JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
J Labelled Cpd Radiopharm 2001; **44**: 587–601.
DOI: 10.1002/jlcr.488

Synthesis of asymmetric [^{75}Se]selenoethers via carbodiimides

T. Blum, J. Ermert* and H. H. Coenen
Institut für Nuklearchemie, Forschungszentrum Jülich GmbH, D-52425 Jülich, Germany

Summary

A convenient radiosynthesis of asymmetric [^{75}Se]selenoethers was developed using 1,3-disubstituted [^{75}Se]selenoureas as intermediates. These were prepared from appropriate carbodiimides and hydrogen [^{75}Se]selenide, which could only be generated from carrier-added (c.a.) [^{75}Se]selenite in aqueous solution using phosphinic acid as reducing agent. Optimization of this initial labelling step with dicyclohexylcarbodiimide and polymeric *N*-cyclohexylcarbodiimide-*N*′-methyl polystyrene resulted in radiochemical yields (RCY) of 73 and 55% (bound on the polymer), respectively, within 45 min. Treatment of [^{75}Se]selenoureas with alkylbromides led to corresponding [^{75}Se]selenouronium salts in nearly quantitative yields. Hydrolysis under basic conditions provided the [^{75}Se]selenolates and a second alkylation yielded asymmetric [^{75}Se]selenoethers. Thus, within 90 min benzylmethyl[^{75}Se]selenide, benzylbutyl[^{75}Se]selenide, benzylisopropyl[^{75}Se]selenide and 1-phenyl-l-(methyl[^{75}Se]seleno)ethane were synthesized with respective RCY of about 59, 55, 10 and 60%. Furthermore, the ^{75}Se-labelled alkylating agent 3-(methyl[^{75}Se]seleno)-1-propanyl *p*-toluenesulfonate and [^{75}Se]selenomethionine were obtained with radiochemical yields of 51 and 41%, respectively. Copyright © 2001 John Wiley & Sons, Ltd.

Key Words: ^{75}Se-labelling; substituted [^{75}Se]selenoureas; asymmetric [^{75}Se]selenoethers; ^{75}Se-labelled alkylating agent; [^{75}Se]selenomethionine

*Correspondence to: J. Ermert, Institut für Nuklearchemie, Forschungszentrum Jülich GmbH, D-52425 Jülich, Germany.

Copyright © 2001 John Wiley & Sons, Ltd.

Received 8 January 2001
Revised 6 April 2001
Accepted 14 April 2001
Published online ▮▮▮▮

Introduction

Selenium-75 ($T_{1/2}$ =120.4 d; 100% EC) has often been used as a gamma-ray emitting label in various radiopharmaceuticals.[1–4] The decay characteristics of ^{75}Se are not ideal for *in vivo* application, because the radiation absorbed dose delivered to the patient in a study is high and the images with conventional γ-cameras are of poor quality.[5] The nuclear properties of ^{73}Se ($T_{1/2}$ = 7.1 h; 65% β^+; 35% EC) suggest that it may be a more useful label for *in vivo* medical applications using positron-emission-tomography (PET). This radioisotope can be produced in sufficient amounts via the ^{75}As(p, 3n)^{73}Se reaction.[6] For the development of radiosyntheses, however, the more easily available radioisotope ^{75}Se is generally used.[7] The most common ^{75}Se-labelled compounds are selenoethers, as the selenium atom can form two stable covalent bonds to carbon, where selenium is stabilized in the oxidation state −2. A widely used method for the preparation of symmetric carrier-added (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. Subsequent alkylation yields the appropriate ^{75}Se-labelled symmetric selenoethers.[8,9] Basmadijan *et al.*[10] suggested the formation of asymmetric c.a. [^{75}Se]selenoethers via reductive cleavage of ^{75}Se-labelled dialkyl diselenides or by basic hydrolysis of [^{75}Se]selenouronium salts yielding alkyl[^{75}Se]selenolates, which can be alkylated *in situ*.

For clinical diagnosis it would be desirable to achieve a general pathway for the rapid radiochemical and fully automated synthesis of [^{73}Se]selenoethers in order to handle high activities and to minimize the radiation dose to the operators. An attractive tool for automated synthesis is solid-phase chemistry which allows purification of the products with minimum effort. The first microscale synthesis suitable for introduction of no-carrier-added (n.c.a.) radioselenium using a solid-phase support was reported by Schmaljohann.[11] For this purpose polymeric bound n.c.a. [^{75}Se]triphenylphosphineselenide was synthesized, subsequently methylated and then hydrolysed to the methylselenide anion. Nucleophilic substitution on alkyl bromides allowed the synthesis of asymmetric n.c.a. [^{75}Se]methylselenoethers.

The aim of this study was the preparation of asymmetric [^{75}Se]selenoethers via 1,3-disubstituted [^{75}Se]selenoureas as intermediates starting from [^{75}Se]H_2Se and carbodiimides. While the alkylation of [^{75}Se]-triphenylphosphineselenide is limited to methyltriflate, [^{75}Se]selenoureas

Copyright © 2001 John Wiley & Sons, Ltd.

would be expected to make a wide variety of symmetric as well as asymmetric [^{75}Se]selenoethers accessible, because the alkylation of selenoureas can be easily accomplished by alkylbromine or -tosylate derivatives.[10] Furthermore, a polymer bound carbodiimide is available as starting material to fulfil the requirements of a solid-phase synthesis.

Preparation of different [^{75}Se]selenoethers as model compounds shows the versatility of this method. The radioselenium-labelled amino acid selenomethionine can be used for the measurement of protein synthesis in the pancreas[12] and possibly even in brain.[13] This radiopharmaceutical was previously obtained in carrier-added form by biosynthesis within 30 h with a RCY of 20–40%[14] or by a radiochemical synthesis within 3 h with a RCY of 80%.[4]

Experimental

General

Unless otherwise mentioned, all chemicals were purchased from Aldrich (Steinheim, Germany) and Fluka (Buchs, Switzerland) and used without further purification. *N*-cyclohexylcarbodiimide-*N*′-methyl polystyrene was obtained from Novabiochem (Schwalbach, Germany), 2-amino-4-bromobutyric acid hydrobromide was obtained from Acros Organics (Geel, Belgium). Further compounds were synthesized according to literature methods: benzylmethylselenide,[15] benzylbutylselenide,[16] benzylisopropylselenide,[16] 3-(methylseleno)-1-propanol,[17] 3-(methylseleno)-1-propanyl *p*-toluenesulfonate[11] and ethyl *N*-tert.-butoxycarbonyl-2-amino-4-bromobutyrate **3a**.[11] All selenocompounds were prepared under an argon atmosphere.

Melting point determinations employed a Mettler FP 61 and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 200 spectrometer with samples dissolved in $CDCl_3$, CD_2Cl_2 or d_6-DMSO. All chemical shifts are reported in δ ppm using the signals of the appropriate solvent as a reference. IR spectra were recorded on a Shimadzu IR-460 spectrophotometer and mass spectra were obtained using a Finnigan Automass Multi mass spectrometer with an electron beam energy of 70 eV. High-resolution mass spectra were recorded on a Finnigan MAT 900 ST apparatus (University of Cologne, Germany).

Analytical radio-HPLC was performed on a system consisting of a Knauer pump 6400 and a Knauer UV/vis photometer 3060 with a

Copyright © 2001 John Wiley & Sons, Ltd.

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, Münster, Germany). Separation of c.a. [^{75}Se]selenomethionine was performed using a Nucleosil^{TM} 100-5 NH_2 (250 × 4 mm) column (CS-Chromatographie Service) with a mobile phase of acetonitrile/0.04 M KH_2PO_4 (aq.) (77/23) (v/v) at a flow rate of 1.0 ml/min. HPLC of aliquots of all other labelled products and standards was performed using a μ-Bondapack C 18 (250 × 4 mm) column (CS-Chromatographie Service GmbH, Langerwehe, Germany) and a mobile phase consisting of acetonitrile/H_2O (40/60) (v/v), 0.01% trifluoroacetic acid and 0.01% triethylamine at a flow rate of 1.0 ml/min. Radio-TLC was performed on Merck silica gel plates with the solvent system diethyl ether/*n*-hexane in various concentrations. The developed TL-chromatograms were measured for radioactivity on an Instant Imager^{TM} (Packard).

Standards and precursors

1,3-Dicyclohexylselenourea. Hydrogen selenide, generated by adding sulfuric acid (20 ml, 18 N) to powdered aluminium selenide (1.5 g, 5.2 mmol), was passed into a solution of 1,3-dicyclohexylcarbodiimide (824 mg, 4 mmol) in diethyl ether (20 ml) for 1 h using a method developed by Klayman and Shine.[18] The solution was stirred at room temperature for 4 h. The solvent was removed by distillation and the residue taken up in dichloromethane. After filtration the solvent was evaporated yielding 1,3-dicyclohexylselenourea as a grey solid. Yield: 440 mg (38%); m.p.: 172°C; ^{1}H-NMR (CD_2Cl_2): δ 5.85 (b, 2H, NH), 3.85 (b, 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); mass spectrum: 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.

1-Phenyl-1-(methylseleno)ethane. 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 while stirring, resulting in a pale yellow solution. 1-(Bromoethyl)benzene (0.78 ml, 5.7 mmol) was added and the mixture further stirred for 1 h at room temperature. The

Copyright © 2001 John Wiley & Sons, Ltd.

solution was hydrolysed with water (10 ml), the organic phase separated, washed with $NaHCO_3$ and brine and dried (Na_2SO_4) according to Gulliver *et al.*[17] The solvent was evaporated and the residue fractionated *in vacuo* to give a pale yellow oil. Yield: 0.63 g (55%); n_D^{20}: 1.5783; ^{1}H-NMR ($CDCl_3$): δ 7.29 (m, 5 H, 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 (Se–CH), 22.5 (CH–$\underline{C}H_3$), 4.9 (Se–CH_3); mass spectrum: m/e 200 (M^+, 5%), 105 (100), 95 (24), 77 (82); HR-MS calculated for $C_9H_{12}Se$: 200.0104, found: 200.0101.

N-tert.-butoxycarbonylselenomethionine ethyl ester ***6***. Dry hydrogen chloride was passed through a suspension of selenomethionine (196 mg, 1 mmol) in ethanol (50 ml) for 2 h with external cooling by ice according to Deimer *et al.*[19] The solution was evaporated to dryness, the residue dissolved in ethanol and evaporated to dryness. The procedure was repeated once more to obtain the crude selenomethionine ethyl ester as a colourless oil without any trace of hydrochloric acid, which was dissolved in CH_2Cl_2 (50 ml). Di-tert.-butyl dicarbonate (218 mg, 1 mmol) and triethylamine (0.28 ml, 2 mmol) were added and the mixture was stirred for 12 h at room temperature according to Itoh *et al.*[20] The solvent was removed under vacuum and the crude product obtained was purified via silica gel column chromatography (hexane/diethyl ether: 1/1) to give 207 mg (64%) *N*-tert.-butoxycarbonylselenomethionine ethyl ester as colourless crystals. M.p.: 43°C; ν_{max} (KBr): 1748, 1684 (C=O); ^{1}H-NMR ($CDCl_3$): δ 5.15 (d, 1H, NH), 4.40 (q, 1H, CH), 4.20 (q, 2H, O–CH_2), 2.55 (t, 2H, Se–CH_2), 2.10 (m, 2H, CH_2–C$\underline{H}_2$–CH), 2.00 (s, 3H, Se–CH_3), 1.45 (s, 9H, C–CH_3), 1.25 (t, 3H, CH_2–C$\underline{H}_3$); ^{13}C-NMR ($CDCl_3$): δ 172.6 (C(O)O), 155.7 (C(O)N), 80.3 ($\underline{C}(CH_3)_3$), 61.8 (O–CH_2), 54.0 (CH), 33.7 (Se–CH_2), 28.7 (C($\underline{C}H_3)_3$), 20.7 (CH_2–$\underline{C}H_2$–CH), 14.6 (CH_2–$\underline{C}H_3$), 4.6 (Se–CH_3); mass spectrum: m/e 325 (M^+, 9%), 269 (18), 224 (8), 174 (27), 147 (14), 101 (18), 57 (100); HR-MS calculated for $C_{12}H_{23}O_4NSe$: 325.0792, found: 325.0784.

Radiosyntheses

Production of [^{75}Se]selenite. [^{75}Se]Selenium was produced using 20 MeV protons at the compact cyclotron CV-28 at the Forschungszentrum Jlich GmbH via the ^{75}As(p,n)^{75}Se reaction on a solid Cu_3As-target. After thermochromatographic separation n.c.a. selenium-75 is

Copyright © 2001 John Wiley & Sons, Ltd.

available in its oxidized form as [^{75}Se]SeO_3^{2-} in water.[21] All experiments were performed using 10 μmol $^{nat}SeO_3^{2-}$ as carrier and were conducted under argon in a conical 5 ml reaction vessel equipped with a magnetic stirring bar and a teflon rubber septum.

1,3-Dicyclohexyl[^{75}Se]selenourea **2**. Reduction of the c.a. [^{75}Se]selenite solution (0.5 ml, typically containing 370 kBq (10 μCi)) was performed at the desired temperature (55–95°C) with 0.1 ml of 32% hydrochloric acid and 0.4 ml of 50% phosphinic acid in water. Via a stream of argon the generated hydrogen [^{75}Se]selenide **1** was slowly led into a solution of 100 mg 1,3-dicyclohexylcarbodiimide (DCC) in 3 ml of diethyl ether. For this purpose a device is used (cf. Figure 1), which separates the reduction from the acid-sensitive DCC. Aliquots were analysed for the [^{75}Se]selenourea by radio-HPLC under the conditions given above ($k' = 5.0$) and radio-TLC (diethyl ether/*n*-hexane 3/2 (v/v), $R_f = 0.52$). Alternatively, 500 mg of *N*-cyclohexylcarbodiimide-*N'*-methyl polystyrene were used instead of DCC for polymer-supported formation of *N*-cyclohexyl[^{75}Se]selenourea-*N'*-methyl polystyrene **8**.

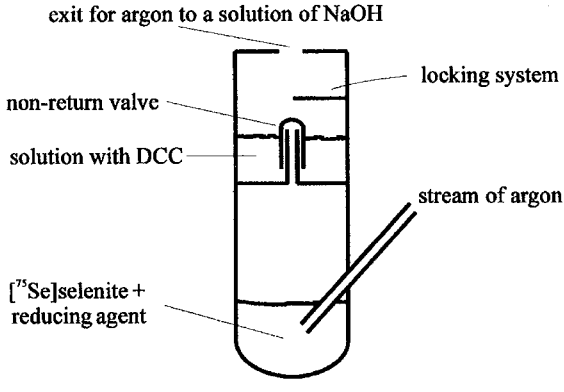


Figure 1. Sketch of apparatus for the synthesis and reaction of [^{75}Se]H_2Se

[^{75}Se]Selenouronium salts. The [^{75}Se]selenouronium salts could be prepared in various solvents (0.3 ml) such as acetonitrile, *N*,*N*-dimethylformamide and ethanol by reaction of **2** with the appropriate alkyl bromide (0.2 mmol), respectively. The alkylation reactions were monitored by radio-TLC (diethyl ether/*n*-hexane 3/2 (v/v), $R_f = 0.1$–0.2). By-products and adducts were removed by silica gel column chromatography using diethyl ether and the purified [^{75}Se]selenouronium salts were eluted from the column with acetone.

Copyright © 2001 John Wiley & Sons, Ltd.

[^{75}Se]Selenoethers. The asymmetric [^{75}Se]selenoethers were prepared by treatment of the purified [^{75}Se]selenouronium salts with tetrabutylammonium hydroxide (0.1 mmol) in the presence of appropriate bromine- or iodine-compounds (0.2 mmol) in 0.3 ml acetonitrile at room temperature within 1 min. The [^{75}Se]selenoethers obtained were analysed by radio-HPLC and radio-TLC.

3-(Methyl[^{75}Se]seleno)-1-propanyl p-toluenesulfonate. Alkylation of **2** with 3-bromo-1-propanol (17 µl, 0.2 mmol) in 0.3 ml acetonitrile was performed within 40 min. After purification, the resulting [^{75}Se]selenouronium salt was treated with a solution of 0.1 mmol tetrabutylammonium hydroxide in 0.3 ml acetonitrile. Subsequently iodomethane (12 µl, 0.2 mmol) was added yielding 3-(methyl[^{75}Se]seleno)-1-propanol. Without purification this compound was allowed to react by addition of toluene-4-sulfonyl chloride (28.5 mg, 0.15 mmol) in 0.1 ml acetonitrile, triethylamine (30 µl, 0.2 mmol) and trimethylamine hydrochloride (8 mg, 0.01 mmol) in 0.2 ml acetonitrile at 0°C for 1 h. Radio-TLC-analysis was performed with diethyl ether/*n*-hexane 1/1 (v/v) as eluent, $R_f = 0.6$.

[^{75}Se]selenomethionine. The labelled amino acid was prepared as described for [^{75}Se]selenoethers, starting with alkylation of 1,3-dicyclohexyl[^{75}Se]selenourea **2** with ethyl *N*-tert.-butoxycarbonyl-2-amino-4-bromobutyrate **3a** (62 mg, 0.2 mmol) in 0.3 ml acetonitrile. The resulting [^{75}Se]selenouronium salt was purified according to the method described above and hydrolysed with tetrabutylammonium hydroxide (0.1 mmol) in 0.3 ml acetonitrile. The obtained [^{75}Se]selenolate was alkylated *in situ* by iodomethane (12 µl, 0.2 mmol). After ester hydrolysis and Boc-cleavage using 16% hydrochloric acid (0.3 ml) at 70 and 90°C, respectively, [^{75}Se]selenomethionine was obtained and analysed by radio-HPLC as detailed above ($k' = 3.16$).

Results and discussion

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

According to the literature, 1,3-disubstituted selenoureas can be prepared from carbodiimides and hydrogen selenide in several organic solvents.[22] We investigated the formation of hydrogen [^{75}Se]selenide **1**

Copyright © 2001 John Wiley & Sons, Ltd.

1 2

Scheme 1. Formation of hydrogen [^{75}Se]selenide 1 and its subsequent conversion to 1,3-dicyclohexyl[^{75}Se]selenourea 2

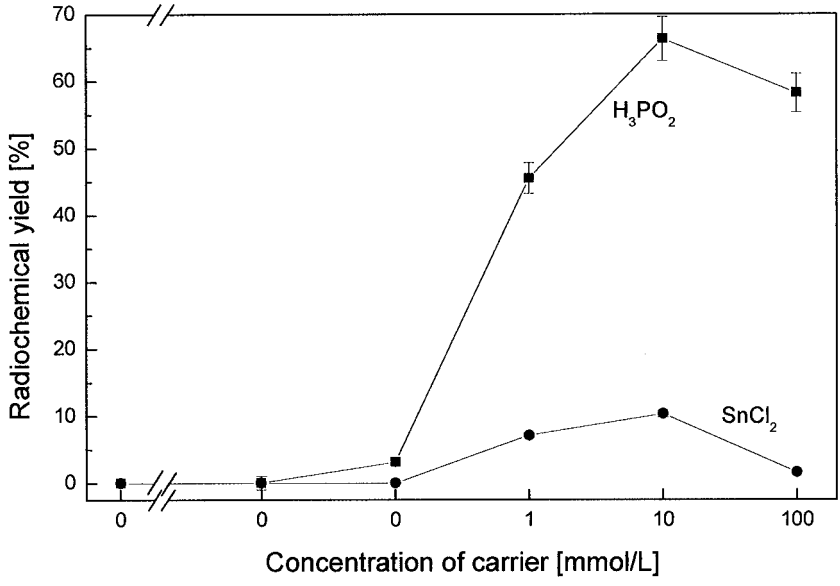


Figure 2. Radiochemical yield of 1 as a function of Na_2SeO_3 carrier concentration with $SnCl_2$ and H_3PO_2 as reducing agents. General reaction conditions: 370 kBq n.c.a [^{75}Se]SeO_3^{2-}, Na_2SeO_3-carrier, 45 min, 0.5 mmol DCC, 3 ml diethyl ether; conditions for H_3PO_2: 3.6 mmol H_3PO_2, 0.9 ml H_2O, 0.1 ml concentrated HCl, 85°C; conditions for $SnCl_2$: 1.5 mmol $SnCl_2$, 1 ml concentrated H_3PO_4, 300°C

under a variety of different reaction conditions by monitoring its reaction with DCC (Scheme 1). TLC-analyses showed, that all the ^{75}Se-activity in the DCC-solution existed as 1,3-dicyclohexyl[^{75}Se]selenourea within 10 s after starting the reduction, i.e. liberation of [^{75}Se]H_2Se.

For comparison, the reducing agents phosphinic acid[23] and tin(II) chloride in strong phosphoric acid[24] were studied. As graphically depicted in Figure 2 a concentration of 10 mmol/l Na_2SeO_3 carrier proved to be useful for obtaining the highest radiochemical yields of $66 \pm 3\%$ of hydrogen [^{75}Se]selenide within 45 min at 85°C, using

Copyright © 2001 John Wiley & Sons, Ltd.

phosphinic acid as the obviously more suitable reducing agent. In the case of $SnCl_2$, the maximum RCY of only 10% was reached at the same carrier concentration within 45 min at 300°C. Higher concentrations led to a decrease of the RCY in both cases.

Increasing the temperature led to an almost linear increase of the radiochemical yield of **1** from about 10% at 55°C to about 75% radiochemical yield (related to [^{75}Se]selenite) obtained at 95°C with phosphinic acid as reducing agent.

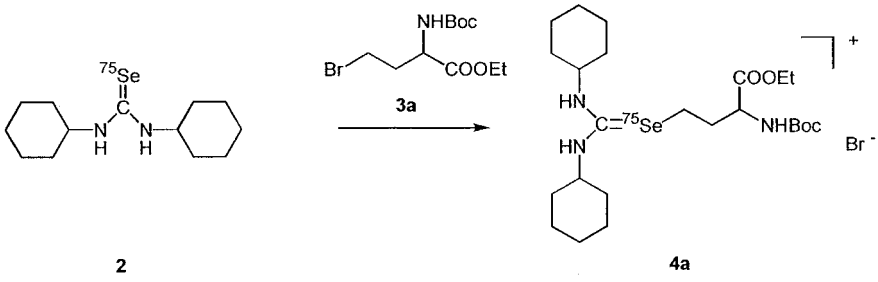
Furthermore, Figure 2 shows that the reduction of [^{75}Se]selenite to [^{75}Se]H_2Se is not possible at the n.c.a. level under the given reaction conditions. All attempts to perform the reduction at the n.c.a. level were unsuccessful. Even the use of various very strong reducing agents such as sodium borohydride, lithium aluminium hydride and sodium as well as of non-isotopic carriers like Na_2SO_3, $Na_2S_2O_3$, Na_2S, Na_2TeO_3 and Na_2Te failed. Under all conditions only n.c.a. $^{75}Se^0$ was obtained. Obviously, the reduction of selenite to hydrogen selenide 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 at the n.c.a. level. 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.

Preparation of [^{75}Se]selenouronium salts

Due to the highly polarized carbon–selenium bond an equimolar reaction of selenourea with various alkyl halides yields the corresponding selenouronium salts as described by Chu and Mautner.[25] In order to apply and optimize this reaction for ^{75}Se-labelled compounds, we chose the preparation of the [^{75}Se]selenouronium salt **4a** starting from **2** and ethyl *N*-tert.-butoxycarbonyl-2-amino-4-bromobutyrate **3a**, the precursor for [^{75}Se]selenomethionine, as model reaction (Scheme 2).

The formation of **4a** was optimized with respect to the effects of reaction time and solvent. Under otherwise fixed reaction conditions (cf. Experimental) saturation yields were obtained in all solvents for **4a** after a reaction time of about 40 min. In dipolar aprotic solvents (acetonitrile, *N*,*N*-dimethylformamide) the formation of **4a** was nearly quantitative related to **2**, whereas the protic solvent ethanol proved not

Copyright © 2001 John Wiley & Sons, Ltd.



Scheme 2. Formation of the [^{75}Se]selenouronium bromide 4a starting from 1,3-dicyclohexyl[^{75}Se]selenourea 2 and ethyl N-tert.-butoxycarbonyl-2-amino-4-bromobutyrate 3a

as suitable since the radiochemical yield of **4a** reached only about 80% (related to **2**).

In order to remove by-products and adducts from the [^{75}Se]selenouronium bromide formed, it was separated by silica gel column chromatography with a recovery rate of about 90% (related to **2**).

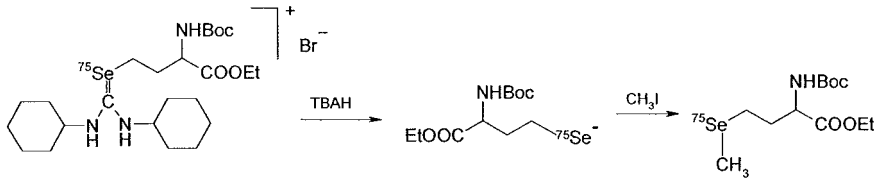
Under the described optimum reaction conditions further alkyl bromides were chosen for the formation of [^{75}Se]selenouronium salts in order to demonstrate the versatility of the reaction sequence described above. The alkyl bromides, benzylbromide, (1-bromoethyl)benzene and 1-bromo-3-propanol served as model compounds to yield the corresponding [^{75}Se]selenouronium salts with a RCY of about 90% (related to **2**) after chromatographic purification.

Preparation of asymmetric [^{75}Se]selenoethers

Treatment of selenouronium salts in the presence of hydroxide yields the corresponding selenolates.[26] Because of rapid autooxidation to diselenides, selenolates are unstable and *in situ* alkylation to selenoethers is recommended. Therefore, iodomethane was added to the purified [^{75}Se]selenouronium bromide in acetonitrile before adding the base, so the intermediate **5** could be methylated *in situ* (Scheme 3).

Using tetrabutylammonium hydroxide (TBAH) as base for hydrolysis, **6** was formed in a radiochemical yield of nearly 90% (related to **4a**) within 1 min using concentrations of at least 0.7 mol/l iodomethane and 0.3 mol/l TBAH. In contrast, the use of potassium hydroxide or pyrrolidine led to unidentified ^{75}Se-labelled by-products. For demonstration of the versatility of the above described reaction sequence,

Copyright © 2001 John Wiley & Sons, Ltd.



4a 5 6

Scheme 3. Formation of N-tert.-butoxycarbonyl[^{75}Se]selenomethionine ethyl ester 6 by hydrolysis of the [^{75}Se]selenouronium bromide 4a and *in situ* methylation of the [^{75}Se]selenolate 5

Table 1. Radiochemical yields of asymmetric [^{75}Se]selenoethers

Compound	RCY (%)	First/second alkylating agent
Benzylmethyl[^{75}Se]selenide	59	Benzylbromide/iodomethane
Benzylbutyl[^{75}Se]selenide	55	Benzylbromide/butylbromide
Benzylisopropyl[^{75}Se]selenide	10	Benzylbromide/isopropylbromide
1-Phenyl-1-(methyl[^{75}Se]seleno)-ethane	60	(1-Bromoethyl)benzene/iodomethane
N-tert.-butoxycarbonyl[^{75}Se]-selenomethionine ethyl ester	59	ethyl *N*-tert.-butoxycarbonyl-2-amino-4-bromobutyrate/iodomethane
3-(Methyl[^{75}Se]seleno)-1-propanol	57	1-bromo-3-propanol/iodomethane

various asymmetric [^{75}Se]selenoethers were prepared, starting from **2** and appropriate alkylating agents (cf. Table 1) and using the optimum reaction conditions described above. Labelling of these compounds resulted in radiochemical yields of 55–60% (related to [^{75}Se]selenite) with nearly quantitative yields for the two alkylation steps. Further investigations with stronger alkylating agents, for example iodine compounds, were therefore not carried out. The relatively low radiochemical yield of benzylisopropyl[^{75}Se]selenide was probably caused by the rapid elimination of isopropylbromide under the given reaction conditions.

Preparation of [^{75}Se]selenomethionine and 3-(methyl[^{75}Se]seleno)-1-propanyl p-toluenesulfonate

N-tert.-butoxycarbonyl[^{75}Se]selenomethionine ethyl ester was hydrolysed by hydrochloric acid to liberate [^{75}Se]selenomethionine. The

Copyright © 2001 John Wiley & Sons, Ltd.

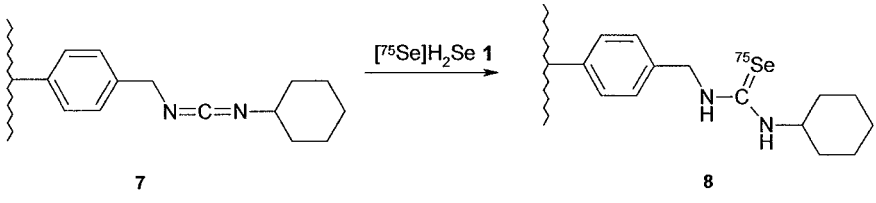
amino acid was obtained with a maximum radiochemical yield of about 70%, related to **6**, and 41%, related to [^{75}Se]SeO_3^{2-}, at a reaction temperature of 90°C within 35 min, whereas at 70°C a RCY of about 55% (related to **6**) was achieved. Longer reaction times decreased the RCY due to decomposition reactions occurring especially at the temperature of 90°C.

Preparation of 3-(methyl[^{75}Se]seleno)-1-propanyl *p*-toluenesulfonate as a small prosthetic group was studied for possible labelling of suitable nucleophilic functional groups via [^{75}Se]selenoalkylation. A radiochemical yield of about 90%, related to 3-(methyl[^{75}Se]seleno)-1-propanol, or 51%, related to [^{75}Se]SeO_3^{2-}, of this ^{75}Se-labelled alkylating agent was achieved at 0°C in acetonitrile, starting with 3-(methyl[^{75}Se]-seleno)-1-propanol and toluene-4-sulfonyl chloride as tosylating agent.

Polymer-supported preparation of [^{75}Se]selenoethers

With the aim of developing an attractive tool for rapid and automated syntheses of radioselenoethers, we synthesized the polymer bound *N*-cyclohexyl[^{75}Se]selenourea-*N*′-methyl polystyrene **8** which was obtained by reaction of hydrogen [^{75}Se]selenide **1** with *N*-cyclohexylcarbodiimide-*N*′-methyl polystyrene **7** (Scheme 4). Using the optimized reaction conditions for the formation of **2** described above, about 55% (related to [^{75}Se]selenite) of the ^{75}Se-activity was bound on the polymer.

The formation of **6** served again as a model reaction of the alkylation procedure. *N*-cyclohexyl[^{75}Se]selenourea-*N*′-methyl polystyrene in acetonitrile was converted with **3a** to the corresponding selenouronium salt. After hydrolysis and reaction with iodomethane, **6** was obtained with a maximum RCY of 13 ± 5% (related to [^{75}Se]selenite), which is low in comparison with 59% using non-polymer carbodiimide. Several attempts to increase the RCY of **6** via the polymer-supported reaction



7 **8**

Scheme 4. Polymer-supported formation of N-cyclohexyl[^{75}Se]selenourea-N′-methyl polystyrene 8 by reaction of N-cyclohexylcarbodiimide-N′-methyl polystyrene 7 with hydrogen [^{75}Se]selenide 1

Copyright © 2001 John Wiley & Sons, Ltd.

pathway by change of solvents or variation of reaction time and temperature remained unsuccessful.

This remarkable difference in the radiochemical yield of **6** could possibly be explained by a hindered formation of polymer-bound **8**. Obviously, only a small part of the 55% of ^{75}Se-activity detected in the polymer existed as the appropriate [^{75}Se]selenourea due to poor permeability of the [^{75}Se]H_2Se dissolved in diethyl ether to the polymer-supported carbodiimide. The larger amount of activity was possibly bound via adsorption as $^{75}Se^0$ after oxidation of **1** by some surface reaction on the resin. Despite the low radiochemical yield of the polymer-supported pathway, the use of a resin offers the advantage of separating and purifying ^{75}Se-labelled intermediates and products in a very convenient way. Therefore, further investigations with different polymer-bound carbodiimides and their reaction with **1** appear attractive.

Conclusion

Various asymmetric carrier-added [^{75}Se]selenoethers can be prepared within a reaction time of 70–90 min in typical radiochemical yields of 55–60% (related to [^{75}Se]selenite) via 1,3-disubstituted [^{75}Se]selenoureas as intermediates. The advantage of the presented reaction sequence is the possibility to synthesize primary and secondary [^{75}Se]selenoethers with different alkyl moieties. A previously described method for n.c.a. ^{75}Se-labelling only led to methyl[^{75}Se]selenoethers.[11] Furthermore, compared to earlier methods for c.a. ^{75}Se-labelling not useful for automated radiosyntheses, improvement of the polymer-supported pathway appears very attractive for automation.

Due to the probable intermediate formation of diselenides during the reduction of Se^0 to Se^{2-}, it is not possible to synthesize hydrogen [^{75}Se]selenide on the n.c.a. level under the applied conditions. The amount of 10 μmol selenium carrier has to be added at the beginning of the radiosynthesis. Thus, the specific radioactivity of ^{75}Se-labelled products can only reach 370 GBq/mmol when starting with 10 mCi [^{75}Se]SeO_3^{2-}. With this given isotopic dilution, results can directly be transferred to labelling with ^{73}Se.

However, investigations are under way to synthesize asymmetric n.c.a. [^{73}Se]selenoethers via [^{73}Se]selenoureas starting from n.c.a. $^{73}Se^0$. This appears promising for radiopharmaceutical research, because the

Copyright © 2001 John Wiley & Sons, Ltd.

sulphur-atom of various pharmaceutically interesting thioethers (e.g. homocysteine thiolactone, thio fatty acids) can be substituted by the positron-emitter selenium-73 in order to obtain longer lived n.c.a. PET-radiotracers suitable for measurement of slow (patho)physiological processes.

Acknowledgements

The authors thank Dr M. Schfer (Institut fr Organische Chemie, Universitt zu Kln) for recording the high-resolution mass spectra, G. Blessing for preparation of n.c.a. selenium-75 and Dr K. Hamacher for useful discussions (both FZ-Jlich).

References

1. Foster SJ, Ganther HE. *Anal Biochem* 1984; **137**: 205.
2 Knapp FF, Butler TA, Ferren LA, Callahan AP, Guyer CE, Coffey JL. *J Med Chem* 1983; **26**: 1538.
3. Sadek S, Basmadijan GP, Patel A. *Appl Radiat Isot* 1987; **38**: 391.
4. Plenevaux A, Cantineau R, Guillaume M, Christiaens L, Tihange G. *Appl Radiat Isot* 1987; **38**: 59.
5. Plenevaux A, Guillaume M, Brihaye C, Lemaire C, Cantineau R. *Appl Radiat Isot* 1990; **41**: 829.
6. Mushtaq A, Qaim SM, Stcklin G. *Appl Radiat Isot* 1988; **39**: 1085.
7. Ermert J, Hamacher K, Coenen HH. *J Labelled Cpd Radiopharm* 1999; **42**(Suppl. 1): 804.
8. Sadek SA, Basmadijan GP, Ice RD. *Nucl Med Commun* 1982; **3**: 247.
9. Kung HF, Blau M. *J Med Chem* 1980; **23**: 1127.
10. Basmadjian GP, Hetzel KR, Ice RD. *Appl Radiat Isot* 1975; **26**: 695.
11. Schmaljohann J. *Report Jl*-3108, 1995, ISSN 0944-2952.
12. Blau M, Manske RF. *J Nucl Med* 1961; **2**: 102.
13. Bergmann R, Brust P, Kampf G, Coenen HH, Stcklin G. *Nucl Med Biol* 1995; **22**: 475.
14. Blau M. *Biochim Biophys Acta* 1961; **49**: 389.
15. Mayer R, Scheithauer S, Kunz D. *Chem Ber* 1966; **99**: 1393.
16. Krief A, Delmotte C, Dumont W. *Tetrahedron* 1997; **53**: 12147.
17. Gulliver DJ, Hope EG, Levason W, Murray SG, Potter DM, Marshall GL. *J Chem Soc Perkin Trans II* 1984; 429.
18. Klayman DL, Shine RJ. *J Org Chem* 1969; **34**: 3549.
19. Deimer KH, In *Houben-Weyl, Methoden der Organischen Chemie*, Mller E. (ed.), Vol. XV/1. Thieme: Stuttgart, 1958; 316.

Copyright © 2001 John Wiley & Sons, Ltd.

20. Itoh M, Hagiwara D, Kamiya T. *Bull Chem Soc Jpn* 1977; **50**: 718.
21. Blessing G, Lavi N, Hashimoto K, Qaim SM. *Radiochim Acta* 1994; **65**: 93.
22. Zetschke F, Pinske H. *Chem Ber* 1941; **74**: 1022.
23. Gutbier A, Rohn E. *Z Anorg Ch* 1903; **34**: 449.
24. Stejskal R, Kronrad L, Cifka J. *Appl Radiat Isot* 1971; **22**: 254.
25. Chu S-H, Mautner HG. *J Org Chem* 1962; **27**: 2899.
26. Segaloff A, Gabbard MD, Gabbard RB. *Steroids* 1965; **5**: 219.

Copyright © 2001 John Wiley & Sons, Ltd.