

## Supporting Information

### Dynamic Combinatorial Chemistry with Diselenides and Disulfides in Water

**Brian Rasmussen, Anne Sørensen, Henrik Gotfredsen, and Michael Pittelkow\***

*Department of Chemistry, University of Copenhagen, Universitetsparken 5, DK-2100 Copenhagen, Denmark,  
E-mail: pittel@kiku.dk, Homepage: www.pittelkow.kiku.dk.*

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## S1 General

Unless otherwise stated, all chemicals were purchased from commercial suppliers and used as received. Solvents were HPLC grade and used as received except THF which was tapped from a Solvent Purification System, Innovative Technology, Inc.

$^1\text{H}$ -NMR and  $^{13}\text{C}$ -NMR spectra were recorded at 500 MHz and 125 MHz, respectively, on a Bruker Ultrashield Plus 500 spectrometer using residual non-deuterated solvent as the internal standard. All chemical shifts ( $\delta$ ) are reported in ppm and all coupling constants (J) are expressed in Hertz (Hz).

The following abbreviations are used for convenience in reporting the multiplicity of NMR resonances: s = singlet, br s = broad singlet, d = doublet, q = quartet, and m = multiplet. Samples were prepared using DMSO- $d_6$  purchased from Cambridge Isotope Labs. The NMR data were processed using MestReNova v. 8.0.2. Assignment of  $^1\text{H}$  and  $^{13}\text{C}$  resonances was achieved using the 2D NMR techniques  $^1\text{H}$ - $^1\text{H}$  COSY and  $^1\text{H}$ - $^{13}\text{C}$  HSQC.

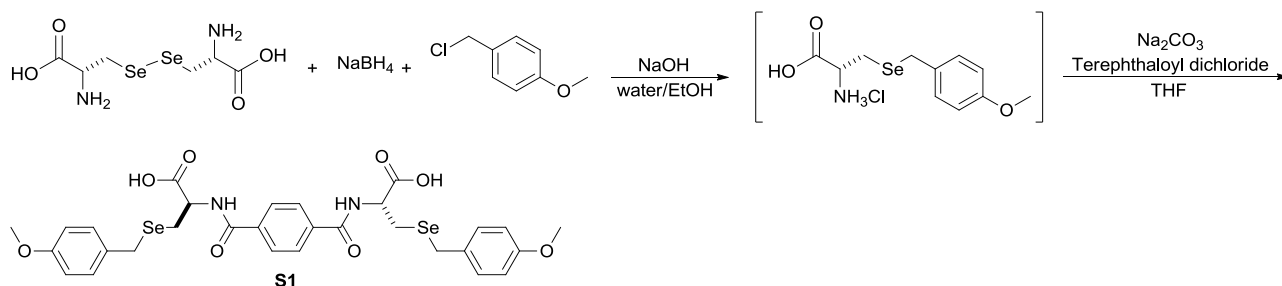
HPLC analyses were performed on a Dionex UltiMate 3000 system coupled to an UltiMate 3000 diode array UV/Vis detector. Separations were achieved using a Dionex Acclaim RSLC 120 C18 2.2  $\mu\text{m}$  120  $\text{\AA}$  2.1  $\times$  100 mm column maintained at 20  $^\circ\text{C}$ . The mobile phase solutions prepared were 0.1 % formic acid in  $\text{H}_2\text{O}$  and 0.1 % formic acid in MeCN. The water used as eluent was purified by a Millipore system. LC/MS was carried out on a Bruker MicroTOF-QII-system with ESI-source with nebulizer 1.2 bar, dry gas 8.0  $\text{L min}^{-1}$ , dry temperature 200  $^\circ\text{C}$ , capillary  $-4500$  V, end plate offset  $-500$  V, funnel 1 RF 200.0 Vpp, ISCID energy 0.0 eV, funnel 2 RF 200.0 Vpp, hexapole RF 100.0 Vpp, quadrupole ion energy 5.0 eV, low mass 100.00  $m/z$ , collision energy 8.0 eV, collision RF 100.0 Vpp, transfer time 80.0  $\mu\text{s}$ , and pre puls storage 1.0  $\mu\text{s}$ . The LC/MS data were processed using DataAnalysis v. 4.0 SP 5. For Se-containing ions in the mass spectrum, only the main selenium isotope ( $^{80}\text{Se}$ ) is reported.

In the processing of HRMS measurements, a sodium formate calibrant solution eluting in the first part of the run was used to calibrate the system in each measurement.

## S2 Synthetic Procedures

Compound (**1**)<sub>2</sub> was synthesized according to the procedure published by Koch *et al.*<sup>1</sup> while the compounds (**2**)<sub>2</sub> and (**3**)<sub>2</sub> were synthesized according to a three step sequence described by Sørensen *et al.*<sup>2</sup> Similarly, 3,5-dimercaptobenzoic acid (**6**) was synthesized following a previously reported literature procedure.<sup>3</sup>

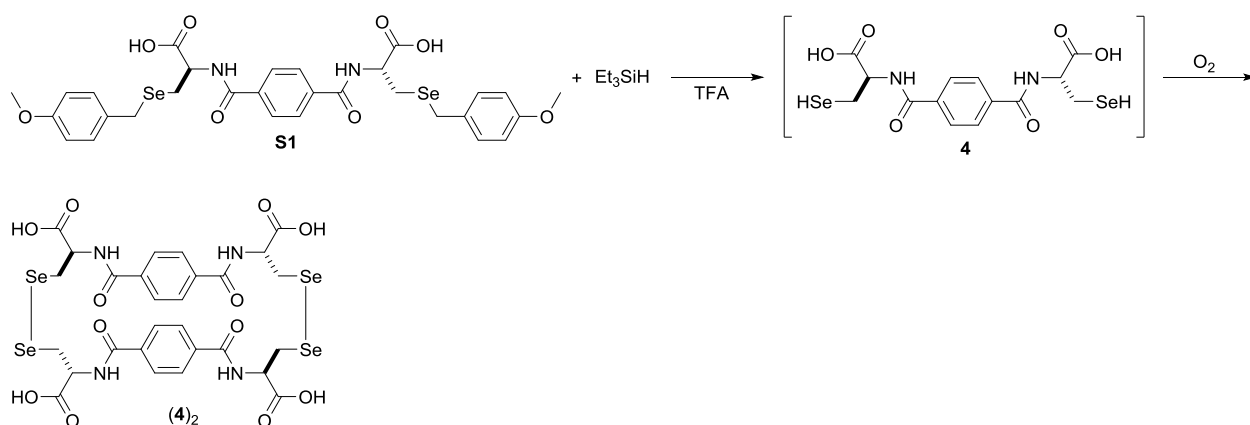
### Compound S1



Selenocystine (0.251 g; 0.751 mmol) was dissolved in a combination of EtOH (10 ml) and 2 M NaOH (3.7 ml) and  $\text{NaBH}_4$  (0.300 g; 7.94 mmol; 10.5 equiv.) was added. The mixture was stirred under  $\text{N}_2$  for 60 minutes and a solution of 4-methoxybenzyl chloride (0.235 g; 1.50 mmol; 1.99 equiv.) in degassed EtOH (7 ml) was added. After stirring for 45 minutes, the solvent was removed, water (5 ml) was added, and the solution was acidified with 2 M HCl at 0 °C. The volatiles were removed and the colorless solids were extensively dried in vacuo before they were suspended in dry THF (10 ml).  $\text{Na}_2\text{CO}_3$  (0.80 g; 7.6 mmol; 5.0 equiv.) and a solution of terephthaloyl dichloride (0.134 g; 0.659 mmol; 0.44 equiv.) in dry THF (8 ml) were added and the mixture was stirred overnight at 25 °C. The mixture was concentrated in vacuo, water was added, and the solution was acidified with 2 M HCl. The formed precipitates were filtered off, dried, and recrystallized from MeOH (30 ml) to give compound **S1** as a colorless solid. Yield 0.225 g (45 %)

$^1\text{H-NMR}$  (500 MHz,  $\text{DMSO-d}_6$ )  $\delta$  = 12.90 (br s, 2H), 8.90 (d,  $J$  = 7.8 Hz, 2H), 7.99 (s, 4H), 7.37 – 7.08 (m, 4H), 6.97 – 6.75 (m, 4H), 4.63 (ddd,  $J$  = 9.7, 7.8, 4.9 Hz, 2H), 3.83 (s, 4H), 3.73 (s, 6H), 3.08 – 2.83 (m, 4H).  $^{13}\text{C-NMR}$  (125 MHz,  $\text{DMSO-d}_6$ )  $\delta$  = 172.16, 165.60, 157.92, 136.25, 131.09, 129.85, 127.41, 113.77, 54.99, 53.17, 26.23, 24.02. HRMS (ESI-TOF)  $m/z$ :  $[\text{M}+\text{H}]^+$  calculated for  $\text{C}_{30}\text{H}_{33}\text{N}_2\text{O}_8\text{Se}_2$  709.0568, found 709.0583.

## Compound (4)<sub>2</sub>

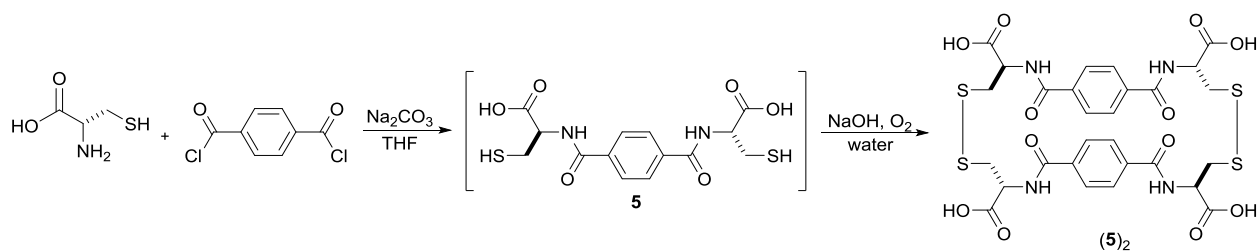


The protected diselenol **S1** (0.168 g; 0.238 mmol) was dissolved in TFA (4 ml) and triethylsilane (1.0 ml; 6.3 mmol; 26 equiv.) was added. The solution was stirred under N<sub>2</sub> at 25 °C for four days. The volatiles were removed, water (5 ml) was added, and the solution was alkalinized with 2 M NaOH. The solution was washed with CH<sub>2</sub>Cl<sub>2</sub> (3×15 ml) and the aqueous phase was poured into ice cold 2 M HCl. The formed precipitates were isolated and washed with water. The crude product was recrystallised from MeOH to give the dimeric macrocycle (4)<sub>2</sub> as a colorless solid. Yield 25 mg (23 %).

<sup>1</sup>H-NMR (500 MHz, DMSO-d<sub>6</sub>) δ = 12.90 (br s, 4H), 8.86 (d, *J* = 7.9, 4H), 7.60 (s, 8H), 4.63 (q, *J* = 7.9, 4H), 2.93 (dd, *J* = 12.2, 7.9, 4H). <sup>13</sup>C-NMR (125 MHz, DMSO-d<sub>6</sub>) δ = 172.36, 165.18, 135.52, 126.95, 53.02, 28.50. HRMS (ESI-TOF) *m/z*: [M-H]<sup>-</sup> calculated for C<sub>28</sub>H<sub>27</sub>N<sub>4</sub>O<sub>12</sub>Se<sub>4</sub> 928.8303, found 928.8329.

The reported signals in the <sup>1</sup>H-NMR spectrum account for five of the six signals from (4)<sub>2</sub>. The <sup>1</sup>H-<sup>1</sup>H COSY spectrum indicates a coupling between the signal positioned at 2.93 ppm and a signal hidden underneath the water signal (see Fig. S8). The existence of this signal is confirmed in the <sup>1</sup>H-<sup>13</sup>C HSQC spectrum where the hidden signal shows a correlation with the carbon signal positioned at 28.50 ppm (see Fig. S9).

## Compound (5)<sub>2</sub>



Terephthaloyl dichloride (0.400 g; 1.97 mmol), cysteine (0.513 g; 4.23 mmol; 2.15 equiv.), and Na<sub>2</sub>CO<sub>3</sub> (10.64 g; 20.95 mmol; 10.6 equiv.) were suspended in dry, degassed THF (25 ml). The suspension was stirred at 25 °C under N<sub>2</sub> overnight where after LCMS analysis of the reaction mixture showed complete conversion to the monomeric dithiol. The solvent was removed *in vacuo*, 1 M NaOH (25 ml) was added, and the solution was stirred for two days in an open flask with access to air. The solution was poured into ice cold 2 M HCl and the precipitates were isolated and washed with water (2×20 ml). The crude product was recrystallized from MeOH to give the dimeric macrocycle (5)<sub>2</sub> as a colorless solid. Yield 0.148 g (20 %).

<sup>1</sup>H-NMR (500 MHz, DMSO-d<sub>6</sub>) δ = 12.90 (br s, 4H), 8.90 (d, *J* = 7.7 Hz, 4H), 7.58 (s, 8H), 4.72 (q, *J* = 7.7, 4H), 3.27 – 3.14 (m, 4H), 2.84 (dd, *J* = 13.6, 7.7, 4H). <sup>13</sup>C-NMR (125 MHz, DMSO-d<sub>6</sub>) δ = 172.07, 165.22, 135.22, 126.93, 51.44, 38.37. HRMS (ESI-TOF) *m/z*: [M+H]<sup>+</sup> calculated for C<sub>28</sub>H<sub>29</sub>N<sub>4</sub>O<sub>12</sub>S<sub>4</sub> 741.0659, found 741.0688.

### S3 Characterization Data

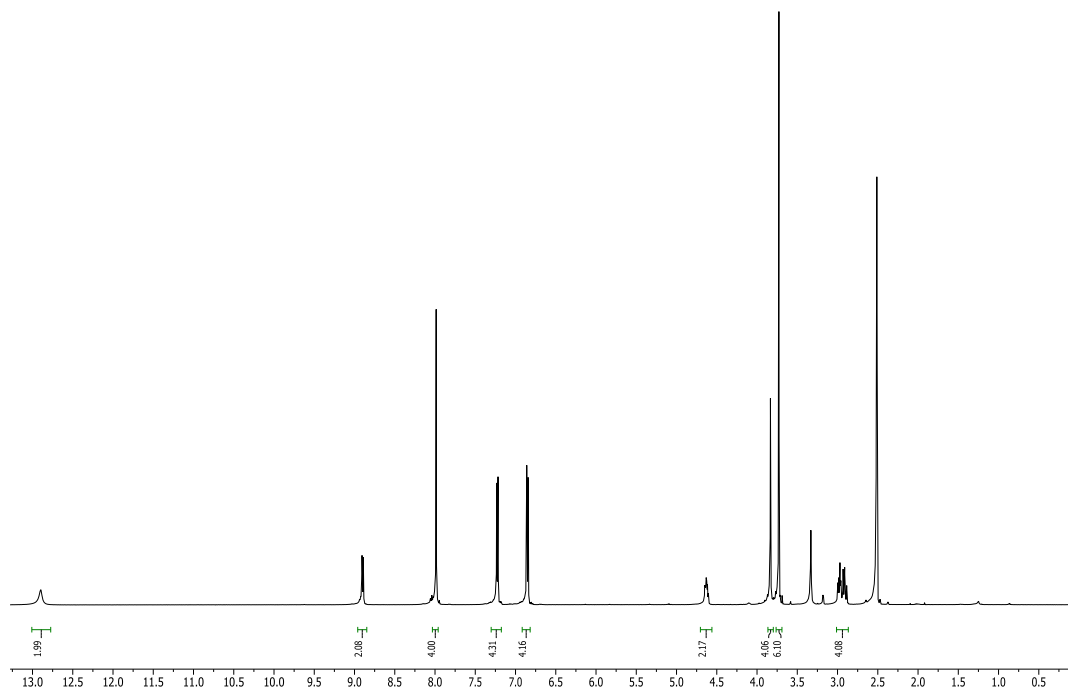


Fig. S1  $^1\text{H}$ -NMR spectrum of compound S1 recorded in DMSO- $d_6$ .

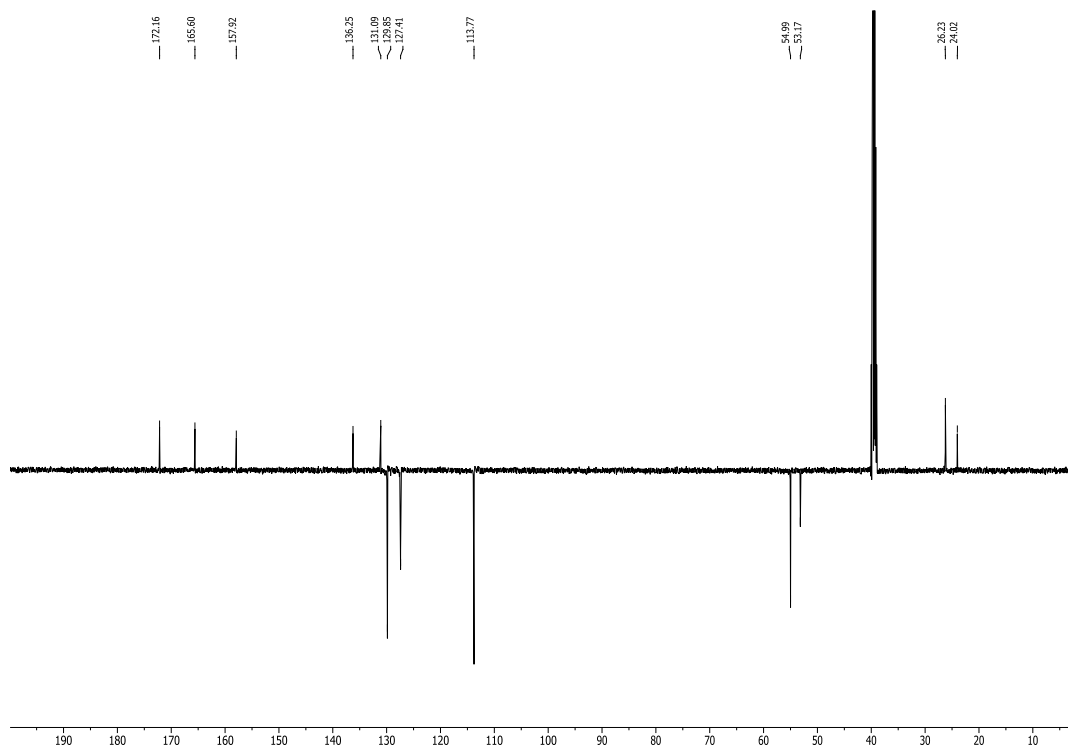


Fig. S2 APT spectrum of compound S1 recorded in DMSO- $d_6$ .

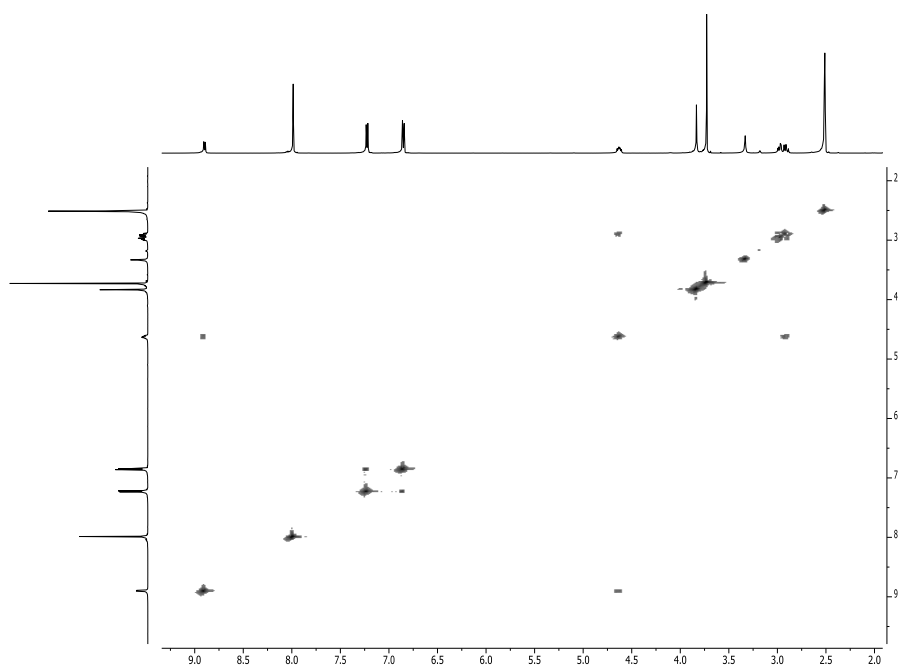


Fig. S3  $^1\text{H}$ - $^1\text{H}$  COSY spectrum of compound S1 recorded in DMSO- $d_6$ .

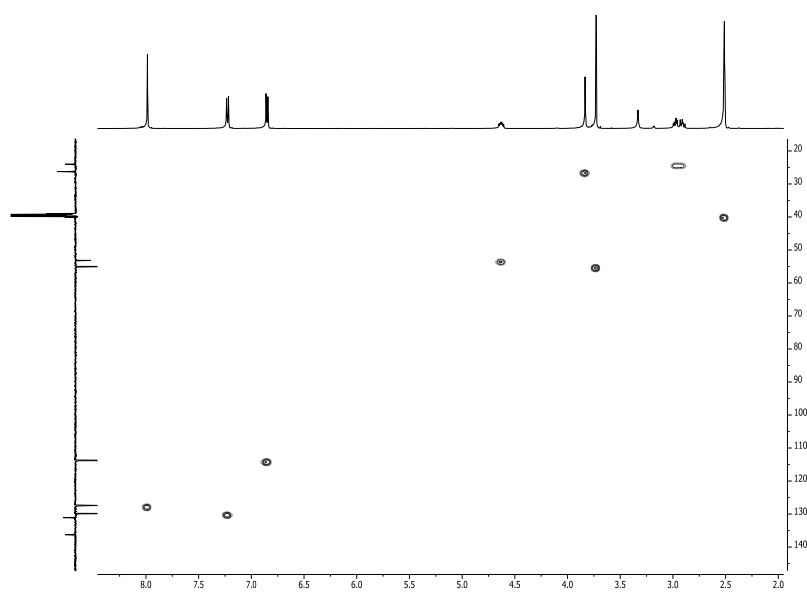


Fig. S4  $^1\text{H}$ - $^{13}\text{C}$  HSQC spectrum of compound S1 recorded in DMSO- $d_6$ .

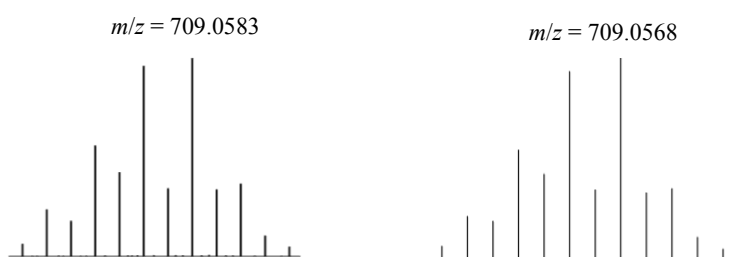


Fig. S5 Measured isotope pattern (left) and calculated isotope pattern (right) for compound S1.

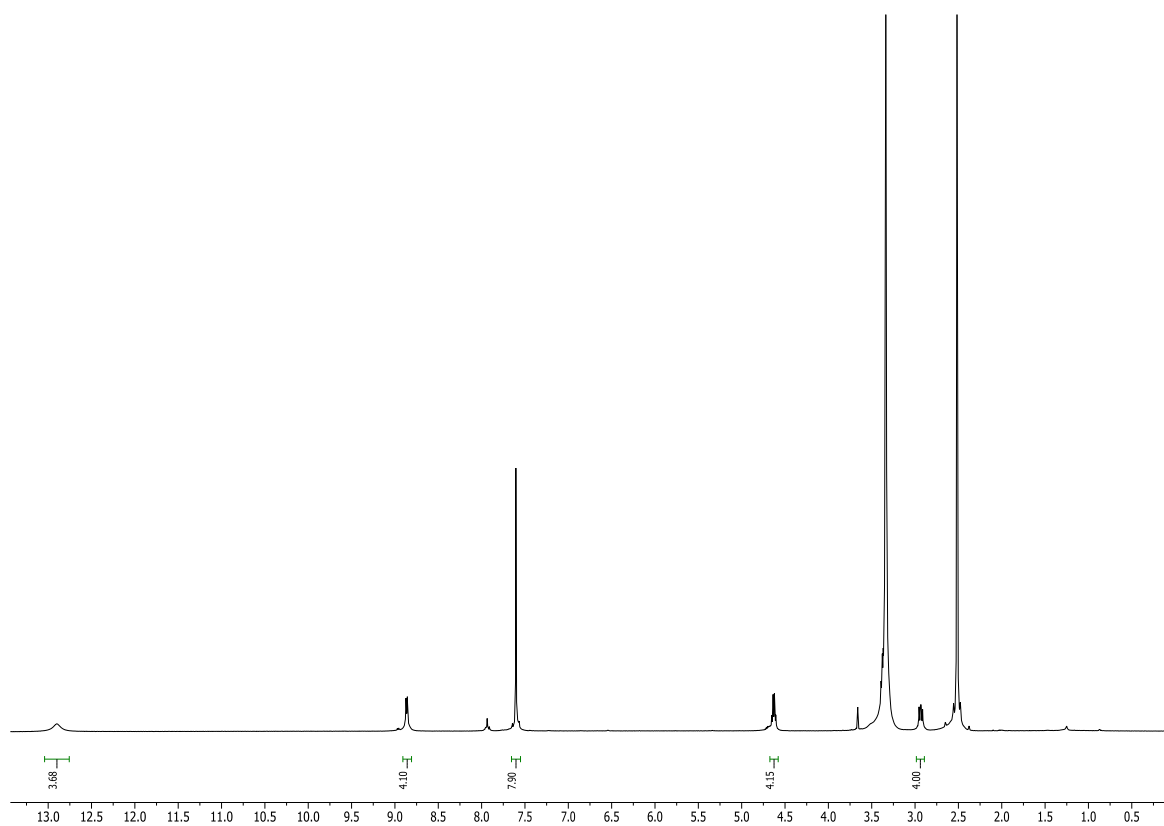


Fig. S6  $^1\text{H}$ -NMR spectrum of compound  $(4)_2$  recorded in  $\text{DMSO-d}_6$ .

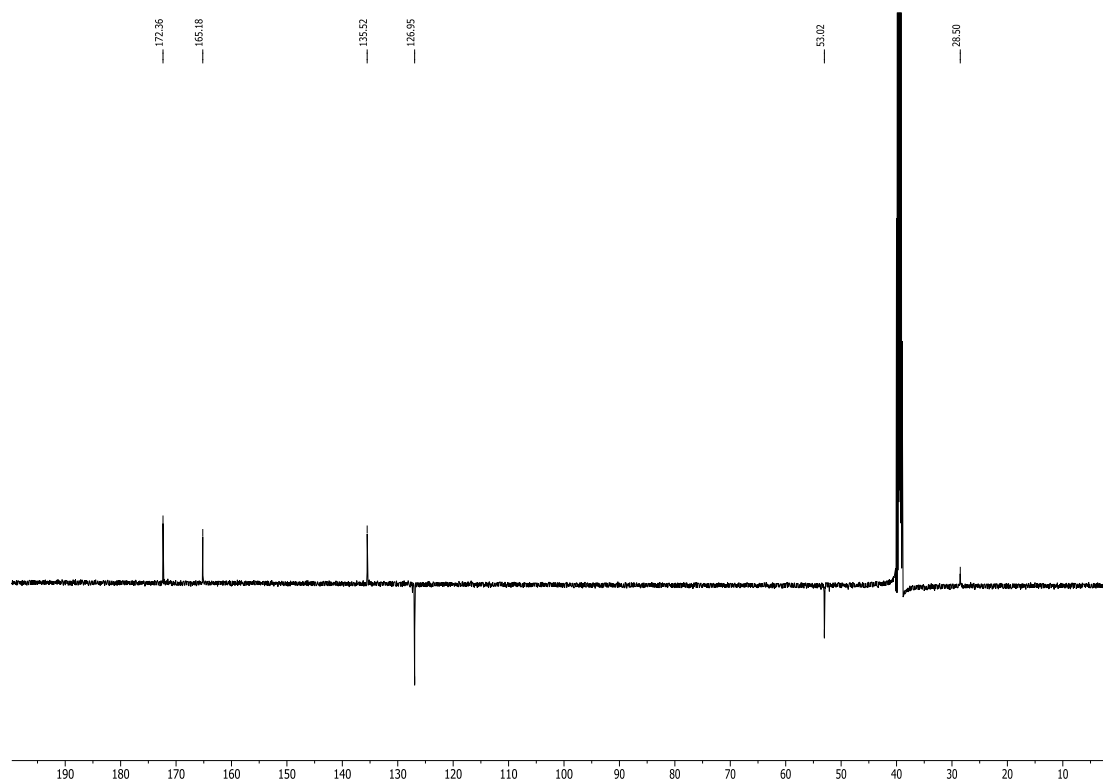
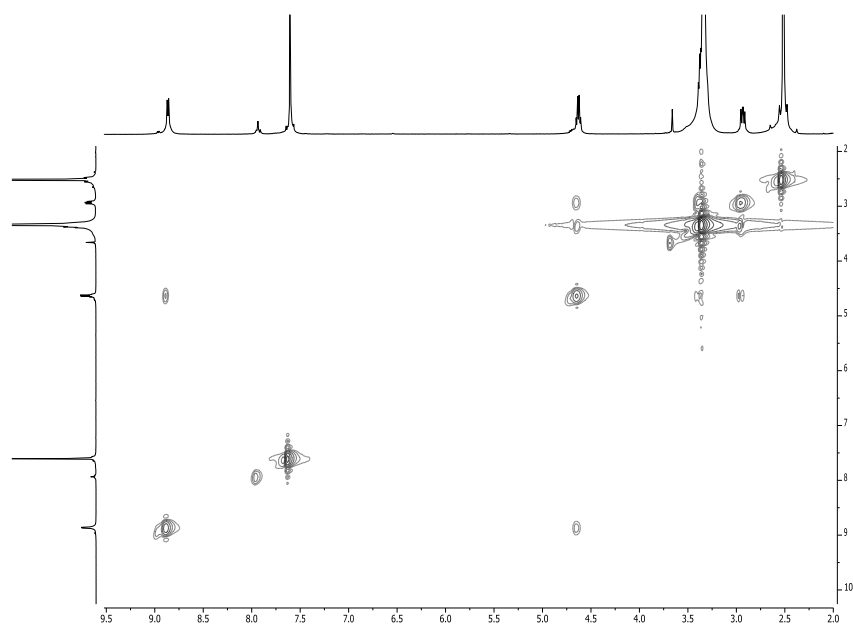
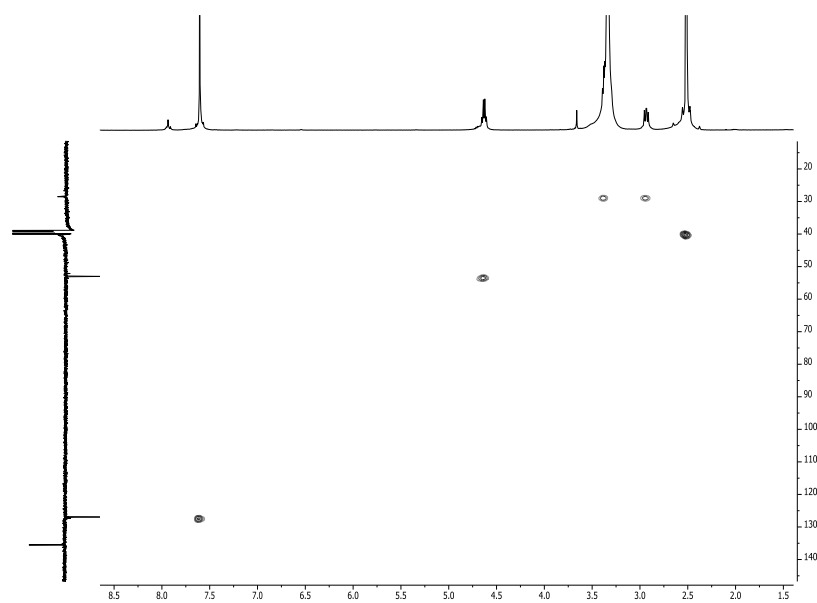


Fig. S7 APT spectrum of compound  $(4)_2$  recorded in  $\text{DMSO-d}_6$ .

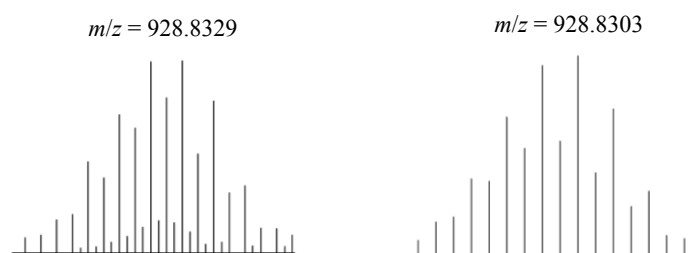




**Fig. S8**  $^1\text{H}$ - $^1\text{H}$  COSY spectrum of compound  $(4)_2$  recorded in  $\text{DMSO-d}_6$ .



**Fig. S9**  $^1\text{H}$ - $^{13}\text{C}$  HSQC spectrum of compound  $(4)_2$  recorded in  $\text{DMSO-d}_6$ .



**Fig. S10** Measured isotope pattern (left) and calculated isotope pattern (right) for compound  $(4)_2$ .

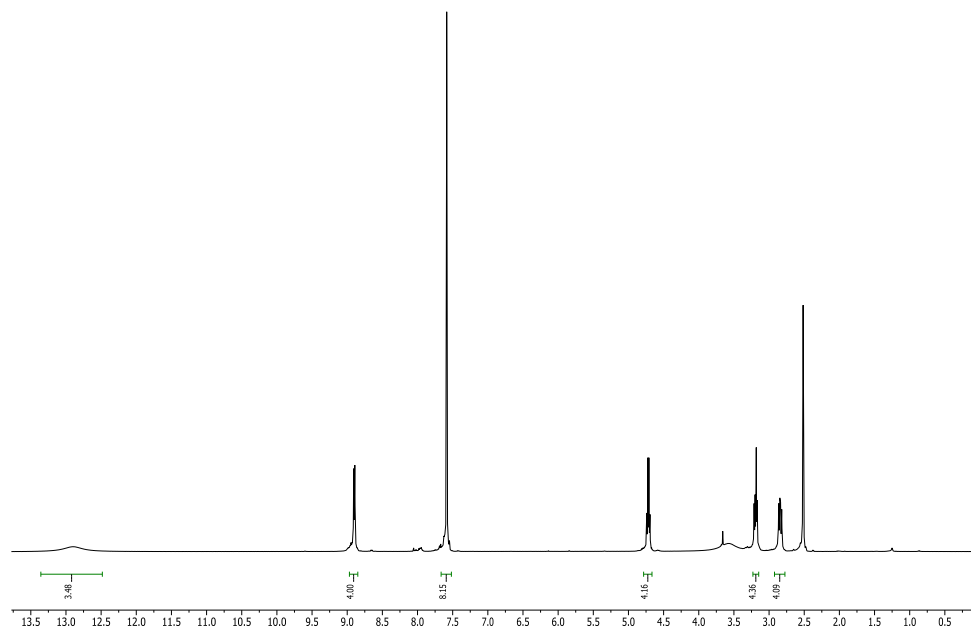


Fig. S11  $^1\text{H-NMR}$  spectrum of compound  $(\mathbf{5})_2$  recorded in  $\text{DMSO-d}_6$ .

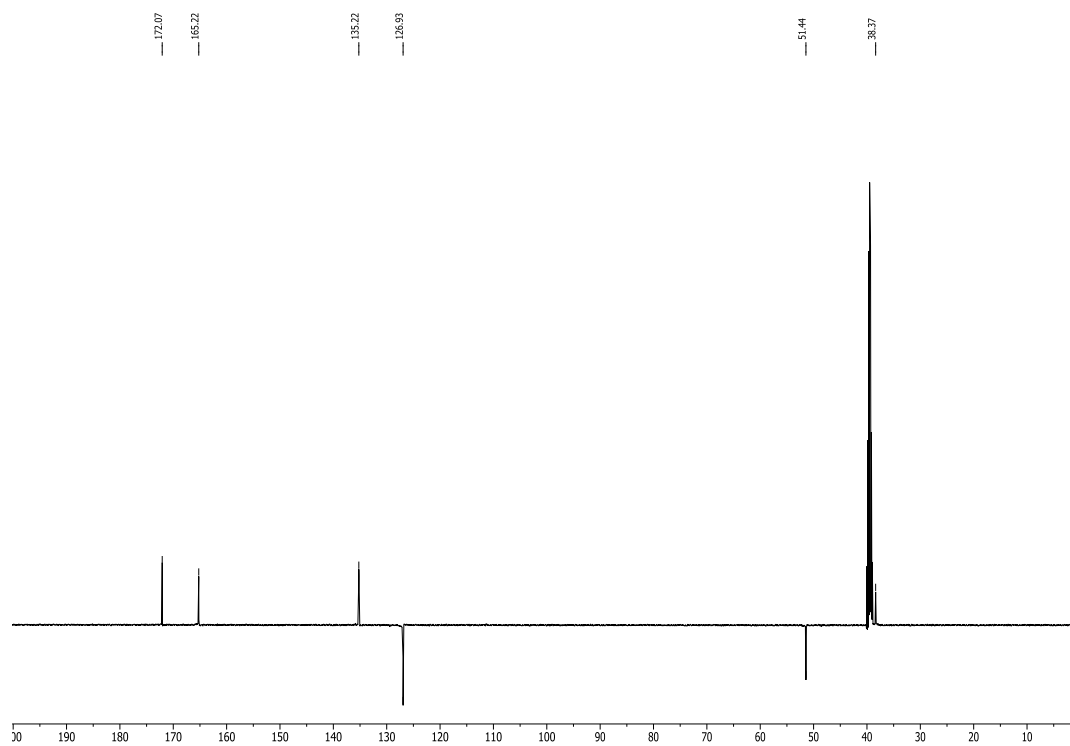
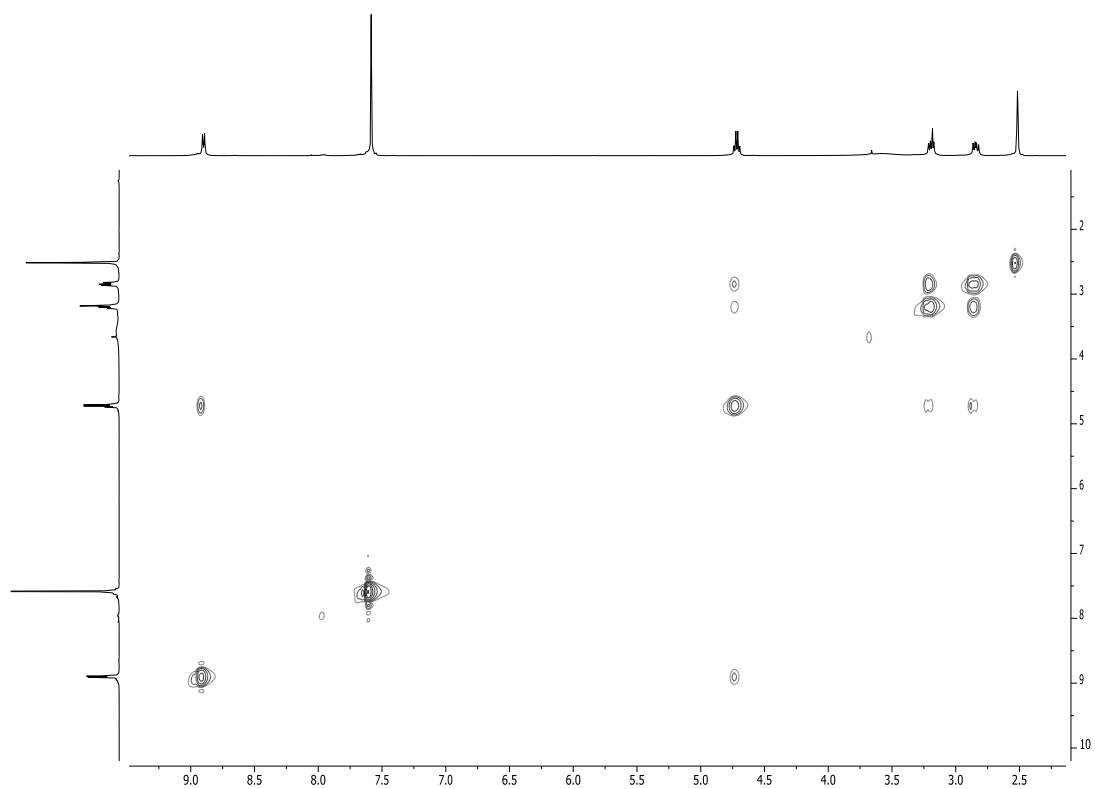
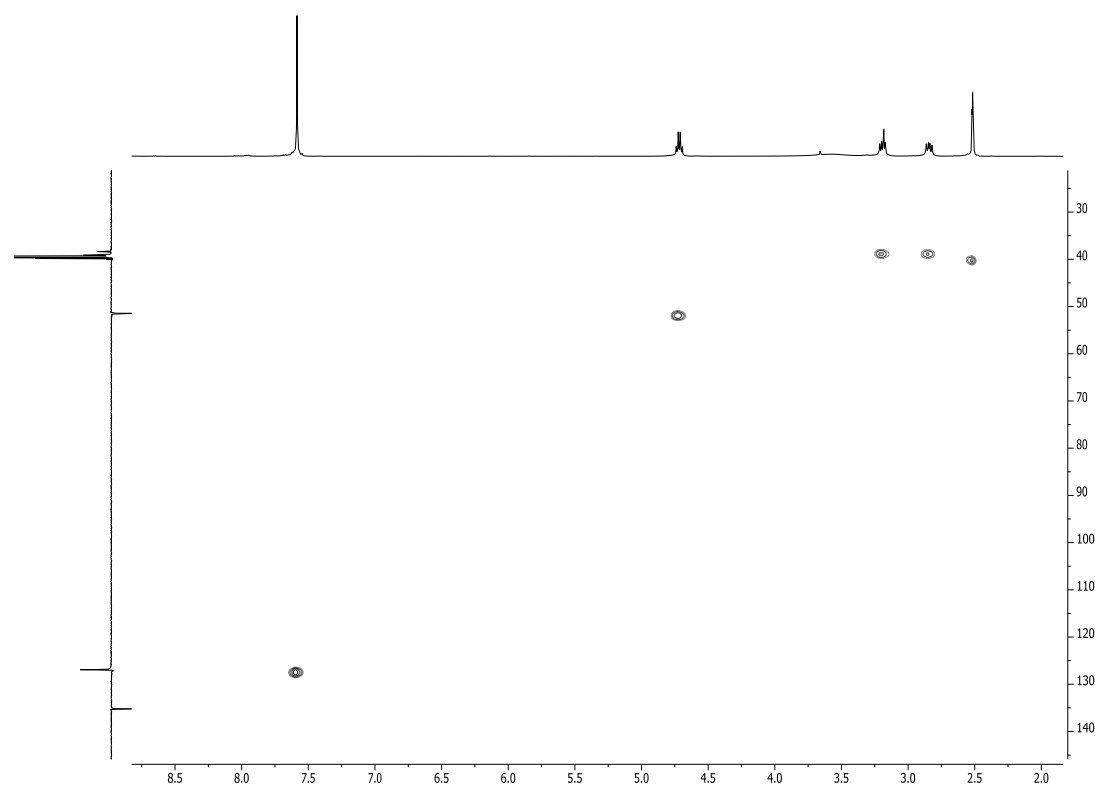


Fig. S12 APT spectrum of compound  $(\mathbf{5})_2$  recorded in  $\text{DMSO-d}_6$ .



**Fig. S13**  $^1\text{H}$ - $^1\text{H}$  COSY spectrum of compound **(5)**<sub>2</sub> recorded in DMSO- $d_6$ .



**Fig. S14**  $^1\text{H}$ - $^{13}\text{C}$  HSQC spectrum of compound **(5)**<sub>2</sub> recorded in DMSO- $d_6$ .

## S4 Diselenide and Selenenyl Based Dynamic Combinatorial Libraries

### HPLC conditions applied to separate the DCLs formed in Section S4.1 and S4.2

Separation of the library members were achieved using a Dionex Acclaim RSLC PolarAdvantage II (PA2) C18 2.2  $\mu\text{m}$  120  $\text{\AA}$  2.1  $\times$  50 mm column together with the following parameters: temperature 20  $^{\circ}\text{C}$ ; flow rate 1.0  $\text{mL min}^{-1}$ ; injection volume 5  $\mu\text{L}$ ; wavelength 290 nm. The mobile phase solutions were 0.1 % formic acid in  $\text{H}_2\text{O}$  (A) and 0.1 % formic acid in MeCN (B). Analysis was achieved using the solvent profile outlined below;

<b>Time [min]</b>	<b>% solution A</b>	<b>% solution B</b>
0.0	10	90
0.1	30	70
9.5	100	0
9.6	10	90

### HPLC conditions applied to separate the DCLs formed in Section S4.3 and S4.4

Separation of the library members were achieved using a Dionex Acclaim RSLC 120 C18 2.2  $\mu\text{m}$  120  $\text{\AA}$  2.1  $\times$  100 mm column together with the following parameters: temperature 20  $^{\circ}\text{C}$ ; injection volume 5  $\mu\text{L}$ ; wavelength 255 nm. The mobile phase solutions were 0.1 % formic acid in  $\text{H}_2\text{O}$  (A) and 0.1 % formic acid in MeCN (B). Analysis of the DCLs was achieved using the solvent profiles outlined below.

#### Solvent profile used in Section S4.3

<b>Time [min]</b>	<b>% solution A</b>	<b>% solution B</b>
0.00	90	10
5.00	10	90
5.10	0	100
6.50	0	100
6.60	90	10

Flowrate 0.6  $\text{mL min}^{-1}$ .

#### Solvent profile used in Section S4.4.

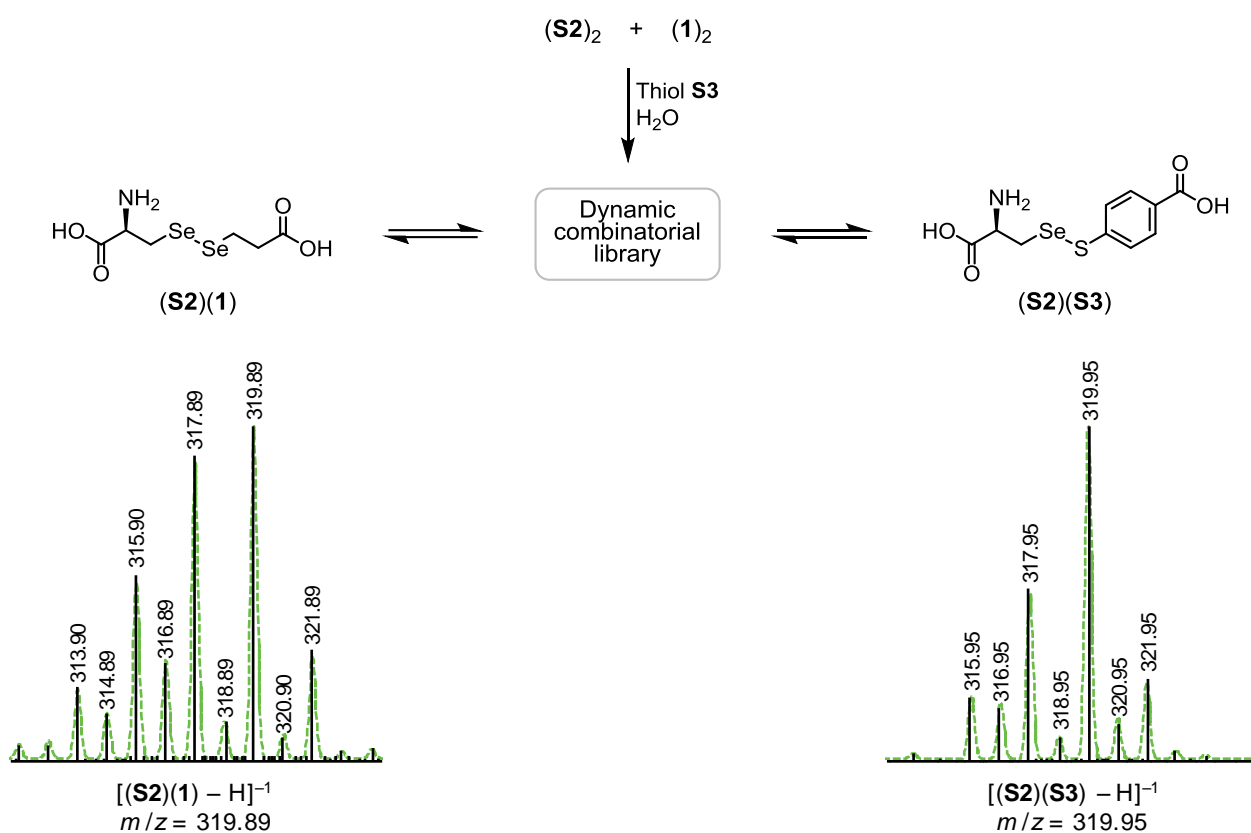
<b>Time [min]</b>	<b>% solution A</b>	<b>% solution B</b>
0.00	90	10
3.00	10	90
3.05	0	100
3.50	0	100
3.60	90	10

Flowrate 1.0  $\text{mL min}^{-1}$ .

### S4.1 Initial Exchange Studies with Simple Diselenides

Selenocystine [(S2)<sub>2</sub>] and (1)<sub>2</sub> were dissolved in a basic aqueous solution and stirred overnight but no significant exchange between the two diselenides was detected by LC/MS analysis. However, by addition of 4-mercaptobenzoic acid (S3) to the mixture of diselenides exchange was effectively enhanced. After only a few hours of equilibration both the scrambled diselenide (S2)(1) and the two possible selenenylsulfides [(S2)(S3) and (1)(S3)] were detected by LC/MS analysis (Fig. S15, top).

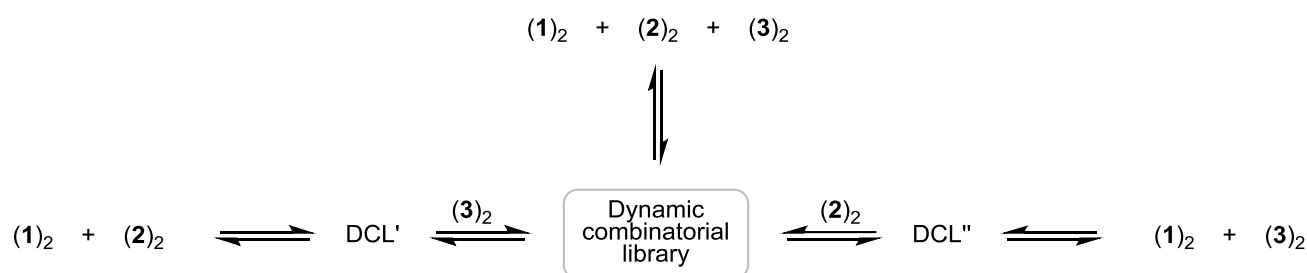
The characteristic isotope distribution of selenium containing molecules in the ESI-MS spectrum provided a convenient and sensitive characterization tool to distinguish between library members with similar masses. Therefore, the exchanged diselenide (S2)(1) and the selenenylsulfide (S2)(S3) were easily and unambiguously identified despite having very similar masses (Fig. S15, bottom). From these early results it could be concluded that it was possible for the two simple diselenides to undergo exchange reaction and that this process was accelerated in the presence of a thiol initiator through the formation of selenenylsulfides.



**Fig. S15** A simple diselenide based DCL starting from the diselenides (S2)<sub>2</sub> and (1)<sub>2</sub> initiated by 4-mercaptobenzoic acid (S3) (top). In the bottom is shown the partial ESI-MS spectra of diselenide [(S2)(1) - H]<sup>-1</sup> and selenenylsulfide [(S2)(S3) - H]<sup>-1</sup>. The calculated isotope patterns are shown as green dotted lines while the black lines are the measured spectra.

## S4.2 Proof of Concept Exchange Studies with Simple Diselenides

To establish that the diselenides equilibrate under thermodynamic control instead of being caught in a kinetic trap DCLs composed of building block  $(1)_2$ ,  $(2)_2$ , and  $(3)_2$  were setup from three different starting points as shown in Fig. S16. In the first library all three building blocks were mixed simultaneously and followed over time (Fig. S16, top). In the second library building block  $(1)_2$  and  $(2)_2$  were combined and allowed to equilibrate, and then combined with  $(3)_2$  (Fig. S16, left). In the third library the order of mixing was reversed as  $(1)_2$  and  $(3)_2$  were combined and allowed to equilibrate before combined with  $(2)_2$  (Fig. S16, right).



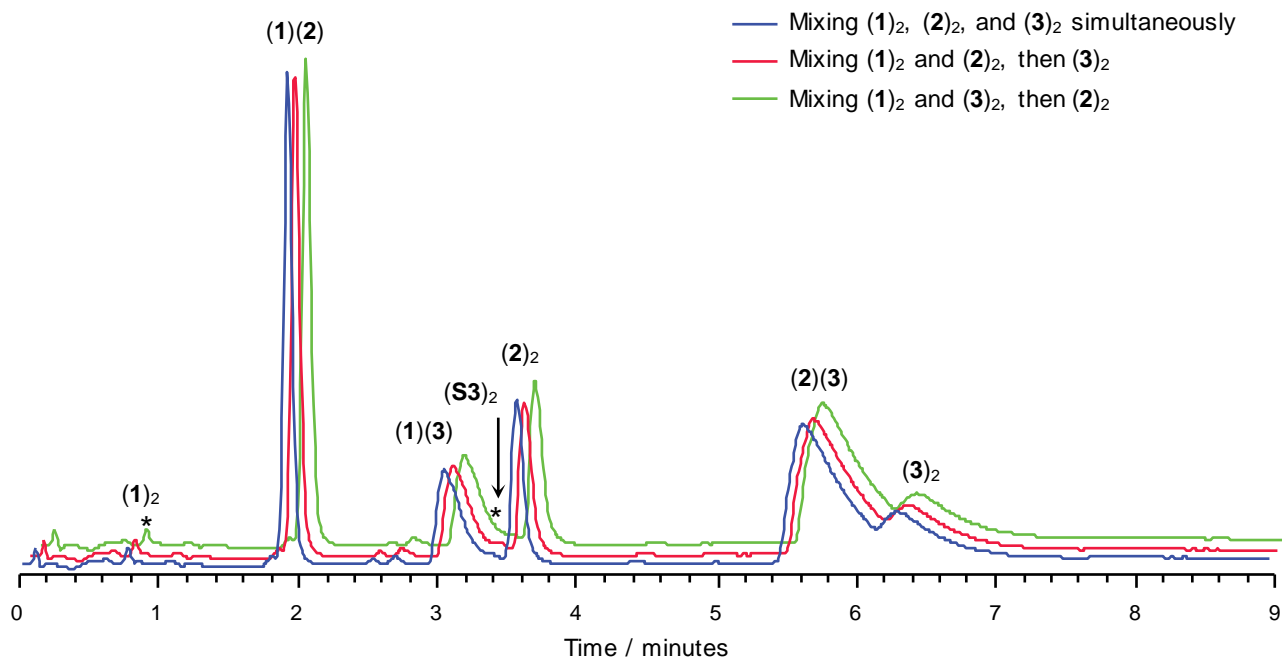
**Fig. S16** The three different approaches used to setup the diselenide based DCL starting from diselenide  $(1)_2$ ,  $(2)_2$ , and  $(3)_2$ . These experiments were performed to verify that the diselenide exchange reaction operated under thermodynamic control.

### Thermodynamic equilibrium in an ammonium acetate buffer

The preparation of the libraries were carried out by dissolving the appropriate amount of diselenide in 45.9 mM ammonium acetate buffer ( $\text{CH}_3\text{COONH}_4/\text{CH}_3\text{COOH}$ ) at pH 7.6 to obtain DCLs with an initial building block concentration of 0.25 mM. To these libraries was added 20 mol% thiol **S3** to act as an initiator. The three libraries were simultaneously prepared without the presence of the initiator to thereby be able to evaluate the effect of this. During the preparation the DCLs had access to air and were afterwards stirred in close-capped vials at 25 °C in-between the measurements that were performed by LC/MS analysis twice a day. When no further changes were observed in the HPLC chromatograms the libraries were concluded to have reached equilibrium (Fig. S17). The various equilibration times are given in Fig. S18 where the green numbers corresponds to the equilibration time for the DCL added thiol while the blue numbers are without addition of thiol. The equilibrated libraries were followed over time and after 40 days the libraries still remained stable and the distribution was found to be unchanged.

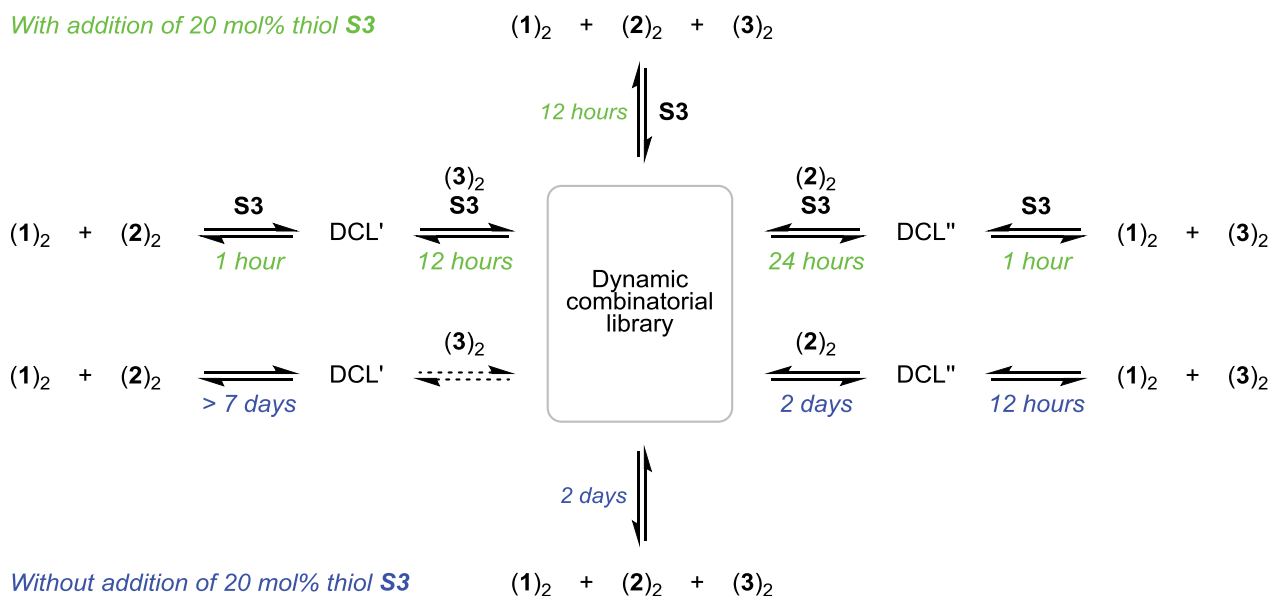
### Thermodynamic equilibrium in a phosphate buffer

The DCLs were prepared by dissolving the three building blocks in a 50.0 mM phosphate buffer ( $\text{Na}_2\text{HPO}_4/\text{NaH}_2\text{PO}_4$ ) at pH 7.9 to obtain DCLs with an initial building block concentration of 0.25 mM. To these libraries were added 20 mol% thiol **S3** to act as an initiator and once more, all the libraries were simultaneously prepared without the thiol to thereby be able to evaluate the effect. The DCLs were again formed from the same three different starting points (Fig. S19) and by following the library formation twice a day by LC/MS analysis it transpired how the equilibrated libraries were found to have an identical equilibrium distribution as the one obtained with the ammonium acetate buffer. The HPLC chromatograms revealed the presence of six different diselenides in the equilibrated libraries together with the disulfide  $(\text{S3})_2$  and it could therefore be concluded that the equilibrium distribution was not affected by the choice of buffer. The equilibrated libraries were followed over time and after 40 days the libraries still remained stable and the distribution was found to be unchanged.



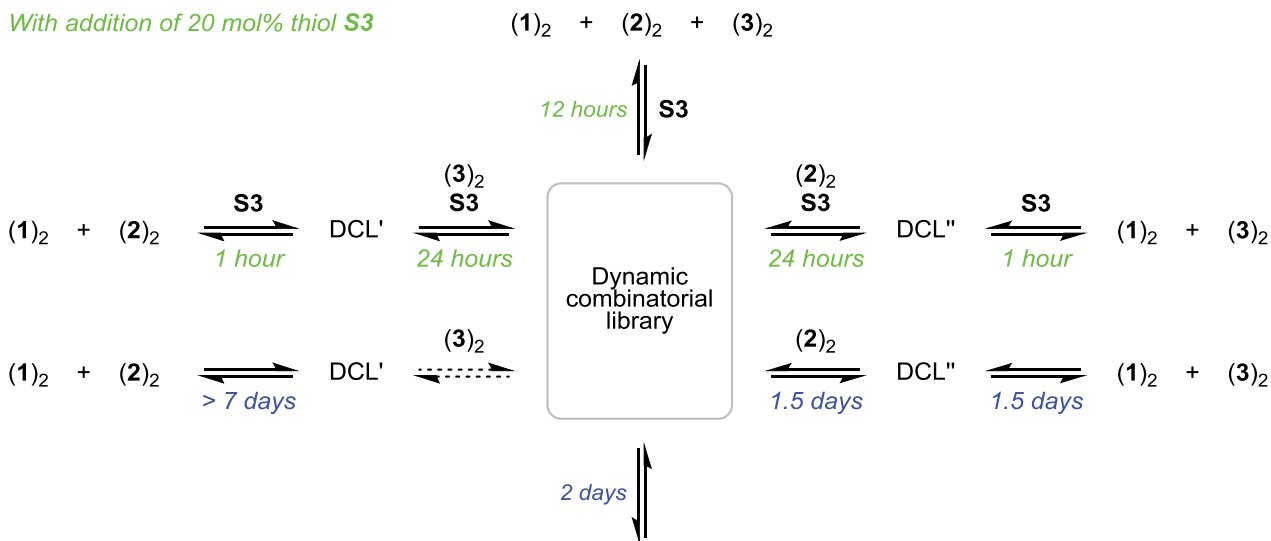
**Fig. S17** Stacked HPLC chromatograms (290 nm) of the 0.25 mM diselenide based DCL at pH 7.6 composed of building block  $(1)_2$ ,  $(2)_2$ , and  $(3)_2$  with addition of 20 mol% thiol **S3** measured after 4 days of equilibration. Blue: mixing  $(1)_2$ ,  $(2)_2$ , and  $(3)_2$  simultaneously; red: mixing  $(1)_2$  and  $(2)_2$ , then added  $(3)_2$ ; and green: mixing  $(1)_2$  and  $(3)_2$ , then added  $(2)_2$ .

With addition of 20 mol% thiol **S3**

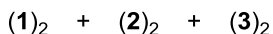


**Fig. S18** The different approaches used to setup the diselenide based DCL starting from diselenide  $(1)_2$ ,  $(2)_2$ , and  $(3)_2$  at pH 7.6 in an ammonium acetate buffer with and without the presence of 20 mol% thiol **S3** as an initiator. The green numbers corresponds to the equilibration time for the DCL added thiol while the blue numbers are without addition of thiol.

With addition of 20 mol% thiol **S3**



Without addition of 20 mol% thiol **S3**



**Fig. S19** The different approaches used to setup the diselenide based DCL starting from diselenide  $(1)_2$ ,  $(2)_2$ , and  $(3)_2$  at pH 7.9 in a phosphate buffer with and without the presence of 20 mol% thiol **S3** as an initiator. The green numbers corresponds to the equilibration time for the DCL added thiol while the blue numbers are without addition of thiol.

### How to halt the selenol/diselenide exchange reaction

A suitable exchange reaction for dynamic combinatorial chemistry must be tamable. That is, the reaction should be reversible under one set of conditions and static under another set of conditions.

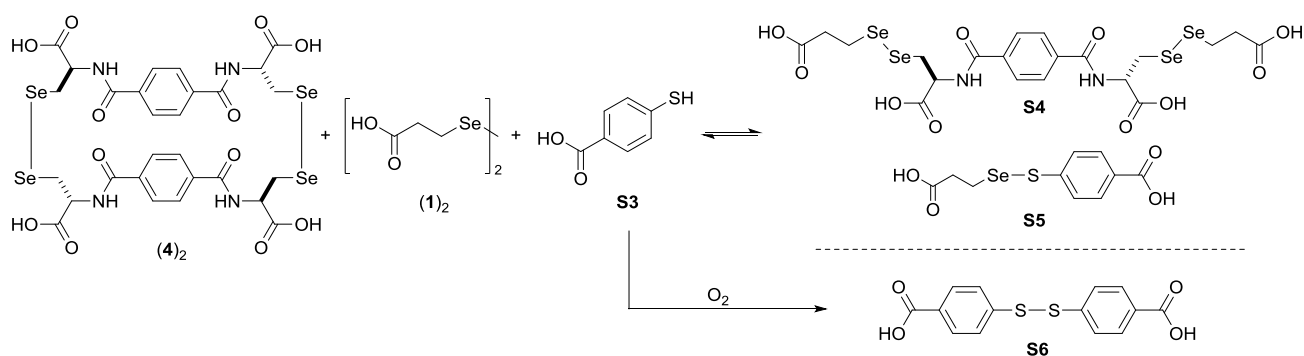
To test if the selenol/diselenide exchange reaction is negligible under acidic conditions two of the building blocks,  $(1)_2$  and  $(3)_2$ , were combined with and without the presence of thiol **S3** and allowed to equilibrate in an ammonium acetate buffer adjusted to pH 7.04, 6.11, 5.05, 4.00, and 2.94. The building blocks  $(1)_2$  and  $(3)_2$  were chosen for these experiments as it was shown above how the equilibration time between  $(1)_2$  and  $(2)_2$  without thiol was considerable longer (> 7 days) than the one between  $(1)_2$  and  $(3)_2$  (~ 1 day). The mixtures at different pH's were then followed by LC/MS analysis over time where it transpired how the diselenide exchange reaction was still active at pH 7.04, 6.11, and 5.05 both with and without the presence of thiol **S3** while no exchange products were observed at pH 4.00 and 2.94 for any of the libraries. From these experiments it was shown how the diselenide exchange reaction was able to proceed in the pH range ~ 5 – 8 while the exchange was hampered below pH 4 – 5.



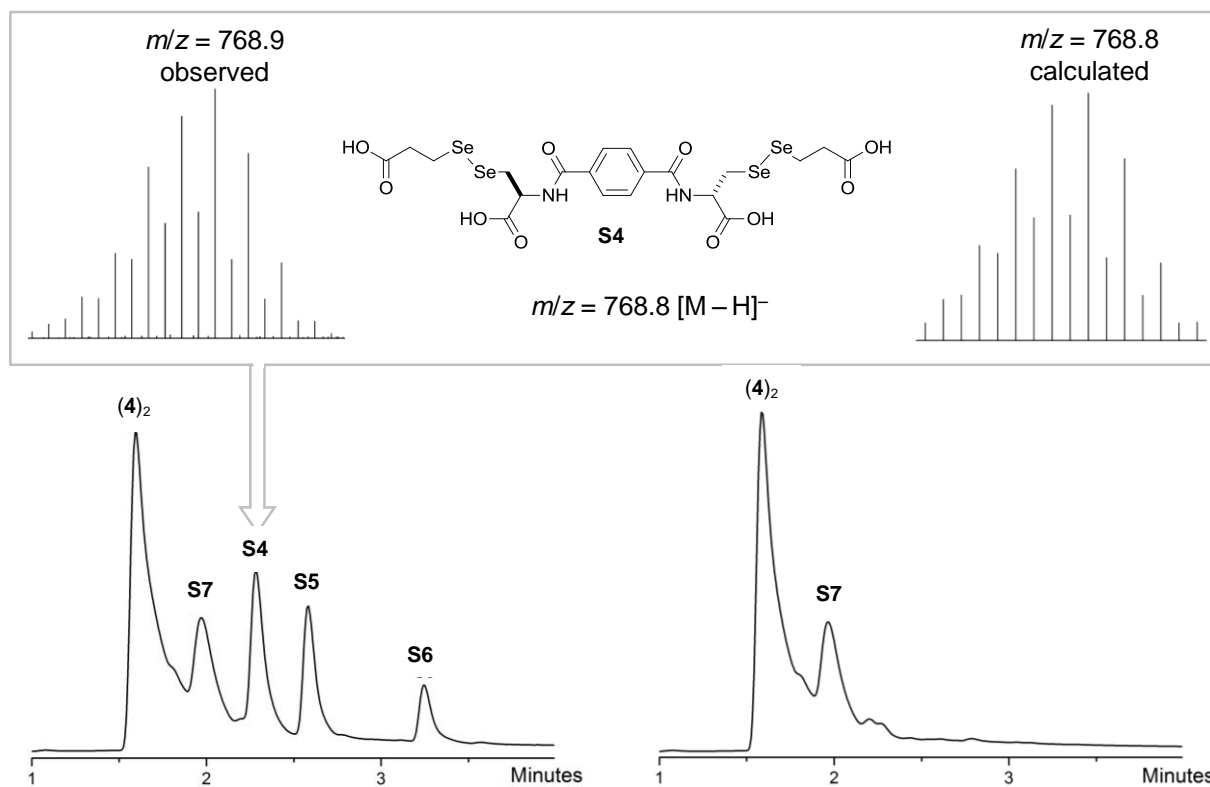
### S4.3 Exchange Studies with (1)<sub>2</sub> and Bis-diselenide (4)<sub>2</sub>

Stock solutions of the diselenides (1)<sub>2</sub> (2 mM) and (4)<sub>2</sub> (1 mM in the dimer; 2 mM in the monomer) and 4-mercaptobenzoic acid (S3, 5 mM) in 46 mM ammonium acetate buffer (pH 8.3) were prepared. Two mixtures were prepared with (1)<sub>2</sub> and (4)<sub>2</sub> in a 1:1 ratio; one with 0.6 equiv thiol S3 and one without.

The formed products are shown in Fig. S20 and their distribution in the DCLs after 1 day of stirring are illustrated in Fig. S21 together with the mass and isotope pattern of the diselenide exchange product S4.



**Fig. S20** Diselenide DCL where the exchange is initiated by 4-mercaptobenzoic acid (S3). The shown structures were all detected in the mixture together with minor amounts of larger diselenides.



**Fig. S21** HPLC chromatograms (255 nm) of the diselenide library starting from (1)<sub>2</sub> and (4)<sub>2</sub> recorded after 1 day of stirring at 298 K. The library containing the initiating thiol 4-mercaptobenzoic acid is shown to the left while the library without the added thiol is shown to the right. S7 is the monomethyl ester of (4)<sub>2</sub>.

#### S4.4 Selenenyl Exchange Studies with (4)<sub>2</sub> and (5)<sub>2</sub>

The bis-diselenide (4)<sub>2</sub> (0.5 mM), bis-disulfide (5)<sub>2</sub> (0.5 mM), and 5-mercaptoisophthalic acid (0.5 mM) were mixed in a 50 mM phosphate buffer (pH 7.8) and the DCL formation was followed over time by use of LC/MS analysis. The different disulfide, diselenide, and selenenylsulfide macrocycles were not found to separate on various HPLC columns and therefore the abundance of each library member was not possible to quantify. However, each library member was unambiguously identified based on the masses in the ESI mass spectrum (Fig. S22) and the measured isotope patterns are shown in Fig. S23 – Fig. S26.

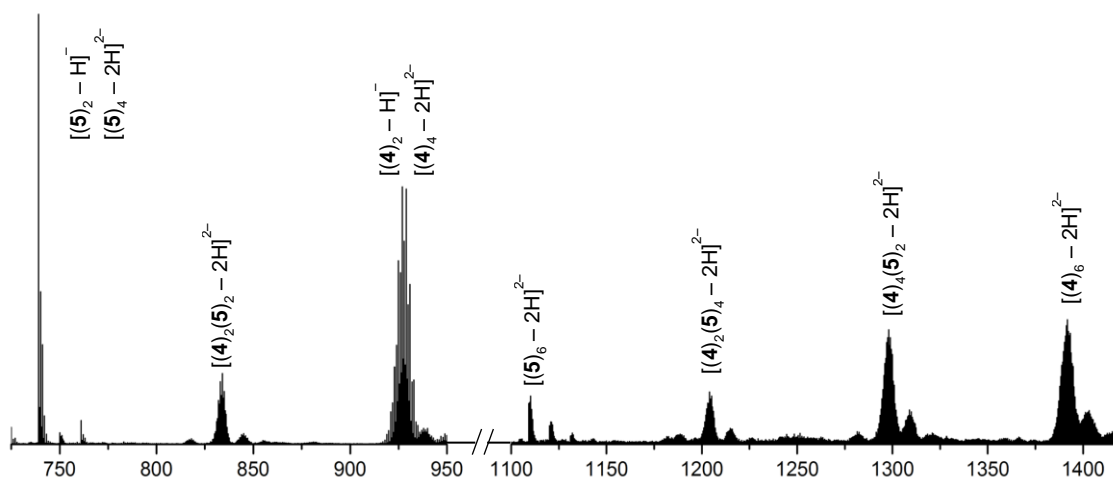


Fig. S22 Partial ESI mass spectrum showing the different macrocyclic diselenide, disulfide, and selenenylsulfide library members.

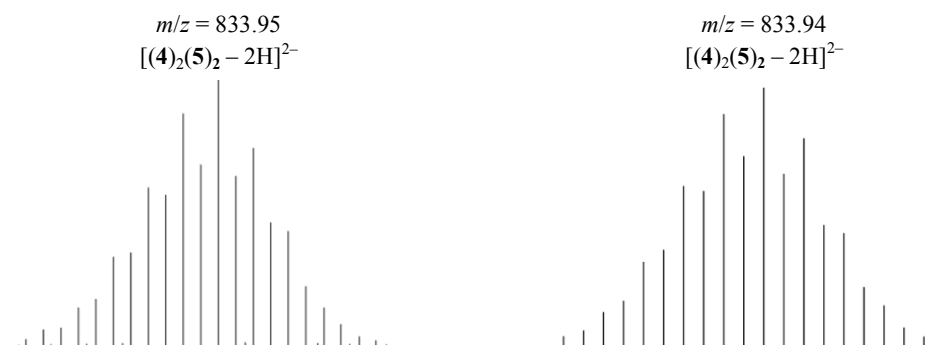


Fig. S23 Measured isotope pattern for the selenenylsulfide tetramer (4)<sub>2</sub>(5)<sub>2</sub> (left) together with the calculated isotope pattern (right).

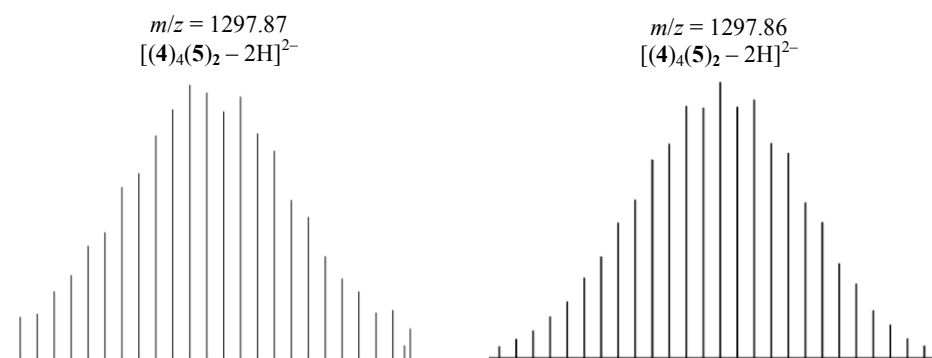
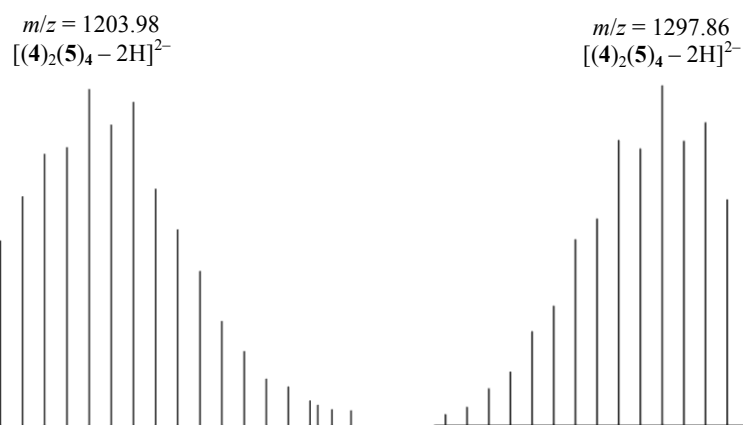
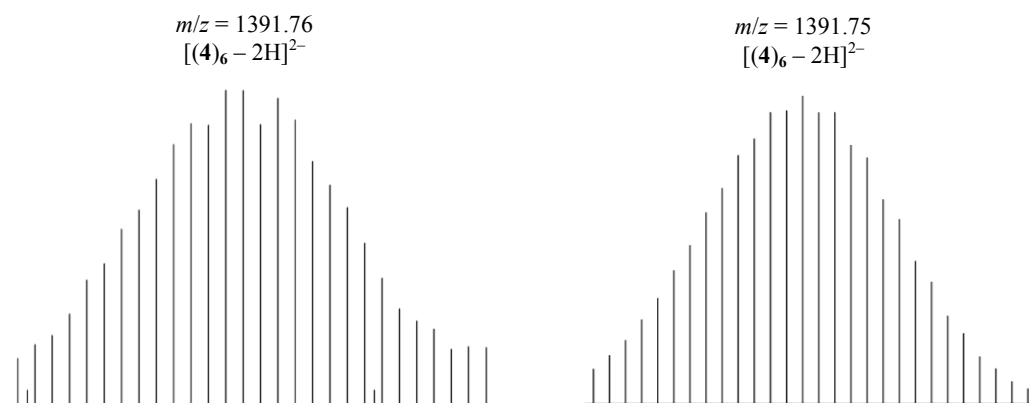


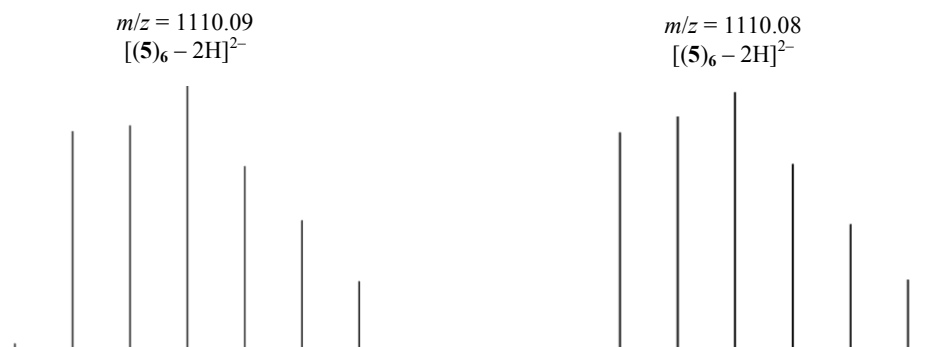
Fig. S24 Measured isotope pattern for the selenenylsulfide hexamer (4)<sub>4</sub>(5)<sub>2</sub> (left) together with the calculated isotope pattern (right).



**Fig. S25** Measured isotope pattern for the selenenylsulfide hexamer  $(\mathbf{4})_2(\mathbf{5})_4$  (left) together with the calculated isotope pattern (right).



**Fig. S26** Measured isotope pattern for the diselenide hexamer  $(\mathbf{4})_6$  (left) together with the calculated isotope pattern (right).



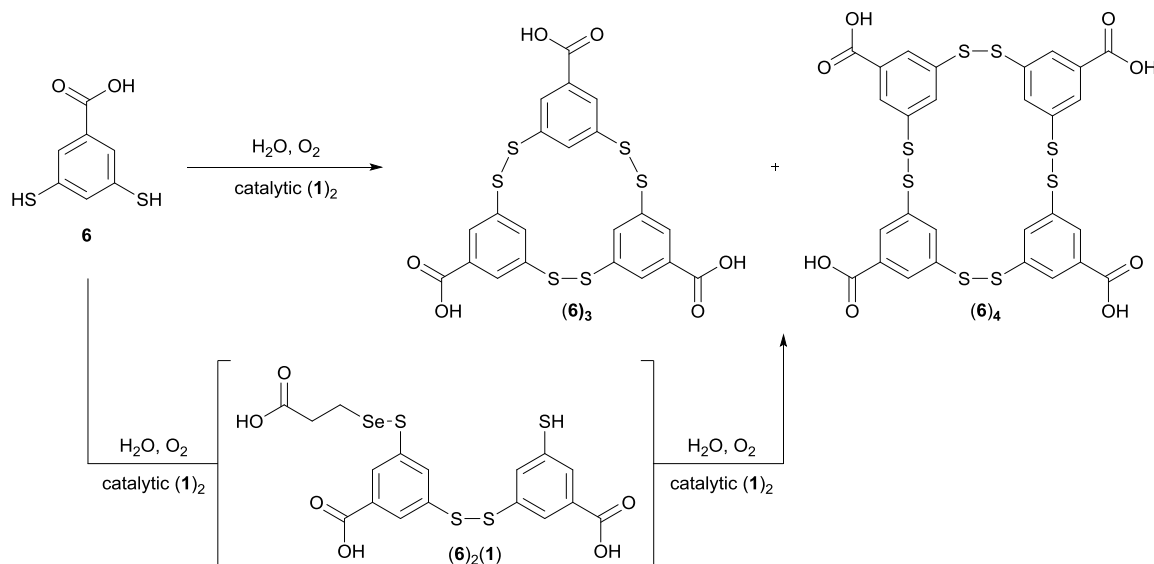
**Fig. S27** Measured isotope pattern for the disulfide hexamer  $(\mathbf{5})_6$  (left) together with the calculated isotope pattern (right).

In addition to the masses of the macrocycles shown in Fig. S23 – Fig. S26 the isotope patterns of the diselenide tetramer,  $(\mathbf{4})_4$ , and the disulfide tetramer,  $(\mathbf{5})_4$ , were observed as double charged species overlapping with the signals for the mono-charged  $(\mathbf{4})_2$  and  $(\mathbf{5})_2$  macrocycles.

The bis-diselenide  $(\mathbf{4})_2$  (0.5 mM), bis-disulfide  $(\mathbf{5})_2$  (0.5 mM), and 5-mercaptoisophthalic acid (5 mol%) were furthermore mixed in a 45.9 mM ammonium acetate buffer (pH 7.6) and the DCL formation was again followed over time by use of LC/MS analysis. The library composition was found to be identical to the one observed when performing the studies in a phosphate buffer and the different disulfide, diselenide, and selenenylsulfide macrocycles were again characterized through ESI-MS analysis.

## S5 Diselenide Catalyzed Dynamic Combinatorial Libraries of Disulfides

To examine the catalytic effect of diselenides on the formation of DCLs with disulfides, a DCL was formed from 3,5-dimercaptobenzoic acid (**6**) in the presence of 3,3'-diselenodipropanoic acid (**1**)<sub>2</sub>, as depicted in Fig. S28.



**Fig. S28** A DCL formed from 3,5-dimercaptobenzoic acid (**6**) in the presence of 3,3'-diselenodipropanoic acid (**1**)<sub>2</sub>.

The DCL was studied by HPLC analysis and separation of the different library members was achieved with the following parameters: temperature 20 °C; flow rate 0.50 mL min<sup>-1</sup>; injection volume 1  $\mu\text{L}$ ; wavelength 255 nm. The mobile phase solutions were 0.1 % formic acid in H<sub>2</sub>O (A) and 0.1 % formic acid in MeCN (B). Analysis of the DCL was achieved using the solvent profile outlined below.

Time [min]	% solution A	% solution B
0.00	98	2
10.0	0	100
12.5	98	2

Initial optimization studies were performed on the disulfide-based DCL without addition of the diselenide (**1**)<sub>2</sub>. A typical DCL was prepared by dissolving thiol **6** in concentrations varying from 0.5–2.0 mM with access to air in 45.9 mM ammonium acetate buffer that was adjusted to the pH given in Table S1. The DCLs were stirred in close-capped vials at room temperature in between the measurements.

**Table S1** Initial optimization studies performed on the disulfide-based DCLs with varying concentrations of thiol **6**.

	pH 6.0	pH 7.0	pH 8.3
0.5 mM	precipitation	DCL	DCL
1.0 mM	precipitation	precipitation	precipitation
2.0 mM	precipitation	precipitation	precipitation

Precipitation in the libraries was observed at all tested concentrations at pH 6.0 and at pH 7.0 and 8.3 with concentrations at 1.0 mM and 2.0 mM. Ultrasonication of these libraries for several hours did not eliminate this issue. No precipitation, however, was observed at pH 7.0 and 8.3 with a thiol concentration of 0.5 mM.

After optimizing the conditions for the disulfide-based DCLs the effect of the diselenide (**1**)<sub>2</sub> was investigated. The preparation of the DCLs were carried out by dissolving thiol **6** in 45.9 mM ammonium acetate buffer at pH 7.0, 7.6, and 8.3 together with varying catalytic amounts of the diselenide (**1**)<sub>2</sub> according to Table S2. The DCLs were ultrasonicated for 15 minutes to ensure complete solvation and were in-between the measurements stirred in close-capped vials at room temperature. All the libraries were simultaneously prepared without the diselenide to evaluate the catalytic effect. When the results were analyzed by HPLC the start intensity of the chromatograms of the catalyzed and uncatalyzed libraries were compared to ensure similar start concentrations of the libraries as the studies were performed at rather low concentration.

**Table S2** Parameters used for the preparation of DCLs with disulfides catalyzed by diselenide (**1**)<sub>2</sub>. All the studies were performed with an initial thiol concentration of 0.5 mM in an ammonium acetate buffer.

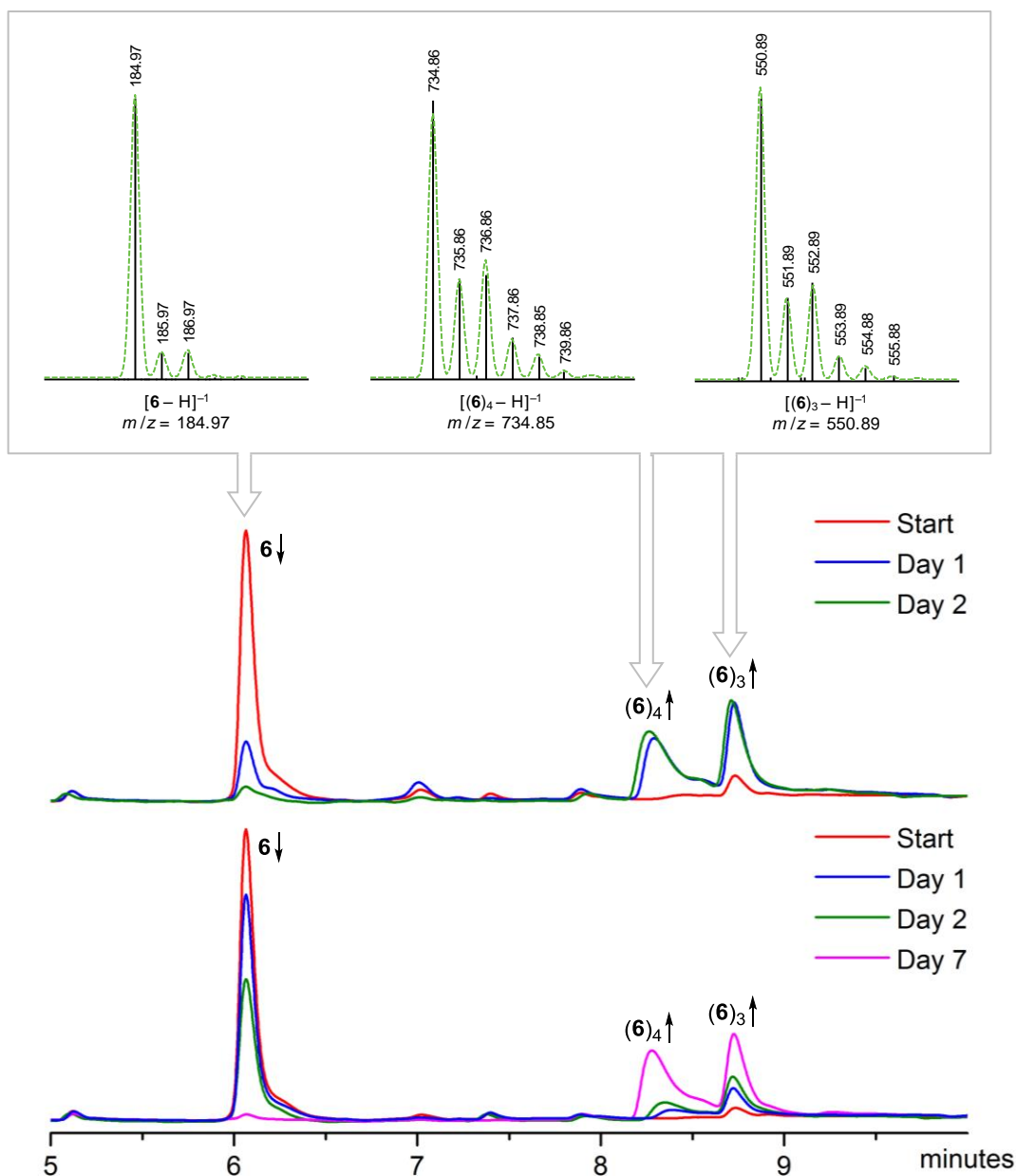
	pH 7.0	pH 7.6	pH 8.3
10 mol% <b>1</b>	DCL catalyzed	DCL catalyzed	DCL catalyzed
1.0 mol% <b>1</b>	DCL catalyzed	–	DCL catalyzed
0.1 mol% <b>1</b>	–	–	DCL not catalyzed

At pH 7.0, 7.6, and 8.3 a catalytic effect was observed both with addition of 10 and 1.0 mol% (**1**)<sub>2</sub> but no effect was observed with 0.1 mol% (**1**)<sub>2</sub> at pH 8.3.

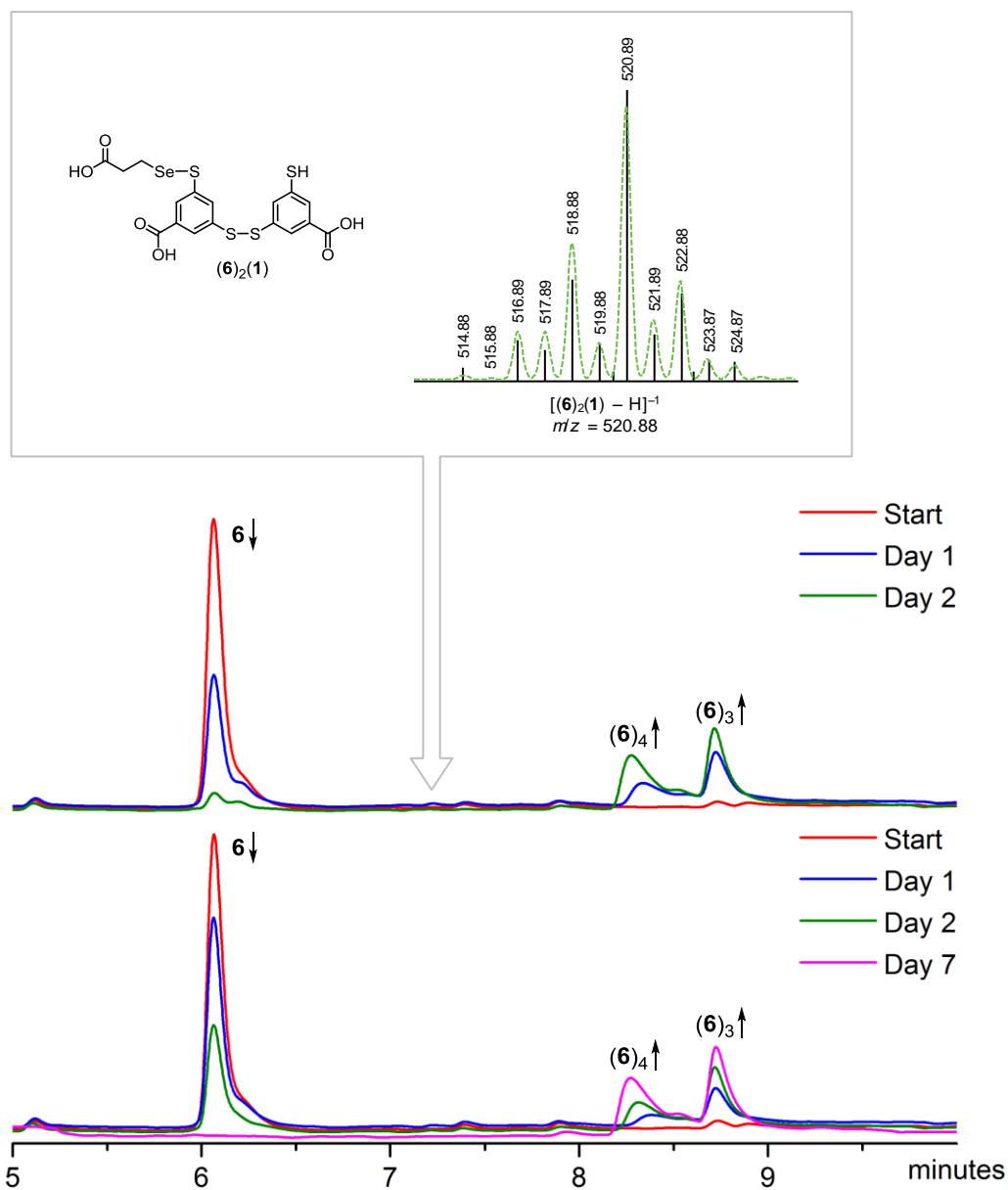
Finally, two additional DCLs were performed at pH 9.4 and 10.0, both of these in 45.9 mM ammonium acetate buffer, with an initial building block concentration of 1.0 mM.<sup>i</sup> No catalyst was added to these libraries. By following these two libraries over time by LC/MS analysis, as done with the other DCLs, it was found that the equilibrium was established after ~ 2 days at pH 9.4 while it only took ~ 1 day at pH 10.0.

Below in Fig. S29 – Fig. S32 are the HPLC chromatograms presented for the different DCLs studied. Fig. S29 shows the library formation at pH 7.0 with 10 mol% (**1**)<sub>2</sub> together with partial MS spectra of the library members. Fig. S30 shows the library formation at pH 7.6 with 10 mol% (**1**)<sub>2</sub> together with a comparison of the observed and calculated isotopic pattern of the selenenylsulfide that forms as an intermediate during library formation. Fig. S31 shows the library formation at pH 8.3 with 10 mol% (**1**)<sub>2</sub> while Fig. S32 shows the library formation at pH 7.0 with 1.0 mol% (**1**)<sub>2</sub>. The HPLC chromatograms obtained from the study at pH 8.3 with 1.0 mol% (**1**)<sub>2</sub> are not presented here as it is similar to the one at pH 7.0. Fig. S33 shows the conversion of thiol building block **6** as a function of time in the different DCLs studied at pH 7.0, 7.6, and 8.3 respectively with and without the addition of 10 mol% diselenide (**1**)<sub>2</sub> while Fig. S34 shows similar graphs from the DCLs studied at pH 7.0 and 8.3 with and without the addition of 1 mol% diselenide (**1**)<sub>2</sub>.

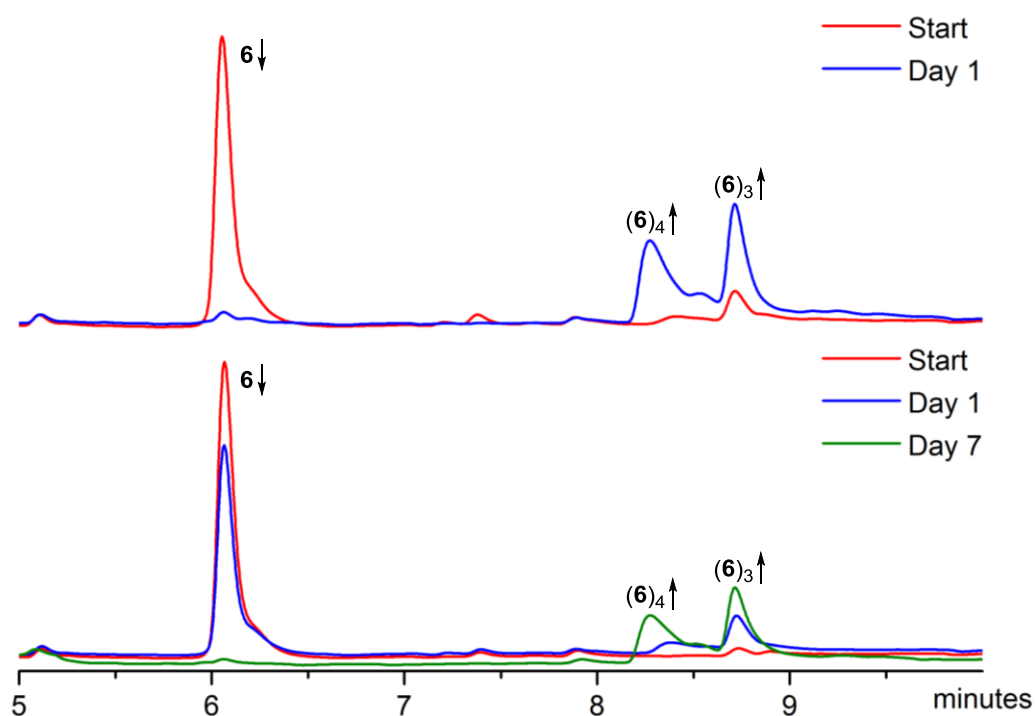
<sup>i</sup> Since the experiments were performed at a higher pH, where the thiol **6** was deprotonated, it was possible to increase the building block concentration to 1.0 mM without observing any precipitation in the DCLs.



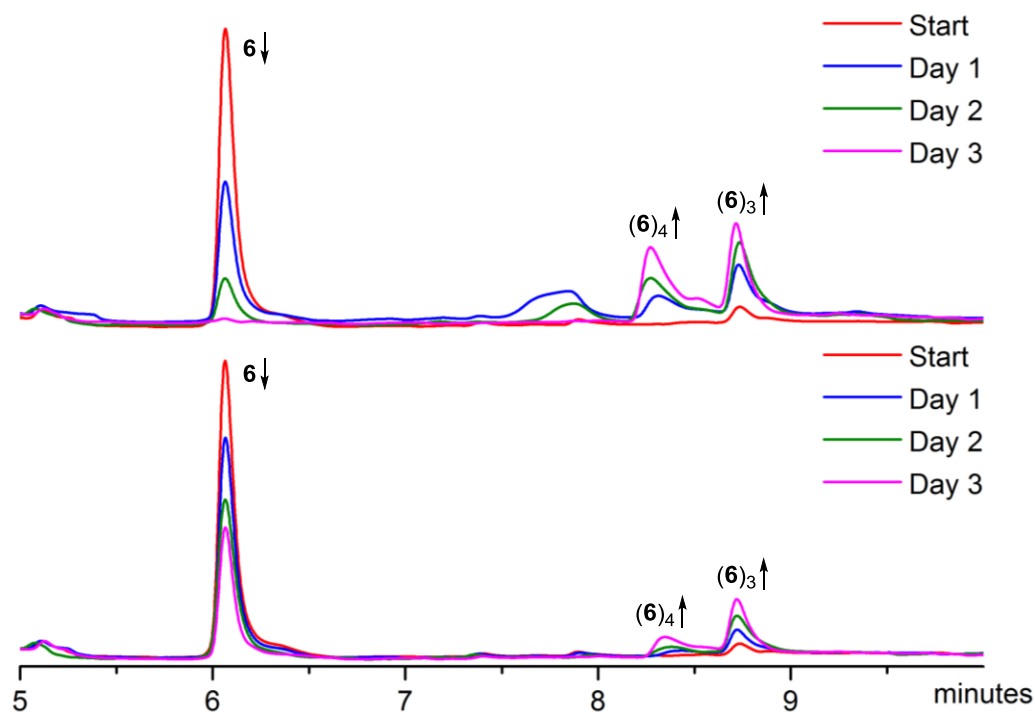
**Fig. S29** HPLC (255 nm) and MS analysis of a 0.5 mM DCL at pH 7.0 at 298 K composed of thiol building block **6** with (top) and without (bottom) addition of 10 mol% diselenide (**1**)<sub>2</sub>. The HPLC chromatograms show the progress of the library formation over time (red – start, blue – day 1, green – day 2, purple – day 7 (only bottom)). The MS analysis shows the peaks from **6**, (**6**)<sub>3</sub>, and (**6**)<sub>4</sub> where the different isotopic patterns transpire depending on the number of sulfur atoms. The calculated isotope patterns are shown as green dotted lines while the black lines are the measured spectra. The HPLC chromatograms are solvent corrected.



**Fig. S30** HPLC (255 nm) and MS analysis of a 0.5 mM DCL at pH 7.6 at 298 K composed of thiol building block **6** with (top) and without (bottom) addition of 10 mol% diselenide  $(\mathbf{1})_2$ . The HPLC chromatograms show the progress of the library formation over time (red – start, blue – day 1, green – day 2, purple – day 7 (only bottom)). The MS analysis shows the observed and calculated isotopic pattern from the selenenylsulfide  $(\mathbf{6})_2(\mathbf{1})$  that forms as an intermediate in the diselenide catalyzed DCL. The calculated isotope pattern is shown as a green dotted line while the black lines are the measured isotope pattern. The HPLC chromatograms are solvent corrected.

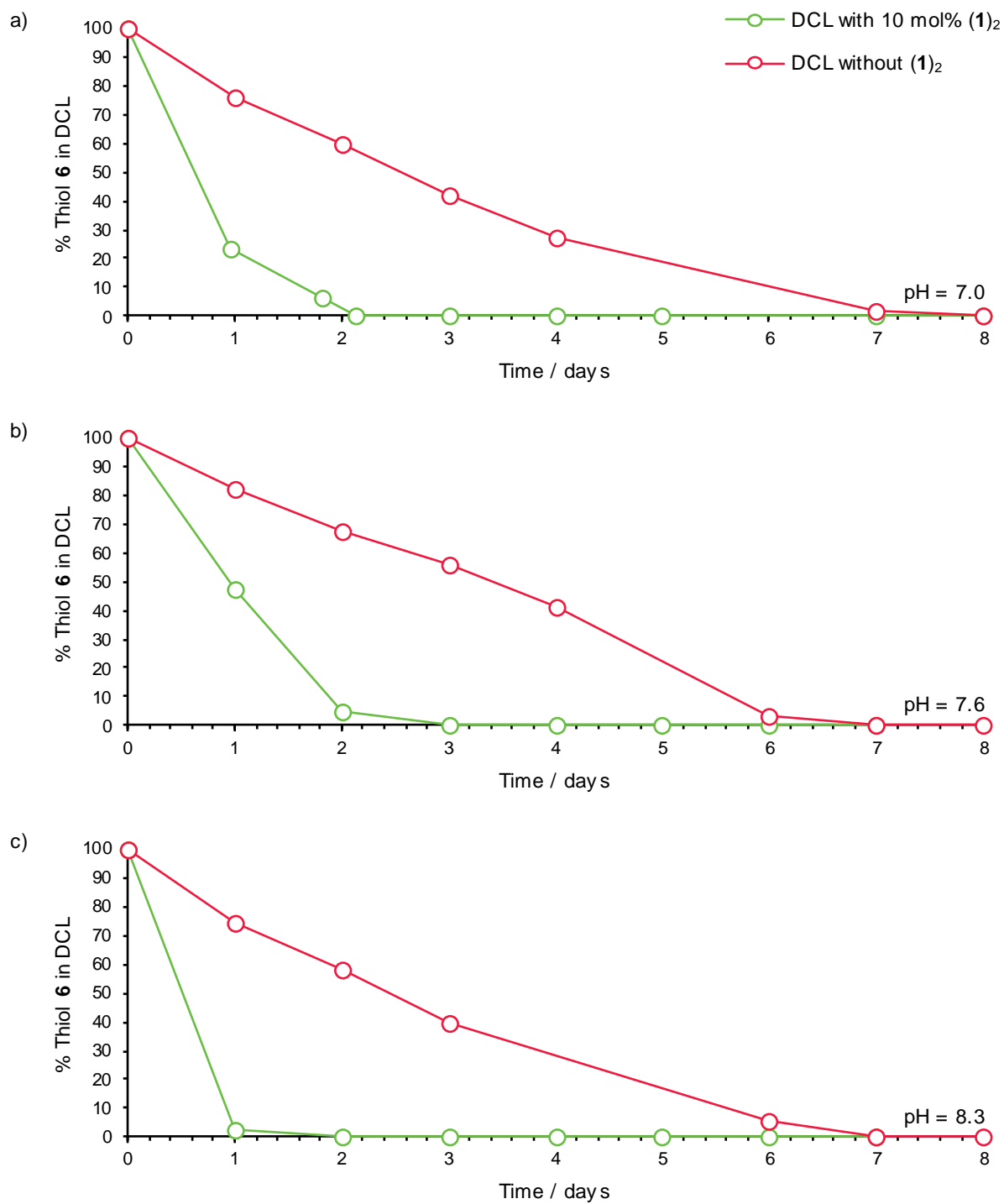


**Fig. S31** HPLC analysis (255 nm) of a 0.5 mM DCL at pH 8.3 at 298 K composed of thiol building block **6** with (top) and without (bottom) addition of 10 mol% diselenide (**1**)<sub>2</sub>. The HPLC chromatograms show the progress of the library formation over time (red – start, blue – day 1, green – day 7 (only bottom)). The HPLC chromatograms are solvent corrected.

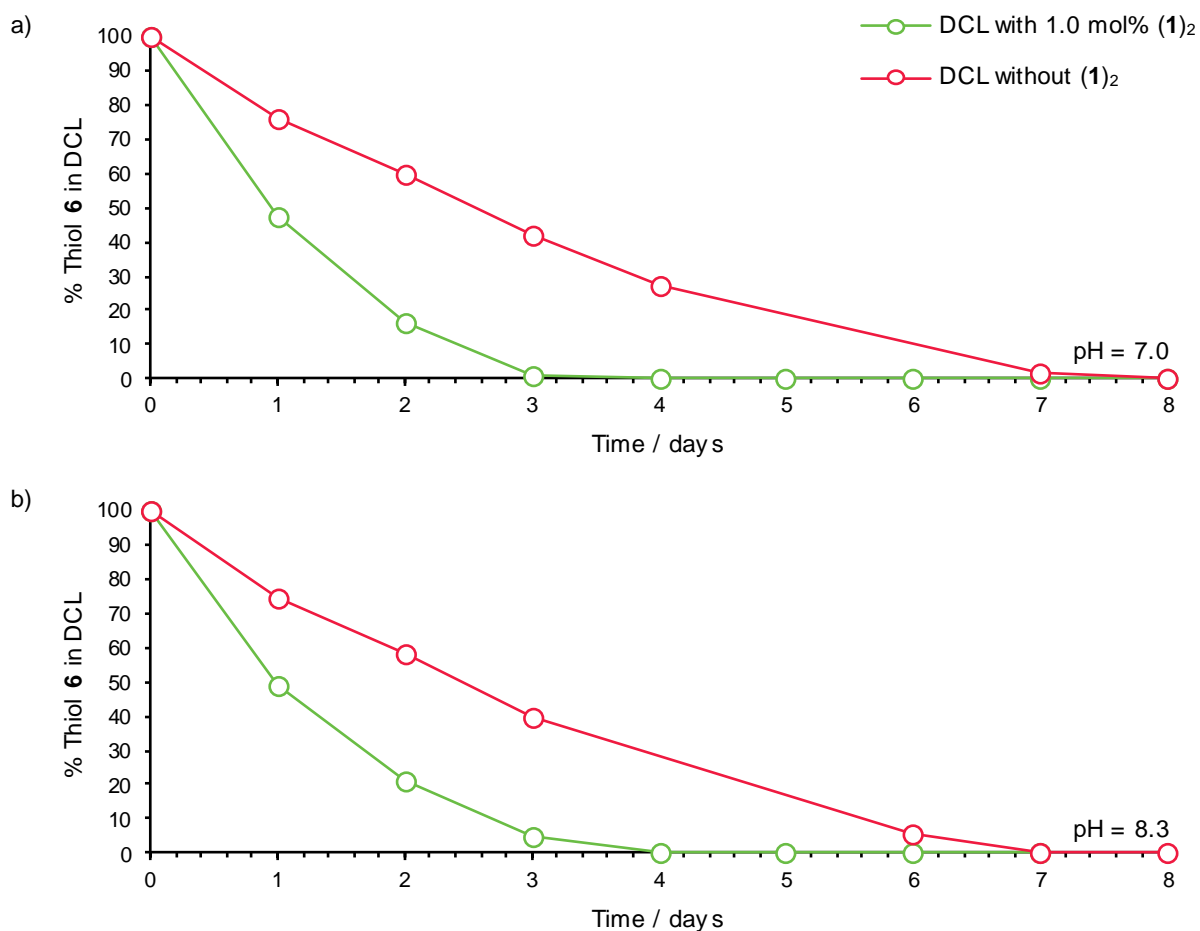


**Fig. S32** HPLC analysis (255 nm) of a 0.5 mM DCL at pH 7.0 at 298 K composed of thiol building block **6** with (top) and without (bottom) addition of 1.0 mol% diselenide (**1**)<sub>2</sub>. The HPLC chromatograms show the progress of the library formation over time (red – start, blue – day 1, green – day 2, purple – day 3). The HPLC chromatograms are solvent corrected.





**Fig. S33** Plots showing the conversion of dithiol building block **6** as a function of time in the 0.5 mM DCLs at a) pH 7.0, b) pH 7.6, and c) pH 8.3 with (green traces) and without (red traces) the addition of 10 mol% catalyst (1)<sub>2</sub>. The percentages are found from the areal of the peak corresponding to **6** in the HPLC chromatograms.



**Fig. S34** Plots showing the conversion of dithiol building block **6** as a function of time in the 0.5 mM DCLs at a) pH 7.0 and b) pH 8.3 with (green traces) and without (red traces) the addition of 1.0 mol% catalyst (**1**)<sub>2</sub>. The percentages are found from the areal of the peak corresponding to **6** in the HPLC chromatograms.

## S6 References

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- 2 A. Sørensen, B. Rasmussen, S. Agarwal, M. Schau-Magnussen, T. I. Sølling and M. Pittelkow, *Angew. Chem. Int. Ed.*, 2013, **52**, 12346-12349.
- 3 S. Otto, R. L. E. Furlan and J. K. M. Sanders, *Science*, 2002, **297**, 590-593.