Electronic Supplementary Material (ESI) for ChemComm.

Template-promoted self-replication in dynamic combinatorial libraries made from a simple building block

Supporting information

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1. Materials and methods

All chemicals, unless otherwise stated, were purchased from commercial suppliers (*Sigma-Aldrich, TCI Europe, Acros Organics, Merck Chemicals or Alfa Aesar*) and used as received. Acetonitrile (ULC-MS grade), water (ULC-MS grade) and trifluoroacetic acid (HPLC grade) were purchased from *Biosolve BV*. Water was doubly distilled prior to use. Anhydrous solvents used in syntheses were freshly collected from a dry solvent purification system prior to use. Flash column chromatography was performed on a Reveleris ® X2 Flash Chromatography System (*Grace Davison Discovery Sciences*, Deerfield IL) on normal phase silica cartridges and on C-18 reversed phase silica cartridges. NMR spectra were taken using a Varian VXR400 spectrometer. High resolution mass spectrometry (HRMS) was carried out on a LTQ ORBITRAP XL spectrometer (*Thermo Scientific*) employing electrospray ionization (ESI) in positive mode.

Library preparation and sampling

Building blocks were dissolved in 1.0 mM concentrations in borate buffer (50 mM, pH 8.5). Where necessary the pH of the solution was adjusted by the addition of a 1.0 M NaOH solution such that the final pH was 8.5. The solutions were kept in a UPLC vial (12 x 32 mm) with a Teflon-lined cap. Stirred samples contained a cylindrical micro-stirrer bar (2 x 5 mm, Teflon-coated, purchased from VWR) and were stirred at 1200 rpm using an IKA RCT basic hotplate stirrer.

Templates were dissolved in 1.0 mM concentrations in borate buffer (50 mM, pH 8.5) and an aliquot corresponding to 0.2 mM of each template was added separately to freshly prepared solutions of building blocks.

UPLC and LC-MS analysis

Samples were homogenized by sonication for 1 minute in an ultrasonic bath prior to sampling. An aliquot of 5.0 µL was taken into a UPLC vial insert and diluted with 95 µL of double distilled water. A volume of 10 µL of the mixture was injected. UPLC analyses were performed on a Waters Acquity H-class equipped with diode array UV/Vis detector. LC-MS analyses were performed on a Xevo G2 UPLC/TOF with ESI ionization, manufactured by Waters. All analyses were performed at 35 °C, with a flow rate of 0.3 mL/min, using a reversed-phase UPLC column (Acquity UPLC HSS T3, 100 Å, 1.8 µm, 2.1 x 150 mm). UV absorbance was monitored at 254 nm. Positive-ion mass spectra were acquired using electro-spray ionization and the same UPLC method (see below). Injection volume was 5.0 µL with dilution as previously described.

UPLC method

Solutions containing building block **1** and its oxidation products were analyzed using the following method (linear gradient):

Solvent A: ULC/MS grade water purchased from Biosolve (0.1% trifluoroacetic acid added).

Solvent B: ULC/MS grade acetonitrile purchased from Biosolve (0.1% trifluoroacetic acid added).

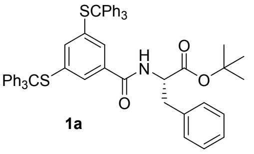
Time (min)	A%	B%
0	60	40
1	60	40
3	35	65
17	10	90
19	5	95
21	5	95
22	60	40
24	60	40

Negative staining transmission electron microscopy

A small drop (5 µL) of sample was deposited on a 400 mesh copper grid covered with a thin carbon film (Agar Scientific). After 30 seconds, the droplet was blotted on filter paper. The sample was then stained twice (4 µL each time) with a solution of 2% uranyl acetate deposited on the grid and blotted on the filter paper after 30 seconds each time. The grids were observed in a Philips CM120 cryo-electron microscope operating at 120 kV. Images were recorded on a slow scan CCD camera.

2. Synthesis of building block **1**

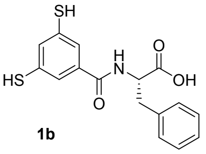
***Tert*-butyl (3,5-bis(tritylthio)benzoyl)-*L*-phenylalaninate (1a)**



1a

3,5-Bis(tritylthio)benzoic acid[S1] (260 mg, 0.388 mmol) was dissolved in 20 mL of dry DCM and the mixture was cooled to 0 °C.) *N*,*N*-diisopropylethylamine (DIPEA; 150 µL, 111 mg, 0.860 mmol), 85 mg (0.44 mmol) of EDC-HCl and 53 mg (0.39 mmol) of anhydrous HOBt were added and the mixture was stirred for 15 minutes. *Tert*-butyl phenylalaninate hydrochloride (100 mg, 0.388 mmol) was added and the mixture was stirred at room temperature for 12 hours. The reaction mixture was washed five times with 20 mL of water and the solvent was removed *in vacuo*. The residue was purified by column chromatography (EtOAc:*n*-hexane, 1:1) or by flash column chromatography (SiO_2, 0% – 50% EtOAc in *n*-heptane). Compound **1a** was obtained as a fluffy white solid (328 mg, 0.375 mmol, 97%). ^1H NMR (400 MHz, $CDCl_3$): δ 7.37 – 6.93 (m, 36H), 6.77 (d, *J* = 1.6 Hz, 2H), 5.69 (d, *J* = 7.7 Hz, 1H), 4.66 (m, 1H), 2.99 (d, *J* = 5.9 Hz, 2H), 1.35 (d, *J* = 1.2 Hz, 9H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 172.89, 168.08, 146.68, 145.18, 138.89, 137.51, 136.39, 135.09, 132.50, 132.18, 130.99, 130.57, 130.42, 129.90, 129.58, 129.47, 84.92, 74.00, 56.49, 40.71, 30.63. HRMS (ESI): Calcd for $C_{58}H_{51}NO_3S_2$ $[M+H]^+$: 874.3383, found 874.3378.

(3,5-Dimercaptobenzoyl)-*L*-phenylalanine (1)



1b

Compound **1a** (100 mg, 0.114 mmol) was dissolved in 3 mL of EtSH under a nitrogen atmosphere and 3 mL of degassed TFA was added. The mixture was stirred for 24 hours after which 0.30 mL of Et_3SiH (0.41 mg, 0.35 mmol) was added. The mixture was stirred for an additional 45 minutes. Volatiles were removed by a stream of nitrogen and the residue was dissolved in 100 mL of a degassed mixture of MeOH/H_2O (9:1) and washed five times with 20 mL of *n*-hexane. MeOH was evaporated and water removed by freeze drying. The product was purified by reverse phase flash column chromatography (RP C18, 10% – 95% MeCN in H_2O with 0.1% TFA) and 17 mg (0.051 mmol, 44%) of a white powder was obtained after the MeCN was evaporated and the water freeze dried. ^1H NMR (400 MHz, $CDCl_3$): δ 7.29 – 7.15 (m, 6H), 7.15 – 7.06 (m, 2H), 6.54 (d, *J* = 7.5 Hz, 1H), 6.29 (s, 1H), 4.98 (m, 1H), 3.43 (s, 2H), 3.33 – 3.10 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 177.43, 168.99, 138.11, 137.71, 135.98, 134.59, 132.01, 131.44, 130.09, 127.42, 56.26, 39.91. HRMS (ESI): Calcd for $C_{16}H_{15}NO_3S_2$ $[M+H]^+$: 334.0566, found 334.0565.

3. Supplementary figures

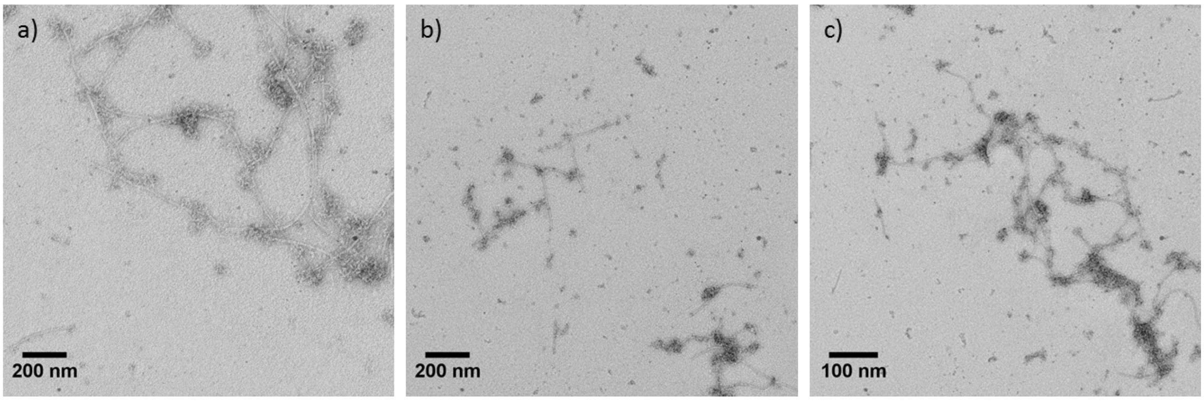


Figure S1. Representative set of TEM micrographs of a fully oxidized DCL made from building block **1** (3.0 mM in 50 mM borate buffer, pH 8.5, stirred at 1200 rpm, room temperature) at day 50. The corresponding library composition is shown in Figure S2.

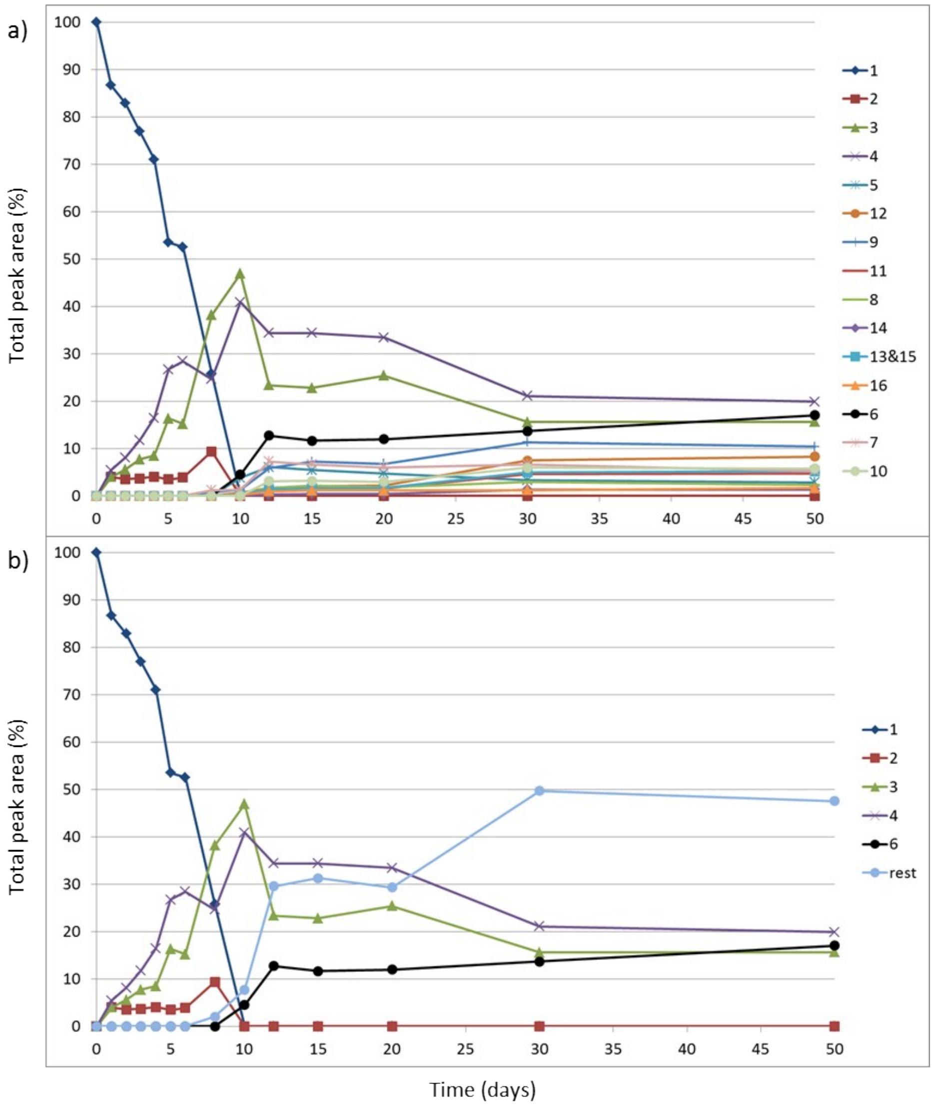


Figure S2. *(a) Change of the product distribution with time for a DCL made from building block* ***1*** *(3.0 mM in 50 mM borate buffer, pH 8.5, stirred at 1200 rpm, room temperature). The numbers in the legend represent the number of monomer units in oligomers made from building block* ***1****. (b) Only the growth curves of* ***1****,* $\mathbf{1}_2$*,* $\mathbf{1}_3$*,* $\mathbf{1}_4$ *and* $\mathbf{1}_6$ *are shown separately, while all the other oligomers are summed together (rest) for clarity.*

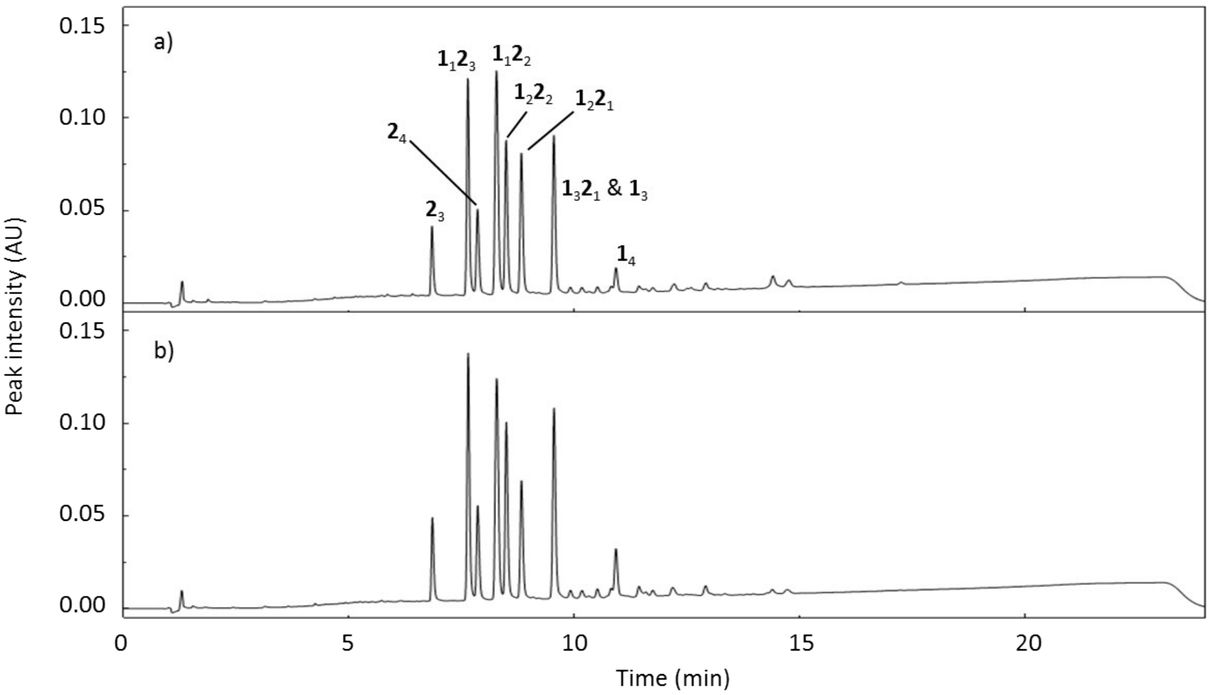


Figure S3. *UPLC-MS analysis of 16 days old DCLs prepared by mixing 1.0 mM solutions of building blocks **1** and **2** in aqueous borate buffer (50 mM, pH 8.5, stirred at 1200 rpm, room temperature) in a 1 to 1 ratio: (a) no template; (b) in the presence of 20% of 1,6-diaminohexane as a template.*

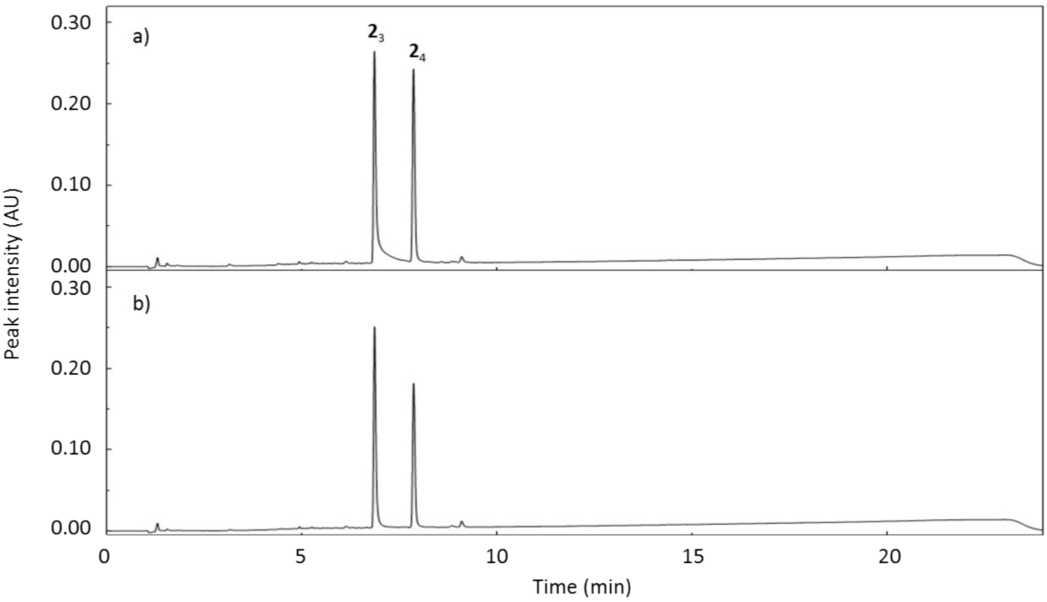


Figure S4. *UPLC-MS analysis of 16 days old 1.0 mM DCLs made from building block **2** in aqueous borate buffer (50 mM, pH 8.5, stirred at 1200 rpm, room temperature): (a) no template; (b) in the presence of 20% of 1,6-diaminohexane as a template.*

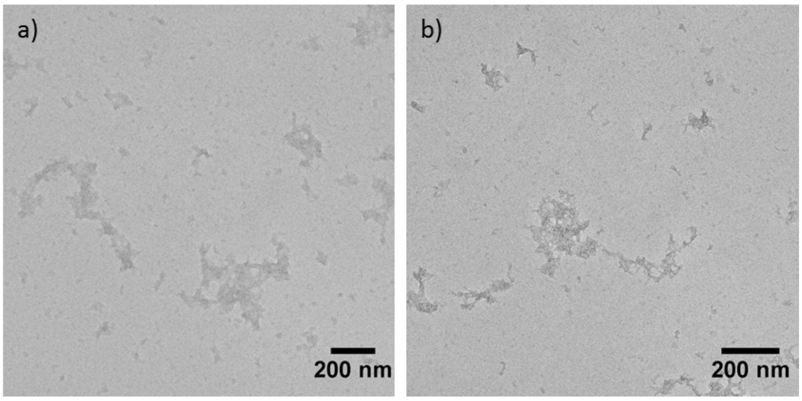


Figure S5. *TEM micrographs of 16 days old DCLs prepared by mixing 1.0 mM solutions of building blocks **1** and **2** in aqueous borate buffer (50 mM, pH 8.5, stirred at 1200 rpm, room temperature) in a 1 to 1 ratio: (a) no template; (b) 20% of 1,6-diaminohexane added as a template. Corresponding UPLC chromatograms shown in Figure S3a and b, respectively.*

4. UPLC-MS characterization of DCLs

LC-MS analysis of DCLs made from building block 1

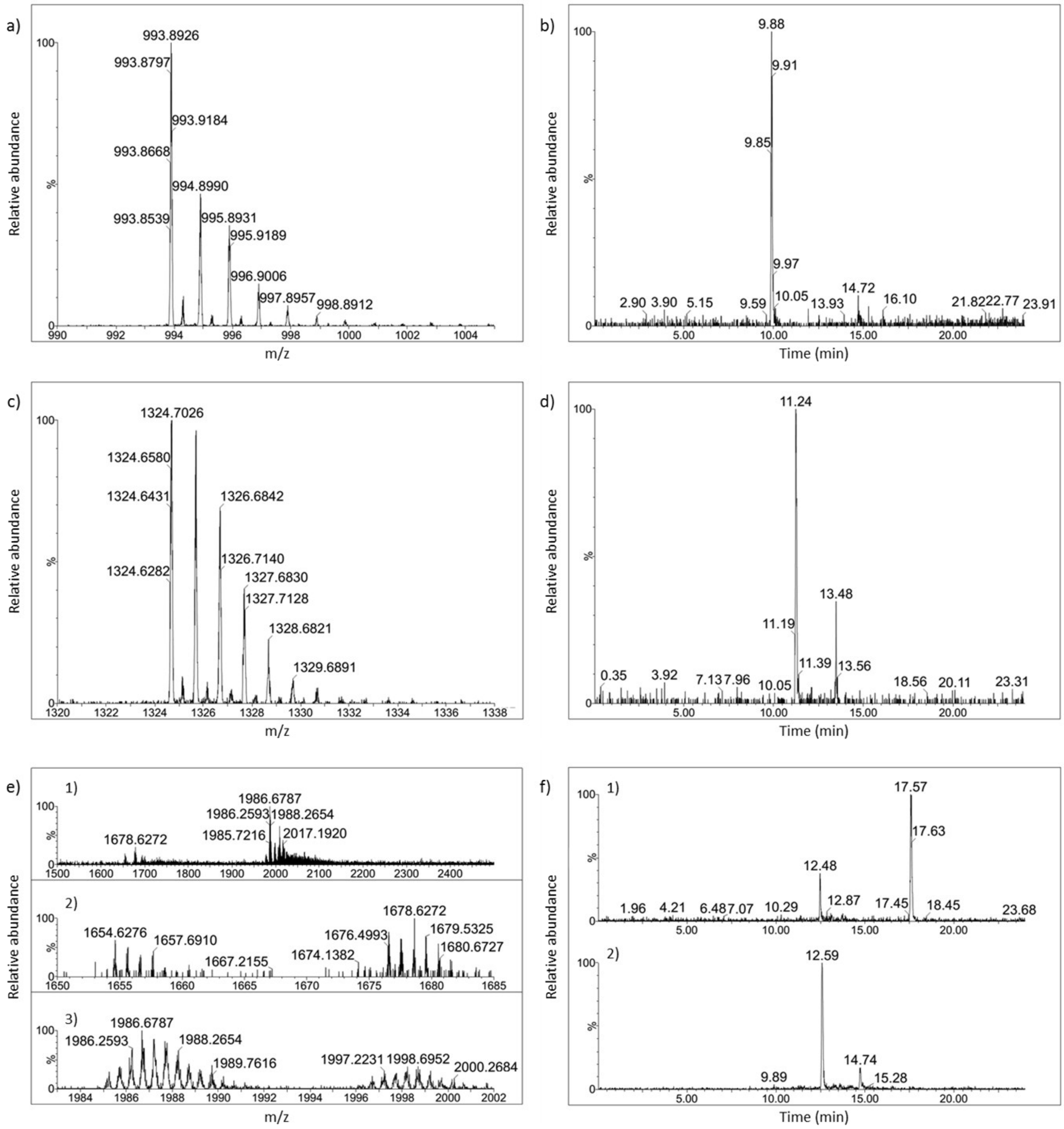


Figure S6. *(a) Isotopic profile of the UPLC peak at 9.88 min, of a DCL made from building block **1**. (b) Extracted ion chromatogram of m/z = 993.0 – 1000.0 corresponding to [$\mathbf{1}_3$+H]$^+$. Expected m/z for [$\mathbf{1}_3$+H]$^+$ = 994.1090. (c) Isotopic profile of the UPLC peak at 11.24 min. (d) Extracted ion chromatogram of m/z = 1324.0 – 1331.0 corresponding to [$\mathbf{1}_4$+H]$^+$. Expected m/z for [$\mathbf{1}_4$+H]$^+$ = 1325.1427. (e) (1) ESI-MS spectrum and isotopic profiles of the UPLC peaks at (2) 12.48 and (3) 12.59 min. (f) Extracted ion chromatogram of (1) m/z = 1653.0 – 1658.0 corresponding to [$\mathbf{1}_5$+H]$^+$*

and (2) m/z = 1984.0 – 1991.0 corresponding to $[\mathbf{1}_{12}+2H]^{2+}$*. Expected m/z for:* $[\mathbf{1}_{5}+H]^{+}$ *= 1656.1763;* $[\mathbf{1}_{12}+2H]^{2+}$ *= 1987.2100. Corresponding UPLC chromatogram shown in Figure 1a.*

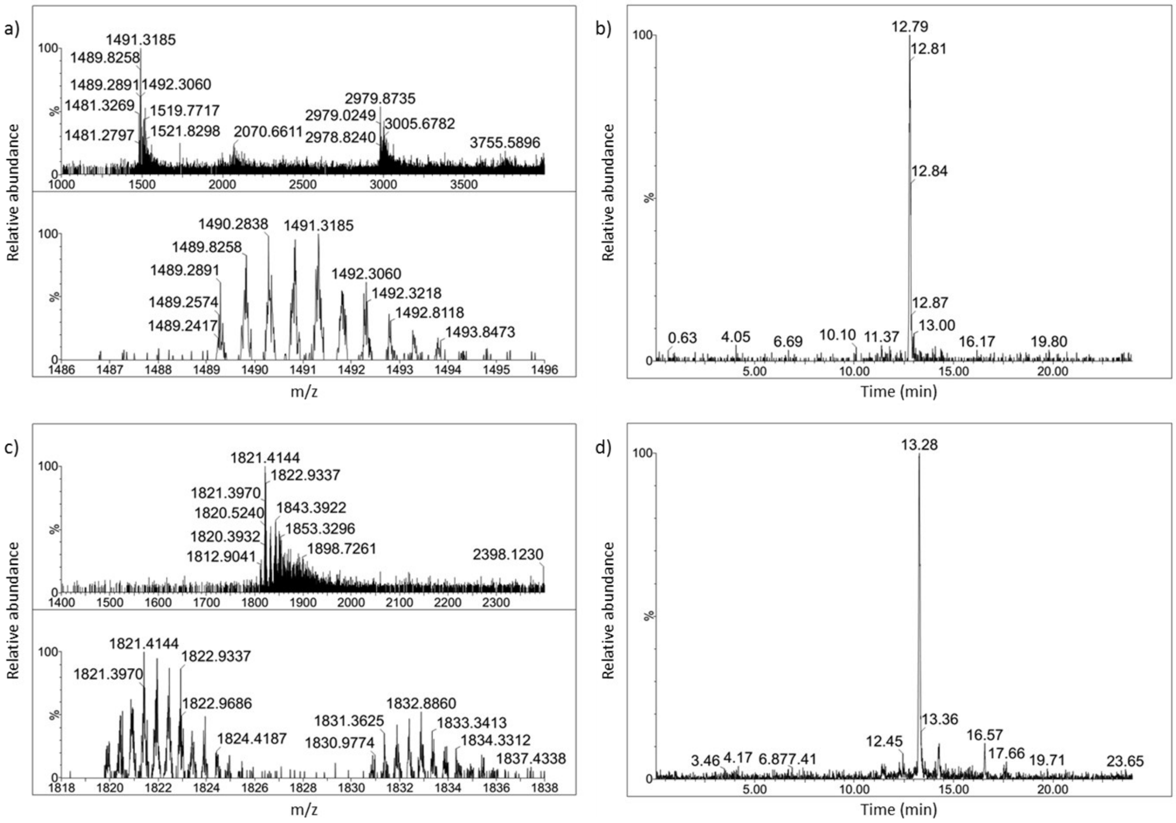


Figure S7. *(a) ESI-MS spectrum and isotopic profile of the UPLC peak at 12.79 min, of a DCL made from building block* ***1****. (b) Extracted ion chromatogram of m/z = 1488.0 – 1494.0 corresponding to* $[\mathbf{1}_{9}+2H]^{2+}$*. Expected m/z for* $[\mathbf{1}_{9}+2H]^{2+}$ *= 1490.6595. (c) ESI-MS spectrum and isotopic profile of the UPLC peak at 13.28 min. (d) Extracted ion chromatogram of m/z = 1818.0 – 1825.0 corresponding to* $[\mathbf{1}_{11}+2H]^{2+}$*. Expected m/z for* $[\mathbf{1}_{11}+2H]^{2+}$ *= 1821.6932. Corresponding UPLC chromatogram shown in Figure 1a.*

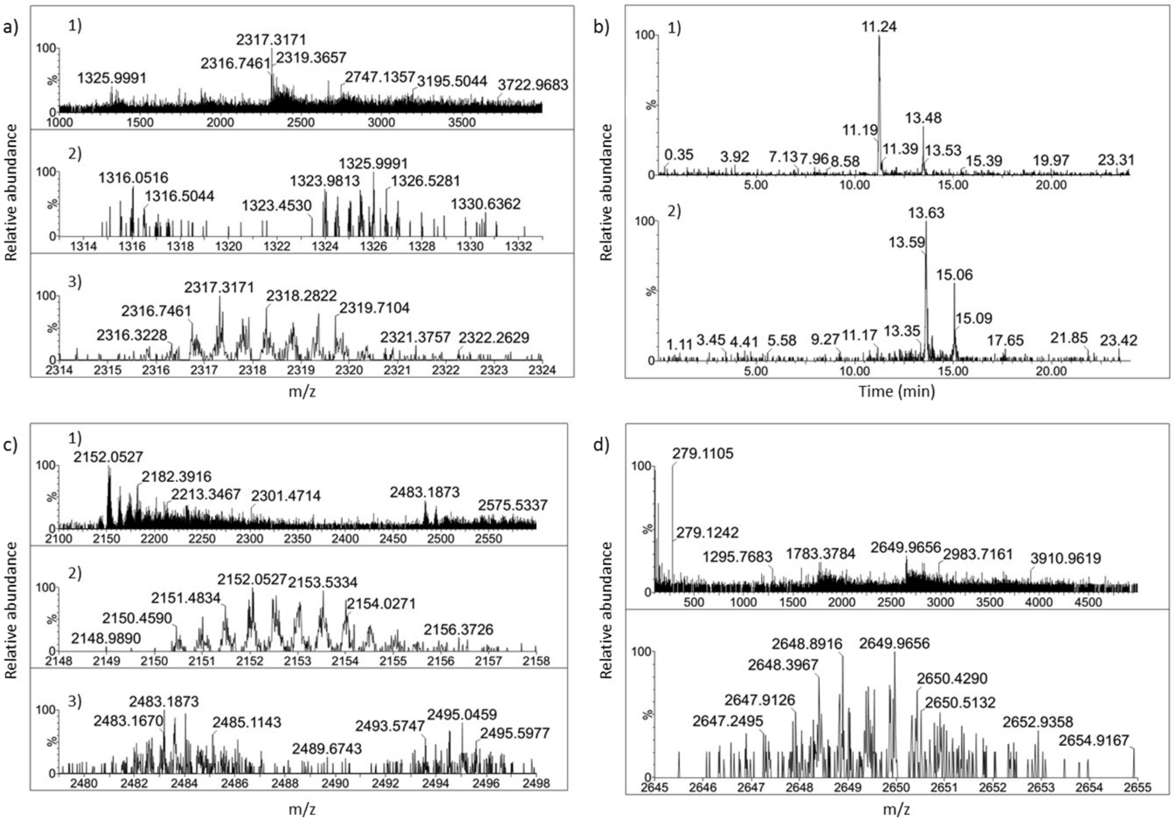


Figure S8. *(a) (1) ESI-MS spectrum and isotopic profiles of the UPLC peaks at (2) 13.48 min and (3) 13.63 min, of a DCL made from building block* ***1****. (b) Extracted ion chromatogram of (1) m/z = 1323.0 – 1328.0 and (2) m/z = 2315.0 – 2320.0, corresponding to* $[\mathbf{1}_8+2H]^{2+}$ *and* $[\mathbf{1}_{14}+2H]^{2+}$*, respectively. Expected m/z for:* $[\mathbf{1}_8+2H]^{2+}$ *= 1325.1427;* $[\mathbf{1}_{14}+2H]^{2+}$ *= 2318.2437. (c) (1) ESI-MS spectrum and (2), (3) isotopic profiles of the UPLC peak at 13.89 min, corresponding to* $[\mathbf{1}_{13}+2H]^{2+}$ *and* $[\mathbf{1}_{15}+2H]^{2+}$ *respectively. Expected m/z for:* $[\mathbf{1}_{13}+2H]^{2+}$ *= 2152.7269;* $[\mathbf{1}_{15}+2H]^{2+}$ *= 2483.7605. (d) ESI-MS spectrum and isotopic profile of the UPLC peak at 14.23 min, corresponding to* $[\mathbf{1}_{16}+2H]^{2+}$*. Expected m/z for* $[\mathbf{1}_{16}+2H]^{2+}$ *= 2649.1774. Corresponding UPLC chromatogram shown in Figure 1a.*

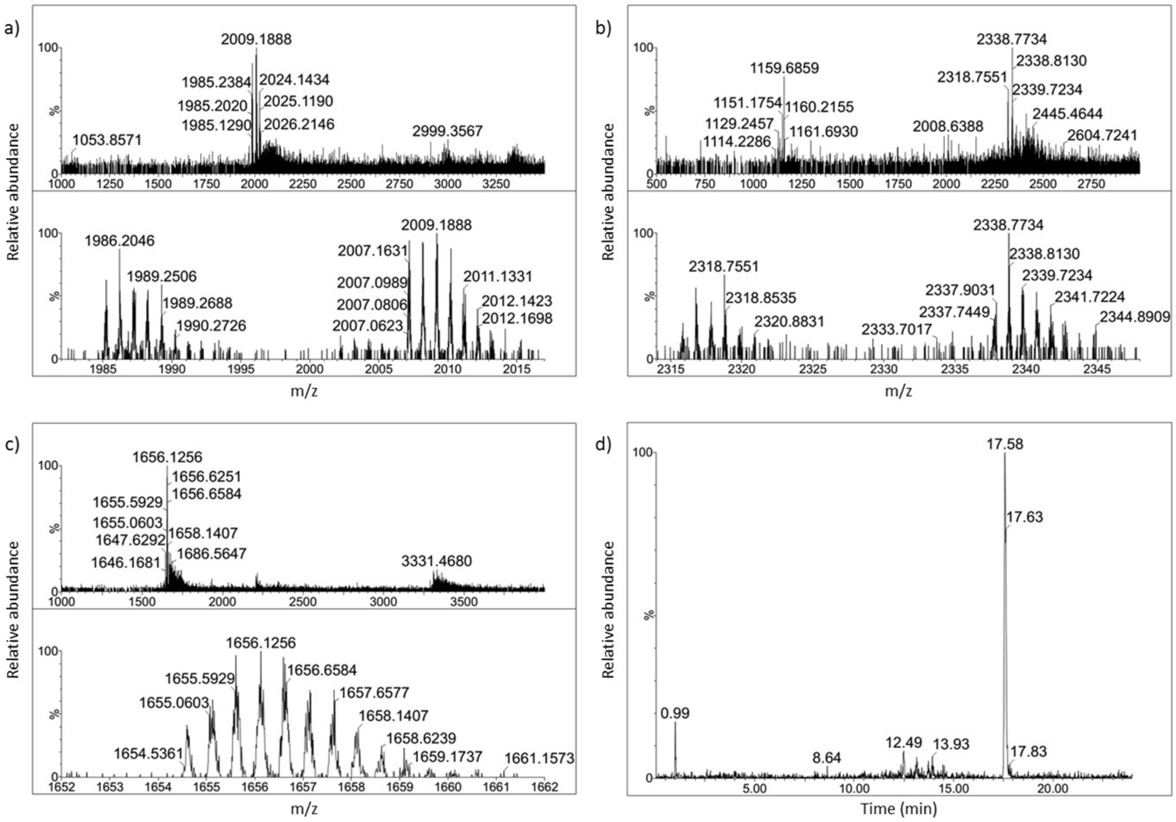


Figure S9. *(a) ESI-MS spectrum and isotopic profile of the UPLC peak at 14.74 min, of a DCL made from building block* ***1****, corresponding to [$\mathbf{1}_6$+H]$^+$. Expected m/z for [$\mathbf{1}_6$+H]$^+$ = 1987.2100. (b) ESI-MS spectrum and isotopic profile of the UPLC peak at 15.03 min, corresponding to [$\mathbf{1}_7$+H]$^+$. Expected m/z for [$\mathbf{1}_7$+H]$^+$ = 2318.2437. (c) ESI-MS spectrum and isotopic profile of the UPLC peak at 17.58 min, corresponding to [$\mathbf{1}_{10}$+2H]$^{2+}$. Expected m/z for [$\mathbf{1}_{10}$+2H]$^{2+}$ = 1656.1763. (d) Extracted ion chromatogram of m/z = 1654.0 – 1660.0 corresponding to [$\mathbf{1}_{10}$+2H]$^{2+}$. Corresponding UPLC chromatogram shown in Figure 1a.*

LC-MS analysis of DCLs made from building block 1 and 2

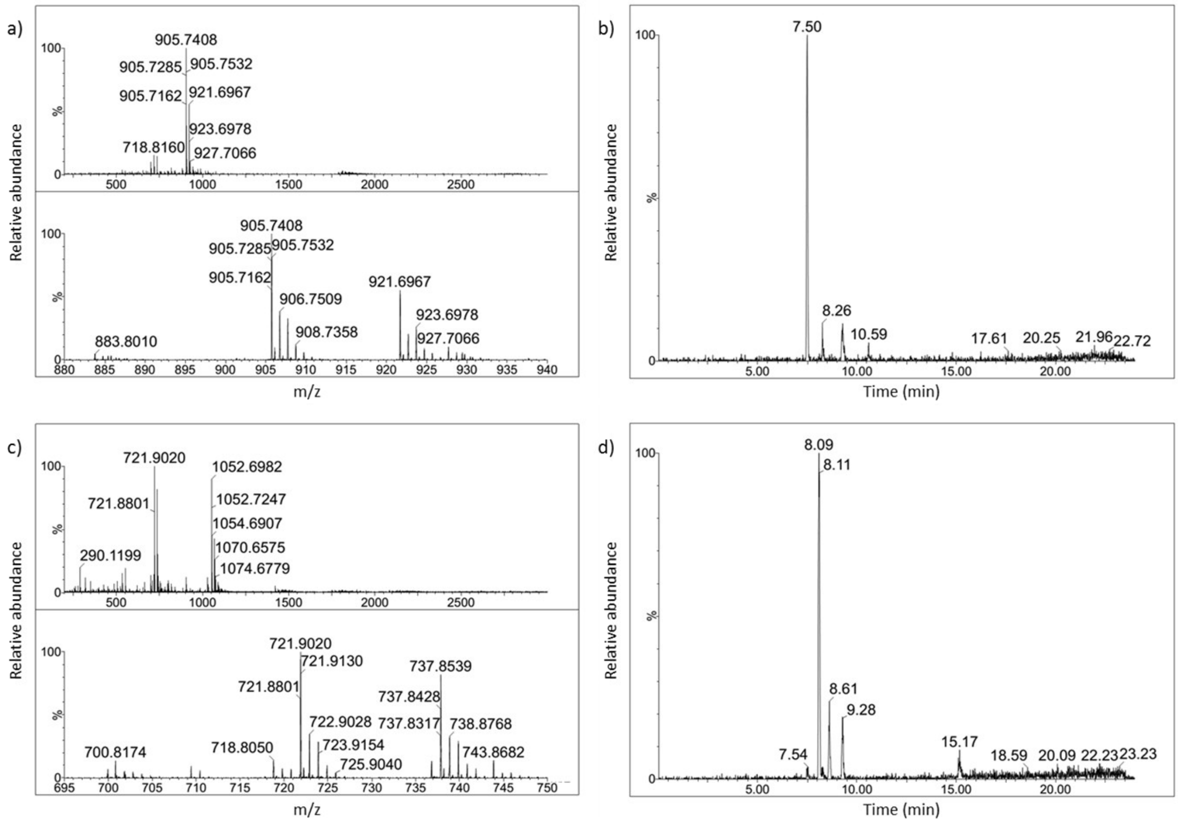


Figure S10. *(a) ESI-MS spectrum and isotopic profile of the UPLC peak at 7.50 min, of a DCL made from building blocks **1** and **2** mixed in a 1 to 1 ratio. (b) Extracted ion chromatogram of m/z = 905.7408 corresponding to* $[\mathbf{1}_1\mathbf{2}_3+\mathrm{Na}]^{1+}$*. Expected m/z for* $[\mathbf{1}_1\mathbf{2}_3+\mathrm{Na}]^{1+}$ *= 905.8923. (c) ESI-MS spectrum and isotopic profile of the UPLC peak at 8.09 min. (d) Extracted ion chromatogram of m/z = 721.9020 corresponding to* $[\mathbf{1}_1\mathbf{2}_2+\mathrm{Na}]^{1+}$*. Expected m/z for* $[\mathbf{1}_1\mathbf{2}_2+\mathrm{Na}]^{1+}$ *= 721.9540. Corresponding UPLC chromatogram shown in Figure S3.*

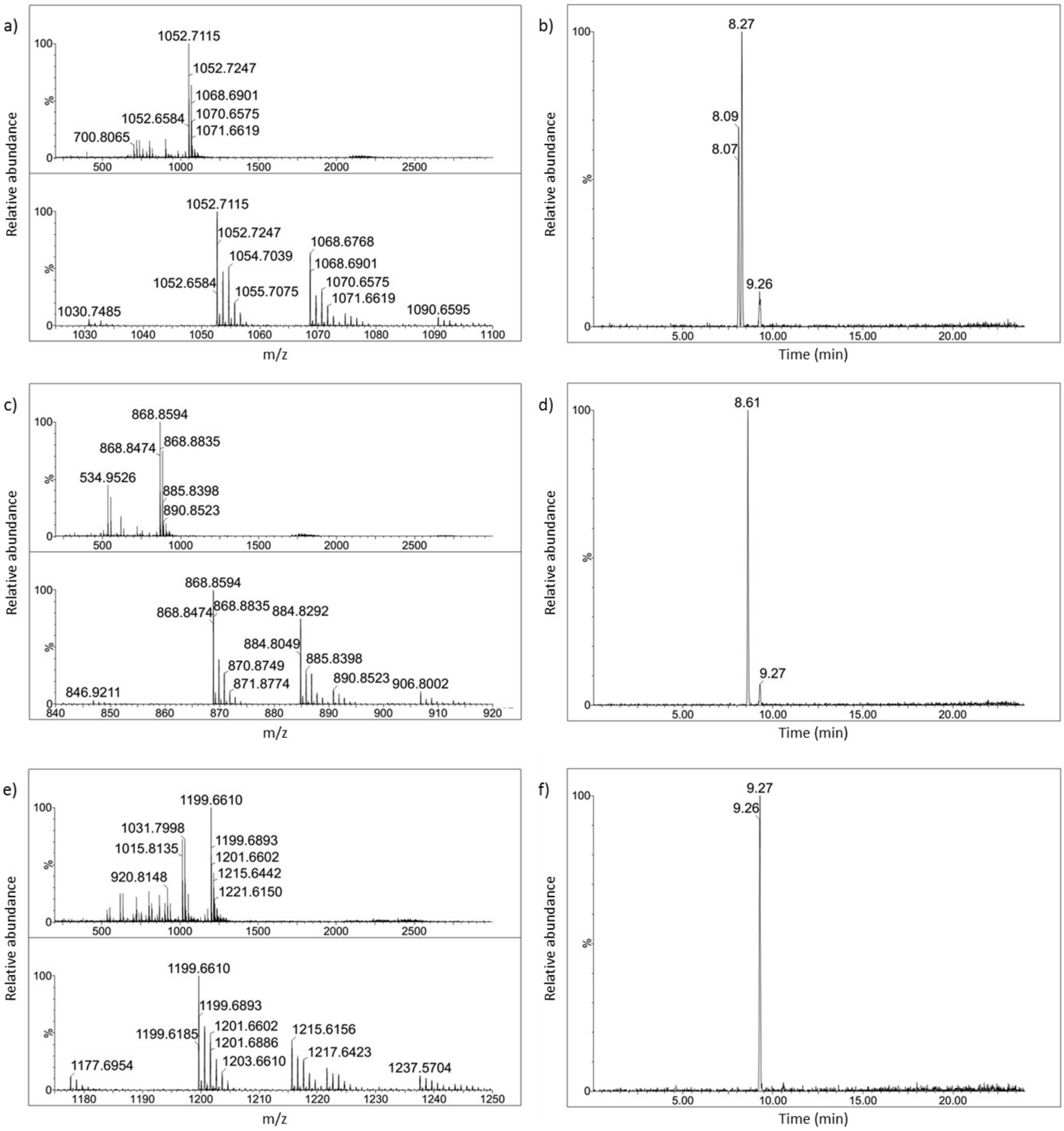
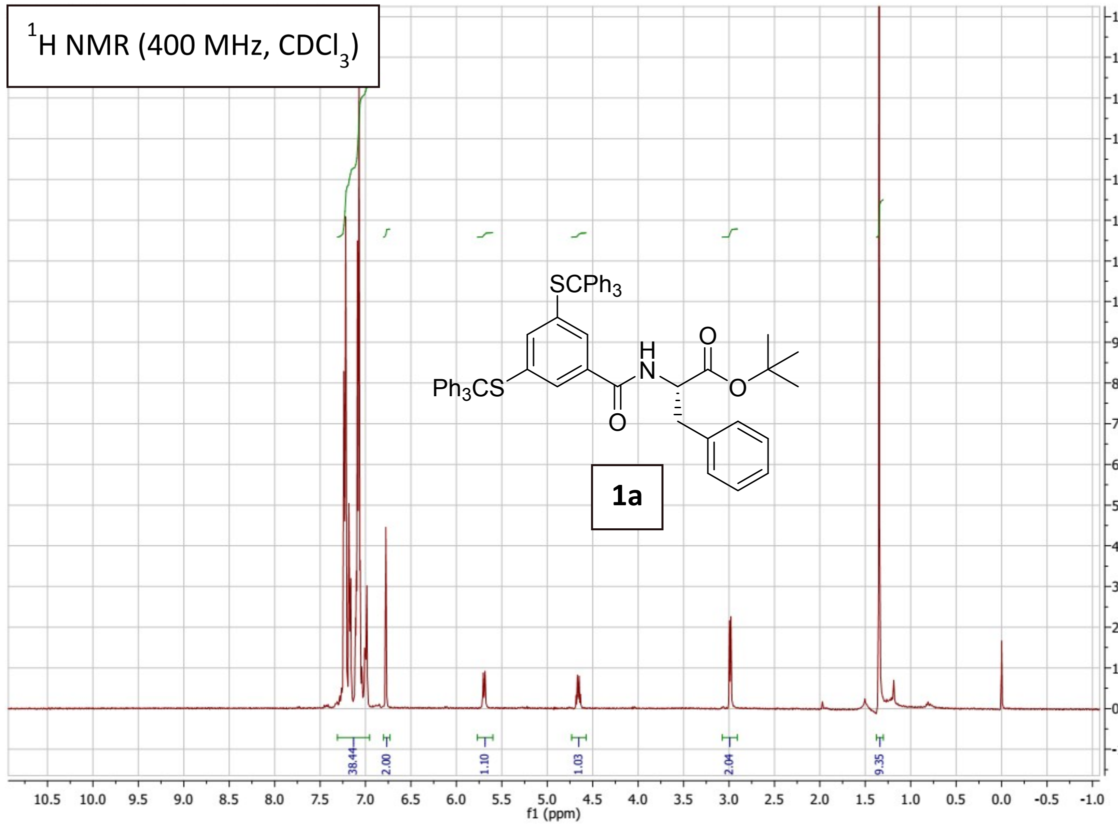
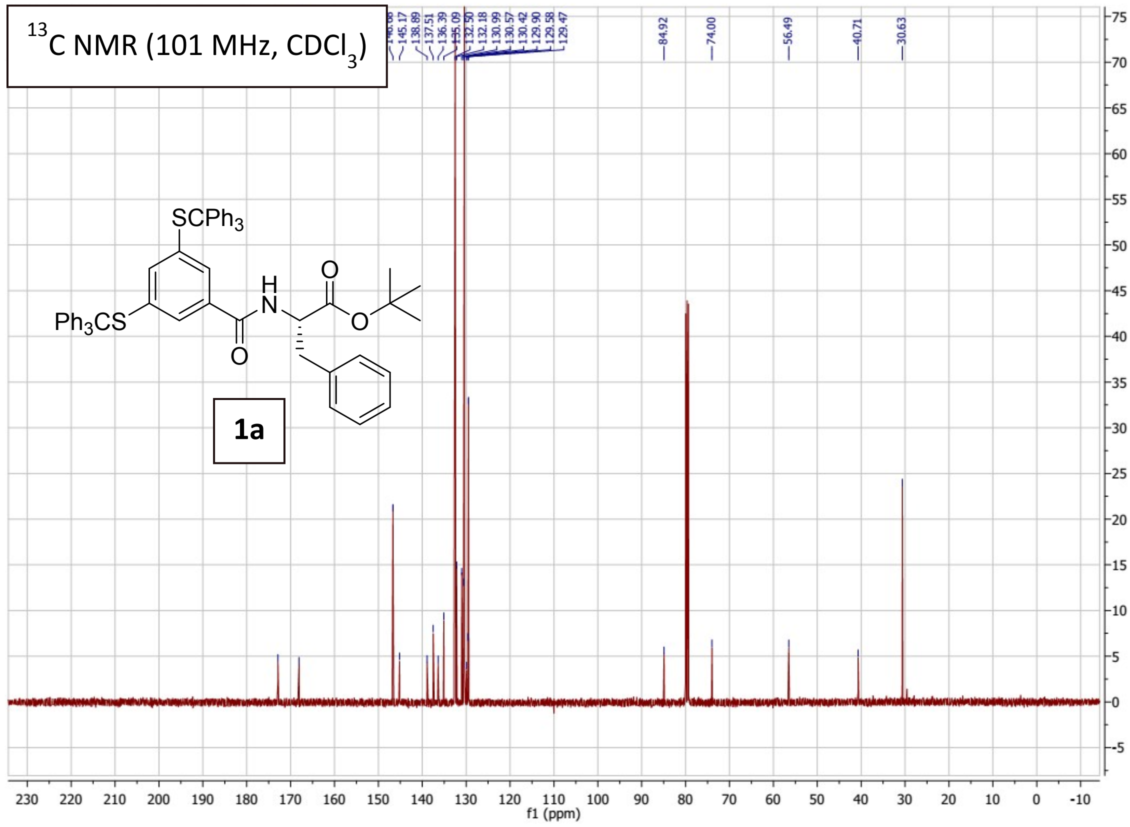
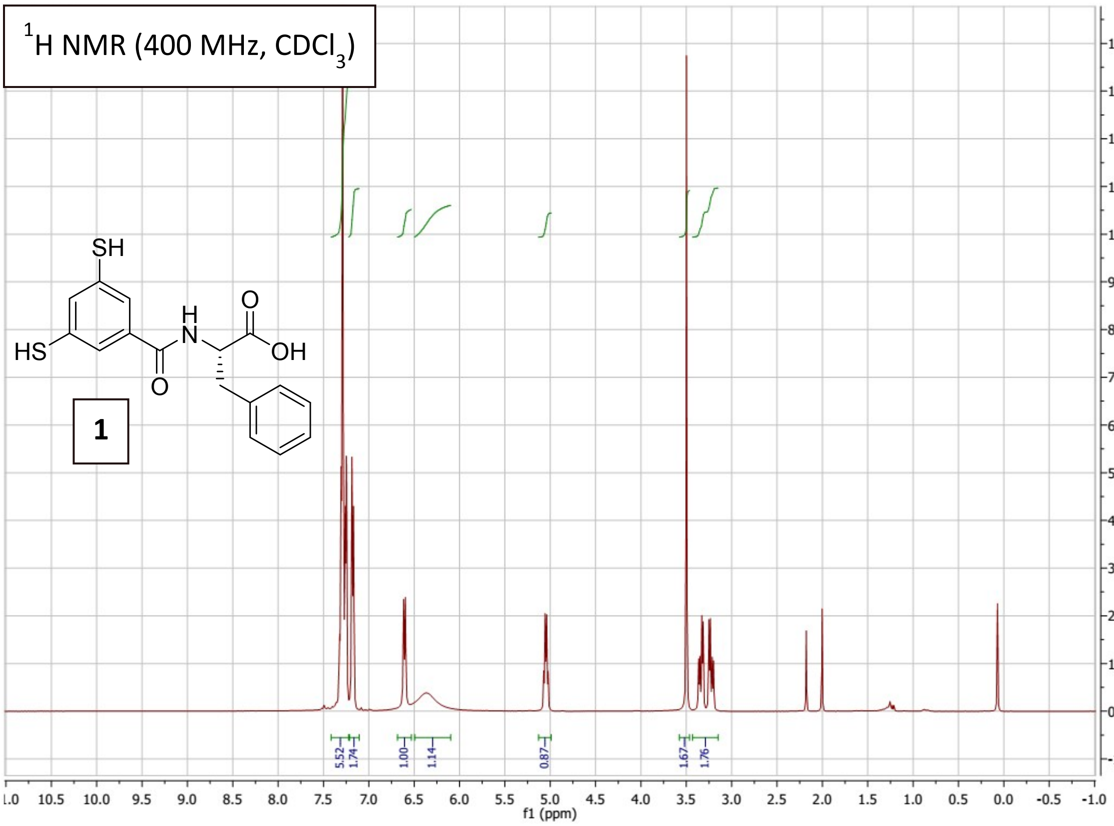


Figure S11. *(a) ESI-MS spectrum and isotopic profile of the UPLC peak at 8.27 min, of a DCL made from building blocks **1** and **2** mixed in a 1 to 1 ratio. (b) Extracted ion chromatogram of m/z = 1052.7115 corresponding to* $[\mathbf{1}_2\mathbf{2}_2\text{+Na}]^{1+}$. *Expected m/z for* $[\mathbf{1}_2\mathbf{2}_2\text{+Na}]^{1+}$ *= 1052.9877. (c) ESI-MS spectrum and isotopic profile of the UPLC peak at 8.61 min. (d) Extracted ion chromatogram of m/z = 868.8594 corresponding to* $[\mathbf{1}_2\mathbf{2}_1\text{+Na}]^{1+}$. *Expected m/z for* $[\mathbf{1}_2\mathbf{2}_1\text{+Na}]^{1+}$ *= 869.0224. (e) ESI-MS spectrum and isotopic profile of the UPLC peak at 9.27 min. (f) Extracted ion chromatogram of m/z = 1199.6610 corresponding to* $[\mathbf{1}_3\mathbf{2}_1\text{+Na}]^{1+}$. *Expected m/z for* $[\mathbf{1}_3\mathbf{2}_1\text{+Na}]^{1+}$ *= 1200.0561. Corresponding UPLC chromatogram shown in Figure S3.*

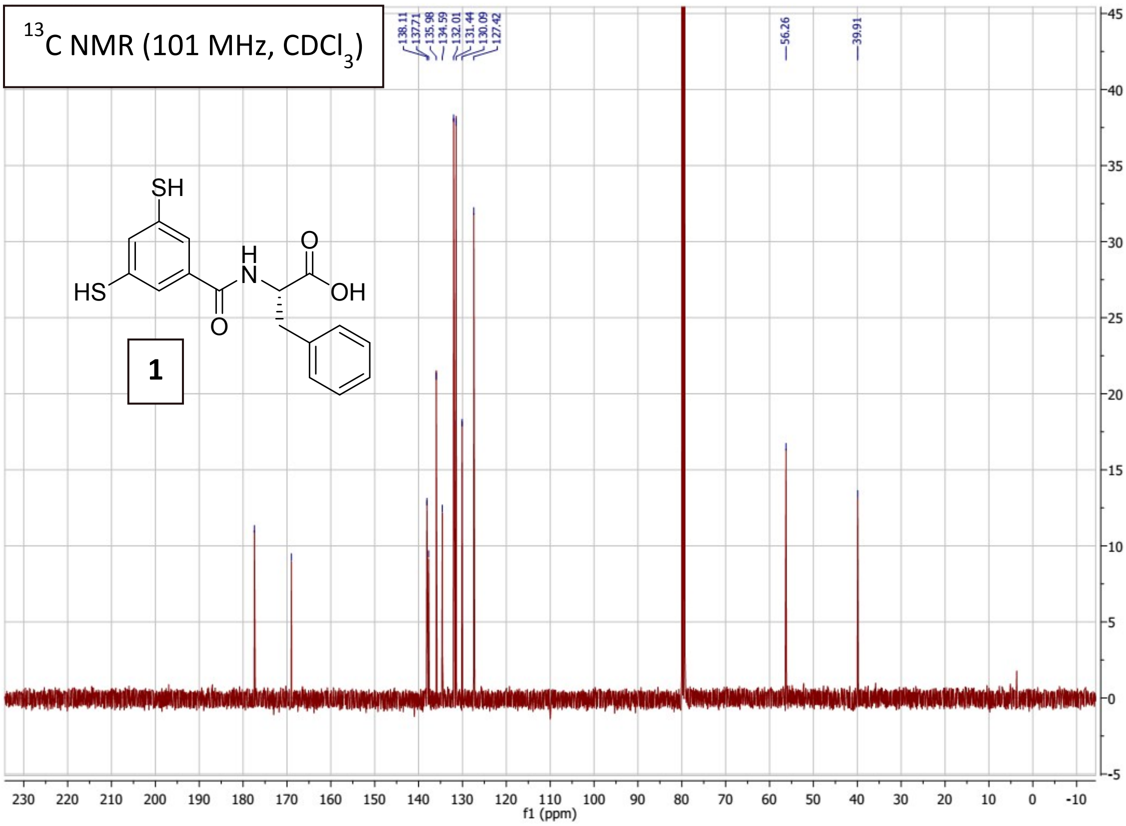
5. NMR spectra







^{1}H NMR (400 MHz, CDCl$_3$)
SH
HS
H
N
O
OH
O
1
5.52
1.74
1.00
1.14
0.87
1.67
1.76
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
-100
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
f1 (ppm)



^{13}C NMR (101 MHz, CDCl$_3$)
138.11
137.71
135.98
134.59
132.01
131.44
130.09
127.42
56.26
39.91
SH
HS
H
N
O
OH
O
1
45
40
35
30
25
20
15
10
5
0
-5
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

6. References

S1 J. M. A. Carnall, C. A. Waudby, A. M. Belenguer, M. C. A. Stuart, J. J. P. Peyralans and S. Otto, *Science*, 2010, **327**, 1502-1506.