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Chemical Synthesis of L-[^{75}Se]Selenomethionine in a High-activity Scale

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A new chemical synthesis of [^{75}Se]selenomethionine elaborated in 1987 has been transferred to a high-activity scale using a remote-handling procedure which is described.

Introduction

Plenevaux *et al.* (1987) described a novel fast chemical synthesis of L-[^{75}Se]selenomethionine of high specific activity. This procedure allowed preparing 100–200 mCi of the title compound according to Scheme 1 in less than 3 h.

$$^{75}\mathrm{Se} + \mathrm{CH_3Li} \rightarrow \mathrm{CH_3{}^{75}SeLi} \qquad (1)$$

$$\mathrm{CH_3{}^{75}SeLi} + \underset{\mathrm{ABB}}{\mathrm{Br{-}(CH_2)_2{-}CH(NH_2){-}COOH}} \rightarrow$$

$$\mathrm{LiBr} + \underset{^{75}\mathrm{SeM}}{\mathrm{CH_3{-}{}^{75}Se{-}(CH_2)_2{-}CH(NH_2){-}COOH}} \qquad (2)$$

Scheme 1. Synthesis of L-[^{75}Se]selenomethionine according to Plenevaux *et al.* (1987).

In our opinion this technique, which is faster and more up-to-date than the biochemical procedure of Blau and Bender (1962) used so far, should be suitable for synthesizing L-[^{75}Se]selenomethionine (^{75}SeM) on a curie scale. Of necessity, a synthesis of ^{75}SeM in a high-activity scale has to be carried out in a box under remote-handling. We have elaborated such a process which is reported here.

Inactive and Tracer Experiments

The synthesis according to Scheme 1 has to be carried out under exclusion of O_2 and moisture. We used dry O_2-free argon. Placing metallic selenium in THF and bubbling argon, methyl lithium in ether solution was added in drops. The disappearance of the black selenium could be well observed. After adding the ethanolic solution of L-α-amino-γ-bromobutyric acid hydro-bromide (ABB·HBr) the quantitative conversion of CH_3SeLi took place within 5 min and, in contrast to Plenevaux *et al.* (1987), without heating the reaction mixture. We decided on a reaction time of 30 min.

In the following work-up, acidification of the alkaline reaction mixture was necessary. After adding HCl, excessive ABB was converted into the hydrochloride of α-amino-γ-hydroxybutyric acid (AHB·HCl). We found that the lithium halides could be advantageously separated from SeM and AHB by using a cation exchange procedure.

Some results of inactive experiments are listed in Table 1. They show that a high yield of about 90% of SeM can be obtained. Quantitative conversion of metallic selenium to CH_3SeLi could be achieved by using CH_3Li in an about tenfold molar amount.

For analyzing t.l.c. on silufol (Kavalier, u.v. 254, CSFR) was used. Because of the sensitivity of CH_3Li and CH_3SeLi to air and moisture the first chromatogram of the reaction mixture was made after adding HCl. The plate was developed in acetone/methylethyl ketone (MEK)/NH_3/H_2O (60:30:20:10). LiI can be visualized under u.v., SeM and AHB as red spots on spraying the chromatogram with ninhydrine solution (1 g ninhydrine + 2.5 g cadmium acetate + 10 mL acetic acid, filled up to 500 mL with ethanol. See "Anfärbereagentien für Dünnschicht-und Papierchromatographie", E. Merck, Darmstadt (1980)] and heating. The following R_f values were found: LiI (0.6), SeM (0.36), AHB (0.2). The second chromatogram was made after the cation exchange process. Now, only SeM and AHB could be detected.

In the tracer experiments natural selenium which had been irradiated in a reactor was worked up as described above. After synthesis and cation exchange

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Table 1. Reaction parameters of the inactive experiments and results of the cation exchange separation process

		According to Plenevaux *et al.* (1987)	Experiment 1	Experiment 2	Experiment 3
Selenium	(mg)	1.05	5.1	5.1	5.6
	(μmol)	13.3	65.0	65.0	70.9
CH_3Li	(μmol)	320	600	600	600
CH_3Li/Se		24	9.2	9.2	8.5
ABB·HBr	(μmol)	30	152	156	152
ABB·HBr/Se		2.25	2.34	2.4	2.1
Total mass Before catex	(mg)[a]	—	198	202	209
SeM + AHB After catex	(mg)[b]	—	20.6	21.4	20.6
Yield (%)[c]		—	90	91	88

[a] The total mass of substances (SeM + AHB + lithium halides) before the cation exchange process was determined after lyophilizing the hydrochloric acid solution.

[b] The mass of the amino acids (SeM + AHB) after the cation exchange process was determined after lyophilizing the ammonia eluate solution. SeM and AHB are present as free acids.

[c] The yield is in relation to quantitative conversion of selenium in reaction (1) and of CH_3SeLi in reaction (2).

separation, radiograms on silufol were obtained as demonstrated in Fig. 1. Labelled SeM represented the main activity. In each experiment a small percentage of activity (2.5–3%) was reproducibly found at a polar by-product which is believed to be an oxidized form of selenomethionine (Cree *et al.*, 1979).

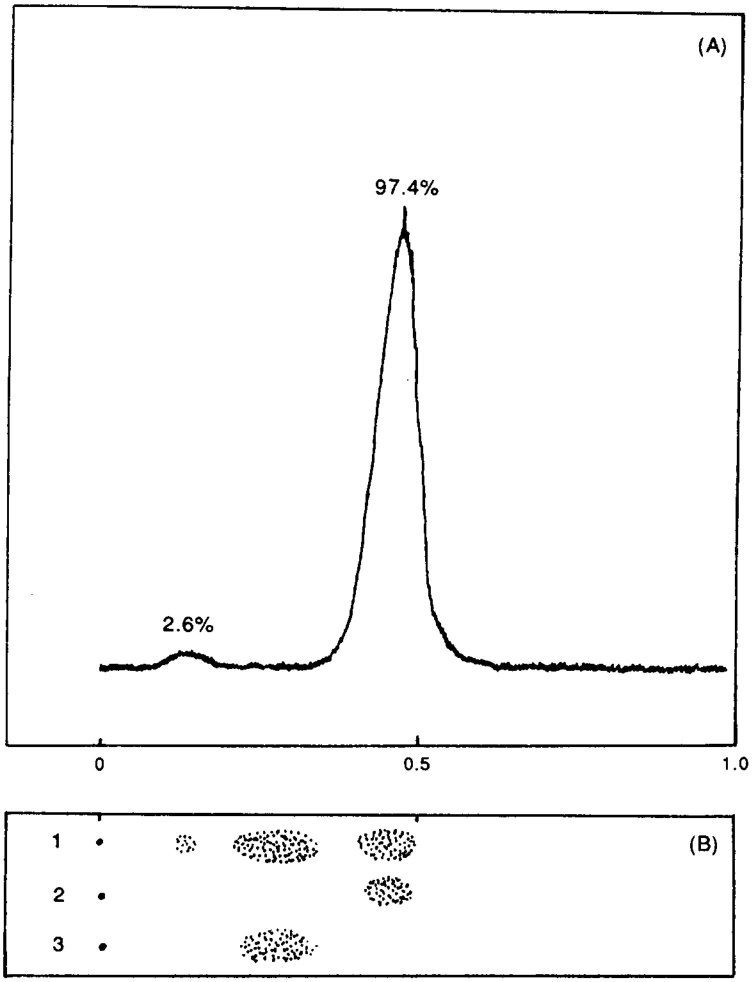

Fig. 1. Radiogram (A) and t.l.c. (B) after cation exchange separation of a tracer experiment. 1, Radioactive solution; 2, inactive selenomethionine; 3, AHB.

Curie Scale Experiments Under Remote-handling

The reactions Se → CH_3SeLi and CH_3SeLi → SeM require the exclusion of air and moisture. Further requirements result from the nuclide properties of ^{75}Se ($t_{1/2} = 120.4$ d; $E(\gamma)_{max} = 0.4$ MeV). All the requirements and conditions important for a successful procedure are listed in the following:

- The reactions have to be carried out under argon.
- Processing ^{75}Se in a curie scale requires remote-handling.
- Under remote-handling only simple procedure steps are realizable.
- The rather small amount of the irradiated selenium powder has to be transferred quantitatively from the irradiation ampoule into the reaction vessel. It is desirable that the arrival of the irradiated selenium in the reaction vessel should be signalled in some fashion.
- Adding CH_3SeLi or ABB through rubber tubings from the outside is not possible.
- It should be advantageous to be able to control on line the course of the synthesis reactions.
- High yield is to be achieved.
- Synthesis, work-up and purification should be carried out within a few hours.
- Radiation protection and a sure handling of high ^{75}Se activity are to be ensured.

Paying attention to these points a new ampoule smashing apparatus was constructed. This apparatus was installed in a box equipped with simple manipulators. The arrangement of the ampoule smashing mechanism and reaction vessel is shown in Fig. 2.

The apparatus consists of a block of metal A and two working cylinders with two pistons S which are working against one another and can be quickly moved pneumatically. The metal block A has a circular opening. On top the opening is closed by a metal cover C having a socket (NS 7.5) for the quartz ampoule which contains the irradiated selenium powder. After breaking the ampoule by the working pistons, the irradiated selenium and quartz fragments fall into the reaction vessel R which is connected with A by a ground joint (NS 29) and held by a pneumatic stroke table LJ. The arrival of the ^{75}Se in the reaction flask is indicated by an activity detector K_1.

The principle of opening the ampoule with the irradiated selenium by the smashing mechanism described above was transferred to the reagent ampoules containing CH_3Li in ether and ABB. HBr in ethanol, respectively. Covers with an NS 14.5 socket were used to hold the ampoules. In order to minimize the amount of broken glass, only the point of the ampoule was destroyed. Before breaking the ampoule point the reagent solution within was frozen in liquid nitrogen. Replacing the cover under flowing argon is not dangerous.

1. Synthesis according to Scheme 1

Tetrahydrofuran (THF, anhydrous, 4 mL) is filled into an absolutely dry two-necked flask R which is introduced into the box, then equipped with an argon inlet tube IT and secured by the stroke table LJ. Argon is turned on and the apparatus is covered by the metal cover C_1 containing the ^{75}Se ampoule. After destroying the ampoule, metallic ^{75}Se (7.5 mg = 100 μmol) and quartz fragments fall into the flask R. Activity is indicated by K_1. Now C_1 is substituted by the cover C_2 containing the ampoule with the frozen etheric CH_3Li solution (900 μmol in about 1 mL dry ether). After smashing the ampoule point and waiting for a moment, CH_3Li solution begins to drop into THF and the black selenium disappears.

Simultaneously, the activity value measured by K_1 decreases because $CH_3{}^{75}SeLi$ is distributed throughout the solution. When K_1 activity value has become constant (after a few minutes), C_2 is substituted by the cover C_3 containing the ampoule with the frozen ethanolic ABB·HBr solution (220 μmol in 1.0 mL EtOH). The ampoule is opened and after a short time ABB·HBr solution starts dropping into the reaction mixture. The start of the reaction (2) is accompanied by brief foaming because of excessive CH_3Li being destroyed by ethanol. On adding ABB·HBr solution the K_1 activity value decreases slightly, followed by an increase in the next few minutes owing to solvent volatilizing. A viscous solution is formed. About 30 min after adding ABB·HBr the reaction (2) is complete.

2. Further work-up

The further work-up can be seen in Fig. 3. The cover C_3 is removed. A glass tube B with a cone (NS 19) is set into the apparatus. HCl (0.1 N, 7.5 mL) is added from the outside via tap 1 and tube B directly into the reaction vessel. Because of the increased volume the K_1 activity value decreases significantly. An acid solution containing lithium halides and the hydrochlorides of ^{75}SeM and AHB is present in the reaction flask. Argon is turned off. Then the acid solution is transferred by vacuum via the three-way tap 2 into the storage vessel D, with tap 3 being opened. From there it is passed on without vacuum via tap 4 onto the top of the column E, which is filled with cation exchange resin (Wofatit KPS, 8% DVB, 160–320 μm, H^+-form, neutral, 15 mmol capacity). Li^+ and the protonated amino acids are bound to the resin with HCl, HBr and HI being eluted. The resin is washed with water repeatedly. The water is added from the outside via tap 1 and glass tube B into the flask R, followed by the same procedure as with the radioactive solution. Eluted washing solutions are discarded via the three-way tap 5 and vessel G. After washing 2.5% NH_3 solution is filled into the flask R and transferred to the column E. The weakly bound amino acid cations are neutralized and substituted

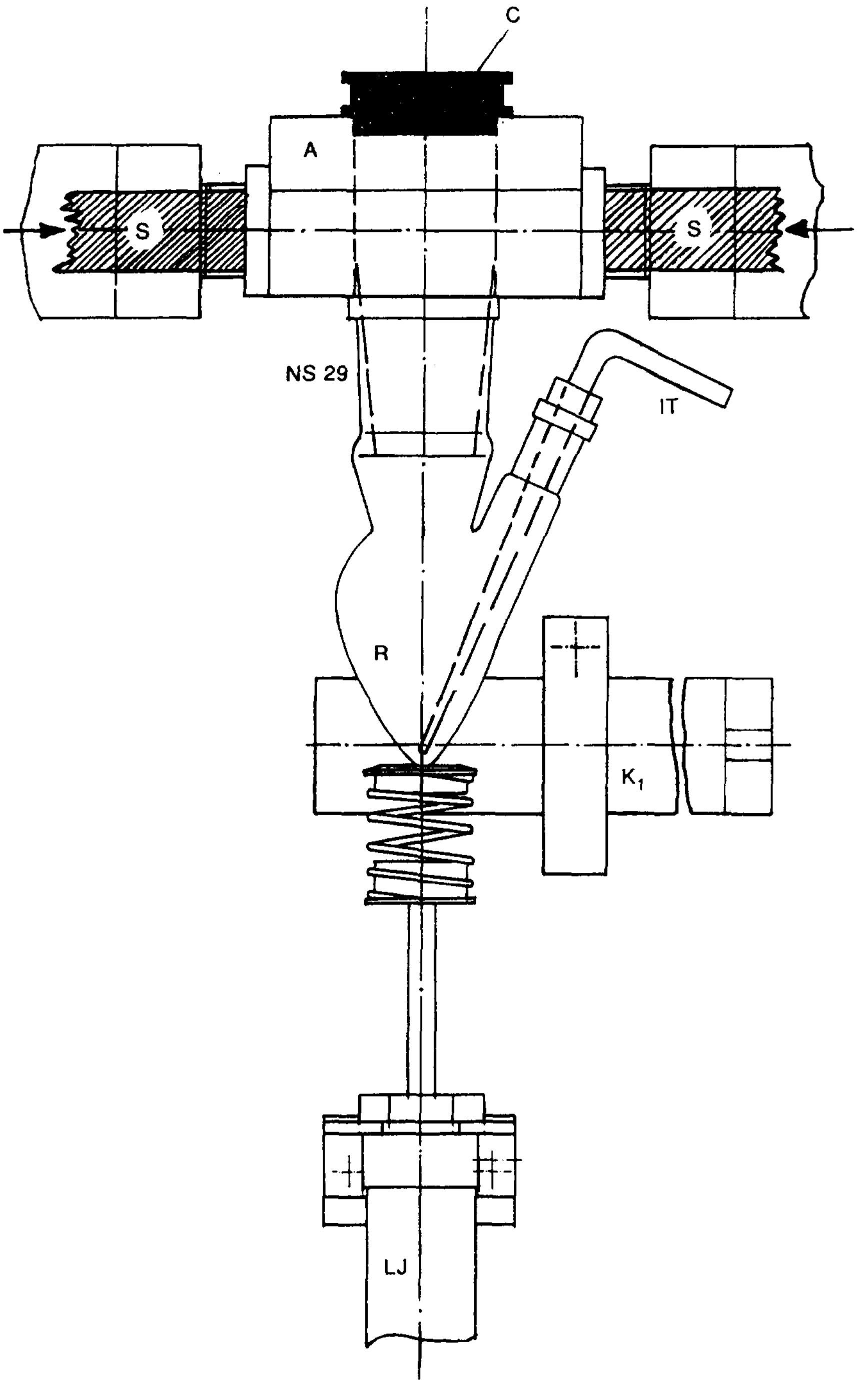

Fig. 2. Ampoule smashing apparatus. A, Metal block; C, metal cover for holding ampoule; S, pistons of the working cylinders; R, reaction bulb; IT, argon inlet tube; K_1, activity detector; LJ, stroke table.

by NH_4^+. Free amino acids (^{75}SeM, AHB) are eluted. Their elution is indicated by the detector K_2. The radioactive solution is collected in a storage vessel H. The solution is then heated by an electric stove ES. Ammonia is volatilized and water is evaporated into vessel I so that only a few milliliters are left. The evaporated solution is transferred by vacuum via the three-way tap 6 into the storage vessel J, with tap 7 being opened. The ^{75}SeM solution is transferred via tap 8 onto the chromatographic column CC for fine purification. The pure final product solution is collected in the flask F. Radioactive by-products eluted from CC (the activity of which is also indicated by the detector K_2) are discarded via the three-way tap 11 into the waste vessel WV. Waste solutions from the vessels G and I are discarded via the taps 9 and 10 into the same waste vessel WV.

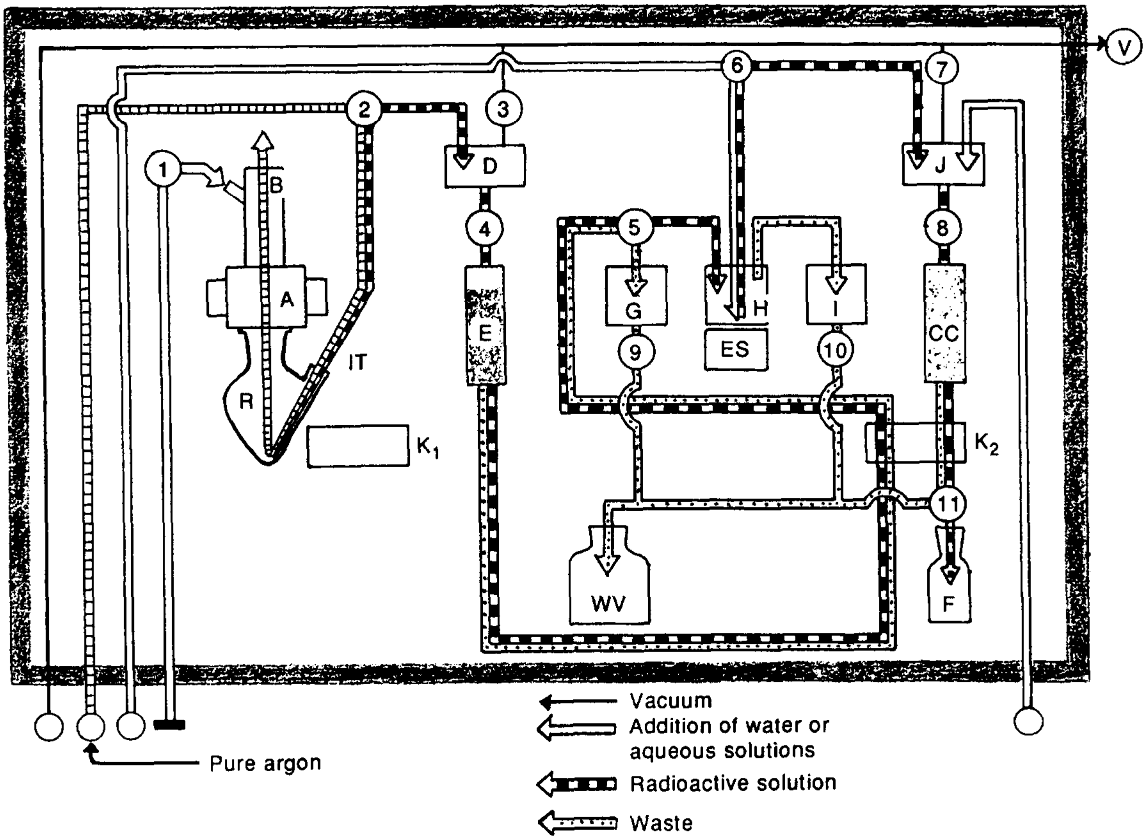

Fig. 3. Scheme of work-up and purification of the high-activity scale [^{75}Se]selenomethionine. 1, 2, 3, . . . , 11, Taps; A, ampoule smashing apparatus; B, glass tube for adding water or aqueous solutions; CC, chromatographic column filled with LiChroprep RP-18; D, storage vessel; E, cation exchange column; ES, electric stove; F, final solution; G, vessel for separated halides; H, storage vessel for radioactive solution to be heated; I, condenser; IT, inlet tube for argon and suction pipe for radioactive and other aqueous solutions; J, storage vessel; K, activity detectors; R, reaction bulb; V, vacuum pump; WV, waste vessel.

3. Reversed-phase chromatographic purification of ^{75}SeM

In tracer experiments only a small percentage of a radioactive by-product (2.5–3%) was registered in the ^{75}SeM final solution. In the experiments with ^{75}Se of high activity, however, the by-product was found to be about 7%. Therefore, a further purification process was necessary.

LiChroprep RP-18 (Merck) is frequently used as column material for HPLC. Its usefulness for our separation problem was investigated under conditions of normal pressure.

A column (15 mm dia) with a G2 frit at the bottom was filled with LiChroprep RP-18 (40–63 μm; suspended in ethanol) up to a bed height of about 200 mm. Then the ethanol was replaced by water.

Three experiments were performed under identical conditions. ^{75}SeM from a highly-active test (content of by-product 7.1%) was used. The column was fed with 5 mL of a solution containing ^{75}SeM (about 1 mCi), inactive selenomethionine (10 mg) and AHB (10 mg). Except for the amount of activity, these conditions are present in working up the highly-active ^{75}SeM to be purified. The column was eluted with water, and the eluate was collected in 10 min fractions.

Column and fractionating conditions:

Bed height	205 mm
Column dia	15 mm
Drop time	0.19 min
Fractionating time	10 min
Fractionating volume	3 mL

The activity values of the fractions measured are represented in a diagram (Fig. 4). Fractions belonging together were collected as fraction collections 1 and 2 (FC 1, FC 2). FC 1 and FC 2 were evaporated and their radiochemical purity was determined by radio t.l.c. The activities of FC 1 and FC 2 were measured and compared with the starting activity.

The elugram in Fig. 4 shows that the fractionating conditions mentioned above allow a quantitative separation of the by-product (FC 1) from ^{75}SeM (FC 2). This fact is also demonstrated by the radio t.l.c. of FC 1 and FC 2. The radiograms are shown in Fig. 5. Pure substances are present. As can be further seen from Fig. 5, AHB is mainly present in FC 1.

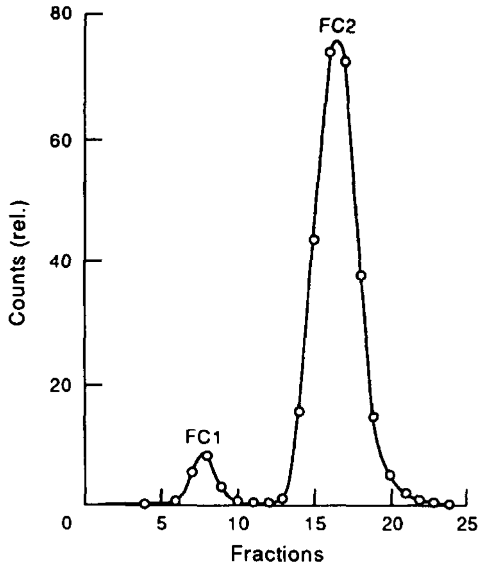

Fig. 4. Elugram of an aliquot of high-activity scale [^{75}Se]selenomethionine. FC1, Fraction collection of the by-products; FC2, fraction collection of [^{75}Se]selenomethionine. For column and fractionating conditions see text.

This fact allowed for the first time an exact determination of the specific activity of ^{75}SeM. This was proved in some tracer experiments with ^{75}Se of known specific activity. The specific activity of the RP-purified ^{75}SeM obtained here was in a good agreement with that of ^{75}Se.

4. *Activity balance, specific activity, activity yield*

After the reaction mixture and washing water have been transferred to the cation exchange column in our experiments no unreacted ^{75}Se was detected in the reaction bulb R.

The results of three experiments in half curie scale are summarized in Table 2. As can be seen, ^{75}Se activities in half curie scale are easily reached by irradiating some few milligrams ^{74}Se (90% enrichment) over a period of some weeks at a high neutron flux. The specific activity of the ^{75}Se and the ^{75}SeM obtained was 8–10 Ci/mmol.

Materials

For preparing ^{75}Se in the reactor, enriched ^{74}Se (90%) was used as a metallic powder. CH_3Li was

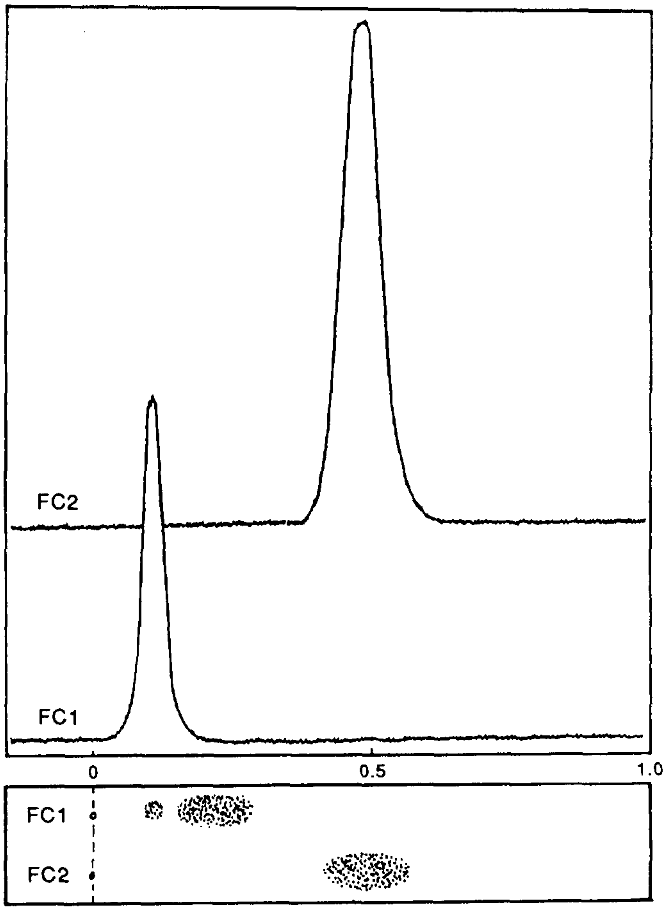

Fig. 5. Radiograms and t.l.c. of the two fraction collections from Fig. 4.

Table 2. Results of three experiments in half curie scale

No.	Place of irradiation	Irradiation time (h)	^{74}Se irradiated (mg)	^{75}Se A_0(mCi)	Activity A(mCi)	Yield (%)
1	Belgium[a]	200	5.0	730	480	65.7
2	Rossendorf[b]	400	7.0	810	580	71.6
3	Rossendorf[b]	400	7.5	780	540	69.0

[a] $5 \cdot 10^{14}$ n/cm^2 s.
[b] $2 \cdot 10^{14}$ n/cm^2 s.

prepared according to data by Schöllkopf summarized in Houben-Weyl (Schöllkopf, 1970). The synthesis of L-ABB · HBr was carried out as published by Jost and Rudinger (1967). The fact that pure L-SeM had actually been synthesized was confirmed by using the new chiral derivation reagent $S(-)$-N-1-(2-naphthyl-sulphonyl)-2-pyrrolidinecarbonyl chloride, synthesis of which was published by Nishi *et al.* (1989), and by chiral-phase HPLC. For cation exchange separations Wofatit (8% DVB, 160–320 μm dia) in the H$^+$-form was used. The column for chromatographic purification of ^{75}SeM was filled with LiChroprep RP-18 (Merck, Fed. Rep. Germany, 40–63 μm).

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