Radiochim. Acta **89**, 863–866 (2001)

Alternative syntheses of [^{73,75}Se]selenoethers exemplified for homocysteine[^{73,75}Se]selenolactone

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Dedicated to Prof. Dr. Dr. h.c. S. M. Qaim on the occasion of his 60th birthday

(Received January 10, 2001; accepted in final form July 27, 2001)

Selenium-73,75 / *Homocysteine*[^{73,75}Se]*selenolactone* / [^{73,75}Se]*Selenomethionine* / [^{73,75}Se]*Selenoether* / *Radioselenation*

Summary. The present work describes two radiosynthetic pathways to prepare homocysteine[^{75}Se]selenolactone **1** starting from n.c.a. [^{75}Se]selenite **2**. It was achieved either by alkylation reaction of n.c.a. methyl[^{75}Se]selenide **4** or by hydrolysis of alkylated 1,3-dicyclohexyl[^{75}Se]selenourea **11**.

N.c.a. methyl[^{75}Se]selenide **4** is available using sulfur as non-isotopic carrier. However, the radiochemical yield of the substitution of 2-tert.-butoxycarbonylamino-4-bromobutyric acid ethylester **5** with n.c.a. methyl-[^{75}Se]selenide is only in the range of 15%–20%. Birch reduction of protected n.c.a. [^{75}Se]selenomethionine **6** formed leads to a RCY of 5%–10% homocysteine[^{75}Se]selenolactone **1**.

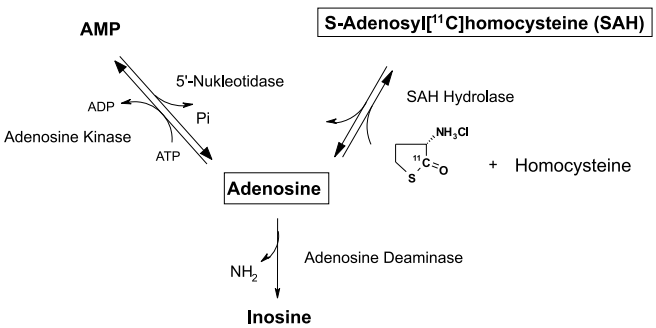
Alternatively, the synthesis of homocysteine[^{75}Se]selenolactone **1** is possible by hydrolysis of the corresponding [^{75}Se]selenouronium salt **11** available by addition of 2-tert.-butoxycarbonylamino-4-bromobutyric acid ethylester **5** to 1,3-dicyclohexyl[^{75}Se]selenourea **10**. A method was developed for the synthesis of 1,3-dicyclohexyl[^{75}Se]selenourea **10** by addition of c.a. [^{75}Se]SeH_2 to 1,3-dicyclohexylcarbodiimide, which leads to 20%–30% RCY of c.a. homocysteine[^{75}Se]selenolactone **1**.

Introduction

Due to its analogous chemical properties, selenium can be a suitable substitute for sulfur in thioamino acids, proteins and sulfur containing pharmaceuticals. The nuclear properties of selenium-73, with a half-life of 7 h and positron branching of 65% suggest that it is useful for *in vivo* medical application using positron emission tomography [1]. The half-life of ^{73}Se offers the possibility to label and study selenated radiotracers with relatively slow pharmacokinetics using PET. A disadvantage of selenium-73, however, is its longer-lived daughter arsenic-73 which adds to the radiation dose. Homocysteine[^{73}Se]selenolactone shall be used as a longer-lived alternative of [^{11}C]homocysteine thiolactone, which can only be obtained as racemic mixture due to the short half-life of carbon-11 [2]. [^{11}C]Homocysteine thiolactone was used as a sensitive indicator for ischemic myocardial tissue [3], 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 which leads to an increased formation of intracellular S-adenosyl homocysteine catalyzed by the enzyme SAH hydrolase (cf. Scheme 1). In order to image regional cardiac adenosine production by positron emission tomography, labelled homocysteine thiolactone is administered, which can readily penetrate cell membranes and take part in the conversion of adenosine into S-adenosyl-homocysteine. According to the chemical homology of sulfur and selenium, it is the aim to substitute sulfur by the positron emitter ^{73}Se to get a longer lived radiotracer, to test the possibility of enzymatic conversion of adenosine into [^{73}Se]selenoadenosine homocysteine for future radiopharmacological studies.

For convenience, all radiosyntheses described here were performed using the longer lived selenium-75 ($T_{1/2}$ = 120 d), which was generated *via* the ^{75}As(p, n)^{75}Se reaction, yielding n.c.a. [^{75}Se]selenite after thermochromatographic separation [4].

Since homocysteine thiolactone can easily be prepared by reductive demethylation of methionine [5], a method was developed to produce [^{75}Se]selenomethionine (2-amino-4-(methyl[^{75}Se]seleno)butyric acid), which can consecutively be converted into the corresponding [^{75}Se]seleno-



Scheme 1. Imaging of regional ischemia with 1-[^{11}C]homocysteine thiolactone [cf.3].

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lactone *via* demethylation. Labelling at the n.c.a. level was desired regarding the possible toxicity of selenium compounds. The first chemical synthesis of c.a. [^{75}Se]selenomethionine was developed by Plenevaux *et al*. [6]. Elemental radioselenium was transformed into methyl selenide by methyl lithium, which reacts with L-2-amino-4-bromobutyric acid to give c.a. L-[^{75}Se]selenomethionine. The radiochemical yield was about 40%, with a reaction time of 2 h. The major drawback was the limitation of specific activity due to the unavoidable addition of selenium carrier.

Alternatively, since Basmadijan *et al*. [7] suggested the formation of asymmetric c.a. [^{75}Se]selenoethers *via* basic hydrolysis of [^{75}Se]selenouronium salts, this synthetic pathway was modified here for the synthesis of n.c.a. homocysteine[^{75}Se]selenolactone.

Experimental

Materials and methods

All chemicals were purchased from Aldrich (Steinheim, Germany) and Fluka (Buchs, Switzerland) except 2-amino-4-bromobutyric acid hydrobromide, which was obtained from Acros Organics (Geel, Belgium). All chemicals were used without further purification. 2-tert.-Butoxycarbonylamino-4-bromobutyric acid ethylester **5** [8] and homo-cysteine selenolactone **1** [9] were synthesized according to literature methods. Their identity was confirmed by ^{1}H and ^{13}C NMR, IR and mass spectra. All preparations were conducted under an atmosphere of argon. Analytical 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 was processed by the software system Raytest Ramona MCS (Nuclear Interface, Münster, Germany). HPLC of aliquots of selenomethionine and [^{75}Se]selenomethionine for determination of the specific activity was performed using a Nucleosil 100-5 NH_2 (250 × 4 mm) column (CS-Chromatographie Service, Langerwehe, Germany) with a mobile phase of acetonitrile/0.04 M KH_2PO_4 (aq.) (77/23) at a flow rate of 1.0 mL/min. Radio-TLC was performed on Merck silica gel plates. The developed radiochromatograms were measured on an Instant Imager™(Packard).

Radiosyntheses

Production of [^{75}Se]selenite

[^{75}Se]Selenium was produced using 20 MeV protons at the compact cyclotron CV-28 at the Forschungszentrum Jülich GmbH *via* the $^{75}As(p, n)^{75}Se$ reaction on a Cu_3As-target. After thermochromatographic isolation and separation *via* described procedures [4], n.c.a. selenium-75 is available in its oxidized form as [^{75}Se]SeO_3^{2-} **2** in water.

Synthesis of homocysteine[^{75}Se]selenolactone 1 *via* methyl lithium

100 μL of a 5% hydrochloric acid and 100 μL of an aqueous solution of 30 mg (1.2 mmol) of sodium thiosulfate were added to a solution of about 10 μCi of n.c.a. [^{75}Se]selenite **2**. The precipitate was centrifuged for 15 min. The water phase was decanted and the sulfur matrix washed three times with dry tetrahydrofuran. The dry sulfur matrix containing the radioselenium **3** was weighed (∼ 6–7 mg (0.1–0.2 mmol) sulfur). It was then transformed into a mixture of methylsulfide **4a** and methyl[^{75}Se]selenide **4** by addition of methyl lithium in the range of 0.05–0.1 mmol under an atmosphere of argon and stirring for 15 min until a yellow to white suspension of methyl sulfide appeared. The mixture was transferred into a conical 5 mL reaction vessel equipped with a magnetic stirring bar and a teflon-rubber septum under an atmosphere of argon. Then 0.1 mmol 2-tert.-butoxycarbonylamino-4-bromobutyric acid ethylester **5** dissolved in 300 μL DMF was added and heated for 30 min. The products were isolated by chromatography on a silica gel column (1 × 10 cm) using acetone as eluent. The organic phase was evaporated to dryness under a stream of argon.

A stream of ammonia was introduced into the reaction vessel containing the dry residue. The flask was cooled to about −40 °C in order to condense liquid ammonia (2–4 mL). Elemental sodium (0.1–0.2 mmol) was added to the solution while gaseous ammonia was continuously led through the deep blue reaction mixture. After 15–20 min at −40 °C ammonium chloride was added to destroy the sodium amide formed. Subsequently, the cooling bath was removed and the liquid ammonia evaporated by a gentle flow of argon followed by the addition 5 mL of concentrated hydrochloric acid. For hydrolysis of protecting groups the solution was heated under reflux for about 30 min in an inert atmosphere and evaporated to dryness under a stream of argon. The residue was suspended in ethanol and filtered over a Sep-Pak™C-18 cartridge (Waters). Homocysteine[^{75}Se]selenolactone **1** was analyzed *via* radio-TLC with *n*-butanol/ethanol/formic acid 5/1/1 (v/v/v) as eluent.

Synthesis of homocysteine[^{75}Se]selenolactone 1 *via* [^{75}Se]selenourea 10

The c.a. [^{75}Se]selenouronium salt **11** was synthezised by reaction of c.a. 1,3-dicyclohexyl[^{75}Se]selenourea **10** (0.1 mmol) (prepared *via* published methods [10]) with 2-tert.-butoxycarbonylamino-4-bromobutyric acid ethylester **5** (0.2 mmol) in acetonitrile (0.3 mL). The alkylation reactions were monitored by radio-TLC (diethyl ether/*n*-hexane 3/2 (v/v)). By-products and educts were removed by column chromatography (10 × 1 cm) on silica gel using diethyl ether and the purified [^{75}Se]selenouronium salt **11** was eluted from the column with acetone.

Hydrolysis of the purified [^{75}Se]selenouronium salts **11** with tetrabutylammonium hydroxide (0.1 mmol) in 2 mL methanol was done within 5 min. After removal of the solvent, 5 mL of concentrated hydrochloric acid 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™C-18 cartridge (Waters). Homocysteine[^{75}Se]selenolactone 1 was analyzed on radio-TLC with *n*-butanol/ethanol/formic acid 5/1/1 (v/v/v) as eluent.

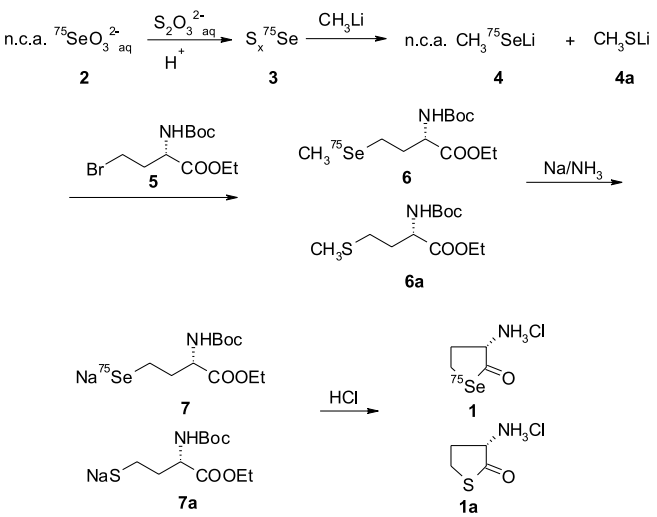
Results and discussion

The first labelling method presented (cf. Scheme 2) is based on the original chemical synthesis of c.a. [^{75}Se]selenomethionine, described by Plenevaux *et al*. [6]. However, the selenium carrier was substituted by elemental sulfur as non-isotopic tracer. N.c.a. [^{75}Se]selenite **2**, obtained after thermochromatographic isolation from the target [4], was reduced under acidic conditions with sodium thiosulfate leading to the precipitation of sulfur inserting more than 90% of the n.c.a. ^{75}Se in the elemental form, probably inserted as [^{75}Se]SeS_8 rings. The sulfur matrix **3** containing the elemental [^{75}Se]selenium prevents the rapid oxidation of the radiotracer. It was transformed with methyl lithium into a mixture of lithium methyl [^{75}Se]selenide **4** and lithium methyl mercaptide **4a**. The optimum amount of methyl lithium ranges from 50 to 100% of the equimolar amount related to precipitated sulfur. An excess of methyl lithium should be avoided in order to minimize side reactions, especially the formation of volatile radioselenium products. Subsequent reaction with 2-tert.-butoxycarbonylamino-4-butyric acid ethylester **5** in DMF leads to the formation of n.c.a. 2-tert.-butoxycarbonylamino-4-(methyl[^{75}Se]seleno)butyric acid ethylester **6** and the corresponding methionine derivative **6a**.

Fig. 1 shows the temperature dependence of the formation of n.c.a. 2-tert.-butoxycarbonylamino-4-(methyl[^{75}Se]seleno)butyric acid ethylester **6** which leads at 80 to 100 °C to a 15 to 20% RCY after 30 min reaction time in DMF.

[^{75}Se]Selenomethionine is available after hydrolysis of 2-tert.-butoxycarbonylamino-4-(methyl[^{75}Se]seleno)butyric acid ethylester **6** with hydrochloric acid and can be separated from methionine by HPLC analysis. The specific activity of the n.c.a. product was > 185 GBq/mmol. For convenience it was determined at the step of [^{75}Se]selenomethionine since isotopic dilution can be excluded during its conversion into homocysteine[^{75}Se]selenolactone **1**.

The Birch reduction of the N-Boc-protected aminoacid esters **6** and consecutive cleavage of the protection groups under simultaneous lactonisation in presence of hydrochloric acid leads to the formation of n.c.a. homocysteine



Scheme 2. Radiosynthesis of non-isotopic carrier added homocysteine [^{75}Se]selenolactone **1** *via* methyl lithium.

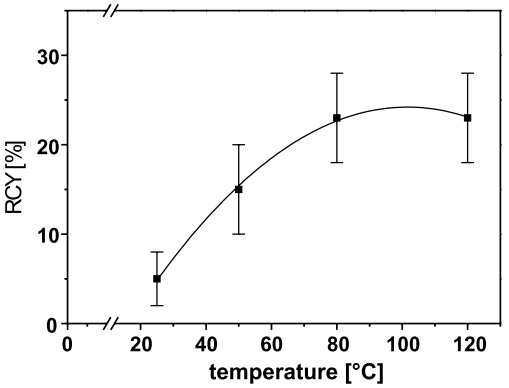


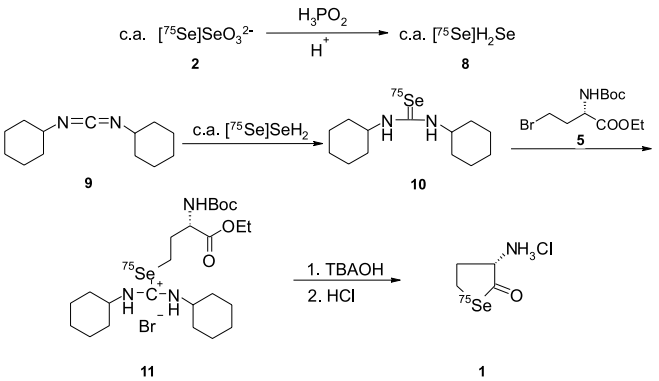
Fig. 1. Temperature dependence of the formation of n.c.a. 2-tert.-butoxycarbonylamino-4-(methyl[^{75}Se]seleno)butyricethylester **6** starting with Li[^{75}Se]$SeCH_3$. *Reaction conditions: c(2-tert.-Butoxycarbonylamino-4-bromobutyric acid ethylester **5**)* = 0.5 mol/l, 0.2 ml *DMF*, 0.2 ml *THF*, 80 °C, *c(Sulfur)* = 0.5 mol/l, *c(MeLi)k* = 0.25–0.5 mol/l, *reaction time:* 30 min.

[^{75}Se]selenolactone **1** with a radiochemical yield of 5%–10% (related to n.c.a. [^{75}Se]selenite) and natural homocysteine thiolactone **1a** within 180 min total synthesis time.

The major drawback of this method is the large loss of volatile selenium activity (presumably dimethyl[^{75}Se]-selenide in the reaction with methyl lithium and corresponding dimethylthio[^{75}Se]selenide, respectively) and the formation of unknown side products.

An alternative radiosynthesis was developed starting with the formation of selenouronium salts **11** (cf. Scheme 3). 1,3-Dicyclohexyl[^{75}Se]selenourea **10**, a useful synthon to produce [^{75}Se]selenols *via* [^{75}Se]selenouronium salts **11**, can be obtained by addition of hydrogen[^{75}Se]selenide **8** to 1,3-dicyclohexylcarbodiimide **9** [10]. However, the formation of [^{75}Se]SeH_2 **8** using hypophosphorous acid or stannous chloride for reduction only takes place in presence of carrier selenite. Accordingly, only c.a. selenoethers can be obtained by this method. However, the alkylation step is not limited to the synthesis of methylselenoethers like in the previous procedure *via* Li[^{75}Se]$SeCH_3$ **4** (also used by Plenevaux *et al*. [6]).

The alkylation of c.a. 1,3-dicyclohexyl[^{75}Se]selenourea **10** with 2-tert.-butoxycarbonylamino-4-bromobutyric acid ethyl ester **5** in acetonitrile leads to a nearly quantitative



Scheme 3. Radiosynthesis of homocysteine[^{75}Se]selenolactone **1** *via* [^{75}Se]selenourea.

formation of the corresponding selenouronium salt **11**, which can be transformed in presence of tetrabutylammonium hydroxide and subsequent acidification with concentrated hydrochloric acid into homocysteine[^{75}Se]selenolactone **1**. The total RCY starting from [^{75}Se]selenite **2** is 20%–30%; the total synthesis time about 90 min. The advantage of this strategy is its versatile application to the synthesis of various asymmetric radioselenoethers. The methods described recently for ^{75}Se-labelling [6, 8] only lead to methyl[^{75}Se]selenoethers and do not offer the possibility to synthesize a broad variety of symmetric as well as asymmetric [^{75}Se]selenoethers. The radiosynthesis of homocysteine[^{75}Se]selenolactone **1** *via* the selenourea-strategy is more convenient, because a Birch reduction, which is not suitable for automated radiosyntheses, is avoided. However, starting with hydrogen [^{75}Se]selenide the synthesis of 1,3-dicyclohexyl[^{75}Se]selenourea is so far only possible in presence of hydrogen selenide carrier.

Conclusion

Radioselenoethers have been synthesized *via* two pathways, either by alkylation of n.c.a. methylselenide **2** or by hydrolysis of alkylated dicyclohexyl[^{75}Se]selenourea **11**. N.c.a. methyl[^{75}Se]selenide is available using sulfur as non-isotopic carrier. This is necessary to enable labelling reactions on the n.c.a. level. However, the radiochemical yield is only in the range of 15%–20% within 60 min (related to starting [^{75}Se]selenite), because the formation of volatile side products, probably dimethyl[^{75}Se]selenide, could not be avoided.

The synthesis of homocysteine[^{75}Se]selenolactone **1** is possible *via* Birch reduction of 2-tert.-butoxycarbonylamino-4-(methyl[^{75}Se]seleno)butyric acid ethylester **6** and subsequent hydrolysis and cyclisation using hydrochloric acid leading to a RCY of 5%–10% of the title compound **1** within 180 min total synthesis time starting from n.c.a. [^{75}Se]selenite **2**.

Alternatively, the synthesis of homocysteine[^{75}Se]selenolactone **1** is possible by hydrolysis of the corresponding [^{75}Se]selenouronium salt **11**. The RCY of c.a. homocysteine[^{75}Se]selenolactone **1** *via* this route compared to the previous one is three times higher with nearly half of the reaction time because the demethylation step (Birch reduction) on [^{75}Se]selenomethionine is avoided. Furthermore, no volatile radio- selenium side products were observed. However, the choice of [^{75}Se]SeH_2 **8** as starting material limits the maximum possible specific activity since it can only be formed in presence of carrier [10].

Nevertheless, in contrast to the recently described method for n.c.a. ^{75}Se-labelling *via* triphenylphosphine which only provides methyl[^{75}Se]selenoethers [8], the method *via* 1,3-dicyclohexyl[^{75}Se]selenourea **10** offers the possibility to synthesize a broad variety of symmetric as well as asymmetric [^{75}Se]selenoethers.

In summary, the selenourea strategy is more convenient for the synthesis of the title compound **1**, because it results in higher RCY, is easier to handle and offers the possibility of automated radiosyntheses. With regard to potential toxicity of selenium compounds, further studies have to be performed in order to synthezise homocysteine [^{75}Se]selenolactone **1** *via* n.c.a. [^{75}Se]selenoureas starting from n.c.a. $^{75}Se^0$.

Acknowledgment. The authors thank G. Blessing (Institut für Nuklearchemie, Forschungszentrum Jülich) for preparation of n.c.a. selenium-75.

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