Supporting information for:

# "Diverse topologies in dynamic combinatorial libraries from tri- and mono-thiols in water: sensitivity to weak supramolecular interactions"

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Contents:

**1.** General

Chemicals were purchased from commercial suppliers and used as received. Water and MeOH for LC-MS were purchased from Romil or Rathburn. HPLC/LC-MS was performed on HP1050 or Agilent 1100LC/MSD trap XCT systems coupled to a diode array detector and the data processed using ChemStation software. Mass spectra (negative mode) were acquired in ultra scan mode using drying temperature of 325°C, nebulizer pressure of 55 psi, drying gas flow of 10 L/min, capillary voltage of 4,000 V, an ICC target of 200,000 ions, and target mass of 1,000. Analytical separations were achieved by injecting 5 $\mu$L (for 5 mM DCL, scaled accordingly for DCL at different concentrations) of DCL solution on to a Symmetry C8 reverse phase column (250 mm 4.6 cm, 3 $\mu$m particle size) with an gradient elution of 10 % MeOH in water to 100 % of MeOH at 20 min. (with 0.1% formic acid) at 35°C and a flow rate of 1 mL/min. All mono-thiols were purchased from commercial suppliers and used as received. The synthesis and characterisation of the tri-thiol **1** was described in our previous report.[5b] A typical analytical DCL was prepared on a 0.8 ml scale by dissolving an equimolar mixture of **1** (5 mM) and monothiol **2** (40 mM) in 10 mM aqueous NaOH, followed by titration with 100 mM aqueous NaOH/HCl to pH = 8. In untemplated DCL 0.2 ml of pure water was added to 0.8 ml. Where appropriate, guests (each at 5 mM in 0.2 ml of $H_2O$) were added which was then followed by titration with 100 mM aqueous NaOH/HCl to pH = 7.5. The DCL was stirred in a close-capped vial at room temperature until being analysed. The pH of each library was checked before and after equilibration process to make sure it remained unchanged. The HPLC traces remained unchanged after 5 days indicating that thermodynamic equilibrium had been reached. To confirm this the separate building blocks **1** and mono-thiols **2** were oxidised independently and subsequently mixed together followed by addition of 15 mol% dithiothreitol to initiate disulfide exchange. Product distributions for DCLs formed through this procedure were essentially identical to those obtained starting directly from the thiol building blocks.

The choice of pH = 7-8 was dictated by following reasons:

1) pH around 7-8 was found to be optimum for the disulfide exchange

2) to ensure that the all of carboxylates are in their deprotonated form

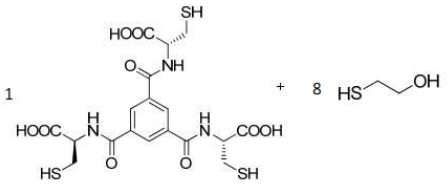
3) to ensure that the highest possible number of amines (for mono-thiol **2a**) are in their protonated from

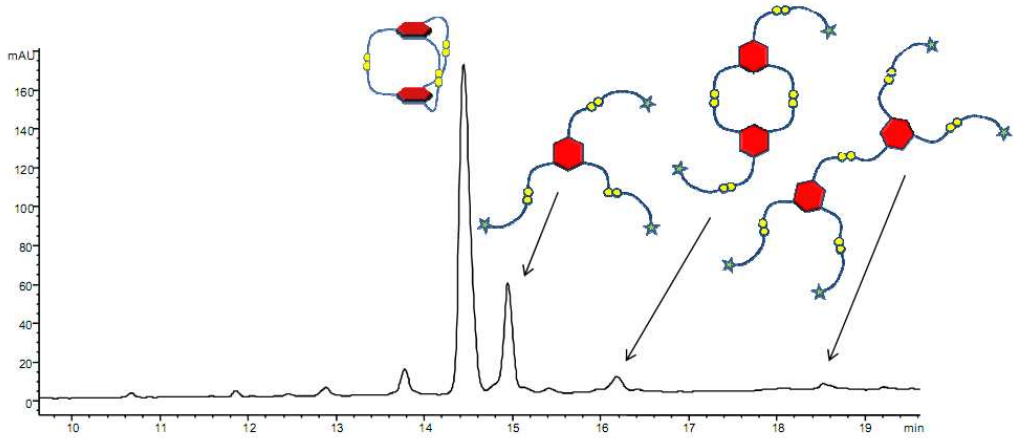
4) solubility of building blocks used is optimum at pH around 7-8

For calculation of the library composition in DCLs is it assumed that each compound investigated has the same molar extinction coefficient and the percentages shown refer to the calculated peak area.

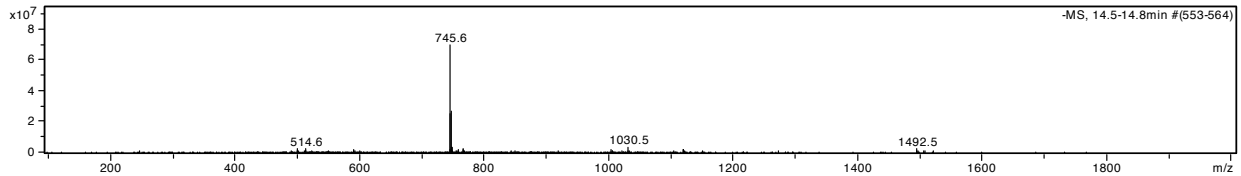
**2.** HPLC and ESI-MS data showing DCLs composition prepared from tri-thiol **1** and monothiols **2b**-**2g**.

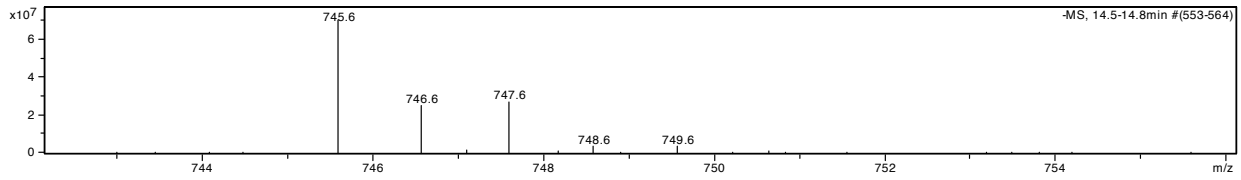
a) DCL composed of **1** (5 mM) and **2b** (40 mM) in water at pH = 8, after 5 days incubation.



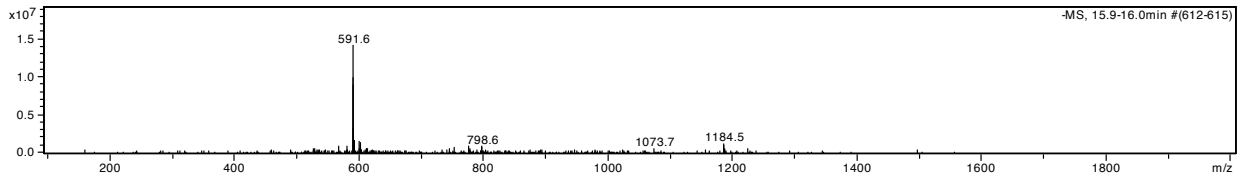



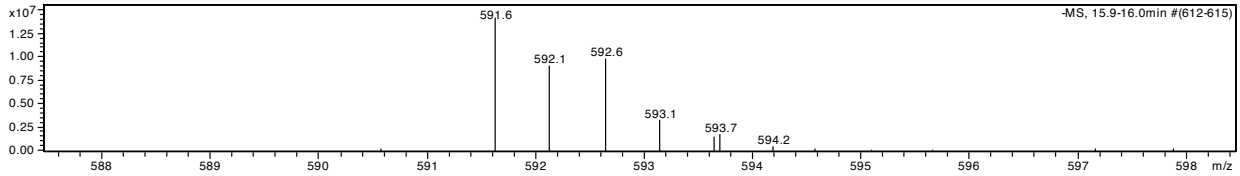

- ESI-MS of tetramer **1**·3×**2b**



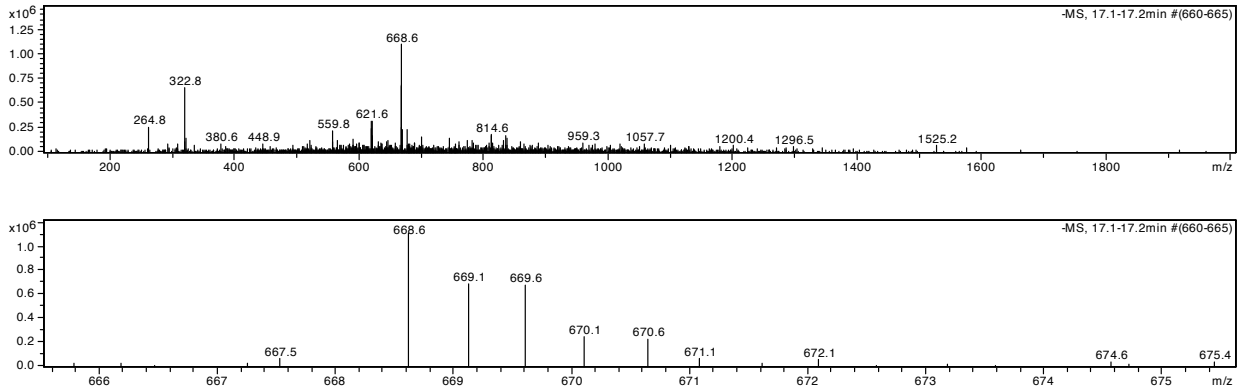




- ESI-MS of tetramer 2×**1**·2×**2b**

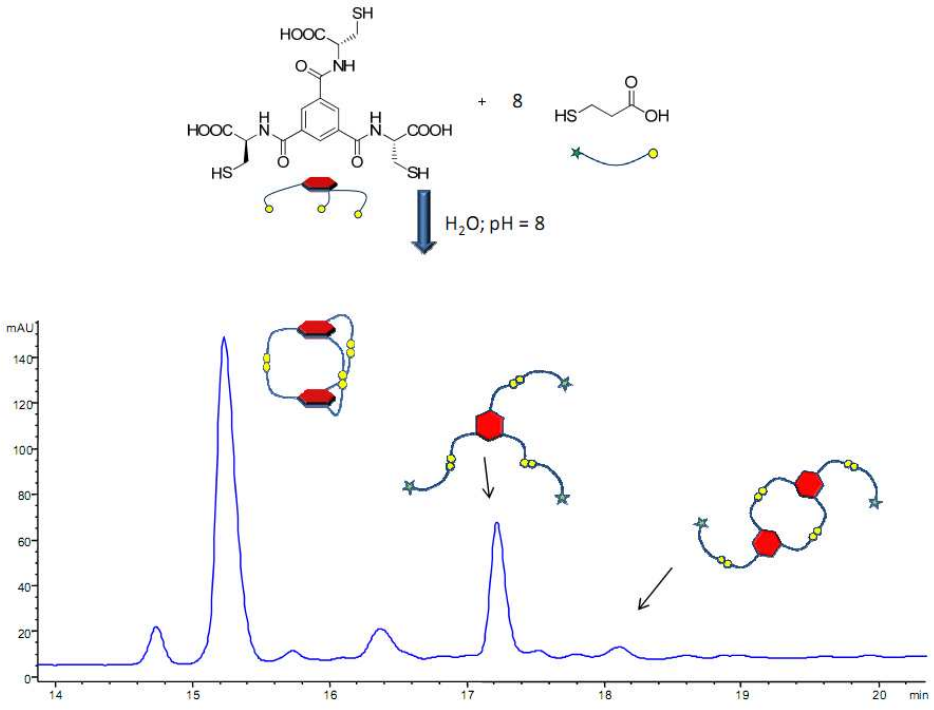




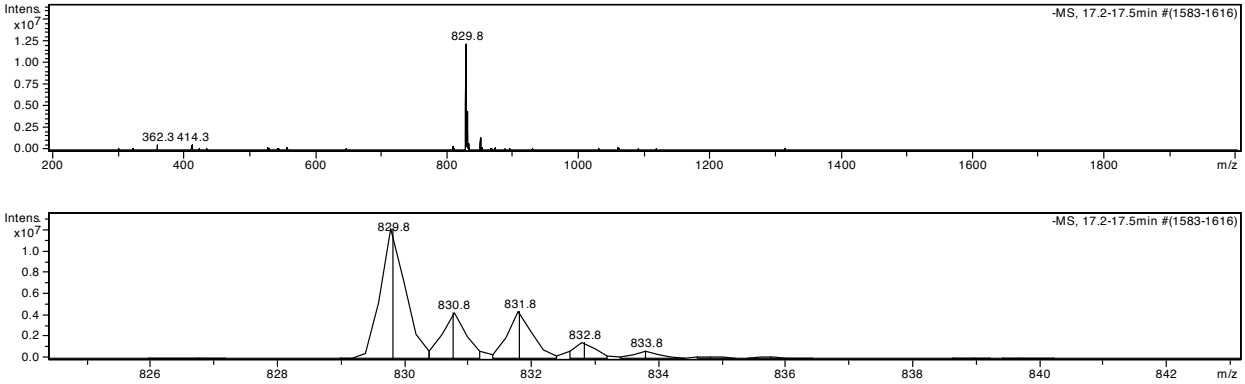

- ESI-MS of hexamer 2×**1**·4×**2b**



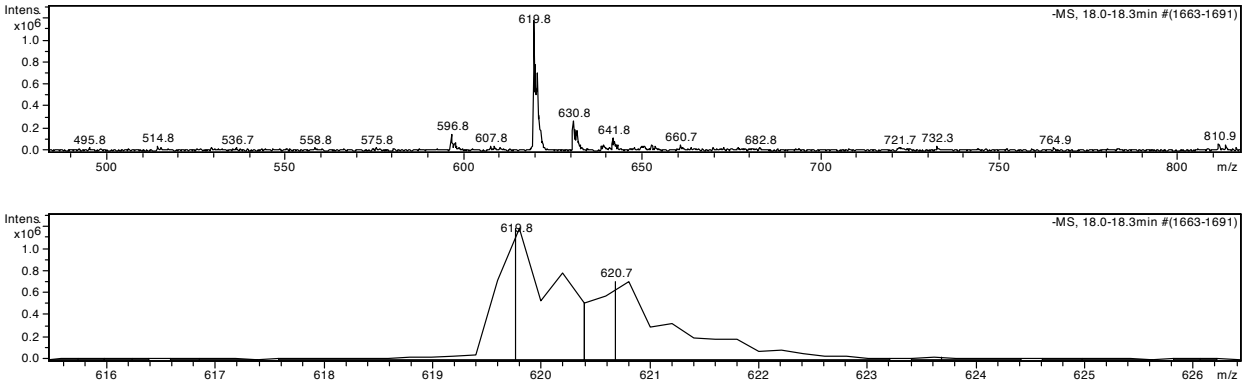

b) DCL composed of **1** (5 mM) and **2c** (40 mM) in water at pH = 8, after 5 days incubation.



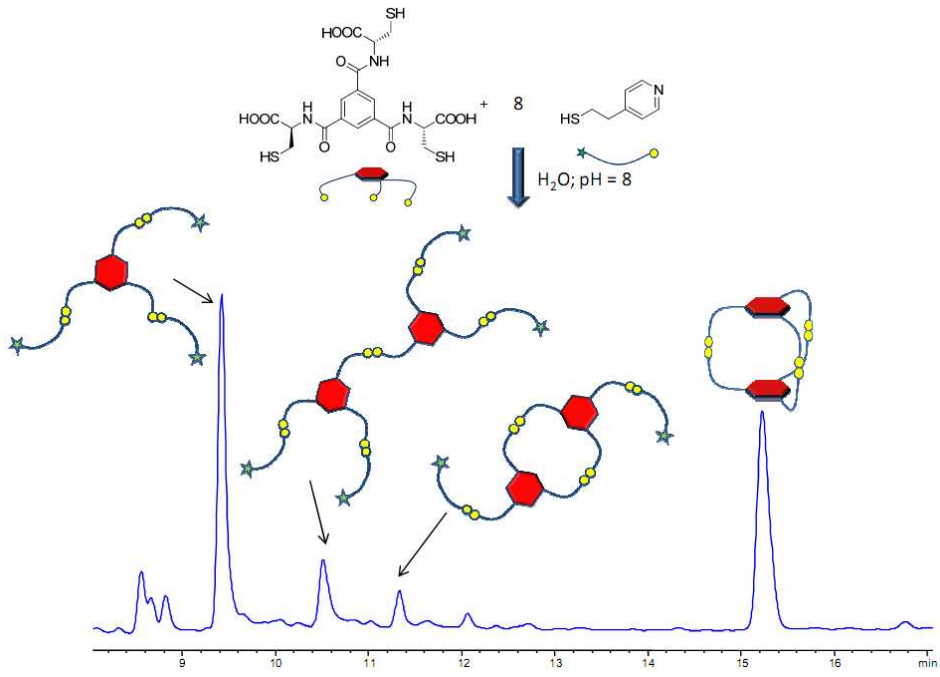
- ESI-MS of tetramer **1**·3×**2c**



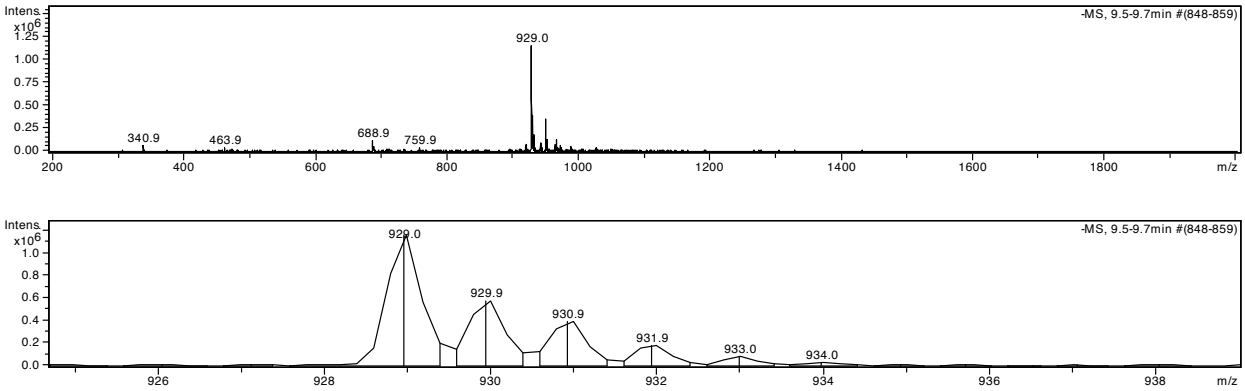

- ESI-MS of tetramer 2×**1**·2×**2c**



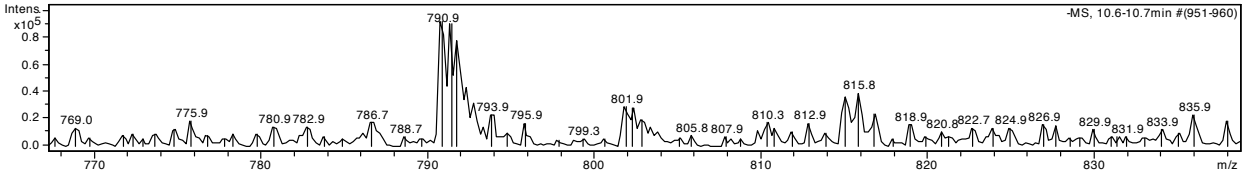

c) DCL composed of **1** (5 mM) and **2d** (40 mM) in water at pH = 8, after 5 days incubation.



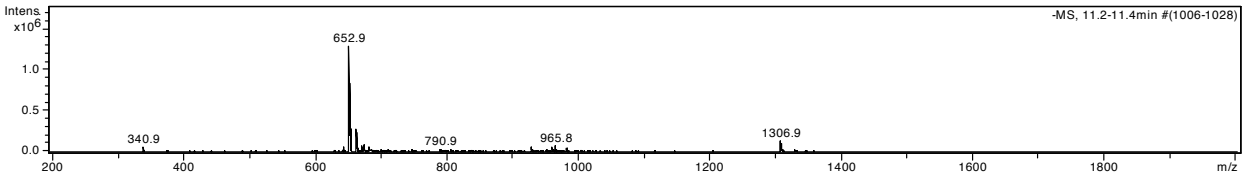
- ESI-MS of tetramer **1**·3×**2d**



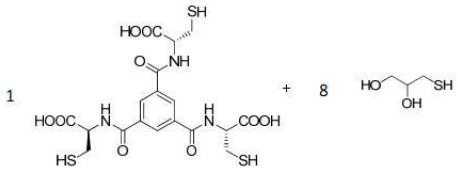

- ESI-MS of hexamer 2×**1**·4×**2d**

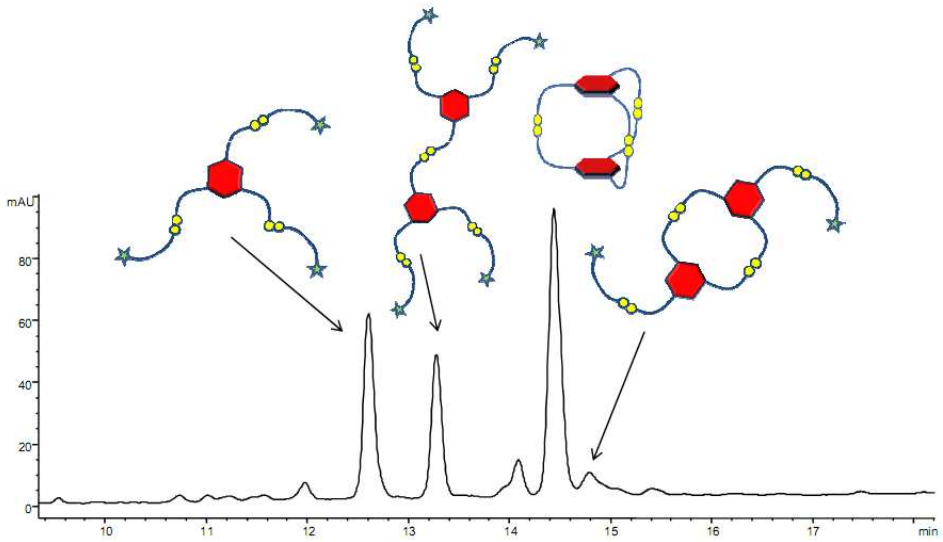



- ESI-MS of tetramer 2×**1**·2×**2d**

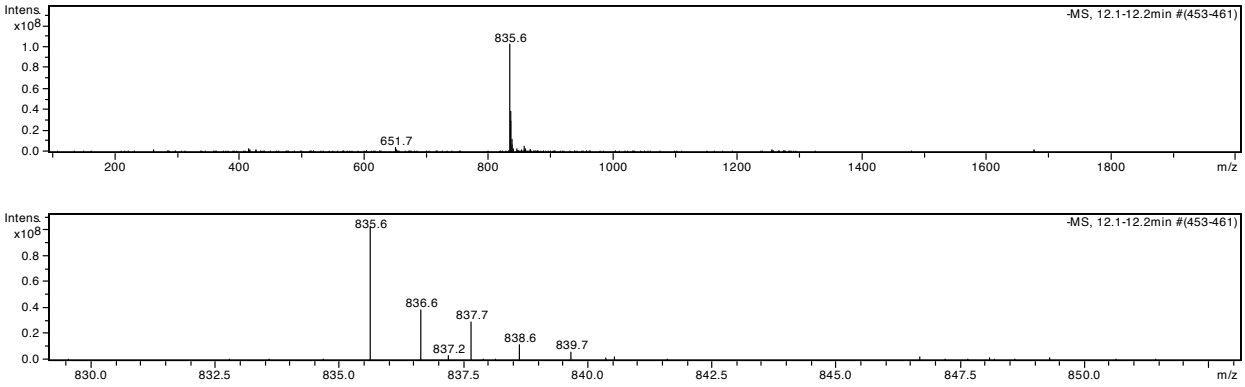


d) DCL composed of **1** (5 mM) and **2e** (40 mM) in water at pH = 8, after 5 days incubation.

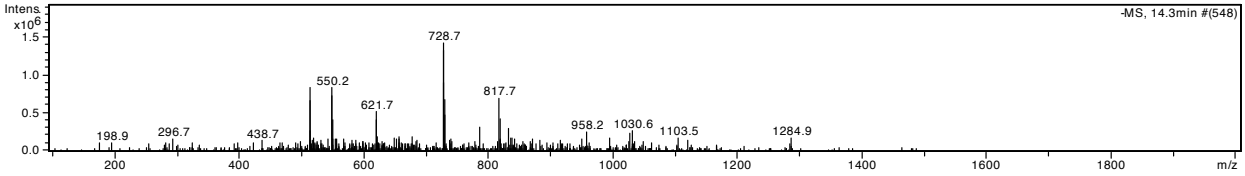


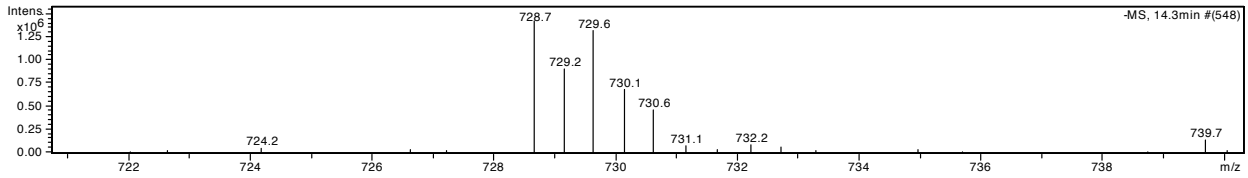




- ESI-MS of tetramer **1**·3×**2e**

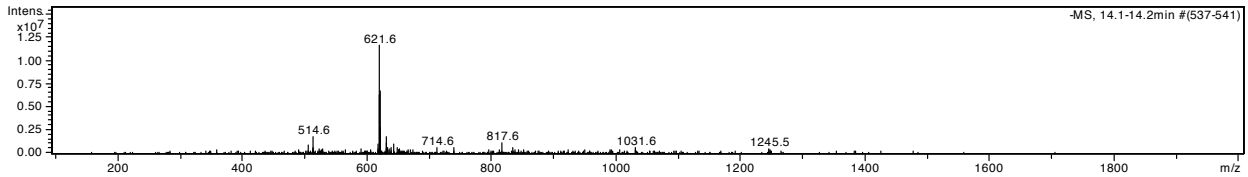


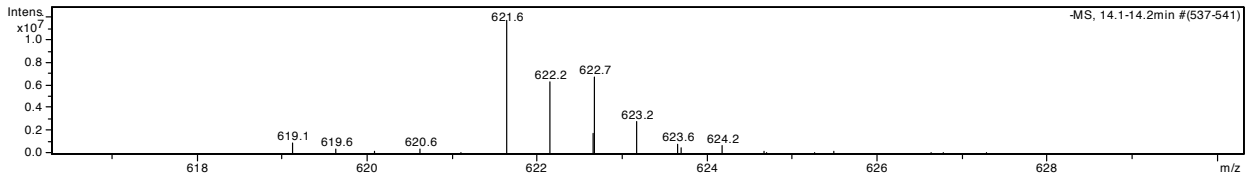

- ESI-MS of hexamer 2×**1**·4×**2e**



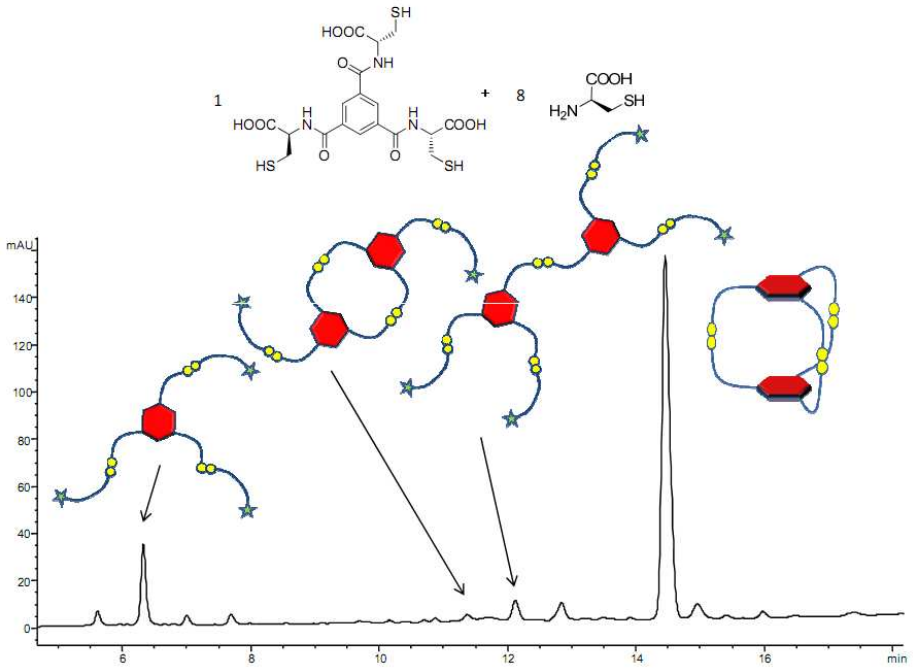



- ESI-MS of tetramer 2×**1**·2×**2e**

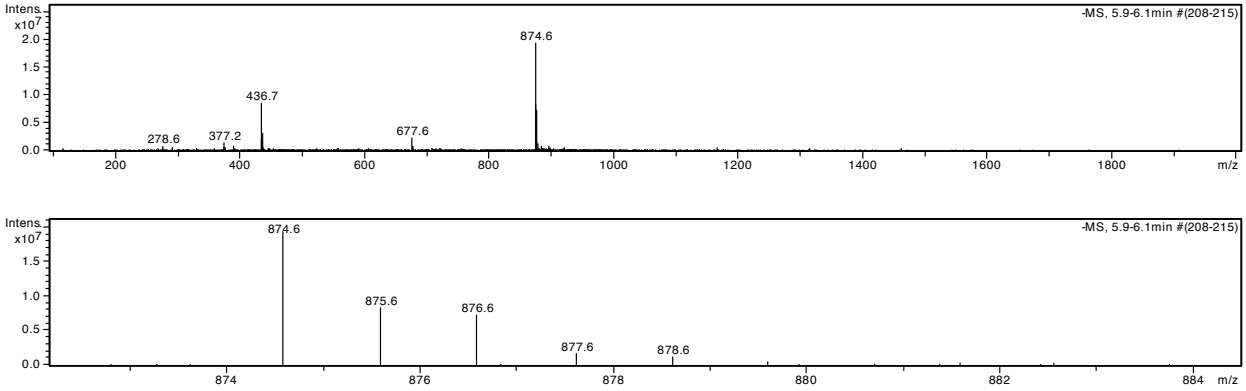


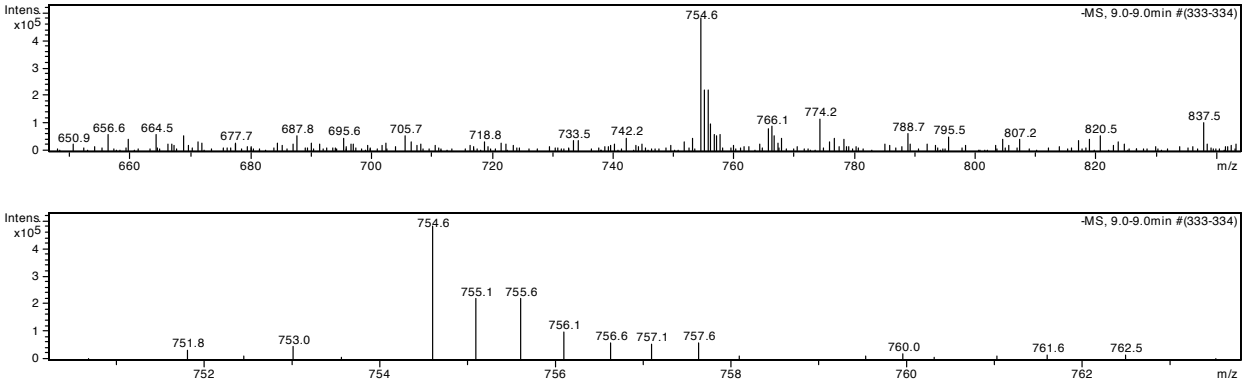

e) DCL composed of **1** (5 mM) and **2f** (40 mM) in water at pH = 8, after 5 days incubation.



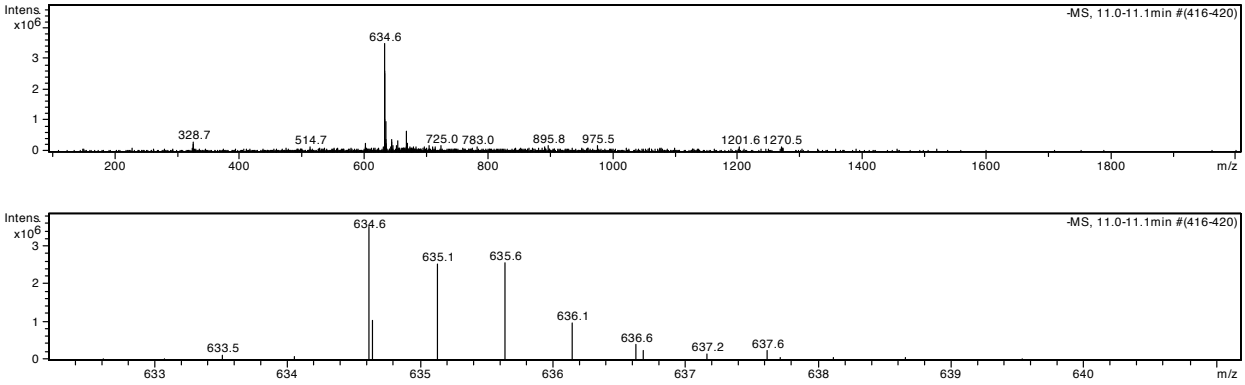
- ESI-MS of tetramer **1**·3×**2f**



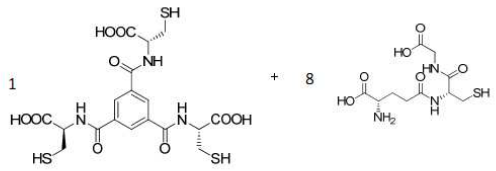

- ESI-MS of hexamer 2×**1**·4×**2f**

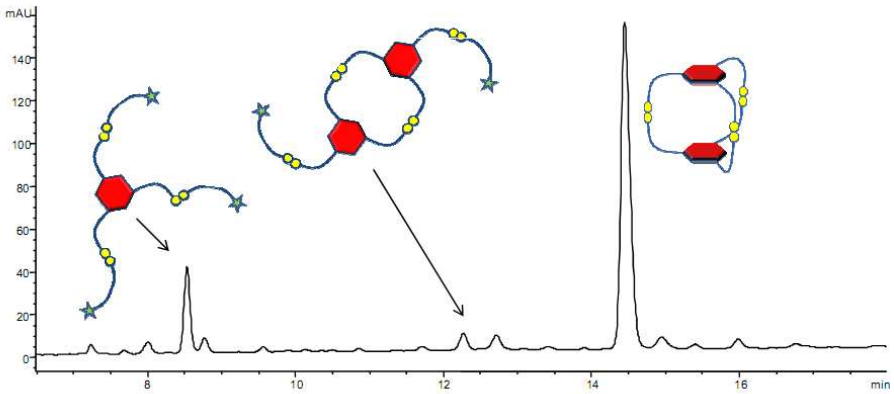



- ESI-MS of tetramer 2×**1**·2×**2f**

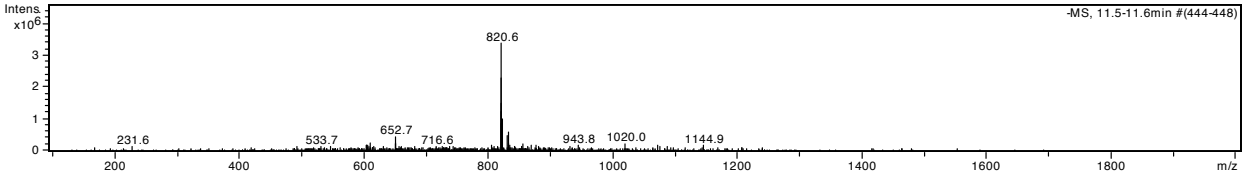


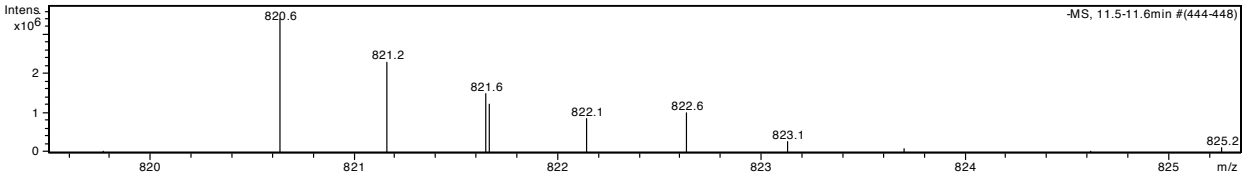
f) DCL composed of **1** (5 mM) and **2g** (40 mM) in water at pH = 8, after 5 days incubation.



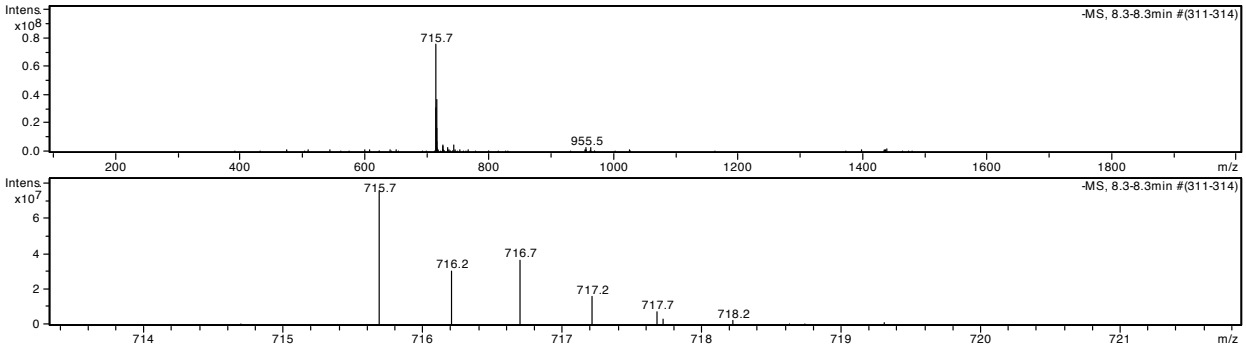




- ESI-MS of tetramer 2×**1**·2×**2g**



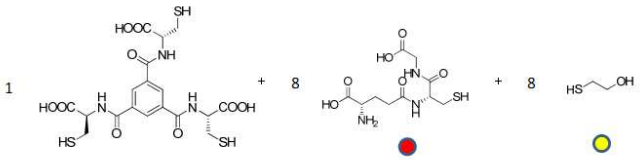



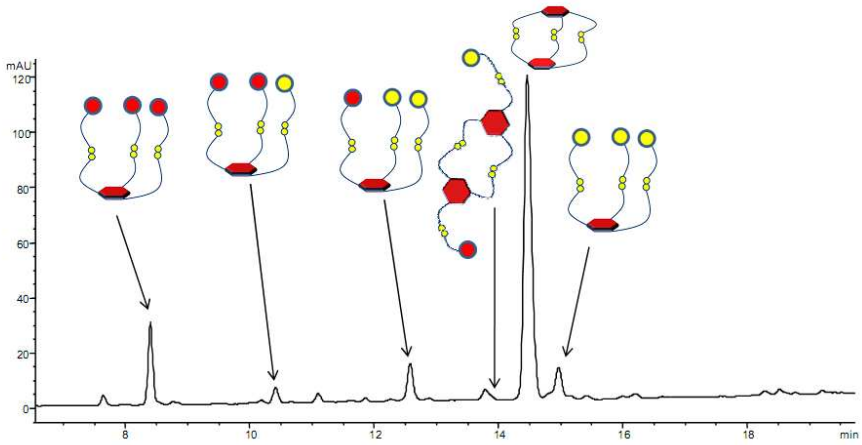

- ESI-MS of tetramer **1**·3×**2g**



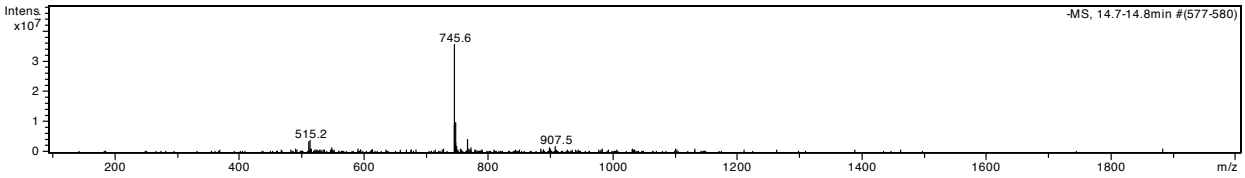
**3.** HPLC and ESI-MS data showing DCLs prepared from tri-thiol **1** and mixture of monothiols.

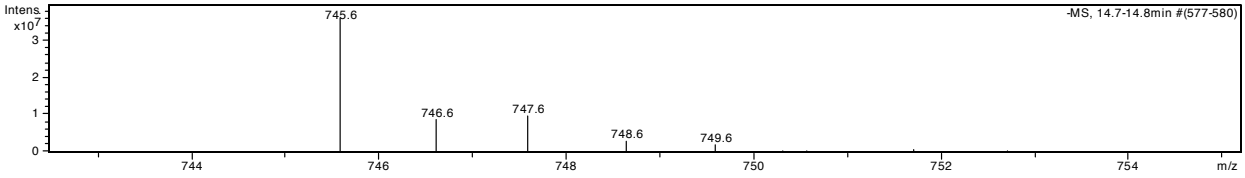
a) DCL composed of **1** (5 mM) and **2b** and **2g** (40 mM, each) in water at pH = 8, after 5 days incubation.



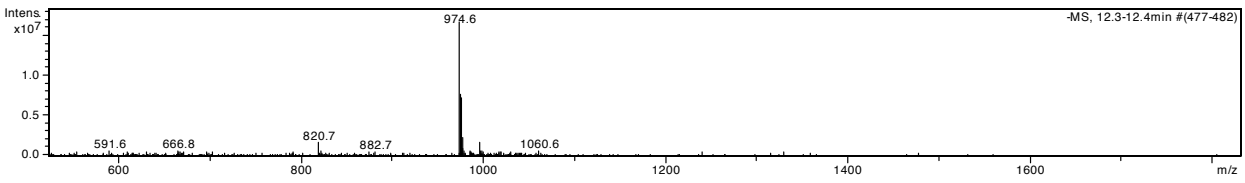



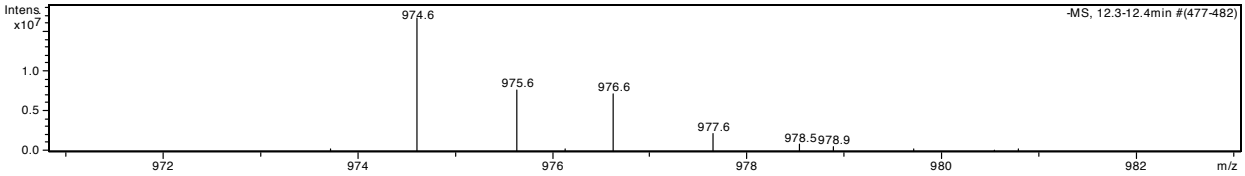

- ESI-MS of tetramer **1**·3×**2b**



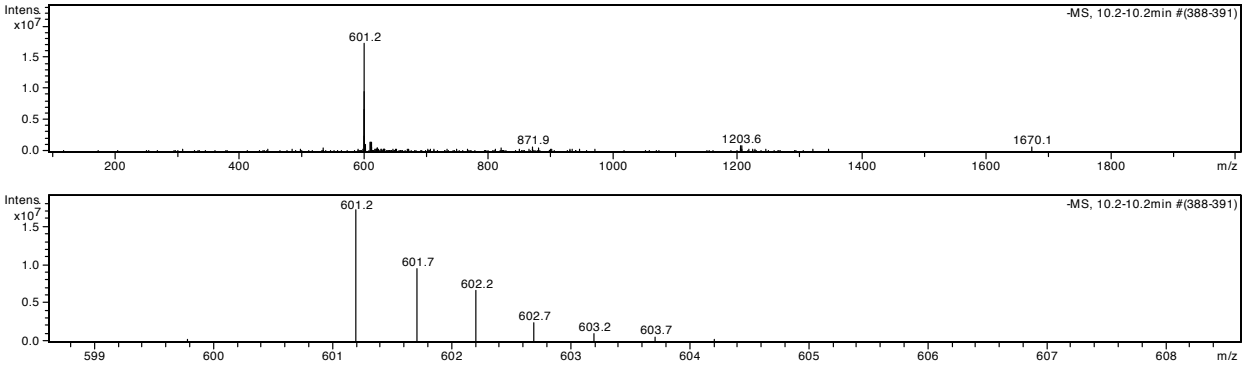




- ESI-MS of tetramer **1**·2×**2b**·1×**2g**

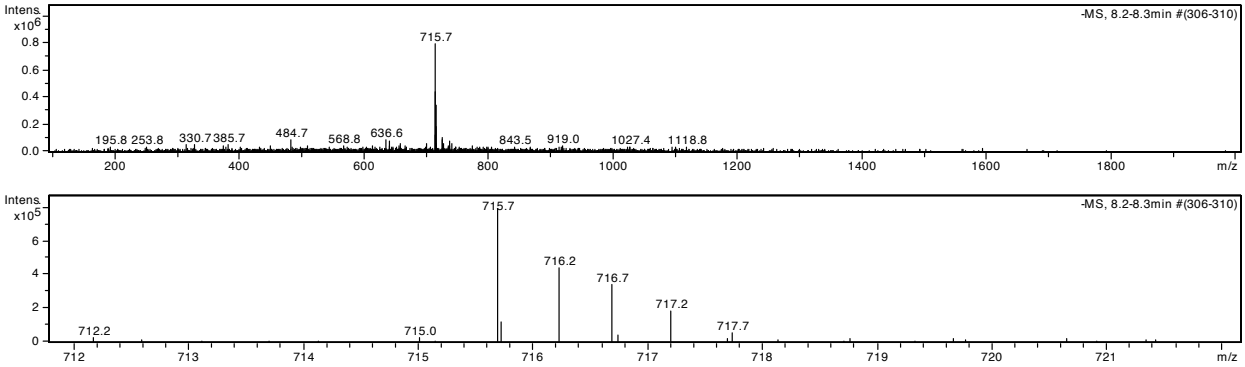




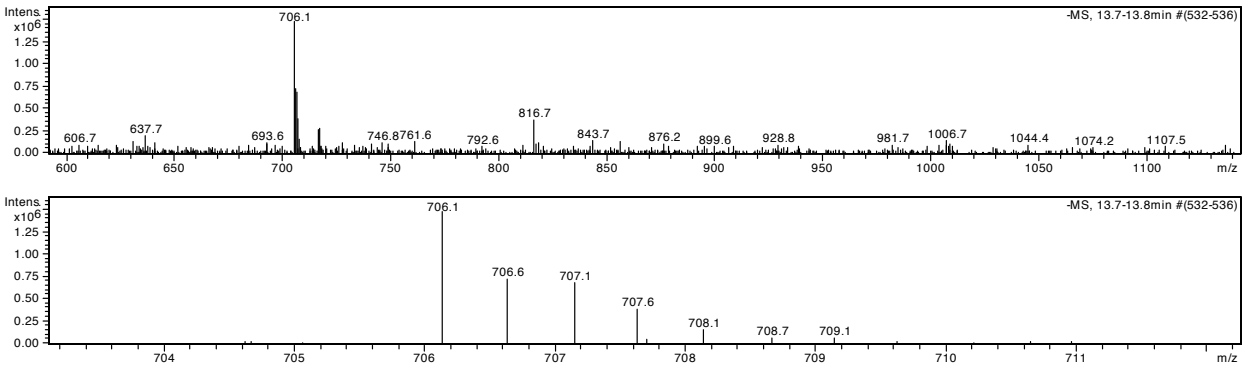

- ESI-MS of tetramer **1**·2×**2b**·2×**2g**



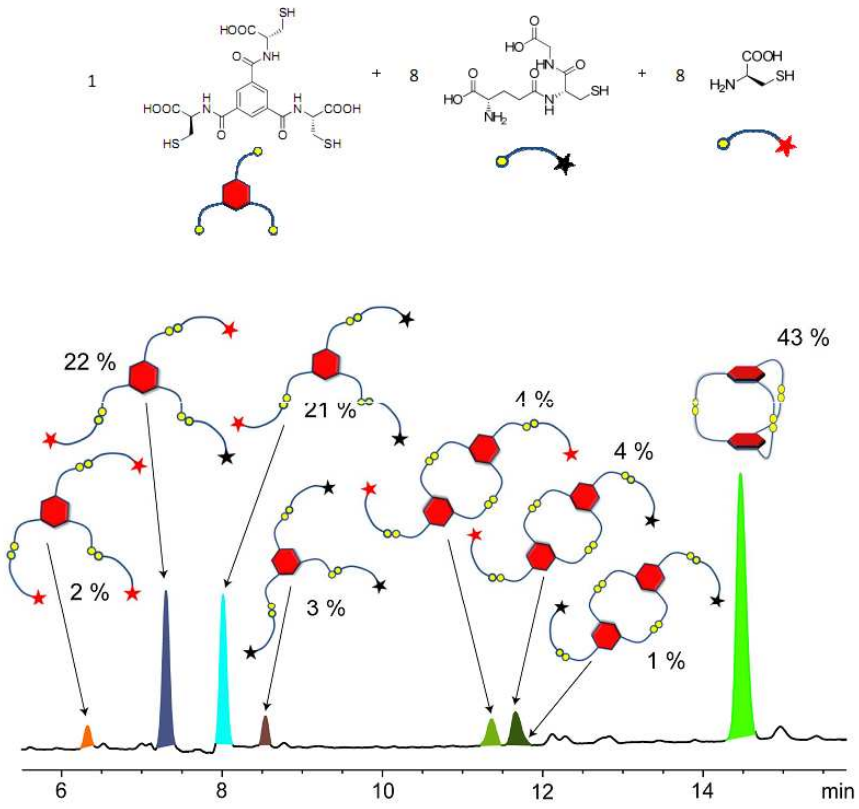

- ESI-MS of tetramer **1**·3×**2g**



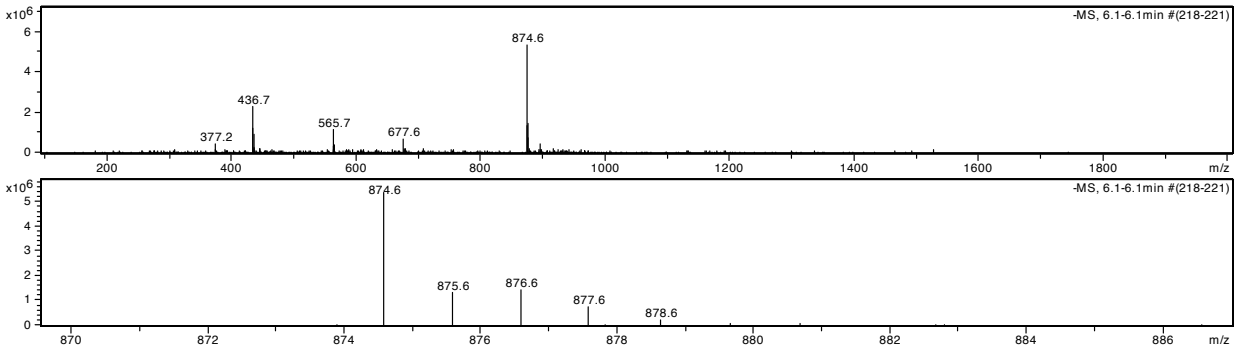

- ESI-MS of tetramer 2×**1**·1×**2g**·1×**2b**



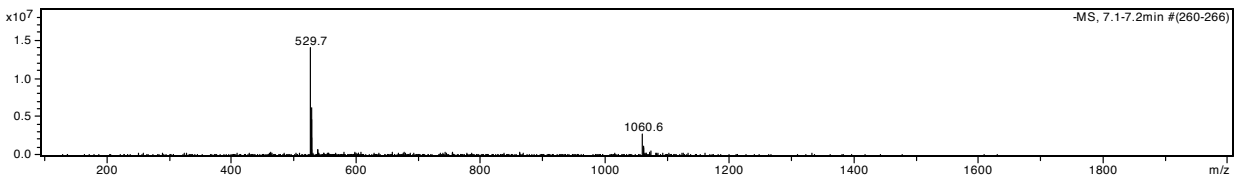
b) DCL composed of **1** (5 mM) and **2f** and **2g** (40 mM, each) in water at pH = 8, after 5 days incubation.

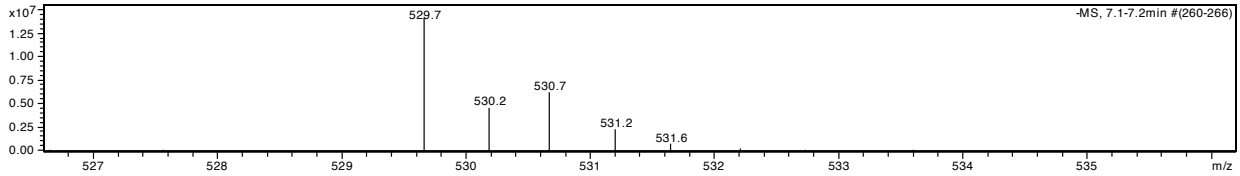



- ESI-MS of tetramer **1**·3×**2f**

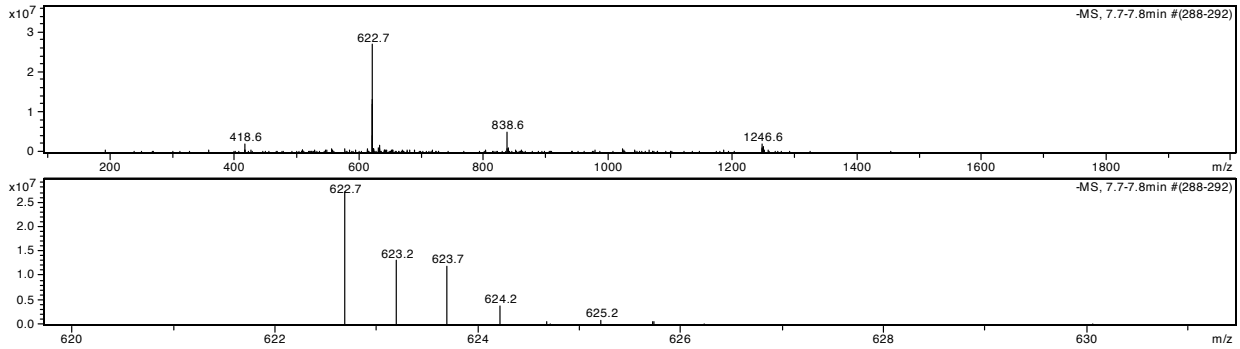



- ESI-MS of tetramer **1**·2×**2f**·1×**2g**

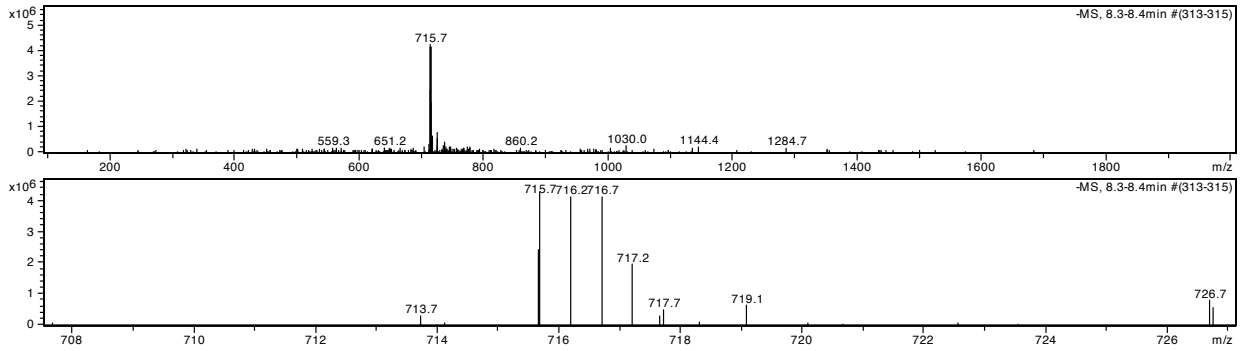




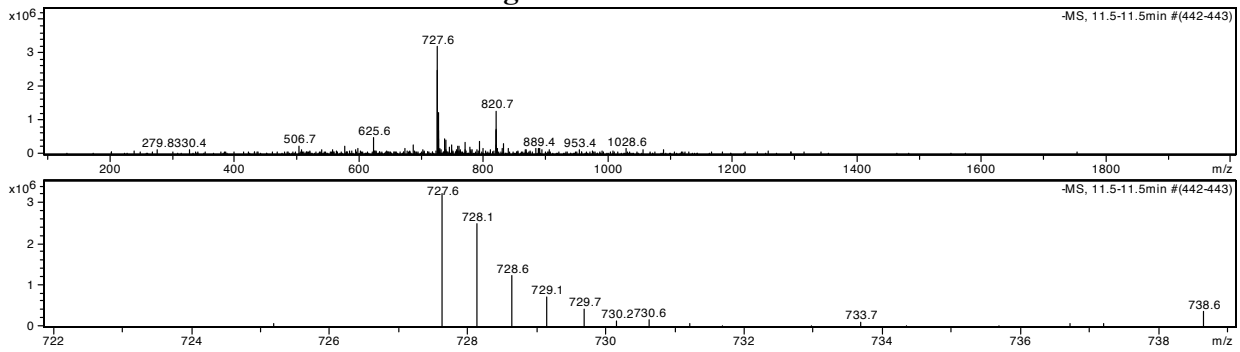

- ESI-MS of tetramer **1**·1×**2f**·2×**2g**



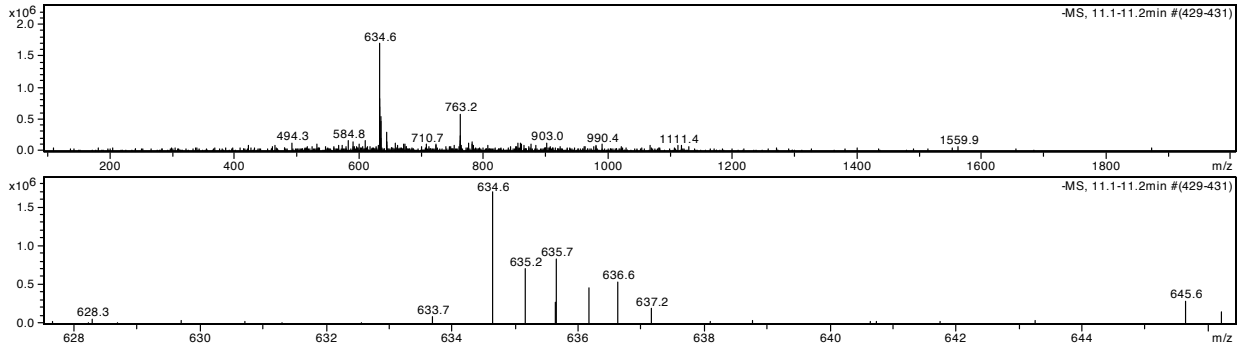

- ESI-MS of tetramer **1**·3×**2g**



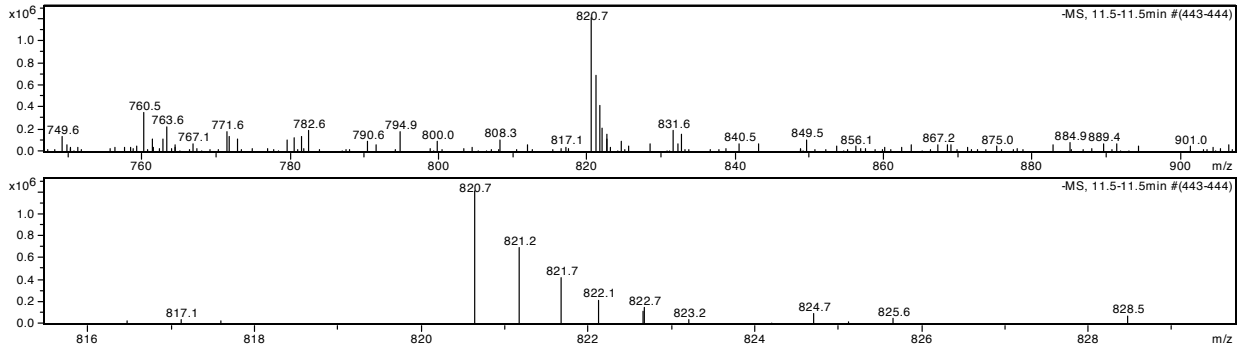

- ESI-MS of tetramer 2×**1**·1×**2f**·1×**2g**




- ESI-MS of tetramer 2×**1**·2×**2f**

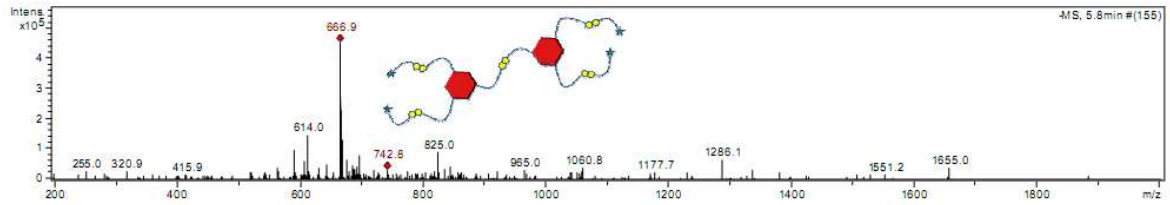



- ESI-MS of tetramer 2×**1**·2×**2g**

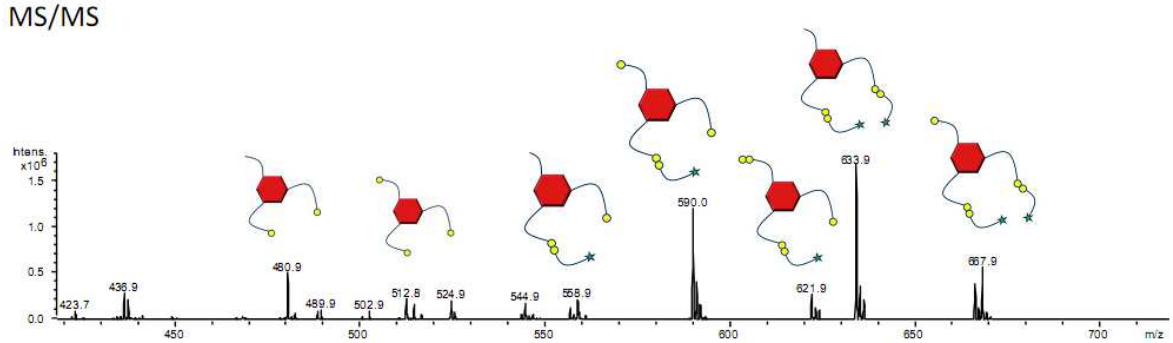


**4.** ESI-MS and MS/MS data of DCLs prepared from tri-thiol **1** and mono-thiol **2a**.

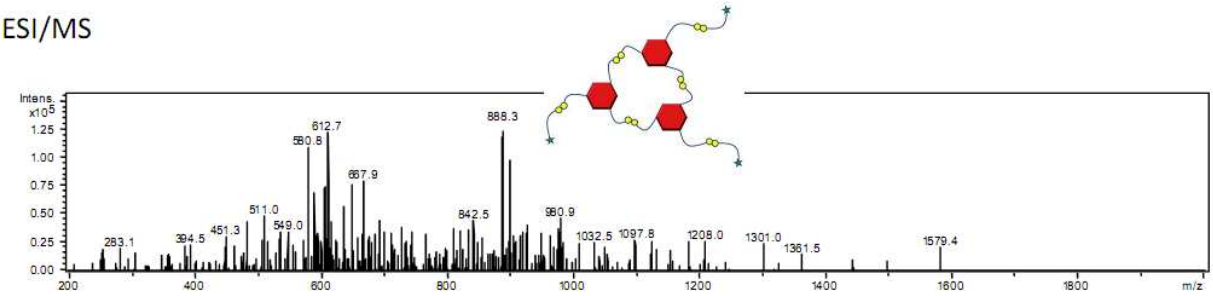
ESI/MS



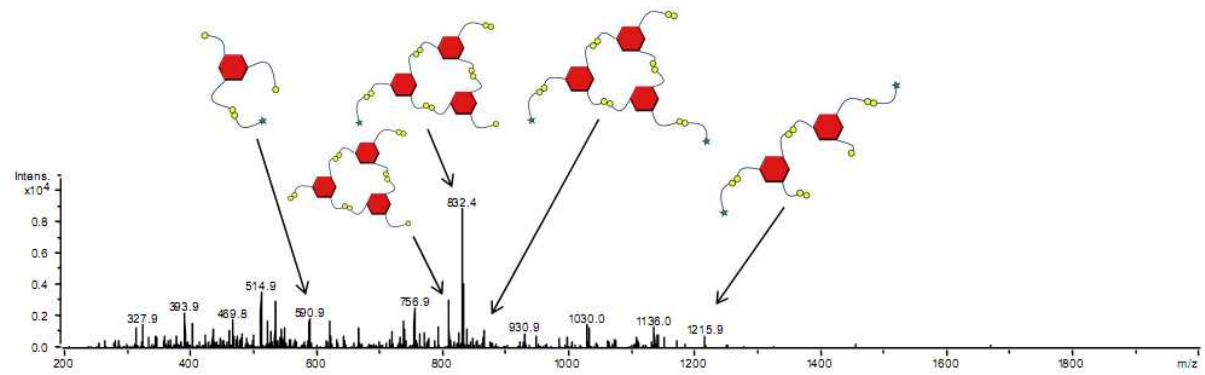

MS/MS



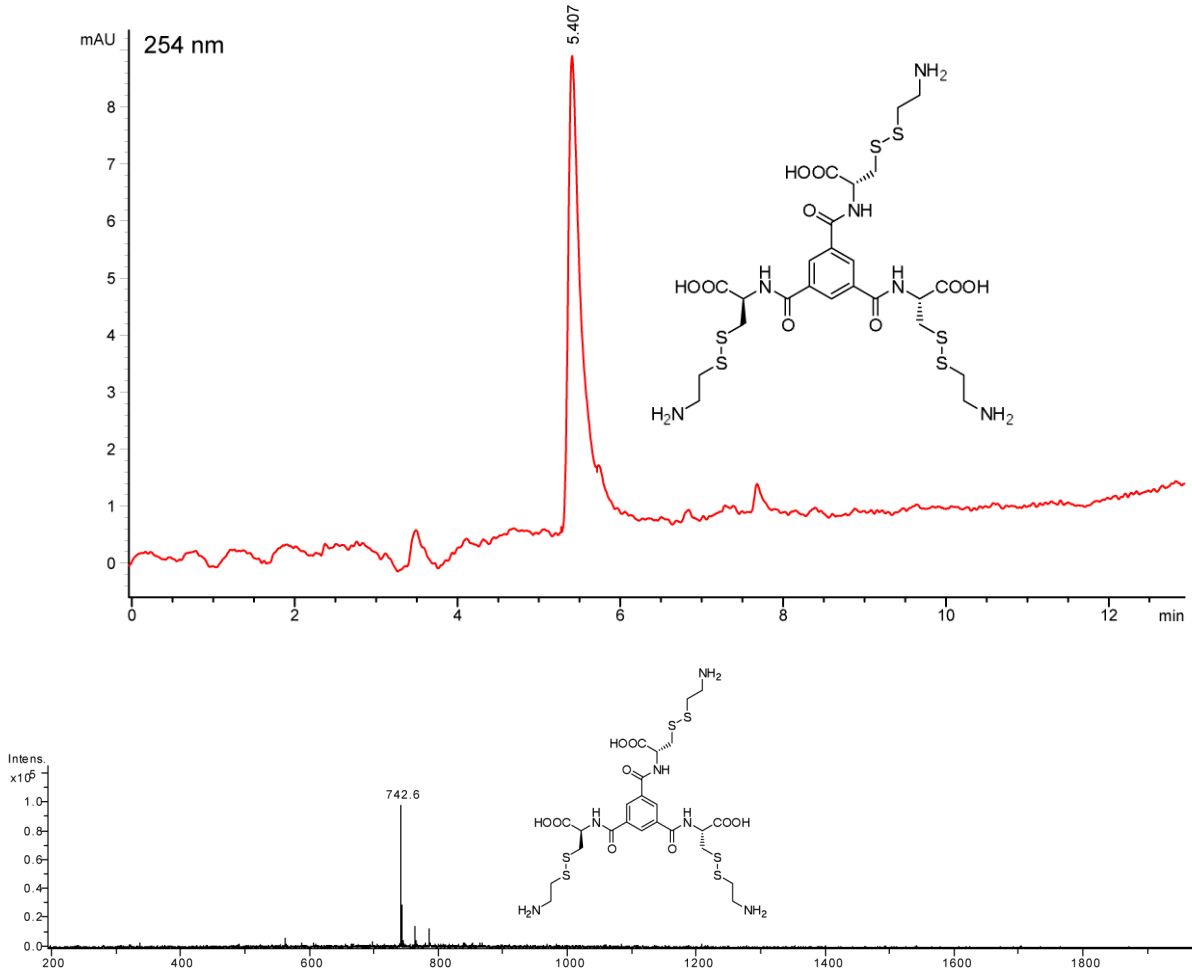

ESI/MS



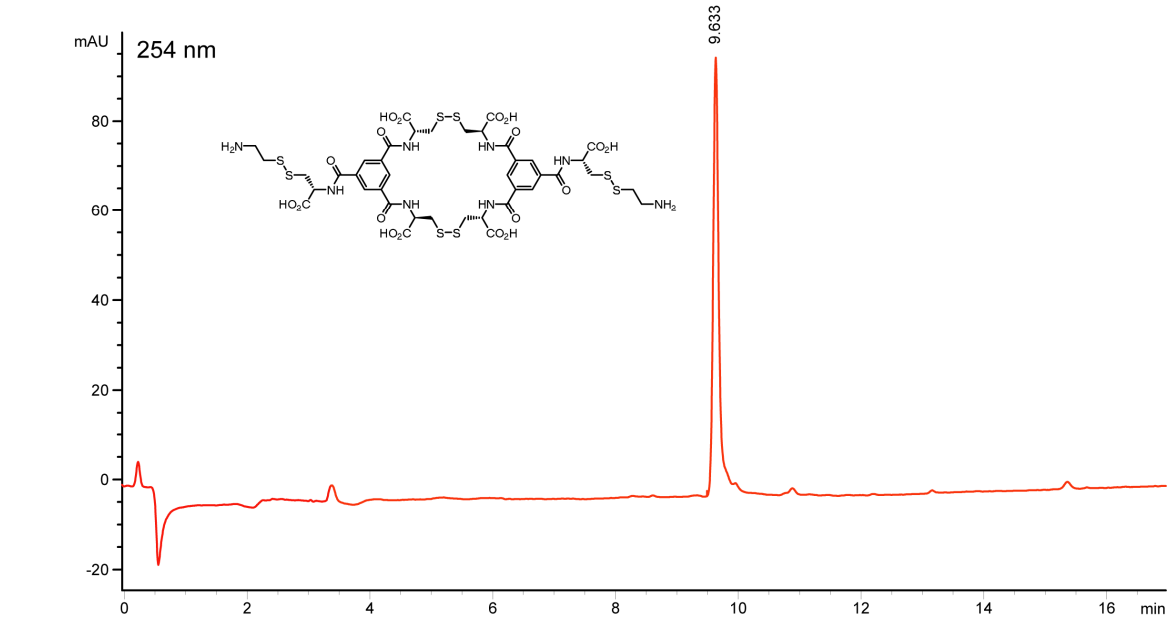

MS/MS

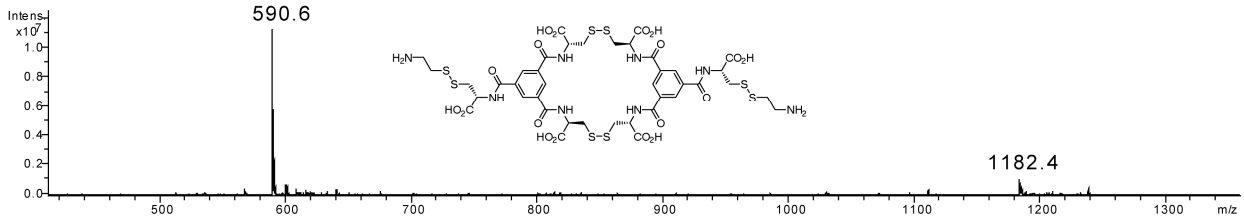


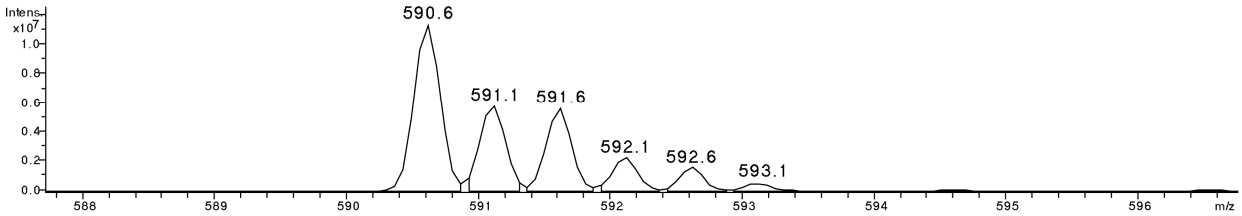
**5.** ESI-MS and HPLC data of the tetramer **1**·3×**2a** after isolation.

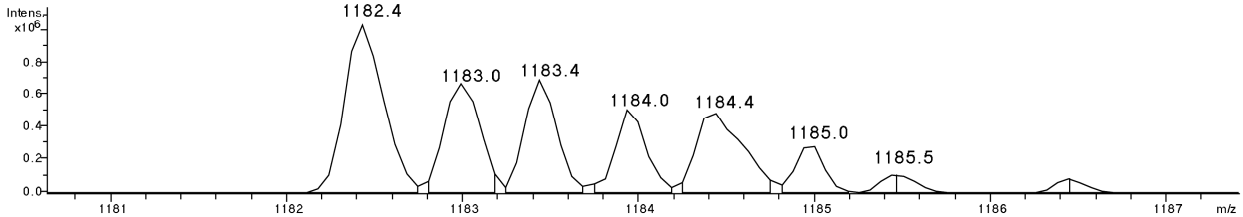


**6.** ESI-MS and HPLC data of the tetramer 2×**1**·2×**2a** after isolation.



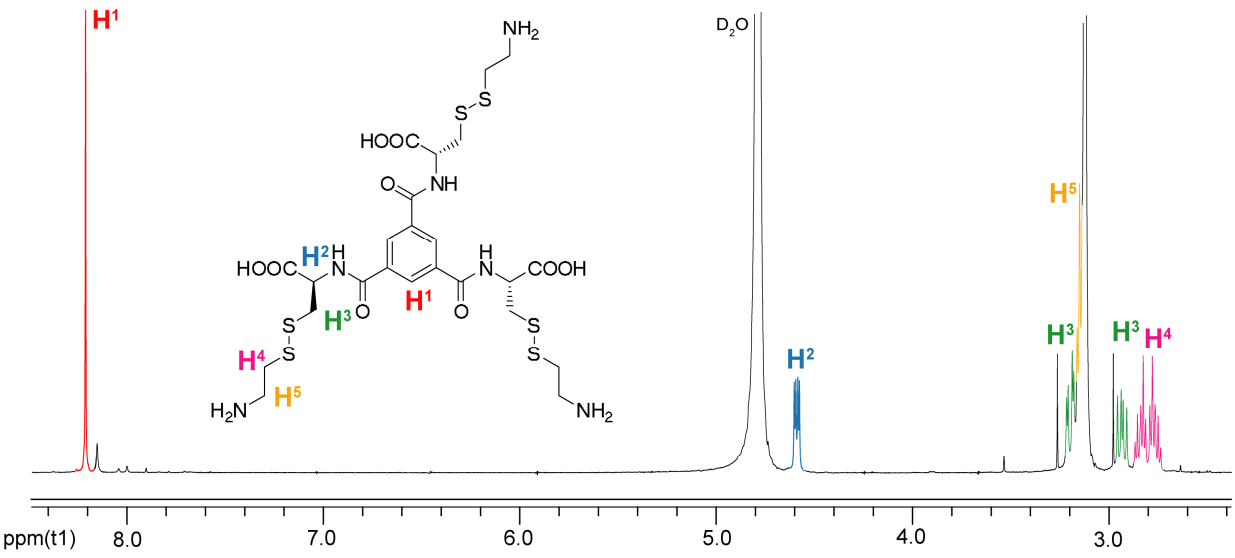



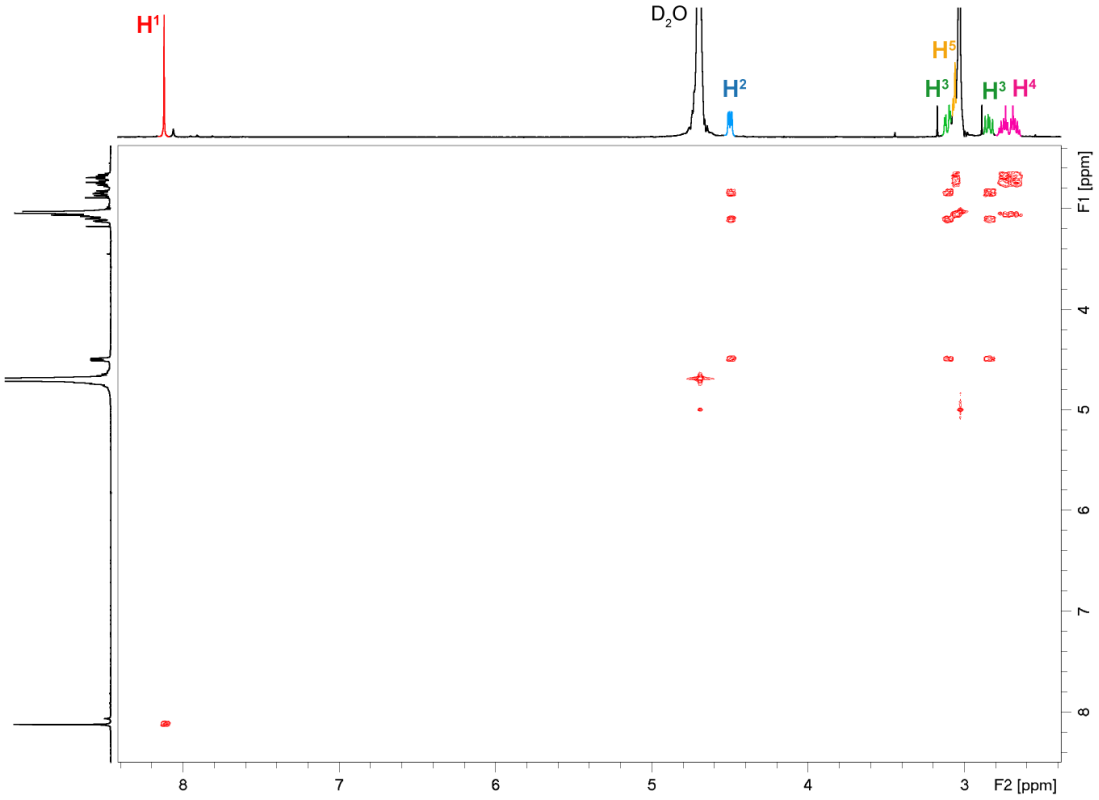



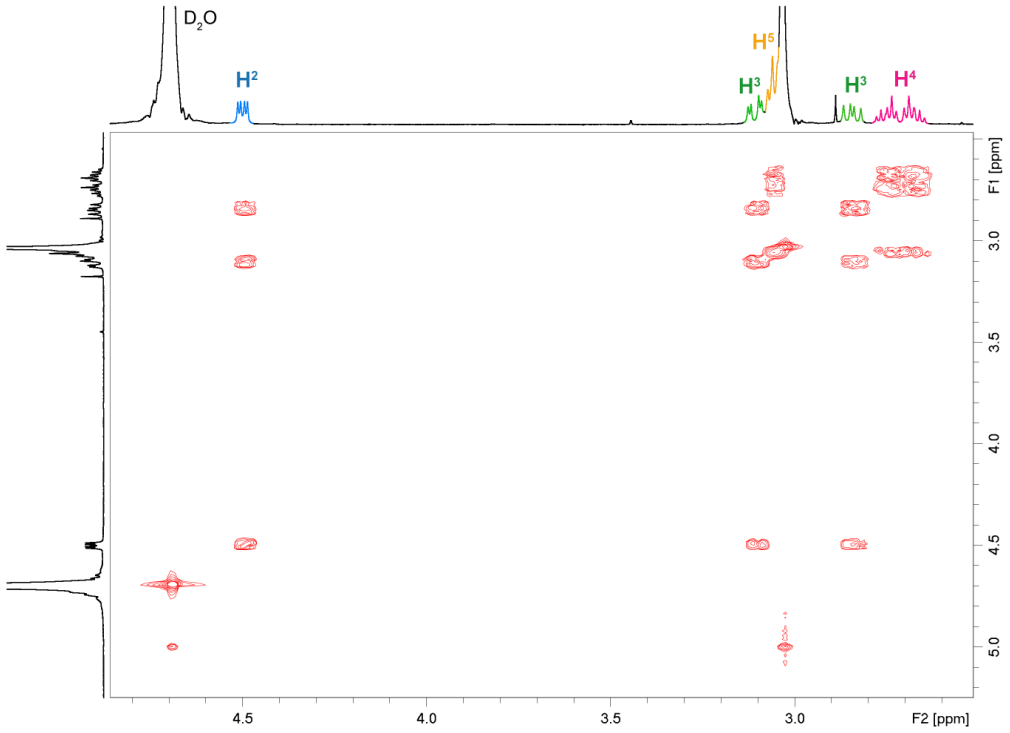
**7.** The linear and macrocyclic tetramers were isolated/purified using preparative HPLC. A preparative DCL was made in a 10 ml scale using the same method as the analytical libraries. Preparative separations were performed on a HP 1050 system coupled to a single variable wavelength UV detector. Samples were injected onto a reverse phase Symmetry Prep C18 column (300 x 7.8 mm, 7 $\mu$m particle size) by using a Gilson 234 auto-injector. The same elution profile as that for the analytical separation was used, with HPLC grade methanol (Fischer), MilliQ water and formic acid (Romil). Fractions were manually collected and combined. Solvents were removed from the combined fractions by a rotary evaporator. The isolated samples were dried in vacuo and stored in the fridge.

$^1$H, and COSY NMR spectra of the tetramer **1**·3×**2a** after isolation. The spectra were recorded in $D_2O$ at 298 K (500 MHz NMR spectrometer).

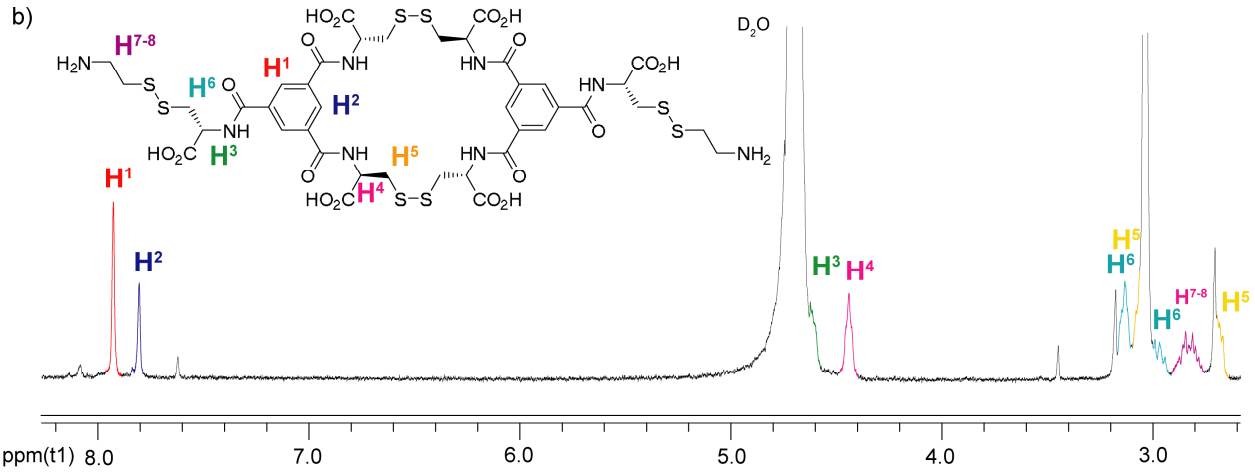
a)

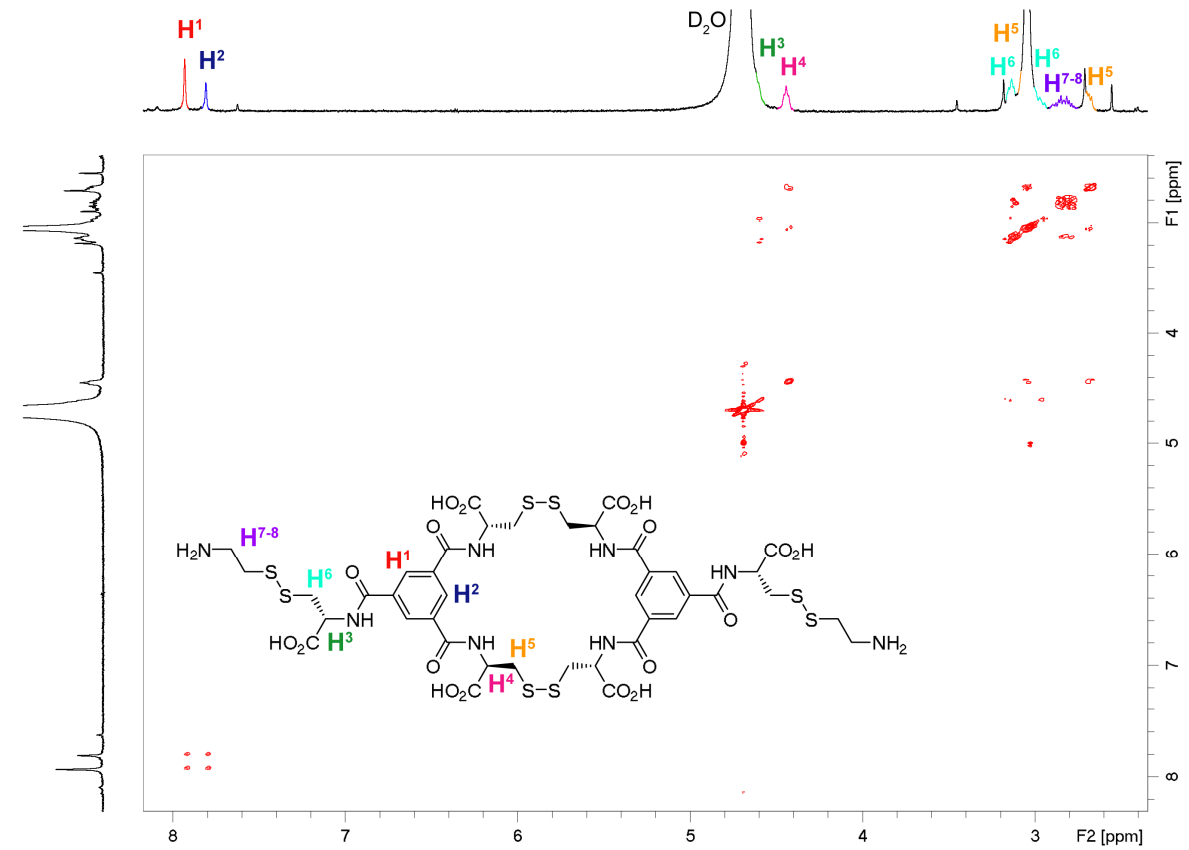


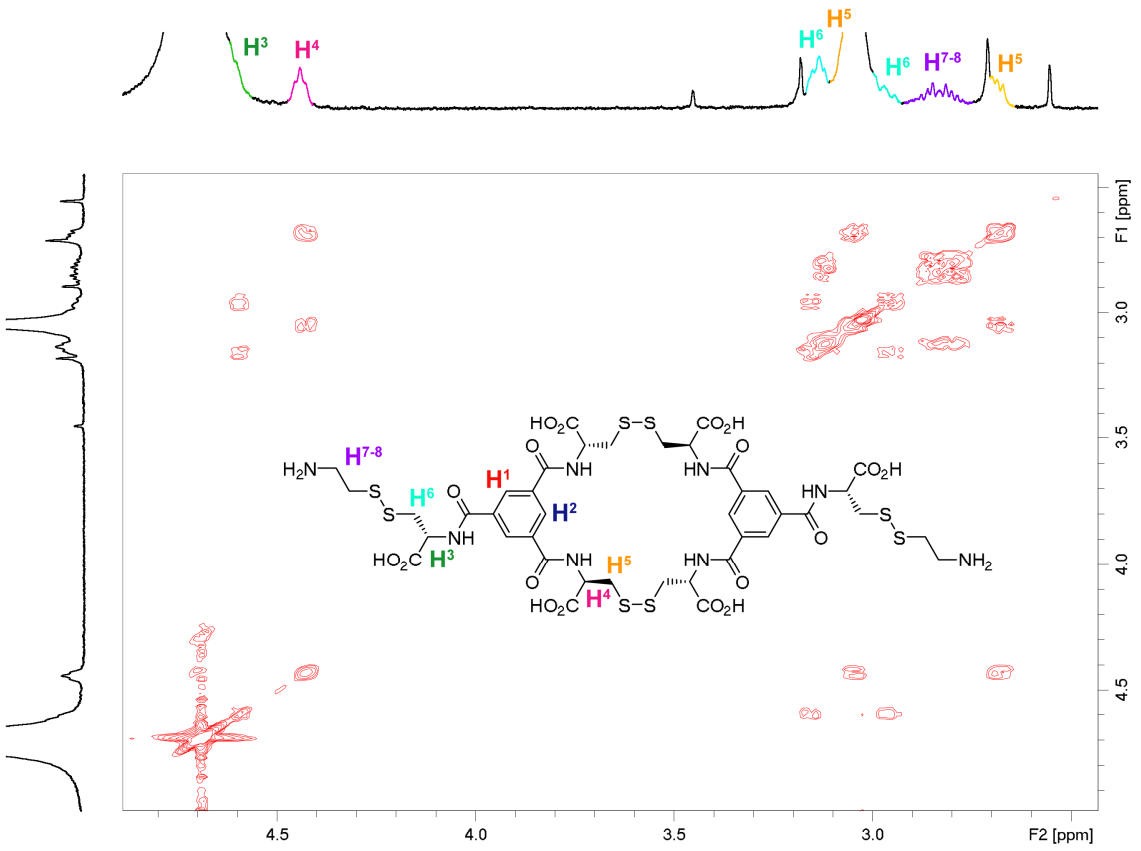

H1
D2O
H2
H5
H3
H3
H4
F1 [ppm]
F2 [ppm]

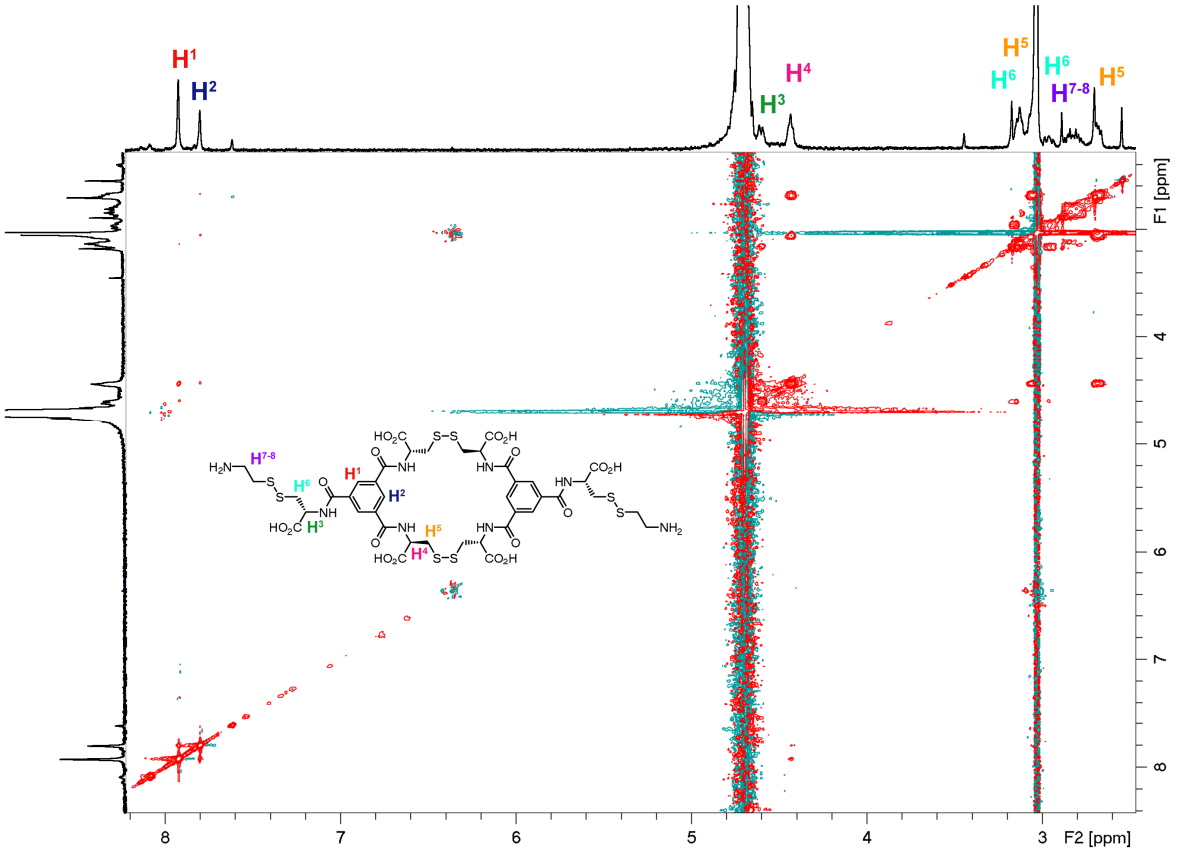

D2O
H2
H5
H3
H3
H4
F1 [ppm]
F2 [ppm]

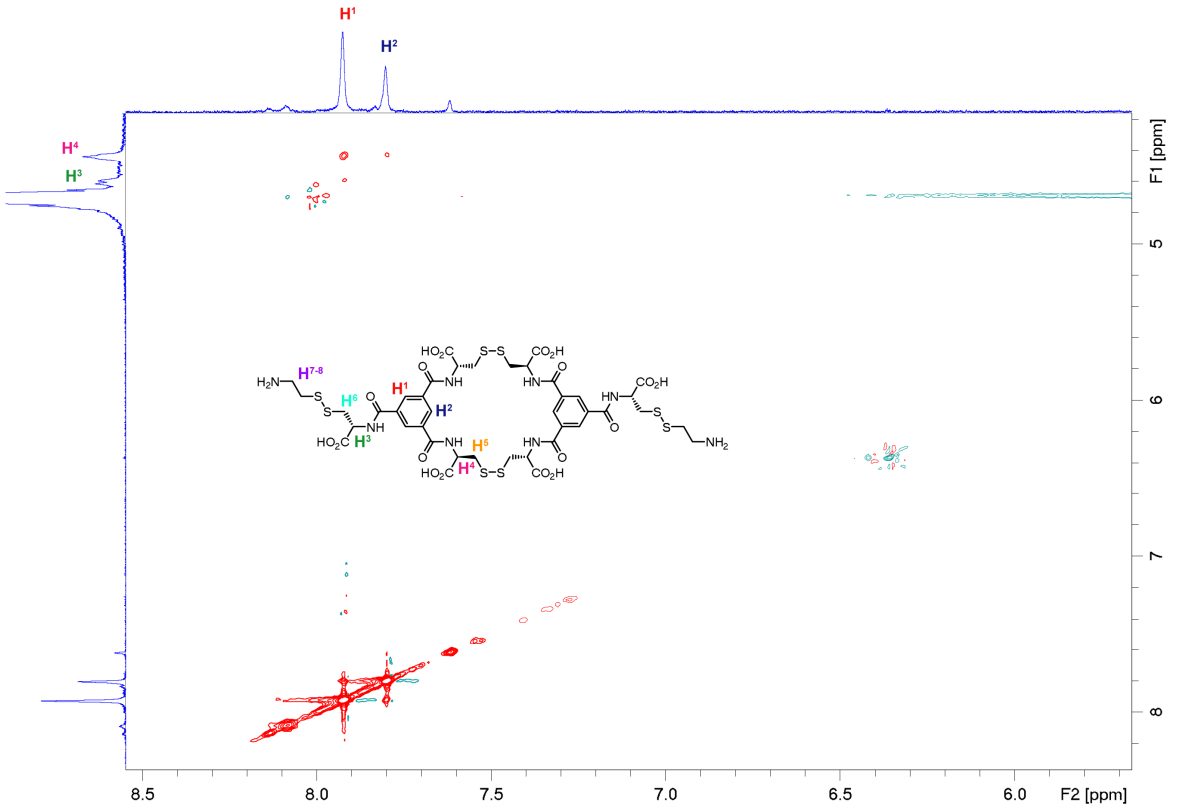
**8.** $^{1}$H, and COSY and NOESY NMR spectra of the tetramer 2×**1**·2×**2a** after isolation. The spectra were recorded in $D_2O$ at 298 K (500 MHz NMR spectrometer).

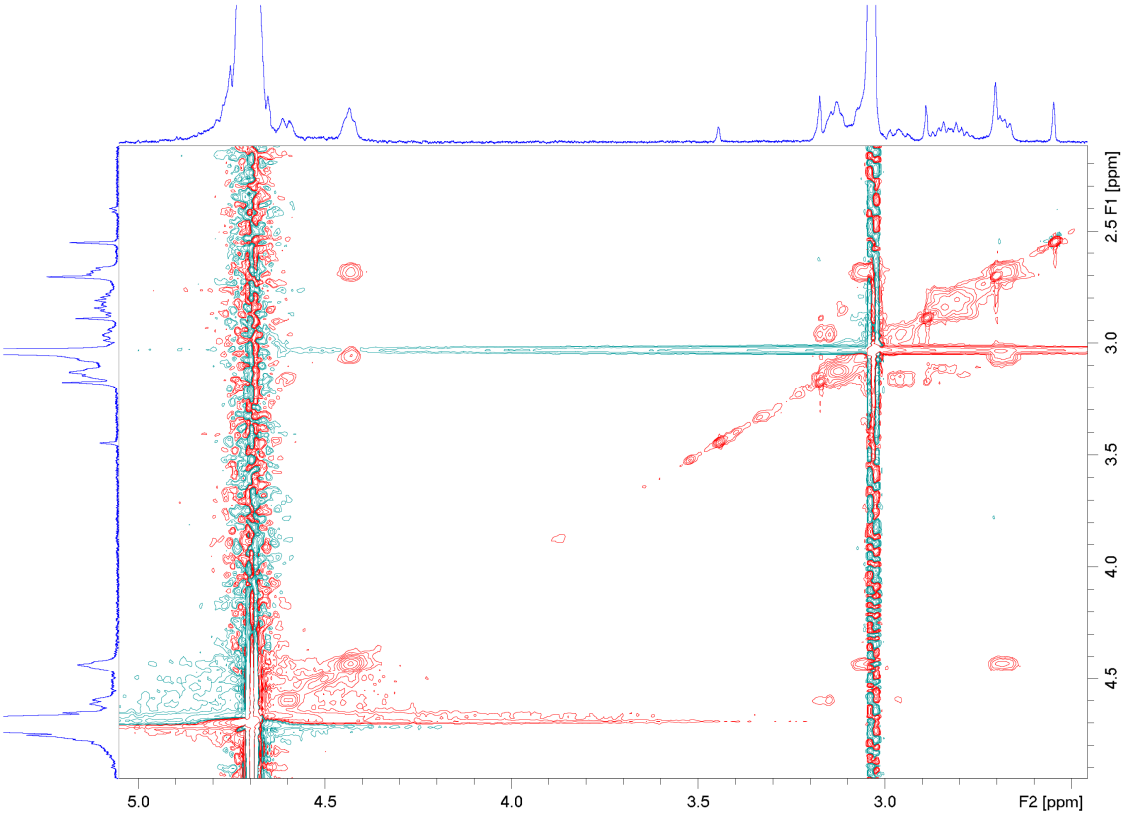



H1
H2
D2O
H3
H4
H5
H6
H6
H7-8
H5
F1 [ppm]
F2 [ppm]
H7-8
H6
H1
H2
H3
H5
H4
HO2C
S–S
CO2H
NH
HN
O
H2N
S
NH2

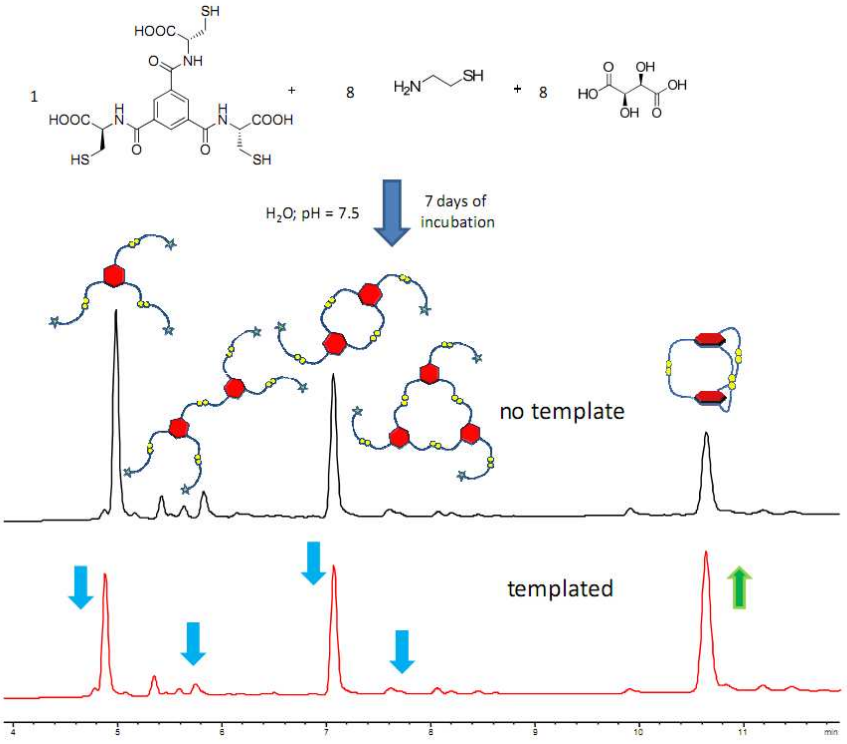

H3
H4
H5
H6
H6
H7-8
H5
F1 [ppm]
F2 [ppm]
H7-8
H6
H1
H2
H3
H5
H4
HO2C
S–S
CO2H
NH
HN
O
H2N
S
NH2

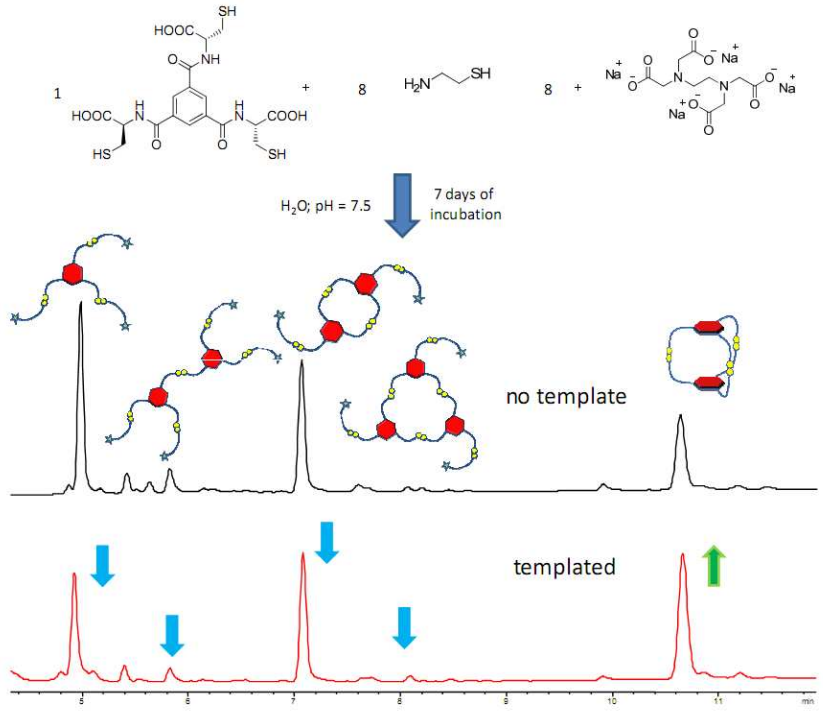

H1
H2
H4
H3
H5
H6
H6
H7-8
H5
F1 [ppm]
F2 [ppm]
8
7
6
5
4
3

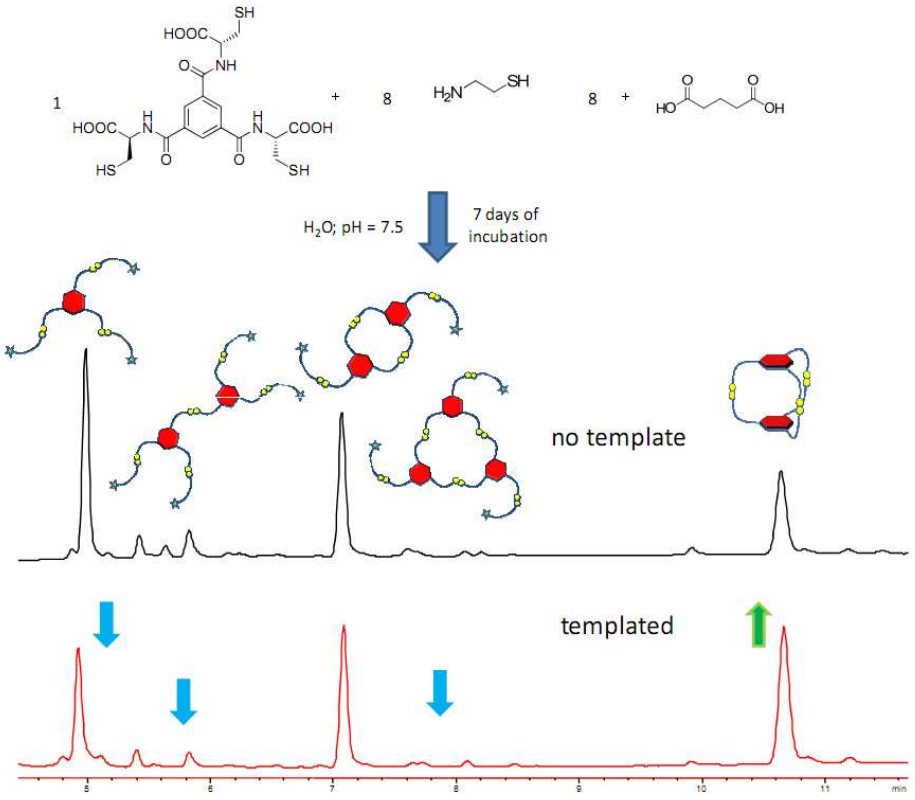

H1
H2
H4
H3
F1 [ppm]
F2 [ppm]
8.5
8.0
7.5
7.0
6.5
6.0

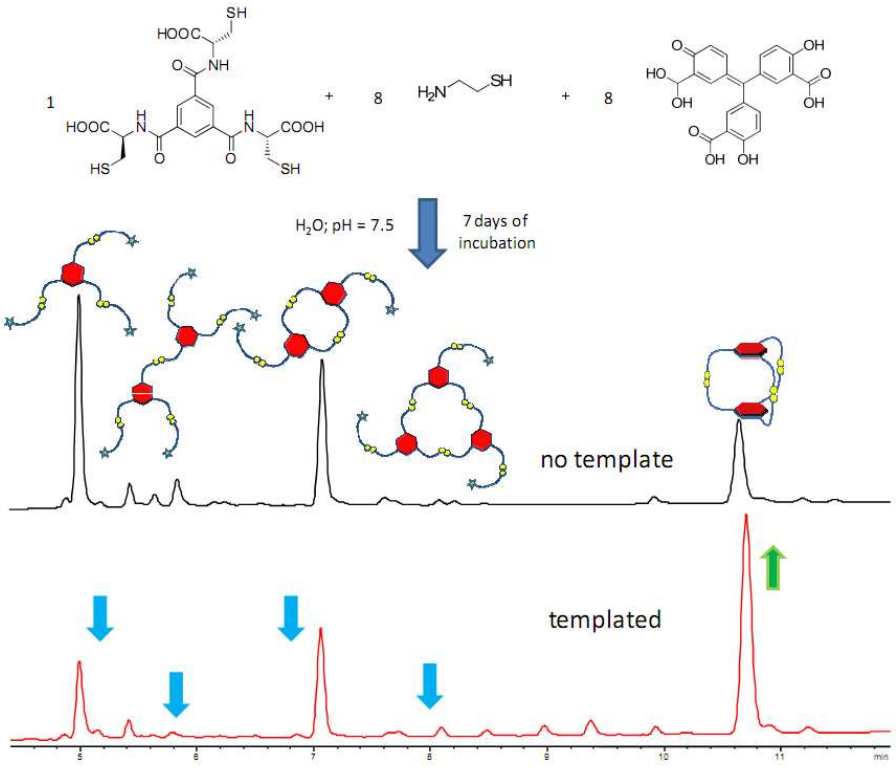

2.5 F1 [ppm]
3.0
3.5
4.0
4.5
5.0
4.5
4.0
3.5
3.0
F2 [ppm]

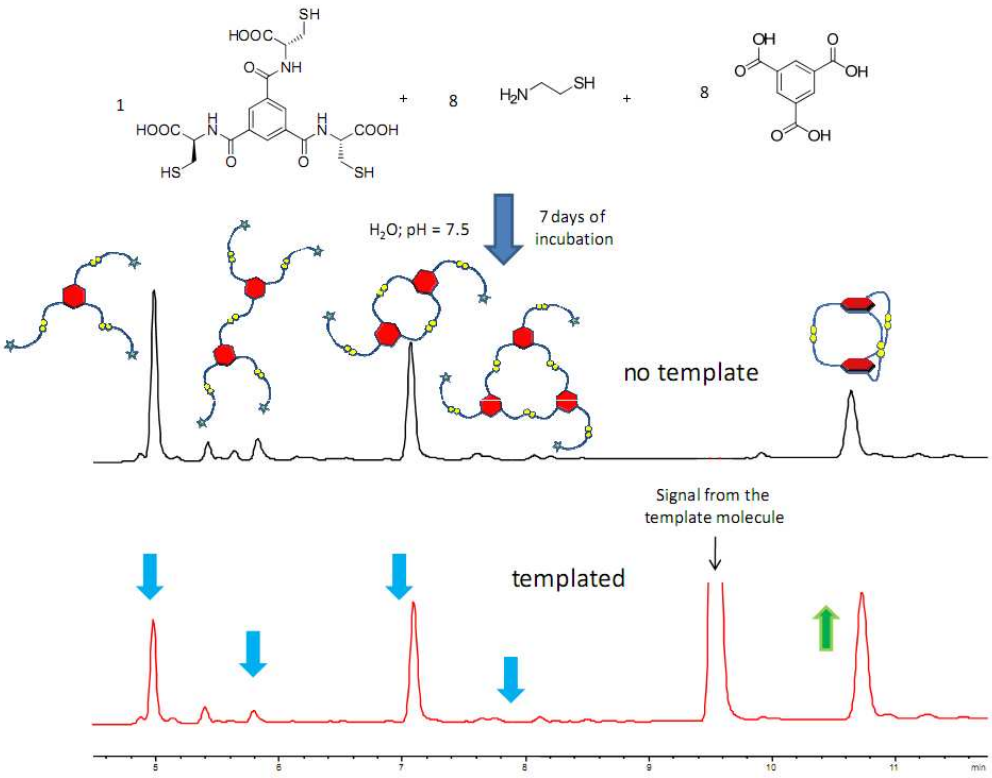
**9.** Templation experiments with different polycarboxylates (each at 5 mM) in DCLs composed of **1** (5 mM) and **2a** (40 mM) in water at pH = 7.5 after 7 days of incubation in the closed capped vials.



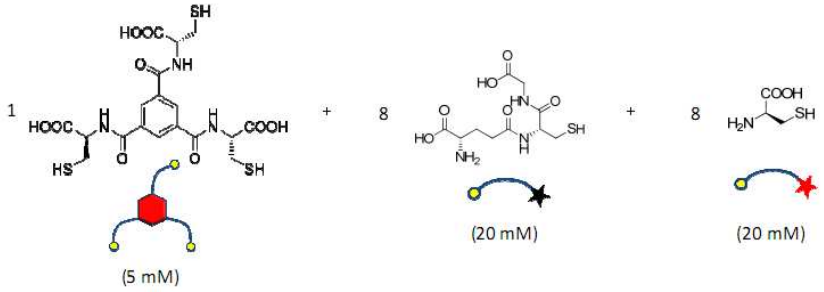



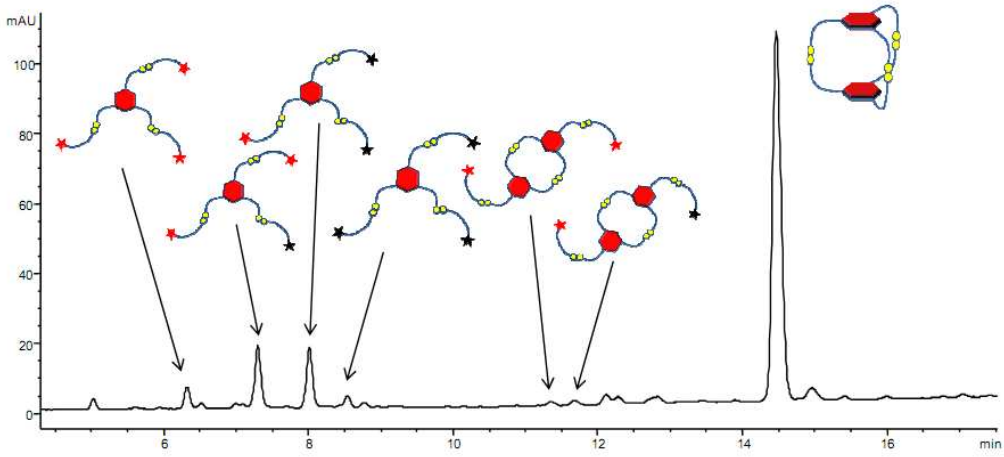

1
8
8
$H_2O$; pH = 7.5
7 days of incubation
no template
templated


1
8
8
$H_2O$; pH = 7.5
7 days of incubation
no template
templated


1
8
8
$H_2O$; pH = 7.5
7 days of incubation
no template
Signal from the template molecule
templated
min

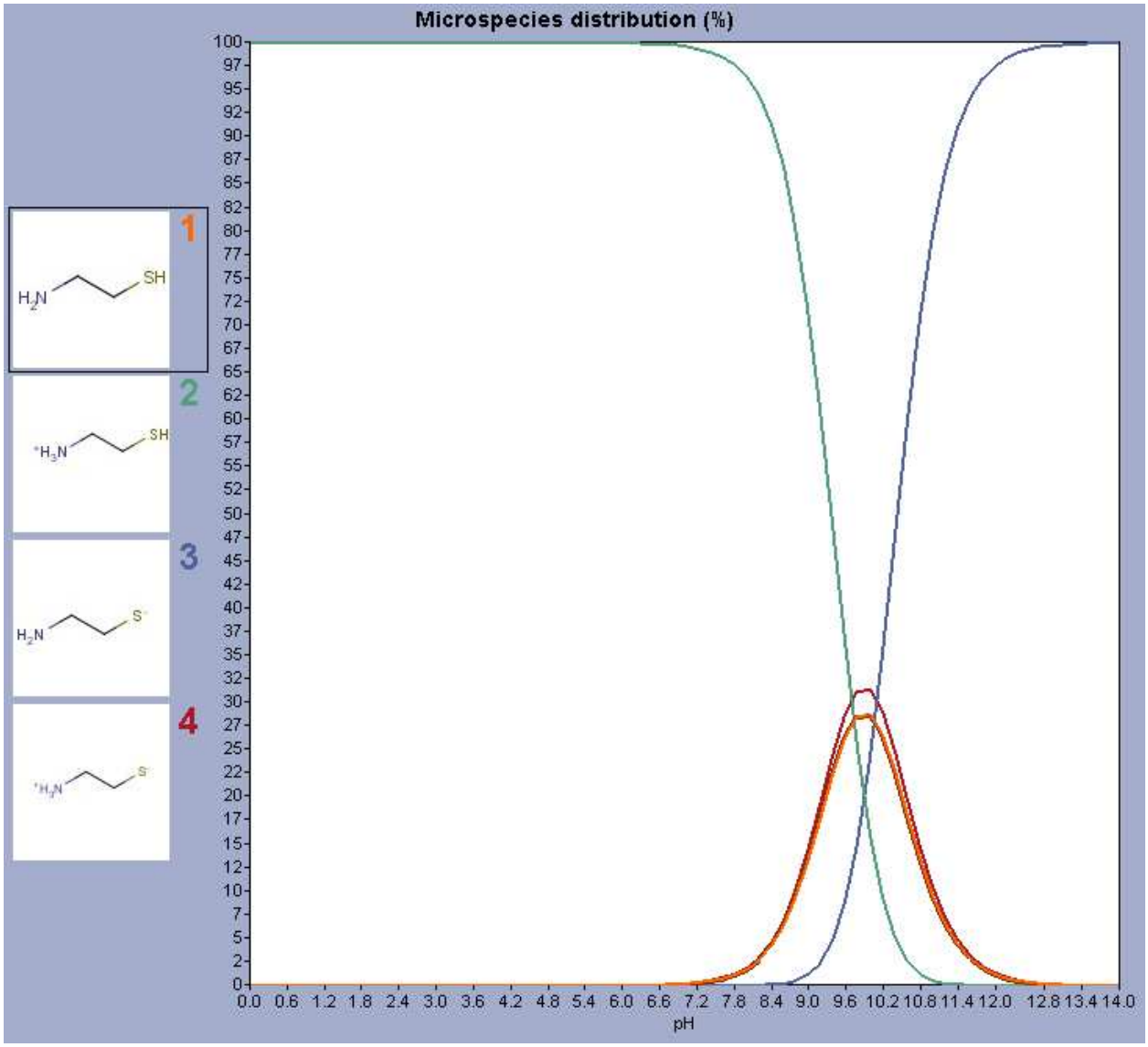
**10.** DCL composition prepared from tri-thiol **1** (5 mM) and the mixture of mono-thiols **2f** and **2g** (each at 20 mM) in water at pH = 7.5 after 7 days of incubation in the closed capped vials. The DCL composition was found to be essentially identical to the one obtained from the mixture of **1** (5 mM) and **2f** and **2g** at higher concentrations (each at 40 mM) except the higher yield of the homo-dimer of **1** when lower concentrations of mono-thiols were used.



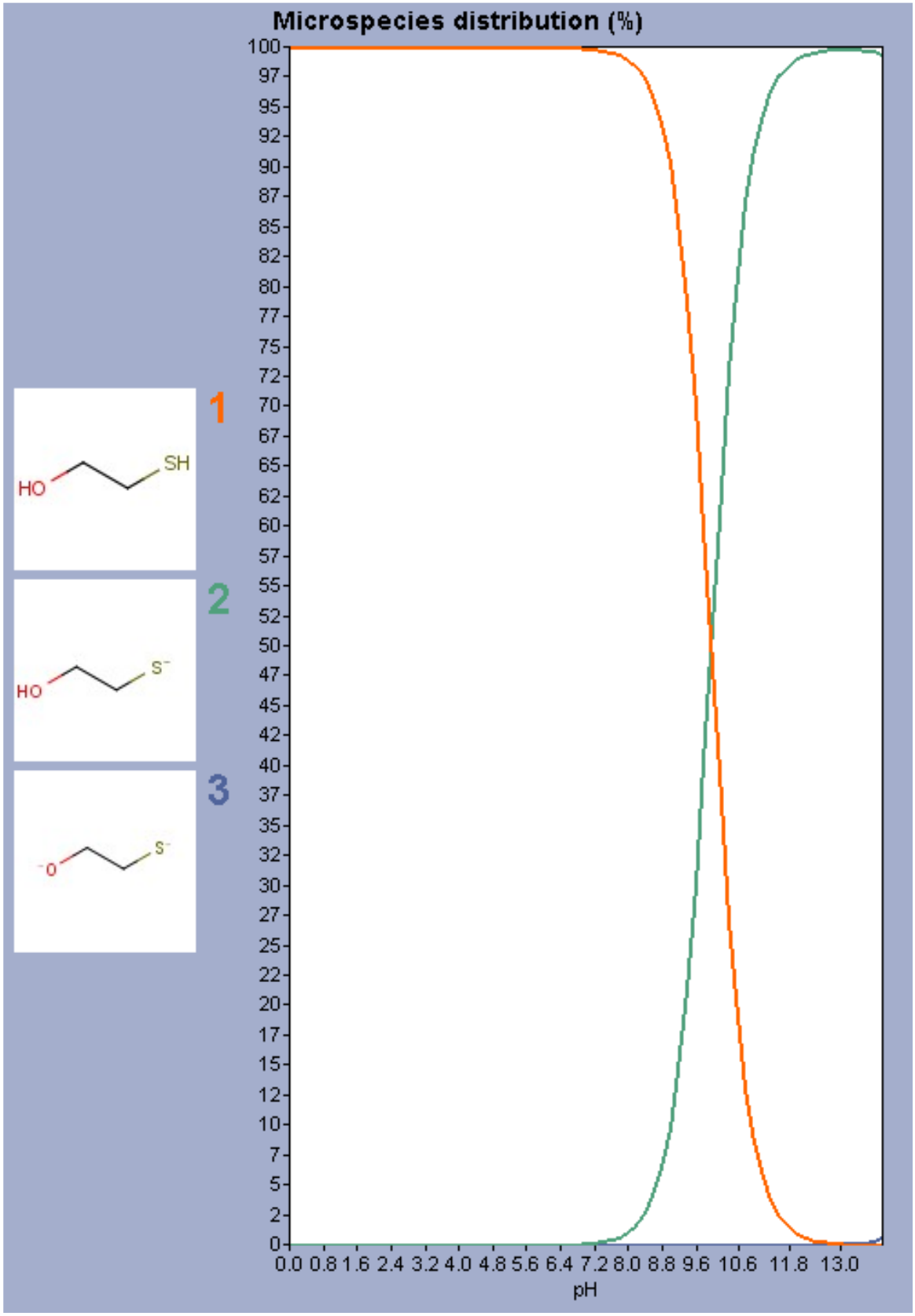



**11.** Speciation plots, were generated using ChemAxon – Marvin Suite 5.11.5 software. The data obtained shows the ionization processes of the mono-thiols **2a**-**g** in aqueous solution. The distribution fraction (in %) for various mono-thiols species vs. pH.

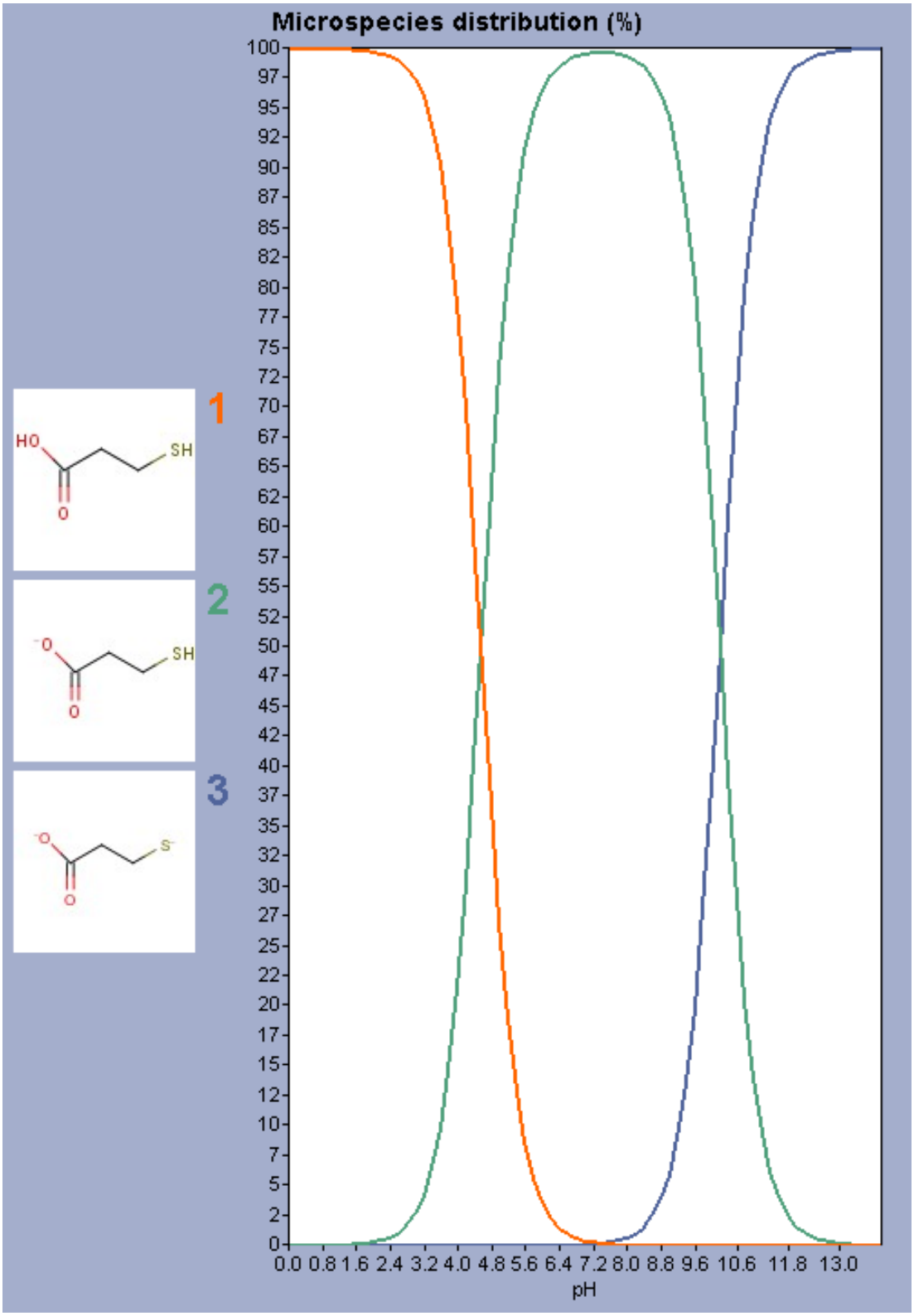
a) **2a**



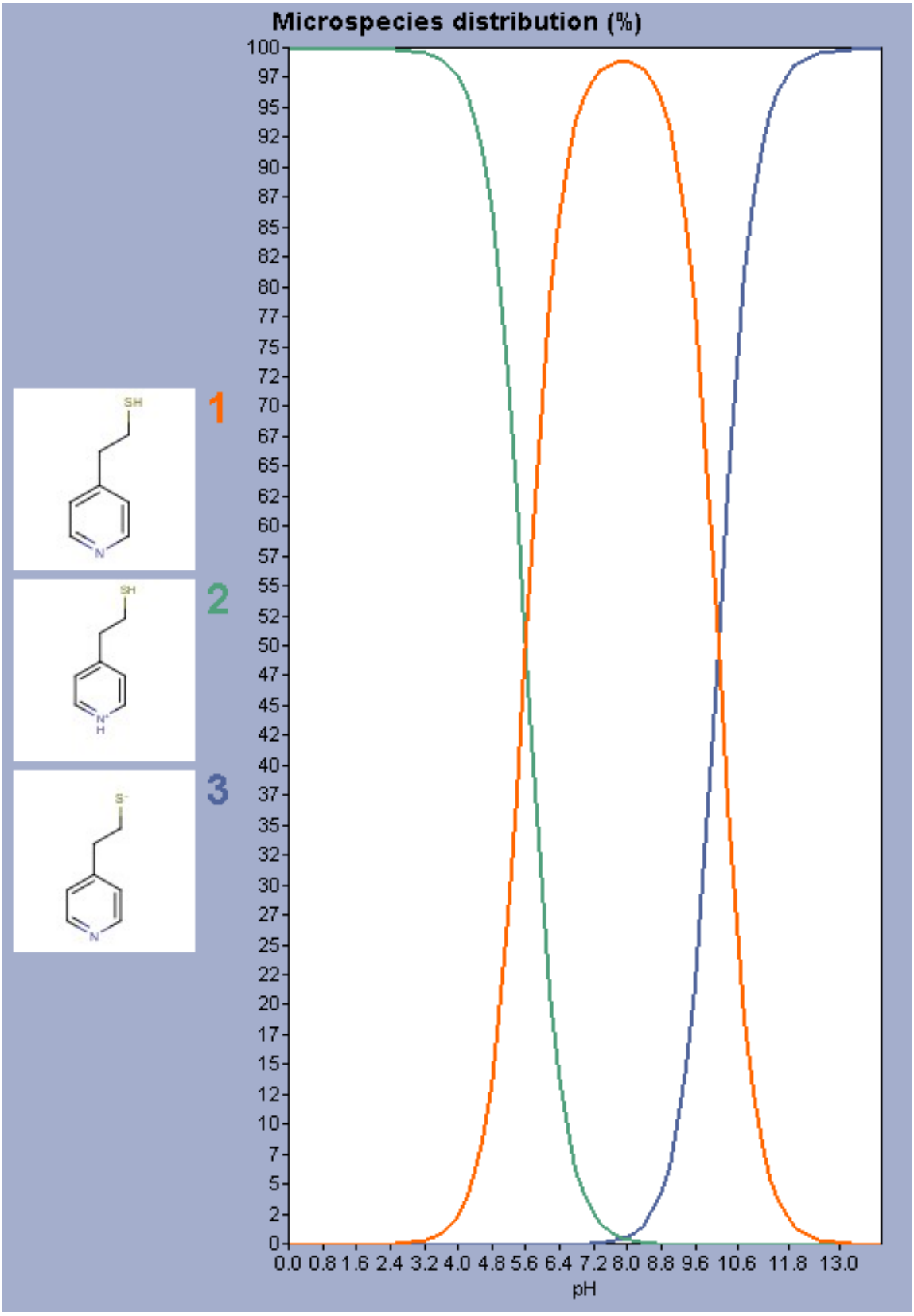
b) **2b**


Microspecies distribution (%)
100
97
95
92
90
87
85
82
80
77
75
72
70
67
65
62
60
57
55
52
50
47
45
42
40
37
35
32
30
27
25
22
20
17
15
12
10
7
5
2
0
0.0 0.8 1.6 2.4 3.2 4.0 4.8 5.6 6.4 7.2 8.0 8.8 9.6 10.6 11.8 13.0
pH
1
HO
SH
2
HO
S⁻
3
⁻O
S⁻

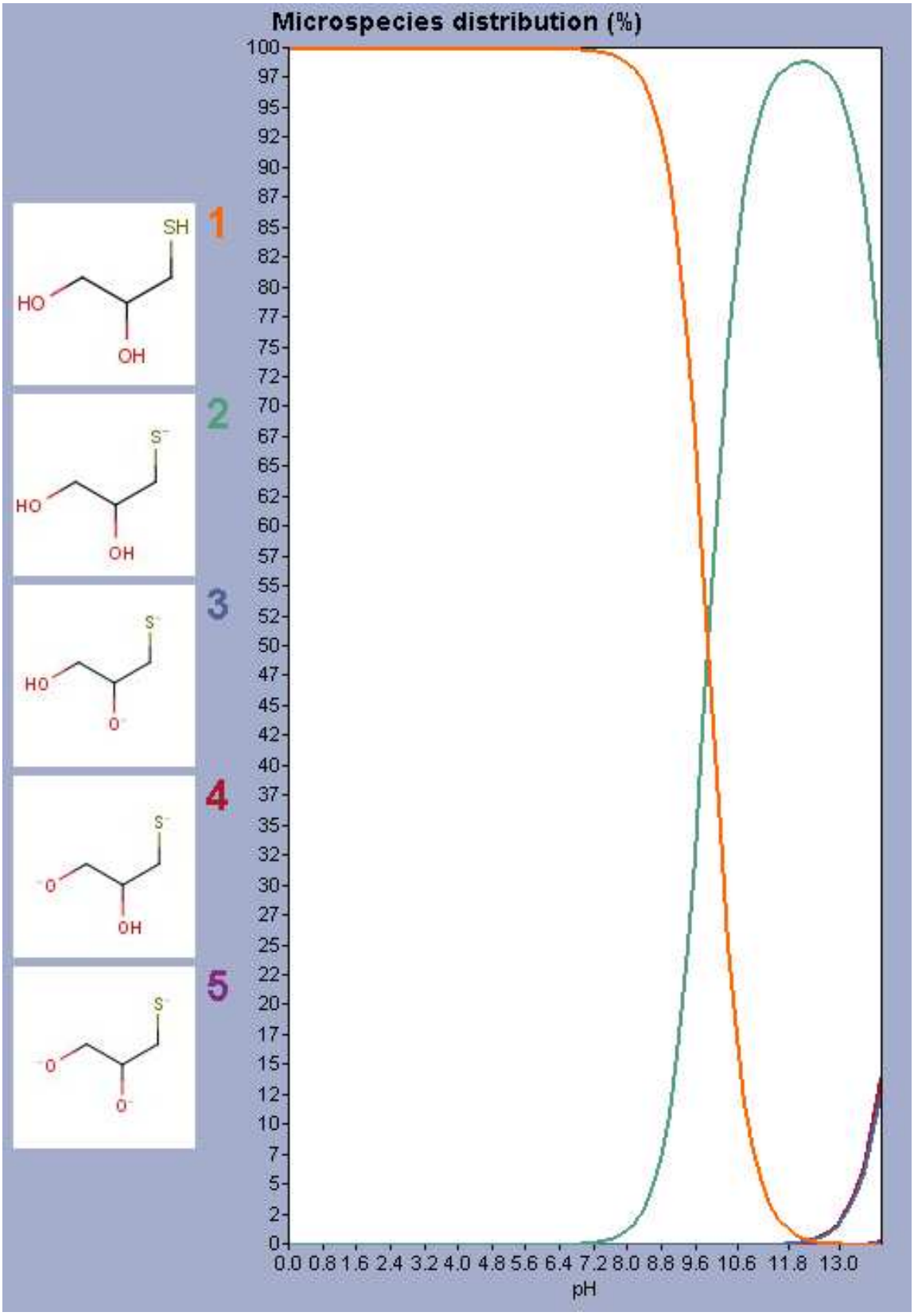
c) **2c**


Microspecies distribution (%)
100
97
95
92
90
87
85
82
80
77
75
72
70
67
65
62
60
57
55
52
50
47
45
42
40
37
35
32
30
27
25
22
20
17
15
12
10
7
5
2
0
0.0 0.8 1.6 2.4 3.2 4.0 4.8 5.6 6.4 7.2 8.0 8.8 9.6 10.6 11.8 13.0
pH
1
2
3
HO
SH
O
O
SH
O
O
S
O

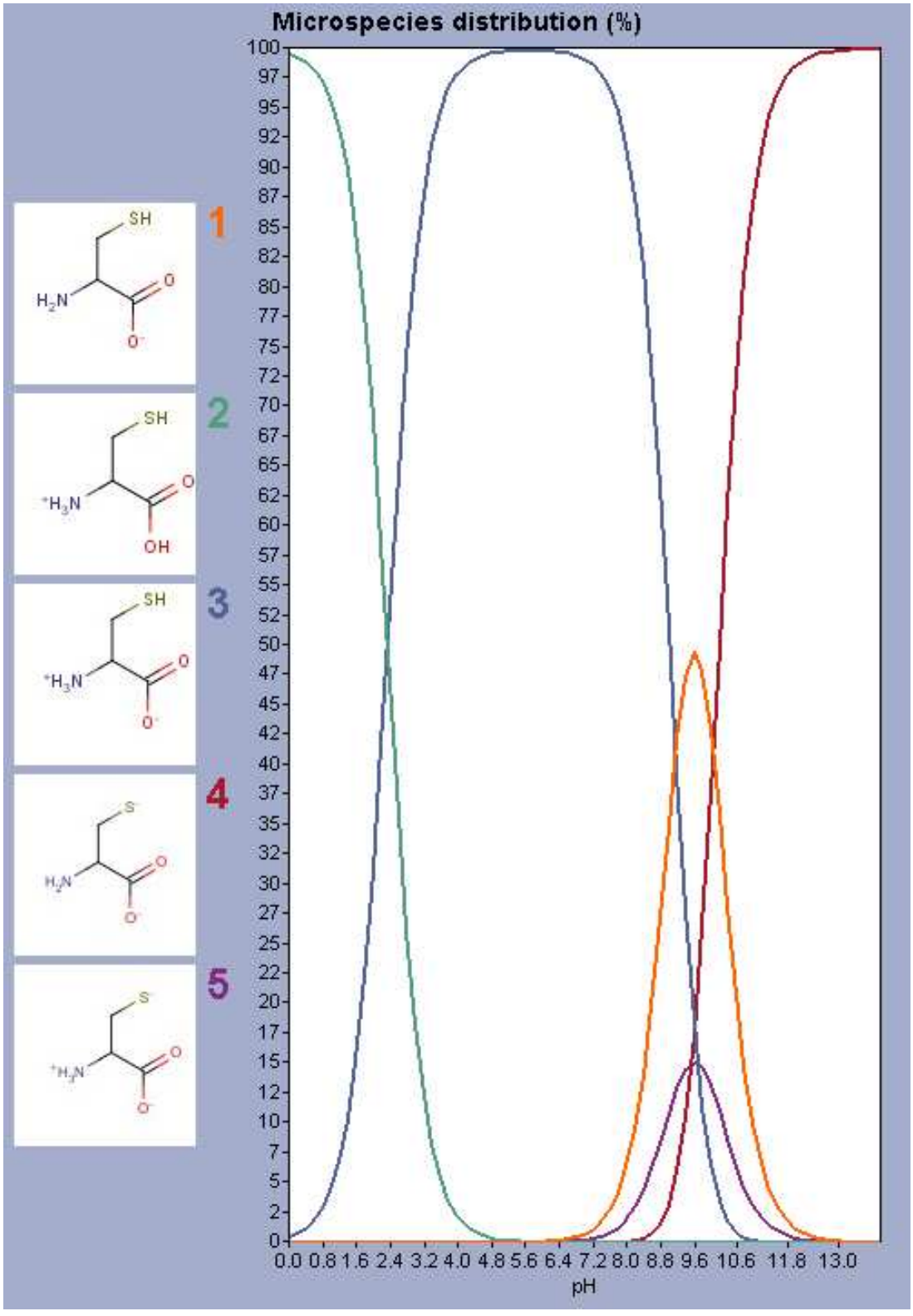
d) **2d**


Microspecies distribution (%)
100
97
95
92
90
87
85
82
80
77
75
72
70
67
65
62
60
57
55
52
50
47
45
42
40
37
35
32
30
27
25
22
20
17
15
12
10
7
5
2
0
0.0 0.8 1.6 2.4 3.2 4.0 4.8 5.6 6.4 7.2 8.0 8.8 9.6 10.6 11.8 13.0
pH
1
2
3

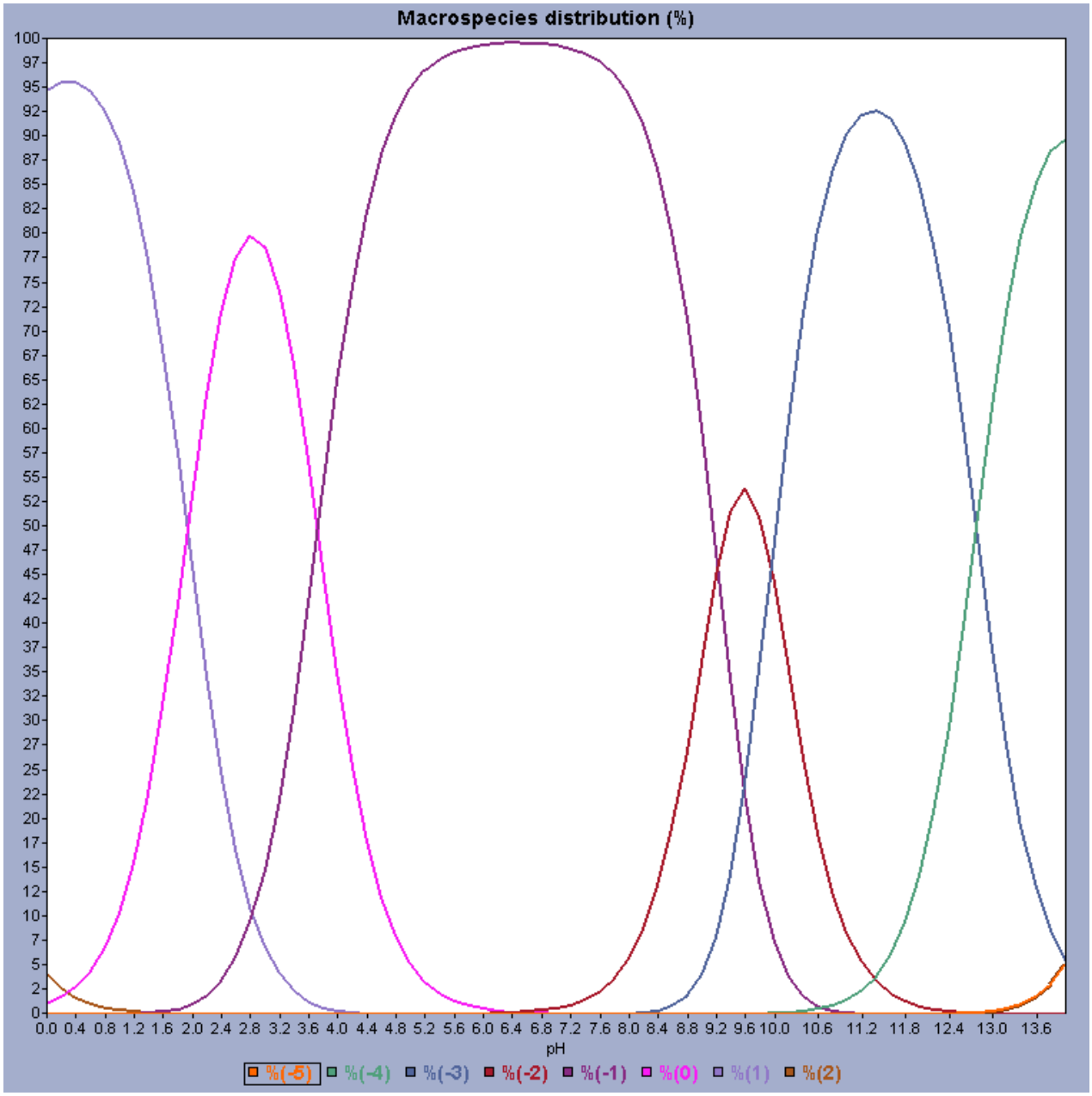
e) **2e**

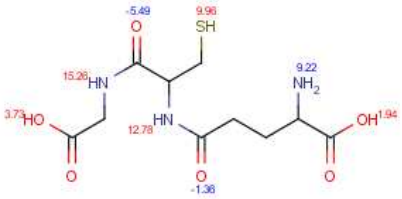

Microspecies distribution (%)
100
97
95
92
90
87
85
82
80
77
75
72
70
67
65
62
60
57
55
52
50
47
45
42
40
37
35
32
30
27
25
22
20
17
15
12
10
7
5
2
0
0.0 0.8 1.6 2.4 3.2 4.0 4.8 5.6 6.4 7.2 8.0 8.8 9.6 10.6 11.8 13.0
pH
1
SH
HO
OH
2
S⁻
HO
OH
3
S⁻
HO
O⁻
4
S⁻
O⁻
OH
5
S⁻
O⁻
O⁻

f) **2f**

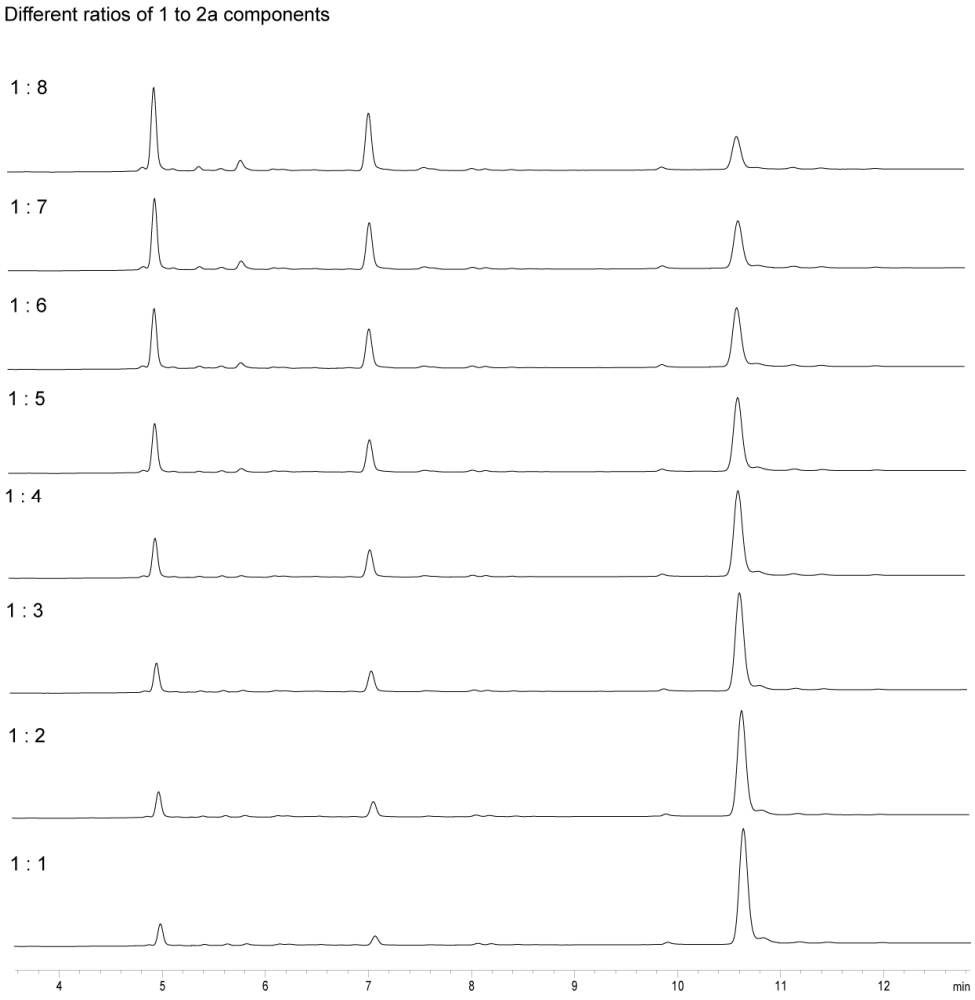

Microspecies distribution (%)
100
97
95
92
90
87
85
82
80
77
75
72
70
67
65
62
60
57
55
52
50
47
45
42
40
37
35
32
30
27
25
22
20
17
15
12
10
7
5
2
0
0.0 0.8 1.6 2.4 3.2 4.0 4.8 5.6 6.4 7.2 8.0 8.8 9.6 10.6 11.8 13.0
pH
1
2
3
4
5
SH
$H_2N$
O
$O^-$
$^+H_3N$
OH
S
$^-S$

g) **2g**


Macrospecies distribution (%)
pH
%(-5)
%(-4)
%(-3)
%(-2)
%(-1)
%(0)
%(1)
%(2)

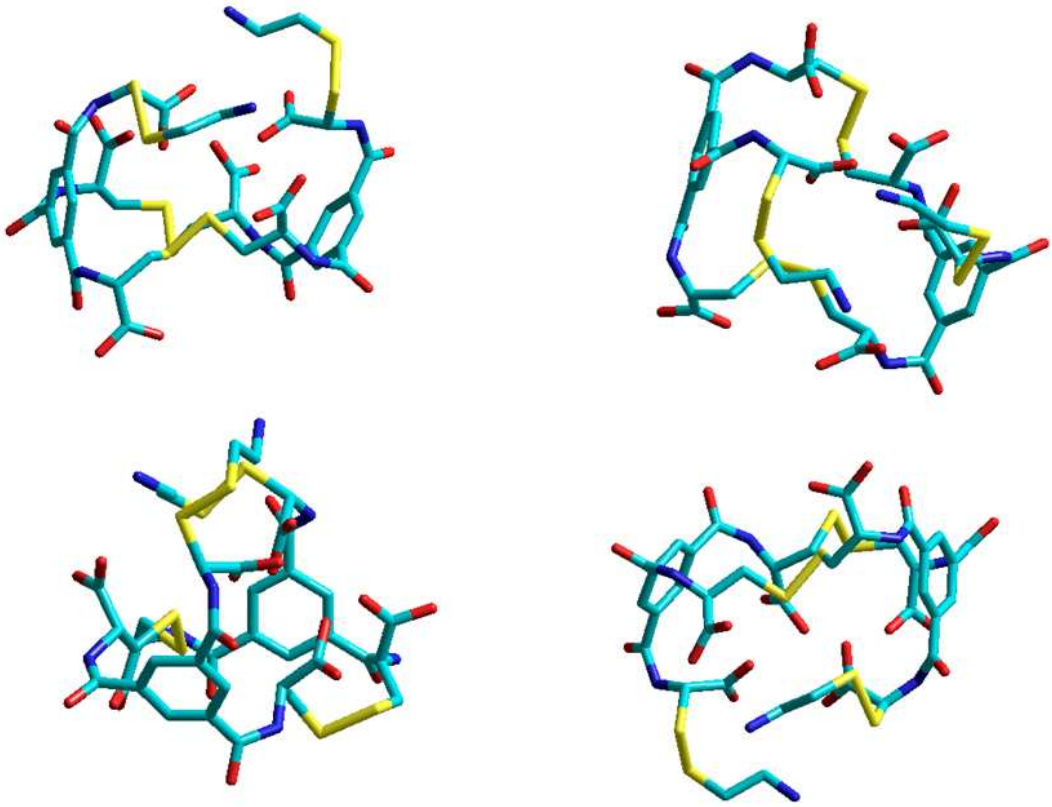

-5.49
O
9.96
SH
15.26
HN
9.22
NH2
3.73
HO
12.78
HN
OH
1.94
O
O
-1.36
O

**12.** HPLC traces of DCLs consisted of **1** and **2a** at different ratios (from 1:8 to 1:1 of **1** to **2a**, respectively).



13. In order to gain insight into the structural details of the macrocyclic tetramer, molecular modelling was performed. The macrocyclic tetramer was assembled using HyperChem Professional 8.0 for Windows. The structure was optimised with MM+ force field calculation. All atoms in supramolecular structure were free to move in each equilibration.

a) without the hydrogen atoms



b) with the hydrogen atoms