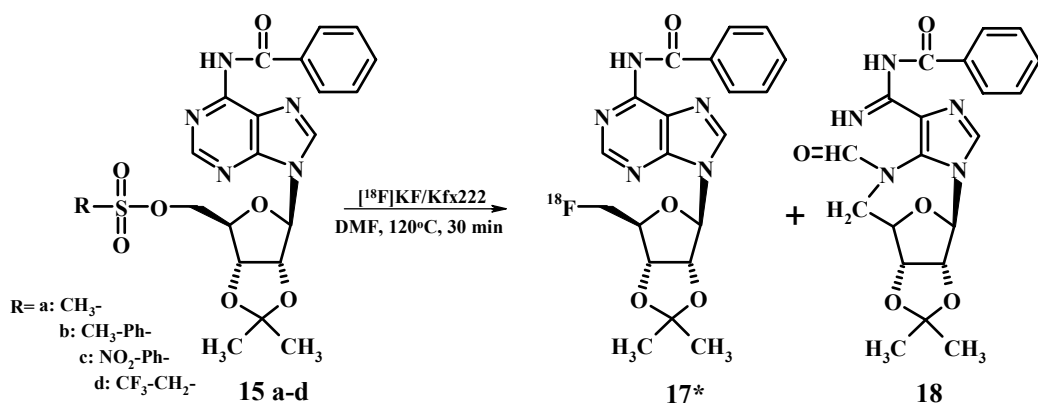
5'-Deoxy-5'-iodoadenosine seemed to be the most active in radiofluorination reaction; however, compared to other iodine-fluorine-18 exchange reactions (e.g. CH_3F production⁸⁴) the yield in this case is far from the expectation.

To make a completely firm identification of the common radioactive product, the radiofluorination reaction mixture and the reference material were spotted together onto a TLC-sheet and developed. The spot derived from the reference material (visualised under UV-lamp) and the radioactivity from the fluorine-18 labelled product were found at the same R_f value.

As the conversion has proved to be rather low, the nucleophilic fluorination of 5'-halogenated adenosines cannot be regarded as an advantageous route to produce 5'-deoxy-5'-[^{18}F]fluoro-adenosine. For a more efficient production of 5'-deoxy-5'-[^{18}F]fluoro-adenosine, precursors possessing more reactive leaving group should be starting material.

4.1.2. Synthesis starting from N⁶-benzoyl 2',3'-isopropylideneadenosine-5'-sulfonates (Paper III.)

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.* N⁶-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 3. Reaction of [^{18}F]KF/Kryptofix2.2.2 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. However, according to published data^{85,86}, 2', 3'-isopropylidene-adenosine-5'-sulfonates are not suitable precursors in the nucleophilic substitution reactions. This finding is consistent with results of our preliminary study: the product of the reaction of this type of precursors with [^{18}F] fluoride ion proved to be N^3 , 5' anhydro-nucleoside, which arose via nucleophilic attack of N^3 of adenosine ring upon 5'-sulfonyl group. The acylation of N^6 amino functional group of the adenine moiety, however, drastically decreases the nucleophilicity of the N^3 nitrogen atom⁸⁷; thus, benzoyl group was chosen to avoid such a cyclisation.

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.* elimination⁸⁸ 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 N^3 is still nucleophilic enough to form the N^3 ,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.

	Acetone-hexane(v/v) 6:4	Acetonitrile-water(v/v) 95:5
Mesylate	0.57	0.68
Tosylate	0.55	0.65
Nosylate	0.53	0.63
Tresylate	0.58	0.68
Cycled product	0.71	0.74
[¹⁸ F]KF/kryptofix2.2.2	0.00	0.00
N ⁶ -Benzoyl -5'-deoxy -5'-fluoro 2',3'-isopropylideneadenosine (reference material)	0.76	0.89
Common radioactive product	0.75	0.89

Table VI. R_f values for nucleosides in two different solvent systems

The radiofluorination reactions were carried out under usual conditions (Table VI.). The common radioactive product was identified by comparing its R_f-value with authentic inactive material on TLC using solvent systems as specified in Table VI. This 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 could be easily visualised under UV lamp and identified to be compound **18**.

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

Table VII. Yield of the radiofluorination (30 min incubation, 120°C)* corrected with the amount of impurity **18**

Table VII. 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. 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 cycled compound.

4.1.3. Radioiodination of N⁶-benzoyl 2',3'-isopropylideneadenosine-5'-tosylate⁸⁹

As we saw in the previous chapter, fluorine-18 can be introduced to the N⁶-benzoyl 2',3'-isopropylideneadenosine-5'-sulfonates with very low yield. Tosylate **15b**, one of these sulfonates, however, served as an appropriate starting material in radioiodination reactions.

After our unsuccessful attempts to introduce fluoride ion to sulfonates **15a-d** in appropriate yield, we started to investigate the reactions of these molecules with other nucleophiles, especially with iodine ion. Indeed, **15a-d** were expected to be suitable reactants, on which nucleophilic substitution reactions ought to take place. Being a stable crystalline solid, tosylate **15b** was chosen for thorough investigation in this replacement reaction. Accordingly, the more powerfully nucleophilic and less basic iodine ion do readily react with **15b**.

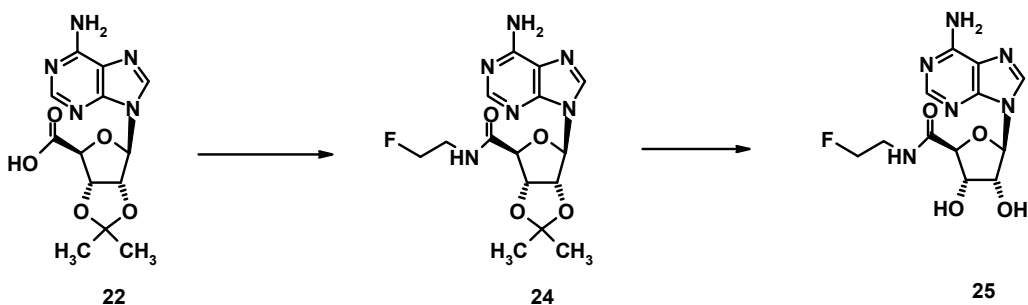
On the basis of preliminary studies conducted in our laboratory, as **15b** reacted with NaI to form the appropriate iodide, we suppose that **15b** could be an ideal precursor in radioiodination reactions. Within the scope of the collaboration with Labelled Compounds Department, Hot Laboratory Centre, Cairo, Egypt, **15b** was tested in radioiodination reactions with [¹²⁵I]NaI in the absence, as well as in the presence of a phase transfer catalyst. The results obtained during this study showed, that **15b** can be labelled with ¹²⁵I⁻ ion in as high radiochemical yield as 45 %; using crown ether (benzo-18 crown-6) as catalyst, the radiochemical yield proved to be higher: 72 %. Other type of phase transfer catalysts (e.g. quaternary ammonium bromides), however, decreased the yield to around 20%.

Although ref. 89 includes the characterisation of the kinetics for the above radioiodination reactions, the thorough discussion is not the subject of this dissertation.

4.2. FNECA (Paper II.)

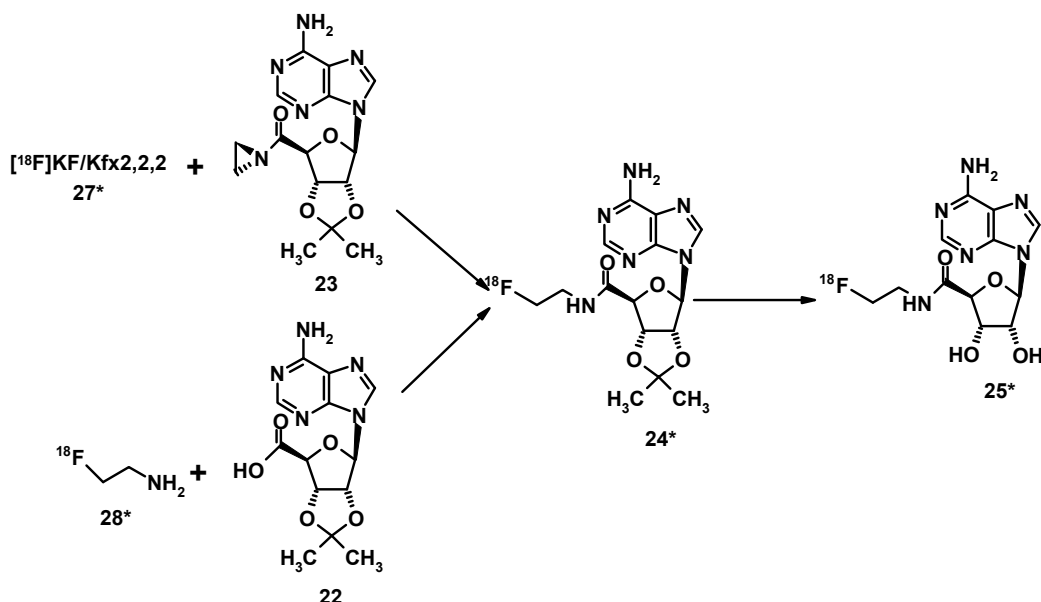
Inactive 5'-N-(2-fluoroethyl)-carboxamidoadenosine **25**, the reference material, was synthesised starting from acid **22** and hydrochloride salt of 2-fluoroethylamin in pyridine, and the hydrolysis of **24** gave **25**, as shown in scheme 4. Its structure was confirmed by ¹H-NMR.

5'-N-(2-[¹⁸F]Fluoroethyl)-carboxamidoadenosine ([¹⁸F]FNECA), this promising ¹⁸F-labelled adenosine agonist has been prepared in two different synthesis routes.



Scheme 4. Synthesis of non-radioactive FNECA

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 5.

Scheme 5. Synthesis of 5'-N-(2-[^{18}F]Fluoroethyl)-carboxamidoadenosine in two different ways

3.2.1 Synthesis starting from precursor of aziridine type

As the strain of the three-membered ring makes aziridines highly reactive compounds, **23** was expected to react readily with fluorine-18. Our preliminary study

conducted in our laboratory also demonstrated that **23** readily reacted with HF.py⁹⁰, as confirmed by TLC analysis.

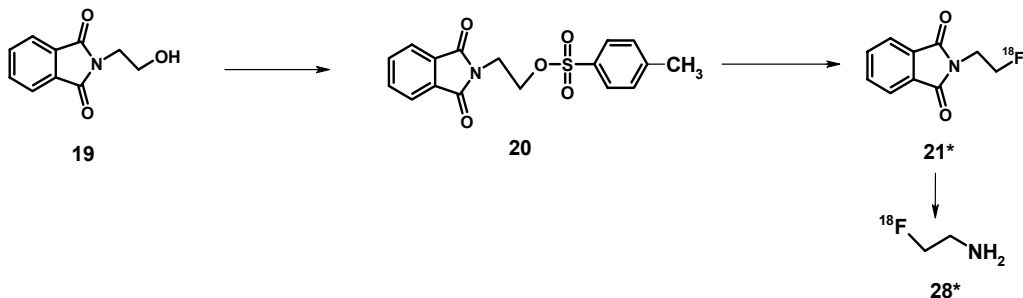
In first approach, thus, the aziridine moiety of compound **23** was reacted with [¹⁸F]F⁻ to form, after ring opening, the desired labelled compound **24**^{*} containing the corresponding 2-[¹⁸F]fluoroethylamino group. According to our experimental data, this reaction, however, took place to only a very small extent: labelled compound **24**^{*} was obtained with a yield of 1±1% in terms of [¹⁸F]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 under UV-light.

As we were unsuccessful in preparing [¹⁸F]FNECA **25**^{*} in a one-step synthesis starting from aziridine **23**, we changed our strategy to a multistep reaction including synthesis of a properly functionalised fluorine-18 labelled molecule, namely 2-[¹⁸F]fluoroethylamine.

4.2.2. Synthesis through 2-[¹⁸F]fluoroethylamine

In this second route, 2-[¹⁸F]fluoroethylamine **28**^{*} was synthesized according to ref. 70 with a yield of 27±11 % based on [¹⁸F]fluoride activity (scheme 6.) 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 DICI⁹¹, a carbodiimide coupling agent.



Scheme 6. Synthesis of (2-[¹⁸F]Fluoroethylamine

The yield of this coupling reaction was as high as 94 ± 13 % based on the activity of the distilled 2-[^{18}F]fluoro-ethylamine. The acyl chloride derivative of **22** in pyridine had been also tested in the coupling reaction. However, the extent of the conversion proved to be lower, and not as reproducible as with the acid **22**.

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 % ($n=5$). Table VIII. summarises the circumstances of preparative and analytical HPLC.

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

Table VIII. RP-HPLC retention times (min)

The overall decay corrected yield based on [^{18}F]fluoride activity was 17 ± 9 %, and the specific activity was determined to be higher than 1.04-3.11 TBq/mmol (28-84 Ci/mmol) at the end of synthesis. (As the concentration of FNECA was proven to be under the quantitation limit, thus the specific activity was assessed by its lower limit.)

Because of the rather large number of steps involved, the entire procedure was accomplished in 4 hours. Yields were mostly affected by the process of introduction of [^{18}F]fluoride-ion into the precursor **20**, and the distillation of **28***. Introduction of [^{18}F]fluoride was performed on the same automated synthesis panel, where 2-[^{18}F]FDG was synthesised as a daily routine. The shape of the reaction chamber of 2-[^{18}F]fluoroethylamine slightly differed from that of 2-[^{18}F]FDG, which resulted in

high deviation in fluorine-18 uptake and less reliability. The use of vessel with altered shaped (conical), however, was essential because of the special problems occurred during the following step, namely distillation.

2- ^{18}F]Fluoroethylamine was purified by *distillation*, which had some pitfalls. At lower temperature **28**^{*} did not distil, at elevated temperature, however, hydrazine monohydrate co-distilled with **28**^{*} and reacted competitively with acid **22**.

When the less volatile phenyl-hydrazine was used for the deprotection of amino group of **21**^{*}, the yield of the distillation drastically decreased. Finally, small amount (100 μl) of hydrazine was used, which required a conical reaction vessel, and distillation was carried out at 50 °C with slow (1-2 ml/min) stream of nitrogen. Although the maintenance of such a gentle condition was complicated, it gives almost quantitative yield in most cases (see figure 6.); the optimal duration of distillation proved to be around 5 minutes.

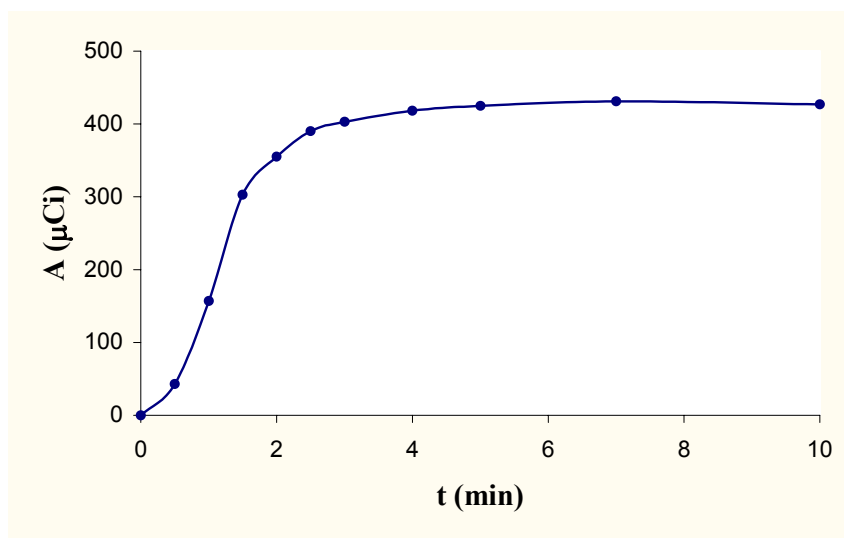


Figure 6. Distillation of 2- ^{18}F]fluoroethylamine

Unfortunately, the incorrectly conducted distillation resulted in the same activity curve showed in figure 6., however, being not as reactive as the co-distilled hydrazine monohydrate, 2- ^{18}F]fluoroethylamine **28**^{*} could not react with acid **22**, and under conditions applied **28**^{*} boiled out of reaction vessel indicated by the drastic loss of activity.

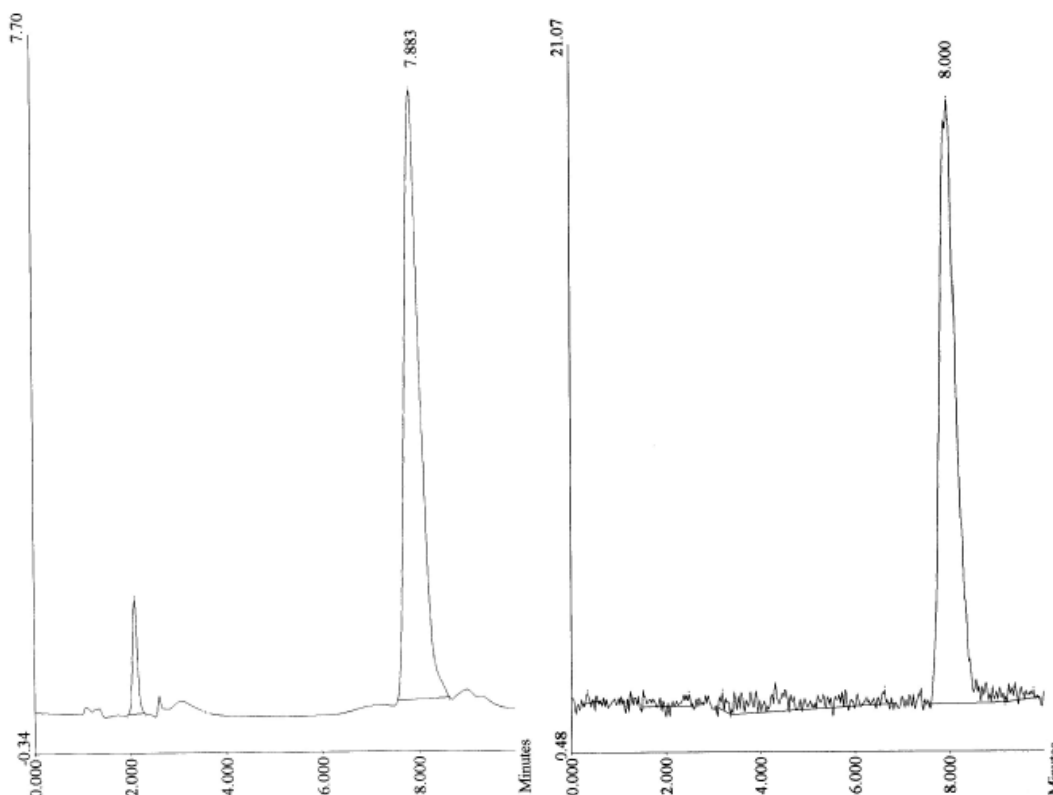


Figure 7. Chromatograms of [^{18}F]FNECA using UV detector (first chromatogram) and radioactivity detector (second chromatogram) coupled in series

Figure 7. shows the chromatogram of [^{18}F]FNECA. To make a completely firm identification of the obtained radioactive product, authentic inactive FNECA was added to its sample and applied for HPLC equipped with UV- and radioactivity detector coupled in series. It can be seen that the UV peak (first chromatogram) derived from the reference material and the radioactivity from the fluorine-18 labelled product (second chromatogram) were found to be at same retention time proving their identity.

4.3. Biological studies (Paper IV.), ref. 92

In vitro autoradiographic investigations were carried out to map the A_1 receptor distribution in the mouse brain and heart (see A.4.). High accumulation of the radio-labelled ligand was observed both on brain slices and heart slices. Particularly high [^{18}F]FNECA uptake was detected in cortex, in hippocampus and in cerebellum. The pattern of brain slices mainly shows the A_1 receptor-distribution of the brain, because, among P_1 receptors expressed in brain, the A_1 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 [^{18}F]FNECA accumulation equivalent to a fraction of the control value was observed.

In the dynamic PET investigation, accumulation of the receptorligand in tissues was monitored for 58 min after administering [^{18}F]FNECA. From the subsequent expositions, the tissue accumulation (TAC) kinetics of the ligand was determined in various tissue regions. The TAC curves for ^{18}F -NECA accumulation of the organs and blood are shown in Figure 8 and 9. Time dependent accumulation was observed in the heart, lung, liver, kidney, brain and testis. The initial uptake of radiotracer was rapid with the level of radioactivity reaching maximum in less than two minutes in heart and testis, while maximum radioactivity in brain, lung and liver was attained 6 minutes after i.v. injection of the radiopharmaceutical. Increased ^{18}F -NECA accumulation was detected in the intestines as well, which is in agreement with the high A_1 adenosine-receptor density of the layers of smooth-muscle for the intestinal wall.

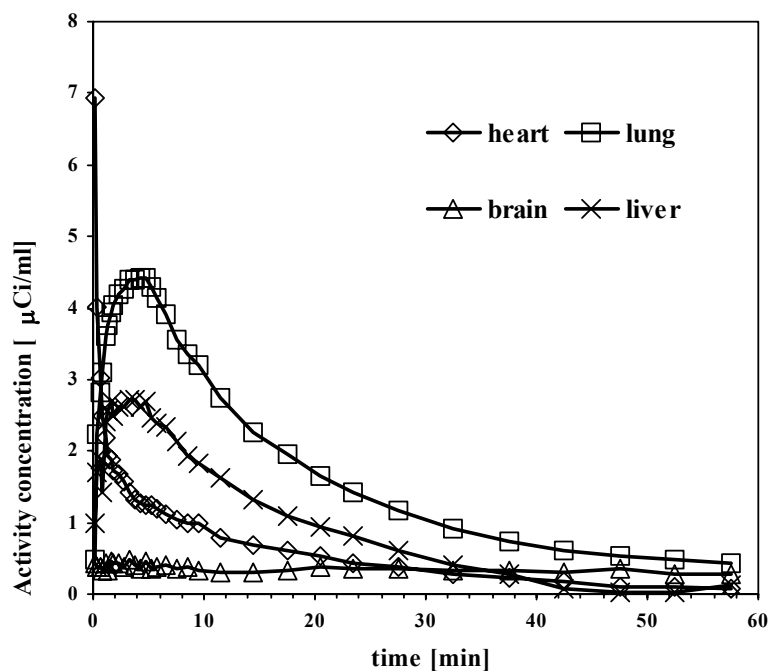


Figure 8. Dynamic ^{18}F -NECA-accumulation in the organs of rabbit

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 [^{18}F]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*.

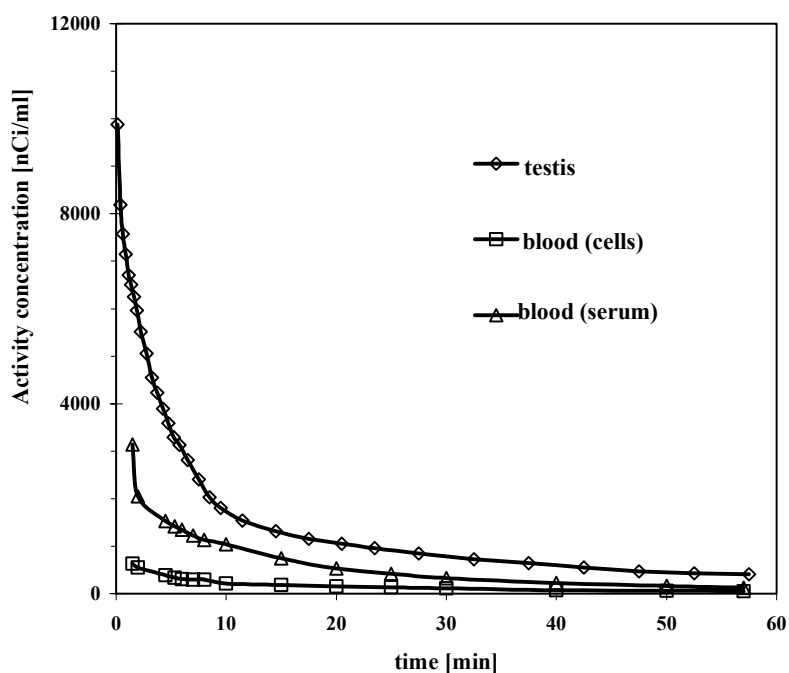


Figure 9. Dynamic PET scan of rabbit – ^{18}F -NECA-accumulation in the blood

5. CONCLUSIONS

The author has taken part in development of the production for some [^{11}C]-labelled caffeine derivative using [^{11}C]methyl iodide as labelling agent. Results obtained showed that [^{11}C]-labelled form of ten adenosine receptor specific 8-styryl-7-methyl-xanthine derivatives were synthesised by N-methylation of the corresponding 8-styryl-xanthine derivatives under optimised conditions. The [^{11}C]methylation in each case can be conducted with high chemical yield (35-93%). These labelled ligands may facilitate the PET investigation of A_{2A} receptors. These investigations have been in progress at our Centre and their results have been published continuously. Results of these investigations are not included into this thesis.

Besides of these carbon-11 labelled adenosine receptor antagonists, however, synthesis of labelled 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. Moreover, one of our target molecules (FNECA) has been expected to possess some selectivity toward the A_{2B} receptor subtype.

The first molecule is 5'-deoxy-5'-[^{18}F]fluoro adenosine, whose synthesis the paper I and III deal with. The results obtained in **paper I** demonstrate that the halogen exchange reaction takes place to some extent for 5'-deoxy-5'-haloadenosines. The conversion (*that is* fluorine-18 uptake) 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. Therefore, the nucleophilic radiofluorination of 5'-halogenated adenosines cannot be regarded as an advantageous route to produce 5'-deoxy-5'-[^{18}F]fluoro-adenosine.

The results obtained in **paper III** showed that fluorine-18 can be introduced into the N⁶-benzoyl 2',3'-isopropylideneadenosine-5'-sulfonates, 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 under the conditions applied.

These yields are so low and insufficient, that the protocol after the hydrolysis and purification steps cannot result in an acceptable amount of the desired 5'-deoxy-5'-[^{18}F]fluoro adenosine.

According to **paper I.** and **III.**, we concluded that 5'-deoxy-5'-[^{18}F]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'-[^{18}F]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.

Paper II. presents that our second molecule of purpose, namely 5'-N-(2-[^{18}F]fluoroethyl)-carboxamidoadenosine ([^{18}F]FNECA), a promising ^{18}F -labelled adenosine agonist, has been prepared in two different synthesis routes.

In the first way, [^{18}F]fluoride was reacted with 5'-N,N-ethylene-2',3'-O-isopropylidencarboxamido-adenosine and after removing the protective group, [^{18}F]FNECA was obtained in a low radiochemical yield ($1\pm 1\%$).

In the second way, 2-[^{18}F]fluoroethylamine 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. 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). The total synthesis took 200 min and the decay-corrected radiochemical yield based on [^{18}F]F $^-$ activity was $17\pm 9\%$ with more than 99.9 % radiochemical purity. This second route provides sufficient amount of [^{18}F]FNECA for the subsequent biological evaluation in PET studies.

Results presented in **paper IV.** showed that [^{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.

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