Supporting information for:

Template-directed synthesis of multi-component aqueous organic cages

Artur R. Stefankiewicz,*[a] Mark R. Sambrook,[b] and Jeremy K. M. Sanders*[a]

[a]University Chemical Laboratory, University of Cambridge, Lensfield Road, Cambridge, CB2 1EW, United Kingdom.
[b] Detection Dept., Defence Science & Technology Laboratory, Porton Down, Salisbury, Wiltshire, UK SP4 0JQ

Email: jkms@cam.ac.uk or as2044@cam.ac.uk ; fax: +44 1223 336017

Contents:

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 µL (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 µm particle size) with an isocratic elution of 58% MeOH in water (with 0.1% formic acid) at room temperature and a flow rate of 1mL/min.

A typical analytical DCL was prepared on a 0.8 ml scale by dissolving an equimolar mixture of **1** and **2** (molar ratio 1 : 2) 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, polyamine guests (each at 5mM in 0.2 ml of H_2O) were added which was then followed by titration with 100 mM aqueous NaOH/HCl to pH = 8. The DCL was stirred in a close-capped vial at room temperature until being analyzed. The pH of each library was checked before and after equilibration process to make sure it remained remained unchanged.

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

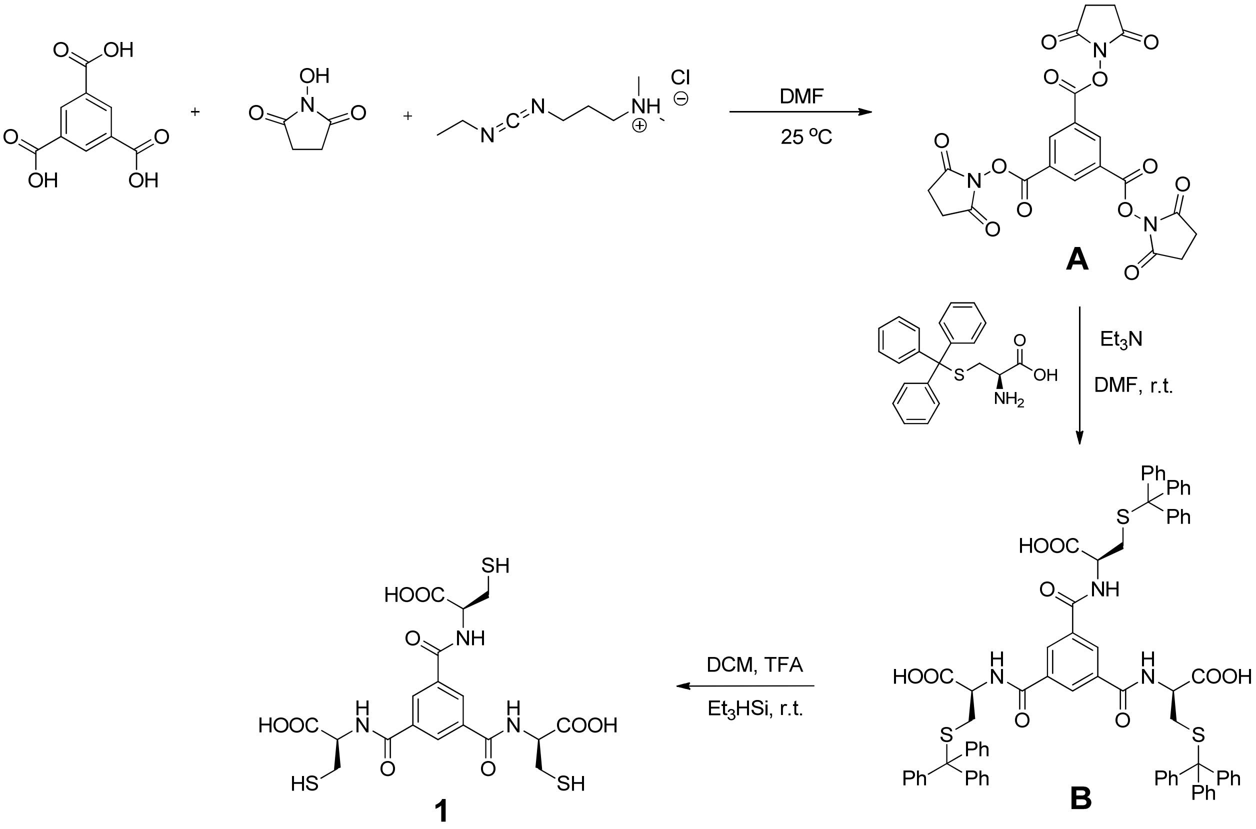
1) pH around 8 was found to be optimum for the disulfide exchange

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

3) to ensure that the highest possible number of amines are in their protonated from

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

Synthesis and characterization data

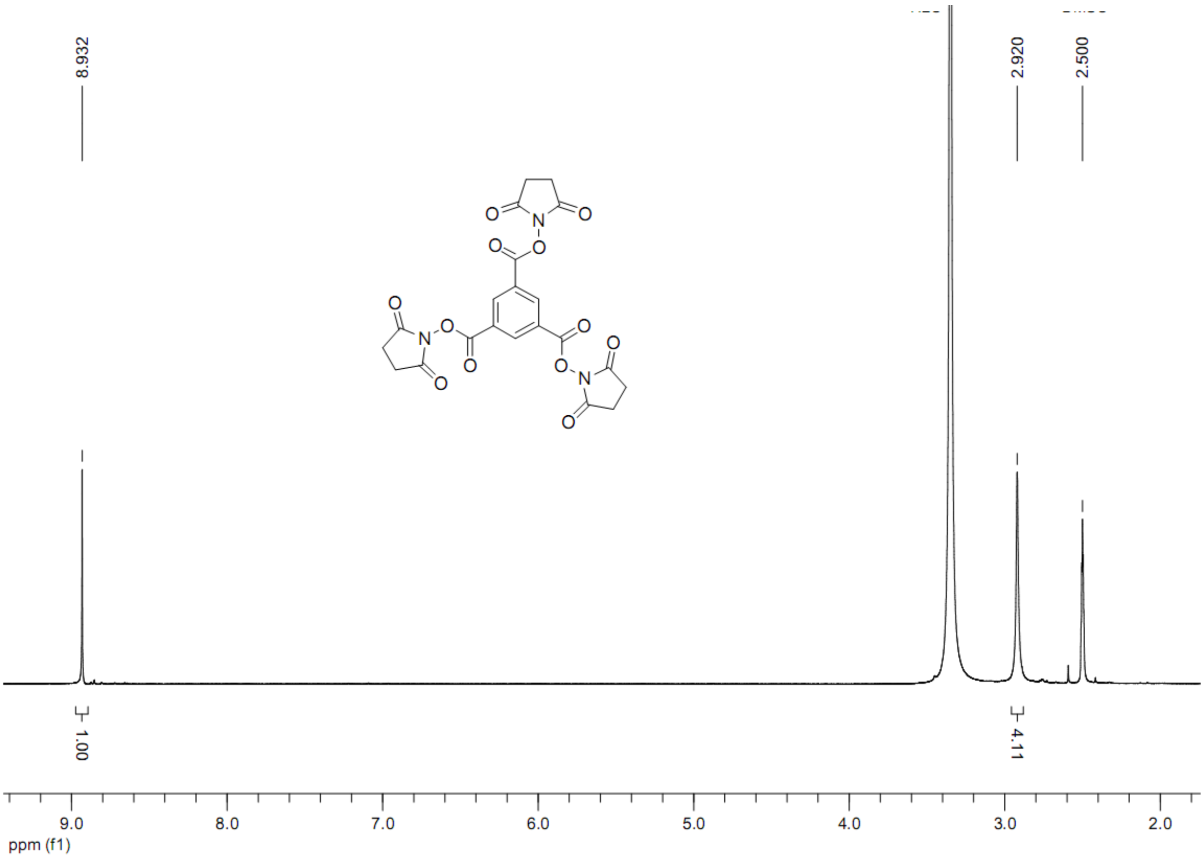


*Synthesis of compound **A**:* To a solution of benzene-1,3,5-tricarboxylic acid (0.78 g, 3.70 mmol) in anhydrous DMF (20 mL), *N*-hydroxysuccinimide (2.55 g, 22.2 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (4.26 g, 22.2 mmol) were added. The mixture was stirred at room temperature for 24 h under a nitrogen atmosphere. After solvent was removed the residue was redissolved in acetone (25 mL) and after poured into 1M HCl (200 mL). White solid precipitated, which was after filtered off, washed with H2O (50 mL) and hot isopropanol (50 mL) and dried under the high vacuum. The product was obtained as a white solid in 70 % yield. ^{1}H-NMR (500 MHz, DMSO-d_6) δ(ppm): 8.93 (s, 1H), 2.92 (s, 4H); ^{13}C NMR (125.75 MHz, DMSO-d_6) δ(ppm): 170.33, 160.12, 136.72, 127.77, 25.99. HRMS (ESI+) calcd. for: $C_{21}H_{15}N_3O_{12}Na$ $[M + Na]^+$ (m/z): 524.0621, found: 524.0528.

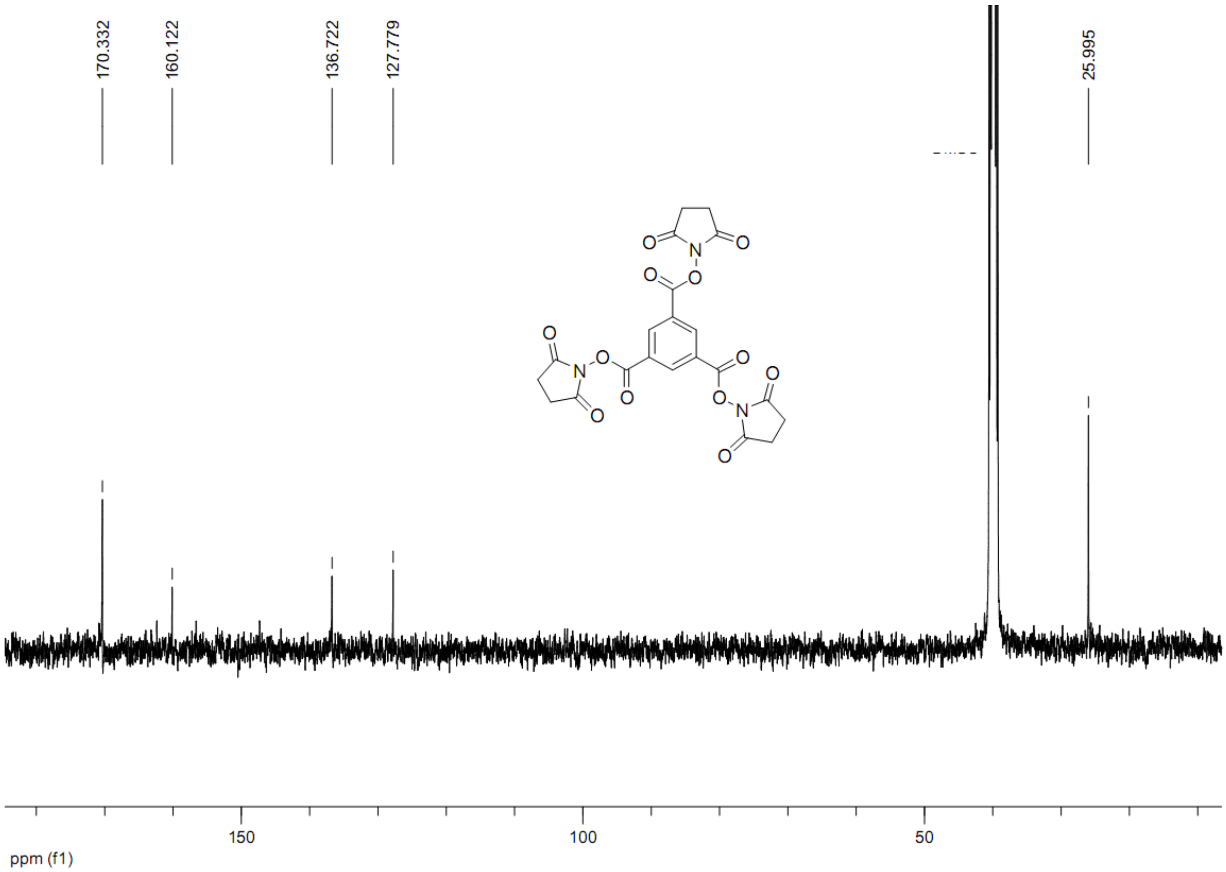
*Synthesis of compound **B**:* To a solution of **A** (0.50 g, 0.99 mmol) in anhydrous DMF (35 mL), S-Trityl-L-cysteine (2.17 g, 5.98 mmol) and triethylamine (0.60 g, 5.98 mmol) were added. The mixture was stirred at room temperature for 24 h under a nitrogen atmosphere. After solvent was removed the residue was redissolved in acetone (15 mL) and after poured into 1M HCl (150 mL). Light yellow solid precipitated, which was after filtered off, washed with H2O (50 mL) and dried under the high vacuum. The product was obtained as a light yellow solid in 78 % yield. ^{1}H-NMR (500 MHz, DMSO-d_6) δ(ppm): 9.14 (d, J = 8 Hz, 1H), 8.46 (s, 1H), 7.31 (m, 15H) 4.34 (m, 1H) 2.79 (t, J = 11 Hz, 1H), 2.55 (m, 1H); ^{13}C NMR (125.75 MHz, DMSO-d_6) δ(ppm): 171.44, 165.28, 147.68, 144.16, 134.21, 129.02, 128.02, 126.75, 80.46, 66.28, 52.21; HRMS (ESI+) calcd. for: $C_{75}H_{64}N_3O_9S_3$ $[M + H]^+$ (m/z): 1246.6821, found: 1246.3772 and calcd. for: $C_{75}H_{63}N_3O_9S_3Na$ $[M + Na]^+$ (m/z): 1268.3629, found: 1268.3583.

*Synthesis of compound **1**:* To a Schlenk flask charged with **B** (200 mg, 0.16 mmol) were added degassed TFA (3 mL, 40 mmol) and DCM (3 mL). The solution was stirred under N_2 at room temperature for 2 hours. Volatiles were removed *in vacuo*. The solid left was washed with Et_2O (30 mL) and collected by filtration. The product was obtained as a light brown solid in 85 % yield. ^{1}H-NMR (500 MHz, DMSO-d_6) δ(ppm): 9.04 (d, J = 9.5 Hz, 1H), 8.52 (s, 1H), 4.58 (m, 1H) 3.03 (m, 1H) 2.93 (m, 1H), 2.61 (t, J = 10.5 Hz, 1H); ^{13}C NMR (125.75 MHz, DMSO-d_6) δ(ppm): 171.57, 165.75, 134.33, 129.29, 64.85, 25.04.

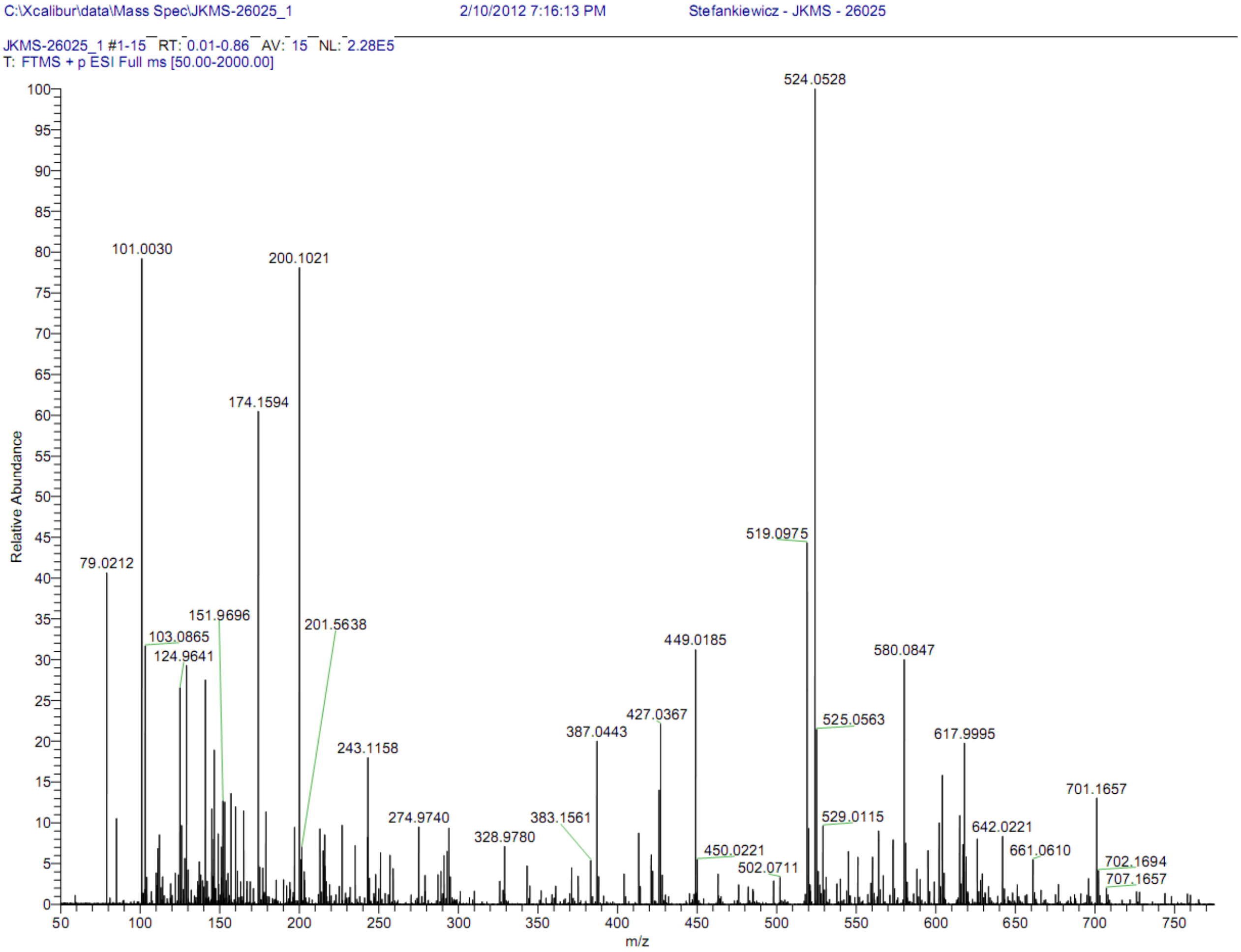
^{1}H NMR in d^6 DMSO of molecule **A**



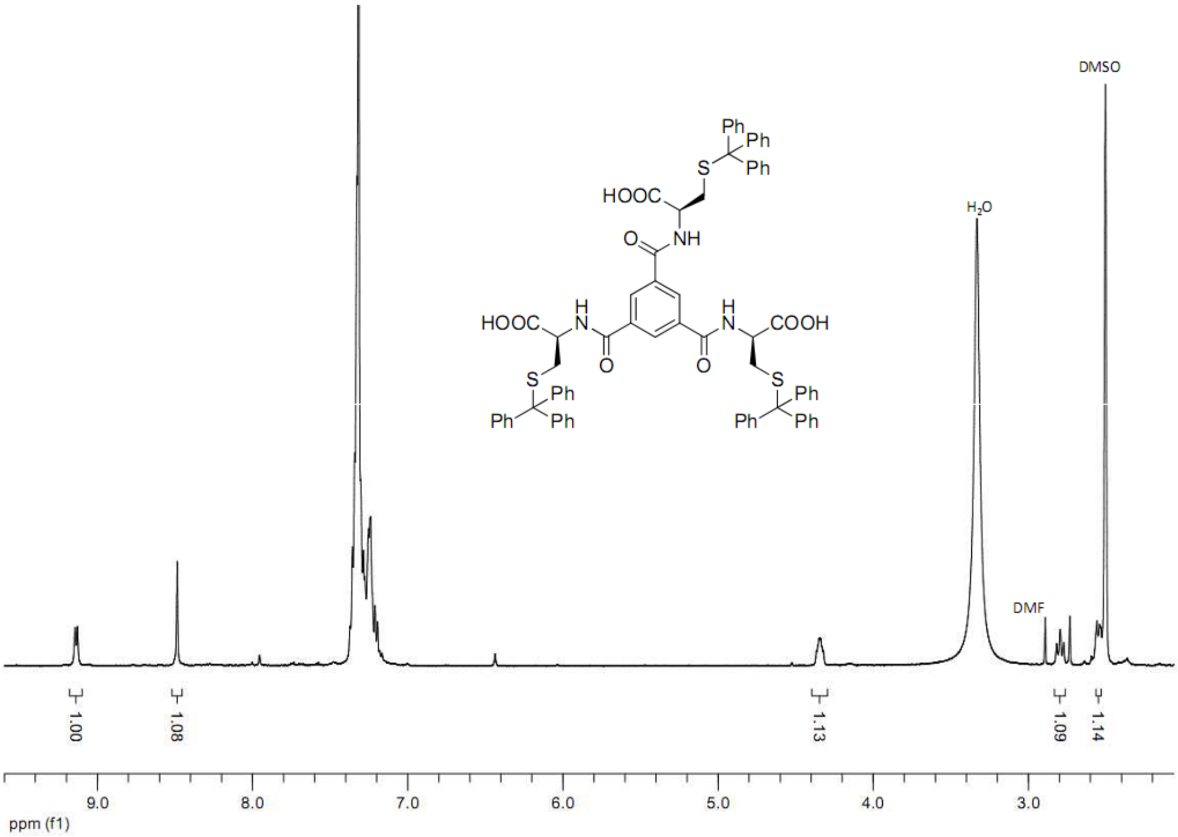
^{13}C NMR in d^{6} DMSO of molecule **A**



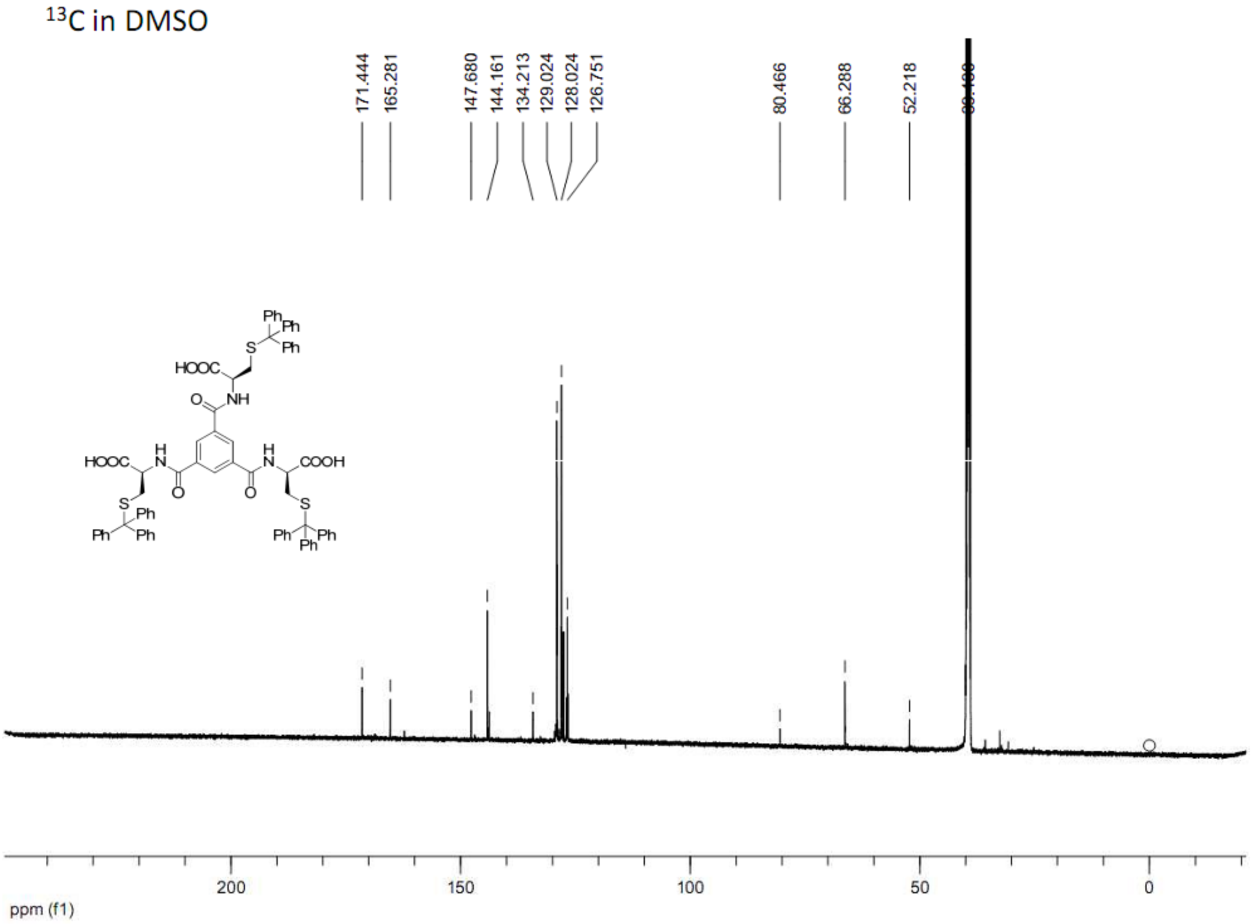
HR-ESI-MS of molecule **A + Na**$^+$ **(524.0528)**



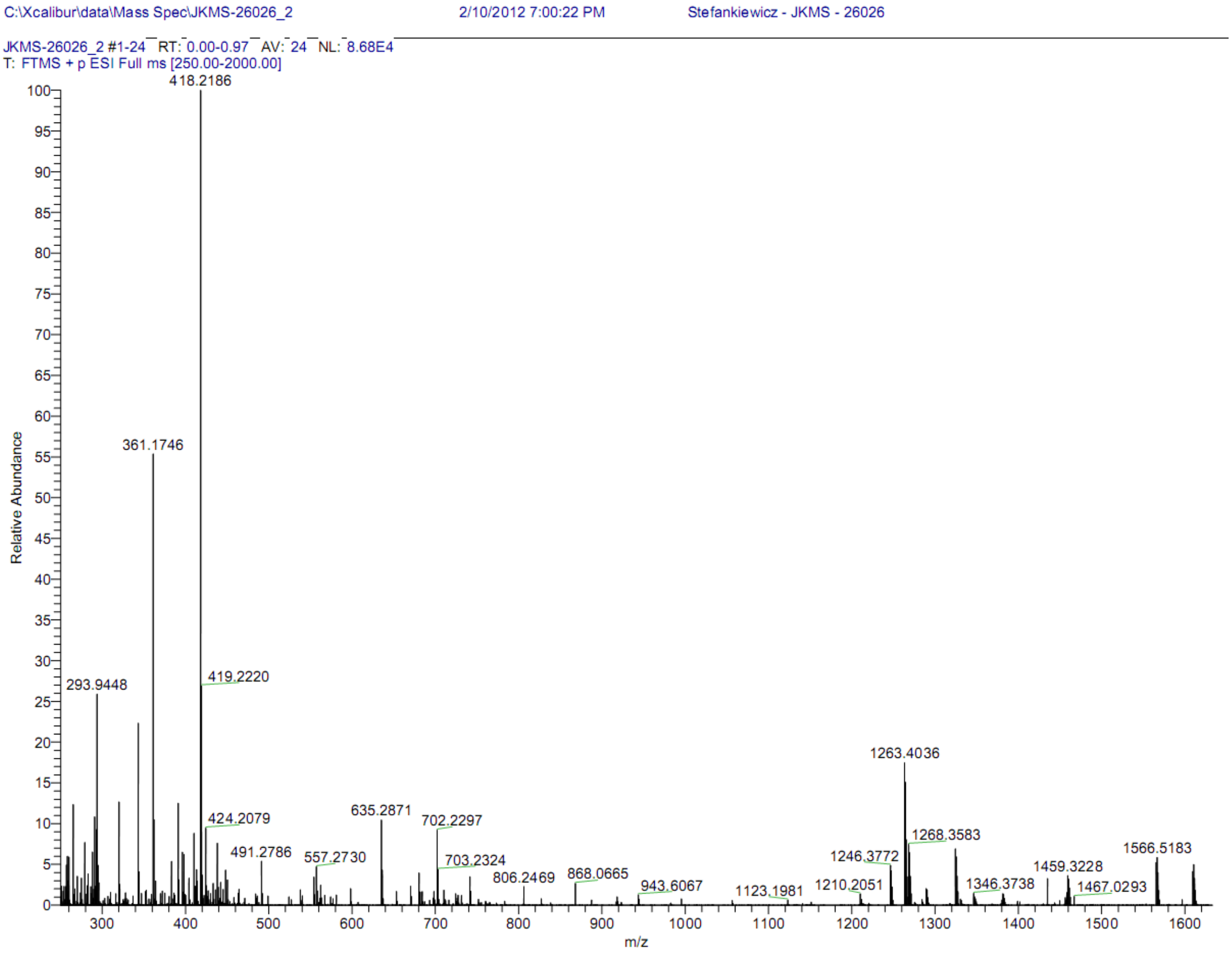
^{1}H NMR in d^{6} DMSO of molecule **B**



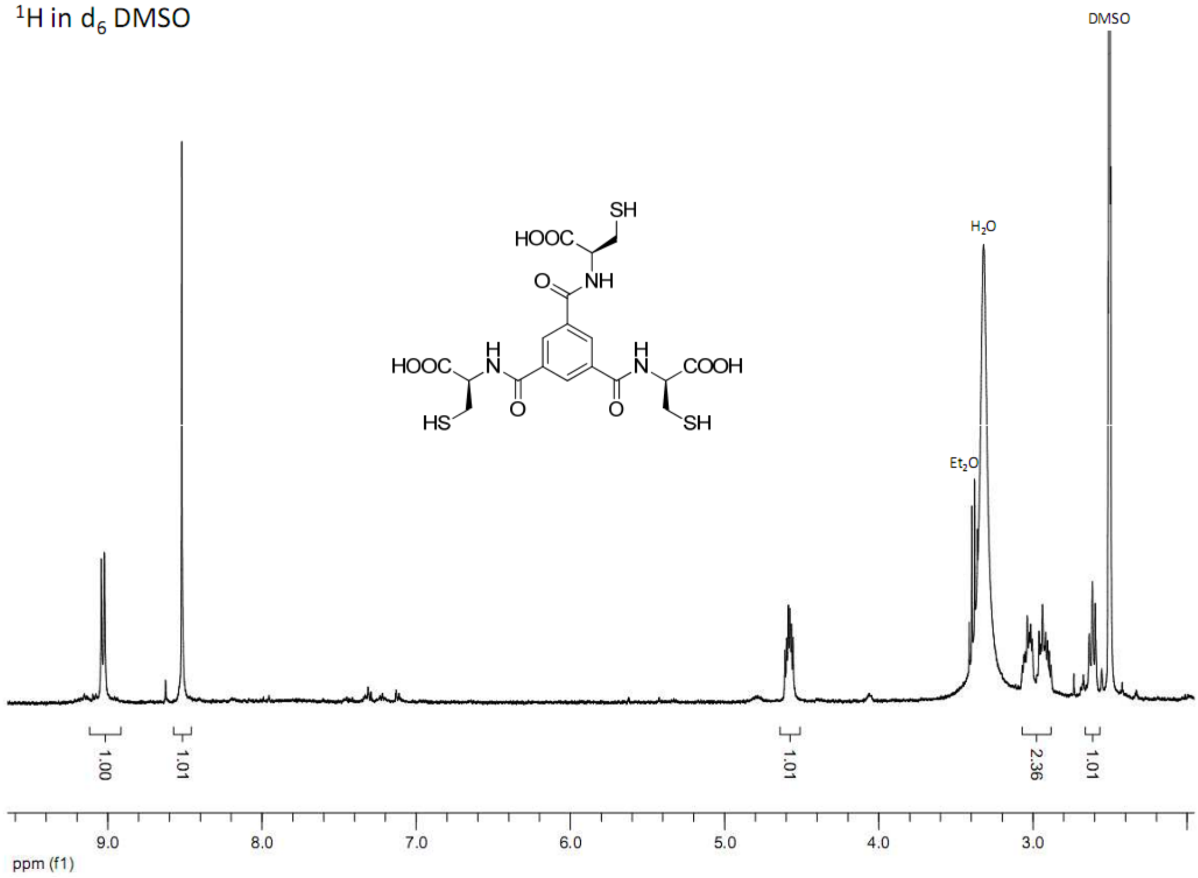
^{13}C NMR in d^6 DMSO of molecule **B**



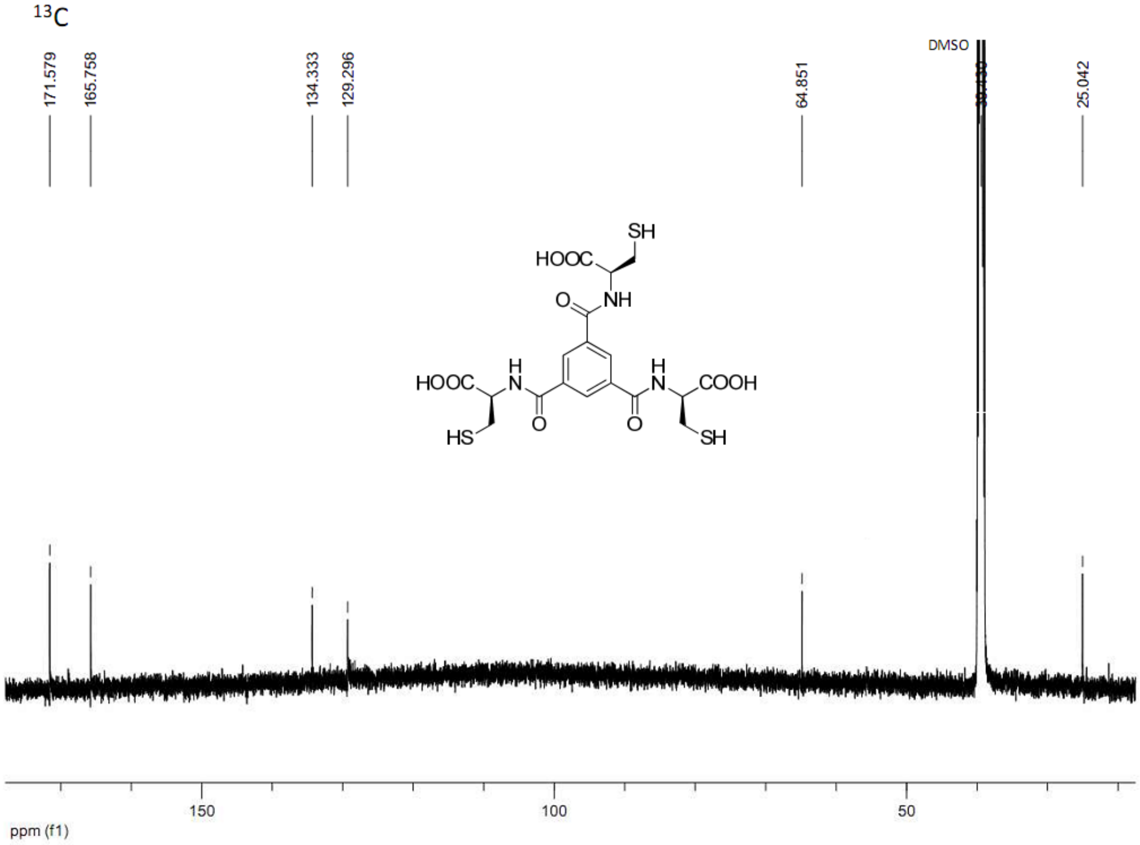
HR-ESI-MS of molecule **B (1246.3772.) and B + Na$^+$ (1268.3583)**



^{1}H NMR in d^6 DMSO of molecule **1**

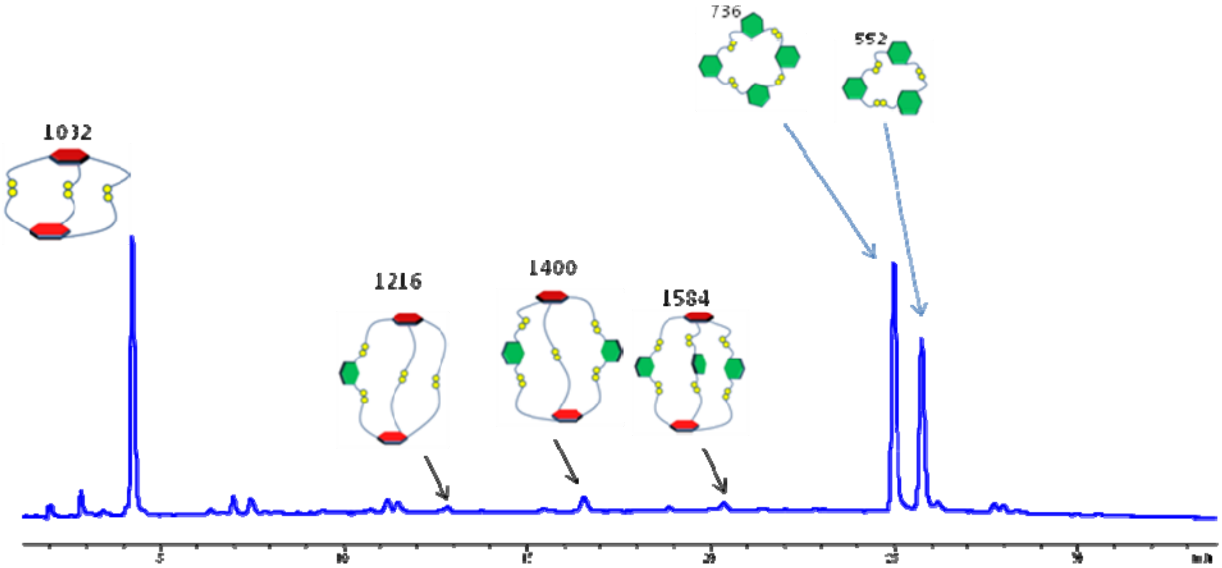


^{13}C NMR in d^6 DMSO of molecule **1**

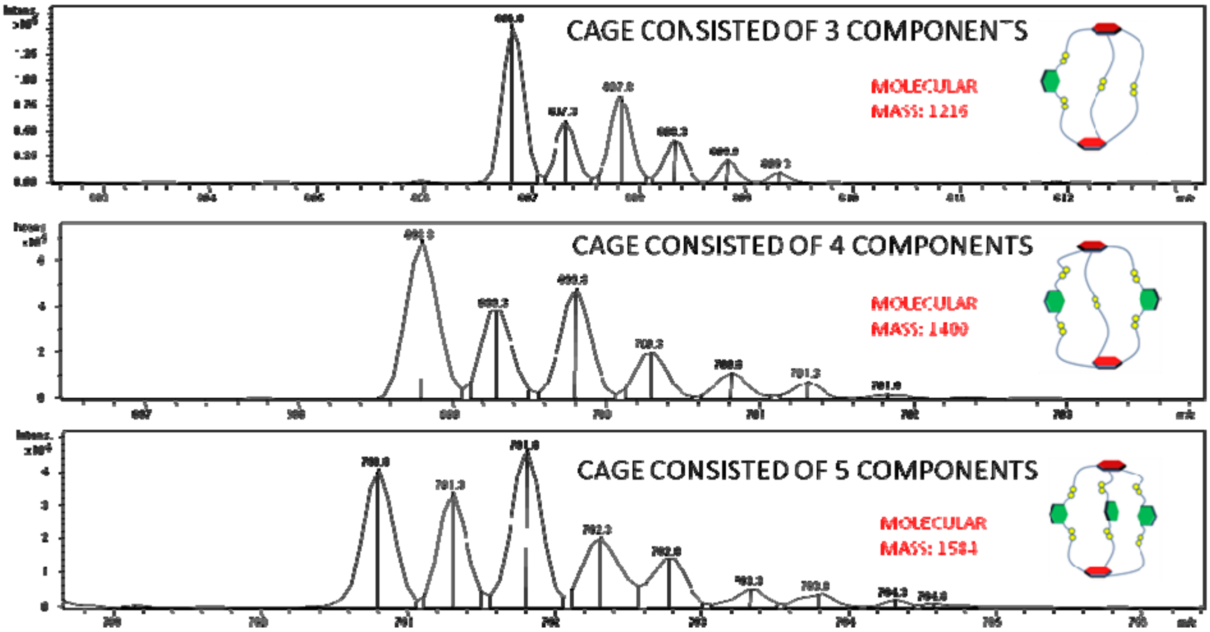


ESI-MS and HPLC DATA

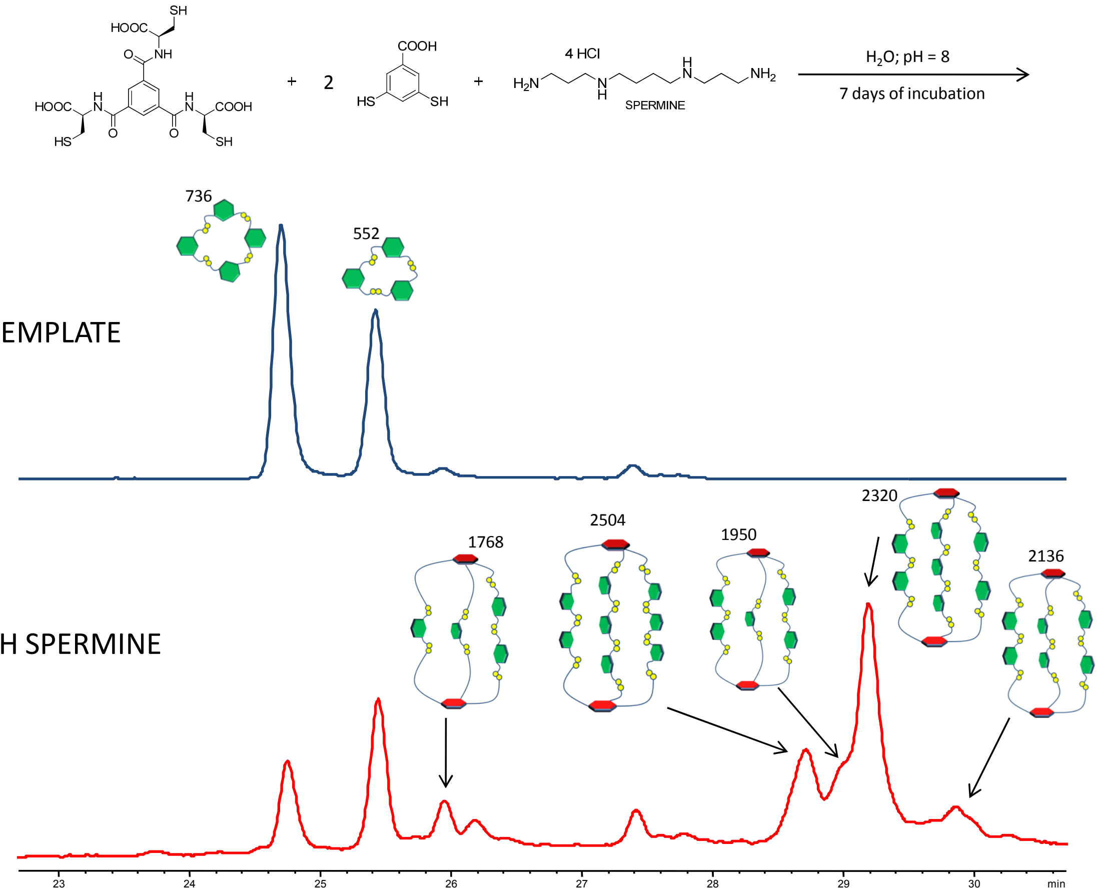
1) in the case of untemplated DCL minor signals assigned to small cages were detected. The signals from these structures disappeared after templation with polyamines.

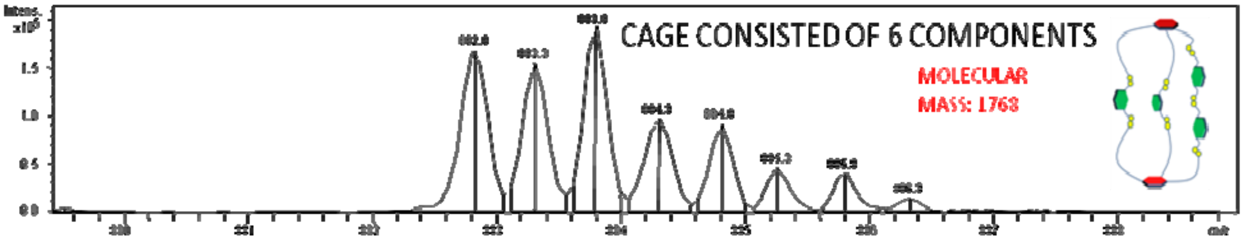


In the ESI-MS spectra all of the small cages appear as doubly charged anions:

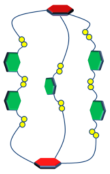


2) in the case of templated libraries all cages observed exist as a doubly charged anions:

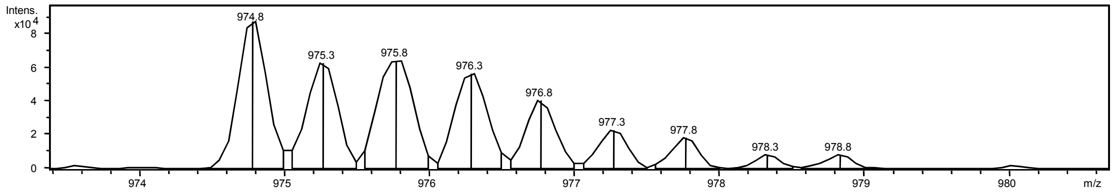

SH
HOOC
NH
O
H
N
HOOC
O
O
H
N
COOH
HS
SH
+
2
COOH
HS
SH
+
4 HCl
H₂N
N
H
H
N
NH₂
SPERMINE
H₂O; pH = 8
7 days of incubation
736
552
NO TEMPLATE
WITH SPERMINE
1768
2504
1950
2320
2136
23
24
25
26
27
28
29
30
min


Intens.
1.5
1.0
0.5
CAGE CONSISTED OF 6 COMPONENTS
MOLECULAR
MASS: 1768

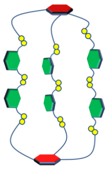
CAGE CONSISTED OF 7 COMPONENTS



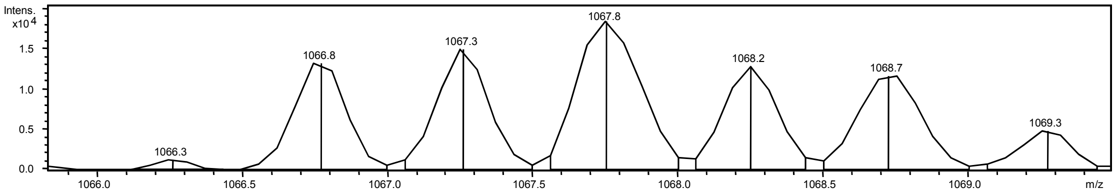
MOLECULAR MASS: 1950



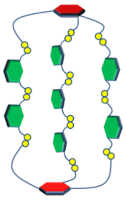
CAGE CONSISTED OF 8 COMPONENTS



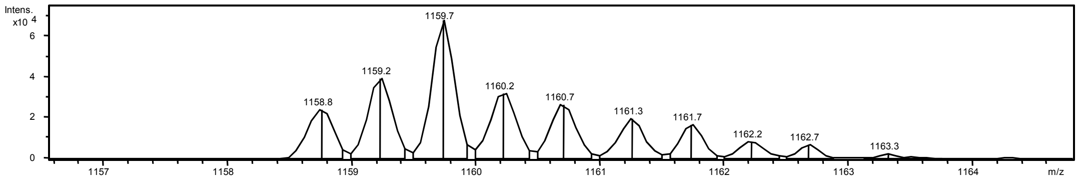
MOLECULAR MASS: 2136



CAGE CONSISTED OF 9 COMPONENTS

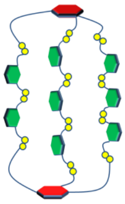


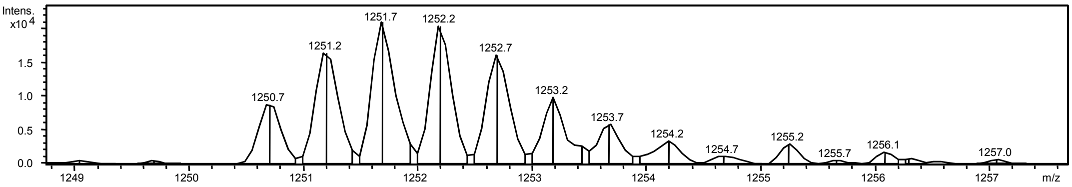
MOLECULAR MASS: 2304



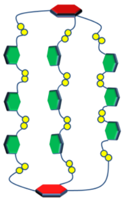
MOLECULAR MASS: 2504

CAGE CONSISTED OF 10 COMPONENTS

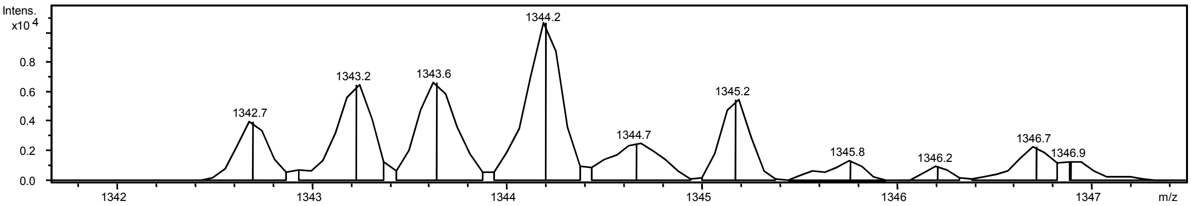




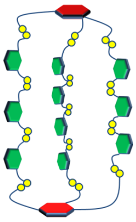
CAGE CONSISTED OF 11 COMPONENTS



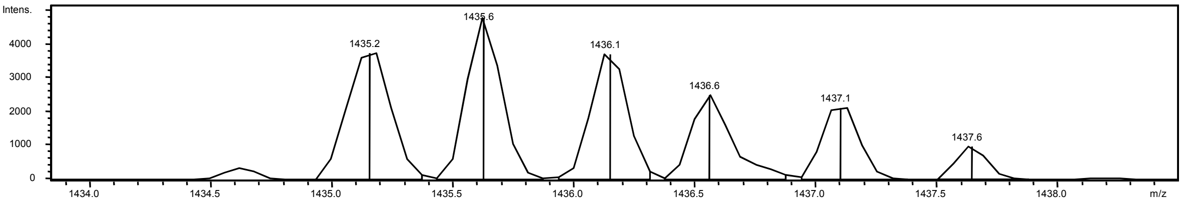
MOLECULAR
MASS: 2688



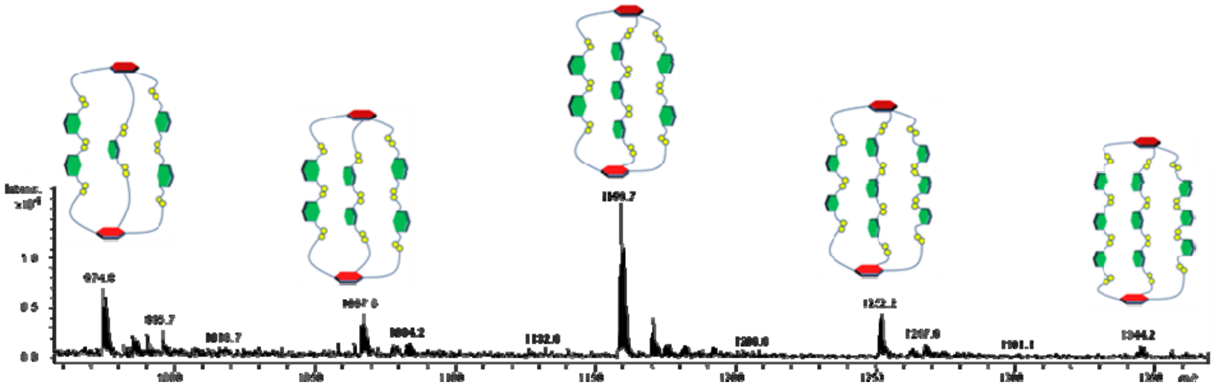
CAGE CONSISTED OF 12 COMPONENTS



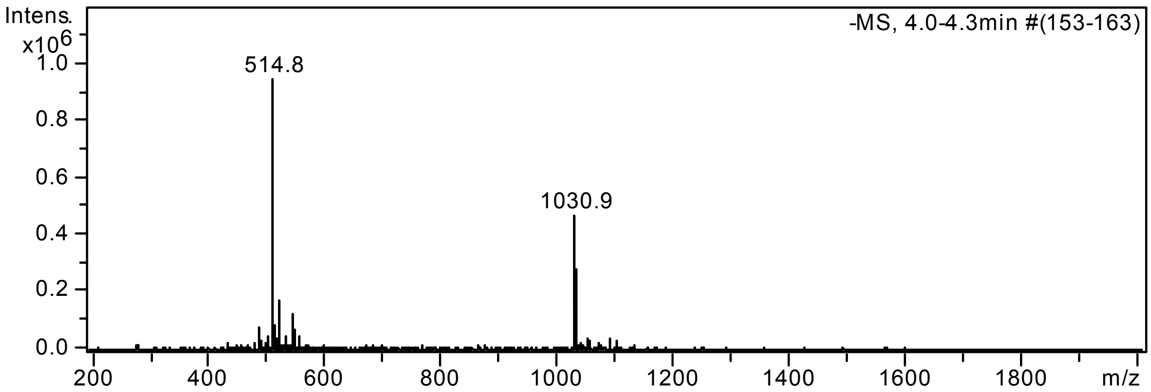
MOLECULAR
MASS: 2872

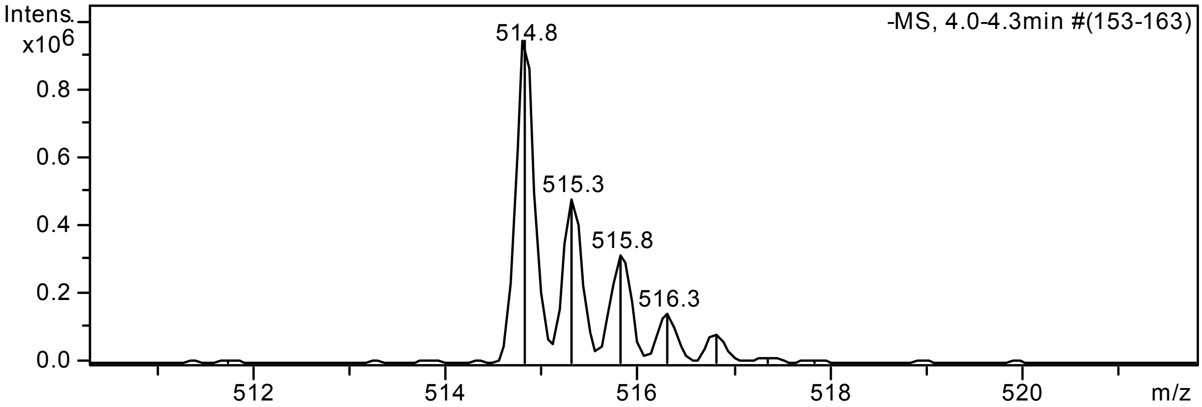


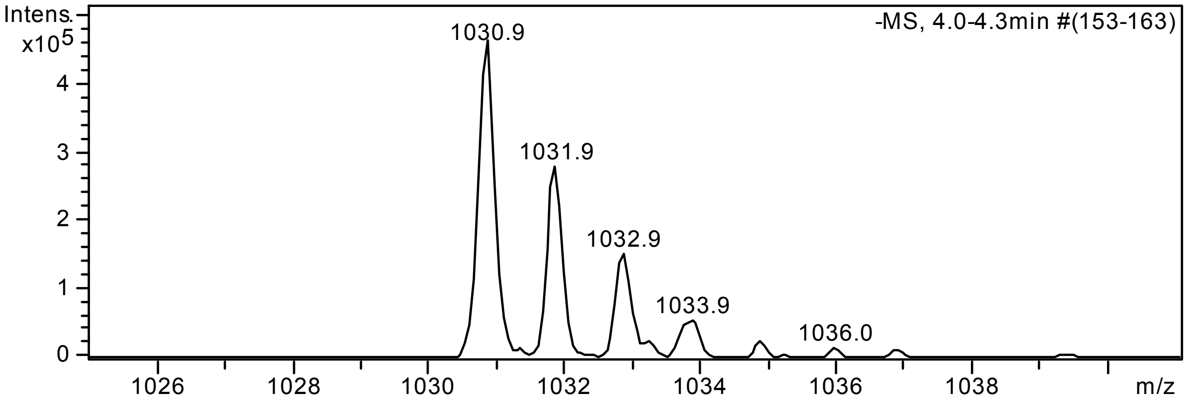
Part of the ESI-MS data of spermine templated library showing series of five cages:



ESI-MS of the homo-dimeric cage consisted of two molecules of **1**. All signals from cage-like archtectures appeared as the doubly charged dianions. This is presumably due to the high number of carboxylates/carboxylic acids, which varies from 6 (for the homo-dimeric cage of **1**) through 10 (in the smallest hetero-cage) to 15 (for the biggest cage).



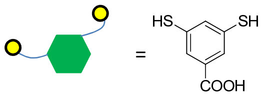


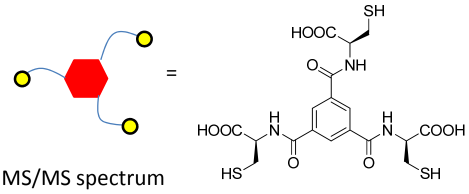


Speciation of polyamines

- based on the data published by *A. Bencini et al.* in *Coordination Chemistry Reviews* 188 (1999) 97–156

	Log K_1	Log K_2	Log K_3	Log K_4	Average number of protonated NH_2 centres at the pH ~ 8	Average % of protonation at the pH ~ 8
spermine	10.80	10.02	8.85	7.96	4	100 %
triethylenetetramine	9.74	9.07	6.59	3.27	2	50%
spermidine	10.89	9.81	8.24	-	3	100%
1,4-butanediamine	10.72	9.44	-	-	2	100%
ethylenediamine	9.87	7.08	-	-	1	50%
tris(2-aminoethyl)amine	No data available					
1, 4, 8, 11 tetraazacyclotetradecane	11.58	10.62	1.61	2.41	2	50%

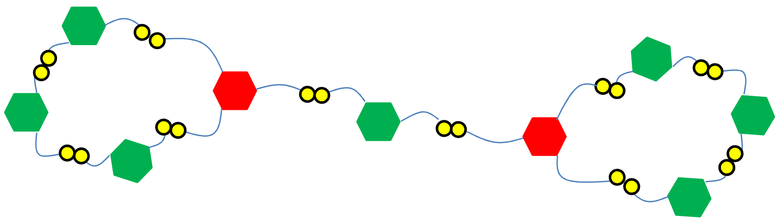




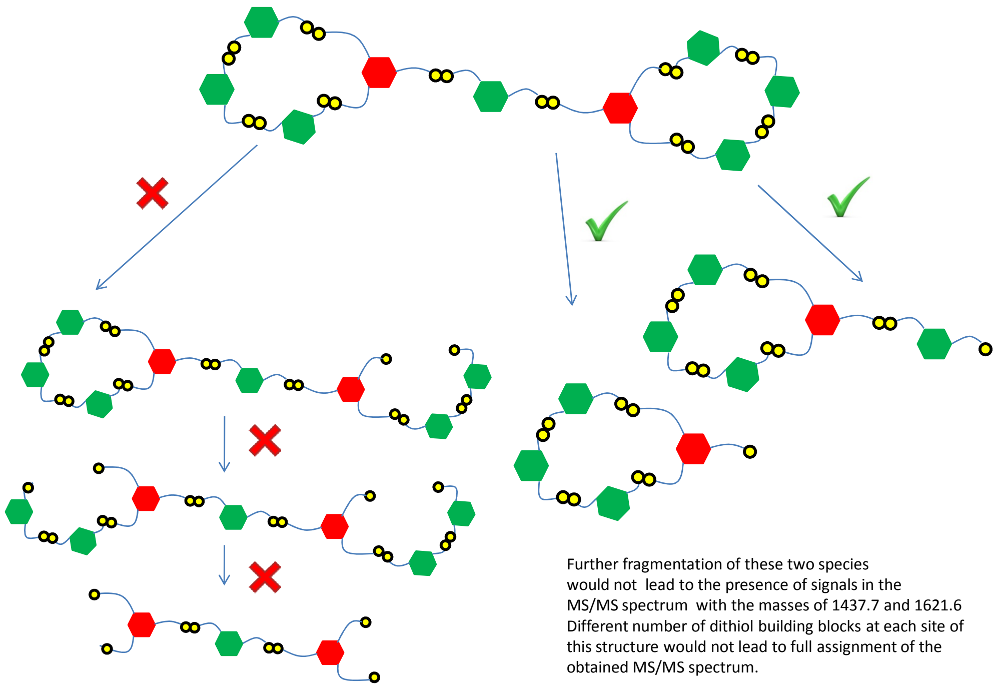
✓ = mass present in the obtained MS/MS spectrum

✗ = mass absent in the obtained MS/MS spectrum

Possible structural isomers for nonameric cage
Isomer nr. 1

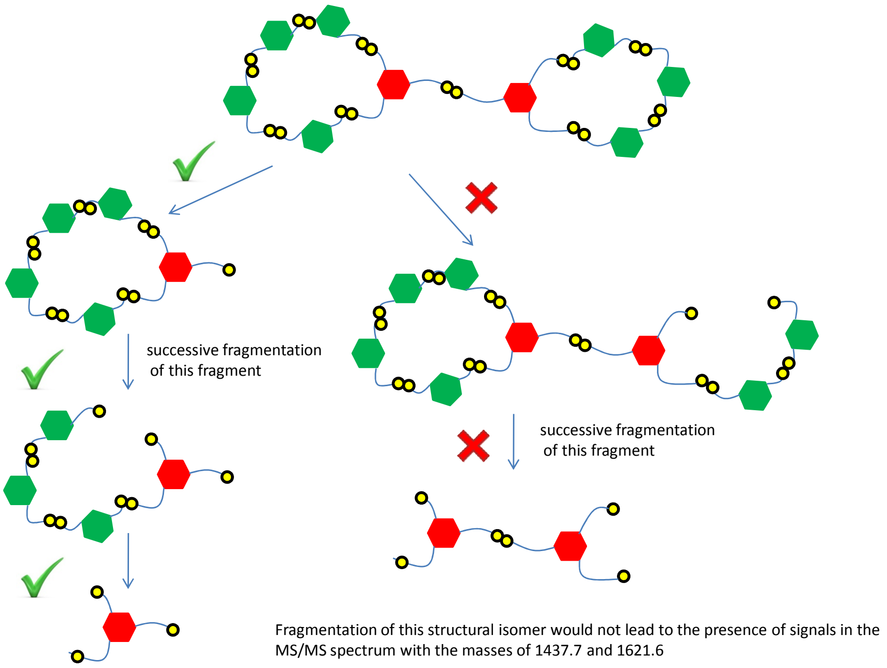


Fragmentation of this molecule would lead to the presence of the following ions in the MS/MS spectra



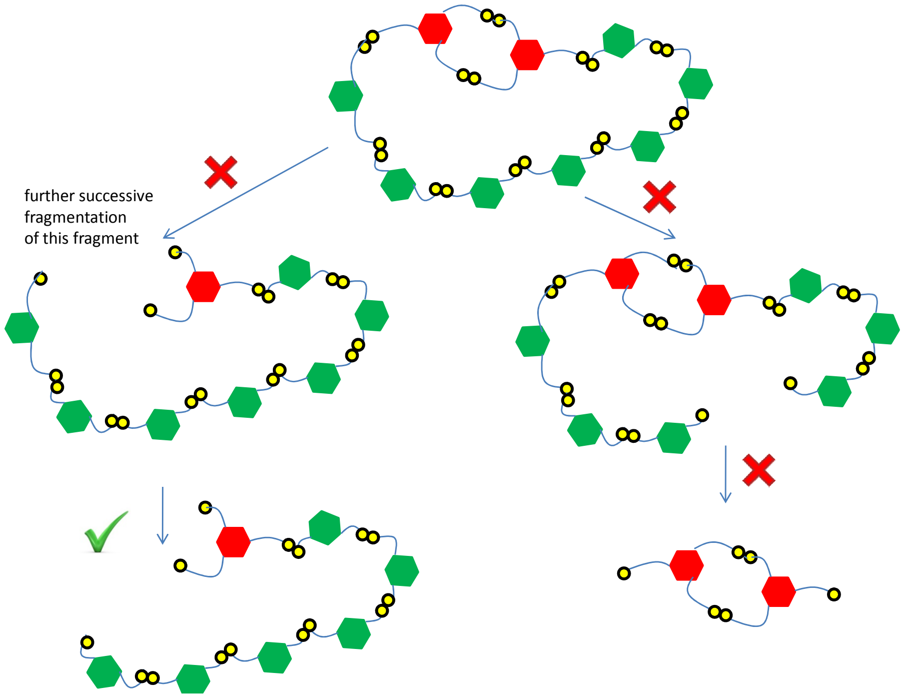
Further fragmentation of these two species would not lead to the presence of signals in the MS/MS spectrum with the masses of 1437.7 and 1621.6 Different number of dithiol building blocks at each site of this structure would not lead to full assignment of the obtained MS/MS spectrum.

Possibile structural isomers for nonameric cage
Isomer nr. 2

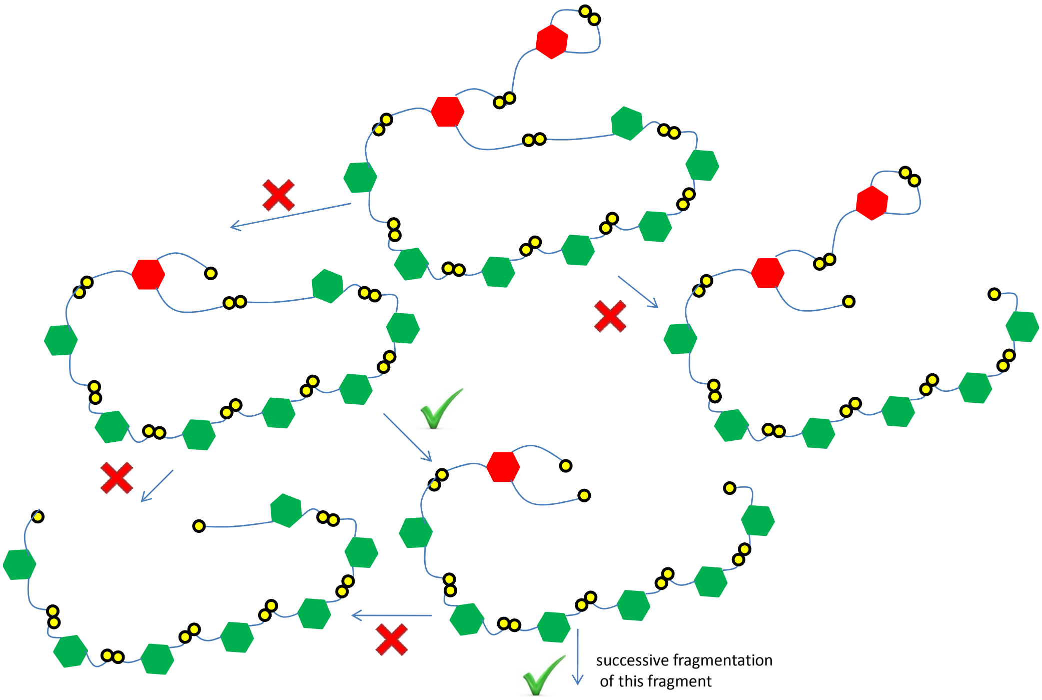


Fragmentation of this structural isomer would not lead to the presence of signals in the MS/MS spectrum with the masses of 1437.7 and 1621.6

Possibile structural isomers for nonameric cage
Isomer nr. 3



Possibile structural isomers for nonameric cage
Isomer nr. 4



Possibile structural isomers for nonameric cage
Isomer nr. 5