ATTACHMENT

7. EXPERIMENTAL PART

All chemicals were purchased from Sigma-Aldrich Chemie GmbH (Schnelldorf, Germany).

For purification by column chromatography, silica gel 60 (0.063–0.2 mm) with particle size 70–230 mesh from Macherey-Nagel was chosen. Mobile phases are described as part of the individual procedures.

Progress of the reaction and product purity were monitored via TLC on plates *SILUFOL UV 254/366*. Mobile phases are mentioned in the individual reactions. UV detection was performed at a wavelength of 254 nm.

Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance 500 spectrometer. The measuring frequency was 500 MHz for ^{1}H-NMR, 125 MHz for ^{13}C-NMR. Measurements were performed at room temperature and $CDCl_3$ or DMSO-d_6 was used as solvent.

A triple Quadrupole LC/MS/MS System from AB Sciex API 2000, Mundelein, Illinois 60060 USA, was used for recording of mass spectra. The mass spectrometer was operated in the positive ion mode. The compounds were dissolved in HPLC grade acetonitrile prior the analysis. This system was used to characterise compound 34.

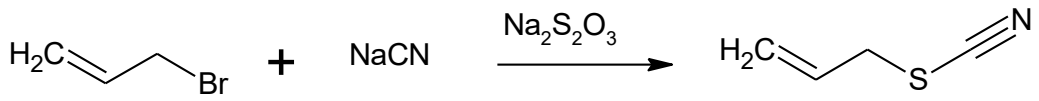
Liquid chromatography coupled with mass spectrometry (LC/MS) was performed on a SpectraSYSTEM™ HPLC and Thermo Scientific™ MSQ Plus™ Single Quadrupole Mass Spectometer using a Bischoff Lambda 1000 UV/VIS at a wavelength of 275 nm, YMCC 18 column and methanol/water (85:15) as mobile phase at a flow rate of 1.0 ml/min. This system was used to characterise compound 41.

7.1 Synthesis of 5-[(prop-2-en-1-yl)sulfanyl]-1*H*-tetrazole (compound 25)

7.1.1 Synthesis of allyl thiocyanate (compound 23)

First, 13 ml of absolute ethanol was added to sodium thiosulfate pentahydrate (9.93 g, 0.04 mol) in a 250-ml round-bottom flask. The flask was attached to a reflux condenser and heated on an oil bath to 100 °C. After 30 min, when the sodium thiosulfate pentahydrate had dissolved, allyl bromide (compound 22) (1.21 g, 0.01 mol) was added. Over a period of 45 min 100 ml of distilled water were added through the top of the reflux condenser in approximately 25 ml segments. The heating was stopped after 2 h, the flask closed with a stopper and externally cooled in an ice bath to promote rapid cooling.

Thereafter a solution of sodium cyanide (1.96 g, 0.04 mol) in 150 ml of ice cold water was added. The stoppered flask was allowed to stand in an ice bath for 30 min with occasional swirling. The mixture was being continuously extracted three times, each time with 40 ml of diethyl ether. The organic extract was dried over anhydrous magnesium sulfate until the solution became clear and was then filtrated and evaporated under reduced pressure. The yellow liquid product (compound 23) was stored in the refrigerator in a dark flask for further use, because of the danger of oxidation. The product yield was 0.85 g.



22 **23**

Mw: 99.15 g/mol

Molecular Formula: C_4H_5NS

Appearance: yellow liquid

Yield: 0.85 g

TLC: R_f = 0.80 (80 % *n*-hexane, 20 % ethyl acetate)

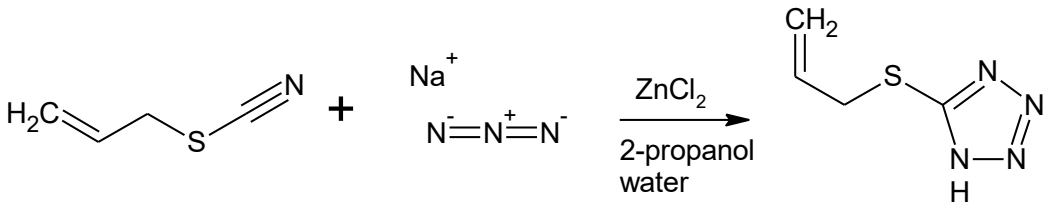
^{1}H NMR (500 MHz, $CDCl_3$) δ 5.96 – 5.85 (m, *J* = 17.0, 9.8, 7.3 Hz, 14H), 5.41 – 5.30 (m, 29H), 5.30 – 5.23 (m, 2H), 3.55 (d, *J* = 7.3, 1.2, 0.6 Hz, 28H) ppm.

^{13}C NMR (125 MHz, $CDCl_3$) δ 130.81 (s), 130.61 (s), 121.76 (s), 112.05 (s), 37.01 (s) ppm.

7.1.2 Click reaction of allyl thiocyanate

Sodium azide (compound 24) (0.33 g, 0.005mol), water and 2-propanol (7ml and 2 ml) and zinc chloride (0.68 g, 0.005 mol) were stirred overnight and then allyl thiocyanate (compound 23) (0.02 g, 0.0002 mol) was added. The solution was again stirred overnight at room temperature.

The mixture was afterwards extracted three times, each time with 20 ml of ethyl acetate. The organic layer was dried over anhydrous magnesium sulfate and filtrated. After that the ethyl acetate was evaporated under reduced pressure. The yield of product 5-[(prop-2-en-1-yl)sulfanyl]-1*H*-tetrazole (compound 25) was 0.023 g.



23 **24** **25**

Mw: 142.18 g/mol

Molecular Formula: $C_4H_6N_4S$

Appearance: yellow liquid

Yield: 0.023 g

TLC: R_f = 0.88 (50 % *n*-hexane, 50 % ethyl acetate)

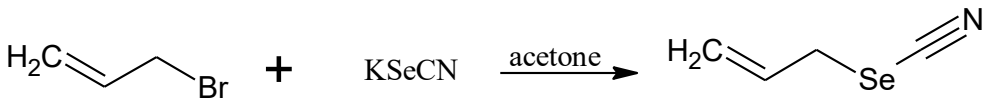
^{1}H-NMR (500 MHz, $CDCl_3$) δ 5.90 (ddt, *J* = 17.0, 9.8, 7.3 Hz, 1H), 5.41 – 5.28 (m, 2H), 3.54 (ddd, *J* = 7.3, 1.2, 0.7 Hz, 2H) ppm.

^{13}C-NMR (125 MHz, $CDCl_3$) δ 130.53 (s), 121.39 (s), 111.75 (s), 36.67 (s) ppm.

7.2 Synthesis of 5-(allylselanyl)-1*H*-tetrazole (compound 27)

7.2.1 Synthesis of allyl selenocyanate (compound 26)

To the allyl bromide (compound 22) (0.36 g, 0.003 mol) potassium selenocyanate (KSeCN) (0.43 g, 0.003 mol) dissolved in 10 ml of acetone was added. The mixture was stirred for 72 h at room temperature in the fume hood. Acetone was then air-dried overnight and a gray solid product of allylselenocyanate (compound 26) with a strong odor of allyl was obtained. The product yield was 0.30 g. The product was immediately used without further purification.



22 **26**

Mw: 146.05 g/mol

Molecular Formula: C_4H_5NSe

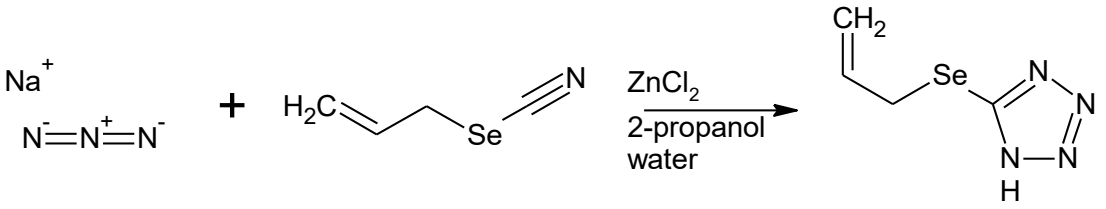
Appearance: gray solid

Yield: 0.30 g

TLC: R_f= 0.66 (50 % *n*-hexane, 50 % diethyl ether)

7.2.2 Click reaction of allyl selenocyanate

Allyl selenocyanate (compound 26) (0.03 g, 0.0002 mol), sodium azide (compound 24) (0.33 g; 0.005 mol), 7 ml of distilled water and 3 ml of 2-propanol was stirred for 48 h at room temperature. The solvent was evaporated under reduced pressure to gain 5-(allylselanyl)-1*H*-tetrazole (compound 27).



24 **26** **27**

Mw: 189.08 g/mol

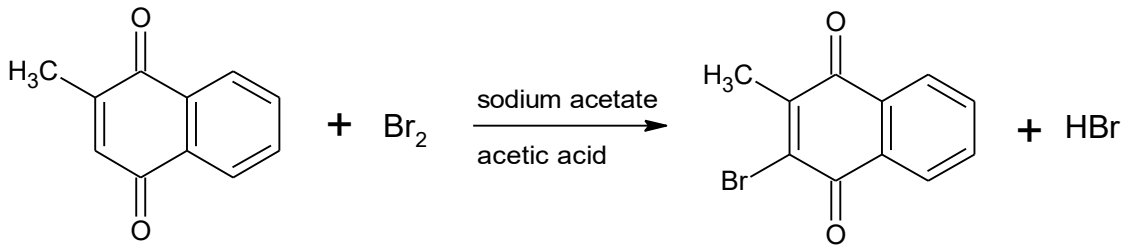
Molecular Formula: $C_4H_6N_4Se$

7.3 Synthesis of 2-(4-(((4-chlorophenyl)selanyl)methyl)-1*H*-1,2,3-triazol-1-yl)-3-methylnaphthalene-1,4-dione (compound 34)

7.3.1 Synthesis of 2-bromo-3-methylnaphthalene-1,4-dione (compound 29)

2-methylnaphthalene-1,4-dione (compound 28) (5.5 g, 0.03 mol) was dissolved in 50 ml of acetic acid, then freshly fused sodium acetate (10.66 g, 0.13 mol) was added and the solution was cooled to incipient crystallization before adding 2 ml (10 % in excess) of dry bromine. The flask was stoppered and allowed to stand in the dark for 72 h.

A yellow crystalline mass then separated. The entire content was poured into 300 ml of distilled water and the precipitate was filtered off. It was purified by recrystallization from methanol and bright yellow needles of compound 29 were obtained. The product yield was 6.40 g.



28 **29**

Mw: 251.08 g/mol

Molecular Formula: $C_{11}H_7BrO_2$

Appearance: yellow needles

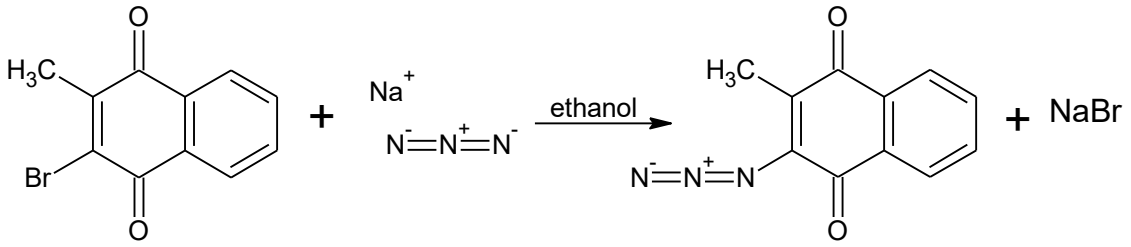
Yield: 6.40 g

TLC: R_f= 0.62 (98 % *n*-hexane, 2 % ethyl acetate)

7.3.2 Synthesis of 2-azido-3-methylnaphthalene-1,4-dione (compound 30)

2-bromo-3-methylnaphthalene-1,4-dione (compound 29) (2.00 g, 0.008 mol) was dissolved in 75 ml of 95% ethanol. Sodium azide (compound 24) (0.72 g, 0.01 mol) dissolved in 30 ml of ethanol was then added to the solution. The solution was heated for a few min in order to obtain a clear solution and then stirred in the dark at room temperature for 3 h.

Cooling the reaction solution with ice precipitated 0.51 g of 2-azido-3-methylnaphthalene-1,4-dione (compound 30). By adding 15 ml of distilled water to the filtrate, another 0.42 g of compound 30 precipitated. The total product yield was 0.93 g.



29 **24** **30**

Mw: 213.19 g/mol

Molecular Formula: $C_{11}H_7N_3O_2$

Appearance: dark yellow solid

Yield: 0.93 g

TLC: R_f= 0.80 (98 % *n*-hexane, 2 % ethyl acetate)

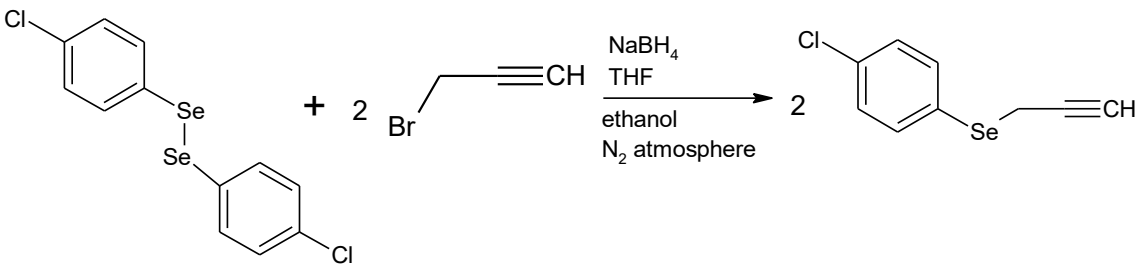
^{1}H NMR (500 MHz, $CDCl_3$) δ 8.24 – 8.06 (m, 2H), 7.82 – 7.65 (m, 2H), 2.40 (s, 3H) ppm.

^{13}C NMR (125 MHz, $CDCl_3$) δ 182.68 (s), 177.64 (s), 149.65 (s), 135.32 (s), 133.59 – 130.73 (m), 127.20 (s), 18.59 (s) ppm.

7.3.3 Synthesis of (4-chlorophenyl)(prop-2-yn-1-yl)selane (compound 33)

A two neck round bottom flask containing sodium borohydride (0.38 g, 0.01 mol) was purged with nitrogen. With a syringe 10 ml of absolute ethanol and 1,2-*bis*(4-chlorophenyl)diselane (compound 31) (0.66 g, 0.002 mol) dissolved in 6 ml of DMF were added. The solution was stirred for 30 min at 70°C and had turned yellow as a result. Subsequently, propargyl bromine (compound 32) (1 ml, 0.01 mol) dissolved in 10 ml of THF was added to the reaction mixture and the mixture was stirred for another 3 h at 70°C.

The solution was poured into 15 ml of distilled water and the product was extracted three times, each time with 15 ml of diethyl ether. Product was dried over $MgSO_4$, filtrated and evaporated under reduced pressure. Product was purified by column chromatography using a gradient mixture of *n*-hexane and ethyl acetate with increasing polarity as eluent. The yield of compound 33 was 0.34 g.



31 **32** **33**

Mw: 229.56 g/mol

Molecular Formula: C_9H_7ClSe

Appearance: brown solid

Yield: 0.34 g

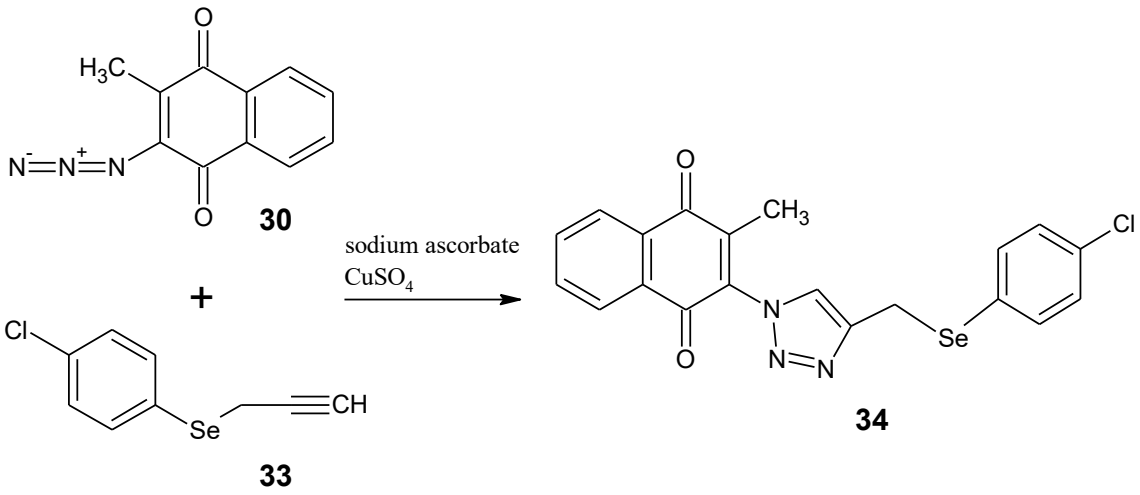
TLC: R_f= 0.65 (80 % *n*-hexane, 20 % ethyl acetate)

^{1}H NMR (500 MHz, DMSO-d_6) δ 7.57 – 7.52 (m, *J* = 1.3 Hz, 100H), 7.39 – 7.24 (m, 157H), 3.12 (t, *J* = 2.7 Hz, 46H), 1.98 (s, 2H) ppm.

^{13}C NMR (125 MHz, DMSO-d_6) δ 131.98 (s), 129.96 (s), 129.51 (s), 127.40 (s), 81.65 (s), 74.33 (s), 11.62 (s) ppm.

7.3.4 Click reaction of (4-chlorophenyl) (prop-2-yn-1-yl)selane

The alkyne, (4-chlorophenyl) (prop-2-yn-1-yl)selane (compound 33) (0.459 g, 0.002 mol) and the 2-azido-3-methylnaphthalene-1,4-dione (compound 30) (0.416 g, 0.002 mol) were added to a round bottom flask and dissolved in 10 ml of DCM. $CuSO_4$ (0.079 g, 0.0005 mol) and sodium ascorbate (0.118 g, 0.0006 mol) were dissolved in 10 ml of distilled water and added to the reaction mixture. The solution was stirred at room temperature for 72 h. The product was poured into 10 ml of distilled water and extracted three times, each time with 10 ml of DCM. The combined organic layers were dried over anhydrous Na_2SO_4 and the solvent was evaporated under reduced pressure. The yield of 2-(4-(((4-chlorophenyl)selanyl)methyl)-1*H*-1,2,3-triazol-1-yl)-3-methylnaphthalene-1,4-dione (compound 34) was 0.16 g.



Mw: 442.76 g/mol

Molecular Formula: $C_{20}H_{14}ClN_3O_2Se$

Appearance: dark red solid

Yield: 0.16 g

TLC: R_f= 0.74 (20 % *n*-hexane, 80 % ethyl acetate)

^{1}H-NMR (500 MHz, Chloroform) δ = 8.01 (s, 1H), 7.82 (m, 2H), 7.73 (q, 2H), 7.35 (m, 2H), 7.10 (m, 2H), 4.05 (m, 3H), 2.42 (s, 2H) ppm.

^{13}C-NMR (125 MHz, Chloroform) δ = 148.9, 135.7, 134.9, 132.2, 129.4, 125.6, 120.8, 30.0, 25.9, 20.3 ppm.

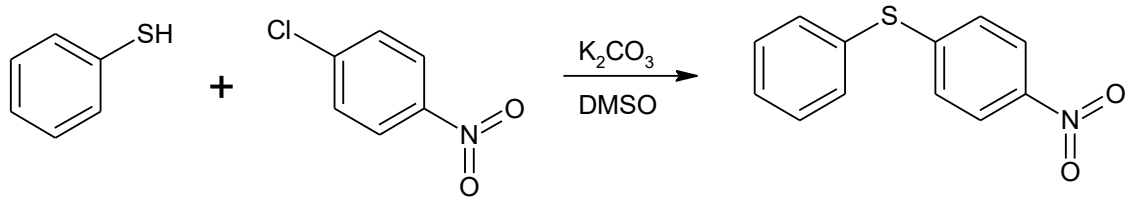
MS (ESI): m/z calculated for $C_{20}H_{14}ClN_3O_2Se$ $[M + H + acetonitrile]^+$: 484.81; found 484.9.

7.4 Synthesis of phenyl(4-selenocyanatophenyl)sulfane (compound 40)

7.4.1 Synthesis of 1-nitro-4-(phenylsulfanyl)benzene (compound 37)

To a solution of benzenethiol (compound 35) (2.64 g, 0.024 mol) in 10 ml DMSO, K_2CO_3 (4.97 g, 0.036 mol) was added. The mixture was stirred for 30 min and then a solution of-1-chloro-4-nitrobenzene (compound 36) (3.78 g, 0.024 mol) in 10 ml DMSO was added dropwise over a period of 5 min at room temperature. The resulting mixture was stirred under reflux for another 5 h at 90°C. After cooling to room temperature, the reaction mixture was poured into 100 ml of distilled water.

The product was filtered off and dried under vacuum to gain 1-nitro-4-(phenylsulfanyl)benzene (compound 37) as light-yellow solid. The product yield was 5.20 g. This compound was used immediately without further purification.



35 **36** **37**

Mw: 231.27 g/mol

Molecular Formula: $C_{12}H_9NO_2S$

Appearance: light-yellow solid

Yield: 5.20 g

TLC: R_f= 0.90 (90 % *n*-hexane, 10 % ethyl acetate)

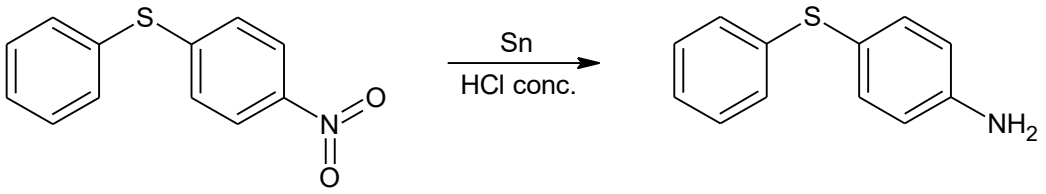
^{1}H-NMR: (500 MHz, DMSO-d_6) δ = 8.20 (d, 2H), 7.72 (d, 2H), 4.95 (s, 1H) ppm.

7.4.2 Synthesis of 4-(phenylsulfanyl)aniline (compound 38)

To a slurry mixture of tin powder (0.83 g, 0.007 mol) in 15 ml concentrated HCl a solution of 1-nitro-4-(phenylsulfanyl)benzene (compound 37) (1.9 g, 0.006 mol) dissolved in 10 ml concentrated HCl was added dropwise over a period of 10 min at 0°C. The resulting mixture was stirred under reflux for 24 h at 80 °C.

The reaction mixture was carefully diluted with NaOH (18.75 g) dissolved in 10 ml of distilled water and a yellow oily product was formed.

The flask was kept at -20 °C for two h to precipitate the product and then filtered to obtain the crude product. The product was purified by column chromatography with a mixture of petroleum ether and ethyl acetate (10:1) as the mobile phase. 4-(phenylsulfanyl)aniline (compound 38) was afforded as a yellow solid. The product yield was 0.98 g.



37 **38**

Mw: 201.29 g/mol

Molecular Formula: $C_{12}H_{11}NS$

Appearance: yellow solid

Yield: 0.98 g

TLC: R_f = 0.85 (90 % *n*-hexane, 10 % ethyl acetate)

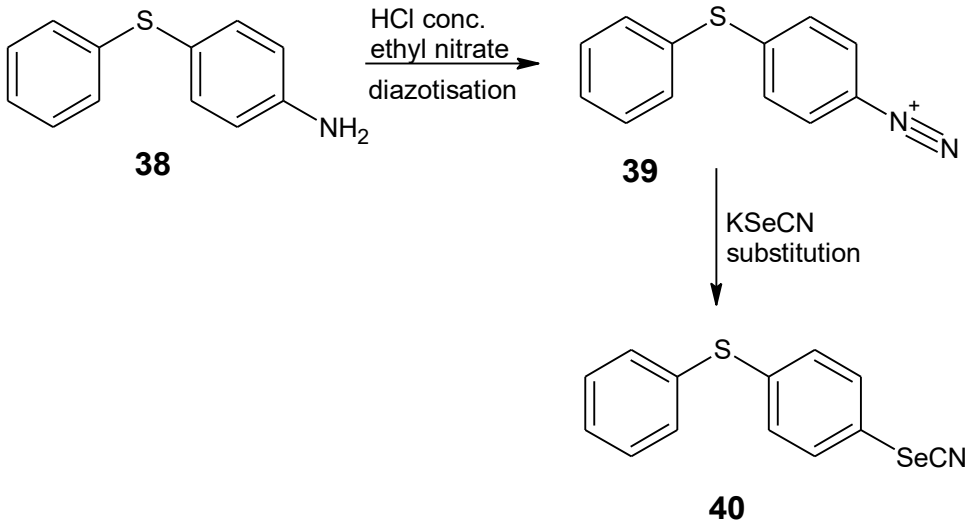
^{1}H-NMR: (500 MHz, DMSO-d_6) δ = 7.33 (m, 5H), 7.20 (q, 2H), 7.01 (d, 2H), 3,92 (d, 2H) ppm.

7.4.3 Synthesis of phenyl(4-selenocyanatophenyl)sulfane (compound 40)

In the next step, 4-(phenylsulfanyl)aniline (compound 38) (2.01 g, 0.01 mol) and concentrated HCl (0.7 ml, 0.02 mol) were mixed slowly. The product, 4-(phenylsulfanyl)anilinium chloride, was collected, air-dried, and dissolved in 30 ml of absolute ethanol. The stirred solution was cooled to between 5 ˚C to 0 ˚C and diazotized by careful dropwise addition (over a period of 30 min) of ethyl nitrite (1 ml, 0.01 mol). Both the solution and ethyl nitrite (boiling point 17 ˚C) had to be cold (-5 to 0 ˚C) during the addition.

After 30 min, a solution of KSeCN (1.44 g, 0.01 mol) in 30 ml of absolute ethanol was added to the 4-(phenylsulfanyl)benzene-1-diazonium (compound 39).

The precipitate obtained was recrystallized from absolute ethanol to yield the final product phenyl(4-selenocyanatophenyl)sulfane (compound 40).



Mw: 290.24 g/mol

Molecular Formula: $C_{13}H_9NSSe$

Appearance: dark red solid

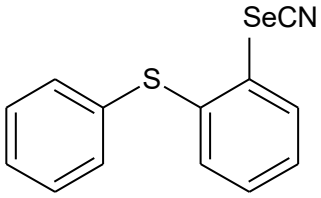
TLC: R_f= 0.65, 0.73, 0.78 (80 % *n*-hexane, 20 % ethyl acetate)

^{1}H-NMR: (500 MHz, DMSO-d_6) δ = 7.6 (m, 5H), 7.4 (d, 2H), 7.3 (q, 2H) ppm.

^{13}C-NMR (125 MHz, DMSO-d_6) δ = 157.1, 135.8, 135.5, 133.3, 131.2, 130.6, 126.8. 126.6, 116.3 ppm.

7.5 Synthesis of phenyl(2-selenocyanatophenyl)sulfane (compound 41)

1-nitro-2-(phenylsulfanyl)benzene (Sigma-Aldrich Chemie GmbH) was used to synthesize 2-(phenylsulfanyl)aniline and subsequently phenyl(2-selenocyanatophenyl)sulfane (compound 41). Compounds were prepared by the same procedures as described for the synthesis of compound 40.



41

Mw: 290.24 g/mol

Molecular Formula: $C_{13}H_9NSSe$

Appearance: dark red solid

TLC: R_f= 0.54, 0.61, 0.69 (90 % *n*-hexane, 10 % ethyl acetate)

^{1}H NMR (500 MHz, DMSO-d_6) δ 7.33 – 7.24 (m, 6H), 7.22 – 7.16 (m, 3H), 7.14 – 7.08 (m, 2H), 7.06 – 7.03 (m, 2H) ppm.

^{13}C NMR (125 MHz, DMSO-d_6) δ 136.74 (s), 130.99 – 130.73 (m), 130.38 (s), 129.12 – 128.44 (m), 126.37 – 125.91 (m), 125.14 (s), 114.74 (s) ppm.

MS (ESI): m/z calculated for C13H9NSSe [M + H + acetonitrile]$^+$: 332,30; found 332,24 and [M + acetonitrile]:331,29, found 331,19.

8. RESULTS AND DISCUSSION

Allyl thiocyanate was followed by TLC, ^{1}H-NMR and ^{13}C-NMR. Apparently allylthiocyanate rearranges to isothiocyanate even at room temperature within a few hours.[1] It was noticeable when the colour had changed from yellow to brown. For this reason it was important to proceed with the click reaction of allyl thiocyanate immediately. In the click reaction tetrazole (compound 25) was created. Even though the sample was sent immediately for testing by TLC, ^{1}H-NMR and ^{13}C-NMR, the product contained impurities.

We tried to prepare tetrazole from allyl selenocyanate. Allyl selenocyanate (compound 26) was synthesized according to the *Encyclopedia of Reagents for Organic Synthesis*[2]. The product was characterised by TLC. The click reaction of allyl selenocyanate was unsuccessful. 5-(allylselanyl)-1*H*-tetrazole (compound 27) was not created in these condition.

$ZnCl_2$ was used as catalyst in both click reactions. Production of tetrazole with azide and organic nitriles were described by *Himo et al.*[3] The zinc ion is bound to the nitrile and the energy barrier of reaction is lower by 5 to 6 kcal/mol.

Huisgen 1,3-dipolar cycloaddition of (4-chlorophenyl)(prop-2-yn-1-yl)selane (compound 33) with 2-azido-3-methylnaphthalene-1,4-dione was found to be successful by TLC, ^{1}H-NMR, ^{13}C-NMR and MS. Sodium ascorbate and $CuSO_4$ were used as catalyst in this click reaction.

2-azido-3-methylnaphthalene-1,4-dione (compound 30) was synthesized from 2-methylnaphthalene-1,4-dione (compound 28) by bromination using dry bromine, acetic acid with sodium acetate followed by azidation with sodium azide to yield 2-azido-3-methylnaphthalene-1,4-dione (compound 30).

(4-chlorophenyl)(prop-2-yn-1-yl)selane (compound 33) was prepared from 1,2-*bis*(4-chlorophenyl)diselane (compound 31) with propargyl bromine, $NaBH_4$ and ethanol under nitrogen atmosphere. The product was purified by column chromatography by using a gradient mixture of *n*-hexane and ethyl acetate with increasing polarity of eluent.

The last two products (compounds 40, 41) contain selenium and sulfur in the structure. They were synthesized in the same way. First, the reduction of the NO_2 group had been performed. The best results were obtained by the reduction with tin and

concentrated HCl and purification by column chromatography with a mixture of *n*-hexane and ethyl acetate. Diazotisation with concentrated HCl and ethyl nitrate following by substitution of the SeCN group as the second step. Both products appeared to face problems with purification.

It was unsuitable to purify compounds by recrystallization. Products contained impurities, because of their reactivity and instability after recrystallization with acetone and ethyl acetate.

Purification by column chromatography with silica gel as stationary phase had also proven to be insufficient, though this approach was better than recrystallization. ^{1}H-NMR and ^{13}C-NMR and MS showed impurities even when the column chromatography was carried out done twice. We assume that the selenocyanate group became partly hydrolysed by the acidic silica.

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1. EMERSON, David W. The preparation and isomerization of allyl thiocyanate. An organic chemistry experiment. *J. Chem. Educ.,* **1971**, *48*(1), 81–82.

2. 41. KRISHNAMURTHY, Venkata Ramanan. Allylselenocyanate. *Encyclopedia of Reagents for Organic Synthesis*. Chichester, UK: John Wiley & Sons, 2001. ISBN 0471936235.

3. 42. HIMO, Fahmi, et al. Why is tetrazole formation by addition of azide to organic nitriles catalyzed by zinc(II) salts? *J. Am. Chem. Soc.,* **2003**, *125*, 9983–9987.