Development of no-carrier-added radioselenation methods for the preparation of radiopharmaceuticals

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Abstract

The radioisotope selenium-73 (half-life: 7.1 h, positron-branching: 65 %) is an interesting label for application in positron emission tomography, serving as a possible substitute of sulfur in thio compounds of interest. Furtheron, the half-life of selenium-73 offers the possibility to study relatively slow pharmacokinetics of selenated radiotracers. Previous methods for labelling with radioselenium suffered from the drawback of an indispensable addition of (nat)Se-carrier, resulting in tracers of low specific activity. In consideration of the possible toxicity of selenium compounds, two new radiosynthetic pathways were developed in this work for the preparation of Se-73-labelled compounds at the no-carrier-added (n.c.a.) level. Selenium-75 (half-life: 120.4 d) was used for the development and optimization of these radiosyntheses.

The first method developed started with a homogenous or polymer-supported reaction of elemental n.c.a. Se-75 with an isocyanide and subsequently an amine. Radioselenoureas formed were alkylated via alkyl triflates to yield the corresponding Se-75-labelled selenouronium salts, which were purified afterwards. Hydrolysis under basic conditions and a subsequent second alkylation yielded various asymmetric n.c.a. Se-75-labelled dialkyl selenoethers with a radiochemical yield of 13 to 56 % (related to elemental Se-75) depending on the substituents. The use of the polymer-supported pathway provides the advantages of a shorter reaction time (35 min in comparison to 130 min in homogenous phase) and a more convenient separation of Se-75-labelled intermediates, thus appearing very attractive for automation. Subsequently, Se-73-labelled model compounds such as benzylmethylselenide and 1-phenyl-1-(propylseleno)ethane were synthesized using the optimized reaction conditions.

The second labelling method was based on the initial reaction of elemental n.c.a. Se-75 with sodium cyanide, yielding sodium radioselenocyanate. Treatment of this intermediate *in situ* with alkyl bromides resulted in Se-75-labelled alkyl selenocyanates. After separation via reversed phase cartridges, the alkyl radioselenocyanates reacted with organic lithium or Grignard compounds to asymmetric Se-75-labelled selenoethers with a radiochemical yield of 9 to 55 % (related to elemental Se-75) depending on the substituents. This pathway offers the advantage to generate both aliphatic and aromatic radioseleno compounds by using appropriate alkyl or aryl lithium or Grignard compounds.

The new approaches to n.c.a. labelling with radioselenium developed in this work extensively enlarge the availability of Se-73,75-labelled compounds. In particular, a method is described for the first time to prepare n.c.a. aryl radioselenoethers. For proof of identity via radioanalysis appropriate radio high performance liquid chromatography and radio thin layer chromatography methods were developed. Several non-radioactive seleno compounds, which served as reference substances, were prepared for the first time.

Besides various simple asymmetric selenoethers, two more complex, physiologically relevant molecules, i.e. the c.a. Se-75-labelled amino acid derivative L-homocysteine selenolactone and the n.c.a. Se-75-labelled adenosine A(1) receptor ligand 5'-(methylseleno)-N(6)-cyclopentyladenosine, were synthesized using preferentially the selenourea method with total radiochemical yields of 20 – 30 % (related to elemental Se-75).

Kurzzusammenfassung

Selen-73 (Halbwertzeit: 7.1 h, Positronenemission: 65 %) ist ein vielversprechendes Radionuklid für die Anwendung in der Positronen-Emissions-Tomographie, das als Substitut für Schwefel in physiologisch relevanten Thioverbindungen fungieren kann. Außerdem ermöglicht seine Halbwertzeit die Untersuchung relativ langsamer Pharmakokinetiken entsprechend selenierter Radiotracer. Etablierte Markierungstechniken mit Radioselen besitzen jedoch den Nachteil, dass aufgrund des unvermeidlichen (nat)Se-Trägerzusatzes Tracer mit nur geringer spezifischer Aktivität hergestellt werden können. Im Hinblick auf die mögliche Toxizität von Selenverbindungen wurden im Rahmen dieser Arbeit zwei neue Radiosynthesen im trägerarmen (n.c.a.) Bereich etabliert. Hierbei wurde das Isotop Selen-75 (Halbwertzeit: 120.4 d) für die Entwicklung und Optimierung eingesetzt.

Das erste Radiosynthesekonzept basierte auf der Reaktion von elementarem n.c.a. Se-75 mit einem Isonitril und einem Amin in homogener oder an fester Phase. Die resultierenden Radioselenoharnstoffe wurden mittels Alkyltriflaten zu entsprechenden Se-75-markierten Selenoniumsalzen umgesetzt. Nach deren Aufreinigung, basischer Hydrolyse und anschließendem zweiten Alkylierungsschritt erhielt man verschiedene asymmetrische, Se-75-markierte n.c.a. Dialkylselenoether in radiochemischen Ausbeuten von 13 bis 56 % (bezogen auf elementares Se-75) abhängig von den Alkylsubstituenten. Die Reaktionsführung an fester Phase bietet die Vorteile einer erheblich geringeren Gesamtsynthesedauer (35 min im Vergleich zu 130 min in homogener Phase) und einer einfacheren Aufreinigung der Se-75-markierten Zwischenprodukte und bietet sich daher für automatisierte Synthesen an. Mit Hilfe der optimierten Reaktionsbedingungen ließen sich Se-73-markierte Modellverbindungen wie Benzylmethylselenoether und 1-Phenyl-1-(propylseleno)ethan synthetisieren.

Die zweite Markierungstechnik basierte auf der Reaktion von elementarem n.c.a. Se-75 mit Natriumcyanid zu Natriumradioselenocyanat, das *in situ* mit Alkylbromiden zu Se-75-markierten Alkylselenocyanaten umgesetzt wurde. Nach Aufreinigung mittels Festphasenfixierung wurden die Alkylradioselenocyanate mit organischen Lithium- oder Grignard-Verbindungen zu asymmetrischen, Se-75-markierten Selenoethern umgesetzt, die man abhängig von den Substituenten in radiochemischen Ausbeuten von 9 bis 55 % (bezogen auf elementares Se-75) erhielt. Diese Radiosynthese bietet den Vorteil, dass sowohl aliphatische als auch aromatische Se-73,75-markierte Verbindungen mittels entsprechender Alkyl- oder Aryl-Lithium- bzw. Grignard-Verbindungen synthetisiert werden können.

Durch diese neuen trägerarmen Radioselenierungstechniken wurde im Rahmen dieser Arbeit die Klasse der zugänglichen Se-73,75-markierten Verbindungen erheblich erweitert. Insbesondere wurde erstmals eine Methode zur Synthese von n.c.a. Arylradioselenoethern entwickelt. Zur Identifizierung der Reaktionsprodukte mittels Radioanalytik wurden im Rahmen dieser Arbeit entsprechende RadioHPLC- und RadioTLC-Methoden entwickelt. Mehrere nicht-radioaktive Selenverbindungen wurden erstmalig synthetisiert.

Neben verschiedenen einfacheren asymmetrischen Selenoethern gelang zudem die Synthese des Se-75-markierten Aminosäurederivates L-Homocysteinselenolacton und des Se-75-markierten Adenosin A(1)-Rezeptorliganden 5'-(Methylseleno)-N(6)-cyclopentyladenosin. Diese zwei komplexeren, physiologisch relevanten Moleküle konnten mittels der Selenoharnstoff-Methode in radiochemischen Gesamtausbeuten von 20 bis 30 % (bezogen auf elementares Se-75) synthetisiert werden.

Contents

Chapter 1

Introduction

1.1 Radionuclides in Life Sciences

Building on the discovery of radioactivity by H. Becquerel in 1896, the science of nuclear chemistry was born in 1898 when the Curies carried out the very first radiochemical investigations, leading to the discovery of polonium and radium. Since those years the work of many chemists made possible the rapid development of this new branch of natural science [1].

Among those chemists the Hungarian G. de Hevesy and the Austrian F. Paneth distinguished themselves by the idea to use radionuclides as indicators. The simple purpose of this radiotracer chemistry is to follow the behaviour of an element in a chemical reaction using radiolabelled atoms, which can be easily detected [2]. The first application (1912) was in the determination of the solubilities of lead chromate and sulfide [3], and Hevesy subsequently made use of this technique in work on chemical reactions, for example, isotopic exchange and electrolytic deposition. In 1920 he carried out the first biological experiments showing the distribution of lead atoms in a plant [4], thus discovering the possible application of this technique for the study of metabolic turnover. Further biological studies followed, and much of tracer studies in general and modern nuclear medicine in particular takes advantage of Hevesy's realization that the state of radioactivity of radionuclides affects in no way their biochemical behaviour because the chemical properties of all isotopes of the same element are identical. He saw that a radioactive isotope might be used as a "representative" tracer of stable atoms of the same element whenever and wherever it accompanies them. This concept was followed by several other scientists and has become an important tool in life sciences. Thus, Hevesy is often attributed as "Grandfather of Nuclear Medicine", and he received a Nobel Prize in 1943 as acknowledgement of his life's work [5].

The number of elements, however, for which radioactive isotopes were available, was comparatively small in the 1920s and possible applications of the method were in consequence strictly limited. But with the invention of the cyclotron by E.O. Lawrence (1930) and the construction of the first nuclear reactor by E. Fermi (1942) a wealth of artificial radionuclides became available, and their applications in life sciences have rapidly increased during the last century [6]. Because of their decay properties, their achievable chemical and radiochemical purity as well as their biochemical usefulness, only a small part of the more than 2400 radionuclides known today can be used in nuclear medicine. They can be classified as diagnostic or therapeutic radioisotopes. The former are mostly short-lived single photon and positron emitters for *in vivo* tracer studies [7] and the latter longer-lived corpuscular radiation emitting radionuclides for brachytherapy and endoradiotherapy [8]. Apart from these *in vivo* medical applications, many radioactive *in vitro* methods (e.g. radioimmunoassay and auto-radiography) have been developed. The radiotracer technique now extends over large fields of life sciences and has played a key role e.g. in the discovery of metabolic principles, of the function of receptor systems and in the development of pharmaceuticals.

1.2 Emission tomography of radiotracers

During the last three decades new powerful non-invasive imaging procedures have been developed to improve the diagnosis of diseases. The methods differ in their underlying physical basics, the achievable image with respect to temporal and spatial resolution as well as in the kind of information they produce. Whereas the strengths of computed tomography (CT), magnetic resonance tomography (MRT) and ultrasound (US) lie in the display of structural information, the attractive characteristics of nuclear medicine methods are their ability to reflect a variety of metabolic and physiological processes all over the body using, dependent on the radionuclide involved, single photon emission computed tomography (SPECT) or positron emission tomography (PET). For these purposes, substrates, which take part in metabolic and physiological processes, are labelled with radioisotopes and used as *in vivo* indicators [9 – 11], according to the radiotracer principle introduced by Hevesy. The use of radioactive tracers in clinical diagnosis and pharmaceutical research is still increasing because of their advantage of detection from outside the body. Because the radiotracer concentrations are generally extremely low, often in the subnanomolar range, these agents exert no pharmacodynamic effects and do not disturb the biological system in any kind [12].

SPECT is the more widely available and routinely used tomographic tool of nuclear medicine due to its lower cost, providing qualitative images. The success of SPECT is strongly coupled to the artificial single photon emitter ^{99m}Tc. It emits a monoenergetic radiation of 140 keV and has a convenient half-life of about 6 h. Furthermore, it is available with a generator system, thus making SPECT independent of a nearby radionuclide production. Over the years the radiopharmaceutical industry has developed many kits containing precursor compounds to be labelled with Tc-99m. In addition, other photon emitters such as ^{123}I and ^{201}Tl are also used routinely.

On the other hand, PET is regarded as an advanced method with a better sensitivity of a factor up to 100 over SPECT and therefore able to record shorter time frames in dynamic studies. Moreover, accurate transmission measurement and exact attenuation correction lead to quantitative interpretation of the PET images. As a result, this technique offers the possibility to quantify tracer concentrations and kinetics in organs and allows the application of biomathematical models in order to calculate physiological reactions [13].

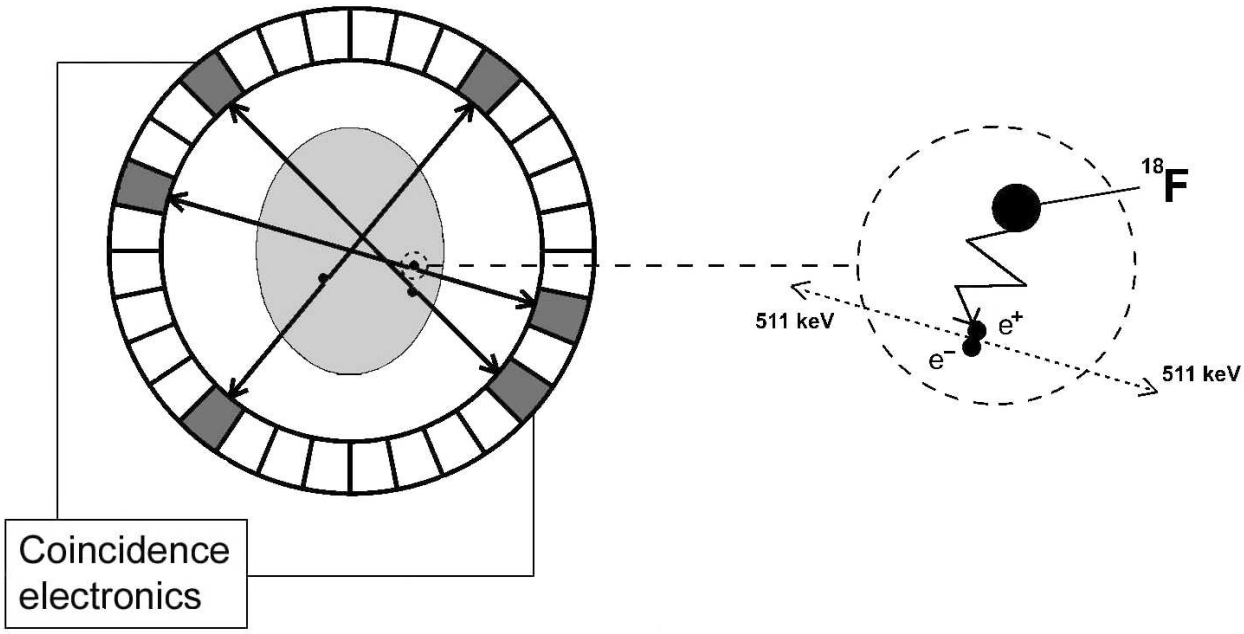


Fig. 1.1: Principle of coincidence measurement; the zoomed part scheme indicates the positron emission of ^{18}F and the subsequent annihilation process

Figure 1.1 shows the physical principles underlying PET. A positron is the primary result of the radioactive decay of an appropriate radiolabel used in PET. Depending on its energy, which is specific for the individual radionuclide, the positron travels a short distance (between less

than 1 mm to up to several mm) until it is nearly at rest and now able to interact with an electron, its anti-particle, in the surrounding material. As a result, both particles annihilate and produce a pair of tissue-penetrating photons that are emitted in 180° apart both with a characteristic energy of 511 keV, corresponding to the rest energy of one electron [14].

This event can be detected externally by a PET device, consisting of a circular array of scintillation detectors. If the two photons of a photon pair hit two opposite detectors of the ring within a very short time window (≈12 ns), it is assumed that these photons belong to the same annihilation process (coincidence measurement). In this case, the positron emitter is located inevitable on the connecting line between the two detectors, and the spatial resolution is widely determined by the size of the detector crystal pair. Due to the fact that the 511 keV γ-rays will completely penetrate the body when measured, an attenuation factor can be determined by an independent transmission measurement performed with ^{68}Ge prior to the tracer injection in order to correct for scattering and absorption. This allows an accurate measurement of the distribution of radioactivity in the body.

Nevertheless, the intrinsic loss of spatial resolution due to the uncertainty in distance between the origin of positron emission and of positron-electron annihilation may be a disturbing factor in applications requiring high resolution. The larger the positron energy, the larger is the average distance that it travels before annihilating and, consequently, the larger the loss of spatial resolution. This intrinsic effect is characteristic for the radionuclide and is independent of the design and imaging properties of the PET cameras. Therefore, nuclides for PET should ideally emit positrons having low energy.

In vivo functional imaging with PET has been regarded mostly as a research tool, too expensive to become of general importance for routine clinical use. Indeed, in the late 1970s first PET devices were introduced mainly in research environments where necessary cyclotrons for the production of the short-lived neutron-deficient positron emitters were available [15]. Since then the sensitivity and image resolution of PET systems have been drastically improved. In addition, the rapid development of radiochemistry, in particular with regard to labelling with short-lived radionuclides at the no-carrier-added level played a key role in this context. The establishment of reliable labelling techniques, reasonable costs and clinical significance have led to a rapid world-wide increase of PET scanners from several dozens in 1990 to more than 300 in 2000, used for routine medical imaging and pharmaceutical research. Modern PET cameras consist of up to 30 rings of detectors with a spatial resolution down to 3 – 5 mm and are sometimes combined with CT or MRT systems. This fusion of PET and MRT/CT facilitates three-dimensional images with exact attribution of physiological to morphological information. In addition, high resolution PET for animals [16] opens the possibility to perform repeatable drug trials in one animal, i.e. without the need for vivisection. This reduces the expense for such

experiments, saving animal lives and cutting costs for the development of new radiopharmaceuticals.

1.3 Radiotracers for PET application

Due to the high sensitivity and the good spatial resolution together with the precise quantitation obtained by PET technique one can acquire more (patho)physiological information about the nature of tissue function at the biochemical level than by any other non-invasive imaging procedure. This metabolic imaging or rather the measurement of regional biochemical functions requires endogenous or exogenous labelled substrates that participate in a metabolic process. Thus, the design of such tracers (termed as radiopharmaceuticals) is based on physiological concepts such as:

- metabolic turnover of biological substrates and drugs,
- perfusion,
- enzyme function,
- neurotransmitter biochemistry and
- immuno reactions.

Biomolecules (e.g. glucose, amino acids, fatty acids) and drugs mainly consist of carbon, hydrogen, oxygen, nitrogen, sulfur and phosphorus; therefore short-lived positron emitting isotopes of these elements lend themselves as authentic labels for those substances because of the unchanged chemical and physiological properties of the labelled molecules. In addition, a short half-life is advantageous for imaging in humans since large amounts of radioactivity can be administered for good counting rates while keeping the total absorbed radiation dose below a tolerably low rate.

The so-called organic radionuclides ^{11}C, ^{15}O, ^{13}N and ^{30}P meet these demands, but due to their very short half-lives ranging from two to twenty minutes their applications are quite limited. In case of N-13 ($T_{1/2}$ = 10 min) and O-15 ($T_{1/2}$ = 2 min), simple molecules such as $[^{13}N]NH_3$, $[^{15}O]H_2O$ and $[^{15}O]C_4H_9OH$ act as perfusion tracers and allow the measurement of blood flow and volume; molecular oxygen metabolism can be studied using $[^{15}O]O_2$. The half-life of carbon-11 (20.4 min), on the other hand, is more suitable for complex radiosyntheses and more extended PET studies. Several primary precursors such as the most important $[^{11}C]CO_2$, $[^{11}C]CH_4$ and $[^{11}C]CO$ [17] and the very significant secondary precursor $[^{11}C]CH_3I$ are readily

available and offer the possibility to label a wealth of organic molecules with carbon-11 with high specific activity [18]. These radiosyntheses have been automated to facilitate the production of ^{11}C-labelled compounds, thus enabling diagnostic nuclear medicine to use some radiotracers (e.g. [methyl-^{11}C]methionine [19], [^{11}C]flumazenil [20] and [^{11}C]raclopride [21]) routinely as PET radiopharmaceuticals.

The short half-lives of the organic positron emitting radioisotopes, however, do not allow studies of slow biochemical processes. These require labels with longer half-lives than the available organic PET isotopes. The idea is to introduce a radioisotope of an element not present in the original biomolecule. This is referred to as foreign labelling resulting in a so-called analogue tracer. Radiohalogens (^{18}F, ^{75,76}Br and ^{124}I) play a key role in this approach to radiopharmaceutical development [22, 23]. Structural analogues are obtained when a halogen atom replaces a hydrogen atom [e.g. 24], a hydroxyl or a methyl group. While structural changes are negligible with a fluorine atom substituting a hydrogen atom or a bromine or iodine atom formally replacing a methyl group, electronic changes can be significant, particularly in the case of fluorine. Substitution with a halogen also leads to an increase in lipophilicity (besides in case of aliphatic fluorination), which often results in an increased non-specific binding or even leads to a blood flow tracer, particularly in the case of iodine. So the biochemical properties of the halogenated analogue can be quite different from those of the original molecule. Since the suitability of a radiopharmaceutical is based on its ability to trace a physiological or biochemical response in specific tissue, this characteristic has to be tested in each individual case [12, 25, 26].

Apart from those standard positron emitters, the increasing significance and spreading of PET stimulate the evaluation of alternative PET radionuclides (e.g. ^{73}Se, see subchapter 1.6) to supplement the commonly used labels for two reasons mainly. First, studies of slow biochemical processes may require access to radionuclides that are more long-lived than the conventional PET nuclides. Secondly, due to a possible shipment of longer-lived positron emitters, satellite PET imaging units would benefit and would be more cost-effective. Furthermore, the newest generation of PET scanners makes increased use of long-lived radionuclides more acceptable, since much lower amounts of injected radioactivity are required for comparable images.

Metallic positron emitters represent the third group of available PET isotopes. Unlike the organic and analogue counterparts, a number of cationic radionuclides can be produced by generators (e.g. ^{82}Rb, ^{62}Cu and ^{68}Ga), thus being readily accessible even in facilities lacking a cyclotron [27]. For *in vivo* investigations they are applied as free cationic tracers or in

complexed form. In addition, the cyclotron-produced positron emitters Y-86 and Tc-94m are of some interest; the former primarily for the possibility of using PET to quantitatively assess the pharmacokinetics of ^{90}Y-labelled agents used in therapeutic treatment and the latter to use PET for quantitative estimation of uptake of gamma emitting ^{99m}Tc-labelled SPECT radiopharmaceuticals [28].

Tab. 1.1: Nuclear properties of important PET radionuclides [from 7, 12]

Nuclide	Half-life	Decay mode (%)	E(β^+_{max}) [MeV]
organic			
^{11}C	20.4 min	β^+ (99.8); EC (0.2)	0.96
^{13}N	10 min	β^+ (100)	1.19
^{15}O	2 min	β^+ (99.9); EC (0.1)	1.72
^{30}P	2.5 min	β^+ (99.8)	3.25
analogue			
^{18}F	109.6 min	β^+ (97); EC (3)	0.64
^{73}Se	7.1 h	β^+ (65); EC (35)	1.30
^{75}Br	98 min	β^+ (75.5); EC (24.5)	1.74
^{76}Br	16 h	β^+ (57); EC (43)	3.90
^{120}I	1.35 h	β^+ (64); EC (36)	4.10
^{124}I	4.2 d	β^+ (25); EC (75)	2.14
metallic			
^{38}K	7.6 min	β^+ (100)	2.68
^{62}Cu	9.7 min	β^+ (98); EC (2)	2.93
^{68}Ga	68 min	β^+ (90); EC (10)	1.90
^{82}Rb	1.3 min	β^+ (96); EC (4)	3.35
^{86}Y	14.7 h	β^+ (34); EC (66)	1.30
^{94m}Tc	52 min	β^+ (72); EC (28)	2.47

For several years, the most important and most used PET radionuclide for routine as well as research application has been fluorine-18. This is due to its very suitable nuclear and chemical properties, i.e.:

- production in good yields, even with low-energy cyclotrons,
- convenient half-life, allowing extended radiosyntheses and PET studies and even shipment within a range of at least 100 km,
- low positron energy, being advantageous with respect to high resolution PET and minor radiation dose to the patient,
- high specific activity, due to low natural occurrence of ^{19}F,
- several facile labelling reactions, developed to replace a C-H bond with a C-^{18}F bond and
- stability of the C-^{18}F bond.

As mentioned before, the introduction of fluorine into a biomolecule can alter its primary physiological properties. This is very useful in the case of 2-[^{18}F]fluoro-2-deoxy-D-glucose (FDG), which is the most widely used fluorine-18 labelled radiotracer for PET. *In vivo*, FDG undergoes carrier-mediated uptake as a glucose analogue and serves as a substrate for hexokinase. But structural differences of FDG from the parent molecule (glucose) lead to no further metabolic acceptance. Since the labelled molecules remain in the cell, this is referred to as metabolic trapping, which allows quantitation of the regional glucose metabolism with the help of a three-compartment model [29]. FDG is used predominantly in the study of cerebral and myocardial metabolism and in the diagnosis of tumours due to the increased glucose metabolism characteristic of tumour cells [30, 31].

The importance of ^{18}F-labelled PET tracers is still spreading thanks to the facile introduction of the label by fully automated black boxes or robots. Routine *in vivo* applications of such radiopharmaceuticals become more and more significant in medical imaging providing a new window to brain, heart and tumour in particular [32 – 34].

1.4 Basic aspects of no-carrier-added radio-labelling

Radiopharmaceuticals are administered in the no-carrier-added state if possible, i.e. at doses in the subnanomolar range. Due to these chemically and physiologically insignificant quantities of radioactive material, pharmacodynamic as well as toxic and immunological reactions do not arise. Therefore, a decisive criterion for the quality of a radiotracer is its specific activity, which

depends on the amount of a carrier, i.e. an element or a compound with identical (isotopic carrier) or very similar (non-isotopic carrier) chemical properties to the radionuclide or the radiolabelled molecule, respectively. In radiochemical practice, radiosyntheses can be classified as

- carrier-free (c.f.),
- no-carrier-added (n.c.a.) or
- carrier-added (c.a.).

The absolute lack of a carrier is ideally only achieved when artificial radioelements (e.g. astatine) are used and the presence of longer-lived radioisotopes of the element can be excluded. This is the only case one can correctly term a carrier-free radiosynthesis. In contrast, when performing labelling reactions with cyclotron-produced radioisotopes of natural-occuring elements, traces of stable isotopes of these elements are omnipresent and act as isotopic carriers, provided that they are in the same chemical state. Possible sources of such contaminations are the air, target and reaction vessels, chemicals and solvents. The stable isotopes present in the reaction mixture are far in excess of the radioactive species but still below the limits of purification by normal chemical methods. In this case one speaks of a no-carrier-added radiosynthesis. Under several circumstances, weighable quantities of the natural-occuring element are added to the system in order to increase the radiochemical yield or even to make certain labelling methods possible. This is termed as a carrier-added radiosynthesis.

In the origin sense, the specific activity A_s is the activity per unit mass of an element or compound containing the radioactive nuclide [35]. But since molar activities are more convenient and informative than specific activities, A_s is usually expressed on a molar basis:

$$A_s = \frac{A}{n} \quad [Bq/mol] \qquad \textbf{Eq. 1.1}$$

where A is the activity and n is the amount of substance. In the absence of stable isotopes of a radionuclide, the maximum molar activity $A_{s,max}$ is given by:

$$A_{s,max} = N_A \frac{ln2}{T_{1/2}} \quad [Bq/mol] \qquad \textbf{Eq. 1.2}$$

where N_A is Avogadro's number and $T_{1/2}$ the half-life of the radionuclide. Many applications in nuclear medicine require high molar activities. For that reason it is useful to know the maximum values that are theoretically attainable. For example, the maximum molar activities of the

routinely used positron-emitters ^{18}F and ^{11}C are $6.3x10^{10}$ GBq/mol and $3.4x10^{11}$ GBq/mol, respectively. However, the ubiquity of stable elements has to be taken into account and the actual molar activities are much smaller than theoretically possible.

As mentioned above, short-lived radionuclides of high specific activity represent micro-amounts (< 1 µg) of matter. Handling of such minute amounts requires special precautions, because their physico-chemical behaviour may be quite different from that observed for components present at macroscopic scale. The most prominent difference is the possible sorption of a large part of n.c.a. radionuclides and radiolabelled products on the walls of the vessel during the reaction and on the exchange material during separation via chromatographic methods (e.g. HPLC, GC) or solid-phase systems. Besides, small quantities of impurities even in chemicals and solvents of high purity grade may be responsible for unexpected side-reactions and losses. In consequence, the radiochemical yield may decrease in comparison to the yield of the corresponding macroscopic reaction and chemically very similar by-products may be produced.

Furthermore, the course of a reaction at the n.c.a. level may strongly differ from that of the "classical" case due to the non-stoichiometric ratios and the non-equilibrium conditions. Whereas in reactions of macroscopic chemistry higher kinetic orders prevail, syntheses at the n.c.a. level, on the other hand, normally proceed according to pseudo-first-order kinetics. In the case of a reaction of the form A + B $\rightarrow$ C, the concentration of the precursor A is in great excess over the n.c.a. radionuclide B, thus it is a good approximation to take [A] as constant throughout the reaction. Assumed the reaction starting with $[B]_0$, the concentration of product C after the reaction time t can be written as:

$$[C]_t = [B]_0 (1 - e^{-kt}) \quad [mol/l] \qquad \textbf{Eq. 1.3}$$

where k is the rate coefficient of pseudo-first-order type and contains the constant [A] [36]. Accordingly, with increasing reaction time t as well as with increasing precursor concentration [A] the radiochemical yield of C rises and can be plotted as a hyperbolic curve. The saturation yield finally reached corresponds in the ideal case to $[B]_0$ and is often achieved within a few minutes due to the high excess of the precursor. Furtheron, consecutive labelling reactions of the product can be excluded, since the radionuclide and the radiolabelled product are both components at the tracer scale which do not interact for statistical reasons.

Considering the differences between "classical" macroscopic chemistry and radiochemistry several further important points have to be taken into account for the development of a

synthesis of a radiopharmaceutical at the n.c.a. level. The synthesis, separation and quality control of a radiopharmaceutical should be completed within three half-lives of the radionuclide used. Because of the sensitivity to impurities, it is necessary to purify reagents and solvents with special care. The so-called “last-step labelling”, i.e. the introduction of the radioactive label into the compound at the latest possible stage, is desirable for routine production in order to avoid complex syntheses with radioactive intermediates which will minimize the radiochemical yield and increase the radiation burden.

1.5 Selenium

Selenium was discovered and isolated in 1817 by J. J. Berzelius and J. G. Gahn, having noticed and analyzed a reddish-brown deposit in the lead chambers of a sulfuric acid plant [37]. It was named after the Greek *selene*, the moon, since the new element resembled tellurium (Latin *tellus*, earth), discovered some 35 years before [38].

Selenium is a comparatively rare element and has a natural abundance of about 0.05 ppm of the earth's crust, similar to that of Ag, Hg and Pd. It is widely but unevenly distributed in rocks and soils, often found in association with sulfur. Many sulfides of chalcophilic metals (e.g. Cu, Fe, Ag) contain Se as selenide and partly in its oxidized form as selenite [39].

Selenium has six stable isotopes, of which the most abundant one is ^{80}Se (49.6 %). They all generate distinct lines in mass spectra of selenium compounds, thus forming a characteristic group, making it easy to identify selenium-containing fragments. The isotope ^{77}Se has a nonzero magnetic moment allowing nuclear magnetic resonance studies on selenium compounds [40].

Selenium exists as several structurally definite forms [41, 42]: The red polymorphs (α, β and γ), consisting of Se_8 rings, are comparable with sulfur and often deposit during reactions involving organoselenium compounds especially by air-oxidation of reduced forms of Se. Grey (or “metallic”) selenium (m.p. 217°C, b.p. 685°C) is thermodynamically the most stable form showing metallic properties and can be obtained crystalline from molten selenium. Its structure of helical polymeric chains contains strong Se-Se bonds between adjacent Se atoms in the chain and weak metallic interactions between the neighbouring atoms of different chains. Grey selenium is of interest because of its photoconductive properties and therefore used in photoelectric devices and xerography. Finally, vitreous black selenium, the ordinary commercial

form of the element, comprises an extremely complex and irregular structure of large polymeric rings with up to 1000 atoms per ring.

The main source of selenium is the anode slime accumulated during the electrolytic refining of Cu. These water-insoluble selenides obtained have to be separated from other components (Cu, Ag, Au, Te) of the deposit. The typical sequence for isolation and purification involves oxidation by roasting with soda ash in air resulting in water-soluble sodium selenite as well as sodium tellurite as by-product. Separation of Se and Te can be achieved by neutralizing the alkaline selenite and tellurite leach with sulfuric acid; this precipitates the tellurium as a hydrous dioxide and leaves the selenous acid (H_2SeO_3) in solution from which pure selenium can be precipitated by reduction with SO_2 [43].

Several industrial applications [44] for selenium have been developed since its discovery. Glass and ceramic industries need significant quantities of selenium, which is used for decolorizing the green tint in glass products, caused by iron impurities. During World War I, this was especially practised due to critically shortage of manganese. On the other hand, selenium was found to impart a ruby-red colour to glass obtained by incorporating solid particles of cadmium sulfoselenide.

Xerography is another very important application of Se. This invention by C. F. Carlson some 60 years ago led to a rapid, cheap and dry process for direct document copying using vacuum-deposited selenium as photoconductor [45]. The use of Se in photoelectric cells and semi-conductor devices represents related functions.

As an essential element of life, selenium is added in trace amounts to animal feeds to prevent certain diseases and to increase growth and of course utilized as human diet supplement. In medicine, a few Se-containing compounds act as fungicides.

1.5.1 General chemical properties

Selenium, a group VI A element (chalcogen) in the fourth period, exhibits many similarities of chemical properties to sulfur and tellurium. In its family it bridges between the nonmetals oxygen and sulfur and the metals tellurium and polonium, while in its period it is located between the metal (or metalloid) arsenic and the nonmetal bromine. Therefore, Se can be classified as both a metal and a nonmetal and occupies a unique position in the Periodic Table [46]. Like sulfur and tellurium, Se exists in the valence states –2 to +6 depending on the corresponding

compounds. As shown in Scheme 1.1, selenium can be reduced to selenide, oxidized to selenite and further oxidized to selenate.

Se^{2-} ⟷ Se^{0} ⟷ SeO_3^{2-} ⟷ SeO_4^{2-} **Sch. 1.1**

The most stable compounds are the selenides formed with strongly electropositive elements (e.g. Na, K, Ca) as well as the compounds with strongly electronegative elements such as O, F and Cl. But compared to sulfur, the higher oxidation states of Se are not that stable and Se oxides are relatively strong oxidizing agents. Another property, which distinguishes Se from sulfur, is its ability to form compounds with a coordination number greater than four [47].

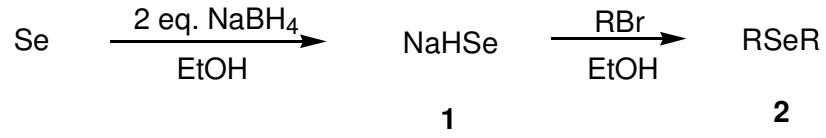
1.5.2 Organoselenium chemistry

Although the first organic compound containing selenium, ethylselenol, was prepared in 1847, organoselenium chemistry was a neglected field for many years [48], not at least because of the bad odour of Se-containing compounds, which are far more evil-smelling than their thio analogues. It was only in 1931 when the first selenium reagent was introduced into organic synthesis: Riley *et al.* observed the specific nature of the oxidizing action of selenium dioxide (SeO_2) on organic compounds [49]. Since that time SeO_2 has been used as a versatile oxidant for olefins and aldehydes [50]. Apart from selenium dioxide as oxidizing agent, elemental selenium and potassium selenocyanate, used for the introduction of Se into organic compounds, were the only selenium containing reagents employed for the next forty years.

The period between 1970 and 1975 represents a turning point in the field of selenium chemistry. We can take this time as the birth of modern organoselenium chemistry: In 1970, Jones *et al.* observed that certain steroidal selenoxides rapidly decompose to produce the corresponding olefins [51]. Shortly thereafter it was shown that eliminations of selenoxides represent a very mild general olefin-forming reaction [52]. A few years later Sharpless *et al.* described a method for the conversion of epoxides to allylic alcohols that utilizes a selenium nucleophile [53]. What followed was a worldwide explosion of interest in this new field of organic chemistry. Many chemical reactions were described allowing a large variety of synthetically useful processes to be accomplished in high yields under mild conditions. In particular, transformations such as allylic oxidations, nucleophile-induced cleavage reactions, regiospecific alkylations and other manipulations of functional groups can be readily achieved [54, 55].

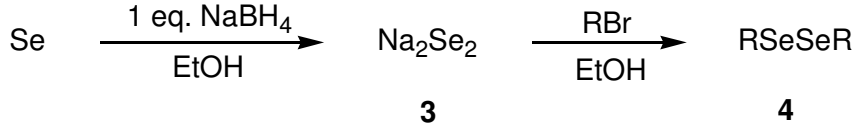
Often chemists refer to the properties of organosulfur compounds in order to rationalize the properties of organoselenium compounds. This is a reasonable approach, considering the chemistry of these elements is qualitatively similar. However, their properties often present marked differences. Usually, organoselenium compounds are more reactive than their sulfur analogues presumably because of the slightly greater polarity and lower bond strength of the C-Se σ-bond as well as other σ-bonds such as the N-Se and O-Se bonds [56]. The Se-Se bond is less stable than the S-S bond and is easily cleaved in various reversible reactions. When exposed to light, selenium compounds are often coloured red by decomposition to the free element.

The introduction of selenium into an organic structure can be performed in several ways [40, 57]. Since the selenium atom is mainly bivalent in organic derivatives and stabilized in the oxidation state –2, a widely used method for the preparation of organoselenium compounds starts with the reduction of selenium by two equivalents of sodium borohydride [58] yielding sodium hydrogen selenide (**1**), which can be utilized directly in typical nucleophilic displacement reactions forming symmetric selenoethers (**2**) (Scheme 1.2).



Sch. 1.2: Preparation of symmetric selenoethers (**2**)

Furthermore, diselenides, the selenium counterpart of organic peroxides and disulfides, play a key role in organoselenium chemistry since they are stable, easily handled and reactive enough to produce electrophilic and nucleophilic species [59], thus serving as useful precursors of various selenium functional groups. They can be produced by reduction of selenium with one equivalent of $NaBH_4$ resulting in sodium diselenide (**3**) which can be transformed to diselenides (**4**) by reaction with alkylating agents (Scheme 1.3).



Sch. 1.3: Preparation of diselenides (**4**)

Other methods for the introduction of selenium into organic molecules use the reaction of elemental selenium with organolithium compounds [60], isocyanides [61] or potassium cyanide and following nucleophilic displacement [62] to name a few only.

1.5.3 Selenium biochemistry

First attention to the biochemical properties of selenium and its compounds was turned in the 1930s when Se was identified as a potent toxic substance for cattle and other livestock. Animal diseases known as “blind staggers” and “alkali disease” were put down to grazing on pastures with soil of a high Se content, especially in parts of the United States of America [63]. Since that time, it has been discovered that selenium produces many toxic events in a wide variety of cell types at excessively high levels of intake [64]. This Se toxicity causes severe cases of poisoning and may even lead to death.

On the other hand, in 1957 the biological essentiality of selenium was recognized [65] and we now know that it is an essential trace element that serves as an integral component of several enzymes and acts as an antioxidant and anticarcinogenic agent [64]. This finding led to the recognition that several diseases are actually selenium deficiency syndromes; for example in certain parts of China, where an increased risk of heart diseases, bone and joint disorders and liver cancer is attributed to Se deficiency [66].

The complex and dynamic role, played by selenium in many biological processes has led to a very broad spectrum of research since the 1950s, still presenting many interesting problems. An effort is stimulated to discover and more thoroughly understand the role of selenium in mammalian organisms.

Humans have an estimated dietary intake of selenium of approximately 250 µg/day mainly coming from cereals, grain, fish, meat and poultry either in the form of selenoamino acids or Se-containing proteins and as selenate and selenite. The latter are reduced after ingestion to deprotonated hydrogen selenide (HSe^-) probably by the same enzymes that metabolize sulfate. Selenium exists in biological systems mainly in the anionic form of selenols ($R\text{-}Se^-$) or as selenoethers analogous to thioamino acids. The highest concentrations of selenium in humans are found in liver and pancreas, followed by kidneys. These organs are the sites of the major pathways of Se-metabolism [67].

The incorporation of Se into selenoproteins stands out due to two general pathways. On the one hand, in a highly specific fashion, selenium is incorporated into some functional proteins

during their biosynthesis in the form of the amino acid selenocysteine [68]. On the other hand, to a lesser degree, unspecific incorporation takes place with selenium replacing sulfur in already existing thioproteins. Therefore thioproteins always contain small amounts of Se. This is the main reason for the toxicity of selenium: When organisms receive more than micromolar concentrations of Se, too much sulfur is substituted. This may lead to inter- and intra-cellular dysfunction and eventually to death.

Selenoenzymes play important roles in oxygen metabolism, detoxification processes and the immune system. Especially their antioxidant function, primarily associated with glutathione peroxidase, suggests that selenium is a significant anticarcinogenic and antimutagenic agent [69].

The best-known selenoprotein is the mentioned glutathione peroxidase, which was identified as a selenium-containing enzyme in 1973 [70]. Many studies showed that most tissues contain this selenoprotein, which catalyzes the reduction of H_2O_2 and organic hydroperoxides to the corresponding alcohols, thus protecting the cells from oxidative damage [71, 72]. The protein is a tetramer with four identical subunits each of them possessing one selenium atom (present as selenocysteine) at the active site [73].

However, the majority of the element is not bound to glutathione peroxidase and in tissues of rats at least 12 other Se-containing proteins or protein subunits have been detected [74]. To name only a few, selenium was found in the selenoprotein P [75] and W [76] and in deiodinase enzymes [77].

1.6 Radioselenium in nuclear medicine

1.6.1 General applications

The role of Se in mammals could not have been detected without the use of radioselenium. Here, neutron activation analysis benefits from the radioactive nuclide selenium-75 and its application has rendered very good service in metabolic studies. Furthermore, ^{75}Se was used as a gamma-ray emitting label in a few radiopharmaceuticals despite unsuitable decay characteristics for *in vivo* medical application. Nowadays, ^{75}Se is mainly used in biochemical *in vitro* studies and for the development of radiosyntheses because of its conveniently long half-life and easy availability.

Apart from ^{75}Se, the positron emitting radionuclide ^{73}Se is an interesting alternative label for application in PET. Due to the lack of suitable positron emitters of sulfur for *in vivo* application in

nuclear medicine, foreign radionuclides have to be used for labelling thio compounds. Due to the chemical homology of sulfur and selenium, radioselenium lends itself as a possible substitute for sulfur to obtain radiotracers for *in vivo* application. The physical half-life of selenium-73 permits PET studies to follow slow metabolism over extended periods. In addition, Se-73 would allow enough time for preparation and delivery of ^{73}Se-labelled radiopharmaceuticals over long distances.

1.6.2 Nuclear properties and production of selenium-75 and selenium-73

As mentioned above, selenium-75 and -73 are the relevant radioisotopes of selenium to be used for the development of radiosyntheses and for application in nuclear medicine, respectively. Their nuclear properties are shown in Table 1.2.

Tab. 1.2: Nuclear properties of selenium-75 and -73 [from 78]

	Selenium-75	**Selenium-73**
Half-life	120.4 d	7.1 h
Decay mode	EC (100 %)	β^+ (65 %), E_{max}: 1.3 MeV; EC (35 %)
γ-ray energy [keV]	265 (58 %)	511 (130 %); 361 (97 %)
Daughter	arsenic-75	arsenic-73
Decay mode of daughter	stable	EC ($T_{1/2}$ = 80 d)

Selenium-75 decays via electron capture (EC) with a half-life of 120.4 d; selenium-73, on the other hand, decays both via positron emission (65 %) and EC (35 %) with a half-life of 7.1 h. ^{75}Se as a gamma-ray emitting label in various radiopharmaceuticals [79 – 81] proved insufficient for *in vivo* application due to the high internal radiation exposure to the patient when injected for diagnostic purpose. Furthermore, only poor-quality images are obtained with conventional gamma-cameras due to background interferences [82]. The advantage of ^{73}Se over ^{75}Se for application in nuclear medicine is its ability to be used as a PET label with two important physical properties. The relatively long half-life of selenium-73 offers the possibility to study selenated radiotracers with relatively slow pharmacokinetics. Secondly, its maximum positron

energy of 1.3 MeV results in an intrinsic spatial resolution of far less than 2 mm, which is very advantageous in PET studies. Besides, an apparently low total absorbed radiation dose is maintained in spite of the formation of ^{73}As as radioactive daughter [83].

The commercially available radioisotope ^{75}Se is generally produced via the (n,γ)-reaction on ^{nat}Se or enriched ^{74}Se in a nuclear reactor, yielding c.a. ^{75}Se with low specific activity. Since the 1970s studies have been carried out to produce Se-75 and Se-73 in the n.c.a. state via a cyclotron. The most relevant nuclear reactions for the production of n.c.a. selenium-75 and -73, respectively, are given in Table 1.3.

Tab. 1.3: Production routes for selenium-75 and -73

Nuclear Reactions	Optimum energy range [MeV]	Yield [MBq (mCi)/µAh]	Reference
Selenium-75			
^{75}As(p,n)	17 → 6	2.8 (0.075)	[78]
^{75}As(d,2n)	24 → 10	2.6 (0.07)	[78]
Selenium-73			
^{75}As(p,3n)	40 → 30	1406 (38)	[78]
^{75}As(d,4n)	45 → 33	651 (17.6)	[78]
^{nat}Ge(^{3}He,xn)	36 → 13	37 (1)	[84, 85]
^{nat}Ge(^{4}He,xn)	28 → 13	26 (0.7)	[84]
^{nat}Br(p,x)	62 → 42	81 (2.2)	[86]

Nuclear data measurements showed that the most suitable method for the production of n.c.a. Se-75 is the ^{75}As(p,n) process over the energy range of E_p = 17 → 6 MeV. This radioisotope can thus be produced even at a small cyclotron. But generally the production of ^{75}Se in no-carrier-added form has been handled in the context of ^{73}Se production, i.e. as an impurity rather than an isotope of utility.

First studies on the production of n.c.a. selenium-73 were carried out in the early 1970s using the ^{nat}Ge(^{3}He,xn) reaction [85]. Later, ^{3}He- and α-particle induced nuclear reactions on natural and enriched germanium were examined in detail and various production routes for ^{73}Se were evaluated at medium-sized compact cyclotrons [84, 87]. Besides, the nuclear reaction ^{nat}Br(p,x)^{73}Se was investigated, obtaining ^{73}Se with only minimal impurities [86]. Nevertheless,

the method of choice for the production of n.c.a. ^{73}Se is the ^{75}As(p,3n) process over the energy range of $E_p = 40 \rightarrow 30$ MeV [78]. Although a cyclotron is needed providing higher energies compared to the production routes via germanium as target material, the obvious advantage is the very high production rate of the ^{75}As(p,3n)^{73}Se reaction.

The separation of radioselenium from the irradiated target can be performed in general by three methods: Solvent extraction [85], anion-exchange chromatography [78] or thermo-chromatography [88], depending on the specific target used, since very different materials are applicable.

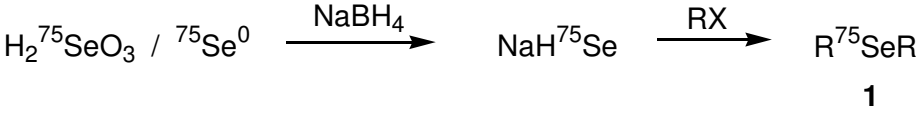
1.6.3 Radioselenium-labelling and radioselenium-labelled tracers

The most common radioselenium-labelled compounds are selenoethers, as the selenium atom can form two stable covalent bonds to carbon. Stabilized in this chemical form (oxidation state -2) radioselenium may serve in general as an authentic label in selenium-containing bio-molecules or as a foreign label in thio compounds of interest (e.g. thioamino acids, sulfur-containing pharmaceuticals). Furthermore, an indirect labelling can be performed by coupling a radioselenium-containing moiety (prosthetic group) to an appropriate compound. Only a few methods for introducing radioselenium into organic molecules have been developed during the past 40 years, which are covered by this chapter.

Carrier-added radiosyntheses and tracers

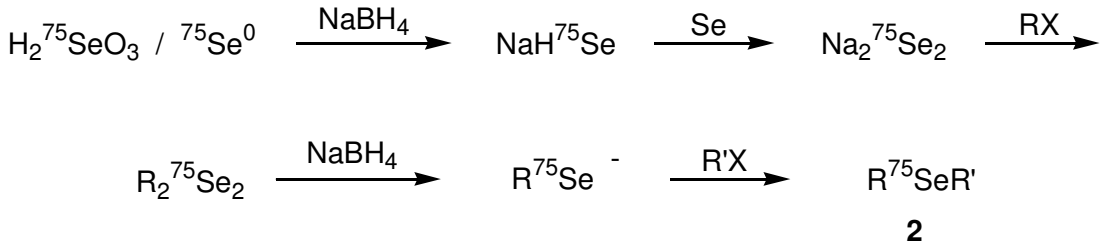
Since the commercially available radioselenium-species is ^{75}Se produced via the (n,γ)-reaction on highly enriched ^{74}Se, most of the radiosyntheses are based on carrier-added selenium-75 as starting material. In case selenium-75 is produced at the n.c.a. level via a cyclotron, ^{nat}Se-carrier is added before beginning of the radiosynthesis.

The most commonly used method for the preparation of symmetric c.a. [^{75}Se]selenoethers starts with the reduction of [^{75}Se]selenious acid or elemental ^{75}Se with an excess of $NaBH_4$ to obtain the [^{75}Se]NaHSe nucleophile. This is a versatile reagent for the synthesis of a variety of ^{75}Se-labelled compounds, since it can readily displace halides and tosyloxy groups (X) attached to carbon by S_N2 processes, yielding the appropriate ^{75}Se-labelled symmetric selenoethers (**1**) according to Scheme 1.4 [89]. This strong nucleophilicity is mainly due to the softness of [^{75}Se]NaHSe, which possesses donor orbitals of quite high energy levels [90].



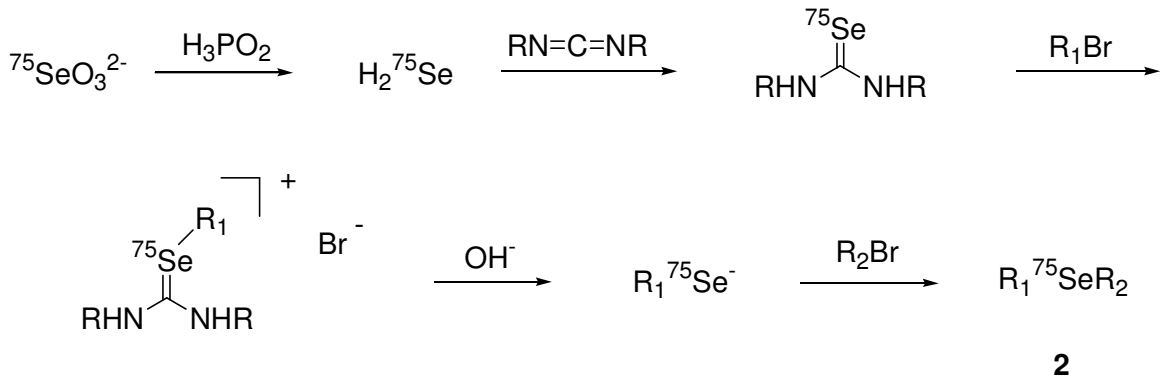
Sch. 1.4: Preparation of symmetric c.a. [^{75}Se]selenoethers (**1**)

On the other hand, asymmetric [^{75}Se]selenoethers can be obtained through intermediate [^{75}Se]selenols or [^{75}Se]selenolates using two general synthetic pathways. As depicted in Scheme 1.5, sodium [^{75}Se]diselenide is formed from $NaH^{75}Se$ by adding an equimolar quantity of elemental selenium to the ethanolic solution of $NaH^{75}Se$. Afterwards, ^{75}Se-labelled dialkyl diselenides can be prepared by adding an alkylating agent to $Na_2{}^{75}Se_2$. A reduction using $NaBH_4$ yields alkyl [^{75}Se]selenols, which may be converted with alkyl halides to asymmetric alkyl [^{75}Se]selenides (**2**) [91].



Sch. 1.5: Preparation of asymmetric c.a. [^{75}Se]selenoethers (**2**) via dialkyl [^{75}Se]diselenides

Alternatively, the synthesis of asymmetric [^{75}Se]selenoethers is possible via 1,3-disubstituted [^{75}Se]selenoureas as intermediates, resulting from 1,3-disubstituted carbodiimides and hydrogen [^{75}Se]selenide, which can be generated from c.a. [^{75}Se]selenite and phosphinic acid as reducing agent.



Sch. 1.6: Preparation of asymmetric c.a. [^{75}Se]selenoethers (**2**) via [^{75}Se]selenoureas

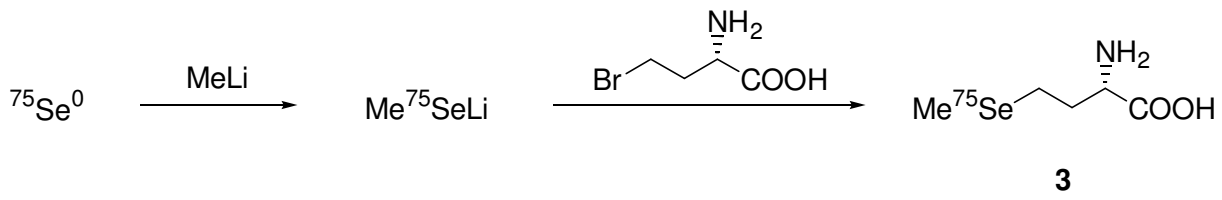
Treatment of c.a. [^{75}Se]selenoureas with alkyl bromides leads to corresponding [^{75}Se]selenouronium salts. Hydrolysis under basic conditions provides the [^{75}Se]selenolates and a second alkylation yields asymmetric [^{75}Se]selenoethers (**2**) (cf. Scheme 1.6) [92].

The irradiation of Se-containing molecules in nuclear reactors in order to obtain directly the corresponding ^{75}Se-labelled compounds via (n,γ) reaction represents another possibility of radioselenium-labelling [93]. Later observations, however, showed that the (n,γ) process involves Se-C bond rupture and Se-Se bond recombination, resulting in several degradation products [94]. Thus, this labelling method has never gained any significance, also with respect to purification problems.

Three commercially available ^{75}Se-labelled radiopharmaceuticals were earlier used for routine *in vivo* functional imaging [95]: [^{75}Se]selenomethionine, tauro-23-[^{75}Se]selena-25-homocholic acid and [^{75}Se]Scintidren. They could be used at the carrier-added level without any problem because they did not disturb the physiological systems examined. Since all the following compounds were asymmetric [^{75}Se]selenoethers, their syntheses were performed via diselenides as depicted in Scheme 1.5, unless otherwise mentioned.

The amino acid analogue L-[^{75}Se]selenomethionine (2-amino-4-methylselanyl-butyric acid) was the most prominent radioselenium-labelled radiopharmaceutical, being used as a pancreatic-imaging agent from the 1960s to the 1980s. That is because the pancreas is the site of high amino acid incorporation into proteins, for example digestive enzymes, and storage in secretory vesicles. Thus, L-[^{75}Se]selenomethionine was a successful agent for detecting pancreatic tumours [79].

The usual method for the preparation of L-[^{75}Se]selenomethionine was via biochemical radiosynthesis using Baker's yeast, followed by peptide hydrolysis and isolation by ion-exchange chromatography. The procedure takes more than 30 h and gives an overall yield of 20 – 40 % [96]. Alternatively, a fast chemical synthesis was developed using ^{75}Se^{0}, methyl lithium and L-α-amino-γ-bromobutyric acid as starting material, giving L-[^{75}Se]selenomethionine (**3**) in a radiochemical yield of more than 80 % in less than 3 h (Scheme 1.7) [97].



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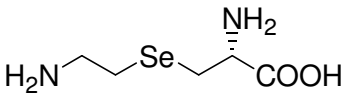
Sch. 1.7: Chemical synthesis of L-[^{75}Se]selenomethionine (**3**)

Apart from radioselenium-labelled selenomethionine, various [^{75}Se]selenoamino acids have been synthesized as potential pancreatic imaging agents. Those include L-4-selenalysine (2-amino-3-(2-amino-ethylselanyl)-propionic acid (**4**)) [98] and β-2/3-selenienylalanine (2-amino-3-selenophen-2/3-yl-propionic acid (**5**)) [99] (Scheme 1.8), which were tested in rats. Although the pancreas to liver ratio of the latter is twice as good as that for L-selenomethionine, it never gained any importance in nuclear medicine.



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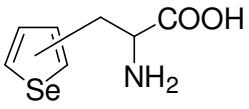

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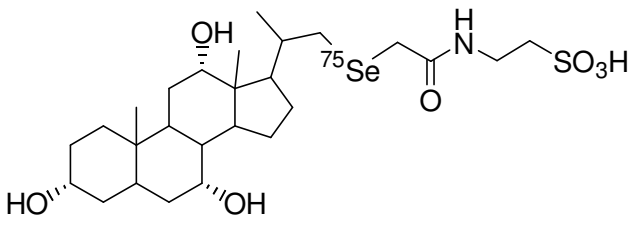
Sch. 1.8: L-4-selenalysine (**4**) and β-2/3-selenienylalanine (**5**)

^{75}Se-labelled compounds have been used in clinical investigations of the enterohepatic circulation (gut, liver and biliary tree), thus simplifying the examination of the functional state of the terminal ileum. For this purpose, various ^{75}Se-labelled bile acids were synthesized and their absorption and excretion were studied in animals [100]. The most promising compound tauro-23-[^{75}Se]selena-25-homocholic acid (**6**) (Scheme 1.9) was afterwards evaluated in man and showed very good results regarding absorption, secretion into the bile, reabsorption from the small intestine and resecretion [80]. This radiopharmaceutical was therefore used in the 1980s as a tracer of the complete enterohepatic circulation.

Further, steroids labelled with selenium-75 have been effective non-invasive probes for the diagnosis of many types of adrenal disease. [^{75}Se]Scintidren (6β-[(methyl[^{75}Se]seleno)methyl]-19-norcholest-5(10)-en-3β-ol (**7**), Scheme 1.9) was the agent of choice for the clinical diagnosis of adrenal pathologies [101].



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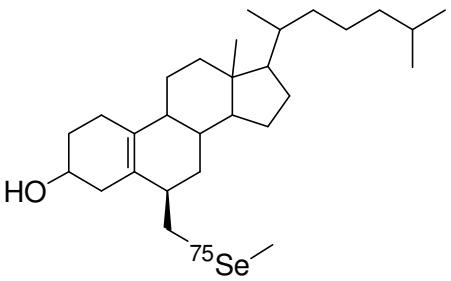

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Sch. 1.9: Tauro-23-[^{75}Se]selena-25-homocholic acid (**6**) and [^{75}Se]Scintidren (**7**)

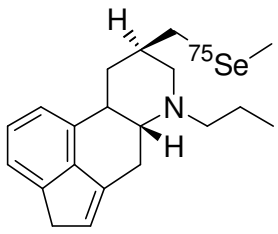
Beside these three earlier used and commercially available ^{75}Se-labelled tracers, various other radioselenium-labelled compounds were synthesized. Two outstanding examples are given below.

Certain types of central nervous system diseases such as Parkinsonism, Huntington's Chorea and Schizophrenia are associated with changes in the density of dopaminergic receptors. These observations have generated considerable interest in the development of techniques for the non-invasive detection and quantification of dopaminergic receptor sites, proving ligands with high affinity for these receptors and labelled with gamma- or positron-emitting radionuclides as ideal agents for mapping dopamine receptor sites [102].

In this context, radiolabelled ergoline derivatives were synthesized and studied for some 20 years. Pergolide, a synthetic, sulfur-containing ergoline, is a potent dopamine agonist in man [103]. Since pergolide contains a methylthio-moiety, which can be substituted by a methyl-[^{75}Se]seleno group, the synthesis of [^{75}Se]Selenopergolide ((8β)-8-[(methyl[^{75}Se]seleno)methyl]-6-propyl ergoline (**8**), Scheme 1.10) produced a new dopamine agonist with a gamma-emitting radionuclide [104].

[^{75}Se]Selenopergolide was evaluated for its ability to cross the blood-brain-barrier of rats and showed a high uptake in the brain, adrenal and heart with good organ to blood ratios, so that **8** might be clinically useful as a brain imaging radiopharmaceutical [102, 104].

An example for an authentic labelled tracer is [^{75}Se]Ebselen (2-phenyl-1,2-benziso[^{75}Se]selenazol-3(2H)-one (**9**), Scheme 1.10). The non-radioactive Ebselen (PZ 51) is a synthetic organoselenium drug with low toxicity and anti-inflammatory properties [105]. Synthesis of the ^{75}Se-labelled compound supported investigations of the metabolism and the pharmacokinetics of **9** in animal experiments [106] and first studies showed several similarities between Ebselen and glutathione peroxidase, resulting in the *in vivo* capability of the compound to catalyse the conversion of hydroperoxides to alcohols [107].



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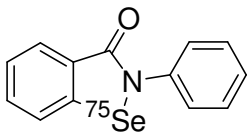


9

Sch. 1.10: [^{75}Se]Selenopergolide (**8**) and [^{75}Se]Ebselen (**9**)

Due to the unsuitable decay characteristics of selenium-75 for *in vivo* medical application and improvements in the field of alternative methods for diagnosis (US, MRT, CT), these ^{75}Se-labelled radiotracers are no longer in use.

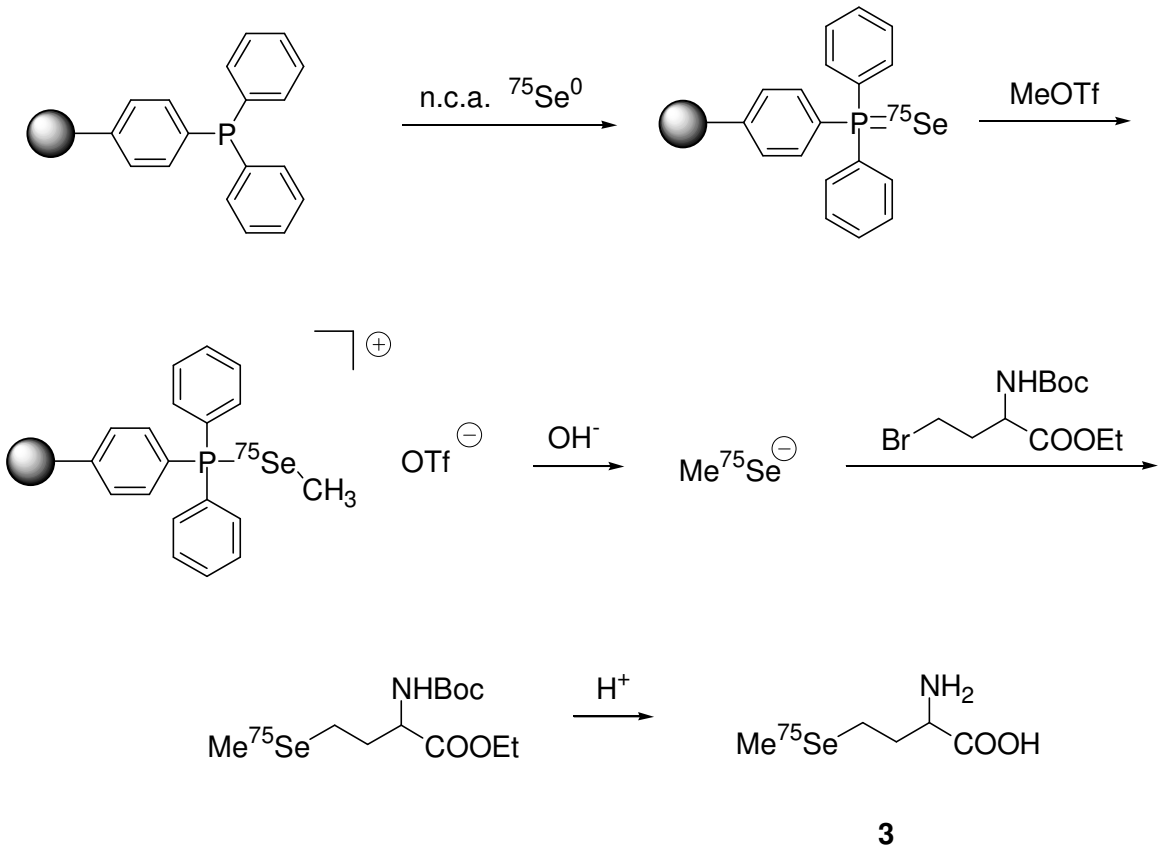
By taking advantage of the positron-emitter selenium-73 instead of the gamma-emitting label ^{75}Se, several radioselenium-labelled tracers could be useful as imaging agents for future PET studies. For example, first human PET investigations with L-[^{73}Se]selenomethionine, prepared by chemical synthesis described above, showed a very good delineation between liver and pancreas and this radiotracer may prove to be clinically valuable [82]. Moreover, it has been suggested that the use of L-[^{73}Se]selenomethionine may even be appropriate to measure brain protein incorporation in humans with PET [108].

Apart from L-[^{73}Se]selenomethionine, radioselenium-labelled steroids were studied as adrenal imaging agents [81] and these investigations showed that the use of several Se-73-labelled steroids would be a potentially powerful tool for the diagnosis of adrenal disease using PET.

No-carrier-added radiosyntheses and tracers

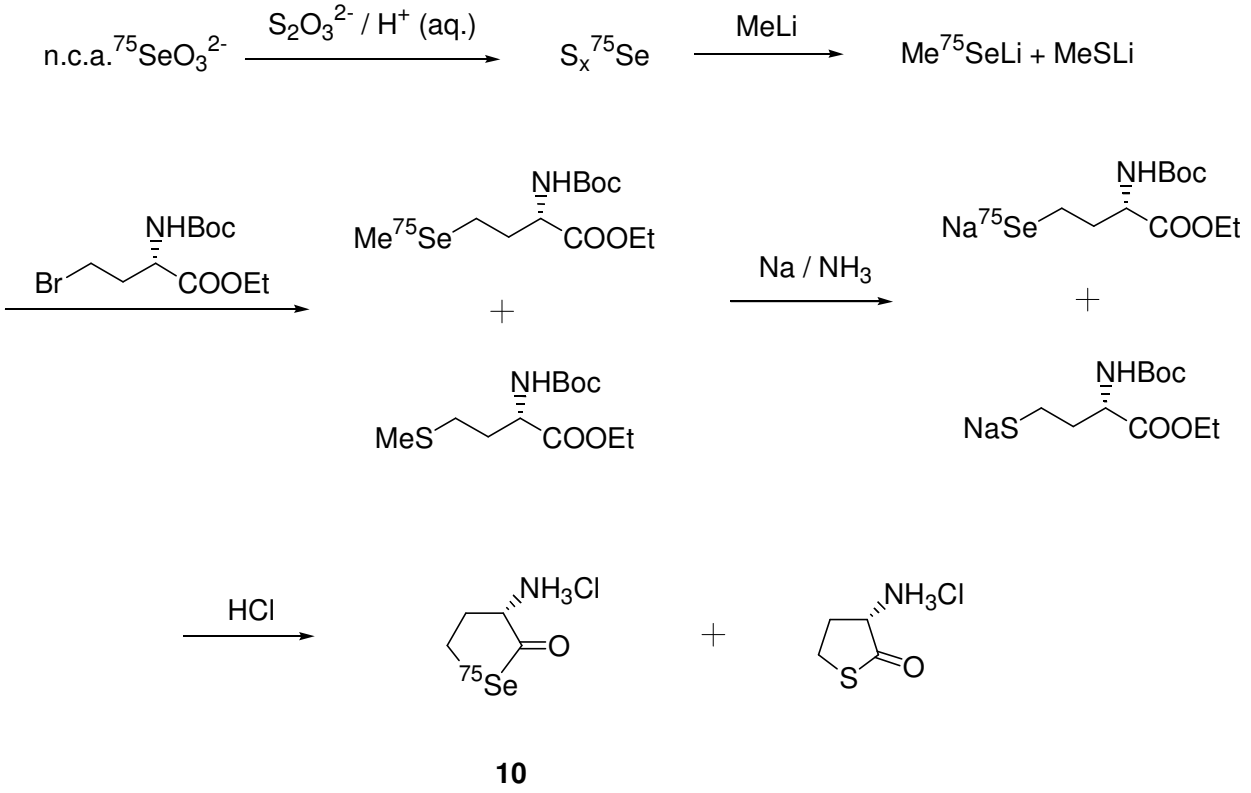
The drawback of the radiosynthetic pathways mentioned above is the indispensable addition of ^{nat}Se-carrier, resulting in products of low specific activity and the risk of possible selenium toxicity. All attempts made to perform these radiosyntheses at the n.c.a. level failed either because of poor reaction kinetics at subnanomolar levels [86] or because the necessary formation of intermediate diselenide-species during the reaction is not possible without ^{nat}Se-carrier [92]. Two completely new labelling approaches have been developed recently to achieve no-carrier-added radioselenium-tracers. In these studies, ^{75}Se was only used as model-nuclide for the development of radiolabelling methods. Possible future *in vivo* application for PET studies requires the use of Se-73.

Schmaljohann reported the first synthesis of n.c.a. D/L-[^{75}Se]selenomethionine (**3**) by a solid phase approach (Scheme 1.11) [109]. The reaction of elemental n.c.a. selenium-75 with polymer-supported triphenylphosphine led to the formation of the corresponding [^{75}Se]triphenyl-phosphineselenide. An alkylation with methyl triflate formed the [^{75}Se]methylselenotriphenyl-phosphonium salt. This phosphonium salt was hydrolized under alkaline conditions yielding the [^{75}Se]methylselenide anion. Alkylation using D/L-2-*tert.*-butoxycarbonylamino-4-bromobutyric acid ethylester as precursor led to the formation of protected D/L-[^{75}Se]selenomethionine. Acid hydrolysis gave the n.c.a. labelled D/L-selenomethionine in a radiochemical yield of 30 %.



Sch. 1.11: Preparation of n.c.a. D/L-[^{75}Se]selenomethionine (**3**) [cf. 109]

In 2001, Ermert *et al.* prepared n.c.a. homocysteine [^{75}Se]selenolactone (L-3-amino-dihydro-[^{75}Se]selenophen-2-one (**10**)) by using sulfur as non-isotopic carrier (Scheme 1.12) [110]. This labelling method is based on the original chemical synthesis of c.a. [^{75}Se]selenomethionine, described by Plenevaux *et al.* [97], substituting the selenium carrier by elemental sulfur. The reduction of n.c.a. [^{75}Se]selenite under acidic conditions with sodium thiosulfate led to the precipitation of sulfur, inserting n.c.a. ^{75}Se in the elemental form. This sulfur matrix was transformed with methyl lithium into a mixture of lithium methyl [^{75}Se]selenide and lithium methyl mercaptide. Subsequent reaction with L-2-*tert.*-butoxycarbonylamino-4-bromobutyric acid ethylester resulted in the formation of the protected L-[^{75}Se]selenomethionine and the corresponding methionine derivative. Following Birch reduction and consecutive cleavage of the protective groups under simultaneous lactonisation in presence of hydrochloric acid led to the formation of n.c.a. L-homocysteine [^{75}Se]selenolactone hydrolchloride (**10**) with a RCY of 5 – 10 % and L-homocysteine thiolactone hydrochloride. L-homocysteine [^{73}Se]selenolactone could be used as a longer-lived alternative of [^{11}C]homocysteine thiolactone, a sensitive indicator of adenosine production in ischemic myocardial tissue [111].



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Sch. 1.12: Radiosynthesis of n.c.a. L-homocysteine [^{75}Se]selenolactone hydrochloride (**10**) with sulfur as non-isotopic carrier

1.7 Radioligands for cerebral adenosine receptors

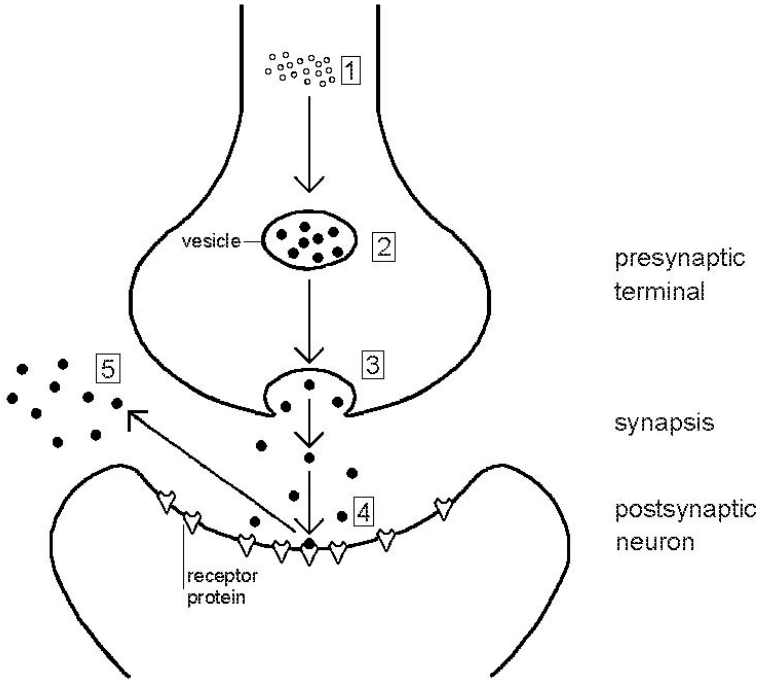
1.7.1 Synaptic transmission

The central nervous system (CNS) consists of a highly complex aggregation of cells, part of which is a communication network and another part a supportive matrix (glial cells). The communication network is formed by neurons, which are the functional cellular units consisting of a cell body (soma) with extensions (dendrites and an axon). Axons and dendrites emerging from different neurons intercommunicate by means of specialized junctional complexes known as synapses which are clefts (gaps) of between 15 and 20 nm separating the presynaptic and the postsynaptic sites [112, 113].

Almost all the synapses used for signal transmission in the CNS are chemical synapses. Whereas information is transmitted in a neuron by conduction of an electrical signal (also referred to as nerve impulse or action potential) from the soma or dendritic region along the

axon to the synaptic ending, a chemical neurotransmitter serves to signal information from the presynaptic end to the next cell. On release from nerve terminals, neurotransmitters act on receptor proteins at the postsynaptic membranes to produce either excitation, inhibition or modification of sensitivity of the target cell. Chemically mediated transmission involves the following processes (cf. Scheme 1.13):

1. synthesis of the neurotransmitter in the soma of the presynaptic cell,
2. storage of the neurotransmitter or its precursor in vesicles in the presynaptic terminal,
3. release of the substance into the synaptic extracellular space in response to an appropriate electrical signal coming from the soma,
4. recognition and binding of the compound by postsynaptic receptors and
5. termination of the action of the neurotransmitter by inactivation, degradation or re-uptake.



Sch. 1.13: Schematic depiction of a chemically mediated synaptic transmission

More than 120 transmitter substances have been discovered thus far. Some of them (e.g. amino acids, acetylcholine) have an “inherent” biological activity. They bind to ligand-activated ion channels at the postsynaptic membrane, thus causing a direct increase in conductance of certain ions into the postsynaptic neuron. Other neurotransmitters are referred to as neuro-modulators (e.g. biogenic amines, peptides); they have no direct activity but act indirectly via second messenger systems (e.g. G protein-coupled receptors) to bring about the postsynaptic

response, mostly being slower but longer-lasting in their effects compared with those of the former [114].

Receptors, which can be classified in several types and subtypes, are those constituents of a cell that have the ability to recognize a drug, a hormone or a neurotransmitter. The membrane of the postsynaptic neuron contains large numbers of those receptor proteins. The molecules of these receptors have two important components: (1) a binding component that protrudes outward from the membrane into the synaptic cleft and (2) an ionophore component that passes all the way through the membrane to the interior of the postsynaptic neuron. The protein surface of the binding component is a complicated shape containing hollows, ravines and ridges and somewhere amidst this complicated geography there is an area which has the correct shape to accept the incoming messenger. When the chemical messenger fits into this site, either the neurotransmitter acts as an agonist and “switches on” the receptor molecule and a postsynaptic action is induced, or the substance acts as an antagonist thus inhibiting a postsynaptic action. The binding of the neurotransmitter to its receptor is not based on a chemical reaction to obtain a completely new molecule, but rather on other interactions (ionic bonding, hydrogen bonding, van der Waals interactions, dipole-dipole interactions and induced dipole interactions). These specific binding interactions between messenger and receptor result in a change of the shape of the receptor protein and, in the case of an agonistic transmitter, subsequently affect other components of the cell membrane (e.g. ion channels, G proteins) leading to a biological effect [115].

1.7.2 Binding assays with radioligands

Though the basic principles governing the binding of neurotransmitters and drugs to receptors were elucidated many years ago, it was not until the 1960s that binding processes were studied directly. This was only possible by the use of radioactive labelled drugs. In the early 1960s, the binding of atropine to muscarinic receptors in smooth muscles was measured in this way. Over the next years, quantitative radioligand binding assays were developed for receptors for a variety of drugs and neurotransmitters, and ligands radiolabelled with ^{3}H, ^{14}C or ^{125}I are now available for the study of many classes of receptors. This widespread availability of suitable ligands has led to a rapid expansion in the use of binding assays with radioligands to characterize receptors and receptor subtypes [116].

Radioligands provide precise probes that permit specific examinations of the initial interaction between a drug and its binding site. For example, the kinetics of association and dissociation of a receptor-radioligand complex can be accurately determined by using simple tissue homo-

genates. A pharmacological profile of the radioligand that is based on the equilibrium dissociation constants of a series of unlabelled ligands can be defined by measuring the inhibition of the binding of a radioligand by these unlabelled compounds. The use of radioligands also permits characterization of receptors in the absence of a measurable biological response. This is important in the study of CNS receptors, where the effects of neurotransmitters are complex and isolated tissue preparations are difficult to obtain. The use of binding assays with radioligands can result in meaningful estimates of the number or density of receptors in a particular tissue. Consequently, changes in the density of receptors resulting from pathological conditions or pharmacological interventions can be monitored. Binding assays can also be used to discriminate multiple classes of receptors in a single tissue and to estimate their relative proportions [117].

Two basic types of assays utilize radioligands. The first, direct binding assay, measures the direct interaction of a radioligand with a receptor. Direct binding assays permit determination of both kinetic and equilibrium properties and provide estimates of the receptor density. They are also used to choose appropriate conditions and radioligands to determine the properties of receptors.

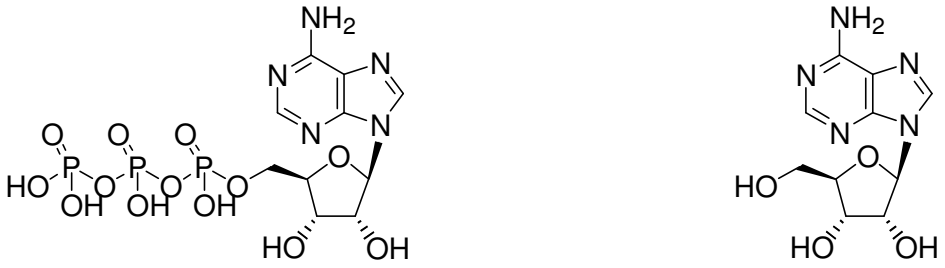
The second, indirect binding assay, measures the inhibition of the binding of a radioligand by an unlabelled ligand to deduce indirectly the affinity of receptors for the unlabelled ligand. This approach is particularly useful in the characterization of receptors and, *vice versa*, of drugs.

1.7.3 Purinergic system

Purines such as adenosine 5'-triphosphate (ATP) (**11**) and adenosine (**12**) (Scheme 1.14) play a central role in the energy metabolism of all life forms, but it is also recognized that purines are released from neurons and produce widespread effects on multiple organ systems by binding to cell-surface purinergic receptors. ATP is a classical neurotransmitter that is packaged into neuronal secretory granules and released in quanta in response to action potentials. In addition, ATP is released from non-neuronal sources such as platelets, and large amounts escape from damaged cells. A number of ectoenzymes are involved in the rapid catabolism of ATP to adenosine in the extracellular space.

Adenosine is not a classical neurotransmitter because it is not stored in neuronal synaptic vesicles. It is generally thought of as a neuromodulator that gains access to the extracellular space in part from the breakdown of extracellular ATP, and in part by translocation from the cytoplasm of cells by nucleoside transport proteins. Adenosine thus acts as a metabolic

messenger that imparts information about the intracellular metabolism of a particular cell to extracellular-facing receptors on the same and adjacent cells. Extracellular adenosine is rapidly removed, in part by reuptake into cells and in part by degradation to inosine. Receptors for both ATP and adenosine are widely distributed in the central and in the peripheral nervous system as well as in other tissues [118].



11 **12**

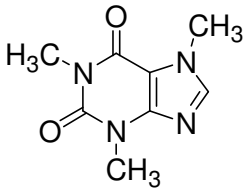
Sch. 1.14: ATP (**11**) and adenosine (**12**)

Based on physiological responses of various tissues to purines, Burnstock deduced that there are distinct receptors that bind adenosine (and analogues) or ATP (and analogues), designated P_1 and P_2 purinergic receptors, respectively [119]. It is known that there are families of both adenosine and ATP receptors, and these subtypes produce physiological responses in various organ systems upon activation by adenosine and ATP analogues, respectively [120].

Adenosine receptors

One of the criteria initially used to distinguish P_1 and P_2 receptors was selective blockade of the former by xanthines such as caffeine (**13**) and theophylline (**14**) (Scheme 1.15). These xanthines occur naturally in coffee, tea and chocolate, and their well-known stimulant action has been attributed to blockade of P_1 receptors in the CNS [121]. In addition, adenosine produces effects on cellular function, some of which are opposite to those produced by ATP. Thus, ATP functions as an excitatory transmitter in the CNS, whereas adenosine inhibits CNS excitability.

Four P_1 receptors, A_1, A_{2A}, A_{2B} and A_3 have been defined pharmacologically and cloned. All adenosine receptors are G protein-coupled, activated by adenosine and antagonized by xanthines [122].



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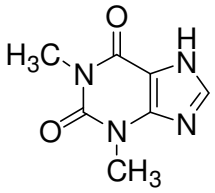


14

Sch. 1.15: Caffeine (**13**) and theophylline (**14**)

A_1 adenosine receptors are widely distributed in the CNS. These receptors have been extensively characterized in brain where they are expressed in high density (0.5 – 1 pmol/mg membrane protein). In the periphery, adenosine A_1 receptors are found in the heart, in adipose tissue and in kidney. A_1 receptor activation leads to inhibition of adenosine 3',5'-cyclic monophosphate (cAMP) formation and N-channel-mediated calcium-conductances.

A_2 adenosine receptors are divided into A_{2A} and A_{2B} subtypes based on substantial differences in tissue distribution and in binding affinity for adenosine, the former having high, the latter lower affinity to adenosine. In the CNS, A_{2A} receptors are restricted to the striatum, nucleus accumbens and olfactory tubercle, whereas A_{2B} adenosine receptors are distributed more ubiquitously throughout the CNS and the periphery. Both A_{2A} and A_{2B} are functionally coupled to activation of cAMP formation.

The A_3 receptor is present in the cerebral cortex, striatum and olfactory bulb with the highest concentration in the testis. It is negatively coupled to cAMP formation.

Ligands for the adenosine receptors have a broad therapeutic potential because of the wide organ and tissue distribution of adenosine receptor subtypes [121]. Agonists for the adenosine receptors could, for example, be useful as sedatives and in the diagnosis of diseases of coronary arteries [123]. However, severe cardiovascular side effects can be expected, caused by the strong hypotensive effects of the adenosine agonists [124]. These side effects are major drawbacks in the therapeutic use of adenosine receptor agonists. Partial agonists could have less pronounced cardiovascular effects and may act more selectively [125]. Another advantage of partial agonists would be that they probably induce less receptor downregulation and desensitization.

1.7.4 Adenosine receptor ligands in medical imaging

The discovery of adenosine receptor subtypes opened up new avenues for potential drug treatment of a variety of conditions such as neurodegenerative disorders, psychosis, anxiety and many other pathophysiological states that are believed to be associated with changes of adenosine levels [126 – 128]. Selective and potent agonists and antagonists at the human adenosine receptor subtypes are needed for such therapeutic intervention. Thus, the particular clinical importance of the A_1 and the A_{2A} adenosine receptors makes them attractive targets for radionuclide *in vivo* imaging.

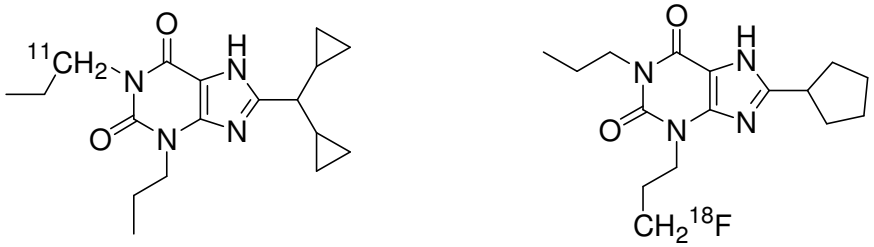
Whereas the radioligand binding assays described in subchapter 1.7.2 are *in vitro* methods, which require biopsy samples or post-mortem tissue in the case of human receptor studies, the technique of *in vivo* receptor imaging permits both, the detection of receptor abnormalities in various disease states and the control of drug treatment. PET is the imaging procedure of choice, offering the opportunity to study not only the distribution of the receptors via suitable radioligands, but also to obtain a pharmacological information equivalent, for example, to the *in vitro* measurement of B_{max} (total number of binding sites).

Though, there are several limiting factors for the use of radioligands of cerebral receptors [129]. Basic requirement is the possibility to label a suitable molecule with a positron-emitter (e.g. carbon-11, fluorine-18). The labelled compound has to be stable *in vivo* and should be transported to the target receptor without being metabolized. The logP-value should be ideally in the range of 2.2 – 2.4 to cross the blood-brain barrier, if the compound is not actively transported into the brain via a carrier-mediated system. The ratio of specific to non-specific binding, the (subtype) selectivity and the affinity to the target receptor (subtype) should be high. In addition, the specific activity of the radioligand should be as high as necessary (mostly at the n.c.a. level) in order to avoid a saturation of the receptor system by the compound administered.

The adenosine receptor ligands under development for PET are all antagonists. There are mainly two reasons why adenosine antagonists, but not agonists, are used in PET. The first is that high affinity agonist binding requires that the receptor is coupled to a G protein. Since usually only a fraction of the receptors are coupled, labelled antagonists serve as better radioligands, because they bind to both, coupled and uncoupled receptors. Second, and perhaps most important, several metabolic pathways exist for adenosine and its analogues, possibly reducing the amount of radioligand available for binding to extracellular receptors, thus diminishing the ability of PET to detect specific binding to receptors.

At present, both A_1 and A_{2A} adenosine receptors antagonists are under evaluation as PET ligands, all labelled with ^{11}C or ^{18}F [130]. The first A_1 receptor ligand developed for PET was [^{11}C]KF15372 (8-dicyclopropylmethyl-1-(1-[^{11}C]propyl)-3-propylxanthine (**15**), Scheme 1.16), which was synthesized with a radiochemical yield of 5 % within 45-55 min [131]. Several studies in mice and monkeys [131 – 133] showed that the radioligand distribution in the brain was heterogeneous, predominantly to the cerebellum, striatum, thalamus and cerebral cortex, sites of known high adenosine A_1 receptor density. The specific binding of [^{11}C]KF15372 to A_1 receptors supports the use of this ligand for imaging CNS receptors, although a few drawbacks like rapid metabolism, high unspecific binding to organs such as the heart and the short half-life of ^{11}C indicate that [^{11}C]KF15372 is not the ideal radioligand.

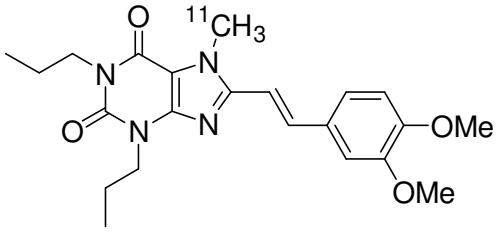
[^{18}F]CPFPX (8-cyclopentyl-3-(3-[^{18}F]fluoropropyl)-1-propylxanthine (**16**), Scheme 1.16) has subnanomolar affinity to the bovine adenosine A_1 receptor [134] and can be synthesized in radiochemical yields of about 55 % [135]. Quantitative autoradiography showed that distribution of [^{18}F]CPFPX within the brain was to regions which were reported [136] to have a high density of A_1 receptors. This radioligand is more advantegeous for the use in PET studies because of its longer half-life.



15 **16**

Sch. 1.16: [^{11}C]KF15372 (**15**) and [^{18}F]CPFPX (**16**)

The first A_{2A} adenosine receptor ligand for PET was [^{11}C]KF17837 ((E)-8-(3,4-dimethoxy-styryl)-1,3-dipropyl-7-[^{11}C]methylxanthine (**17**), Scheme 1.17), which can be synthesized in radiochemical yields ranged between 19 and 50 % [137]. Biodistribution studies in mice and *ex vivo* autoradiography of rat brain showed that the accumulation of the radioligand in the striatum was higher than in cerebellum or cortex [138]. A comprehensive comparison of KF17837 and three other styrylxanthines in mice and rats identified [^{11}C]KF18446 ((E)-8-(3,4,5-trimethoxy-styryl)-7-[^{11}C]methylcaffeine (**18**), Scheme 1.17) as a ligand with superior ability to label the striatum [139].



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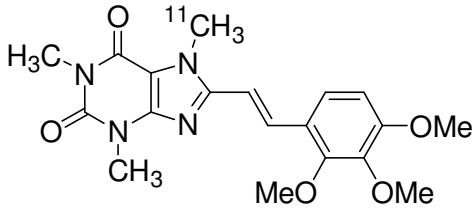


18

Sch. 1.17: [^{11}C]KF17837 (**17**) and [^{11}C]KF18446 (**18**)

There is some question about whether styrylxanthines such as [^{11}C]KF17837 or [^{11}C]KF18446 specifically label adenosine A_{2A} receptors in central neurons, because in primate brain regional distribution does not match receptor densities measured by other methods, notably quantitative autoradiography. Another recently developed potential radioligand for A_{2A} receptors is [^{11}C]MSX-2 (3-(3-hydroxypropyl)-8-(*m*-methoxystyryl)-7-(methyl-1-propargylxanthine), which binds to A_{2A} receptors but also shows very high unspecific binding [140]. Several non-xanthine compounds are therefore at the beginning of evaluation.

The main clinical application of PET studies of receptors is the determination of the density and pathophysiological changes of those receptors. Since the adenosine A_1 receptor is neuromodulatory, it plays a critical role in the regulation of CNS activity, which explains its high density in areas such as the cerebral and cerebellar cortices, the basal ganglia and the thalamus. Accordingly, PET could be a useful tool in testing the hypothesis that defects of neuromodulation such as epilepsy owe to a decreased density of adenosine A_1 receptors [141]. Since experimental models show that cerebral ischemia rapidly decreases A_1 receptor density in brain [142], PET might have a role in mapping the extent of ischemic injury, for example, that is caused by stroke.

The adenosine A_{2A} receptor is found in high density in the basal ganglia of the brain, where substantial evidence indicates they act reciprocally with the dopamine D_2 receptor in the control of movement [143]. Accordingly, A_{2A} adenosine receptor PET ligands might be useful investigational or even diagnostic tools in Parkinsonism and other movement disorders [144 – 146]. Preliminary evidence [147, 148] suggests that the A_{2A} adenosine receptor may play a role in the pathogenensis of schizophrenia; PET could play a role in testing that hypothesis and in given case be of diagnostic help.

Chapter 2

Aims and Scope

Selenium-73 is considered as a candidate for application in positron emission tomography as already outlined in the Introduction (cf. subchapter 1.6). This positron-emitting radionuclide (65 % β^+) can be produced in sufficient amounts via the $^{75}As(p,3n)^{73}Se$ nuclear reaction. Its half-life of 7.1 h enables to perform PET studies over extended periods in order to follow slow kinetics in a given case. Due to a lack of a suitable radioisotope of sulfur for *in vivo* medical application in nuclear medicine, ^{73}Se may serve as a possible substitute for sulfur in thio compounds of interest according to the chemical homology of sulfur and selenium. Moreover, selenium-containing biomolecules could be labelled and utilized as radiotracers for PET studies.

The toxicity of selenium compounds on the one hand, and conceivable future applications of ^{73}Se-labelled molecules, e.g. as radioligands for the determination of receptor density and occupancy, on the other hand, require the use of no-carrier-added products. The availability of potential ^{73}Se-labelled radiopharmaceuticals at the n.c.a. level, though, is quite limited because only two methods for introducing n.c.a. radioselenium into organic compounds have been developed thus far. The disadvantage of these radiosyntheses, however, is their limitation to the class of n.c.a. labelled methyl selenoethers.

The aim of this work was to enlarge the range of available n.c.a. ^{73}Se-labelled compounds to a wide variety of asymmetric alkyl and aryl selenoethers, in which selenium is stabilized in the oxidation state –2 and forms two stable covalent bonds to carbon. For convenience, the development and optimization of the radiosyntheses were to be performed using the longer-lived selenium-75 ($T_{1/2}$ = 120.4 d). Some of the labelling routes developed were to be repeated with

selenium-73 afterwards in order to compare the radiochemistry of selenium-73 and selenium-75.

The main focus should be on the development of straightforward synthetic strategies leading to n.c.a. radioselenoethers within the shortest possible time and with high radiochemical yield.

Based on a recent research project, in which carrier-added 1,3-disubstituted [^{75}Se]selenoureas served as intermediates for the preparation of c.a. asymmetric radioselenoethers, a new radiosynthesis should be developed in order to obtain radioselenium-labelled products at the no-carrier-added level. The conversion of n.c.a. elemental ^{75}Se to an appropriate n.c.a. 1,3-disubstituted [^{75}Se]selenourea is the key step of the underlying radiosynthetic strategy and should be investigated in detail using an alkyl isocyanide and an alkyl amine. Subsequent reaction steps, including alkylation of the [^{75}Se]selenourea, hydrolysis and a final alkylation were to be examined, thus generating various asymmetric n.c.a. [^{75}Se]selenoethers. Furthermore, in consideration of a future automated synthesis for clinical routine use, a polymer-supported radiosynthesis was to be developed using the outlined pathway.

Alternatively, a route via alkyl [^{75}Se]selenocyanates as intermediates, their effective synthesis using n.c.a. $^{75}Se^{0}$, sodium cyanide and alkylating agents as starting materials was to be investigated. The conversion of alkyl [^{75}Se]selenocyanates to the desired n.c.a. radioselenium-labelled products was to be performed via suitable reagents.

Special emphasis should be on the optimization of every single reaction step of each radiosynthesis regarding various reaction conditions in order to obtain labelled products in a highly efficient manner. Preparation of different [^{75}Se]selenoethers as model compounds should show the versatility of the radiosynthetic strategies developed.

In addition, two more complex, physiologically relevant radioselenium-labelled compounds should be prepared to demonstrate the utility of the radiosyntheses developed. The first one, L-homocysteine [^{75}Se]selenolactone, is a potential *in vivo* indicator for ischemic myocardial tissue and is of great interest as a longer-lived alternative of [^{11}C]homocysteine thiolactone. The second one is 5'-(methyl[^{75}Se]seleno)-N^{6}-cyclopentyladenosine, which is a radioligand with high affinity for the adenosine A_1 receptor. The evaluation of the no-carrier-added radiochemistry is particularly important for future labelling with selenium-73.

In order to identify ^{73,75}Se-labelled intermediates and products, suitable conditions for radio high performance liquid chromatography and radio thin layer chromatography needed to be developed. In addition, standard compounds and precursors had to be synthesized.

Chapter 3

Results and Discussion

Selenoethers, sometimes also referred to as selenides, are one of the most stable classes of selenium-containing organic compounds [54]. They do not decompose in dilute acids or strongly alkaline solutions and are resistant to mild oxidizing or reducing agents. Furthermore, they possess a satisfactory *in vivo* stability [89]. Of particular interest are asymmetric selenoethers, since they cover a broad range of Se-containing compounds. Therefore, a potential approach for labelling of organic, pharmacologically effective substances with radioselenium is the synthesis of appropriate asymmetric radioselenoethers, which may possibly act as tracers in nuclear medicine. Special emphasis is laid on the preparation of radioselenium-labelled compounds at the no-carrier-added level to obtain products of high specific activity. This is due to the fact that, firstly, *in vivo* toxic effects can be excluded and, secondly, suitable selenoethers can be used as radioligands for the determination of receptor occupancy and density.

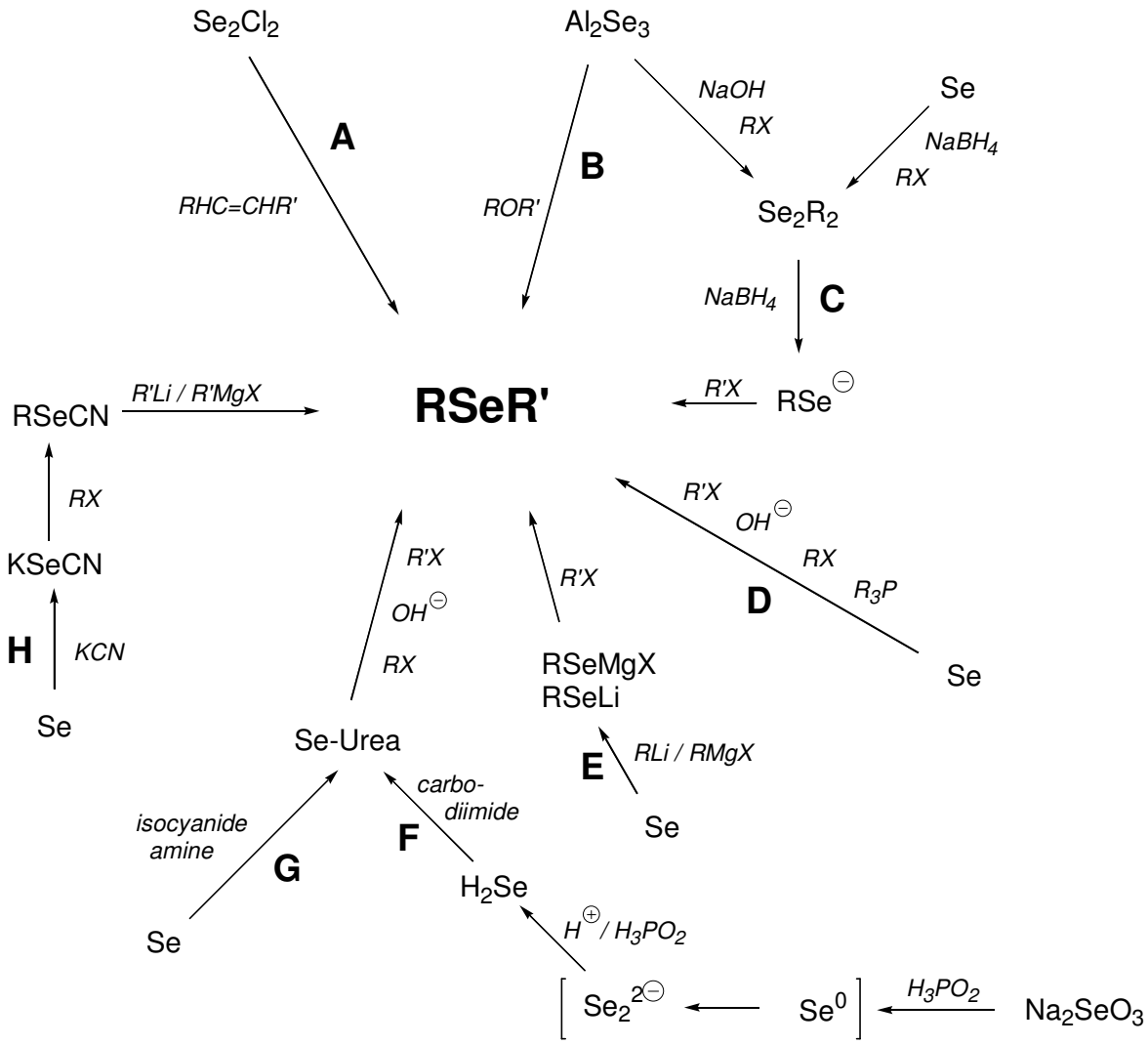
Several synthetic strategies have been developed for the preparation of asymmetric selenoethers [40, 46, 54, 56]. Some of the more important of these are outlined in Scheme 3.1. The direct application to radiolabelling, especially at the n.c.a. level, though, is not possible for all of them.

The commonly used synthetic pathways **A**-**C** do not seem suitable for the preparation of n.c.a. radioselenoethers, since they require the use of dimeric or trimeric selenium species as starting material (**A**, **B**) or as intermediate (**C**). For statistical reasons, a reaction between two components at the n.c.a. level can be excluded [149]. Thus, a formation of n.c.a. radioseleno oligomers is not likely.

Therefore, alternative reaction sequences for introducing n.c.a. radioselenium into organic compounds appear necessary. The first synthesis of a n.c.a. ^{75}Se-labelled organic compound

([^{75}Se]selenomethionine) was reported by Schmaljohann using n.c.a. selenium-75 in its elemental form as starting material (synthetic pathway **D**) [109]. However, using strategy **E**, repeated attempts to prepare n.c.a. [^{75}Se]selenomethionine failed [82, 97].

The use of a selenourea as intermediate for the synthesis of asymmetric selenoethers seems to be another promising approach (concepts **F**, **G**). The reduction of selenite to hydrogen selenide, however, does not occur directly, but proceeds via the intermediates Se^0 and Se_2^{2-}. The first reduction step from selenite to elemental selenium can be carried out without any problem. Further reduction from Se^0 to hydrogen selenide most probably requires the interim formation of diselenide-species. Since these cannot be formed at the n.c.a. level, the preparation of n.c.a. hydrogen [^{75}Se]selenide does not appear possible, and n.c.a. selenoureas cannot be obtained via the synthetic pathway **F** [92].



Sch. 3.1: Synthetic concepts for the preparation of asymmetric selenoethers

Alternatively, the strategies **G** and **H** offer principally the possibility to synthesize asymmetric n.c.a. radioselenoethers via selenoureas and selenocyanates, respectively, which is examined in this work in detail.

Both, selenium-73 and selenium-75 are the relevant radioisotopes in this study. They were produced in the no-carrier-added state at appropriate cyclotrons using the $^{75}As(p,3n)^{73}Se$ and the $^{75}As(p,n)^{75}Se$ nuclear reactions, respectively [78, 88]. Thermochromatographic workup of the target, following reduction by sulfur dioxide and extraction provided n.c.a. $^{73}Se^0$ and n.c.a. $^{75}Se^0$, respectively, in benzene [88]. The alternative method for the reduction of n.c.a. [^{73,75}Se]selenite to $^{73,75}Se^0$ via sodium thiosulfate [110] was only used in few preliminary studies of the development of radiosyntheses, because the presence of thio compounds could have undesired effects on the radiosyntheses.

Throughout this work selenium-75 served as a model-nuclide for the development of radiosyntheses because of its more suitable physical properties for this purpose than Se-73:

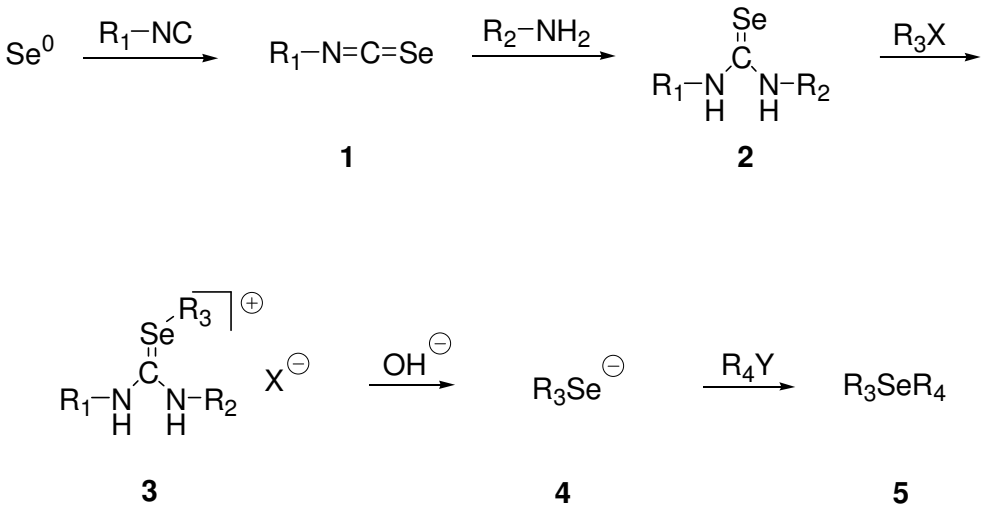
- longer half-life (120.4 d vs. 7.1 h),
- easier accessibility (20 MeV protons vs. 45 MeV protons),
- lower radiation exposure to the experimentalist (265 keV γ-ray vs. 1.3 MeV β^+-particles),
- non-radioactive daughter nuclide (^{75}As vs. ^{73}As).

In view of the fact that selenium-73 is the required radionuclide for PET studies, several ^{73}Se-labelled model compounds were prepared after development and optimization of the radiosynthetic strategies with selenium-75 in order to compare the radiochemical yields of the corresponding ^{73}Se- and ^{75}Se-labelled compounds, thus confirming that the radiosyntheses developed are also suitable for the preparation of n.c.a. [^{73}Se]selenoethers.

3.1 No-carrier-added radioselenoethers via 1,3-disubstituted [^{73,75}Se]selenoureas

Recently, it was shown that carrier-added (c.a.) 1,3-disubstituted [^{75}Se]selenoureas, which were obtained by reaction of c.a. [^{75}Se]H_2Se and carbodiimides, are attractive intermediates for the synthesis of various asymmetric c.a. [^{75}Se]selenoethers [92]. The drawback of this synthesis, however, is the indispensible addition of ^{nat}Se-carrier, resulting in products of low specific activity. Using the concept of selenoureas as radioselenium-labelled intermediates as basis, this project led to a new labelling method at the n.c.a. level [150].

It is known that the reaction of selenium with alkyl isocyanides produces alkyl isoselenocyanates (**1**), which can be converted to the desired selenoureas (**2**) by adding corresponding amines [61, 151]. The alkylation of **2** can be done by various alkylating agents due to the highly polarized carbon-selenium bond [152] yielding corresponding selenouronium salts (**3**). Hydrolysis under basic conditions provides the alkyl selenolates (**4**) [153] and subsequent *in situ* alkylation yields asymmetric selenoethers (**5**) [40] (Scheme 3.2).



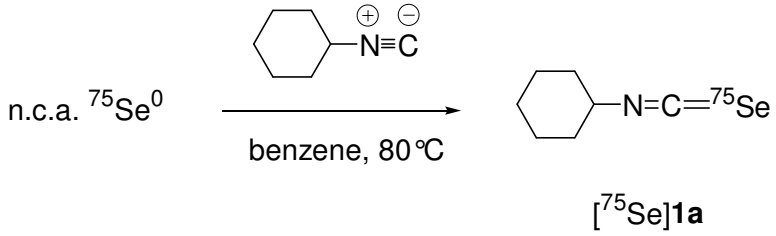
Sch. 3.2: Synthesis of selenoethers (**5**) via substituted selenoureas (**2**) (R_1 = R_2 = cyclohexyl; R_3 = methyl, ethyl, propyl; X = triflate; R_4 = various alkyl moieties; Y = bromine)

In the preliminary stages of the development of the radiosynthesis, this synthetic pathway was followed using $^{nat}Se^0$ as starting material in order to check for general feasibility of this concept. It also served to synthesize the standard compounds used as reference for radio high performance liquid chromatography (radio-HPLC) and radio thin layer chromatography (radio-TLC). All non-radioactive seleno compounds **1**-**5** were obtained in satisfactory yields (cf. subchapter 4.2.1).

The development and optimization studies of the n.c.a. radiosynthesis via 1,3-disubstituted [^{75}Se]selenoureas using n.c.a. $^{75}Se^0$ as starting material are described in the following.

3.1.1 Cyclohexyl iso[^{75}Se]selenocyanate via cyclohexyl isocyanide

According to resonance theory, the carbon atom of an alkyl isocyanide bears a lone electron pair and may act as a nucleophile. The radiosynthetic strategy to be developed takes advantage of this property by performing a nucleophilic attack on n.c.a. $^{75}Se^0$ to give an alkyl iso[^{75}Se]selenocyanate ([^{75}Se]**1**). This introduction of n.c.a. radioselenium into an organic compound is the initial key-step of the radiosynthesis; the investigation of this reaction sequence is therefore essential. Optimization studies regarding solvent and temperature were performed using commercially available cyclohexyl isocyanide as precursor yielding the model compound cyclohexyl iso[^{75}Se]selenocyanate ([^{75}Se]**1a**) (Scheme 3.3).



Sch. 3.3: Synthesis of cyclohexyl iso[^{75}Se]selenocyanate ([^{75}Se]**1a**)

Since the nucleophilic attack of the carbon atom on [^{75}Se]selenium takes place only at higher temperatures according to literature [154], the impact of various solvents on this reaction was examined at 80 °C. As graphically depicted in Figure 3.1 the maximum radiochemical yield (RCY) of [^{75}Se]**1a** (all RCYs given in subchapter 3.1.1 are related to n.c.a. $^{75}Se^0$) was obtained within 45 min using ethanol, benzene or acetonitrile as the solvent (with 57 ± 6 %, 54 ± 5 % and 47 ± 7 %, respectively). The subsequent slight decrease of the RCYs can be attributed to decomposition of [^{75}Se]**1a** due to further heating. Ethanol as a protic, benzene as a relatively unpolar and acetonitrile as a dipolar aprotic solvent have the same effect on the RCY of [^{75}Se]**1a**. They are equally suitable solvents, whereas the use of dimethyl sulfoxide led to a maximum RCY of 19 ± 4 % only within 15 min. Apparently, interactions between dimethyl sulfoxide and the product led to a rapid decomposition of [^{75}Se]**1a**, yielding unidentified ^{75}Se-labelled byproducts, as shown by radio-TLC.

Although ethanol appeared as the most suitable solvent because of the largest RCY obtained, benzene was used in subsequent optimization studies in order to avoid a change of solvent after extraction of elemental selenium-75 into benzene during isolation.

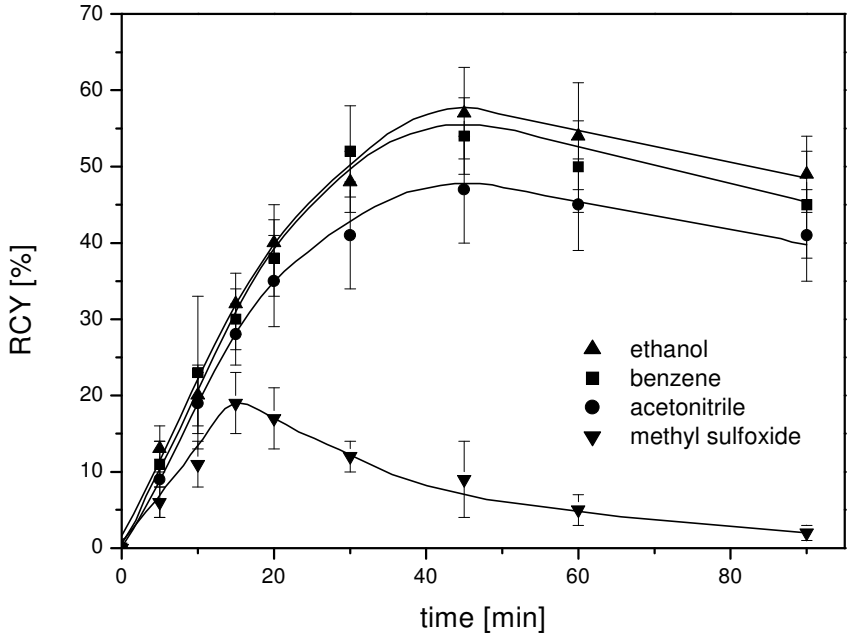


Fig. 3.1: Radiochemical yield of cyclohexyl iso[^{75}Se]selenocyanate ([^{75}Se]**1a**) as a function of time and solvent
Reaction conditions: 370 kBq n.c.a. $^{75}Se^{0}$, cyclohexyl isocyanide (10 µL, 80 µmol), 0.5 mL solvent, 80 °C.

The data given in Figure 3.2 show that the RCY of [^{75}Se]**1a** decreased with decreasing reaction temperature. The maximum RCYs were obtained within 45 min and reached 54 ± 5 %, 47 ± 5 % and 32 ± 3 % at 80, 60 and 40 °C, respectively. Since there is no detectable reduction of the RCY of [^{75}Se]**1a** at 60 or 40 °C after reaching the saturation yield of about 50 and 30%, respectively, the process of decomposition of [^{75}Se]**1a** seems to be effective only at temperatures above 60 °C.

The concentration of the precursor cyclohexyl isocyanide was also optimized. Such studies showed that the reagent is needed in an amount of at least 80 µmol per 0.5 mL benzene to provide [^{75}Se]**1a** with an optimum RCY of 54 ± 5 %. These optimization studies showed that the reaction sequence illustrated in Scheme 3.3 is best carried out in 0.5 mL benzene at 80 °C within 45 min using at least a 160 mM solution of cyclohexyl isocyanide.

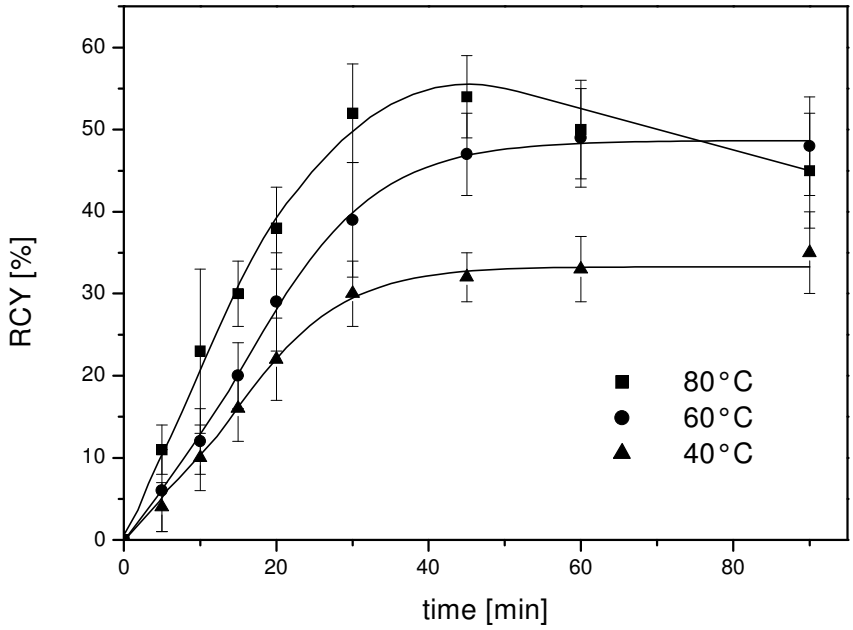
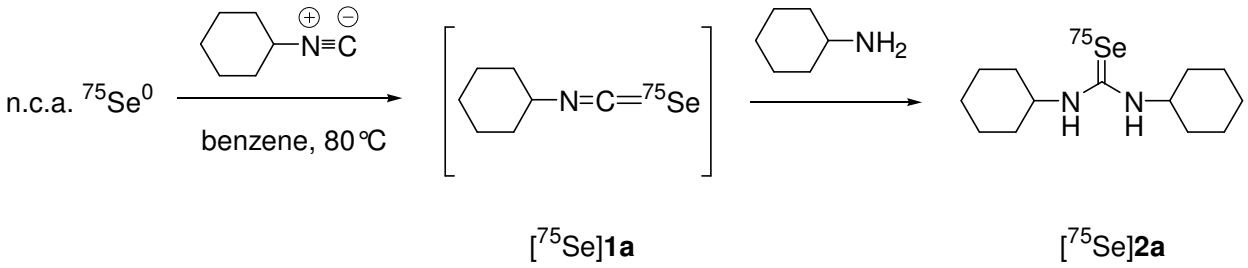


Fig. 3.2: Radiochemical yield of cyclohexyl iso[^{75}Se]selenocyanate ([^{75}Se]**1a**) as a function of time and temperature
Reaction conditions: 370 kBq n.c.a. $^{75}Se^{0}$, cyclohexyl isocyanide (10 µL, 80 µmol), 0.5 mL benzene, various reaction temperatures (80, 60, 40 °C).

3.1.2 Formation of 1,3-dicyclohexyl [^{75}Se]selenourea using a one-pot procedure

Primary amines easily add to alkyl isoselenocyanates (**1**) to give 1,3-disubstituted selenoureas (**2**) by forming a new C-N bond between the nitrogen of the amino group and the carbon atom of the isoselenocyanate group [61, 151]. This reaction sequence was used to synthesize 1,3-dicyclohexyl [^{75}Se]selenourea ([^{75}Se]**2a**) as model compound starting from [^{75}Se]**1a** and cyclohexylamine. Using the optimal reaction conditions described above for the preparation of [^{75}Se]**1a**, it was demonstrated by radio-TLC that [^{75}Se]**2a** was obtained immediately upon adding cyclohexylamine to the reaction mixture. Accordingly, a one-pot procedure was examined as model reaction for the preparation of [^{75}Se]**2a** with interim formation of [^{75}Se]**1a** starting from a mixture of n.c.a. $^{75}Se^{0}$, cyclohexyl isocyanide and cyclohexylamine (Scheme 3.4).



[^{75}Se]**1a** [^{75}Se]**2a**

Sch. 3.4: One-pot synthesis of 1,3-dicyclohexyl [^{75}Se]selenourea ([^{75}Se]**2a**)

For convenience, this reaction step was optimized in benzene as explained in subchapter 3.1.1. An optimal RCY of [^{75}Se]**2a** (92 ± 5 %; RCYs given in subchapter 3.1.2 are related to n.c.a. $^{75}Se^0$) was obtained via the one-pot synthesis at 80 °C within 90 min as graphically depicted in Figure 3.3.

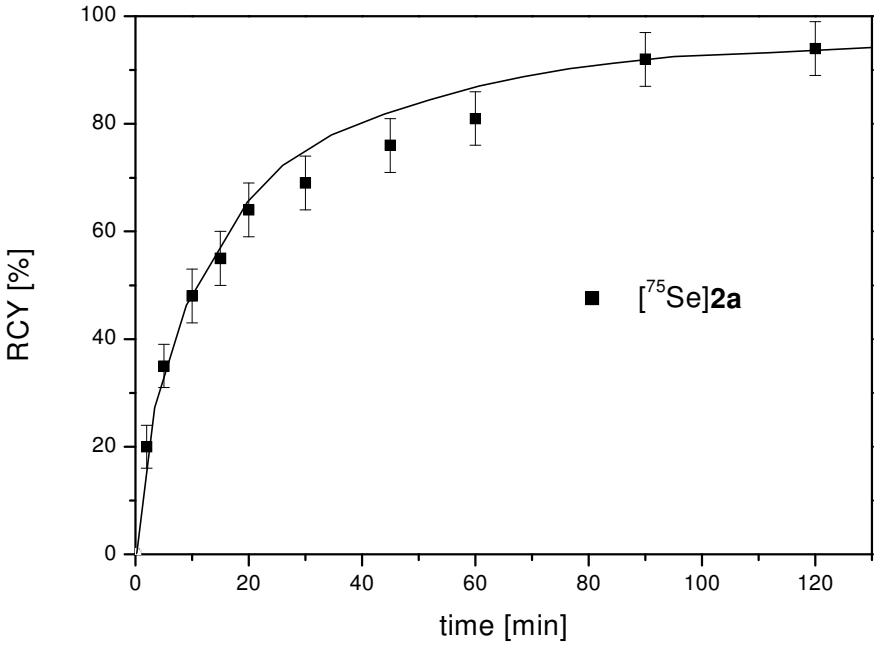


Fig. 3.3: Radiochemical yield of 1,3-dicyclohexyl [^{75}Se]selenourea ([^{75}Se]**2a**) as a function of time
Reaction conditions: 370 kBq n.c.a. $^{75}Se^0$, cyclohexyl isocyanide (10 µL, 80 µmol), cyclohexylamine (10 µL, 90 µmol), 0.5 mL benzene, 80 °C.

Surprisingly, the plot shows that the reaction rate of the synthesis of [^{75}Se]**2a** was much faster than that of [^{75}Se]**1a**. This unexpected observation can be explained as follows. Firstly, the radiosynthesis of [^{75}Se]**1a** is the rate-determining step of the one-pot procedure. Apparently,

[^{75}Se]**1a**, once formed, is immediately converted to [^{75}Se]**2a**. In this case, the rate of the synthesis of [^{75}Se]**2a** should be identical with that of the synthesis of [^{75}Se]**1a**. A possible explanation of the difference between the reaction rate of [^{75}Se]**1a** and [^{75}Se]**2a** is, that the rapid conversion of [^{75}Se]**1a** to [^{75}Se]**2a** prevents the decomposition of [^{75}Se]**1a**, which is much slower. Therefore, one can suggest that the reaction rate of [^{75}Se]**2a** is also the actual rate of the formation of [^{75}Se]**1a**, while the experimentally determined reaction rate of [^{75}Se]**1a** is the overall rate of both, formation and decomposition of [^{75}Se]**1a** and is therefore smaller. Due to the fact that the intermediate [^{75}Se]**1a**, once formed, is immediately transformed to [^{75}Se]**2a**, the decomposition does not affect the RCY of [^{75}Se]**2a**, which explains the larger RCY of [^{75}Se]**2a** than that of [^{75}Se]**1a** at any reaction time.

As outlined in subchapter 1.4, n.c.a. syntheses of the form A + B → C proceed according to pseudo-first-order kinetics and thus, the concentration (or activity) of the labelled target molecule can be written as:

$$[C]_t = [B]_0 (1 - e^{-kt}) \quad [mol/l] \qquad \textbf{Eq. 3.1}$$

with k: rate constant, $[B]_0$: initial activity of $^{75}Se^0$ and $[C]_t$: activity of [^{75}Se]**1a** and [^{75}Se]**2a**, respectively, at reaction time t.

Both, the synthesis of [^{75}Se]**1a** as well as the synthesis of [^{75}Se]**2a** are of the form A + B → C. The former is straightforward and contains A (cyclohexyl isocyanide), B (n.c.a. $^{75}Se^0$) and C ([^{75}Se]**1a**); the latter is more complex and can be written as A + B → C + D → E with A: cyclohexyl isocyanide, B: n.c.a. $^{75}Se^0$, C: [^{75}Se]**1a**, D: cyclohexylamine and E: [^{75}Se]**2a**. Since the first stage of this sequence is rate-determining and C and D were converted immediately to E after formation of C, this synthesis can roughly be simplified by leaving out the intermediate C and the second precursor D, which is in large excess. Thus, the synthesis can be written as "A + B → E", now representing a pseudo-first-order reaction.

In order to determine the rate constants k of [^{75}Se]**1a** and [^{75}Se]**2a**, one can convert Equation 3.1 to the formula $\ln([B]_0/([B]_0-[C]_t)) = kt$. The corresponding plots of this term as a function of time can be seen in Figure 3.4. Only the first 20 min were taken into account. As expected for reactions which proceed according to pseudo-first-order kinetics the plots can be fitted resulting in corresponding straight lines. The slopes of these regression lines give the rate constants k, being 0.048 min^{-1} and 0.024 min^{-1} for the synthesis of [^{75}Se]**2a** and [^{75}Se]**1a**, respectively.

From these k-values one can suggest that the decomposition reaction considerably affects the overall reaction rate of the formation of [^{75}Se]**1a** and that the consecutive step from [^{75}Se]**1a**

to [^{75}Se]**2a** seems to prevent the decomposition of [^{75}Se]**1a** by the instant conversion to [^{75}Se]**2a**.

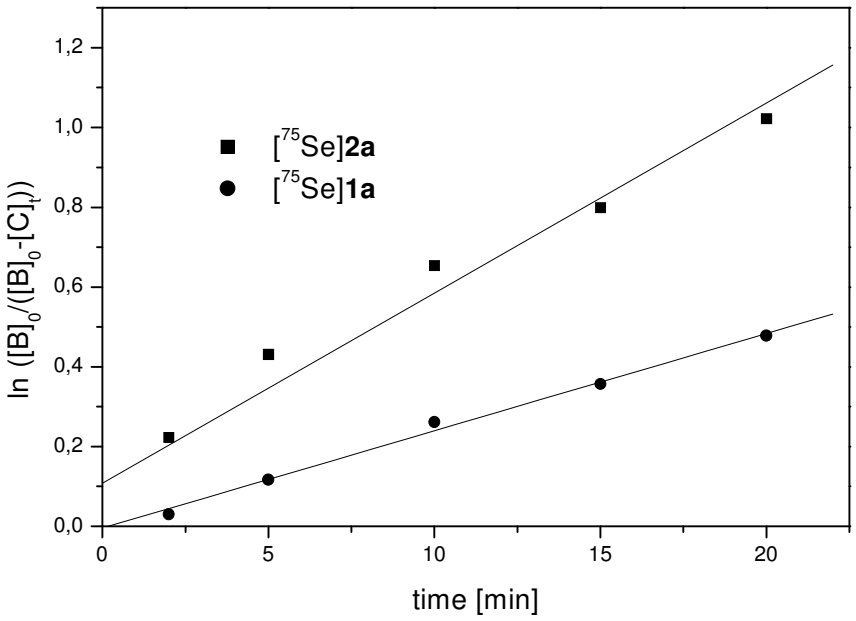


Fig. 3.4: Plot of $\ln([B]_0/([B]_0-[C]_t))$ as a function of time for the determination of the relative rate constant k for the synthesis of [^{75}Se]**2a** and [^{75}Se]**1a**
Reaction conditions: see Fig. 3.3.

After additional optimization studies with respect to the amount of precursor, the radio-selenium-labelled intermediate [^{75}Se]**2a** was conveniently generated in benzene at 80°C via a one-pot procedure with a RCY of 92 ± 5 % within 90 min using cyclohexyl isocyanide and cyclohexylamine each with a concentration of at least 0.16 mol/L.

3.1.3 Alkylation of 1,3-dicyclohexyl [^{75}Se]selenourea

The nature of the selenourea group and in particular the position of its double-bond is responsible for the advantegeous use of selenoureas as suitable intermediates for the preparation of selenoethers. Resonance structures of the selenourea group can be depicted by **I** and **II** in Scheme 3.5. From experimental [155] and semi-theoretical [156] investigations it is concluded that **II** is the more important resonance form. The increased contribution of **II** is

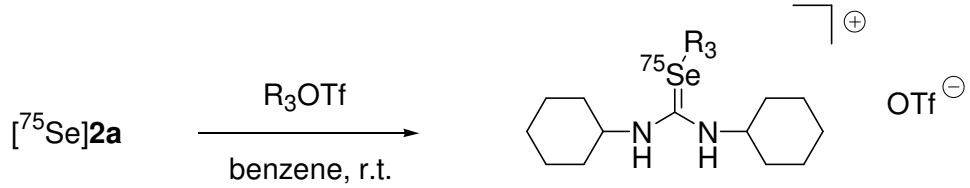
explained as due to a decrease in orbital overlap between the carbon 2p and the selenium 4p orbitals, which leads to a reduction of the C=Se double-bond character, thus polarizing the selenium atom by imparting a negative charge.

I **II**

Sch 3.5: Resonance structures of the selenourea group

Therefore, alkylation of selenoureas never occurs at the N atom but rather at the Se atom [157] to give the corresponding selenouronium salts (**3**) [152], which are sometimes also referred to as isoselenouronium salts or selenopseudourea salts. They show ionic character, the cation containing the tetravalent selenium atom, and are more stable than the selenourea precursor [40]. Commonly used agents for the alkylation of selenoureas are alkyl halides or tosylates. For reasons of slow reaction kinetics, this alkylation step takes place only at high reaction temperature and a prolonged reaction time [92].

In this study, attempts to obtain n.c.a. [^{75}Se]selenouronium salts ([^{75}Se]**3**) using alkyl halides or tosylates failed under a variety of different reaction conditions because of an unexpectedly rapid decomposition of n.c.a. [^{75}Se]**2a** to elemental selenium-75. Therefore, trifluoromethane-sulfonic acid alkyl esters (R_3OTf) were used as much stronger alkylating agents, which reacted even at room temperature (Scheme 3.6).



[^{75}Se]**3**

Sch. 3.6: Synthesis of [^{75}Se]selenouronium salts ([^{75}Se]**3**)
(R_3 = methyl ([^{75}Se]**3a**), ethyl ([^{75}Se]**3b**) and propyl ([^{75}Se]**3c**))

For optimization of the RCY of this alkylating reaction, the synthesis of [^{75}Se]**3a** was used as model reaction and it was optimized with respect to the amount of methyl triflate and to reaction time.

As expected, the effect of the amount of methyl triflate on the RCY of [^{75}Se]**3a** initially shows pseudo-first-order kinetics, as can be seen in Figure 3.5. A quantitative yield of [^{75}Se]**3a** was obtained with 0.1 mmol methyl triflate. Increasing amounts of alkylating agent led to a slight decrease of the RCY of [^{75}Se]**3a**, which is probably due to side reactions between [^{75}Se]**3a** formed and the increasing excess of methyl triflate.

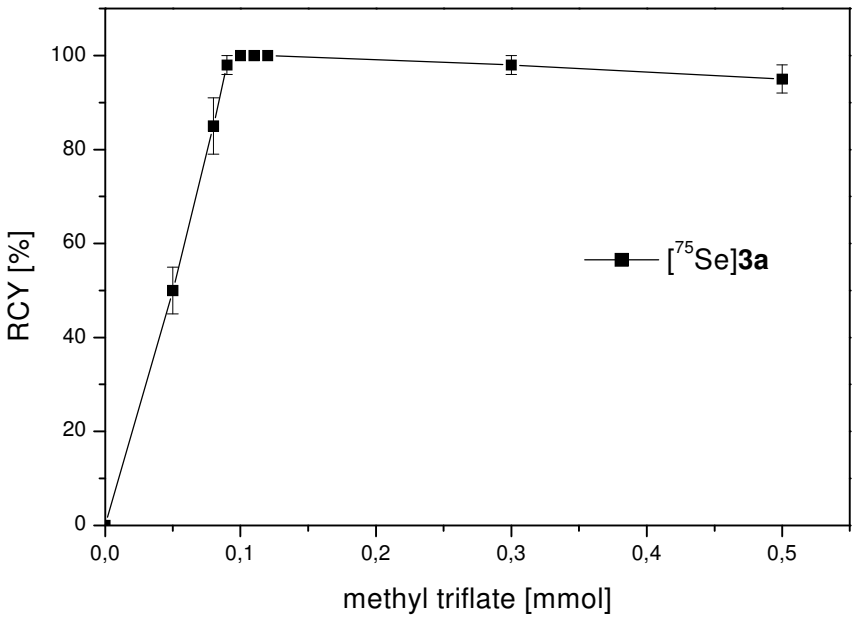


Fig. 3.5: Radiochemical yield of methyl selenouronium salt ([^{75}Se]**3a**) as a function of amount of methyl triflate
Reaction conditions: 185 kBq [^{75}Se]**2a**, various amounts of methyl triflate, 0.5 mL benzene, 1 min, r.t.

The dependence of the RCY of [^{75}Se]**3a** as a function of time shows that the reaction is quantitatively finished within 1 min at room temperature (Figure 3.6). Using longer reaction times the RCY decreased, probably due to the same side reactions that occurred with amounts of more than 0.1 mmol methyl triflate (see above).

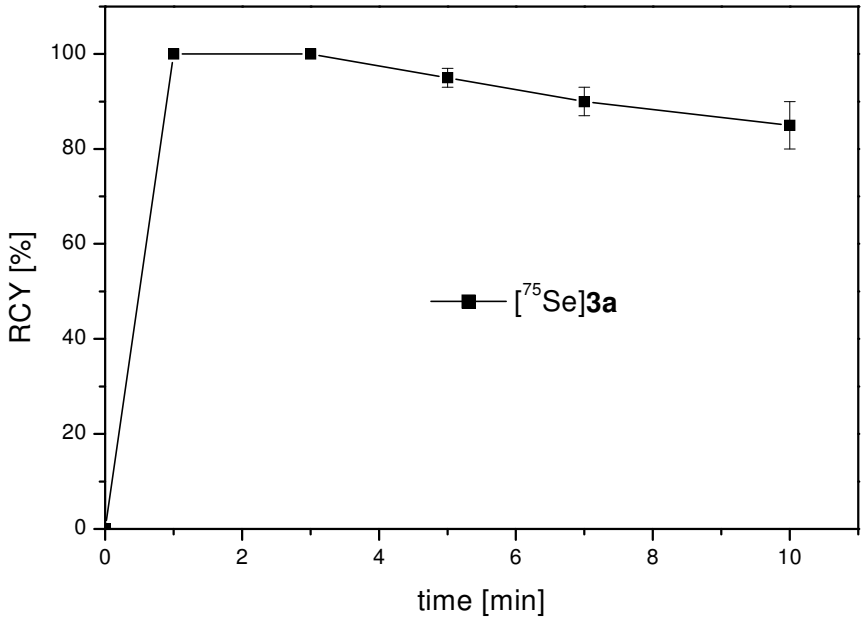
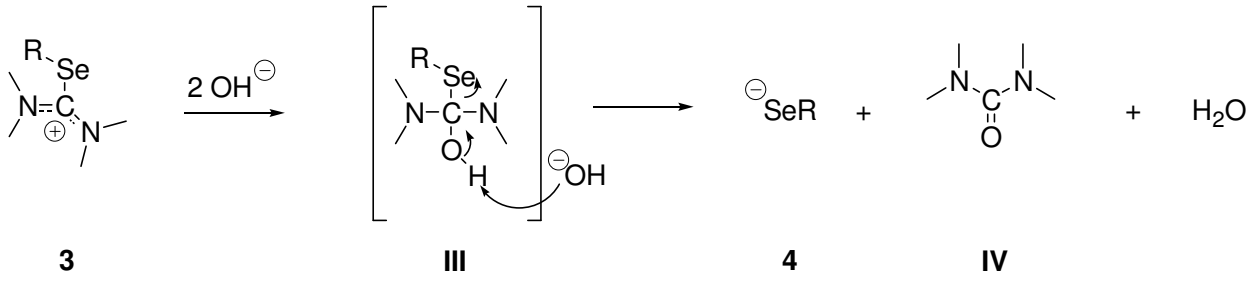


Fig. 3.6: Radiochemical yield of methyl selenouronium salt ([^{75}Se]**3a**) as a function of time Reaction conditions: 185 kBq [^{75}Se]**2a**, methyl triflate (10 µL, 0.1 mmol), 0.5 mL benzene, r.t.

Therefore, an instant and complete separation of [^{75}Se]**3** formed from surplus alkyl triflate was extremely important in order to avoid side reactions. Furthermore, even minor traces of this strong alkylating agent could lead to undesired byproducts in the following stages of the radiosynthesis. Small scale silica gel column chromatography proved to be the most effective method for purification, and [^{75}Se]**3a**-**c** were separated with a recovery rate of 87 ± 3 % (related to [^{75}Se]**2a**).

3.1.4 Synthesis of asymmetric dialkyl [^{75}Se]selenoethers

Treatment of selenouronium salts (**3**) with hydroxide yields the corresponding free selenolate (**4**) [153, 158]. The tentative mechanism, leading to the displacement of the alkylseleno group from **3** by the hydroxide ion, is presented in Scheme 3.7.



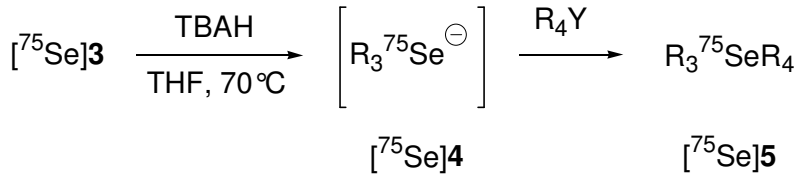
3 **III** **4** **IV**

Sch. 3.7: Tentative mechanism for the formation of selenolate (**4**)

A C-O single-bond is formed through a nucleophilic attack of the hydroxide anion on the positively polarized selenourea group, as can be seen in the transition state (structure **III**). It is likely that the second stage of the reaction is an internal electron movement, thus strengthening the C-O bond and weakening the C-Se bond. Subsequently, the proton is transferred to a second hydroxide anion and the carbon-selenium bond is cleaved heterolytically, resulting in the corresponding selenolate (**4**), an urea (**IV**) and water.

Selenols (also referred to as selenomercaptans) are the selenium analogues of alcohols and thiols (mercaptans). They are quite acidic (pK_a = 3.73) and are deprotonated in basic solvent, forming selenolates (**4**). This species is a strong nucleophile and is rapidly oxidized by atmospheric oxygen [46]. In order to obtain selenoethers (**5**), *in situ* alkylation of **4** can be performed using appropriate alkylating agents [54]. This last step can be considered as a modified Williamson ether synthesis, since **4** is the seleno-analogue of the alkoxide ion used in Williamson syntheses.

The transfer of this reaction sequence to the n.c.a. level was investigated as described in this subchapter. As already mentioned in literature [92], tetrabutylammonium hydroxide (TBAH) is a suitable base for hydrolysis of [^{75}Se]**3**, since the tetrabutylammonium cation acts as phase transfer catalyst that makes the hydroxid anion soluble in organic solvent systems. Using TBAH in tetrahydrofuran (THF) the corresponding [^{75}Se]selenolates ([^{75}Se]**4**) were liberated and subsequently alkylated *in situ*, yielding the desired [^{75}Se]selenoethers ([^{75}Se]**5**) (Scheme 3.8).



Sch. 3.8: Synthesis of asymmetric [^{75}Se]selenoethers ([^{75}Se]**5**) via one-pot procedure starting from [^{75}Se]**3**
(R_3 = methyl, ethyl, propyl; R_4 = various alkyl moieties; Y = bromine, tosylate)

Further investigations showed again that this reaction step was most effective using a one-pot procedure with simultaneous addition of TBAH and the alkylating agent. Attempts to perform hydrolysis and alkylation in separate steps resulted in poorer yields of [^{75}Se]**5**. This is because [^{75}Se]**4** is not stable and is very reactive. Thus, the liberation of [^{75}Se]**4** always yielded [^{75}Se]**5** as well as an unidentified polar ^{75}Se-labelled byproduct, probably an oxidized species of [^{75}Se]**4**.

This observation illustrates the reactivity-selectivity principle, which states that the greater the reactivity of a species, the less selective it will be, in other words [^{75}Se]**4** reacted with all possible electrophilic “precursors” and not only with the added alkylating agent.

The effect of the concentration of TBAH on the formation of [^{75}Se]**4** is an important parameter of the synthesis of [^{75}Se]**5**. On the one hand, the hydroxide anions are necessary to liberate [^{75}Se]**4** from [^{75}Se]**3**, but on the other hand, OH^- is a nucleophile and can therefore act as a competitor of [^{75}Se]**4**, which may lead to a decrease of precursor R_4Y. For optimization studies, the synthesis of benzylmethyl[^{75}Se]selenide ([^{75}Se]**5a**) was used as model reaction starting from [^{75}Se]**3a**, various concentrations of TBAH and benzyl bromide as precursor.

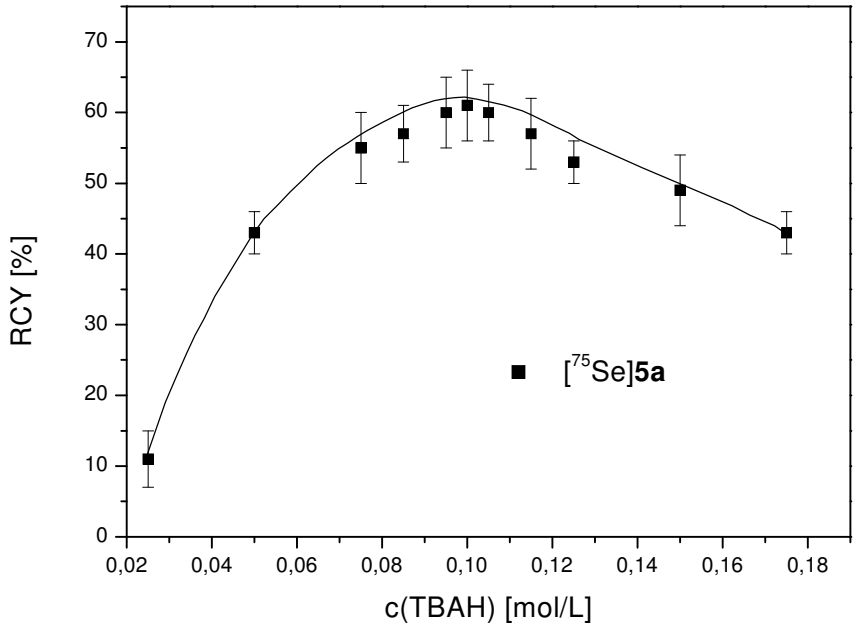


Fig. 3.7: Radiochemical yield of benzylmethyl[^{75}Se]selenide ([^{75}Se]**5a**) as a function of concentration of TBAH
Reaction conditions: 185 kBq [^{75}Se]**3a**, various concentrations of TBAH, benzyl bromide (12 µL, 0.1 mmol), 0.6 mL THF, 5 min, 70°C.

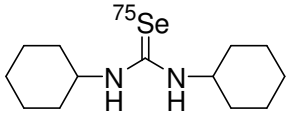
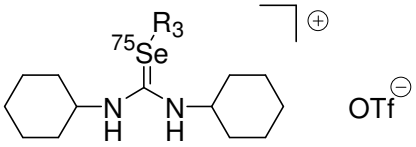
As can be seen in Figure 3.7 the larger the concentration of TBAH in the range of 0.025 – 0.1 mol/L, the more methyl [^{75}Se]selenolate ([^{75}Se]**4a**) was formed, which was instantly converted to benzylmethyl[^{75}Se]selenide ([^{75}Se]**5a**). The maximum RCY of [^{75}Se]**5a** (61 ± 5 %, related to methyl selenouronium salt ([^{75}Se]**3a**)) was obtained using a concentration of 0.1 mol/L TBAH. Above 0.1 mol/L of TBAH the RCY of [^{75}Se]**5a** decreased. Since the amount of the precursor obviously dropped due to its increased conversion to the corresponding alcohol as demonstrated by TLC, the liberation of [^{75}Se]**4a** led to an increased formation of the unidentified polar ^{75}Se-labelled byproduct.

Since the reaction of [^{75}Se]**4a** with an alkyl halide proceeds according to an S_N2 mechanism, polar, aprotic solvents other than THF are expected to favour the synthesis of [^{75}Se]**5a** probably leading to an increase of the maximum RCY of [^{75}Se]**5a** and to a decrease of the polar byproduct. Therefore, N,N-dimethylformamide and dimethyl sulfoxide instead of THF were used as solvents. However, a drastic decline of RCY of the model compound [^{75}Se]**5a** was observed. The ^{75}Se-labelled byproduct mentioned above now represented the major product and was obtained in yields of 60 – 90 % (related to [^{75}Se]**3a**).

These and further investigations showed that maximum radiochemical yields of [^{75}Se]**5** (up to 61 % related to [^{75}Se]**3**) were obtained using concentrations of at least 0.1 mol/L TBAH and 0.17 mol/L alkylating agent in THF at 70 °C. In particular, studies regarding the time dependence showed that the optimum RCY was reached within 5 min.

To sum up subchapters 3.1.1 to 3.1.4, the time schedule of the radiosynthesis developed and optimized is depicted in Scheme 3.9. Starting from n.c.a. $^{75}Se^0$, cyclohexyl isocyanide and cyclohexylamine 1,3-dicyclohexyl [^{75}Se]selenourea ([^{75}Se]**2a**) was obtained via one-pot procedure within 90 min with a RCY of 92 ± 5 %. Subsequent alkylation of [^{75}Se]**2a** via alkyl triflates gave rise to a quantitative yield of [^{75}Se]selenouronium salts ([^{75}Se]**3**) which were purified using silica gel column chromatography. Hydrolysis of purified [^{75}Se]**3** under basic conditions and *in situ* alkylation provided asymmetric [^{75}Se]selenoethers ([^{75}Se]**5**) within a total reaction time of 130 min in RCYs of 15 to 48 % (related to n.c.a. $^{75}Se^0$), depending on the nature of the alkyl groups R_3 and R_4.

A survey of n.c.a. [^{75}Se]selenoethers prepared as model compounds via the radiosynthetic pathway described above is given in subchapter 3.1.7.

	time	RCY
n.c.a. $^{75}Se^0$	0 min	
↓ i		
 [^{75}Se]**2a**	90 min	92 ± 5 % (related to n.c.a. $^{75}Se^0$)
↓ ii		
 [^{75}Se]**3**	91 min	100 % (related to [^{75}Se]**2a**)
↓ iii		
purified [^{75}Se]**3**	125 min	87 ± 3 % (related to [^{75}Se]**3**)
↓ iv		
$R_3{}^{75}SeR_4$ [^{75}Se]**5**	130 min	19 - 61 % (related to purified [^{75}Se]**3**) 15 - 48 % (related to n.c.a. $^{75}Se^0$)

Sch. 3.9: Radiosynthesis of [^{75}Se]selenoethers ([^{75}Se]**5**)
Reaction conditions: i) cyclohexyl isocyanide (0.08 mmol), cyclohexylamine (0.08 mmol), benzene (0.5 mL), 80 °C; ii) methyl, ethylor propyl triflate (0.1 mmol), benzene (0.5 mL), r.t.; iii) separation via silica gel column chromatography; iv) TBAH (0.06 mmol), various alkyl bromine or tosylate compounds (0.1 mmol), THF (0.6 mL), 70 °C.

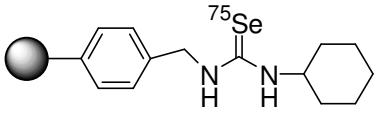
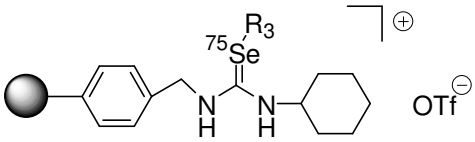
3.1.5 Polymer-supported preparation of n.c.a. [^{75}Se]selenoethers

The method of radioselenoether synthesis described above is extremely time-consuming because of the tedious intermediate purification step required. Further, for routine application it would be desirable to achieve a general pathway for a fully automated synthesis of n.c.a. [^{73}Se]selenoethers in order to prevent undue product handling thus decreasing exposure of personnel. Therefore, the reaction sequence described in Scheme 3.9 was modified for a polymer-supported synthesis in order to separate and purify radioselenium labelled intermediates in a very convenient way while decreasing the overall reaction time.

Starting from n.c.a. elemental selenium-75, cyclohexyl isocyanide and aminomethylated polystyrene, the corresponding polymer-bound [^{75}Se]selenourea ([^{75}Se]**2**$_{resin}$) was synthesized in benzene at 80 °C within 10 min with a radiochemical yield of 80 ± 5 % (related to n.c.a. $^{75}Se^{0}$) (Scheme 3.10). The use of toluene as solvent and increased reaction temperature and time, as well as larger amounts of the precursors and previous swelling of the resin, did not improve the RCY. Surprisingly, the maximum RCY of [^{75}Se]**2**$_{resin}$ was obtained within a much shorter time compared to the preparation of free [^{75}Se]**2a** (10 min vs. 90 min), which is probably due to the high density of amino groups on the resin. The intermediate [^{75}Se]**2**$_{resin}$ was purified by washing with benzene in order to separate starting material not transformed and any byproduct. The RCY was determined as activity bound on the resin of purified [^{75}Se]**2**$_{resin}$, since it is likely that ^{75}Se was present on the polymer in no other form than as in the corresponding selenourea group.

Subsequent alkylation in benzene via alkyl triflates yielded the corresponding [^{75}Se]selenouronium salt ([^{75}Se]**3**$_{resin}$) within 5 min with a radiochemical yield of 92 ± 3 % (activity bound on the purified resin, related to [^{75}Se]**2**$_{resin}$), which was independent from the alkyl triflate used (Scheme 3.10). Reaction time was extended in comparison to the homogenous radiosynthesis of [^{75}Se]**3** (5 min vs. 1 min) to guarantee that most of [^{75}Se]**2**$_{resin}$ was converted to [^{75}Se]**3**$_{resin}$. Purification of [^{75}Se]**3**$_{resin}$ was carried out by repeated washing with benzene and tetrahydrofuran in order to get rid of surplus alkyl triflate and to determine the RCY of the purified [^{75}Se]**3**$_{resin}$.

Using the optimized reaction conditions for the formation of [^{75}Se]**5** described in subchapter 3.1.4, basic hydrolysis of the purified [^{75}Se]**3**$_{resin}$ and *in situ* alkylation of the intermediate anion [^{75}Se]**4** yielded asymmetric radioselenoethers in radiochemical yields of 18 – 76 % (related to [^{75}Se]**3**$_{resin}$) and 13 – 56 % (related to n.c.a. $^{75}Se^{0}$), respectively, within a total reaction time of 35 min (Scheme 3.10). Thus, the overall reaction time was reduced by a factor of almost four while the RCYs remained unchanged.

	time	RCY
n.c.a. $^{75}Se^0$	0 min	
↓ i		
	10 min	
[^{75}Se]**2**$_{resin}$		
↓ ii		
purified [^{75}Se]**2**$_{resin}$	15 min	80 ± 5 % (related to n.c.a. $^{75}Se^0$)
↓ iii		
	20 min	
[^{75}Se]**3**$_{resin}$		
↓ iv		
purified [^{75}Se]**3**$_{resin}$	30 min	92 ± 3 % (related to [^{75}Se]**2**$_{resin}$)
↓ v		
$R_3{}^{75}SeR_4$	35 min	18 - 76 % (related to purified [^{75}Se]**3**$_{resin}$) 13 - 56 % (related to n.c.a. $^{75}Se^0$)
[^{75}Se]**5**		

Sch. 3.10: Polymer-supported radiosynthesis of [^{75}Se]selenoethers ([^{75}Se]**5**)
Reaction conditions: i) cyclohexyl isocyanide (0.08 mmol), aminomethylated polystyrene (40 mg), benzene (0.5 mL), 80°C; ii) washing with benzene; iii) methyl, ethyl or propyl triflate (0.1 mmol), benzene (0.5 mL), r.t.; iv) separation via washing with benzene and tetrahydrofuran; v) TBAH (0.06 mmol), various alkyl bromine or tosylate compounds (0.1 mmol), THF (0.6 mL), 70°C.

A survey and a comparison of RCYs of the various n.c.a. [^{75}Se]selenoethers prepared as model compounds via the polymer-supported and the homogenous radiosynthesis are given in subchapter 3.1.7.

3.1.6 Labelling experiments with n.c.a. selenium-73

After the development and optimization of the homogenous and polymer-supported radiosyntheses of [^{75}Se]**5**, the positron-emitter selenium-73 was used as radiolabel. The preparation of [^{73}Se]selenoethers ([^{73}Se]**5**) was performed via the reaction strategies described in Scheme 3.9 (subchapter 3.1.4) and Scheme 3.10 (subchapter 3.1.5) using n.c.a. $^{73}Se^{0}$ as starting material.

Repeated experiments with Se-73 showed no differences in radiochemical yields, and ^{73}Se-labelled compounds were obtained under the same reaction conditions as their corresponding ^{75}Se-labelled counterparts (listed in subchapter 3.1.7). It was confirmed that there is no difference in the radiochemistry of selenium-73 and selenium-75. Possible radiolytic effects due to higher energies of the γ-rays or the β^{+}-particles of the decaying selenium-73 were not observed.

In this empirical way it was demonstrated that selenium-75 is a suitable model-nuclide for selenium-73 and that radiosyntheses developed via selenium-75 may serve as well for the preparation of ^{73}Se-labelled compounds without any necessity of modifying the reaction conditions.

3.1.7 Comparative discussion of results

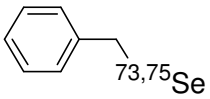
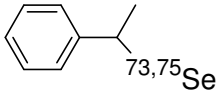
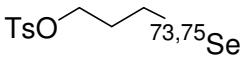
Several asymmetric alkyl [^{73,75}Se]selenoethers ([^{73,75}Se]**5a**-**g**) were prepared as model compounds (cf. Table 3.1) in order to demonstrate the versatility of the polymer- and non-polymer-supported syntheses described above. Labelling of these compounds resulted in radiochemical yields of up to 56% related to elemental radioselenium. Using ethyl or propyl [^{73,75}Se]selenolates, the yields decreased in comparison to methyl [^{73,75}Se]selenolate, using both the homogenous and the polymer-supported method. The radiochemical yields decreased also when using a secondary bromide as second alkylating agent.

Furthermore, the radioselenium labelled alkylating agent methyl[^{73,75}Se]selenopropanyl toluenensulfonate ([^{73,75}Se]**5g**) was obtained with a RCY of 15 ± 4 %. It may serve as a small

longer-lived prosthetic group for labelling of suitable nucleophilic functional groups via [^{73,75}Se]selenoalkylation.

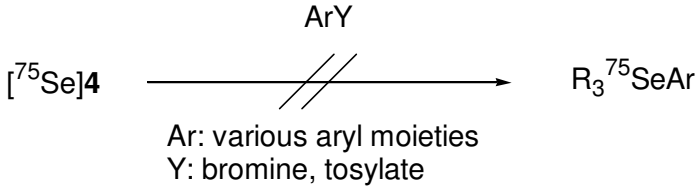
The yields of the homogenous and the polymer-supported syntheses are similar, whereas the total reaction time can be reduced from more than two hours to about half an hour using the polymer-supported method.

Tab. 3.1: Comparison of radiochemical yields* [%] of various asymmetric n.c.a. [^{73,75}Se]selenoethers via homogenous and polymer-supported synthesis

$R_4{}^{73,75}Se$	R_3	Compound No.	homogenous^{+}	polymer-supported^{++}
	Me	[^{73,75}Se]**5a**	48 ± 5	56 ± 5
	Et	[^{73,75}Se]**5b**	41 ± 4	39 ± 4
	Prop	[^{73,75}Se]**5c**	39 ± 4	32 ± 3
	Me	[^{73,75}Se]**5d**	37 ± 4	36 ± 4
	Et	[^{73,75}Se]**5e**	31 ± 3	24 ± 2
	Prop	[^{73,75}Se]**5f**	21 ± 2	17 ± 2
	Me	[^{73,75}Se]**5g**	15 ± 4	13 ± 2

* related to $^{73,75}Se^0$; $^{+}$ 130 min and $^{++}$ 35 min total reaction time, respectively

Although a wide variety of asymmetric n.c.a. alkyl [^{73,75}Se]selenoethers could be obtained demonstrating the versatility of both radiosynthetic pathways via [^{73,75}Se]selenoureas, all attempts made to synthesize aryl radioselenoethers failed. Nucleophilic aromatic substitution (S_NAr) using various aryl bromine or aryl tosylate compounds with strong electron-withdrawing substituents to be attacked by n.c.a. [^{75}Se]selenolates did not occur, and the target molecules ($R_3{}^{75}SeAr$) were not obtained (Scheme 3.11). Experiments using more nucleofugic groups, e.g. fluorine, NR_3^+ or NO_2, as leaving groups in aryl derivatives with strong electron-withdrawing substituents may possibly lead to arylalkylselenides.



Sch. 3.11: Unsuccessful experiments for the preparation of n.c.a. aryl [^{75}Se]selenoethers

3.1.8 Homocysteine [^{75}Se]selenolactone

L-Homocysteine [^{73}Se]selenolactone ([^{73}Se]**6**) is of great interest as a longer-lived alternative of [^{11}C]homocysteine thiolactone ([^{11}C]**7**) (Scheme 3.12). The latter, so far, could only be obtained as racemic mixture due to the short half-life of carbon-11 [159].

[^{73}Se]**6** [^{11}C]**7**

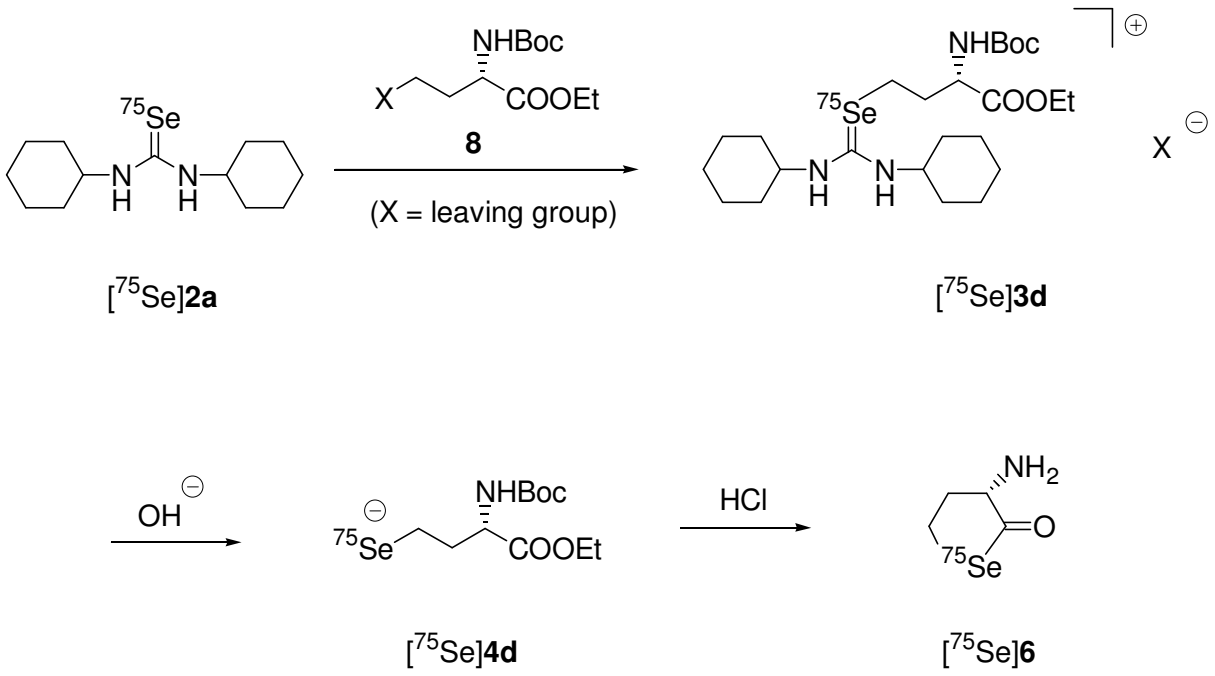
Sch. 3.12: L-Homocysteine [^{73}Se]selenolactone ([^{73}Se]**6**) and D/L-[^{11}C]homocysteine thiolactone ([^{11}C]**7**)

In order to image regional cardiac adenosine production by PET, D/L-[^{11}C]homocysteine thiolactone was used as a sensitive indicator for ischemic myocardial tissue [111], where an increased formation of adenosine is due to the breakdown of adenine nucleotides. The accumulation of adenosine in hypoxic cells can be detected by an excess of homocysteine in presence of [^{11}C]homocysteine thiolactone which leads to an increased formation of intracellular S-adenosyl homocysteine catalyzed by the enzyme SAH hydrolase.

According to the chemical homology of sulfur and selenium, it is the aim to substitute sulfur by radioselenium to get a longer-lived radiotracer, to test the possibility of enzymatic conversion of adenosine into [^{73}Se]selenoadenosine homocysteine for future radiopharmacological studies.

Since homocysteine [^{75}Se]selenolactone can be prepared by reductive demethylation of [^{75}Se]selenomethionine via an appropriate [^{75}Se]selenolate as intermediate [110] (cf. subchapter 1.6.3), a method was developed in this study to generate the appropriate [^{75}Se]seleno-

late ([^{75}Se]**4d**) via basic hydrolysis of the corresponding [^{75}Se]selenouronium salt ([^{75}Se]**3d**). Consecutive cyclization yielded the target molecule [^{75}Se]**6** as depicted in Scheme 3.13.



[^{75}Se]**2a** [^{75}Se]**3d**

[^{75}Se]**4d** [^{75}Se]**6**

Sch. 3.13: Homocysteine [^{75}Se]selenolactone ([^{75}Se]**6**) via the corresponding [^{75}Se]selenouronium salt ([^{75}Se]**3d**)

As examined in subchapter 3.1.3, the alkylation of [^{75}Se]**2a** at the n.c.a. level is only successful using appropriate trifluoromethanesulfonic acid alkyl esters as very strong alkylating agents. Unfortunately, all attempts to generate the precursor **8** with X = triflate failed, and only ethyl N-tert.-butoxycarbonyl-2-amino-4-bromobutyrate (X = Br) could be obtained. Obviously, the triflate compound was too reactive and unstable. Starting from the corresponding alcohol and trifluoromethanesulfonic anhydride according to literature methods [160], all attempts to synthesize this compound failed. Due to the lack of an appropriate triflate precursor, it was not possible to alkylate n.c.a. [^{75}Se]**2a**.

Instead, carrier-added 1,3-dicyclohexyl [^{75}Se]selenourea was produced starting from c.a. $^{75}Se^{0}$, cyclohexyl isocyanide and cyclohexylamine. This radiosynthesis of c.a. disubstituted [^{75}Se]selenoureas is more advantageous than that recently reported via carbodiimides and hydrogen [^{75}Se]selenide [92], since the use of a volatile radioselenium-labelled compound ([^{75}Se]H_2Se) is avoided. The subsequent alkylation with **8** (X = Br) led to a nearly quantitative formation of the desired c.a. [^{75}Se]selenouronium salt ([^{75}Se]**3d**), which was purified according to subchapter 3.1.3. Hydrolysis in presence of TBAH and consecutive acidification with

concentrated hydrochloric acid yielded c.a. homocysteine [^{75}Se]selenolactone by cyclization and simultaneous cleavage of the protecting groups.

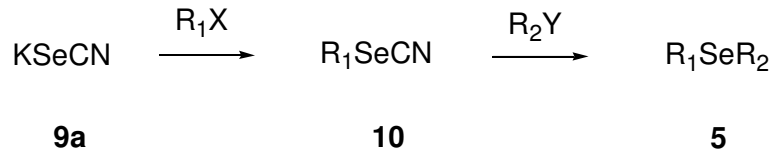
The total RCY starting from c.a. selenium-75 was 20 – 30 %, being three times higher compared to the radiosynthetic strategy via lithium methyl [^{75}Se]selenide by using sulfur as non-isotopic carrier [110] (cf. subchapter 1.6.3). In addition, the selenourea strategy is more convenient because it is easier to handle since the demethylation step (Birch reduction) on [^{75}Se]selenomethionine (cf. Scheme 1.11) is avoided. Furthermore, it offers the possibility of automated radiosynthesis. The indispensable addition of ^{nat}Se-carrier limits the maximum possible specific activity. However, the use of [^{73}Se]**6** in nuclear medicine does not absolutely require that this tracer is administered at the n.c.a. level, provided that the toxicity of this compound is no problem.

3.2 No-carrier-added alkyl and aryl [^{75}Se]selenoethers via [^{75}Se]selenocyanates

With regard to the unsuccessful experiments to obtain aryl radioselenoethers via the selenourea strategy (cf. subchapter 3.1.7), the development of another attractive reaction sequence was performed in order to enlarge the scope of available n.c.a. radioselenium-labelled compounds to aryl selenoethers [161]. This class is of particular interest, because aryl selenoethers are expected to be even more stable than their alkyl analogues. This represents an advantage if appropriate radioselenium-labelled compounds are applied for *in vivo* studies.

The potassium salt of selenocyanic acid (KSeCN (**9a**)) is one of the best-established reagents for the introduction of selenium into organic molecules [40]. The compound is readily prepared from elemental selenium and potassium cyanide [162, 163] and commercially available. Potassium selenocyanate is soluble in water, the lower aliphatic alcohols, acetonitrile and many other polar organic solvents. This high solubility in organic solvent systems earns **9a** a unique position among the readily accessible inorganic selenium derivatives. Like thiocyanate, the selenocyanate anion (**9**) is considered to be a pseudohalide because of resemblance of its chemical behaviour to that of halide ions. With regard to the reactivity of **9**, it was found that it is ten times more nucleophilic than the thiocyanate ion and lies between chloride and bromide anions for substitution reactions on haloacetophenones [57]. The major mode of application of **9a** takes advantage of this high nucleophilicity towards aliphatic halides, yielding alkyl selenocyanates (**10**) [164] as illustrated in Scheme 3.14.

Alkyl selenocyanates have occupied a privileged position in organoselenium chemistry [57] and represent stable compounds which are transformed to valuable organoselenium derivatives [165]. In particular, the selenium atom of alkyl selenocyanates is the preferential site of attack by different nucleophiles due to its electrophilicity. Therefore, selenocyanates react with organometallic compounds producing chemoselectively, in a single step and in almost quantitative yields, aliphatic and aromatic selenoethers (**5**) [40] as depicted in Scheme 3.14.



Sch. 3.14: Synthesis of selenoethers (**5**) via alkyl selenocyanates (**10**)
(R_1 = alkyl moieties, R_2 = alkyl or aryl moieties, X = halides, Y = Li or MgBr)

In the preliminary stages of the development of the radiosynthesis, this synthetic pathway was followed using $^{nat}Se^0$ as starting material to check for general feasibility of this concept and to synthesize the standard compounds used as reference for radio-HPLC and radio-TLC. All non-radioactive seleno compounds (**10** and **5**) prepared via this route were obtained in satisfactory yields (cf. subchapter 4.2.2).

The application of this reaction sequence to the n.c.a. level was examined and optimized in order to prepare n.c.a. alkyl and aryl radioselenoethers as described in the following sections.

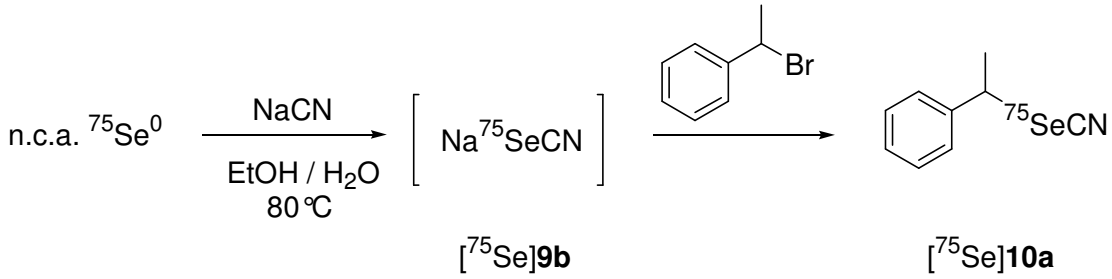
3.2.1 Alkyl [^{75}Se]selenocyanates via sodium [^{75}Se]selenocyanate

The reaction of elemental selenium with potassium cyanide is well-known, resulting in potassium selenocyanate. In this study here sodium cyanide was used for the preparation of the [^{75}Se]selenocyanate anion ([^{75}Se]**9**) due to the easier availability of NaCN in comparison with KCN.

Since the $SeCN^-$ anion is a very nucleophilic agent, it appeared promising to develop a one-pot procedure with interim formation of sodium [^{75}Se]selenocyanate ([^{75}Se]**9b**) and *in situ* conversion to an alkyl [^{75}Se]selenocyanate ([^{75}Se]**10**).

The formation of n.c.a. 1-phenylethyl-1-[^{75}Se]selenocyanate ([^{75}Se]**10a**) was used as a model reaction starting from n.c.a. $^{75}Se^0$, sodium cyanide and (1-bromoethyl)benzene (cf. Scheme 3.15). As demonstrated by radio-TLC, the synthesis of sodium [^{75}Se]selenocyanate was the

rate-determining step in this one-pot reaction sequence, since [^{75}Se]**10a** was formed immediately upon adding benzyl bromide to a solution of [^{75}Se]**9b**, being synthesized using n.c.a. $^{75}Se^0$ and NaCN as starting material.



Sch. 3.15: Synthesis of 1-phenylethyl-1-[^{75}Se]selenocyanate ([^{75}Se]**10a**)

The formation of [^{75}Se]**10a** was optimized with respect to the effects of solvent, amount of water, time, temperature and concentration of sodium cyanide and of the precursor (1-bromoethyl)benzene, as detailed below.

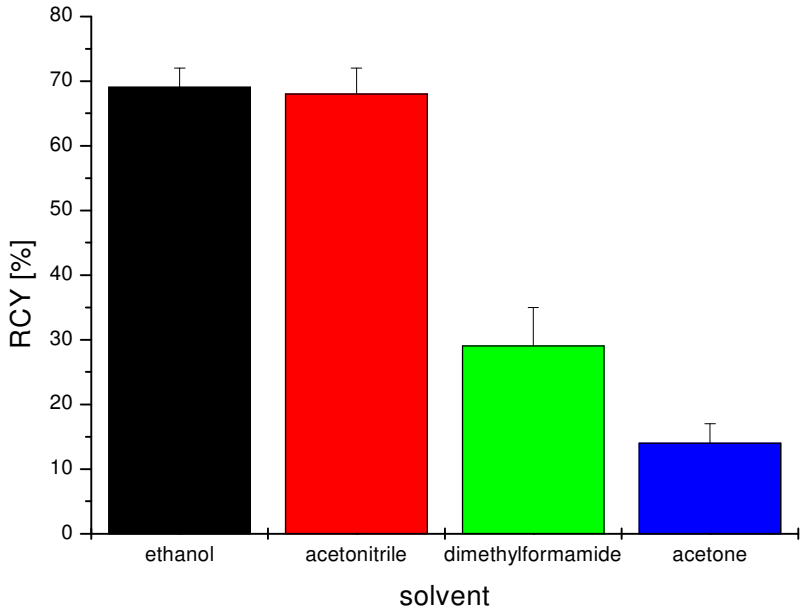


Fig. 3.8: Radiochemical yield of 1-phenylethyl-1-[^{75}Se]selenocyanate ([^{75}Se]**10a**) in various solvents
Reaction conditions: 370 kBq n.c.a. $^{75}Se^0$, sodium cyanide (10 µmol in 0.1 mL H_2O), (1-bromoethyl)benzene (73 µmol), 0.5 mL solvent, 5 min, 80 °C.

Synthesis of [^{75}Se]**10a** was equally successful in either ethanol or acetonitrile with a radiochemical yield of 69 ± 3 % and 68 ± 4 %, respectively. However, the use of ethanol as solvent gave rise to 70 ± 5 % recovery rate after the subsequent purification step, compared to only 50 ± 6 % recovery rate using acetonitrile. Other solvents like N,N-dimethylformamide, acetone and benzene did not prove as suitable since the RCY of [^{75}Se]**10a** reached only 29, 14 and 0 %, respectively, as can be seen in Figure 3.8. Therefore, of those examined ethanol was the solvent of choice in which [^{75}Se]**10a** was obtained in a satisfactory yield and, in addition, which was suitable for the subsequent purification step via reversed phase cartridges.

The effect of water on this labelling step is graphically depicted in Figure 3.9. Working in the presence of some amount of water appeared advantageous for this initial reaction step, since sodium cyanide can be easily added to the reaction mixture if dissolved in H_2O.

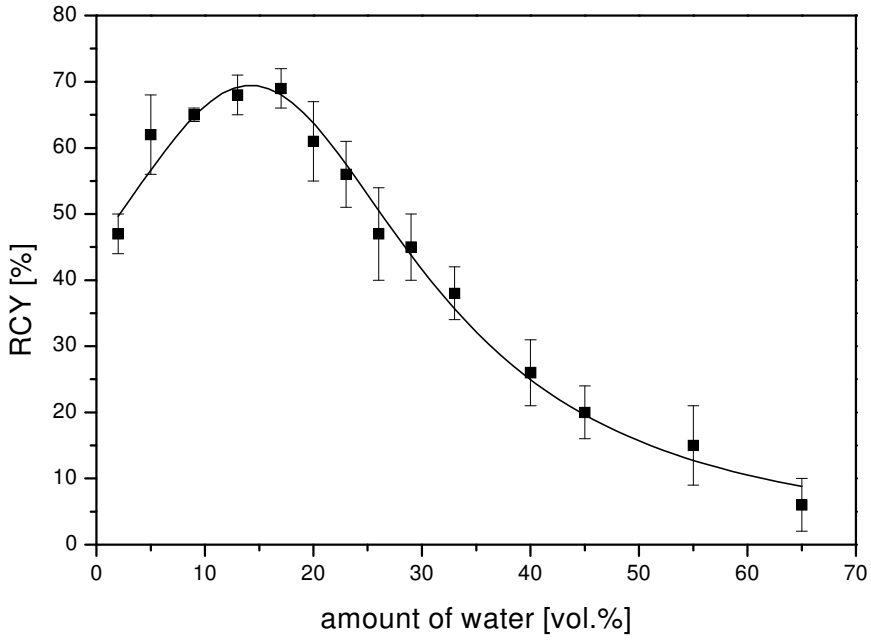


Fig. 3.9: Radiochemical yield of 1-phenylethyl-1-[^{75}Se]selenocyanate ([^{75}Se]**10a**) as a function of added water
Reaction conditions: 370 kBq n.c.a. $^{75}Se^0$, sodium cyanide (10 µmol), (1-bromoethyl)benzene (73 µmol), 0.6 mL solvent (ethanol/water mixture), 5 min, 80 °C.

As illustrated in Figure 3.9, the amount of water was a crucial factor for obtaining a high RCY of [^{75}Se]**10a**. The cyanide- and [^{75}Se]selenocyanate-anion as ionic species seem to prefer an aqueous solution as reaction solvent. The maximum RCY of [^{75}Se]**10a** was observed with

69 ± 3 % at 15 ± 2 vol.% H_2O. The reaction step tolerated a water-amount between 10 and 20 vol.% with no significant decrease of the RCY. Utilization of less than 10 vol.% H_2O led to considerably lower yields, probably because of the lower solubility of sodium cyanide in ethanol. Treatment of n.c.a. $^{75}Se^0$ with NaCN and (1-bromoethyl)benzene in ethanol containing more than 20 vol.% H_2O resulted in lower RCY as well. This decrease can be possibly explained by a growing suppression of the nucleophilic substitution reaction on the alkyl halide caused by increasing inhibition of an S_N2-type exchange by $NC^{75}Se^-$ due to the more protic reaction solvent.

In order to examine the influence of the reaction temperature on the [^{75}Se]selenocyanate formation, the synthesis of [^{75}Se]**10a** was performed at 60°C and 80°C under otherwise stable conditions. The data given in Figure 3.10 indicate that the optimum temperature for the formation of [^{75}Se]**10a** in ethanol was 80°C. The time dependences of the RCY represent hyperbolic curves with a saturation yield, which is typical for pseudo-first-order reactions.

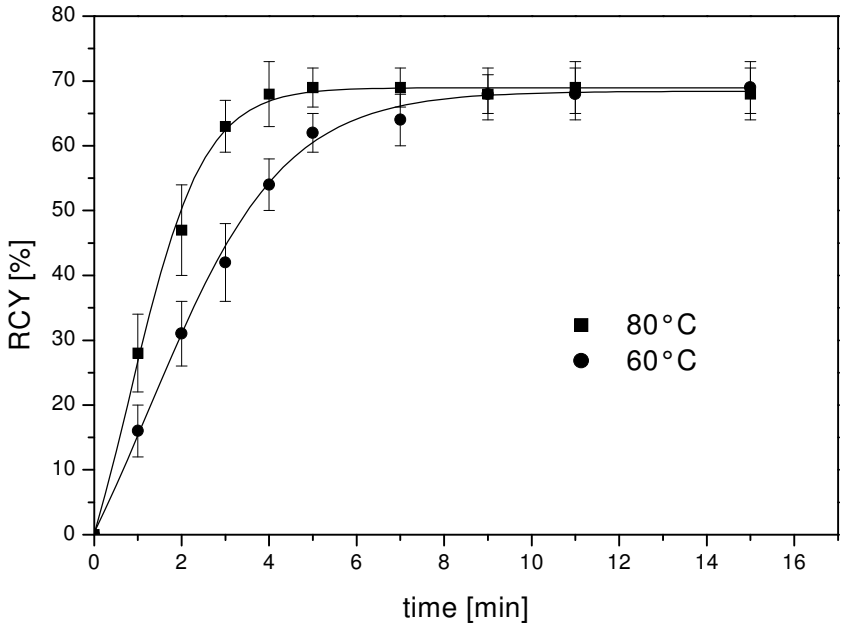


Fig. 3.10: Radiochemical yield of 1-phenylethyl-1-[^{75}Se]selenocyanate ([^{75}Se]**10a**) as a function of temperature and time
Reaction conditions: 370 kBq n.c.a. $^{75}Se^0$, sodium cyanide (10 µmol in 0.1 mL H_2O), (1-bromoethyl)benzene (73 µmol), 0.5 mL ethanol.

The saturation yield was obtained for [^{75}Se]**10a** after a very short reaction time of only 4 min, a fact which is favourable in view of a short radiosynthesis. Although the curves of the time

dependences are very similar and the saturation yields are approximately the same whether the reactions were carried out at 60 or 80°C, the reaction rate is faster at the higher temperature.

As demonstrated by radio-TLC, ^{75}Se-labelled byproducts were formed in amounts of about 30 % independent on the temperatures, which explains the equal saturation yield for both reaction temperatures.

In order to compare the effect of the reaction temperature on the synthesis of [^{75}Se]**10a**, the relative rate constants k of [^{75}Se]**10a** were determined at 60°C and 80°C. As can be seen in Figure 3.11, the term $\ln([B]_0/([B]_0-[C]_t))$ (with $[B]_0$: initial activity of $^{75}Se^0$ and $[C]_t$: activity of [^{75}Se]**10a** at reaction time t) is plotted as a function of time. The slopes of the fitted straight lines give the rate constants k, being 0.28 min^{-1} and 0.2 min^{-1} for the synthesis of [^{75}Se]**10a** at 80°C and 60°C, respectively. From these k-values one can see that an increase of reaction temperature of 20°C increases the rate of reaction by 40%.

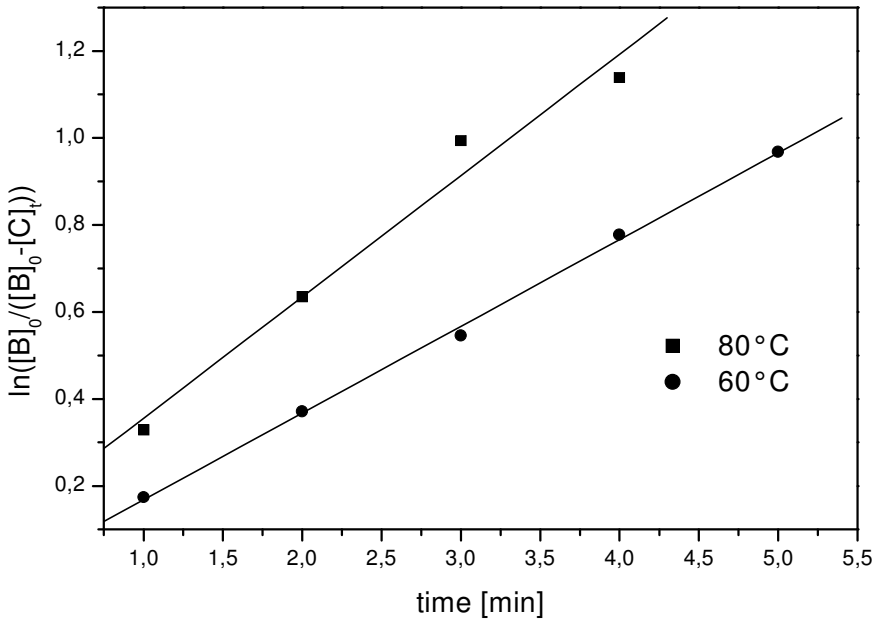


Fig. 3.11: Plot of $\ln([B]_0/([B]_0-[C]_t))$ as a function of time for the determination of the relative rate constants k for the synthesis of [^{75}Se]**10a** at 80°C and 60°C
Reaction conditions: see Fig. 3.10.

The dependence of the RCY of [^{75}Se]**10a** on the amount of sodium cyanide is shown in Figure 3.12 and was determined not only for maximizing the radiolabelling yield but also for minimizing precursor concentration in order to facilitate product separation and purification. With regard to a

future *in vivo* application of a radioselenium-labelled compound being synthesized via selenocyanate, it is extremely important to obtain the target molecule without any traces of sodium cyanide because of its high toxicity. Use of at least 10 µmol sodium cyanide was necessary to obtain the optimum RCY of [^{75}Se]**10a** of about 70 %. A smaller amount of NaCN led to a reduction. In this case, a semilogarithmic amount-RCY plot shows a sigmoidal curve, which represents the typical course of the RCY for a pseudo-first-order reaction.

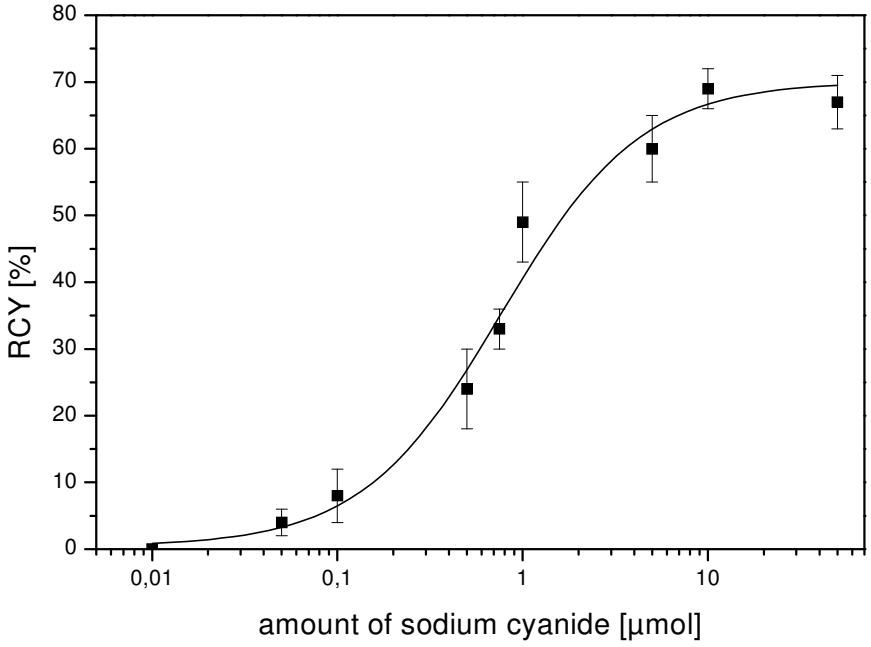


Fig. 3.12: Radiochemical yield of 1-phenylethyl-1-[^{75}Se]selenocyanate ([^{75}Se]**10a**) as a function of the amount of sodium cyanide
Reaction conditions: 370 kBq n.c.a. $^{75}Se^{0}$, sodium cyanide (various amounts in 0.1 mL H_2O), (1-bromoethyl)benzene (73 µmol), 0.5 mL ethanol, 5 min, 80 °C.

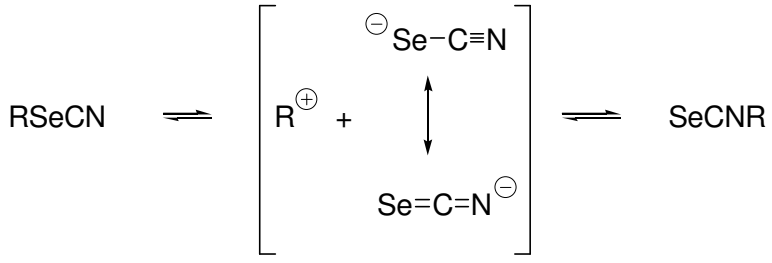
Furthermore, the concentration of the precursor (1-bromoethyl)benzene was optimized keeping all conditions except the one examined as constant. An amount of at least 73 µmol of the alkylating agent per 0.6 mL of solvent proved to be useful for obtaining a high RCY of 69 ± 3 % for [^{75}Se]**10a**, while higher amounts did not lead to a significant increase of the RCY.

These optimization studies showed that the reaction sequence illustrated in Scheme 3.15 is best carried out within 5 min at 80°C using 10 µmol NaCN (dissolved in 0.1 mL H_2O) and 73 µmol alkylating agent in 0.5 mL ethanol.

In order to demonstrate the versatility of this labelling sequence, reactions using the alkylating agents benzyl bromide and methyl trifluoromethanesulfonate (MeOTf) were carried out under the conditions described above, yielding benzyl [^{75}Se]selenocyanate ([^{75}Se]**10b**) and methyl [^{75}Se]selenocyanate ([^{75}Se]**10c**) with a RCY of 15 ± 4 % and 73 ± 8 %, respectively.

The disappointingly low yield of [^{75}Se]**10b** can be probably explained by an isomerization of [^{75}Se]**10b** to its corresponding iso[^{75}Se]selenocyanate as demonstrated by radio-TLC. This assumption bases on the knowledge of the properties of thiocyanates [166]. In general, the isomerization of alkyl thiocyanates to corresponding isothiocyanates is practically complete in the vast majority of cases, and only traces of thiocyanate may be detected at equilibrium. Selenocyanates isomerize more rapidly than the corresponding thiocyanates, but the equilibrium at macroscopic scale is much more shifted towards the “normal” compound, i.e. selenocyanate, than in the case of sulfur [167]. This was particularly confirmed in the case of benzyl selenocyanate via IR spectra. It was found that the reaction of potassium selenocyanate and benzyl bromide resulted in both, benzyl selenocyanate and, to a lesser degree, benzyl isoselenocyanate.

A proposed mechanism of this isomerization reaction is portrayed in simplified terms in Scheme 3.16.



Sch. 3.16: Simplified mechanism for the isomerization reaction of organic selenocyanates (**10**)

This isomerization mechanism is a simple dissociation of organic selenocyanates. The selenocyanate group acts as a good leaving group from aliphatic carbon, thus generating a transition state in which an alkyl cation **V** and the selenocyanate anion **9** are formed by heterolysis. Since **9** can act as a selenium as well as a nitrogen nucleophile, subsequent rearrangement yields isoselenocyanates **VI**. Tracer and stereochemical studies, though, indicate that the dissociation and formation of this simple transition state is only a rough approximation of reality. Detailed

studies rather suggest a more complex internal ion-pair mechanism without complete heterolytic cleavage [167].

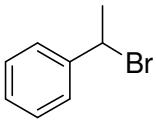
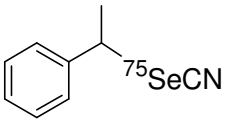
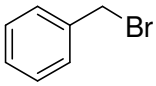
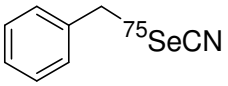
In case of n.c.a. benzyl [^{75}Se]selenocyanate ([^{75}Se]**10b**), this isomerization seems to be favoured without reaching an equilibrium as in the macroscopic scale. This preferred isomerization may be due to the fact that the benzyl cation is relatively stable and thus more easily formed than other aliphatic cations due to the fact that it has delocalized electrons and five resonance contributors. In addition, the benzyl-nitrogen bond is more stable than the benzyl-Se bond, so the iso-derivative is more abundant resulting in the decrease of the RCY of [^{75}Se]**10b**.

In the case of [^{75}Se]**10a** and [^{75}Se]**10c**, on the other hand, satisfactory RCYs were obtained, which can be explained by the fact that these compounds isomerize only to a small degree as demonstrated by radio-TLC.

Surprisingly, [^{75}Se]**10a** does not favour isomerization, although the transition state contains a benzylic cation, which is even more stable than the benzyl cation. Apparently, the mechanism depicted in Scheme 3.16 is too simplified and needs to be more thoroughly studied.

An alternative explanation for the formation of isoselenocyanate derivatives bases on the property of the selenocyanate anion to act directly as a nitrogen nucleophile due to its ambident nucleophilicity. So it is also likely that the bidentate anion **9** reacts with the alkylating agent to give the isoselenocyanate derivative without “detour” via the aliphatic selenocyanate.

Tab. 3.2: Preparative RCY of alkyl [^{75}Se]selenocyanates ([^{75}Se]**10**) after separation via reversed phase cartridges (related to n.c.a. $^{75}Se^{0}$)

Precursor	Product	Compound No.	RCY [%] (a)	RCY [%] (b)
		[^{75}Se]**10a**	69 ± 3	48 ± 5
		[^{75}Se]**10b**	15 ± 4	11 ± 5
MeOTf	Me^{75}SeCN	[^{75}Se]**10c**	73 ± 8	69 ± 8

(a) substitution yield, (b) after Sep-Pak / LiChrolut purification

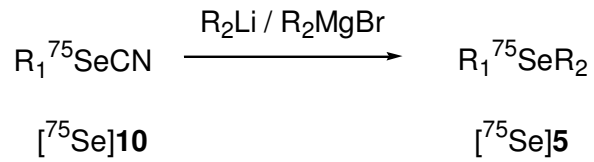
A separation of the n.c.a. alkyl [^{75}Se]selenocyanates from their aqueous/ethanolic solvent system was indispensable due to the following reaction step involving organic lithium or Grignard compounds. Furthermore, it must be taken into account that the starting material sodium cyanide is highly toxic and must completely be removed before use of an appropriate ^{73}Se-labelled target molecule as *in vivo* radiotracer.

For simple performance, reversed phase cartridges were used for the purification of [^{75}Se]**10**. Maximum recovery rates were reached with ethanol as eluant for [^{75}Se]**10a** and [^{75}Se]**10b** using Sep-Pak® Plus C18 cartridges (70 ± 5 %) and for [^{75}Se]**10c** using LiChrolut® EN cartridges (95 ± 3 %), resulting in a total RCY after separation, which is given in Table 3.2 for the various reagents.

3.2.2 Synthesis of asymmetric alkyl and aryl [^{75}Se]selenoethers

Due to its properties as a pseudohalide, the cyano group in alkyl selenocyanates acts as a good leaving group upon nucleophilic attack on the positively polarized selenium-atom. Apparently, the weak cyanocarbon-selenium bond is the reason of the high reactivity of the selenocyanato-substituted compound [168].

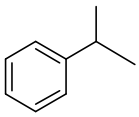
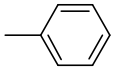
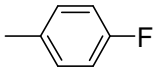
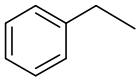
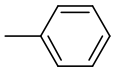
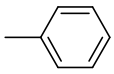
Therefore, the direct reaction of [^{75}Se]**10** with alkyl or aryl lithium derivatives according to Scheme 3.17 leads to asymmetric [^{75}Se]selenoethers ([^{75}Se]**5**). Grignard reagents may be used in place of lithium compounds. This conversion was carried out in anhydrous diethyl ether with typical RCYs of 80 – 90 %. The reaction appears to be quite general, not being impaired by various aromatic or aliphatic compounds used. Optimization studies showed that this reaction step is most effective while using Li- or Grignard-compounds in amounts of 0.1 mmol in 0.5 mL anhydrous diethyl ether, taking place at room temperature within 1 min.



Sch. 3.17: Synthesis of asymmetric [^{75}Se]selenoethers ([^{75}Se]**5**) via organic [^{75}Se]selenocyanates ([^{75}Se]**10**)

Several asymmetric aliphatic and aromatic [^{75}Se]selenoethers were prepared as model compounds (Table 3.3) in order to prove the versatility of the method.

Tab. 3.3: RCY of some asymmetric n.c.a. aryl and alkyl [^{75}Se]selenoethers ([^{75}Se]**5**) formed via [^{75}Se]selenocyanates ([^{75}Se]**10**)

Precursor	Product (R_1-^{75}Se-R_2) R_1	R_2	Compound No.	RCY [%] (related to $^{75}Se^0$)
[^{75}Se]**10a** + MeLi		Me	[^{75}Se]**5d**	40 ± 5
[^{75}Se]**10a** + BuLi		Bu	[^{75}Se]**5h**	39 ± 5
[^{75}Se]**10a** + PhLi			[^{75}Se]**5i**	44 ± 5
[^{75}Se]**10a** + p-FPhMgBr			[^{75}Se]**5j**	43 ± 5
[^{75}Se]**10b** + MeLi		Me	[^{75}Se]**5a**	9 ± 5
[^{75}Se]**10b** + PhLi			[^{75}Se]**5k**	9 ± 5
[^{75}Se]**10c** + PhLi	Me		[^{75}Se]**5l**	55 ± 8

When benzyl bromide was used as alkyl halide to be reacted with [^{75}Se]selenocyanate, the radiochemical yield decreased significantly due to an isomerization to the iso-derivative. Accordingly, the RCYs of [^{75}Se]**5a** and [^{75}Se]**5k** were very low. The overall RCYs of the other model compounds were similar and quite satisfactory. Both, alkyl and aryl organometallic agents added readily to the [^{75}Se]selenocyanates. The use of Grignard compound instead of lithium derivatives had no effect on the RCY.

To sum up Chapter 3.2, the radiosynthesis developed and optimized is depicted in Scheme 3.18. Starting from n.c.a. $^{75}Se^{0}$, sodium cyanide and various alkyl bromine or triflate compounds, several alkyl [^{75}Se]selenocyanates were obtained via a one-pot procedure within 5 min and with a RCY of 15 – 73 %. Subsequent purification using reversed phase cartridges gave rise to recovery rates of 70 – 95 %. Reaction of purified [^{75}Se]**10** with organometallic compounds yielded asymmetric [^{75}Se]selenoethers ([^{75}Se]**5**) within a total reaction time of 20 min in RCYs of 9 to 55 % (related to n.c.a. $^{75}Se^{0}$).

	time	**RCY**
n.c.a. $^{75}Se^{0}$	0 min	
↓ i		
$R_1{}^{75}SeCN$ [^{75}Se]**10**	5 min	15 - 73 % (related to n.c.a. $^{75}Se^{0}$)
↓ ii		
purified [^{75}Se]**10**	19 min	70 - 95 % (related to [^{75}Se]**10**)
↓ iii		
$R_1{}^{75}SeR_2$ [^{75}Se]**5**	20 min	80 - 90 % (related to purified [^{75}Se]**10**) 9 - 55 % (related to n.c.a. $^{75}Se^{0}$)

Sch. 3.18: Radiosynthesis of [^{75}Se]selenoethers ([^{75}Se]**5**) via [^{75}Se]selenocyanates
Reaction conditions: i) sodium cyanide (10 µmol in 0.1 mL H_2O), various alkyl bromine or triflate compounds (73 µmol), 0.5 mL ethanol, 80 °C; ii) purification via reversed phase cartridges, iii) alkyl or aryl lithium or Grignard compounds (0.1 mmol), 0.5 mL anhydrous diethyl ether, r.t.

3.3 Comparison and evaluation of the n.c.a. radiosyntheses developed

Two new radiosynthetic pathways were developed to prepare various aliphatic and aromatic [^{75}Se]selenoethers at the no-carrier-added level, both based on the initial formation of radioselenium labelled intermediates.

The radiosyntheses developed take advantage of the large nucleophilicity of a negatively charged or polarized terminal carbon atom adjacent to a nitrogen via multiple bond. Mainly two compounds exhibit this property: on the one hand alkyl isocyanides and on the other hand the inorganic cyanide anion. In both cases, elemental radioselenium is attacked by the carbon nucleophile, yielding a labelled alkyl isoselenocyanate (RNC^{75}Se) or the inorganic [^{75}Se]selenocyanate anion (NC^{75}Se$^-$), respectively. Apparently, these initial reaction steps for introducing ^{75}Se^0 into an organic or inorganic compound proceed very similar, and they represent the rate-determining steps in their corresponding radiosyntheses.

Both products were prepared and converted *in situ* to give the desired radioselenium labelled intermediates in corresponding one-pot procedures.

In the former case, the alkyl iso[^{75}Se]selenocyanate and a primary amine react to give the corresponding labelled 1,3-disubstituted selenoureas and in the latter case, the [^{75}Se]selenocyanate anion adds to an appropriate alkyl halide, yielding alkyl [^{75}Se]selenocyanates (R^{75}SeCN). These intermediates belong to two completely different classes with regard to the reactivity of the selenium atom. Whereas on the one hand the substituted [^{75}Se]selenourea contains the Se atom as a nucleophile, in alkyl [^{75}Se]selenocyanate, on the other hand, it is an electrophile. However, both of these organic derivatives of selenium have in common that they offer the general possibility for the formation of new C-Se bonds.

In case of [^{75}Se]selenoureas, one can consider the organic moiety as an activating as well as a protecting group, that facilitates the further synthetic sequence and which is removed afterwards by a suitable procedure. While many selenium nucleophilic reagents contain a selenium anion and are generally used in alkaline media, the neutral [^{75}Se]selenourea is readily alkylated in organic solvents to give [^{75}Se]selenouronium salts. After purification, these salts are hydrolyzed under basic conditions to the corresponding selenolates, which are alkylated by various alkylating agents (R'X) *in situ*, yielding the desired asymmetric radioselenoethers (R^{75}SeR').

On the other hand, the alternative intermediate product R^{75}SeCN offers the possibility to obtain the target molecules more straightforward. Since the cyano moiety of this derivative is a good leaving group, a nucleophilic attack of an appropriate organometallic compound (R'Li or

R'MgBr) on the electrophilic selenium atom displaces the cyano group by the organic moiety R', thus obtaining asymmetric radioselenoethers ($R^{75}SeR'$).

Both of these radiosyntheses developed in this work have proved to be valuable methods for the preparation of a broad range of asymmetric radioselenoethers at the no-carrier-added level, but there are some individual differences in their applicability and in their handling.

Of particular interest are the overall reaction times of the respective radiosynthetic strategies. The entire synthesis for the preparation of the desired asymmetric [^{75}Se]selenoether via a free [^{75}Se]selenourea-derivative (i.e. homogenous phase synthesis) as intermediate takes more than two hours, which is due to the long reaction time for the formation of the disubstituted [^{75}Se]selenourea and the tedious purification required.

The use of a polymer-supported primary amine as precursor for the [^{75}Se]selenourea synthesis reduces the total reaction time to about half an hour, being only a quarter of the original reaction time, while the same RCYs are attained. This saving of time is caused firstly by the more rapid formation of polymer-supported [^{75}Se]selenoureas in comparison to the free substituted [^{75}Se]selenourea and, secondly, by a much shorter purification procedure.

An even larger decrease of the total reaction time is achieved using alkyl [^{75}Se]selenocyanates as intermediates. This more straightforward synthesis of asymmetric [^{75}Se]selenoethers is performed within only 20 min, since $^{75}Se^0$ is introduced directly into a part of the target molecule, and no "detour" via an activating or protecting group is necessary.

Since the alkylation of n.c.a. labelled selenourea derivatives can only be carried out using trifluoromethanesulfonic acid alkyl esters as alkylating agents, which are in general unstable compounds and which cannot be synthesized with several more complex alkyl moieties, the range of achievable radioselenoethers is limited.

A disavantage of the radiosynthetic pathway using alkyl [^{75}Se]selenocyanates is the possible isomerization of these intermediates to the corresponding iso[^{75}Se]selenocyanate, which can lead to a drastic decrease of the radiochemical yield in some cases (cf. subchapter 3.2.1).

The huge advantage of the latter radiosynthesis is that asymmetric alkyl as well as aryl [^{75}Se]selenoethers can be obtained, since aliphatic and aromatic lithium or Grignard compounds can be used in the last reaction step.

The radiosynthetic strategy via 1,3-disubstituted [^{75}Se]selenoureas, on the other hand, only offers the possibility to prepare asymmetric dialkyl [^{75}Se]selenoethers. All attempts made to obtain aryl [^{75}Se]selenoethers failed.

The radiochemical yields of the labelled target molecules are similar in both cases and amount up to about 50 – 55 % (related to n.c.a. $^{75}Se^{0}$).

Thus, the spectrum of available n.c.a. radioselenium labelled compounds has been enlarged via these presented reaction sequences to a wide range of asymmetric alkyl and aryl [^{75}Se]selenoethers, which were obtained at the n.c.a. level for the first time.

Furthermore, due to the successful application to a solid-phase synthetic route, the pathway via polymer-supported labelled selenourea-derivatives appears very attractive for automation. In addition, this radiosynthetic strategy avoids the use of highly toxic sodium cyanide as reagent.

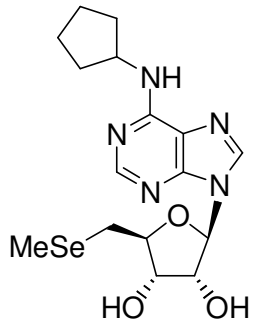
Tab. 3.4: Comparison of radioselenation methods

Synthesis of	**total reaction time**	**RCY**	**References**
n.c.a. alkyl [^{73,75}Se]selenoethers			
via 1,3-disubstituted [^{73,75}Se]selenoureas			
in solution	130 min	15 – 48 %	this work
polymer-supported	35 min	13 – 56 %	this work
n.c.a. alkyl / aryl [^{75}Se]selenoethers			
via alkyl [^{75}Se]selenocyanates	20 min	9 – 55 %	this work
n.c.a. [^{75}Se]selenomethionine			
via triphenylphosphine[^{75}Se]selenide	60 min	30 %	[109]
n.c.a. homocysteine [^{75}Se]selenolactone			
via lithium methyl [^{75}Se]selenide			
using sulfur as non-isotopic carrier	180 min	5 – 10 %	[110]
c.a. alkyl [^{75}Se]selenoethers			
via 1,3-disubstituted [^{75}Se]selenoureas	90 min	10 – 60 %	[92]
c.a. alkyl / aryl [^{75}Se]selenoethers			
via sodium hydrogen [^{75}Se]selenide	> 60 min	up to 86 %	[89, 91]

As listed in Table 3.4, previously described methods for radioselenation only lead either to n.c.a. methyl selenoethers and related compounds [109, 110] or to carrier-added alkyl and aryl radioselenoethers [89, 91, 92].

3.4 Synthesis of a radioselenated ligand for the adenosine A_1 receptor

As already outlined in the Introduction (cf. subchapter 1.7.3) partial agonists for the adenosine A_1 receptor could have an attractive broad therapeutic potential. Previously, van der Wenden *et al.* [169] have synthesized such partial agonists by substituting the 5'-position of N^6-cyclopentyladenosine (CPA). Studies showed that *inter alia* 5'-(methylseleno)-CPA (**19**) (Scheme 3.19) proved selective for the adenosine A_1 receptors, displaying an affinity in the nanomolar range.



19

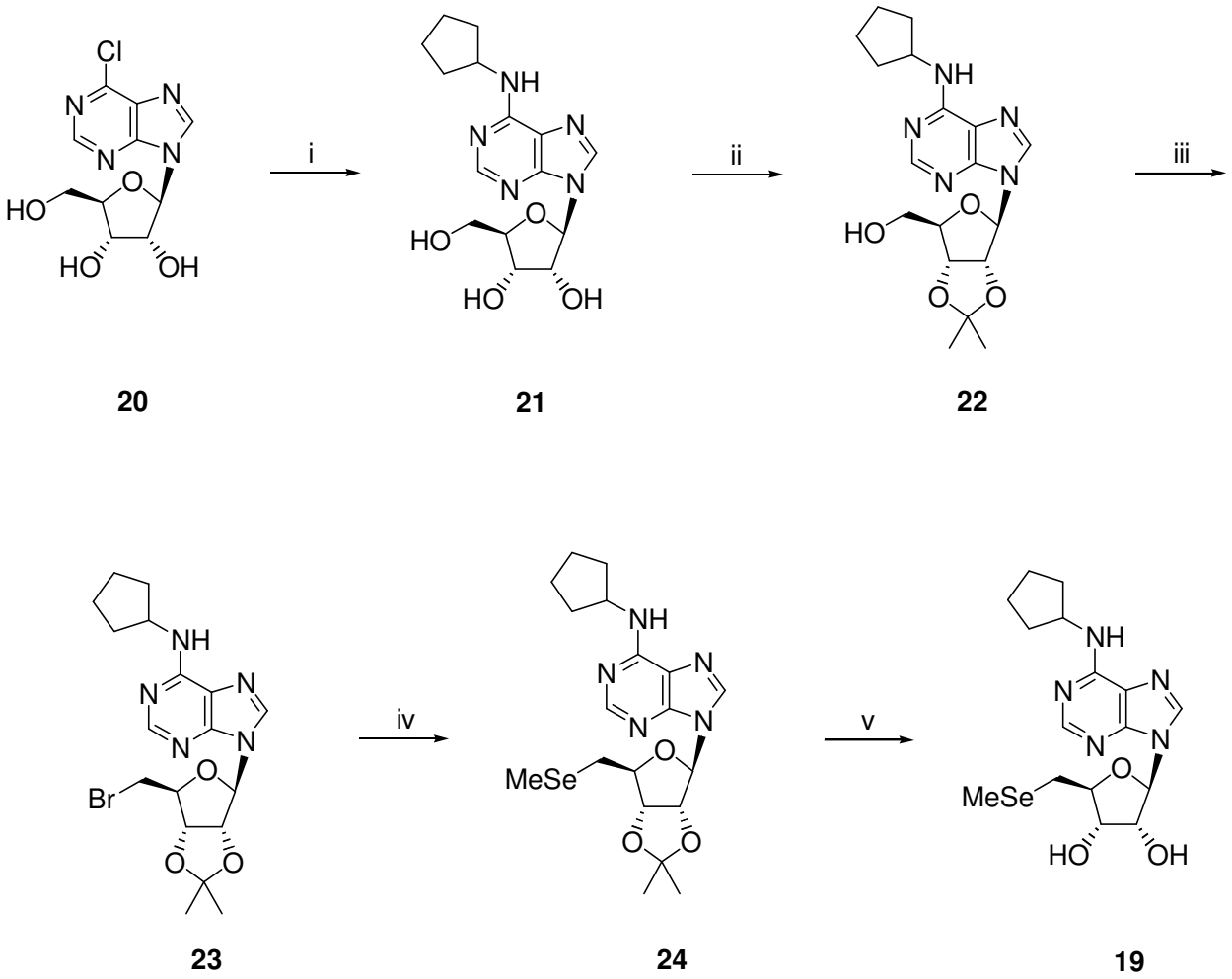
Sch. 3.19: 5'-(Methylseleno)-N^6-cyclopentyladenosine (**19**)

The application of the longer-lived positron emitter selenium-73 could be useful in particular for neuroreceptor work. It has been argued that the only feasible means of accurately studying receptor sites present in low concentrations is by using very high affinity ligands labelled with long-lived radionuclides that permit the "wash-out" phase to be analysed [170]. In late images of long-lived radiotracers the receptor-bound tracer should dominate the images, while with shorter-lived positron-emitters (e.g. ^{11}C, ^{18}F) the non-specific binding and the influence of perfusion on the local distribution may be considerable, thus both must be accounted for.

In order to obtain a ^{73}Se-labelled radioligand, which could be useful for PET studies of the adenosine A_1 receptor system, compound **19** was selected for labelling at the n.c.a level; first with selenium-75 to check for general feasibility of appropriate radioselenation methods.

3.4.1 Standards and precursor

The standard compound 5'-(methylseleno)-CPA (**19**) and the precursor for radioselenation 5'-bromo-2',3'-isopropylidenedioxy-CPA (**23**) were synthesized in five and three steps, respectively, starting from 6-chloropurine riboside (**20**) (Scheme 3.20).



20 **21** **22**

23 **24** **19**

Sch. 3.20: Synthesis of 5'-(methylseleno)-CPA (**19**)
i) cyclopentylamine, EtOH, reflux; ii) sulfuric acid (conc.), acetone, 0 °C; iii) triphenylphosphine, carbon tetrabromide, CH_2Cl_2, r.t.; iv) dimethyldiselenide, sodium borohydride, EtOH, r.t.; v) acetic acid, reflux.

CPA (**21**) was obtained by reacting **20** with cyclopentylamine in ethanol to substitute the chlorine atom [171]. To obtain a 5'-group that is selectively reactive with respect to the other two (2'- and 3'-) positions, the cis vicinal 2'- and 3'-hydroxyl groups of CPA were selectively protected with an isopropylidene moiety by sulfuric acid-catalytic reaction in acetone [172], yielding 2',3'-isopropylidenedioxy-CPA (**22**). Subsequently, the 5'-hydroxyl group of **22** was replaced by a bromine atom as leaving group, by reaction with carbon tetrabromide and triphenylphosphine in CH_2Cl_2 [173], resulting 5'-bromo-2',3'-isopropylidenedioxy-CPA (**23**). This compound was quite unstable and was therefore used directly after purification. A reaction between **23** and methyl selenolate, which was obtained by reductive cleavage of dimethyl-diselenide via sodium borohydride [174], yielded 5'-(methylseleno)-2',3'-isopropylidenedioxy-CPA (**24**). Deprotecting the 2'- and 3'-position of **24** with acetic acid [175] gave the final product 5'-(methylseleno)-CPA (**19**) with an overall yield of about 20 % (related to **20**).

Results of radioligand binding studies for 5'-(methylseleno)-CPA (**19**) in literature [169] are promising regarding selectivity for the adenosine A_1 receptor, since they showed a 150 fold better affinity for the A_1 vs. the A_{2A} receptor, which is probably due to to the presence of the N^6-cyclopentyl substituent. Though, the K_i value of **19** for the adenosine A_1 receptor was not very satisfactory with only 76 nM; the K_i value for the A_{2A} receptor amounted to 11 µM. These literature data were obtained using membranes of rat brain cortex with [^{3}H]DPCPX as the radioligand and using rat striatal membranes with [^{3}H]CGS21680 as the radioligand, respectively.

Nevertheless, **19** seems to be a very attractive selective ligand for the A_1 receptor, therefore radioligand binding studies were carried out in context with this thesis by the Pharmacology Group of the Institute of Nuclear Chemistry under different conditions (cf. subchapter 4.5). Its affinity for the adenosine A_1 receptor was determined by competition with [^{3}H]CPFPX on pig cortical membranes. The K_i value of **19** for the adenosine A_1 receptor was 0.9 nM (0.8 – 1.1 nM, 95 % confidence interval). On the other hand, **19** had a K_i value of 1.3 µM (0.3 – 5.5 µM, 95 % confidence interval) for the adenosine A_{2A} receptor measured by displacement of [^{3}H]CGS21680 from pig striatal membranes. These results suggest, that 5'-(methylseleno)-CPA (**19**) is selective for the adenosine A_1 vs. the A_{2A} receptor with a 1500 fold better affinity. The differences of this study and literature data can be explained by the altered conditions of the radioligand binding studies, i.e. by a different radioligand competitor in case of the A_1 receptor study and by tissues of different animals in the studies.

3.4.2 Synthesis of n.c.a. 5'-(methyl[^{75}Se]seleno)-N^6-cyclopentyladenosine

Since the specific activity of a potential radioligand for *in vivo* studies is a crucial factor and should be as high as possible in order to avoid a saturation of the receptor system by the compound administered, appropriate n.c.a. labelling methods are required. The n.c.a. radioselenation strategies developed in this work were tested for the preparation of n.c.a. 5'-(methyl-[^{75}Se]seleno)-CPA ([^{75}Se]**19**).

Attempts to obtain n.c.a. [^{75}Se]**19** via the selenocyanate method were not successful probably due to isomerization (cf. subchapter 3.2.1) of 5'-[^{75}Se]selenocyanato-2',3'-isopropylidenedioxy-CPA to 5'-iso[^{75}Se]selenocyanato-2',3'-isopropylidenedioxy-CPA.

Using instead the first radiosynthetic concept via free methyl [^{75}Se]selenouronium salt ([^{75}Se]**3a**), 5'-(methyl[^{75}Se]seleno)-2',3'-isopropylidenedioxy-CPA ([^{75}Se]**24**) was obtained in a RCY of 70 % (related to [^{75}Se]**3a**) after hydrolysis of [^{75}Se]**3a** and alkylation of the resulting methyl [^{75}Se]selenolate ([^{75}Se]**4a**) with 5'-bromo-2',3'-isopropylidenedioxy-CPA (**23**) under the optimized reaction conditions given in subchapter 3.1.4. The corresponding reaction sequence utilizing the polymer-supported methyl [^{75}Se]selenouronium salt ([^{75}Se]**3a$_{resin}$**) as starting material (cf. subchapter 3.1.5) gave rise to a RCY of [^{75}Se]**24** of about 70 % (related to [^{75}Se]**3a$_{resin}$**) as well. Subsequent deprotection of [^{75}Se]**24** using hydrochloric acid at 80 °C yielded the final n.c.a. product [^{75}Se]**19** within 5 min and with a RCY of 55 % (related to [^{75}Se]**24**). Thus, 5'-(methyl[^{75}Se]seleno)-N^6-cyclopentyladenosine was obtained at the n.c.a. level with an overall RCY of approximately 30 % (related to $^{75}Se^0$) within a total reaction time of 135 and 40 min using the free and the polymer-supported selenourea method, respectively.

The specific activity of n.c.a. [^{75}Se]**19** was determined to be more than 300 GBq/mmol (8 Ci/mmol) (cf. subchapter 4.4.3). In consideration of this relatively low value and a theoretically $A_{s,max}$ of 40 TBq/mmol (1 Ci/µmol) for carrier-free selenium-75, the actual specific activity will amount to a value in the range between these two limits. Provided production of higher starting activities would not increase the dilution with stable selenium, i.e. the amount of carrier will still remain below 1 nmol, which is the limit of detection (cf. subchapter 4.4.3), one can suggest that the actual specific activity would increase with the same factor as the starting activity. In case of the positron emitter selenium-73, the theoretically $A_{s,max}$ amounts to 1600 TBq/mmol (400 Ci/µmol). Therefore one can suggest that an even higher specific activity will be obtained if the starting activity is in the GBq-range. Thus, it should be possible to obtain a specific activity, which is sufficient to perform *in vivo* receptor studies with ^{73}Se-labelled ligands using PET.

Chapter 4

Experimental

4.1 General

All chemicals and solvents were purchased from Aldrich (Germany), Fluka (Switzerland) and Merck (Germany). They were reagent grade or better and used without further purification. LiChrolut® EN cartridges were obtained from Merck; Sep-Pak® Plus C18 cartridges were obtained from Waters (USA) and aminomethylated polystyrene was obtained from Novabiochem (Germany). All selenocompounds were prepared under a slight positive pressure of argon. Gravity column chromatography was done with Merck silica gel 60 (0.063 – 0.200 mm). Flash chromatography was done with Fluka silica gel 60 (220 – 440 mesh). Mixtures of elution solvents are given as V:V ratios.

Melting point determinations employed a Mettler FP 61 apparatus. Melting points (m.p.) and boiling points (b.p.) are uncorrected. Analytical thin-layer chromatography (TLC) was performed with precoated silica gel plates (5 x 7.5 cm plates Type F_{254S}, Merck, Germany). Visualization of TLC slides was by either iodine, UV or ninhydrin. Infrared spectra (IR) were recorded as KBr pellets on a Shimadzu IR-460 spectrophotometer. ^{1}H, ^{13}C and ^{19}F nuclear magnetic resonance (NMR) spectra were recorded at 200, 50 and 188 MHz, respectively, on a Bruker DPX Avance 200 spectrometer with samples dissolved in $CDCl_3$, CD_2Cl_2 or d_6-DMSO. ^{13}C NMR were routinely run with broad band decoupling. All chemical shifts are reported in δ ppm using the signals of the solvent as a reference. Mass spectra (MS) were obtained using a Thermoquest Automass Multi mass spectrometer using the ionization method indicated for each single compound. High-resolution mass spectra (HR-MS) served as additional identification of compounds prepared for the first time and were determined with electron ionization (EI) using

standard methods on a Finnigan MAT 900 ST apparatus (University of Cologne, Germany). HR-MS was used in place of elemental analysis, since it provided results which were sufficient for this work.

4.2 Standards and precursors

Most of the syntheses described below were carried out as modified versions of the corresponding original literature. In few cases the syntheses were taken directly from the literature without any alteration, and in some cases completely new syntheses were elaborated.

4.2.1 Syntheses via 1,3-dicyclohexyl selenourea

*Cyclohexyl isoselenocyanate (**1a**) [following 154]*

Selenium (0.19 g, 2.4 mmol) was added to a solution of cyclohexyl isocyanide (0.25 mL, 2 mmol) in EtOH (20 mL). The mixture was stirred under reflux for 4 h. After cooling, undissolved selenium was removed by filtration, the solvent evaporated, and the residue was purified via gravity column chromatography (hexane/diethyl ether 20:1) to give the product [176] as an oil after evaporation of the eluent.

Yield: 113 mg (30 %).

R_f: 0.35 (hexane/diethyl ether 100:3).

IR: ν_{max} (KBr): 2120 cm^{-1} (NCSe).

^{1}H-NMR (d_6-DMSO): δ 4.06 (m, 1H, CH), 1.64 (m, 10H, CH_2).

^{13}C-NMR (d_6-DMSO): δ 131.6 (N=C=Se), 56.4 (CH-N), 32.9 (CH_2), 25.3 (CH_2), 23.4 (CH_2).

MS (EI): m/e 189 (M, 18 %), 66 (75), 55 (100).

*1,3-Dicyclohexyl selenourea (**2a**) [following 61]*

Selenium (0.95 g, 12 mmol) was added to a solution of cyclohexyl isocyanide (1.24 mL, 10 mmol) and cyclohexyl amine (1.14 mL, 10 mmol) in EtOH (100 mL). The mixture was stirred under reflux for 16 h. After cooling, undissolved selenium was removed by filtration, the solvent

evaporated, and the residue was purified via gravity column chromatography (hexane/diethyl ether 2:3) to give the product [92] as colourless solid after evaporation of the eluent.

Yield: 2.53 g (88 %).

M.p.: 172°C.

R_f: 0.52 (hexane/diethyl ether 2:3).

IR: ν_{max} (KBr): 3250 cm^{-1} (NH), 2930 cm^{-1} (CH), 1560 cm^{-1} (NH).

^{1}H-NMR (CD_2Cl_2): δ 5.85 (bs, 2H, NH), 3.85 (bs, 2H, CH), 1.65 (m, 10H, CH_2).

^{13}C-NMR (d_6-DMSO): δ 173.0 (C=Se), 54.3 (CH-N), 32.0, 25.0, 24.5 (CH_2).

MS (EI): m/e 288 (M, 25 %), 207 (10), 125 (30), 98 (29), 83 (23), 43 (100).

HR-MS calculated for $C_{13}H_{24}N_2Se$: 289.118, found 289.117.

Trifluoromethanesulfonic acid propyl ester [following 160]

A solution of 1-propanol (1.8 mL, 24 mmol) in CH_2Cl_2 (10 mL) was added dropwise with stirring over a 30-min period to a solution of 2,6-lutidine (3.6 mL, 30 mmol) and trifluoromethanesulfonic anhydride (4.8 mL, 30 mmol) in CH_2Cl_2 (30 mL) at –70°C. After warming to r.t., the solvent was evaporated, and the residue was distilled to give the product as colourless liquid.

Yield: 3.41 g (74 %).

B.p.: 75°C at 20mbar.

^{1}H-NMR ($CDCl_3$): δ 4.58 (t, 2H, CH_2-O), 1.91 (m, 2H, C<u>H</u>$_2$-CH_3), 1.09 (t, 3H, CH_3).

^{13}C-NMR ($CDCl_3$): δ 119.1 (CF_3), 79.6 (CH_2-O), 23.1 (<u>C</u>H_2-CH_3), 9.8 (CH_3).

^{19}F-NMR ($CDCl_3$): δ –75.4 (CF_3).

*Selenouronium salts (**3**)*

1,3-Dicyclohexyl selenourea (**2a**) (575 mg, 2 mmol) was suspended in benzene (50 mL) and heated to 70°C, resulting in a grey solution. The appropriate trifluoromethanesulfonic acid alkyl ester (2.1 mmol) was added and the mixture further stirred for 1 h at room temperature. The solvent was evaporated and the crude product obtained was purified via gravity column chromatography. Byproducts and starting material were removed (hexane/diethyl ether 1:1) and the appropriate purified selenouronium salts were eluted from the column with acetone yielding grey solids after evaporation:

*(Biscyclohexylaminomethylene)methylselenouronium trifluoromethanesulfonate (**3a**)*
("methyl selenouronium salt")

Yield: 750 mg (83 %).

M.p.: 122 °C.

R_f: 0.1 – 0.2 (hexane/diethyl ether 2:3).

MS (ESI): m/e 303 (M-OTf, 100 %).

*(Biscyclohexylaminomethylene)ethylselenouronium trifluoromethanesulfonate (**3b**)*
("ethyl selenouronium salt")

Yield: 820 mg (88 %).

M.p.: 95 °C.

R_f: 0.1 – 0.2 (hexane/diethyl ether 2:3).

MS (ESI): m/e 317 (M-OTf, 100 %).

*(Biscyclohexylaminomethylene)propylselenouronium trifluoromethanesulfonate (**3c**)*
("propyl selenouronium salt")

Yield: 776 mg (81 %).

M.p.: 95 °C.

R_f: 0.1 – 0.2 (hexane/diethyl ether 2:3).

MS (ESI): m/e 331 (M-OTf, 100 %).

*Methylselenoethers (**5a, 5d**)*

An aqueous solution (1.5 M) of tetrabutylammonium hydroxide (0.53 mL, 0.8 mmol) was added to a mixture of **3a** (352 mg, 0.78 mmol) and selected alkylbromides/tosylates (0.8 mmol) in acetonitrile (30 mL). The mixture was stirred for 0.5 h at room temperature. The solvent was evaporated and the residue was purified via gravity column chromatography (hexane/diethyl ether 100:2) to give **5a** and **5d** as pale yellow oils after evaporation of the eluent:

*Benzylmethylselenide (**5a**) [177]*

Yield: 106 mg (73 %).

n_D^{20}: 1.5962 (lit.: 1.5969).

R_f: 0.41 (hexane/diethyl ether 100:3).

^{1}H-NMR ($CDCl_3$): δ 7.33 (m, 5H, Ar-H), 3.77 (s, 2H, CH_2-Ph), 1.95 (s, 3H, CH_3).

^{13}C-NMR ($CDCl_3$): δ 136.8 (C_q), 128.3 (=CH), 25.3 (CH_2), 6.3 (CH_3).

MS (EI): m/e 186 (M, 7 %), 169 (1), 91 (100).

*1-Phenyl-1-(methylseleno)ethane (**5d**) [92]*

Yield: 103 mg (66 %).

n_D^{20}: 1.5783.

R_f: 0.40 (hexane/diethyl ether 100:3).

^{1}H-NMR ($CDCl_3$): δ 7.29 (m, 5H, Ar-H), 4.16 (q, 1H, CH), 1.90 (s, 3H, Se-CH_3), 1.73 (d, 3H, CH-C$\underline{H}_3$).

^{13}C-NMR ($CDCl_3$): δ 144.7 (C_q), 128.0 (=CH), 38.2 (Ar-CH), 22.5 (CH-$\underline{C}H_3$), 4.9 (Se-CH_3).

MS (EI): m/e 200 (M, 5 %), 105 (100), 95 (24), 77 (82).

3-(Methylseleno)-1-propanol [following 60]

Selenium powder (450 mg, 5.7 mmol) was suspended in tetrahydrofuran (20 mL), and methyl lithium in diethyl ether (4.3 mL, 6.9 mmol) was added with stirring. The selenium dissolved to give a pale yellow solution. 3-Bromo-1-propanol (0.52 mL, 5.7 mmol) was added and the mixture stirred for 1 h. The solution was hydrolyzed with water (10 mL), the organic phase separated, washed with $NaHCO_3$ and NaCl solutions and dried (Na_2SO_4). The solvent was removed by distillation and the residue fractionated *in vacuo* to give the product as a pale yellow oil.

Yield: 0.4 g (45 %).

B.p.: 100 °C at 7 mmHg (lit.: 100 °C at 8 mmHg).

R_f: 0.40 (hexane/diethyl ether 1:1).

^{1}H-NMR ($CDCl_3$): δ 3.80 (t, 2H, C$\underline{H}_2$-OH), 2.70 (t, 2H, CH_2-Se), 2.05 (s, 3H, CH_3), 1.93 (m, 2H, CH_2-C$\underline{H}_2$-CH_2).

MS (EI): m/e 154 (M, <1 %), 93 (11), 57 (15), 28 (100).

*3-(Methylseleno)-1-propanyl p-toluenesulfonate (**5g**) [following 178]*

Toluene-4-sulfonyl chloride (285 mg, 1.5 mmol) in acetonitrile (1 mL) was added to a stirred solution of 3-(methylseleno)-1-propanol (153 mg, 1 mmol), triethylamine (0.3 mL, 2 mmol) and trimethylamine hydrochloride (80 mg, 0.1 mmol) in acetonitrile (1 mL) at 0°C, and the mixture was stirred for 1 h. Water was added to the mixture, which was extracted with ethyl acetate. The organic phase was washed with brine, dried (Na_2SO_4) and concentrated. The obtained crude product was purified by gravity column chromatography (hexane/diethyl ether 1:1) to give **5g** [109] as a pale yellow oil after evaporation of the eluent.

Yield: 295 mg (96 %).

n_D^{20}: 1.5506.

R_f: 0.60 (hexane/diethyl ether 1:1).

^{1}H-NMR ($CDCl_3$): δ 7.75 (m, 2H, Ar-H), 7.35 (m, 2H, Ar-H), 4.15 (t, 2H, CH_2-OTos), 2.53 (t, 2H, Se-CH_2), 2.40 (s, 3H, Ph-CH_3), 1.95 (m, 2H, CH_2-C<u>H</u>$_2$-CH_2), 1.90 (s, 3H, Se-CH_3).

MS (EI): m/e 308 (M, 38 %), 213 (8), 155 (2), 95 (21), 91 (100).

*Ethylselenoethers (**5b**, **5e**)*

Ethylselenoethers **5b** and **5e** were prepared from **3b** (365 mg, 0.78 mmol), appropriate alkylbromides (0.8 mmol) and an aqueous solution (1.5 M) of tetrabutylammonium hydroxide (0.53 mL, 0.8 mmol) in acetonitrile (30 mL) in the same way as described for methylselenoethers. Workup as described above afforded pale yellow oils:

*Benzylethylselenide (**5b**)*

Yield: 85 mg (50 %).

n_D^{20}: 1.5777.

R_f: 0.39 (hexane/diethyl ether 100:3).

^{1}H-NMR ($CDCl_3$): δ 7.30 (m, 5H, Ar-H), 3.84 (s, 2H, Ar-CH_2), 2.56 (q, 2H, Se-C<u>H</u>$_2$-CH_3), 1.41 (t, 3H, CH_3).

^{13}C-NMR ($CDCl_3$): δ 140.0 (C_q), 128.1 (=CH), 27.0 (Ar-CH_2), 17.8 (Se-<u>C</u>H$_2$-CH_3), 15.9 (CH_3).

MS (EI): m/e 200 (M, 30 %), 91 (100), 65 (30).

HR-MS calculated for $C_9H_{12}Se$: 200.010, found: 200.010.

*1-Phenyl-1-(ethylseleno)ethane (**5e**)*

Yield: 82 mg (49 %).

n_D^{20}: 1.5623.

R_f: 0.37 (hexane/diethyl ether 100:3).

^{1}H-NMR ($CDCl_3$): δ 7.31 (m, 5H, Ar-H), 4.25 (q, 1H, CH), 2.46 (q, 2H, CH_2), 1.77 (d, 3H, CH-C$\underline{H}_3$), 1.34 (t, 3H, CH_2-C$\underline{H}_3$).

^{13}C-NMR ($CDCl_3$): δ 145.1 (C_q), 128.0 (=CH), 37.1 (Ar-CH), 23.0 (CH-$\underline{C}H_3$), 18.4 (CH_2), 16.0 (CH_2-$\underline{C}H_3$).

MS (EI): m/e 214 (M, 22 %), 105 (100), 79 (18), 77 (24), 51 (27).

HR-MS calculated for $C_{10}H_{14}Se$: 214.026, found: 214.026.

*Propylselenoethers (**5c**, **5f**)*

Propylselenoethers (**5c** and **5f**) were prepared from **3c** (374 mg, 0.78 mmol), appropriate alkylbromides (0.8 mmol) and an aqueous solution (1.5 M) of tetrabutylammonium hydroxide (0.53 mL, 0.8 mmol) in acetonitrile (30 mL) in the same way as described for methylselenoethers. Workup as described above afforded pale yellow oils:

*Benzylpropylselenide (**5c**)*

Yield: 68 mg (41 %).

n_D^{20}: 1.5639.

R_f: 0.45 (hexane/diethyl ether 100:3).

^{1}H-NMR ($CDCl_3$): δ 7.29 (m, 5H, Ar-H), 3.81 (s, 2H, Ar-CH_2), 2.52 (t, 2H, Se-C$\underline{H}_2$-CH_2), 1.69 (m, 2H, C$\underline{H}_2$-CH_3), 1.00 (t, 3H, CH_3).

^{13}C-NMR ($CDCl_3$): δ 140.1 (C_q), 129.1 (=CH), 27.3 ($\underline{C}H_2$-CH_3), 26.7 (Ar-CH_2), 24.0 (Se-$\underline{C}H_2$-CH_2), 15.0 (CH_3).

MS (EI): m/e 214 (M, 27 %), 91 (100), 65 (25).

HR-MS calculated for $C_{10}H_{14}Se$: 214.026, found: 214.026.

*1-Phenyl-1-(propylseleno)ethane (**5f**)*

Yield: 76 mg (43 %).

n_D^{20}: 1.5532.

R_f: 0.39 (hexane/diethyl ether 100:3).

^{1}H-NMR ($CDCl_3$): δ 7.31 (m, 5H, Ar-H), 4.21 (q, 1H, CH), 2.44 (t, 2H, Se-CH_2), 1.77 (d, 3H, CH-CH_3), 1.58 (m, 2H, CH_2-CH_3), 0.96 (t, 3H, CH_3).

^{13}C-NMR ($CDCl_3$): δ 145.1 (C_q), 128.0 (=CH), 37.4 (Ar-CH), 27.1 (CH_2-CH_3), 24.1 (Se-CH_2), 23.1 (CH-CH_3), 15.1 (CH_2-CH_3).

MS (EI): m/e 228 (M, 17 %), 105 (100), 79 (14), 77 (20), 51 (17).

HR-MS calculated for $C_{11}H_{16}Se$: 228.041, found: 228.042.

*Ethyl N-tert.-butoxycarbonyl-2-amino-4-bromobutyrate (**8**) [from 109]*

L-α-Amino-γ-butyrolactone hydrobromide (3.5 g, 22.5 mmol) was dissolved in a solution of hydrobromic acid (5.7 M) in acetic acid (40 mL) and heated at 100°C for 6h in an autoclave. The mixture was cooled overnight, the precipitate was filtered off and washed with diethyl ether to give 2-amino-4-bromobutyric acid hydrobromide as colourless crystals, which were used without further purification.

2-Amino-4-bromobutyric acid hydrobromide (3.3 g, 12.6 mmol) was dissolved in ethanol (50 mL), and gaseous HCl was introduced at 0°C for 2h. The solvent was evaporated to give ethyl 2-amino-4-bromobutyrate as a brown oil, which was used without further purification.

A solution of ethyl 2-amino-4-bromobutyrate, di-tert.-butyl dicarbonate (2.75 g, 12.6 mmol) and $NaHCO_3$ (1.05 g, 12.6 mmol) in ethanol (50 mL) was treated with ultrasound for 2 h. The solvent was evaporated, and the residue was purified via gravity column chromatography (hexane/ethyl acetate 7:3) to give ethyl N-tert.-butoxycarbonyl-2-amino-4-bromobutyrate as colourless crystals after evaporation of the eluent.

Yield: 2.8 g (40 % related to L-α-amino-γ-butyrolactone hydrobromide).

M.p.: 65°C (lit.: 69.5°C).

R_f: 0.78 (hexane/diethyl ether 2:3).

IR: ν_{max} (KBr): 3375 cm^{-1} (NH), 1749 cm^{-1} (CO), 1682 cm^{-1} (CO).

^{1}H-NMR ($CDCl_3$): δ 5.15 (d, 1H, NH), 4.45 (q, 1H, CH), 4.20 (q, 2H, O-CH_2), 3.50 (t, 2H, Br-CH_2), 2.30 (m, 2H, CH_2-CH_2-CH), 1.50 (s, 3H, CH_3), 1.35 (t, 3H, CH_2-CH_3).

^{13}C-NMR ($CDCl_3$): δ 172.2 (C(O)O), 156.7 (C(O)N), 80.9 ($C(CH_3)_3$), 62.2 (O-CH_2), 54.9 (C_{tert}), 28.7 (Br-CH_2), 28.7 ($C(CH_3)_3$), 27.8 (CH_2-CH_2-C_{tert}), 14.6 (CH_2-CH_3).

MS (EI): m/e 311 (M, <1 %), 236 (22), 210 (10), 182 (11), 136 (18), 57 (100).

*Homocysteine selenolactone hydroiodide (**6**) [from 179]*

Selenomethionine (100 mg, 0.51 mmol) was added to hydrogen iodide (5 mL, 57 %) and the mixture was refluxed at 140 °C for 4 h. The resulting solution was extracted several times with diethyl ether, the aqueous phase was diluted with water and then dried by lyophilization. The product was dissolved in hot ethanol and then precipitated by addition of three volumes of diethyl ether. The final product **6** was recovered by centrifugation and was further washed with diethyl ether.

Yield: 94 mg (63 %).

R_f: 0.68 (n-butanol/ethanol/formic acid 5:1:1).

^{1}H-NMR (d_6-DMSO): δ 4.38 (t, 1H, CH), 2.34 (t, 2H, CH_2-Se), 1.65 (m, 2H, CH_2-C<u>H$_2$</u>-CH).

MS (ESI): m/e 164 (M-HI, 100 %).

4.2.2 Syntheses via alkyl selenocyanates

*Alkyl selenocyanates (**10**) [following 180]*

Potassium selenocyanate (**9a**) (1.44 g, 10 mmol) and an appropriate alkylbromide (10 mmol) were heated under reflux in ethanol (50 mL) for 2 h. The solvent was evaporated and the residue was purified via flash chromatography (hexane/diethyl ether 1:4) to give **10** after evaporation of the eluent:

*Benzyl selenocyanate (crystalline) (**10a**) [181]*

Yield: 1.45 g (74 %).

M.p.: 71.7 °C (lit.: 72 °C).

R_f: 0.66 (hexane/diethyl ether 2:3).

IR: ν_{max} (KBr): 2140 cm^{-1} (Se-C≡N).

^{1}H-NMR ($CDCl_3$): δ 7.38 (m, 5H, Ar-H), 4.35 (s, 2H, CH_2).

^{13}C-NMR ($CDCl_3$): δ 135.8 (C_q), 129.4 (=CH), 102.5 (Se-C≡N), 33.2 (CH_2).

MS (EI): m/e 197 (M, 2 %), 91 (100), 65 (76).

*1-Phenylethyl-1-selenocyanate (yellow oil) (**10b**) [182]*

Yield: 1.66 g (79 %).

n_D^{20}: 1.5833.

R_f: 0.77 (hexane/diethyl ether 2:3).

IR: ν_{max} (KBr): 2145 cm^{-1} (Se-C≡N).

^{1}H-NMR ($CDCl_3$): δ 7.43 (m, 5H, Ar-H), 4.96 (q, 1H, Ar-CH), 2.10 (d, 3H, CH_3).

^{13}C-NMR ($CDCl_3$): δ 139.9 (C_q), 128.6 (=CH), 103.1 (Se-C≡N), 48.8 (CH), 23.3 (CH_3).

MS (EI): m/e 211 (M, 1 %), 105 (100), 77 (44).

*Methyl selenocyanate (**10c**)* was obtained by reaction of **9a** (0.014 g, 0.1 mmol) and trifluoromethanesulfonic acid methyl ester (11 µL, 0.1 mmol) in refluxing ethanol (10 mL) within 30 min. It was not separated due to its instability.

R_f: 0.91 (hexane/diethyl ether 2:3).

*Alkylselenoethers (**5a**, **5d**, **5h**) [following 183]*

Alkylselenoethers (**5a**, **5d** and **5h**) were prepared from **10** (1 mmol) and the appropriate alkyl lithium compound (1 mmol) in anhydrous diethyl ether (20 mL) by stirring this mixture for 10 min at r.t. The solvent was evaporated, and the residue was purified via flash chromatography (hexane/diethyl ether 100:2) to give **5a**, **5d** and **5h** as pale yellow oils after evaporation of the eluent:

*Benzylmethylselenide (**5a**) [177]*

Yield: 154 mg (83 %).

Analytical data see above.

*1-Phenyl-1-(methylseleno)ethane (**5d**) [92]*

Yield: 172 mg (86 %).

Analytical data see above.

*1-Phenyl-1-(butylseleno)ethane (**5h**)*

Yield: 193 mg (80 %).

n_D^{20}: 1.5361.

R_f: 0.47 (hexane/diethyl ether 100:3).

^{1}H-NMR ($CDCl_3$): δ 7.31 (m, 5H, Ar-H), 4.21 (q, 1H, Ar-CH), 2.44 (t, 2H, Se-C<u>H</u>$_2$-CH$_2$), 1.76 (d, 3H, CH-C<u>H</u>$_3$), 1.60 (m, 2H, CH$_2$-C<u>H</u>$_2$-CH$_2$), 1.36 (m, 2H, C<u>H</u>$_2$-CH$_3$), 0.89 (t, 3H, CH$_2$-C<u>H</u>$_3$).

^{13}C-NMR ($CDCl_3$): δ 145.1 (C_q), 128.0 (=CH), 37.4 (Ar-CH), 32.9 (Se-CH$_2$), 24.7 (CH$_2$-<u>C</u>H$_2$-CH$_2$), 23.6 (CH-<u>C</u>H$_3$), 23.1 (<u>C</u>H$_2$-CH$_3$), 14.0 (CH$_2$-<u>C</u>H$_3$).

MS (EI): m/e 242 (M, 10 %), 105 (100), 77 (18).

HR-MS calculated for $C_{12}H_{18}Se$: 242.057, found: 242.057.

*Arylselenoethers (**5i**-**k**) [following 183 and 184]*

Arylselenoethers (**5i**-**k**) were prepared from **10** (1 mmol) and the appropriate aryl lithium or Grignard compound (1 mmol) in anhydrous diethyl ether (20 mL) in the same way as described for alkylselenoethers. Workup as described above afforded pale yellow oils:

*Benzylphenylselenide (**5k**) [185]*

Yield: 220 mg (89 %).

n_D^{20}: 1.6277.

R_f: 0.42 (hexane/diethyl ether 100:3).

^{1}H-NMR ($CDCl_3$): δ 7.39 (m, 10H, Ar-H), 4.19 (s, 2H, CH$_2$).

^{13}C-NMR ($CDCl_3$): δ 144.9 (C_q), 136.1 (C_q), 130.7 (=CH), 32.7 (CH$_2$).

MS (EI): m/e 248 (M, 12 %), 157 (3), 91 (100), 65 (16).

*1-Phenyl-1-(phenylseleno)ethane (**5i**) [186]*

Yield: 209 mg (80 %).

n_D^{20}: 1.6183.

R_f: 0.50 (hexane/diethyl ether 100:3).

^{1}H-NMR ($CDCl_3$): δ 7.44 (m, 10H, Ar-H), 4.57 (q, 1H, Ar-CH), 1.86 (d, 3H, CH$_3$).

^{13}C-NMR ($CDCl_3$): δ 144.1 (C_q), 136.0 (C_q), 128.9 (=CH), 42.7 (Ar-CH), 22.7 (CH_3).

MS (EI): m/e 262 (M, 5 %), 157 (5), 105 (100), 77 (21).

*1-Phenyl-1-(4-fluorophenylseleno)ethane (**5j**)*

Yield: 244 mg (87 %).

n_D^{20}: 1.5988.

R_f: 0.42 (hexane/diethyl ether 100:3).

^{1}H-NMR ($CDCl_3$): δ 7.18 (m, 9H, Ar-H), 4.45 (q, 1H, Ar-CH), 1.79 (d, 3H, CH_3).

^{13}C-NMR ($CDCl_3$): δ 160.9 (C-F), 143.8 (C_q), 138.4 (C_q), 122.4 (=CH), 43.3 (Ar-CH), 22.3 (CH_3).

^{19}F-NMR ($CDCl_3$): δ -113.9.

MS (EI): m/e 280 (M, 13 %), 175 (3), 105 (100), 77 (83).

HR-MS calculated for $C_{14}H_{13}FSe$: 280.017, found: 280.016.

*Phenylmethylselenide (**5l**) [following 187]*

A mixture of diphenyl diselenide (0.4 g, 1.3 mmol) and sodium borohydride (0.1 g, 2.7 mmol) in EtOH (50 mL) was stirred at r.t. for 10 min. After adding of methyl iodide (0.17 mL, 2.7 mmol), the solution was refluxed for 30 min. The solvent was evaporated and the residue was purified via flash chromatography (hexane/diethyl ether 100:2) to give phenylmethylselenide [188] as a pale yellow oil after evaporation of the eluent.

Yield: 164 mg (74 %).

n_D^{20}: 1.602.

R_f: 0.46 (hexane/diethyl ether 100:2).

^{1}H-NMR ($CDCl_3$): δ 7.38 (m, 5H, Ar-H), 2.40 (s, 3H, CH_3).

^{13}C-NMR ($CDCl_3$): δ 129.4 (=CH), 7.7 (CH_3).

MS (EI): m/e 172 (M, 100 %), 157 (95).

4.2.3 Synthesis of 5'-(methylseleno)-N^6-cyclopentyladenosine

*N^6-Cyclopentyladenosine (**21**) [from 171]*

A mixture of 6-chloropurine ribonucleoside (5.7 g, 20 mmol), cyclopentylamine (11.8 mL, 120 mmol) and EtOH (100 mL) was refluxed for 15 h, and it was evaporated *in vacuo* to dryness. The residue was triturated with a small amount of EtOH, and colourless crystals of N^6-cyclopentyladenosine were obtained.

Yield: 4.56 g (68 %) (lit.: 75 %).

M.p.: 78.8 °C (lit.: 77 – 81 °C).

R_f: 0.68 (CH_2Cl_2/MeOH 1:4).

^{1}H-NMR (d_6-DMSO): δ 8.36 (s, 1H, H8), 8.21 (s, 1H, H2), 7.80 (d, 1H, N^6H), 5.91 (d, 1H, H1'), 5.47 (m, 2H, OH-2'/OH-3'), 5.21 (m, 1H, OH-5'), 4.62 (m, 1H, H2'), 4.56 (bs, 1H, HCN^6), 4.17 (m, 1H, H3'), 3.99 (m, 1H, H4'), 3.67 (m, 2H, H5'), 1.78 (m, 8H, $H_{cyclopentyl}$).

^{13}C-NMR (d_6-DMSO): δ 155.3 (C4/C6), 153.1 (C2), 144.9 (C8), 140.4 (C5), 88.8 (C1'), 86.8 (C4'), 74.3 (C2'), 71.5 (C3'), 62.6 (C5'), 56.9 ($C1_{cyclopentyl}$), 33.0 ($C2/5_{cyclopentyl}$), 24.3 ($C3/4_{cyclopentyl}$).

MS (ESI): m/e 336 (M+1, 100 %).

*2',3'-Isopropylidenedioxy-N^6-cyclopentyladenosine (**22**) [from 172]*

N^6-cyclopentyladenosine (**21**) (4.36 g, 13 mmol) dissolved in acetone (50 mL) was added dropwise to a stirred mixture of acetone (50 mL) and concentrated sulfuric acid (1.3 mL, 26 mmol) at 0 °C. After complete addition, the mixture was stirred at r.t. for 90 min. The solution was cooled again to 0 °C and was neutralized with $NaOH_{aq}$ (20 mL, 20 %). The mixture was concentrated and partitioned between H_2O and CH_2Cl_2. The aqueous layer was extracted several times with CH_2Cl_2. The combined organic layers were dried (Na_2SO_4), and the solvent was evaporated. The residue was purified via flash chromatography (CH_2Cl_2/MeOH 100:2) to give 2',3'-isopropylidenedioxy-N^6-cyclopentyladenosine as colourless crystals after evaporation of the eluent.

Yield: 3.79 g (78 %).

M.p.: 96 °C.

R_f: 0.35 (CH_2Cl_2/MeOH 25:1).

^{1}H-NMR (d_6-DMSO): δ 8.36 (s, 1H, H8), 8.23 (s, 1H, H2), 7.81 (d, 1H, N^6H), 6.15 (d, 1H, H1'), 5.32 (m, 2H, H2'/OH-5'), 4.99 (m, 1H, H3'), 4.56 (bs, 1H, HCN^6), 4.24 (m, 1H, H4'), 3.57 (m, 2H, H5'), 1.77 (m+s, 11H, $H_{cyclopentyl}$/$CH_{3,\ isopropylidene}$), 1.34 (s, 3H, $CH_{3,\ isopropylidene}$).

^{13}C-NMR (d_6-DMSO): δ 155.8 (C4/C6), 153.4 (C2), 144.9 (C8), 140.2 (C5), 113.9 ($C_{q,\ isopropylidene}$), 90.6 (C1'), 87.3 (C4'), 84.1 (C2'), 82.3 (C3'), 62.5 (C5'), 57.0 ($C1_{cyclopentyl}$), 33.0 ($C2/5_{cyclopentyl}$), 27.9 ($CH_{3,\ isopropylidene}$), 26.0 ($CH_{3,\ isopropylidene}$), 24.3 ($C3/4_{cyclopentyl}$).

MS (ESI): m/e 375 (M, 100 %), 204 (35).

*5'-Bromo-2',3'-isopropylidenedioxy-N^6-cyclopentyladenosine (**23**) [from 173]*

Triphenylphosphine (2.36 g, 9 mmol) in dry CH_2Cl_2 (20 mL) was added dropwise to a stirred solution of **22** (3.38 g, 9 mmol) and CBr_4 (5 g, 15 mmol) in dry CH_2Cl_2 (20 mL) at r.t. After 15 h the solvent was evaporated and the residue was purified via flash chromatography (CH_2Cl_2/MeOH 100:2) to give 5'-bromo-2',3'-isopropylidenedioxy-N^6-cyclopentyladenosine as an oil after evaporation of the eluent.

Yield: 2.09 g (53 %).

R_f: 0.81 (CH_2Cl_2/MeOH 25:1).

^{1}H-NMR (d_6-DMSO): δ 8.38 (s, 1H, H8), 8.28 (s, 1H, H2), 7.75 (d, 1H, N^6H), 6.22 (d, 1H, H1'), 5.51 (m, 1H, H2'), 5.02 (m, 1H, H3'), 4.58 (bs, 1H, HCN^6), 4.48 (m, 1H, H4'), 3.68 (m, 2H, H5'), 1.75 (m+s, 11H, $H_{cyclopentyl}$/$CH_{3,\ isopropylidene}$), 1.24 (s, 3H, $CH_{3,\ isopropylidene}$).

^{13}C-NMR (d_6-DMSO): δ 156.8 (C4/C6), 154.6 (C2), 145.3 (C8), 140.1 (C5), 113.3 ($C_{q,\ isopropylidene}$), 91.7 (C1'), 86.2 (C4'), 85.0 (C2'), 80.8 (C3'), 57.8 ($C1_{cyclopentyl}$), 32.6 ($C2/5_{cyclopentyl}$), 32.5 (C5'), 26.7 ($CH_{3,\ isopropylidene}$), 25.2 ($CH_{3,\ isopropylidene}$), 24.5 ($C3/4_{cyclopentyl}$).

MS (ESI): m/e 440 (M, 100 %).

*5'-(Methylseleno)-2',3'-isopropylidenedioxy-N^6-cyclopentyladenosine (**24**) [following 174]*

5'-Bromo-2',3'-isopropylidenedioxy-N^6-cyclopentyladenosine (**23**) (1.75 g, 4 mmol) dissolved in EtOH (20 mL) was added dropwise to a stirring mixture of dimethyl diselenide (1.07 mL, 8 mmol) and sodium borohydride (2 g) in EtOH (20 mL). After complete addition, the mixture was stirred at r.t. for 30 min. The solution was concentrated and partitioned between H_2O and CH_2Cl_2. The aqueous layer was extracted several times with CH_2Cl_2. The combined organic layers were dried (Na_2SO_4), and the solvent was evaporated. The residue was purified via flash

chromatography (CH_2Cl_2/MeOH 100:2) to give 5'-(methylseleno)-2',3'-isopropylidenedioxy-N^6-cyclopentyladenosine as oil after evaporation of the eluent.

Yield: 1.5 g (83 %).

R_f: 0.35 (CH_2Cl_2/MeOH 25:1).

^{1}H-NMR (d_6-DMSO): δ 8.35 (s, 1H, H8), 8.25 (s, 1H, H2), 7.76 (d, 1H, N^6H), 6.20 (d, 1H, H1'), 5.54 (m, 1H, H2'), 5.05 (m, 1H, H3'), 4.56 (bs, 1H, HCN^6), 4.33 (m, 1H, H4'), 2.80 (m, 2H, H5'), 1.78 (m+2s, 14H, $H_{cyclopentyl}$/$SeCH_3$/$CH_{3,\ isopropylidene}$), 1.35 (s, 3H, $CH_{3,\ isopropylidene}$).

^{13}C-NMR (d_6-DMSO): δ 155.6 (C4/C6), 153.5 (C2), 144.9 (C8), 140.5 (C5), 114.1 ($C_{q,\ isopropylidene}$), 90.1 (C1'), 87.2 (C4'), 84.7 (C2'), 84.2 (C3'), 58.1 ($C1_{cyclopentyl}$), 33.0 ($C2/5_{cyclopentyl}$), 27.8 (C5'), 27.5 ($CH_{3,\ isopropylidene}$), 26.0 ($CH_{3,\ isopropylidene}$), 24.3 ($C3/4_{cyclopentyl}$), 5.0 ($SeCH_3$).

MS (ESI): m/e 454 (M, 100 %), 360 (97).

*5'-(Methylseleno)-N^6-cyclopentyladenosine (**19**) [from 175]*

A solution of **24** (1.36 g, 3 mmol) in acetic acid (60 mL, 65 %) was refluxed for 4 h. The solvent was evaporated and the residue was purified via flash chromatography (CH_2Cl_2/MeOH 100:2) to give 5'-(methylseleno)-N^6-cyclopentyladenosine [169] as a colourless solid. The product recrystallized from EtOH.

Yield: 1.08 g (87 %).

M.p.: 71.3°C (lit.: 66– 72°C).

R_f: 0.22 (CH_2Cl_2/MeOH 25:1).

^{1}H-NMR (d_6-DMSO): δ 8.37 (s, 1H, H8), 8.24 (s, 1H, H2), 7.69 (d, 1H, N^6H), 5.94 (d, 1H, H1'), 5.54 (m, 1H, OH-2'), 5.42 (m, 1H, OH-3'), 4.80 (m, 1H, H2'), 4.62 (bs, 1H, HCN^6), 4.20 (m, 2H, H3'/H4'), 2.91 (m, 2H, H5'), 1.77 (m+s, 11H, $H_{cyclopentyl}$/$SeCH_3$).

^{13}C-NMR (d_6-DMSO): δ 155.2 (C4/C6), 153.4 (C2), 145.0 (C8), 140.3 (C5), 88.2 (C1'), 85.1 (C4'), 74.1 (C2'), 73.7 (C3'), 56.9 ($C1_{cyclopentyl}$), 33.1 ($C2/5_{cyclopentyl}$), 28.2 (C5'), 24.3 ($C3/4_{cyclopentyl}$), 5.4 ($SeCH_3$).

MS (ESI): m/e 414 (M+1, 100 %).

4.3 Radiosyntheses

4.3.1 Production of n.c.a. radioselenium [from 78, 88, 189]

For routine production of n.c.a. [^{73,75}Se]selenium the following established procedure was used. A Cu_3As-layer (0.75 mm thick, As content: 28 %) on a watercooled Cu-backing was irradiated with 20 MeV protons at the compact cyclotron CV-28 of the Forschungszentrum Jülich GmbH. ^{75}Se was produced via the ^{75}As(p,n) process in batch yields of about 37 MBq within 4 h at a nominal beam current of 20 µA.

The n.c.a. selenium-73 was produced through the ^{75}As(p,3n) process on a solid high-current Cu_3As-target using 45 MeV protons in the internal beam of the high energy isochronous cyclotron (COSY Injector) of the Forschungszentrum Jülich GmbH.

4.3.2 Preparation of elemental n.c.a. selenium-75 [from 87, 88, 110]

The following preparations are methods already established and can be performed with Se-73 without any alteration.

Thermochromatography

The chemical processing started two days (one hour in case of ^{73}Se) after irradiation in order to allow the decay of short-lived activities. A two-step thermochromatographic method using O_2 as the purging gas was performed to separate radioselenium from the Cu_3As-target (cf. Figure 4.1).

The irradiated Cu_3As-target was placed in a quartz tube (15 mm diameter) located in an oven. In the first step, thermochromatography was done in an O_2 stream (85 mL/min) at 660°C for 40 min whereby arsenic was removed from the target and the As_2O_3 formed was deposited about 7 cm away from the end of the oven. Thereafter, the quartz tube was shifted back till the As_2O_3 zone was in the middle of the oven. The temperature of the oven was then raised to 750°C for about 5min whereby As_2O_3 was shifted further by 12 cm. The quartz tube was now moved to its original position and the second stage of thermochromatography started. The temperatures used gradually were: 900°C (7min), 1000°C (15min) and 1100°C (10min). A stepwise increase in temperature was mandatory to avoid silicate formation which would lead to a decreased volatility of radioselenium. The radioselenium was deposited as $^{75}SeO_2$ about 11 cm away from the end of the oven. At the end of the thermochromatographic process the

quartz tube was cooled to r.t. and rinsed with warm hydrochloric acid (6 mL, 6 M) whereby >98 % of the radioselenium was dissolved. The radionuclidic purity of the separated ^{75}Se was >99 %, and >95 % of the radioactivity existed as [^{75}Se]selenite as demonstrated via γ-ray spectrometry and radio-TLC, respectively.

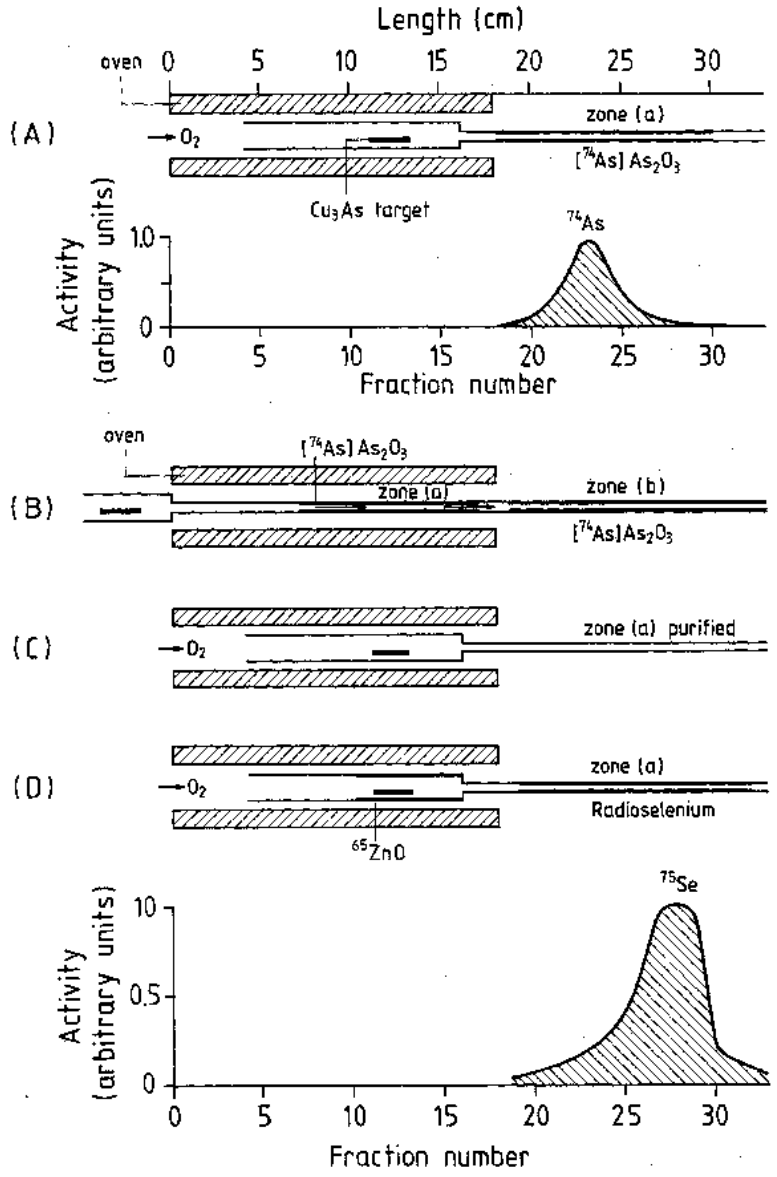


Fig. 4.1: Thermochromatographic procedure for separation of ^{75}Se from the target [from 88]

(A) Removal of [^{74}As]As_2O_3 at 660 °C to zone (a).

(B) Shift of zone (a) to the middle of the oven; transfer of [^{74}As]As_2O_3 at 750 °C to zone (b), thereby purification of zone (a).

(C) Shift of the quartz tube to original position.

(D) Removal of ^{75}Se at 900-1100 °C to zone (a).

Reduction of [^{75}Se]selenite to elemental selenium-75

Since elemental selenium-75 was used as starting material for the radiosyntheses developed, the thermochromatographically separated n.c.a. [^{75}Se]SeO_3^{2-} was reduced to $^{75}Se^0$ in dilute hydrochloric acid by bubbling SO_2 for 5 min. The solution was then heated to 85 °C for 30 min. The subsequent extraction of elemental selenium-75 into benzene was performed using the apparatus shown in Figure 4.2.

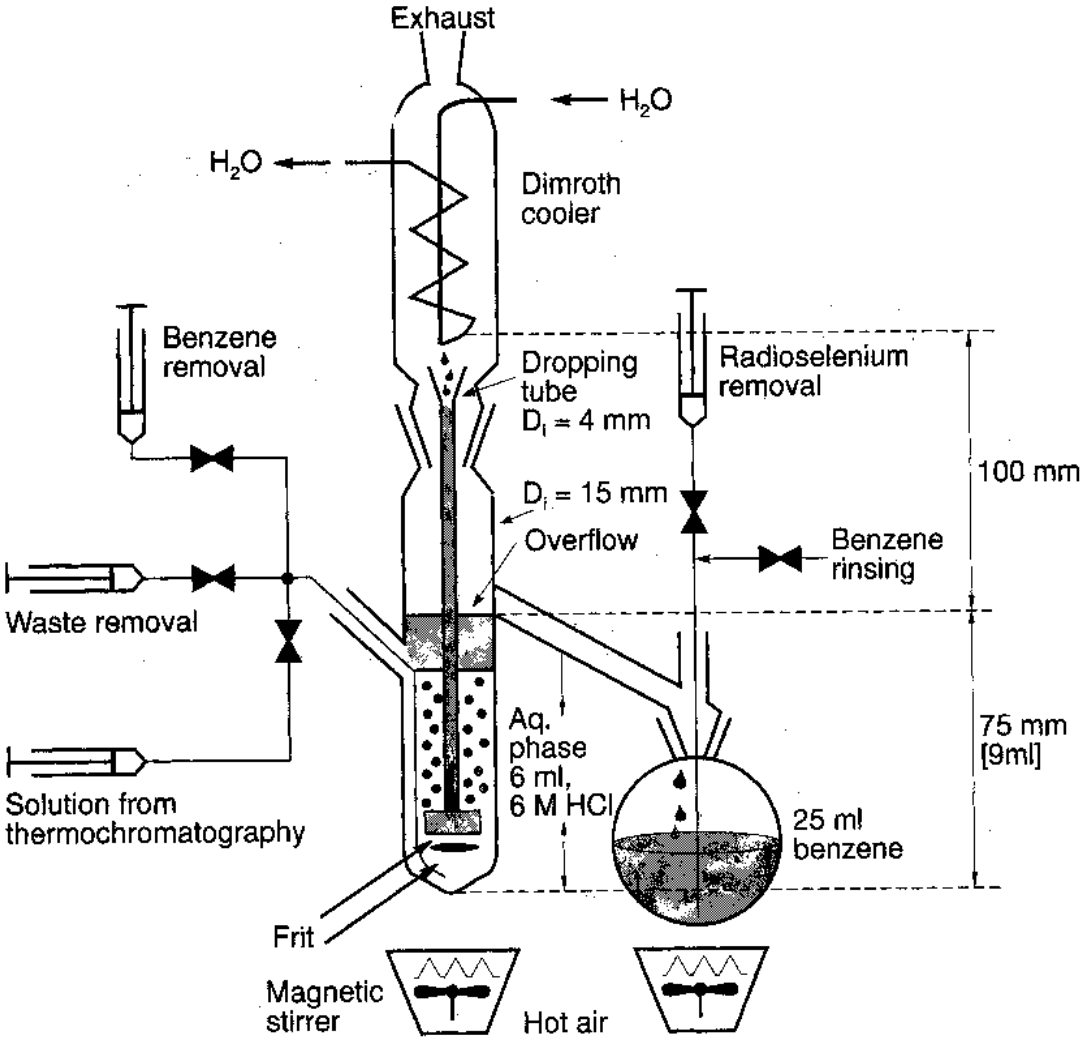


Fig. 4.2: Apparatus for extraction of radioselenium in benzene [from 88]

It consisted of a round bottom distillation flask connected to an extractor in the form of a perforator. The benzene was distilled, condensed in the Dimroth cooler and fell through a funnel into the dropping tube having a frit at the end. While stirring, the reduced radioselenium solution was injected into the extractor. With a benzene dropping rate of 5 mL/min, the elemental ^{75}Se

was extracted quantitatively in the organic phase within 30 min. Thereafter, the aqueous phase was separated via the waste removal part and the radioselenium concentrated in benzene.

Alternatively, n.c.a. [^{75}Se]selenite was reduced to n.c.a. $^{75}Se^0$ with sodium thiosulfate. An aqueous solution of $Na_2S_2O_3$ (2 mL, 0.5 mol/L) was added to n.c.a. [^{75}Se]SeO_3^{2-} in dilute hydrochloric acid. The precipitate was centrifuged for 15 min. The water phase was decanted and the sulfur matrix washed three times with THF. The dry sulfur matrix containing the elemental radioselenium was then dissolved in CS_2 (2 mL).

All experiments with n.c.a. elemental selenium-73,75 mentioned below were conducted under argon in conical 5 mL reaction vessels equipped with a magnetic stirring bar and a teflon rubber septum.

4.3.3 Radiosyntheses via disubstituted n.c.a. [^{73,75}Se]selenoureas

*Cyclohexyl iso[^{75}Se]selenocyanate ([^{75}Se]**1a**)*

Studies on the formation of n.c.a. cyclohexyl iso[^{75}Se]selenocyanate ([^{75}Se]**1a**) were performed using the reaction of n.c.a. [^{75}Se]Se^0 (370 kBq, 10 µCi) and various amounts of cyclohexyl isocyanide in selected solvents (0.5 mL) at various temperatures. Aliquots were analyzed for [^{75}Se]**1a** by radio-HPLC and radio-TLC under conditions given below.

*1,3-Disubstituted [^{73,75}Se]selenoureas ([^{73,75}Se]**2**)*

The preparation of n.c.a. 1,3-dicyclohexyl [^{73,75}Se]selenourea ([^{73,75}Se]**2a**) was performed at 80 °C within 90 min after adding cyclohexyl isocyanide (10 µL, 0.08 mmol) and cyclohexylamine (10 µL, 0.09 mmol) to a solution of n.c.a. [^{73,75}Se]Se^0 in benzene (0.5 mL, typically containing 370 kBq (10 µCi)). Aliquots were analyzed for [^{73,75}Se]**2a** by radio-HPLC and radio-TLC under conditions given below. The solution obtained was used in the following reaction step without any purification.

Alternatively, aminomethylated polystyrene (40 mg) was used instead of cyclohexylamine for polymer-supported formation of N-cyclohexyl [^{73,75}Se]selenourea-N'-methyl polystyrene ([^{73,75}Se]$\mathbf{2_{resin}}$), which was purified by washing with benzene (2 mL) before use in the following reaction step.

*[^{73,75}Se]Selenouronium salts ([^{73,75}Se]**3**)*

The [^{73,75}Se]selenouronium salts ([^{73,75}Se]**3**) were prepared in benzene (0.5 mL) by reaction of [^{73,75}Se]**2a** with the appropriate trifluoromethanesulfonic acid alkyl ester (0.1 mmol) at r.t. within 1 min. The alkylation reactions were monitored by radio-TLC. The surplus of triflate was removed by silica gel column chromatography (10 x 1 cm) using diethyl ether, and the purified [^{73,75}Se]selenouronium salt was eluted from the column with acetone.

Using [^{73,75}Se]$\mathbf{2}_{\mathbf{resin}}$ the alkylation step was performed as described above within 5 min. Purification of [^{73,75}Se]$\mathbf{3}_{\mathbf{resin}}$ was carried out by washing the resin with benzene (2 mL) and tetrahydrofuran (2 mL).

*[^{73,75}Se]Selenoethers ([^{73,75}Se]**5a**-**g**)*

The asymmetric [^{73,75}Se]selenoethers ([^{73,75}Se]**5a**-**g**) were prepared by treatment of [^{73,75}Se]**3a**-**c** or [^{73,75}Se]$\mathbf{3}_{\mathbf{resin}}$ with tetrabutylammonium hydroxide (0.06 mmol) in the presence of the selected bromine- or tosylate-compounds (0.1 mmol) in 0.6 mL tetrahydrofuran at 70 °C within 5 min. The [^{73,75}Se]selenoethers ([^{73,75}Se]**5a**-**g**) obtained were analyzed by radio-HPLC and radio-TLC.

*Homocysteine [^{75}Se]selenolactone ([^{75}Se]**6**)*

C.a. 1,3-dicyclohexyl [^{75}Se]selenourea ([^{75}Se]**2a**) was synthesized by reaction of c.a. $^{75}Se^{0}$ (containing 2 mg (0.025 mmol) $^{nat}Se^{0}$), cyclohexyl isocyanide (10 µL, 0.08 mmol) and cyclohexylamine (10 µL, 0.09 mmol) in acetonitrile (0.5 mL) and used without further purification. The c.a. [^{75}Se]selenouronium salt ([^{75}Se]**3d**) was prepared in acetonitrile (0.5 mL) by reaction of c.a. [^{75}Se]**2a** with **8** (31 mg, 0.1 mmol) at 80 °C within 40 min. The alkylation reaction was monitored by radio-TLC. Surplus of **8** and byproducts were removed by silica gel column chromatography as described above.

Hydrolysis of purified [^{75}Se]**3d** with TBAH (0.1 mmol) in methanol (2 mL) was done within 5 min at 70 °C. After removal of the solvent, concentrated hydrochloric acid (5 mL) was added to the residue and heated for 30 min at 100 °C. The solvent was removed, the residue was suspended in ethanol and filtered over a Sep-Pak® Plus C18 cartridge. Homocysteine [^{75}Se]selenolactone was analyzed on radio-TLC as detailed below.

4.3.4 Radiosyntheses via alkyl [^{75}Se]selenocyanates

*Alkyl [^{75}Se]selenocyanates ([^{75}Se]**10**)*

The preparation of n.c.a. alkyl [^{75}Se]selenocyanates ([^{75}Se]**10**) was performed at 80 °C within 5 min after adding sodium cyanide (10 µmol in 0.1 mL H_2O) and the appropriate alkylating agent (73 µmol) to a solution of n.c.a. [^{75}Se]Se^0 in ethanol (0.5 mL, typically containing 370 kBq (10 µCi)). Aliquots were analyzed for [^{75}Se]**10** by radio-HPLC and radio-TLC.

Purification of [^{75}Se]**10** was carried out using reversed phase cartridges: 10 mL of water were added to the reaction mixture and then passed through an appropriate reversed phase cartridge prewashed with 3 mL of ethanol followed by 10 mL of water. The products adsorbed on the solid phase were eluted with 2 mL of anhydrous diethyl ether and passed through a Na_2SO_4-cartridge.

*Alkyl and aryl [^{75}Se]selenoethers ([^{75}Se]**5a**, **5d**, **5h**-**l**)*

The asymmetric alkyl- and aryl[^{75}Se]selenoethers were prepared within 1 min by treatment of purified [^{75}Se]**10** with a selected lithium or Grignard compound (0.1 mmol) in 0.5 mL anhydrous diethyl ether at room temperature. The [^{75}Se]selenoethers obtained were analyzed by radio-HPLC and radio-TLC.

4.3.5 Radiosynthesis of n.c.a. 5'-(methyl[^{75}Se]seleno)-N^6-cyclopentyladenosine

The labelled adenosine-derivative was prepared as described above for [^{75}Se]selenoethers, starting with alkylation of n.c.a. 1,3-dicyclohexyl [^{75}Se]selenourea (and N-cyclohexyl [^{75}Se]selenourea-N'-methyl polystyrene, alternatively) with trifluoromethanesulfonic acid methyl ester (11 µL, 0.1 mmol) in benzene (0.5 mL). The resulting [^{75}Se]selenouronium salt was purified according to the method described above and hydrolyzed with tetrabutylammonium hydroxide (0.06 mmol) in 0.6 mL tetrahydrofuran in the presence of 5'-bromo-2',3'-isopropylidenedioxy-N^6-cyclopentyladenosine (**23**) (44 mg, 0.1 mmol) resulting in 5'-(methyl[^{75}Se]seleno)-2',3'-isopropylidenedioxy-N^6-cyclopentyladenosine ([^{75}Se]**24**). After hydrolysis using hydrochloric acid (0.3 mL, 1 N) at 80 °C, 5'-(methyl[^{75}Se]seleno)-N^6-cyclopentyladenosine ([^{75}Se]**19**) was obtained and analyzed by radio-HPLC and radio-TLC as detailed below.

4.4 Radioanalytical methods

4.4.1 Radio high performance liquid chromatography

Analytical radio high performance liquid chromatography (radio-HPLC) was performed on a system consisting of a Knauer pump 6400 and a Knauer UV/vis photometer 3060 with a detector wavelength of 220 nm. Sample injection was accomplished by a Rheodyne-Injector block 7125. For measurement of radioactivity the outlet of the UV detector was connected to a NaI(Tl) well-type scintillation detector and the recorded data were processed by the software system Raytest Ramona MCS (Nuclear Interface, Germany).

Tab. 4.1: K'-values of seleno compounds on a Lichrosorb RP Select B column, used as reference substances for co-chromatographic identification

Compound	k'
Selenium (ionic)	0.23[1)]
Cyclohexyl isoselenocyanate (**1a**)	6.44[1)]
1,3-Dicyclohexyl selenourea (**2a**)	4.36[1)]
Benzylmethylselenide (**5a**)	3.73[2)]
1-Phenyl-1-(methylseleno)ethane (**5d**)	4.35[2)]
3-(Methylseleno)-1-propanyl p-toluenesulfonate (**5g**)	3.01[2)]
Benzylethylselenide (**5b**)	2.61[2)]
1-Phenyl-1-(ethylseleno)ethane (**5e**)	3.46[2)]
Benzylpropylselenide (**5c**)	3.80[2)]
1-Phenyl-1-(propylseleno)ethane (**5f**)	5.00[2)]
Benzyl selenocyanate (**10a**)	3.23[1)]
1-Phenylethyl-1-selenocyanate (**10b**)	5.15[1)]
1-Phenyl-1-(butylseleno)ethane (**5h**)	11.14[2)]
Benzylphenylselenide (**5k**)	6.13[2)]
1-Phenyl-1-(phenylseleno)ethane (**5i**)	7.32[2)]
1-Phenyl-1-(4-fluorophenylseleno)ethane (**5j**)	5.97[2)]
Phenylmethylselenide (**5l**)	1.71[2)]
5'-(Methylseleno)-2',3'-isopropylidenedioxy-N^6-cyclopentyladenosine (**24**)	4.22[3)]
5'-(Methylseleno)-N^6-cyclopentyladenosine (**19**)	2.07[4)]

[1)] CH_3CN/H_2O 50:50. [2)] CH_3CN/H_2O 60:40. [3)] MeOH/H_2O 70:30. [4)] MeOH/H_2O 60:40.

HPLC of aliquots of labelled products and standards was performed using a Lichrosorb RP Select B (250 x 4 mm) column (CS-Chromatographie Service GmbH, Germany) and a mobile phase consisting of acetonitrile/H_2O and MeOH/H_2O mixtures, respectively, in various concentration ratios (given as V:V ratios) at a flow rate of 1.0 mL/min. The typical amount of ^{75}Se radioactivity was 37 kBq (1 µCi). All analyses were performed at ambient temperature. Individual k'-values of standards and elution solvents are given in Table 4.1.

4.4.2 Radio thin layer chromatography

Analytical radio thin layer chromatography (radio-TLC) was carried out in order to verify that no radioactive species other than those eluted and detected in the HPLC scans were present. This would include colloidal material or species strongly retained and not eluted from the HPLC column under the conditions employed. Radio-TLC was performed on Merck silica gel plates (Type F_{254S}) with the solvent system hexane/diethyl ether and CH_2Cl_2/MeOH, respectively, in various concentrations (given as V:V ratios). The developed TL-chromatograms were measured for radioactivity on an Instant Imager™ (Packard, USA). Individual R_f-values of standards and solvent systems are given in Table 4.2.

Tab. 4.2: R_f-values of seleno compounds on Merck silica gel plates, used as reference substances for co-chromatographic identification

Compound	R_f
Selenium (elemental, n.c.a.)	0.95[1)]
Selenium (ionic)	0.00[1)]
Cyclohexyl isoselenocyanate (**1a**)	0.35[2)]
1,3-Dicyclohexyl selenourea (**2a**)	0.52[1)]
Selenouronium salts (**3**)	0.1 – 0.2[1)]
Benzylmethylselenide (**5a**)	0.41[2)]
1-Phenyl-1-(methylseleno)ethane (**5d**)	0.40[2)]
3-(Methylseleno)-1-propanyl p-toluenesulfonate (**5g**)	0.60[3)]
Benzylethylselenide (**5b**)	0.39[2)]
1-Phenyl-1-(ethylseleno)ethane (**5e**)	0.37[2)]
Benzylpropylselenide (**5c**)	0.45[2)]

Table 4.2 continued

1-Phenyl-1-(propylseleno)ethane (**5f**)	0.39[2)]
Homocysteine selenolactone (**6**)	0.68[4)]
Potassium selenocyanate (**9a**)	0.00[1)]
Benzyl selenocyanate (**10a**)	0.66[1)]
1-Phenylethyl-1-selenocyanate (**10b**)	0.77[1)]
Methyl selenocyanate (**10c**)	0.91[1)]
1-Phenyl-1-(butylseleno)ethane (**5h**)	0.47[2)]
Benzylphenylselenide (**5k**)	0.42[2)]
1-Phenyl-1-(phenylseleno)ethane (**5i**)	0.50[2)]
1-Phenyl-1-(4-fluorophenylseleno)ethane (**5j**)	0.42[2)]
Phenylmethylselenide (**5l**)	0.46[5)]
5'-(Methylseleno)-2',3'-isopropylidenedioxy-N^6-cyclopentyladenosine (**24**)	0.35[6)]
5'-(Methylseleno)-N^6-cyclopentyladenosine (**19**)	0.22[6)]

[1)] Hexane/diethyl ether 2:3. [2)] Hexane/diethyl ether 100:3. [3)] Hexane/diethyl ether 1:1.
4) n-Butanol/ethanol/formic acid 5:1:1. [5)] Hexane/diethyl ether 100:2. [6)] CH_2Cl_2/MeOH 25:1.

4.4.3 Determination of the specific activity of n.c.a. 5'-(methyl[^{75}Se]seleno)-N^6-cyclopentyladenosine

The analysis of the specific activity of n.c.a. 5'-(methyl[^{75}Se]seleno)-N^6-cyclopentyladenosine ([^{75}Se]**19**) was carried out via HPLC. The applied amount of non-radioactive standard compound is proportional to the integral of the UV-signal obtained by HPLC. The measured peak-area was fitted as a function of amount of **19**, resulting a straight line that was used for calibration (Figure 4.3).

For the determination of the specific activity the solvent of a complete reaction batch containing [^{75}Se]**19** was evaporated, the residue was solved in 100 µL HPLC eluent and injected on HPLC. [^{75}Se]**19** was collected and its activity was ascertained (300 kBq (8 µCi)). Since no corresponding UV signal was detected, only a lower limit of the specific activity A_s of > 300 GBq/mmol (> 8 Ci/mmol) could be determined in consideration of the limit of detection of 1 nmol.

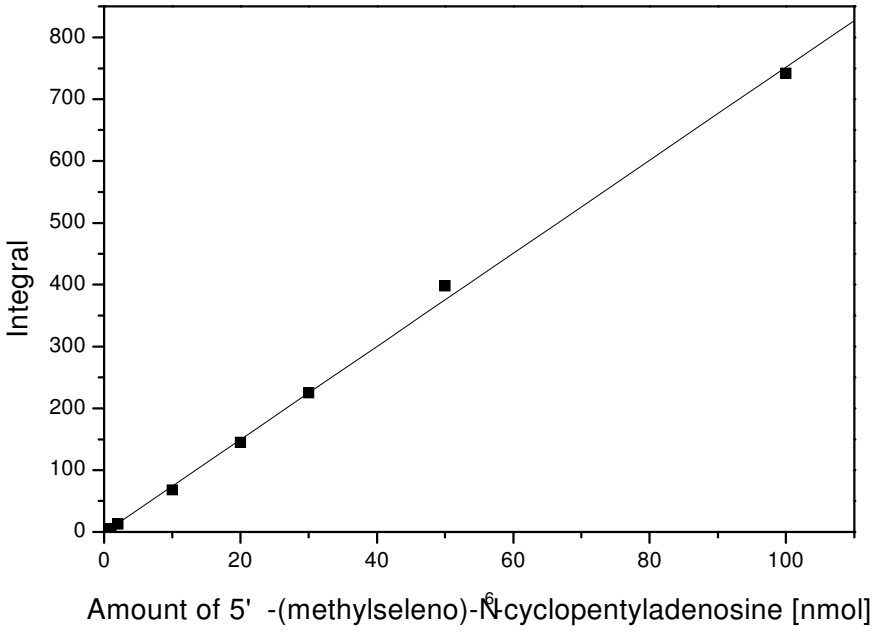


Fig. 4.3: Dependence of the integral as a function of amount of 5'-(methylseleno)-N^6-cyclopentyladenosine ([^{75}Se]**19**)

4.5 Radioligand binding assays

Radioligand binding assays were carried out by the Pharmacology Group of the Institute of Nuclear Chemistry. *Corpora striata* (for A_{2A}AR assays) and frontal *cortices* (for A_1AR assays) were dissected from pig brain and the tissue was homogenized for one minute in 20 volumes of ice-cold 50 mM TRIS-HCl buffer, pH 7.4 containing 10 mM $MgCl_2$, Soybean Trypsin Inhibitor (20 µg/mL), Bacitracin (200 µg/mL), and Benzamidine HCl (160 µg/mL) by means of an Ultra Turrax at 20,000 rpm. The homogenate was centrifuged at 48,000 × *g* for 10 min at 4°C (Beckmann Optima L, SW41Ti rotor). The pellet was suspended in 20 volumes of TRIS-HCl, pH 7.4, containing adenosine deaminase (2 U/mL) and Trypsin Inhibitor (20 µg/mL), then was incubated for 30 min at 37°C. After centrifugation at 48,000× g for 10 min at 4°C the resulting pellet was diluted in 20 volumes of 50 mM TRIS-HCl, pH 7.4, containing 10 mM $MgCl_2$. Aliquots of the homogenate (1 mL) were stored at – 80°C. The assays were performed in triplicate by incubating aliquots of the membrane fractions (87 µg protein/assay for A_1-assays, 68 µg protein/assay for A_{2A}-assys) in TRIS-HCl, pH 7.4, containing adenosine deaminase (2 U/mL).

Cortical homogenates were used for A_1AR- and striatal homogenates for A_{2A}AR-assays. Incubation was carried out at 20°C for 60 min in a total assay volume of 200µL. In A_{2A}AR competition experiments [^{3}H]CGS21680 (A_{2A} receptor radioligand, K_D = 26 nM) was used in a concentration of 5 nM. In A_1AR competition experiments [^{3}H]CPFPX (A_1 receptor radioligand, K_D = 0.62 nM) was used in a concentration of 2 nM. Centrifugation at 48,000 × *g* for 6 min at 8°C separated bound from free ligand. Supernatants were discarded, the pellets washed with 1 mL ice cold buffer and dissolved by incubating in Solvable™ (500 µL, Canberra-Packard) for 120 min at 50°C. Aliquots of 450µL were placed in scintillation vials with scintillation cocktail (10 mL, Ultima Gold XR, Canberra-Packard). Radioactivity was measured in a Liquid Scintillation Analyzer. Protein estimation was performed with a commercial assay (Bio-Rad DC Protein Assay) after solubilization in 15 % NH_4OH containing 2 % SDS (v,w); human serum albumin served as a standard. K_i was calculated by a computer-assisted curve-fitting program (GraphPad Prism, version 3.0) as exemplified in Figure 4.4 for the adenosine A_1 receptor.

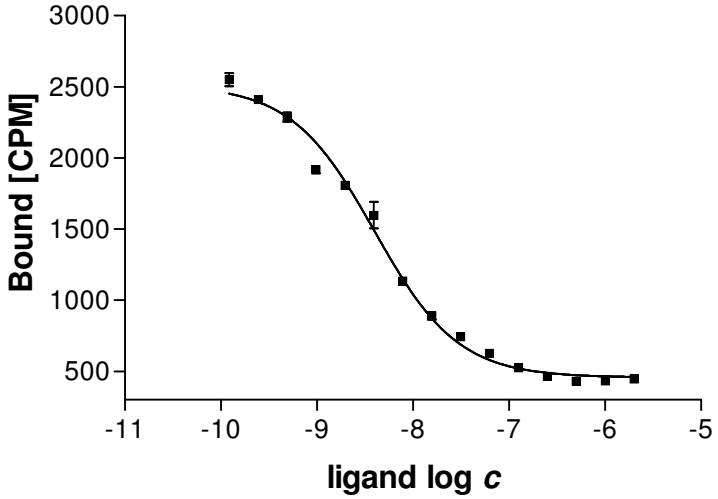


Fig. 4.4: Determination of the K_i value of 5'-(methylseleno)-N^6-cyclopentyladenosine (**19**) for the adenosine A_1 receptor

Chapter 5

Summary

With the increasing use of positron emission tomography as a method of *in vivo* molecular imaging, there has been great interest in the development of methods suitable for the synthesis of compounds labelled with positron-emitting radionuclides, as these can be used as tracers for physiological and pharmacological phenomena. In addition to the commonly used labels carbon-11 and fluorine-18, selenium-73 is an alternative positron emitter with suitable characteristics for tomographic imaging, and its half-life of 7.1 h permits imaging over an extended period of many hours.

For the preparation of ^{73}Se-labelled radiopharmaceuticals, it must be taken into account that selenium compounds may be toxic or may disturb the physiological system to be studied. Both of these drawbacks could be circumvented if no-carrier-added compounds are available, so that pharmacodynamic effects or even toxic events are excluded. This implicit necessity for preparation methods that can avoid the addition of substantial amounts of ^{nat}Se-carrier, however, places constraints on chemical approaches used to prepare selenium-73 labelled compounds. In fact, most of the techniques developed during the last 40 years to introduce radioselenium into an appropriate organic compound work only with the addition of ^{nat}Se-carrier. Very few methods for the preparation of n.c.a. radioselenium-labelled compounds have been investigated thus far and their limitation to quite a small class of selenium-containing compounds is a major disadvantage.

In this thesis work, the scope of available asymmetric n.c.a. radioselenoethers was extensively enlarged by development of two new n.c.a. radioselenation methods. For convenience and costs, these labelling routes were elaborated and optimized using the longer-lived isotope selenium-75 ($T_{1/2}$ = 120.4 d).

The first radiosynthetic concept examined was based on the intermediate formation of a disubstituted [^{75}Se]selenourea derivative. The initial key step, i.e. the introduction of n.c.a. selenium-75 into an organic compound, was performed via nucleophilic attack of cyclohexyl isocyanide on n.c.a. $^{75}Se^{0}$ yielding cyclohexyl iso[^{75}Se]selenocyanate as model compound. Subsequent addition of cyclohexylamine resulted in the formation of 1,3-dicyclohexyl [^{75}Se]selenourea. Optimization studies showed that this intermediate was conveniently produced in a direct one-pot procedure starting from n.c.a. $^{75}Se^{0}$, cyclohexyl isocyanide and cyclohexylamine within 90 min with a radiochemical yield of 95 ± 5 %.

The outstanding chemical property of selenourea derivatives with respect to the reactivity of the selenium atom enables these compounds to act as versatile intermediates for the preparation of asymmetric selenoethers. Due to a reduced C=Se double-bond character the selenium atom is polarized with a negative charge, and alkylation of 1,3-dicyclohexyl [^{75}Se]selenourea via alkyl triflates gave rise to the formation of corresponding (biscyclohexylaminomethylene)alkyl[^{75}Se]selenouronium trifluoromethanesulfonates (alkyl [^{75}Se]selenouronium salts) in nearly quantitative yield. In order to separate the alkyl [^{75}Se]selenouronium salts from surplus alkyl triflates and byproducts, a small scale silica gel column chromatography was developed.

Hydrolysis of a purified alkyl [^{75}Se]selenouronium salt by treatment with hydroxide yielded the corresponding alkyl [^{75}Se]selenolate, which could be alkylated *in situ* with various alkyl halides and tosylates yielding asymmetric n.c.a. [^{75}Se]selenoethers analogous to the Williamson ether synthesis. Studies showed that this reaction sequence was most effective using a one-pot procedure with simultaneous addition of base and alkylating agent to the alkyl [^{75}Se]selenouronium salt. Thus, starting from elemental selenium-75 a wide variety of asymmetric n.c.a. dialkyl [^{75}Se]selenoethers were synthesized as model compounds in radiochemical yields of 15 to 48 %, depending on the substituents, within a total reaction time of 130 min.

This labelling technique is extremely time-consuming due to the tedious purification step required. An improved approach was therefore developed using a polymer-supported pathway. A big advantage of this strategy is that, after each step of the procedure, the polymer-supported radioselenium-labelled intermediates can be purified by washing the resin with an appropriate solvent whereby the impurities are eluted out of the resin. Since the labelled intermediate compounds were covalently attached to the resin, none of them was lost in the purification step, leading to high radiochemical yields of purified selenourea and selenouronium salts, respectively, within a very short time.

Starting from n.c.a. elemental selenium-75, cyclohexyl isocyanide and aminomethylated polystyrene, the corresponding polymer-bound [^{75}Se]selenourea was synthesized within 10 min with a radiochemical yield of 80 ± 5 % (related to n.c.a. $^{75}Se^{0}$). Subsequent alkylation via alkyl triflates yielded the corresponding alkyl [^{75}Se]selenouronium salts. After their purification and basic hydrolysis, the *in situ* alkylation of alkyl [^{75}Se]selenolate yielded the same model compounds as produced via the non-polymer-supported strategy in radiochemical yields of 13 to 56 % (related to $^{75}Se^{0}$) within a total reaction time of 35 min. Besides similar radiochemical yields, these were attained in only a quarter of the reaction time necessary for the free selenourea method. Furthermore, the polymer-supported labelling route lends itself much more to automation, which is desirable in particular for future routine application.

Comparative n.c.a. labelling experiments with the positron emitter selenium-73 showed no differences in radiochemical yields of the same model compounds. Thus, ^{73}Se-labelled compounds at the n.c.a. level can be obtained using both, the free and polymer-supported selenourea method without modifying the reaction conditions.

However, as a major drawback, attempts made to synthesize aryl radioselenoethers via this labelling technique failed.

Therefore, another reaction concept was explored in order to extend the range of available n.c.a. radioselenium-labelled compounds to aryl selenoethers. This radiosynthetic pathway was based on the formation of alkyl [^{75}Se]selenocyanates as intermediates. The reaction of n.c.a. $^{75}Se^{0}$ and sodium cyanide to sodium [^{75}Se]selenocyanate and subsequent alkylation with an alkyl bromide to form the corresponding alkyl [^{75}Se]selenocyanate were tested and optimized by use of (1-bromoethyl)benzene as model alkylating agent yielding 1-phenylethyl-1-[^{75}Se]selenocyanate. These studies showed *inter alia* that this alkyl [^{75}Se]selenocyanate was conveniently produced in a one-pot procedure using an ethanol/water mixture as solvent with a ratio of about 5/1. Obviously, both the cyanide and the [^{75}Se]selenocyanate anions need a certain degree of water to reach their highest possible reactivity. Under these optimized reaction conditions, several alkyl [^{75}Se]selenocyanates were produced in radiochemical yields of up to 73 % (related to $^{75}Se^{0}$) within 5 min. The disappointingly low yield of benzyl [^{75}Se]selenocyanate (15 ± 4 %) can probably be explained by an isomerization of this compound to its corresponding iso[^{75}Se]selenocyanate.

After separation of the n.c.a. alkyl [^{75}Se]selenocyanates from the ethanolic/aqueous solvent mixture via a reversed phase cartridge, these intermediates were converted to the desired n.c.a. [^{75}Se]selenoethers by reacting them with organometallic compounds. Since both, aliphatic and aromatic lithium or Grignard compounds can be used in this last reaction step, the n.c.a. preparation of a wide variety of asymmetric dialkyl and alkylaryl [^{75}Se]selenoethers was

possible as demonstrated for various model compounds, which were synthesized in radiochemical yields of 9 to 55 % (related to $^{75}Se^{0}$), depending on the substituents within a total reaction time of only 20 min.

This is the first method described to obtain n.c.a. aryl radioselenoethers and offers the advantage of a very rapid and efficient labelling technique.

Both the radiosyntheses developed in this work have proven to be valuable methods for the preparation of a broad range of symmetric and asymmetric radioselenoethers at the no-carrier-added level. These new approaches to n.c.a. radioselenium labelling enlarge the availability of ^{73,75}Se-labelled compounds, whereas previous radioselenation methods at the n.c.a. level were limited to methyl radioselenoethers.

For proof of identity via radioanalysis appropriate radio high performance liquid chromatography and radio thin layer chromatography methods were developed. Several non-radioactive seleno compounds, which served as reference substances, were prepared for the first time.

In order to demonstrate the versatility of the selenourea method in particular, two more complex, physiologically relevant molecules were labelled with selenium-75. Firstly, L-homocysteine [^{75}Se]selenolactone, an artificial amino acid derivative for potential measurement of adenosine production in ischemic myocardial tissue, was synthesized with a total radiochemical yield of about 20 – 30 % (related to $^{75}Se^{0}$). Since the appropriate triflate compound to alkylate the [^{75}Se]selenourea derivative could not be obtained, however, this alkylation step had to be performed with c.a. 1,3-dicyclohexyl [^{75}Se]selenourea in order to allow the use of the corresponding bromine derivative of the precursor. Thus, the indispensable addition of ^{nat}Se-carrier limits the maximum possible specific activity of L-homocysteine [^{75}Se]selenolactone. However, this is not a problem in case of this metabolic tracer.

Secondly, the receptor ligand 5'-(methyl[^{75}Se]seleno)-N^6-cyclopentyladenosine was synthesized at the n.c.a. level. This adenosine derivative is a high-affinity partial agonist for the adenosine A_1 receptor and could be applied as radioligand in PET studies if labelled with selenium-73. Using both, the free and the polymer-supported selenourea methods, the final n.c.a. product was obtained with a radiochemical yield of about 30 % (related to $^{75}Se^{0}$), within a total reaction time of 135 and 40 min, respectively and with a specific activity of more than 300 GBq/mmol (8 Ci/mmol). Provided no further isotopic dilution would occur in large scale syntheses, a much higher specific activity will be obtained. If selenium-73 is used as radiolabel, the achievable specific activity would be even higher in case of a large scale synthesis, and one can suggest that the obtainable specific activity would be sufficient to perform *in vivo* receptor studies with ^{73}Se-labelled ligands using PET.

Chapter 6

Zusammenfassung

Aufgrund der wachsenden Bedeutung der Positronen-Emissions-Tomographie auf dem Gebiet der molekularen *in vivo* Bildgebung besteht großes Interesse an der Entwicklung von Markierungstechniken. Hierbei sollen neue, mit Positronenstrahlern markierte Verbindungen zugänglich gemacht werden, die als Tracer für physiologische und pharmakologische Untersuchungen genutzt werden können. Neben den gebräuchlichsten Nukliden Kohlenstoff-11 und Fluor-18 bietet sich dabei das Selen-73 als alternativer Positronenemitter an, da es geeignete Charakteristika für die tomographische Bildgebung aufweist und seine Halbwertzeit von 7.1 h die Durchführung ausgedehnter nuklearmedizinischer Untersuchungen zulässt.

Für die Herstellung ^{73}Se-makierter Radiopharmaka muss die mögliche Toxizität von Se-Verbindungen und eventuelle Störungen der zu untersuchenden physiologischen Systeme in Betracht gezogen werden. Um diese Nachteile umgehen zu können, sind trägerarme (n.c.a.) Verbindungen erforderlich, die pharmakodynamische oder sogar toxische Effekte ausschließen würden. Dies erfordert Markierungstechniken, die keinen ^{nat}Se-Trägerzusatz benötigen, wodurch jedoch die Anzahl der möglichen Methoden zur Synthese von ^{73}Se-markierten Verbindungen stark eingeschränkt wird. So benötigen die meisten der in den letzten 40 Jahren entwickelten Techniken zur Radioselenierung organischer Verbindungen den Zusatz von ^{nat}Se-Träger. Nur sehr wenige Strategien zur Darstellung von trägerarmen Radioselenverbindungen sind entwickelt worden, die zudem auf die sehr kleine Klasse der Methylselenoether beschränkt sind.

Im Rahmen dieser Arbeit konnte die Bandbreite der verfügbaren asymmetrischen n.c.a. Radioselenoether stark erweitert werden, indem zwei neue n.c.a. Radioselenierungsmethoden entwickelt wurden. Aufgrund von praktischen Erwägungen und aus Kostengründen wurden

diese Syntheseverfahren mit dem langlebigeren Isotop Selen-75 ($T_{1/2}$ = 120.4 d) ausgearbeitet und optimiert.

Das erste umgesetzte Radiosynthesekonzept basierte auf der Bildung von disubstituierten [^{75}Se]Selenoharnstoffderivaten als Zwischenprodukte. Der entscheidende initiale Schritt war die Einführung des trägerarmen Selen-75 in eine organische Verbindung und wurde durch einen nukleophilen Angriff von Cyclohexylisonitril auf n.c.a $^{75}Se^0$ realisiert. Das resultierende Cyclohexyliso[^{75}Se]selenocyanat fungierte als Modellverbindung und wurde durch anschließende Reaktion mit Cyclohexylamin zu 1,3-Dicyclohexyl[^{75}Se]selenoharnstoff umgesetzt. Dieses Zwischenprodukt konnte nach Optimierungsstudien ausgehend von n.c.a. $^{75}Se^0$, Cyclohexylisonitril und Cyclohexylamin in einer Eintopf-Reaktion mit einer radiochemischen Ausbeute von 95 ± 5 % innerhalb 90 min hergestellt werden.

Die außerordentliche chemische Eigenschaft von Selenoharnstoffderivaten bezüglich der Reaktivität des Selens ermöglicht den Einsatz dieser Verbindungen als vielseitige Zwischenstufen für die Darstellung asymmetrischer Selenoether. Durch den verminderten C=Se-Doppelbindungscharakter wird das Selen negativ polarisiert, und die Alkylierung von 1,3-Dicyclohexyl-[^{75}Se]selenoharnstoff durch Alkyltriflate führt zu der Bildung von entsprechenden (Biscyclohexylaminomethylen)alkyl[^{75}Se]selenonium Trifluoromethansulfonaten (Alkyl[^{75}Se]selenoniumsalzen) in nahezu quantitativer Ausbeute. Zur Abtrennung der Alkyl[^{75}Se]selenoniumsalze von überschüssigem Alkyltriflat und Nebenprodukten wurde eine miniaturisierte Säulenchromatographie entwickelt.

Die Hydrolyse der aufgereinigten Alkyl[^{75}Se]selenoniumsalze durch Hydroxid-Anionen setzte entsprechende Alkyl[^{75}Se]selenolate frei, die *in situ* mit verschiedenen Alkylbromiden und -tosylaten alkyliert und so in Analogie zur Williamson-Ethersynthese zu asymmetrischen n.c.a. [^{75}Se]Selenoethern umgesetzt werden konnten. Diese Sequenz verlief am effektivsten in einer Eintopf-Reaktion mit gleichzeitiger Zugabe der Base und des Alkylierungsmittels zum Alkyl[^{75}Se]selenoniumsalz. Auf diese Weise konnten verschiedene asymmetrische n.c.a. Dialkyl[^{75}Se]selenoether dargestellt werden. Diese Modellverbindungen erhielt man ausgehend von $^{75}Se^0$ in radiochemischen Ausbeuten zwischen 15 und 48 % abhängig von den Alkylsubstituenten innerhalb von 130 min.

Da diese Markierungstechnik aufgrund des benötigten Reinigungsschrittes sehr zeitaufwendig ist, wurde eine verbesserte Methode entwickelt, welche auf eine festphasengebundene Strategie zurückgreift. Ein großer Vorteil dieses Konzeptes ist, dass nach jedem Reaktionsschritt die polymergebundenen, radioselenmarkierten Zwischenprodukte von Verunreinigungen befreit werden konnten, indem das Harz mit einem geeigneten Lösungsmittel gewaschen wurde. Da

diese Zwischenprodukte kovalent an den polymeren Träger gebunden waren und deshalb nicht vom Harz eluiert wurden, konnten entsprechend der oben beschriebenen Reaktionssequenzen innerhalb kürzester Zeit hohe Ausbeuten an aufgereinigtem Selenoharnstoff und daraus Selenoniumsalzen erzielt werden.

Ausgehend von n.c.a. $^{75}Se^{0}$, Cyclohexylisonitril und aminomethyliertem Polystyrol gelang die Darstellung des entsprechenden polymergebundenen [^{75}Se]Selenoharnstoffs innerhalb von 10 min mit einer radiochemischen Ausbeute von 80 ± 5 % (bezogen auf $^{75}Se^{0}$). Anschließende Umsetzung mit Alkyltriflaten ergab die entsprechenden Alkyl[^{75}Se]selenoniumsalze. Nach ihrer Aufreinigung und basischer Hydrolyse wurden durch *in situ* Alkylierungen der Alkyl[^{75}Se]selenolate die gleichen Modellverbindungen dargestellt wie mit Hilfe der nicht-polymergebundenen Reaktionsführung. Diese erhielt man in radiochemischen Ausbeuten zwischen 13 und 56 % (bezogen auf $^{75}Se^{0}$) innerhalb einer Gesamtsynthesedauer von 35 min. Neben vergleichbaren Ausbeuten konnte die Reaktionszeit gegenüber der Methode über Selenoharnstoffe in homogener Phase auf ein Viertel reduziert werden. Außerdem begünstigt die festphasenfixierte Markierungstechnik eine Automatisierung, was insbesondere für zukünftige Routineanwendungen wünschenswert ist.

Vergleichende Experimente mit dem Positronenstrahler Selen-73 ergaben keine Unterschiede in den radiochemischen Ausbeuten der gleichen Modellverbindungen. Damit wurde gezeigt, dass trägerarme ^{73}Se-markierte Verbindungen sowohl mit Hilfe der freien als auch der festphasengebundenen Selenoharnstoff-Methode synthetisiert werden können, ohne dass die bereits optimierten Reaktionsbedingungen modifiziert werden müssten.

Jedoch schlugen alle Versuche fehl, mit dieser Markierungsmethode Arylradioselenoether herzustellen.

Deshalb wurde ein weiteres Reaktionskonzept entwickelt, um den Bereich der verfügbaren n.c.a. radioselenmarkierten Verbindungen um die Klasse der Arylselenoether zu erweitern. Diese Radiosynthese basierte auf der Bildung von Alkyl[^{75}Se]selenocyanaten als Zwischenprodukte. Die Reaktion von n.c.a. $^{75}Se^{0}$ und Natriumcyanid zu Natrium[^{75}Se]selenocyanat und die anschließende Alkylierung mit einem Alkylbromid zum entsprechenden Alkyl[^{75}Se]selenocyanat wurden anhand von 1-Phenylethylbromid als Modellalkylierungsmittel für die Darstellung von 1-Phenylethyl-1-[^{75}Se]selenocyanat getestet und optimiert. Diese Untersuchungen zeigten unter anderem, dass dieses Alkyl[^{75}Se]selenocyanat am effektivsten in einer Eintopf-Reaktion mit einem Ethanol/Wasser-Gemisch im Verhältnis von 5/1 zugänglich war. Offensichtlich erreichten sowohl das Cyanid- als auch das [^{75}Se]Selenocyanat-Anion ihre höchste Reaktivität bei einem bestimmten Wasseranteil. Verschiedene Alkyl[^{75}Se]selenocyanate wurden unter den

optimierten Reaktionsbedingungen in radiochemischen Ausbeuten von bis zu 73 % (bezogen auf $^{75}Se^{0}$) innerhalb von 5 min dargestellt. Die dabei enttäuschend geringe Ausbeute von Benzyl[^{75}Se]selenocyanat (15 ± 4 %) kann möglicherweise mit der Isomerisierung dieser Verbindung zum entsprechenden Iso[^{75}Se]selenocyanat erklärt werden.

Nach der Abtrennung der n.c.a. Alkyl[^{75}Se]selenocyanate vom Ethanol/Wasser-Lösungsmittelgemisch mittels Festphasenfixierung wurden diese Intermediate durch Reaktion mit organometallischen Verbindungen zu den gewünschten n.c.a. [^{75}Se]Selenoethern umgesetzt. Da sowohl aliphatische als auch aromatische Lithium- oder Grignard-Verbindungen in diesem letzten Reaktionsschritt verwendet werden können, ist die Darstellung vieler unterschiedlicher asymmetrischer Dialkyl- und Alkylaryl[^{75}Se]selenoether möglich. Dies wurde anhand verschiedener Modellverbindungen demonstriert, die in radiochemischen Ausbeuten von 9 bis 55 % (bezogen auf $^{75}Se^{0}$) abhängig von den Substituenten innerhalb einer Gesamtsynthesedauer von nur 20 min dargestellt wurden.

Diese Markierungstechnik ist die erste beschriebene Methode, die trägerarme Arylselenoether zugänglich macht. Außerdem bietet sie den Vorteil einer schnellen und effizienten Radiosynthese.

Beide in dieser Arbeit entwickelten Synthesekonzepte eignen sich sehr gut zur Darstellung einer großen Auswahl von trägerarmen symmetrischen wie auch unsymmetrischen Radioselenoethern. Diese neuen n.c.a. Radioselenierungstechniken vergrößern den Bereich der zugänglichen ^{73,75}Se-markierten Verbindungen erheblich; wohingegen mit bereits etablierten Methoden die Synthesemöglichkeiten auf die Stoffklasse der n.c.a. Methylradioselenoether begrenzt waren.

Zur Identifizierung der Reaktionsprodukte mittels Radioanalytik wurden im Rahmen dieser Arbeit entsprechende Radio Hochleistungschromatographie- und Radio Dünnschichtchromatographie-Methoden entwickelt. Mehrere nicht-radioaktive Selenverbindungen wurden erstmalig synthetisiert.

Um die Vielseitigkeit insbesondere der Selenoharnstoff-Methode zu überprüfen, wurden zwei komplexere, physiologisch relevante Moleküle mit Selen-75 markiert. Das artifizielle Aminosäurederivat L-Homocystein[^{75}Se]selenolacton, das für die Messung der Adenosinfreisetzung in ischämischen Herzgewebe dienen soll, wurde mit einer radiochemischen Ausbeute von 20 – 30 % (bezogen auf $^{75}Se^{0}$) synthetisiert. Da die entsprechende Triflatverbindung zur Alkylierung des n.c.a. [^{75}Se]Selenoharnstoffderivates nicht zugänglich war, musste dieser Reaktionsschritt mit geträgertem 1,3-Dicyclohexyl[^{75}Se]selenoharnstoff und der entsprechenden Bromverbindung des Vorläufers durchgeführt werden. Der unentbehrliche ^{nat}Se-Trägerzusatz führt zu einer

Verminderung der maximal erreichbaren spezifischen Aktivität des L-Homocystein[^{75}Se]selenolactons, was sich aber für diesen metabolischen Tracer als nicht störend auswirken sollte.

Außerdem wurde der Rezeptorligand 5'-(Methyl[^{75}Se]seleno)-N^6-cyclopentyladenosin in trägerarmen Mengen synthetisiert. Dieses Adenosinderivat ist ein hoch-affiner partieller Agonist für den Adenosin A_1-Rezeptor und könnte mit Selen-73 markiert als Radioligand für PET-Studien dienen. Sowohl mittels der freien als auch der festphasengebundenen Selenoharnstoff-Methode konnte dieser n.c.a. Radioligand mit einer radiochemischen Ausbeute von rund 30 % (bezogen auf $^{75}Se^0$), innerhalb von 135 bzw. 40 min und mit einer spezifischen Aktivität von mindestens 300 GBq/mmol (8 Ci/mmol) hergestellt werden. Keine weitere Isotopen-Verdünnung bei Synthesen mit großen Aktivitätsmengen vorausgesetzt, kann eine viel höhere spezifische Aktivität erzielt werden. Bei der Verwendung von Selen-73 als Radionuklid würde die erreichbare spezifische Aktivität im Falle einer Synthese mit entsprechenden Aktivitätsmengen noch höher sein und höchstwahrscheinlich ausreichen, um PET-Untersuchungen an Rezeptorsystemen mit ^{73}Se-markierten Liganden *in vivo* durchführen zu können.

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Abbreviations

A_s	specific / molar activity
ATP	adenosine 5'-triphosphate
b	broad
b.p.	boiling point
Bq	Becquerel (decays per second)
c.a.	carrier-added
cAMP	adenosine 3',5'-cyclic monophosphate
cf.	confer
c.f.	carrier-free
Ci	Curie (1 Ci = 3.7×10^{10} Bq)
CNS	central nervous system
CPA	N^6-cyclopentyladenosine
CT	computed tomography
d	dublet
EC	electron capture
e.g.	for example
EI	electron ionization
Eq.	Equation
eq.	equivalent
ESI	electron spray ionization
EtOH	ethanol
FDG	2-[^{18}F]-2-deoxy-D-glucose
Fig.	Figure
GC	gas chromatography
HPLC	high performance liquid chromatography
HR-MS	high-resolution mass spectrum
i.e.	id est
IR	infrared
lit.	literature
m	muliplet
M	molecule ion
MeOH	methanol
MeOTf	methyl trifluoromethanesulfonate

m.p.	melting point
MRT	magnetic resonance tomography
MS	mass spectrum
n.c.a.	no-carrier-added
NMR	nuclear magnetic resonance
q	quartet
PET	positron emission tomography
ppm	parts per million
RCY	radiochemical yield
r.t.	room temperature
s	singulet
S_N	nucleophilic substitution
Sch.	Scheme
SPECT	single photon emission computed tomography
t	triplet
$T_{1/2}$	half-life
Tab.	Table
TBAH	tetrabutylammonium hydroxide
THF	tetrahydrofuran
TLC	thin layer chromatography
US	ultrasound
UV	ultraviolet
vol.	volume
vs.	versus

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Erklärung

Ich versichere, dass ich die von mir vorgelegte Dissertation selbständig angefertigt, die benutzten Quellen und Hilfsmittel vollständig angegeben und die Stellen der Arbeit einschließlich Tabellen, Karten und Abbildungen, die anderen Werken im Wortlaut oder dem Sinn nach entnommen sind, in jedem Einzelfall als Entlehnung kenntlich gemacht habe; dass diese Dissertation noch keiner anderen Fakultät oder Universität zur Prüfung vorgelegen hat; dass sie abgesehen von unten angegebenen Teilpublikationen noch nicht veröffentlicht worden ist sowie, dass ich eine solche Veröffentlichung vor Abschluss des Promotionsverfahrens nicht vornehmen werde.
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