Supplementary Materials for

Complex Molecules that Fold like Proteins Can Emerge Spontane-ously

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Contents

1. General procedures

All chemicals, unless otherwise stated, were purchased from Sigma-Aldrich and used as received. Acetonitrile (ULC-MS grade), water (ULC-MS grade) and trifluoroacetic acid (HPLC grade) were purchased from Biosolve BV. Anhydrous solvents used in synthesis 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 or reverse phase silica cartridges. NMR spectra were recorded on 400, 500 and 600 MHz, cryo-NMR spectrometers, locked on deuterated solvents and referenced to the solvent peak. HRMS spectra were recorded on a LTQ Orbitrap XL instrument in ESI mode.

1.1 Library Preparation

Building blocks were dissolved in borate buffer (50 mM, pH 8.2) to prepare a library. All libraries were set up in an HPLC vial (12×32 mm) with a Teflon-coated screw cap. All HPLC vials were equipped with a cylindrical stirrer bar (2×5 mm, Teflon coated, purchased from VWR) and were stirred at 1200 rpm using an IKA RCT basic hot plate stirrer. All experiments were performed at ambient conditions.

1.2 UPLC-MS analysis

UPLC-MS analyses were performed using a Waters Acquity UPLC H-class system coupled to a Waters Xevo-G2 TOF. The mass spectrometer was operated in the positive electrospray ionization mode with the following ionization parameters: capillary voltage: 3 kV; sampling cone voltage: 20 V; extraction cone voltage: 4 V; source gas temperature: 120 °C; desolvation gas temperature: 450 °C; cone gas flow (nitrogen): 1 L/h; desolvation gas flow (nitrogen): 800 L/h.

1.3 TOF/TOF analysis

Sample volumes of 1 µL were applied to a MALDI target plate (stainless steel, polished), and mixed on the plate with 1 µL of matrix solution, containing 5 mg/mL α-cyanohydroxycinnamic

acid in water/acetonitrile 50/50 v/v with 0.1% trifluoroacetic acid. After drying of the spots, positive reflector mode MALDI-TOF spectra were recorded between m/z 825 and 6000 with an UltrafleXtreme MALDI-TOF/TOF mass spectrometer (Bruker Daltonics, Bremen, Germany) operated under Bruker flexControl software (version 3.4); 8000 shots were acquired randomly over the spot.

1.4 UPLC analysis

UPLC analyses were performed on a Waters Acquity H-class system equipped with a PDA detector, at a detection wavelength of 254 nm. Samples were injected on an Aeris WIDEPORE 3.6 μm BEH-C18 (150 × 2.1 mm) column, purchased from Phenomenex, using ULC-MS grade water (eluent A) and ULC-MS grade acetonitrile (eluent B), containing 0.1 V/V % TFA as a modifier. A flow rate of 0.3 mL/min and a column temperature of 35 °C were applied.

Methods for the analysis of DCLs made from building block **1**:

Table S1. Method 1:

t / min	% B
0	10
10	40
12	90
13	90
14.5	10
17	10

Table S2. Method 2:

t / min	% B
0	10
20	40
22	90
27	90
28	10
30	10

Table S3. Method for the analysis of DCLs made from building block **2**:

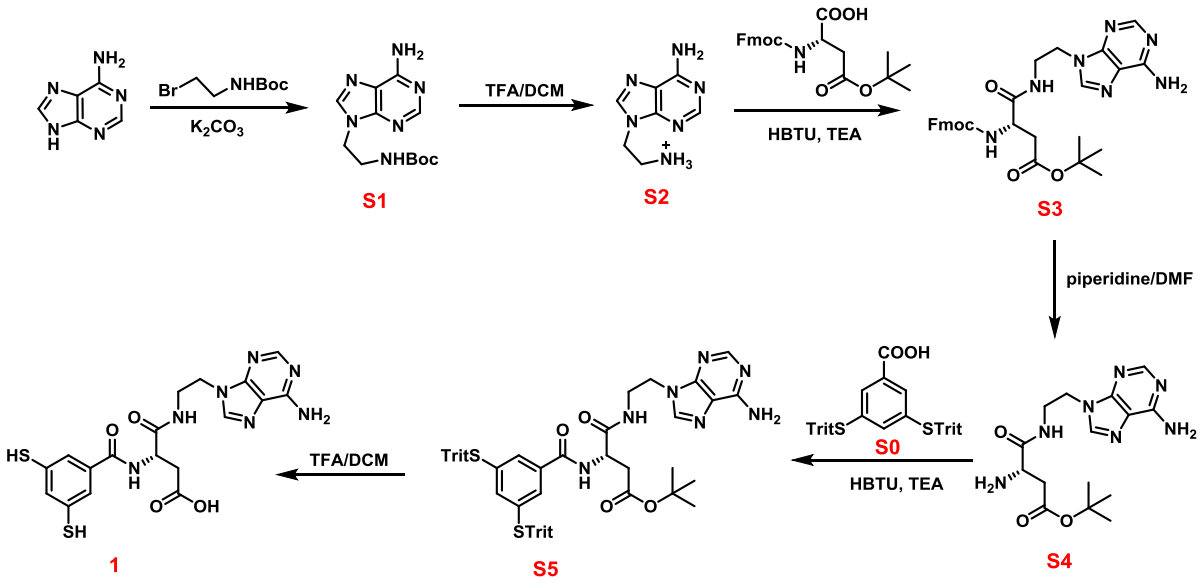
t / min	% B
0	10
2	15
8	20
20	40
22	90
27	90
28	10
30	10

Table S4. Method for the analysis of DCLs made from building block **3**:

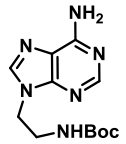
t / min	% B
0	10
1	30
11	45
12	90
13	90
14.5	10
17	10

2. Synthesis and characterization

2.1 Synthesis of building block **1**



S1



tert-Butyl (2-bromoethyl)carbamate (4.2 g, 15 mmol) was added to a dry dimethylformamide (DMF, 120 mL) solution of adenine (2.0 g, 15 mmol) and K_2CO_3 (5.0 g, 36 mmol) and the reaction mixture was stirred at room temperature for 48 hours. Then the solvent was evaporated under vacuum and the crude mixture was purified by flash column chromatography (SiO_2, 0-10% methanol in DCM), followed by removal of solvent to afford product **S1** as a colorless solid.

Yield = 34%, 1.4 g.

^{1}H NMR: (400 MHz, CD_3OD, 298K) δ_H = 1.31 (s, 9H, -Boc), 3.47-3.48 (m, 2H, -CH_2), 4.28-4.29 (m, 2H, -CH_2), 8.02 (s, 1H, adenine H), 8.19 (s, 1H, adenine H). ^{13}C NMR (101 MHz, CD_3OD, 298K) δ_C = 29.9, 42.2, 46.1, 81.5, 121.2, 144.2, 152.1, 154.8, 158.5, 159.4. HRESI-MS calc. for $C_{12}H_{19}N_6O_2^+$ 279.1564, found 279.1593.

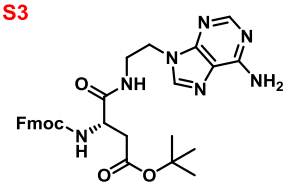
S2

S1 (0.5 g, 1.8 mmol) was dissolved in 10 mL 50% TFA/DCM under a nitrogen atmosphere and the mixture was stirred overnight. After that, the solvents were evaporated under reduced pressure and the crude product was dissolved in methanol and precipitated by addition of diethylether. Colorless solid powder was collected by filtration.

Yield = 98%, 0.49g.

^{1}H NMR: (400 MHz, DMSO-d6, 298K) δ_H = 3.34-3.35 (m, -CH_2, 2H), 4.45 (t, *J* = 6.4 Hz, -CH_2, 2H), 8.09 (br, -NH_3, 3H), 8.35 (s, 1H, adenine H), 8.43 (s, 1H, adenine H), 8.82 (br, -NH_2, 2H). ^{13}C NMR (101 MHz, DMSO-d6, 298K) δ_C = 38.6, 41.9, 118.8, 114.1, 146.3, 149.6, 151.5. HRESI-MS calc. for $C_7H_{11}N_6^+$ 179.1040, found 179.1042.

S3

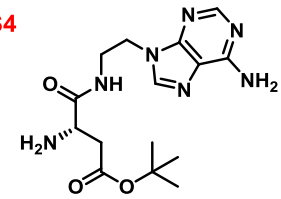


L-Fmoc-aspartic acid alpha-*t*-butylester (411 mg, 1.00 mmol), 2-(*1H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU, 379 mg, 1.00 mmol), **S2** (292 mg, 1.00 mmol) and triethylamine (0.30 mL, 2.15 mmol) were dissolved in 10 mL dry acetonitrile and the mixture was stirred at room temperature overnight under a nitrogen atmosphere. Solvent was evaporated under vacuum and the crude mixture was purified by flash column chromatography (SiO_2, 0-10% methanol in DCM), followed by removal of solvent to afford **S3** as a white powder.

Yield = 58%, 330 mg.

^{1}H NMR: (400 MHz, DMSO-d6, 298K) δ_H = 1.33 (s, 9H, -$(CH_3)_3$), 2.38 (dd, J_1 = 16 Hz, J_2 = 9.6 Hz, 1H, -CH_2), 2.59 (dd, J_1 = 16 Hz, J_2 = 4.8 Hz, 1H, -CH_2), 3.38-3.51 (m, 2H, -CH_2), 4.16-4.33 (m, 6H), 7.18 (s, 2H, NH_2), 7.30 (t, *J* = 7.6 Hz, 2H, ArH), 7.40 (t, *J* = 7.6 Hz, 2H, ArH), 7.61 (d, *J* = 8.4 Hz, 1H, NH), 7.70 (t, *J* = 6.4 Hz, 2H, ArH), 7.86 (d, *J* = 7.6 Hz, 2H, ArH), 8.00 (s, 1H, adenine H), 8.12 (s, 1H, adenine H), 8.16 (t, *J* = 6.0 Hz, 1H, -CONH). ^{13}C NMR (101 MHz, DMSO-d6, 298K) δ_C = 27.8, 37.5, 42.6, 46.1, 46.7, 51.6, 65.8, 80.2, 118.8, 120.2, 125.34, 125.36, 127.1, 127.8, 140.8, 141.1, 143.8, 143.9, 149.7, 152.4, 155.8, 156.0, 169.5, 170.8. HRESI-MS calc. for $C_{30}H_{34}N_7O_5^+$ 572.2616, found 572.2608.

S4

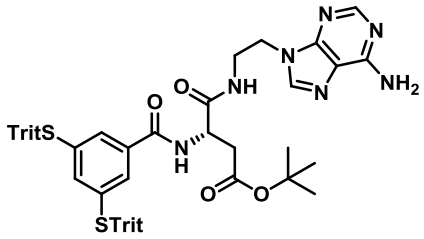


S3 (571 mg, 1.00 mmol) was dissolved in 20 mL 20% piperdine in DMF (20:80/piperidine:DMF) and stirred for 0.5 h at room temperature. Then the solvents were evaporated under vacuum and the crude powder was washed with hexane (30 mL x 3) to afford derivative **S4** as a slightly yellow powder.

Yield = 63%, 220 mg.

^{1}H NMR: (400 MHz, CD_3OD, 298K) δ_H = 1.43 (s, 9H, -$(CH_3)_3$), 2.45 (dd, J_1 = 16.8 Hz, J_2 = 7.2 Hz, 1H, -CH_2), 2.56 (dd, J_1 = 16.4 Hz, J_2 = 5.2 Hz, 1H, -CH_2), 3.51 (dd, J_1 = 6.8 Hz, J_2 = 5.2 Hz, 1H, -CH), 3.64 (dd, J_1 = 6.8 Hz, J_2 = 5.2 Hz, 2H, -CH_2), 4.34 (t, J = 5.6 Hz, 2H, -CH_2), 8.12 (s, 1H, adenine H), 8.21 (s, 1H, adenine H). ^{13}C NMR (101 MHz, CD_3OD, 298K) δ_C = 29.6, 41.5, 42.2, 45.6, 54.1, 83.5, 121.3, 144.3, 152.1, 154.9, 158.5, 173.4, 177.7. HRESI-MS calc. for $C_{15}H_{24}N_7O_3^+$ 350.1935, found 350.1929.

S5

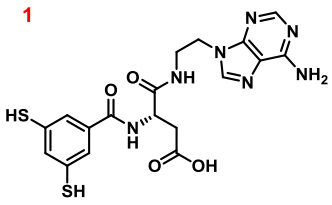


S0 (670 mg, 1.00 mmol), 2-(*1H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU, 379 mg, 1.00 mmol), **S4** (350 mg, 1.00 mmol) and triethylamine (0.30 mL, 2.15 mmol) were dissolved in 10 mL dry DMF and the mixture was stirred at room temperature overnight under nitrogen atmosphere. Solvent was evaporated under vacuum and the crude mixture was purified by flash column chromatography (SiO_2, 0-10% methanol in DCM), followed by removal of solvent to afford **S5** as a white powder.

Yield = 67%, 670 mg.

^{1}H NMR: (400 MHz, DMSO-d6, 298K) δ_H = 1.30 (s, 9H, -$(CH_3)_3$), 2.43 (dd, J_1 = 15.6 Hz, J_2 = 8.8 Hz, 1H, -CH_2), 2.57 (dd, J_1 = 16 Hz, J_2 = 5.6 Hz, 1H, -CH_2), 3.35-3.43 (m, 1H, -CH_2), 3.51-3.56 (m, 1H, -CH_2), 4.20-4.24 (m, 2H, -CH_2), 4.53 (ddd, J_1 = 13.6 Hz, J_2 = 8.4 Hz, J_3 = 4.8 Hz, 1H, -CH), 6.62 (s, 1H, ArH), 7.17-7.23 (m, 32H, ArH), 8.07 (t, J = 6.0 Hz, 1H, NH), 8.13 (d, J = 8.0 Hz, 1H, NH), 8.21 (s, 1H, adenine H), 8.27 (s, 1H, adenine H), 8.85 (br, 2H, -NH_2). ^{13}C NMR (101 MHz, DMSO-d6, 298K) δ_C = 30.8, 40.3, 46.2, 48.9, 53.2, 74.0, 83.2, 121.5, 130.0, 131.0, 132.4, 135.8, 136.5, 136.8, 144.0, 146.0, 146.6, 151.0, 152.2, 155.5, 168.2, 172.4, 173.6. HRESI-MS calc. for $C_{60}H_{56}N_7O_4S_2^+$ 1002.3830, found 1002.3848.

1



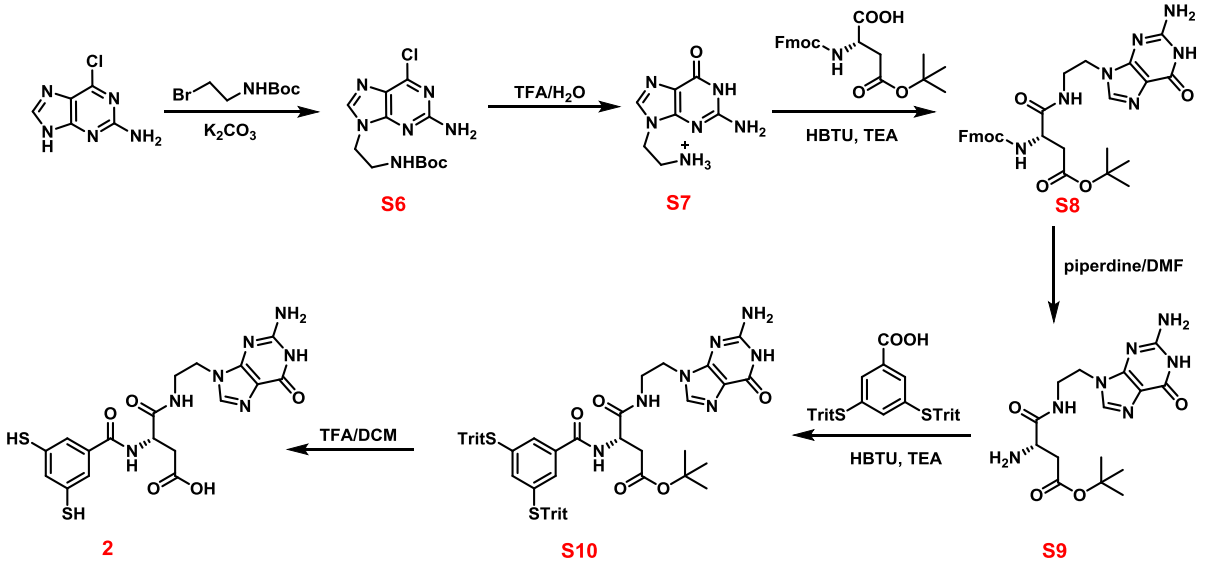
S5 (100 mg, 0.10 mmol) was dissolved in 10 mL 50% TFA in DCM and stirred for 12 h at room temperature under N_2. Et_3SiH (0.50 mL, 3.1 mmol) was added to the reaction mixture and further stirred for another hour. Solvents were evaporated under vacuum and the crude

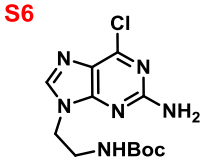
mixture was washed with hexane (10 mL x 2). The product was purified by reverse phase flash column chromatograph (RP C18, 0-90% acetonitrile in water with 0.1% TFA), and the desired product **1** was obtained after lyophilization as a white powder.

Yield = 35%, 16 mg.

^{1}H NMR: (400 MHz, CD_3OD, 298K) δ_H = 2.80 (dd, J_1 = 16.8 Hz, J_2 = 8.0 Hz, 1H, -CH_2), 2.91 (dd, J_1 = 16.8 Hz, J_2 = 8.0 Hz, 1H, -CH_2), 3.75-3.78 (m, 2H, -CH_2), 4.49 (dd, J_1 = 9.6 Hz, J_2 = 4.8 Hz, 2H, -CH_2), 4.78-4.81 (m, 1H, -CH), 7.46-7.47 (m, 1H, ArH), 7.53-7.54 (m, 2H, ArH), 8.39 (s, 1H, adenine H), 8.40 (s, 1H, adenine H). ^{13}C NMR (101 MHz, CD_3OD, 298K) δ_C = 36.4, 40.2, 45.0, 52.0, 119.7, 125.2, 132.0, 135.3, 136.4, 145.5, 145.8, 150.7, 151.9, 168.6, 173.6, 173.9. HRESI-MS calc. for $C_{18}H_{18}N_7O_4S_2^-$ 460.0862, found 460.0850.

2.2 Synthesis of building block **2**

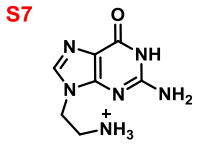




The procedure for the synthesis of **S6** was the same as that described for the synthesis of **S1.**

Yield = 35%, 1.64 g.

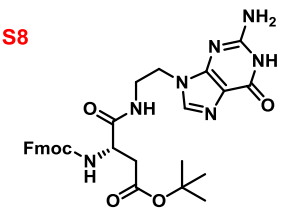
^{1}H NMR: (400 MHz, DMSO-d6, 298K) δ_H = 1.29 (s, 9H, Boc), 3.29-3,33 (m, 2H, -CH_2), 4.06 (t, *J* = 5.9 Hz, 2H, -CH_2), 6.84 (s, 2H, -NH_2), 6.93 (t, *J* = 5.2 Hz, 1H, -CONH), 7.95 (s, 1H, guanine H). ^{13}C NMR (101 MHz, DMSO-d6, 298K) δ_C = 21.6, 22.2, 28.1, 43.1, 77.9, 123.5, 143.3, 149.1, 154.3, 155.5, 159.7. HRESI-MS calc. for $C_{12}H_{18}ClN_6O_2^+$ 313.1174, found 313.1177.



S6 (0.50 g, 1.6 mmol) was dissolved in 10 mL 50% TFA/water under a nitrogen atmosphere and the mixture was stirred for 24 h. After that, the solvents were evaporated under reduced pressure and the crude product was precipitated by addition of diethylether. The white powder was collected by filtration. Yield = 98%, 0.46 g.

^{1}H NMR: (400 MHz, DMSO-d6, 298K) δ_H = 3.32 (q, *J* = 5.5 Hz, 2H, -CH_2), 4.32 (t, *J* = 5.7 Hz, 2H, -CH_2), 7.09 (s, 2H, -NH_2), 8.28 (s, 3H, -NH_3^+), 8.42 (s, 1H, guanine H), 11.46 (s, 1H, guanine NH). ^{13}C NMR (101 MHz, DMSO-d6, 298K) δ_C = 37.8, 42.0, 112.1, 137.7, 150.6, 154.8, 155.2. HRESI-MS calc. for $C_7H_{11}N_6O^+$ 195.0989, found 195.0989.

S8

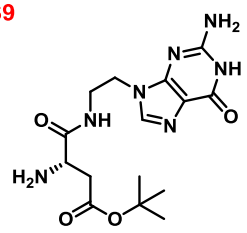


The procedure for the synthesis of **S8** was the same as that described for the synthesis of **S3**.

Yield = 43%, 0.25 g.

^{1}H NMR: (400 MHz, DMSO-d6, 298K) δH = 1.31 (s, 9H, -$(CH_3)_3$), 2.41 (dd, J_1 = 16.0 Hz, J_2 = 9.5 Hz, 1H, -CH_2), 2.59 (dd, J_1 = 16.0 Hz, J_2 = 4.9 Hz, 1H, -CH_2), 3.28-3.49 (m, 2H, -CH_2), 3.98 (t, J = 5.7 Hz, 2H, -CH_2), 4.16-4.36 (m, 4H, -CH&CH_2), 6.50 (s, 2H, -NH_2), 7.29 (t, J = 7.5 Hz, 2H, ArH), 7.39 (t, J = 7.5 Hz, 2H, ArH), 7.57 (s, 1H, guanine H), 7.61 (d, J = 8.3 Hz, 1H, -CONH), 7.69 (t, J = 6.4 Hz, 2H, ArH), 7.86 (d, J = 7.6 Hz, 2H, ArH), 8.11 (s, 1H, -CONH), 10.62 (s, 1H, guanine NH). ^{13}C NMR (101 MHz, DMSO-d6, 298K) δ_C = 27.7, 37.5, 42.2, 45.6, 46.6, 51.6, 65.8, 80.2, 116.5, 120.1, 125.3, 127.1, 127.7, 137.7, 140.7, 143.7, 143.9, 151.2, 153.6, 155.8, 156.8, 169.4, 170.9. HRESI-MS calc. for $C_{30}H_{34}N_7O_6^+$ 588.2565, found 588.2565.

S9

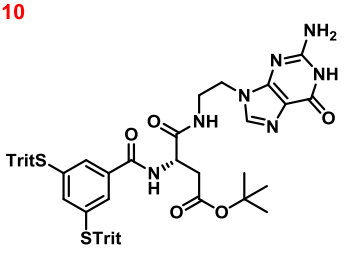


The procedure for the synthesis of **S9** was the same as that described for the synthesis of **S4**.

Yield = 67%, 0.24 g.

^{1}H NMR: (400 MHz, DMSO-d6, 298K) δ_H = 1.39 (s, 9H, -$(CH_3)_3$), 2.68 (qd, J_1 = 17.6 Hz, J_2 = 6.2 Hz, 2H, -CH_2), 3.42 (dd, J_1 = 13.9 Hz, J_2 = 5.5 Hz, 1H, -CH_2), 3.51 (dq, J_1 = 11.7 Hz, J_2 = 5.9 Hz, 1H, -CH_2), 3.95 (dd, J_1 = 8.1 Hz, J_2 = 4.5 Hz, 1H, -CH), 4.02 (t, J = 5.6 Hz, 2H, -CH_2), 6.59 (s, 2H, -NH_2), 7.77 (s, 1H, guanine H), 8.19 (s, 2H, NH_2), 8.57 (t, J = 5.8 Hz, 1H, -CONH), 10.80 (s, 1H, guanine NH). ^{13}C NMR (101 MHz, DMSO-d6, 298K) δ_C = 27.7, 36.1, 42.4, 48.8, 64.9, 81.6, 115.6, 137.6, 151.2, 15.9, 156.5, 167.9, 168.6. HRESI-MS calc. for $C_{15}H_{24}N_7O_4^+$ 366.1884, found 366.1890.

S10



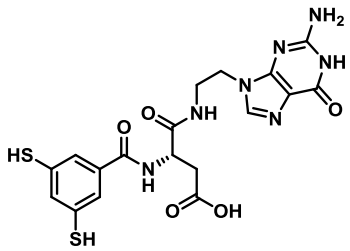
The procedure for the synthesis of **S10** was the same as that described for the synthesis of **S5.**

Yield = 54%, 0.55 g.

^{1}H NMR: (400 MHz, DMSO-d6, 298K) δ_H = 1.30 (s, 9H, -$(CH_3)_3$), 2.46-2.51 (m, 1H, -CH_2), 2.61 (dd, J_1 = 16.0 Hz, J_2 = 4.8 Hz, 1H, -CH_2), 3.32-3.40 (m, 2H, -CH_2), 3.96 (t, J = 5.8 Hz, 2H, -CH_2), 4.61-4.55 (m, 1H, -CH), 6.44 (s, 2H, -NH_2), 6.60 (s, 1H, ArH), 7.14-7.24 (m, 32H, ArH), 7.57 (s, 1H, guanine H), 8.07 (t,

J = 5.8 Hz, 1H, CONH), 8.16 (d, J = 8.1 Hz, 1H, CONH), 10.55 (s, 1H, guanine NH). ^{13}C NMR (101 MHz, DMSO-d6, 298K) δ_C = 27.7, 37.3, 42.2, 50.2, 70.9, 80.1, 126.9, 127.8, 129.3, 132.7, 133.4, 133.7, 137.6, 140.9, 143.5, 151.1, 153.5, 156.7, 164.1, 169.3, 170.5. HRESI-MS calc. for $C_{60}H_{56}N_7O_5S_2^+$ 1018.3779, found 1018.3844.

2

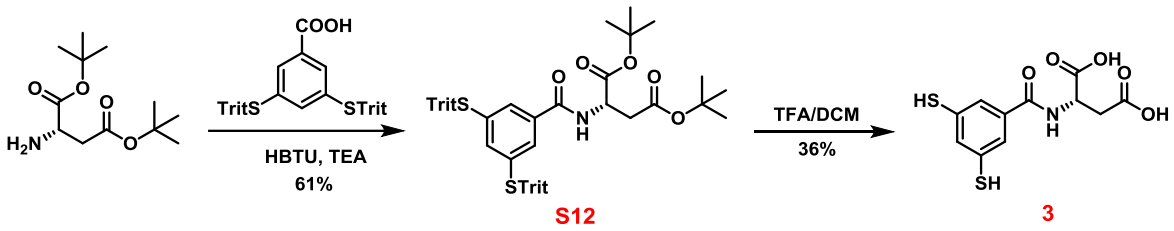


The procedure for the synthesis of **2** was the same as that described for the synthesis of **1**.

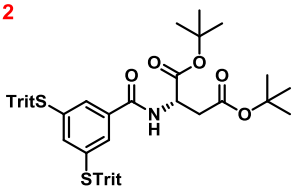
Yield = 34%, 16 mg.

^{1}H NMR: (400 MHz, CD_3OD, 298K) δ_H = 2.77 (dd, J_1 = 16.8 Hz, J_2 = 7.8 Hz, 1H, -CH_2), 2.89 (dd, J_1 = 16.8 Hz, J_2 = 5.9 Hz, 1H, -CH_2), 3.64 (t, J = 5.5 Hz, 2H, -CH_2), 4.28 (t, J = 5.5 Hz, 2H, -CH_2), 4.74 (dd, J_1 = 7.8, J_2 = 5.9 Hz, 1H, -CH), 7.35 (t, J = 1.7 Hz, 1H, ArH), 7.44 (d, J = 1.7 Hz, 2H, ArH), 8.46 (s, 1H, guanine H). No ^{13}C NMR could be obtained due to the poor solubility of **2**. HRESI-MS calc. for $C_{18}H_{20}N_7O_5S_2^+$ 478.0962, found 478.0959.

2.3 Synthesis of building block **3**



S12

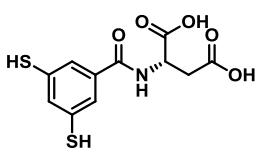


S0 (670 mg, 1.00 mmol), 2-(*1H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU, 379 mg, 1.00 mmol), *L*-aspartic acid di-*tert*-butyl ester hydrochloride (281 mg, 1.00 mmol) and triethylamine (0.30 mL, 2.15 mmol) were dissolved in 10 mL dry DMF and the mixture was stirred at room temperature overnight under N_2. The solvent was evaporated under vacuum and the crude mixture was purified by flash column chromatography (SiO_2, 0-10% methanol in DCM), followed by removal of solvent to afford derivative **S12** as a white powder.

Yield = 61%, 547 mg.

^{1}H NMR: (400 MHz, $CDCl_3$, 298K) δ_H = 1.45 (s, 9H, -$(CH_3)_3$), 1.47 (s, 9H, -$(CH_3)_3$), 2.67 (dd, J_1 = 16.8 Hz, J_2 = 4.4 Hz, 1H, -CH_2), 2.83 (dd, J_1 = 17.2 Hz, J_2 = 4.8 Hz, 1H, -CH_2), 4.65-4.69 (m, 1H, -CH), 6.33 (d, J = 8.0 Hz, 1H, -CONH), 6.92 (d, J = 1.6 Hz, 2H, ArH), 7.06 (t, J = 1.7 Hz, 1H, ArH), 7.14-7.22 (m, 18H, ArH), 7.28-7.35 (m, 12H, ArH). ^{13}C NMR (101 MHz, $CDCl_3$, 298K) δ_C = 29.6, 30.6, 30.8, 40.2, 52.1, 74.1, 83.9, 84.8, 129.5, 130.4, 130.6, 132.5, 135.4, 136.3, 137.4, 145.5, 146.7, 168.2, 172.1, 172.8. HRESI-MS calc. for $C_{57}H_{56}NO_5S_2^+$ 898.3594, found 898.3587.

3

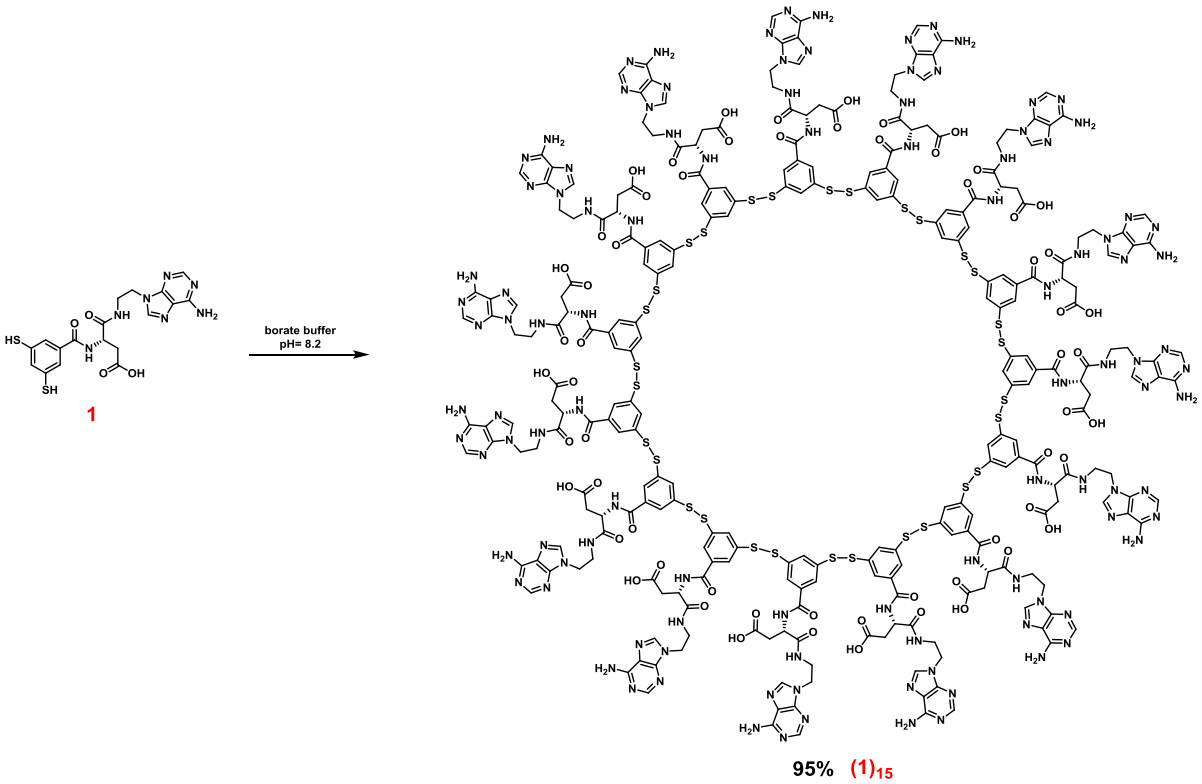


The procedure for the synthesis of **3** was the same as that described for the synthesis of **1**.

Yield = 36%, 12 mg.

^{1}H NMR: (400 MHz, CD_3OD, 298K) δ_H = 2.88 (dd, J_1 = 16.7 Hz, J_2 = 7.5 Hz, 1H, -CH_2), 2.98 (dd, J_1 = 16.7 Hz, J_2 = 5.3 Hz, 1H, -CH_2), 4.92 (dt, J_1 = 7.5 Hz, J_2 = 5.1 Hz, 1H, -CH), 7.37 (t, J = 1.8 Hz, 1H, ArH), 7.46 (d, J = 1.7 Hz, 2H, ArH). ^{13}C NMR (101 MHz, CD_3OD, 298K) δ_C = 37.9, 52.2, 126.4, 133.2, 136.6, 137.9, 175.2, 175.3. HRESI-MS calc. for $C_{11}H_{12}NO_5S_2^+$ 302.0151, found 302.0147.

2.4 Synthesis of foldamer $(\mathbf{1})_{15}$



Building block **1** (50 mg) was dissolved at a 2.0 mM concentration in borate buffer (50 mM, pH=8.2) containing 1.0 M NaCl. The reaction mixture was stirred at room temperature under air and the library was monitored by UPLC. After all of monomer was consumed, the product was purified by reverse phase flash column chromatograph (RP C18, 0-90% acetonitrile in water with 0.1% TFA), and the desired foldamer $(\mathbf{1})_{15}$ was obtained after lyophilization as a white powder. Yield = 94%, 47 mg.

^{1}H NMR: (500 MHz, D_2O, 298K) δ_H = 2.35 (d, *J* = 13.2 Hz, 1H, -CH_2), 2.64 (d, *J* = 17.2 Hz, 1H, -CH_2), 2.74 (d, *J* = 16.3 Hz, 2H, -CH_2), 2.81-2.94 (m, 4H, -CH_2), 3.04-3.21 (m, 2H, -CH_2), 3.46 (d, *J* = 11.5 Hz, 1H, -CH_2), 3.85 (dd, J_1 = 33.0, J_2 = 13.8 Hz, 2H, -CH_2), 3.93-3.98 (m, 3H, -CH_2), 4.13 (s, 1H, -CH), 4.52 (s, 2H, -CH_2), 4.82 (s, 1H, -CH), 5.06 (s, 1H, -CH), 5.72 (s, 1H, ArH), 6.37 (s, 1H, ArH), 6.85 (s, 2H, ArH), 7.05 (s, 1H, ArH), 7.11 (s, 1H, ArH), 7.12 (s, 2H, ArH & adenien H), 7.36 (s, 1H, ArH), 7.45 (s, 1H, ArH), 8.07 (s, 1H, adenine H), 8.13 (s, 1H, adenine H), 8.31 (s, 1H, adenine H), 8.33 (s, 1H, adenine H), 8.42 (s, 1H, adenine H), 8.92 (s, 2H, -CONH), 9.04 (d, *J* = 7.6 Hz, 1H, -CONH).

3. Kinetic profile of the library made from building block 1

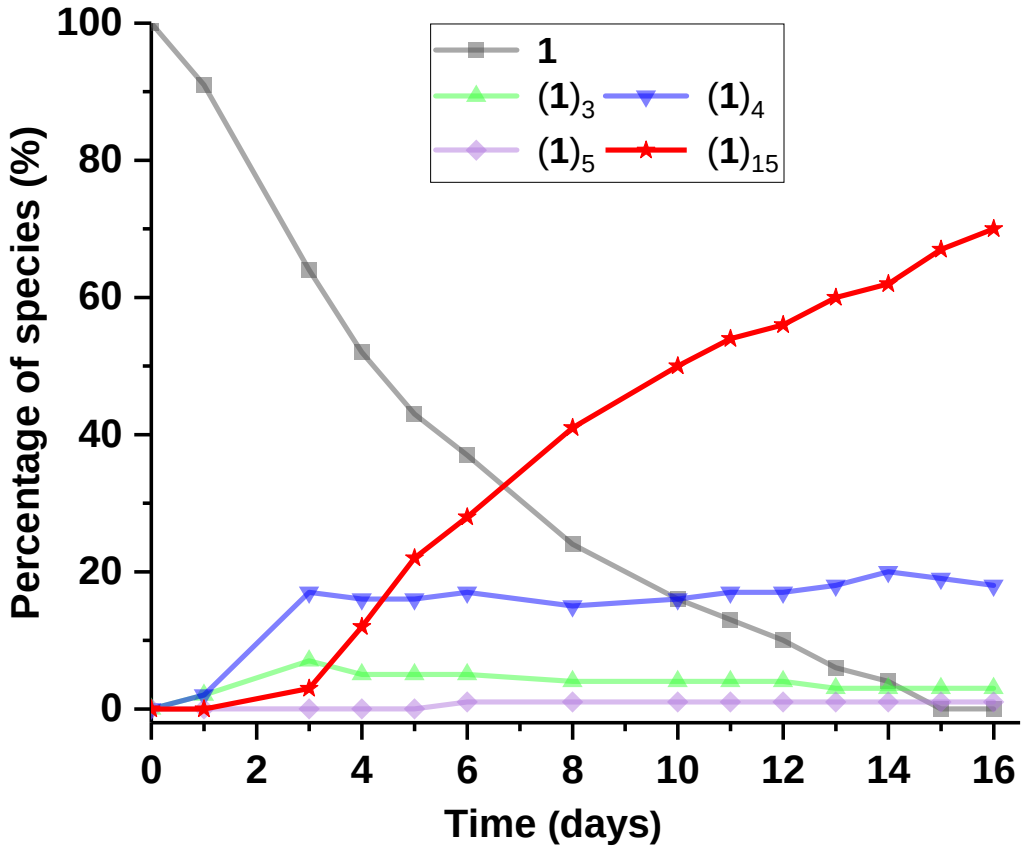


Fig. S1. Kinetic profile of the library made from 1.0 mM building block **1** in borate buffer (pH = 8.2, 50 mM) under continuous stirring.

4. Total peak area of the library made from building block 1

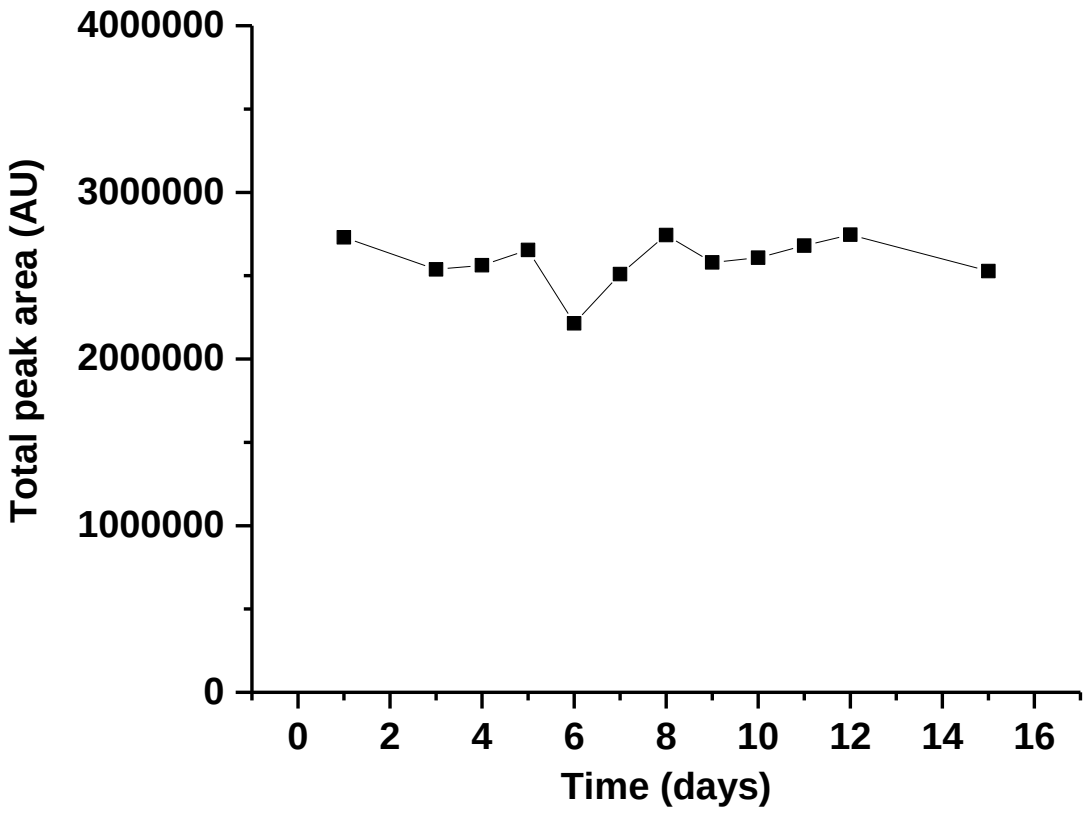


Fig. S2. Total UPLC peak area of library made from building block **1** monitoring the library shown in Fig. S1.

5. Effect of concentration of building block 1 on the product distribution

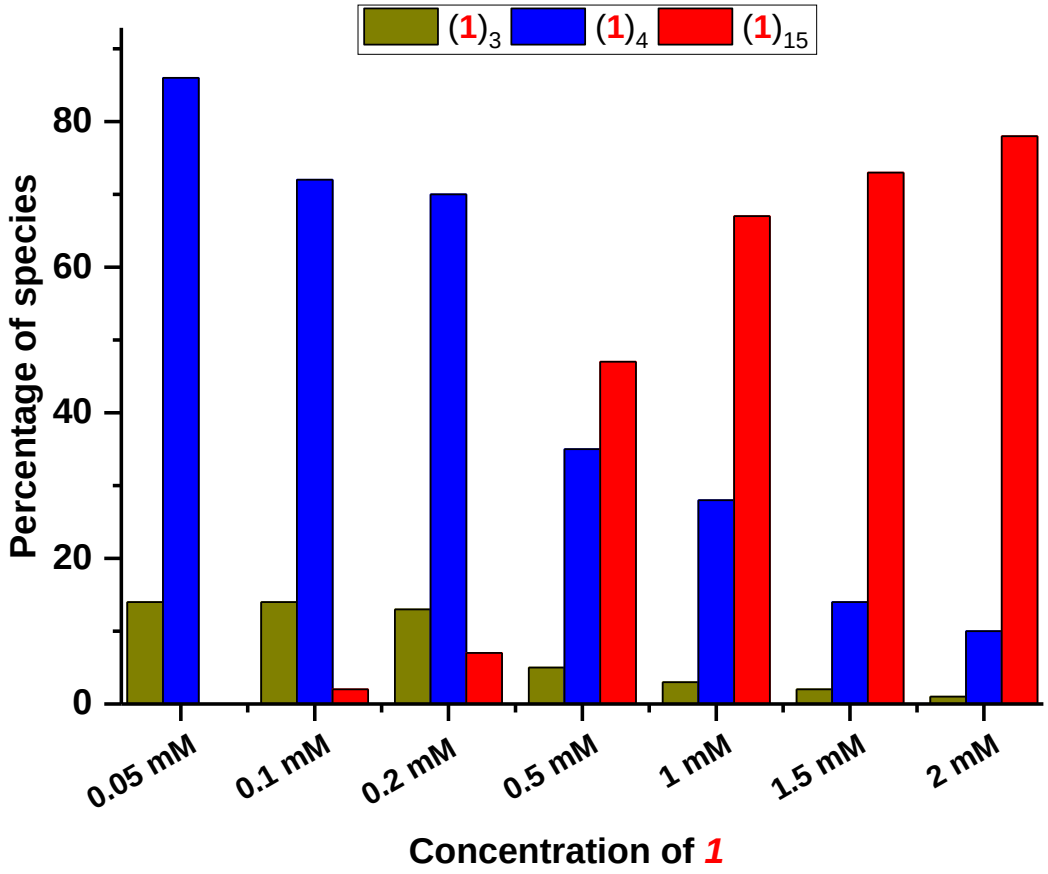
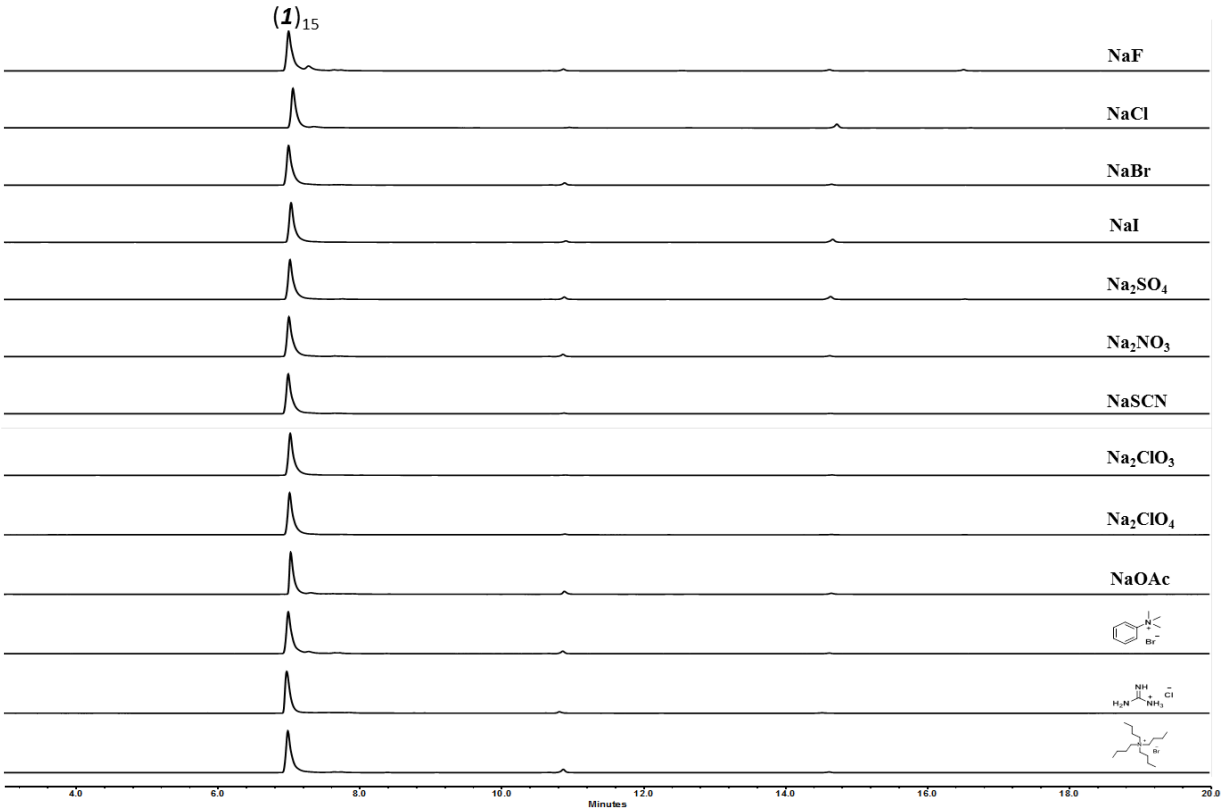


Fig. S3. Effect of the concentration of building block **1** on the product distribution. Libraries were prepared by dissolving different amounts of building block **1** in borate buffer (pH = 8.2, 50 mM). The distribution of the products was monitored by UPLC after the libraries had reached equilibrium.

6. Effect of salts on the formation of foldamer $(1)_{15}$



0.5mM ***1***, Borate buffer (50mM, pH=8.2) + 1M Salt

Fig. S4. Effect of salts on the formation of foldamer $(\mathbf{1})_{15}$. The libraries were prepared by dissolving building block **1** (0.50 mM) in borate buffer containing 1.0 M of a specific salt. After the libraries had reached equilibrium the distribution of the products was monitored by UPLC.

7. Effect of co-solvents on product distribution of the library made from building block 1

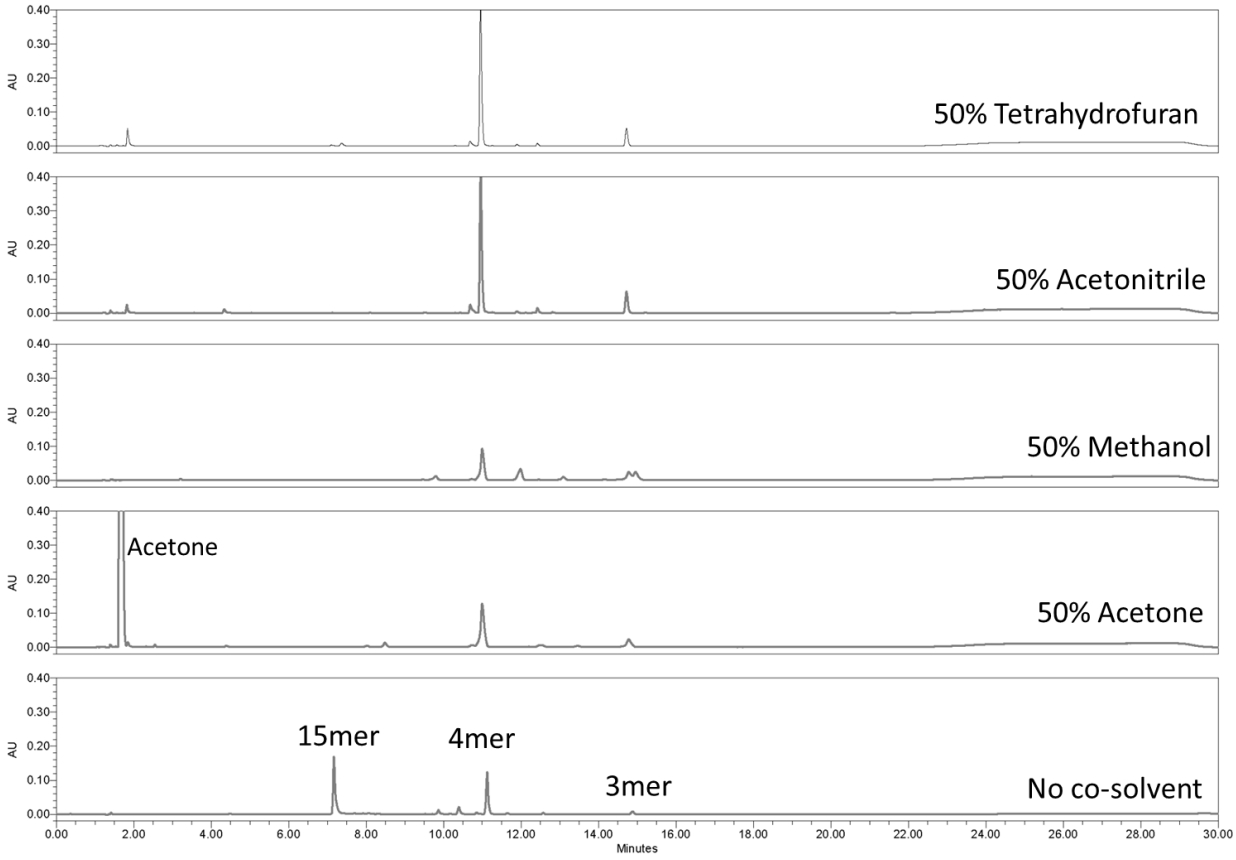


Fig. S5. Effect of co-solvents on prodcut distribution of the library made from building block **1**. The libraries were prepared by dissolving building block **1** (0.50 mM) in borate buffer with 50% (volume) organic co-solvent. UPLC spectra from bottom to the top: No co-solvents, 50% acetone, 50% methanol, 50% acetonitrile and 50% tetrahydrofuran.

8. Reversibility test of library composition of the library made from building block 1

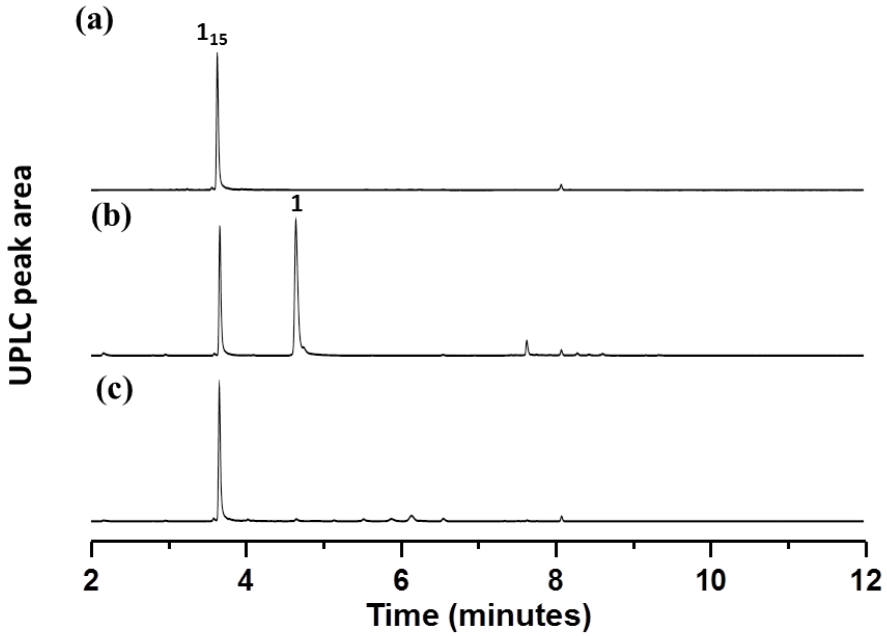


Fig. S6. Reversible formation of 15mer upon reduction and oxidation. UPLC analyses of the product distributions of a library made from building block **1** in borate buffer (pH= 8.2, 50 mM) at 2.0 mM concentration: (a) after reaching equilibrium; (b) upon adding 60 mol% DTT and (c) upon re-oxidation using 60% $NaBO_3$.

9. TOF/TOF analysis of foldamer $(1)_{15}$

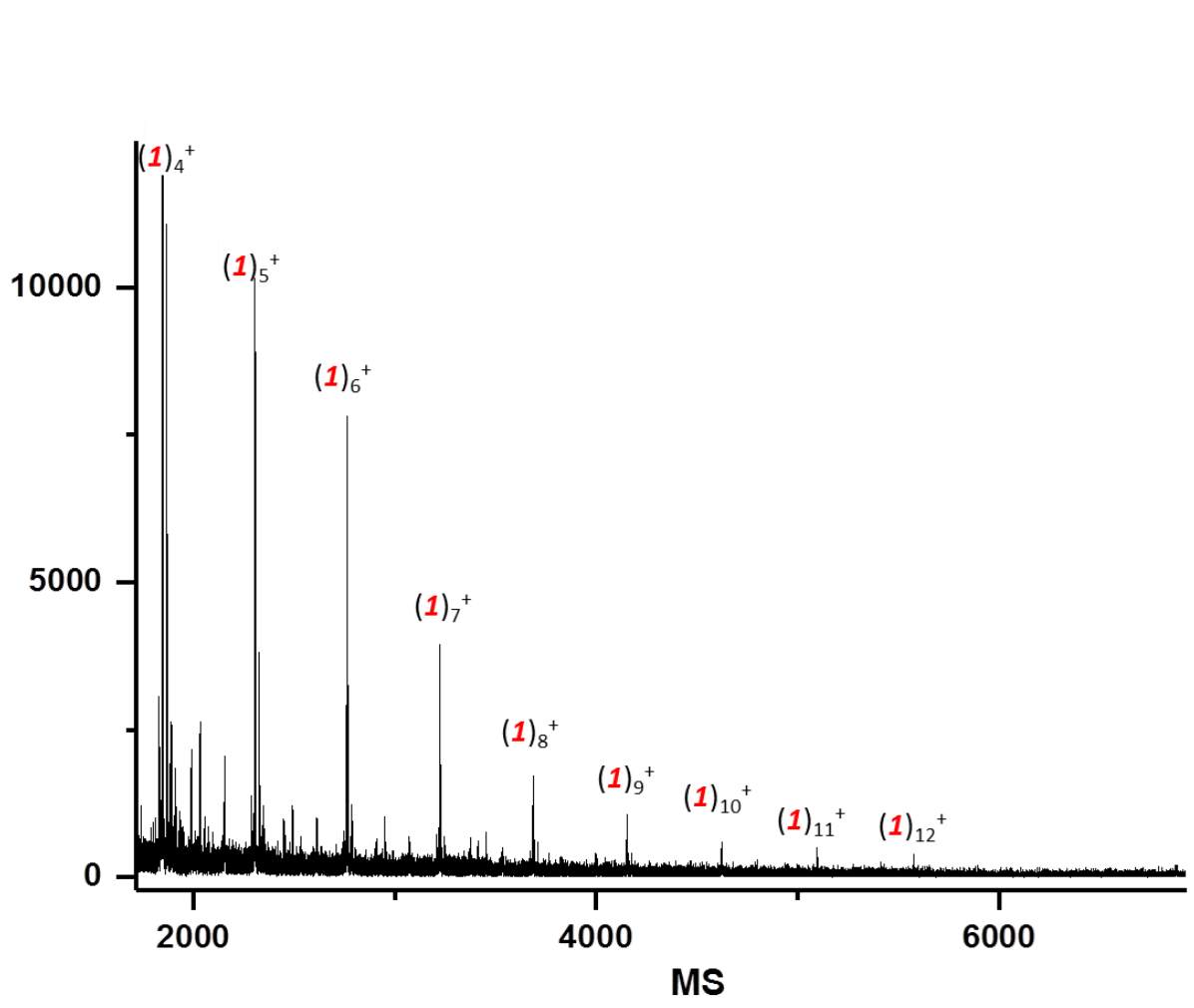


Fig. S7. TOF/TOF analysis of foldamer $(\mathbf{1})_{15}$.

10. Proton NMR assignment of foldamer $(1)_{15}$

The assignment of the proton NMR was based on 2D NMR and the crystal structure of the foldamer.

Experimental details: A 5-8 mg sample of $(\mathbf{1})_{15}$, was dissolved in 0.7 mL of the deuterated solvent (D_2O, H_2O/D_2O 90/10 v/v, DMF-d_7, D_2O/acetone-d_6) in a 5 mm NMR tube. A Bruker Avance III (14 T) spectrometer operating at 600 MHz for ^1H and 150 MHz for ^{13}C NMR spectra was applied. 1D proton spectra were collected at 298 K, except for the variable temperature experiments which were performed over the 273-363 K and 253-373 K temperature ranges for D_2O and DMF-d_7 solutions, respectively. 2D correlation experiments (COSY, NOESY, ROESY, HSQC, and HMBC) were acquired and processed by means of standard software of Bruker library. $^1H,^1H$ COSY, phase-sensitive NOESY, and ROESY spectra were collected over a 5 kHz spectral window with a 2048 x 512 digital resolution of the t_2 x t_1 matrix. Typically, 16 (COSY), 24 (NOESY), and 32 (ROESY) transitions were acquired for each point of the t_1 domains. The same resolutions were applied for $^1H,^{13}C$ heteronuclear experiments collecting 32 or 64 scans for HSQC or HMBC, respectively. Gradient methods were applied for homo- and heteronuclear correlation experiments, except for that of the ROESY. NOESY experiments were conducted with gradient pulses in mixing time of 300 ms. Water suppression pulses were included to the 1D and 2D experiments performed for the solution in H_2O/D_2O.

Details of proton assignment: Proton 1'' is the only proton which is in close contact with two protons belonging to other aryl rings (3 and 1'), in the crystal structure and the NOESY NMR shows the corresponding cross-peaks.

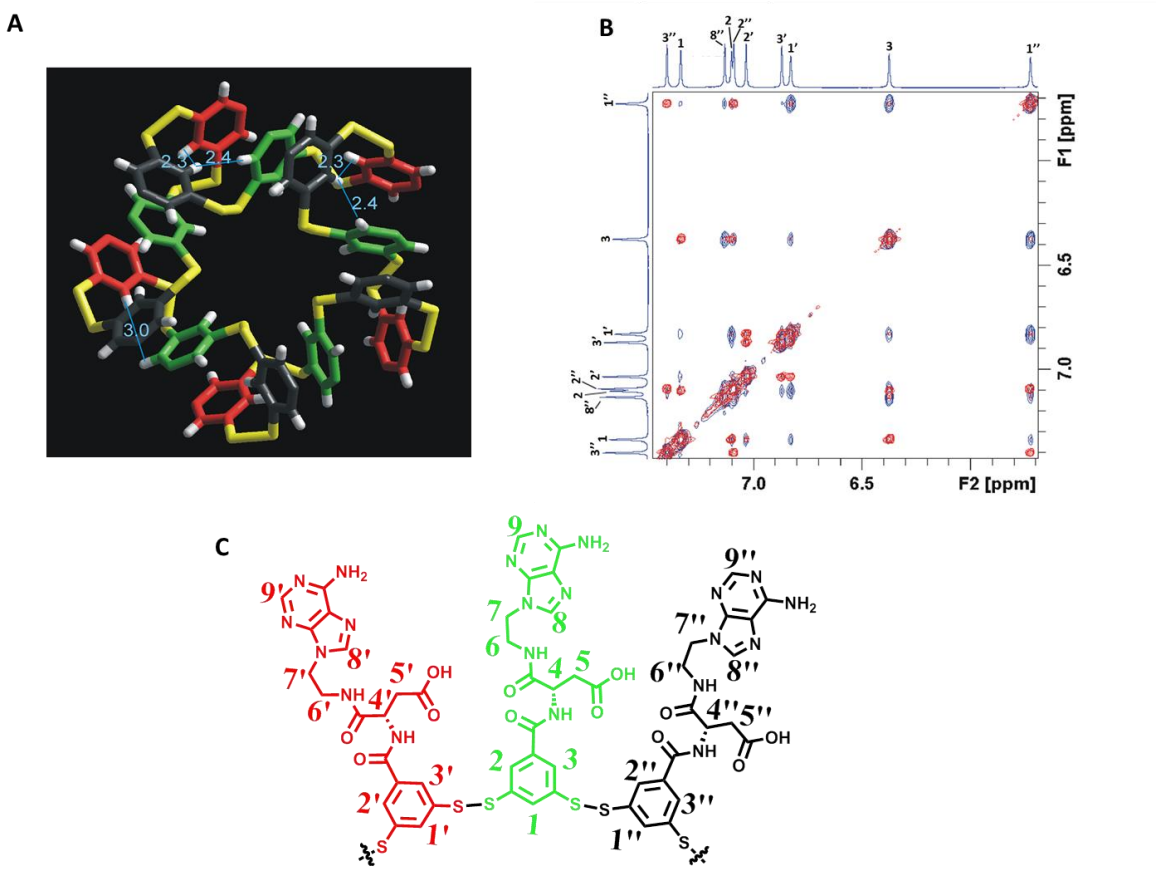


Fig. S8. (A) Core part of the foldamer indicating the distance between protons as observed in the crystal structure. (B) Superimposed COSY (red) and NOESY (blue) spectra (600 MHz, 290 K, D_2O). (C) Repeating unit of $(\mathbf{1})_{15}$ colored as in Fig. S8A.

Assignments of adenine signals to A8/A9 protons were based on the ^{1}H, ^{13}C HMBC experiment: In the structure of adenine, only A8 protons give rise to cross-peaks due to correlations with C-A5 (least shifted on the map) in HMBC spectrum (blue spots). Protons A9 and A8 correlate (pairwise) with the same carbon (C-A4) in the HMBC spectrum (about 150 ppm), and the A9 protons correlate with the C-A6 (most shifted carbons). Taken together, these cross-peaks allowed us to unequivocally assign the proton signals of the adenines.

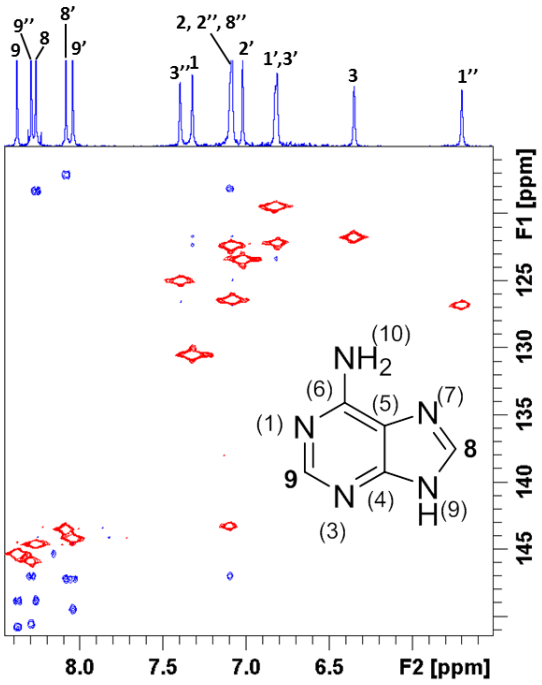


Fig. S9. Part of the HMBC map (blue) and the superimposed HSQC map (red) of $(\mathbf{1})_{15}$ showing the assignment of protons 8 and 9 (D_2O, 298 K).

Distinction between 2 (or 3) and 1 was made on the basis of ^{1}H, ^{13}C-HMBC: Only H2's and H3's give rise to cross-peaks in HMBC with amide C=O carbons. That allowed the assignments of proton signals to either position 1 or 2/3 in each of the aryl rings.

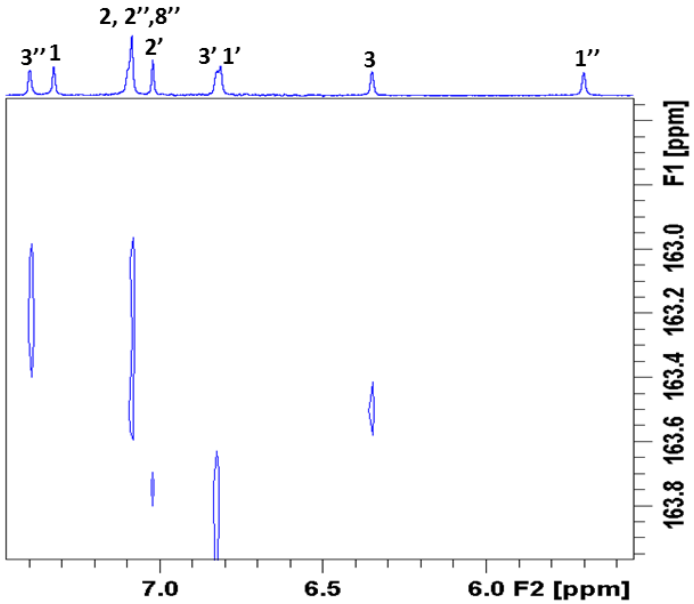


Fig. S10. Part of the HMBC map of $(\mathbf{1})_{15}$ showing the contact between protons 2, 3 and amide C=O.

Assignments of ethylene-bridge protons (6 and 7) were made on the basis of discrete NOE correlations with aryl protons at various conformations available in the structural model. Assignments of these protons to a specific carbon of ethylene was made on the basis of $^{1}H,^{13}C$ HSQC spectra (diastereotopic methylene proton signals, except 6 and 7, correlate with the same carbon). In most cases, the correlations observed in the NOESY map for 6 and 7 protons were with aryl protons of a different monomer unit (different color). Strong correlations of ethylene protons were also observed with adenine 8 protons with the same monomer unit.

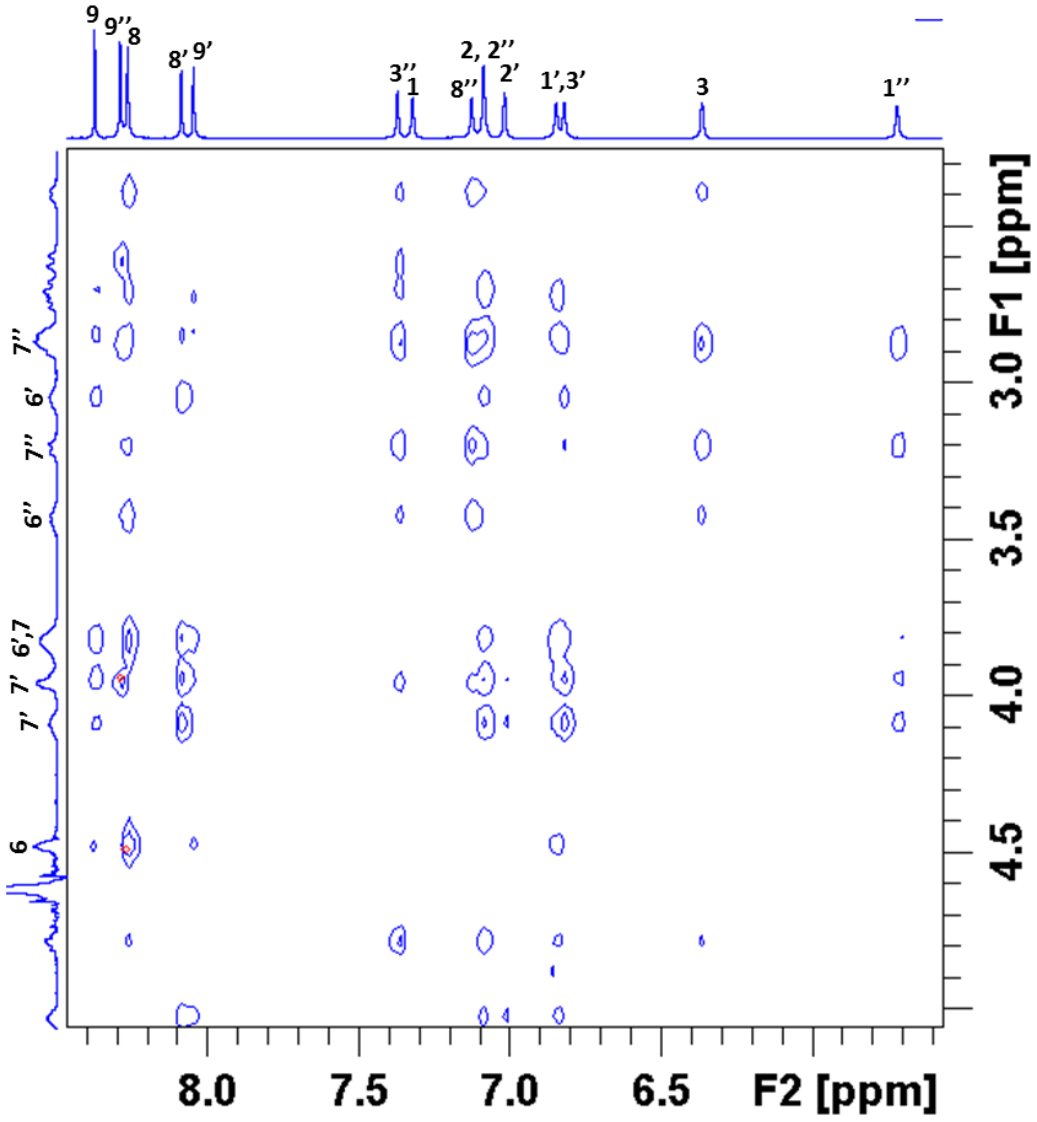


Fig. S11. Parts of the NOESY maps (600 MHz, D_2O, 290 K) of $(\mathbf{1})_{15}$ showing the contact between protons (6 and 7) and aromatic protons.

The Asn 4 and 5 protons (diastereotopic, strongly overlapping with each other) were assigned by various contacts with either ethylene-linker protons 6, 7 or adenine proton 8. One NH of Asn appeared to be most deeply buried inside the foldamer and, thus, exchanged only slowly with D_2O. Indeed, its signal correlates with that of proton 4 in the COSY spectrum. The other NH's are either exchanged completely, or do not correlate with proton 4, neither in COSY nor NOESY experiments, even when they are recorded in H_2O solution. Although the α-β-β'-triads were assigned for each subunit, attempts to do it on the basis of H-C correlations failed, despite several approaches, due to lack of any correlations between α-protons with amide C=O in the HMBC spectrum.

11. Variable temperature ^{1}H-NMR of foldamer $(1)_{15}$

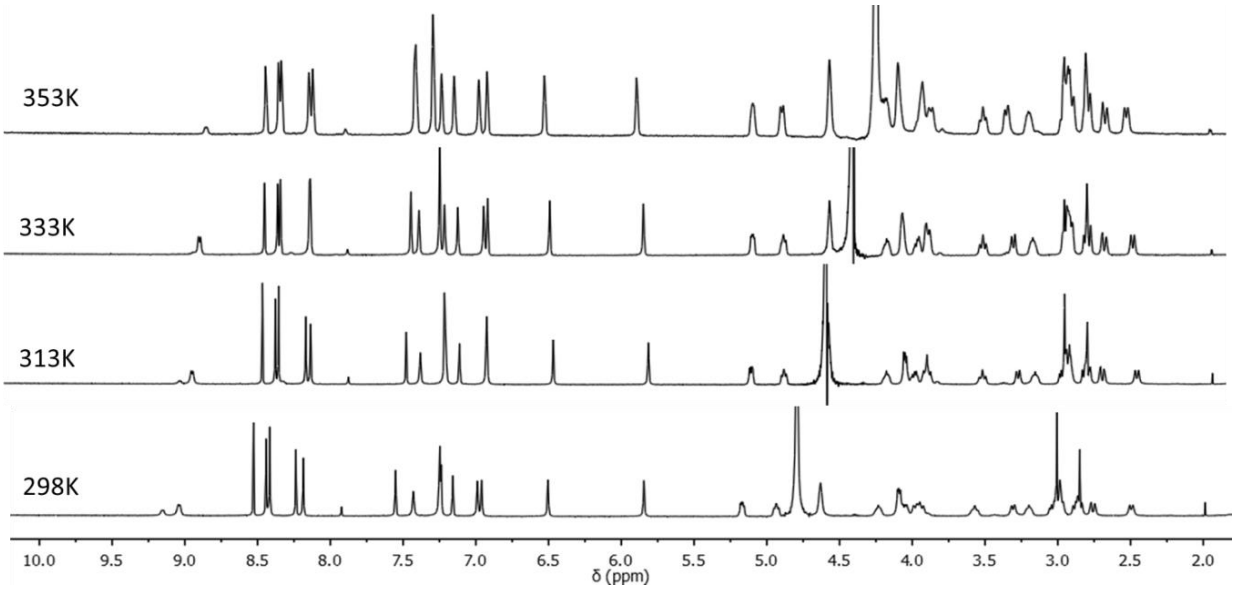


Fig. S12. Variable temperature ^{1}H-NMR spectra of foldamer $(\mathbf{1})_{15}$ in D_2O at 353 K, 333 K, 313 K and 298 K (600 MHz).

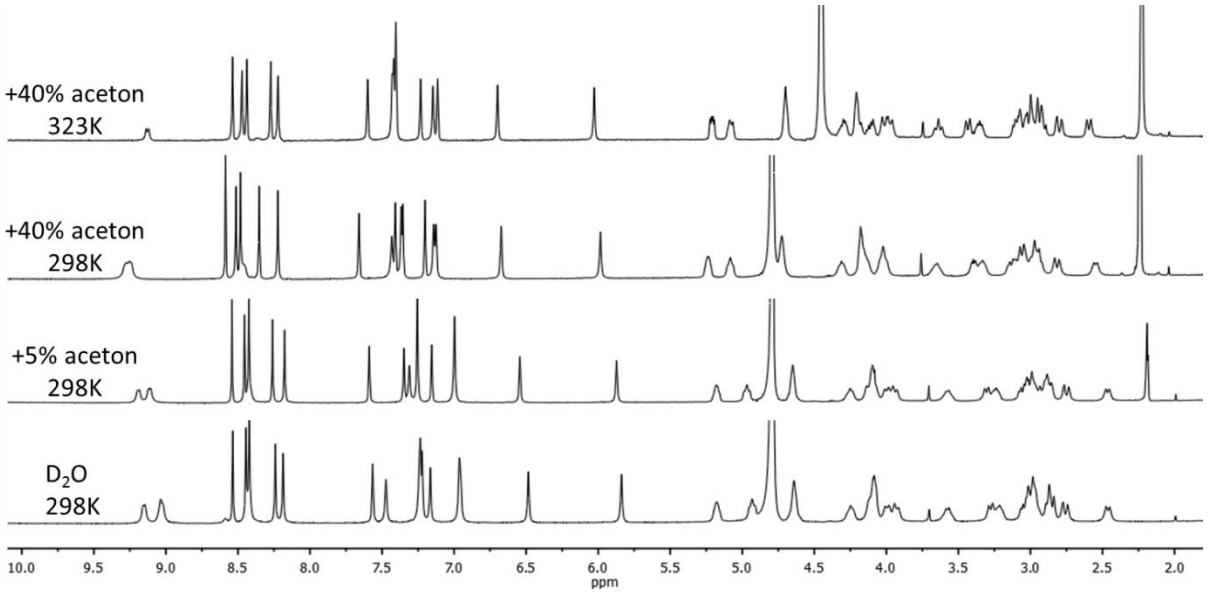


Fig. S13. Variable temperature ^{1}H-NMR spectra of the foldamer $(\mathbf{1})_{15}$ in D_2O at 323 K with 40% acetone; 298 K with 40% acetone; 298 K with 5% acetone and 298 K in D_2O (500 MHz).

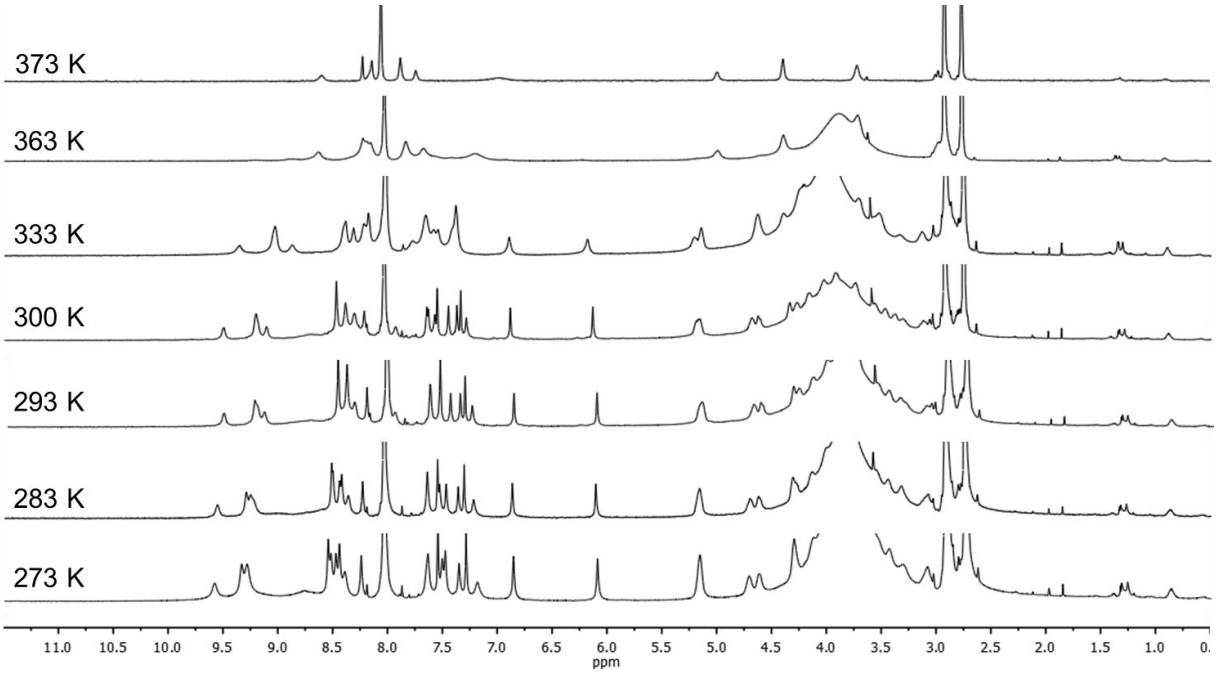


Fig. S14. Variable temperature ^{1}H-NMR spectra of the foldamer $(\mathbf{1})_{15}$ in DMF-d_7 at 373 K, 363 K, 333 K, 300 K, 293 K, 283 K and 273 K (600 MHz).

12. CD spectra of foldamer $(1)_{15}$

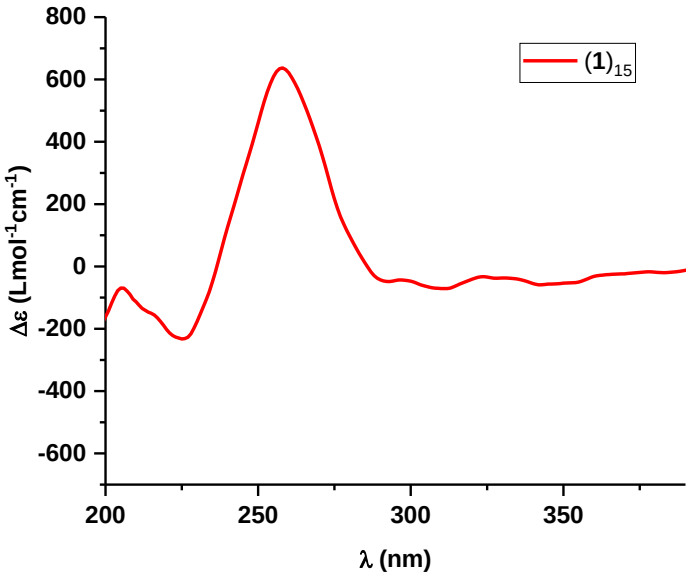


Fig. S15. CD spectrum of foldamer $(\mathbf{1})_{15}$ in water at 298 K.

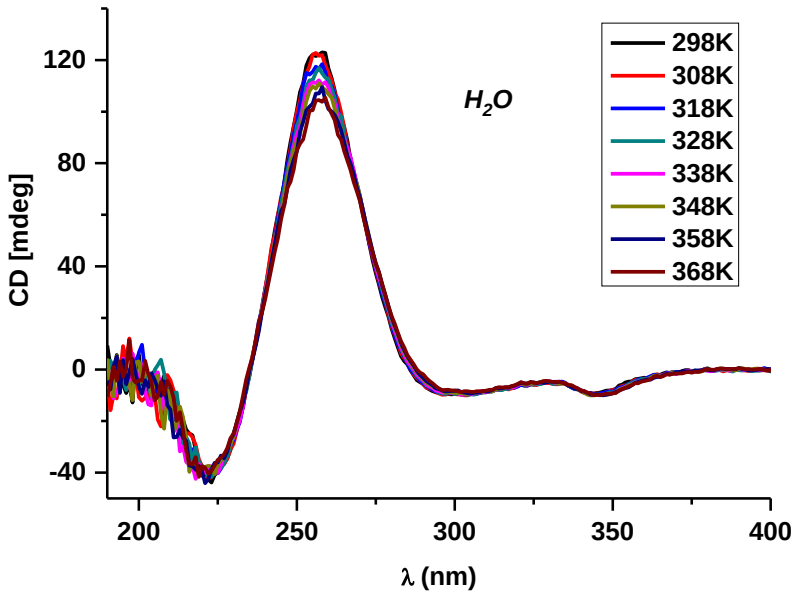


Fig. S16. CD spectra of foldamer $(\mathbf{1})_{15}$ in water at different temperatures.

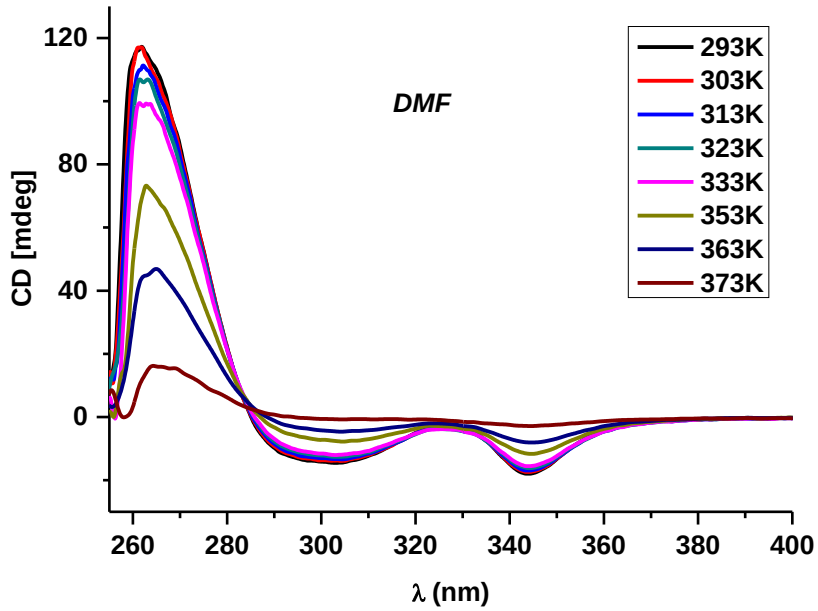


Fig. S17. CD spectra of foldamer $(\mathbf{1})_{15}$ in DMF at different temperatures.

13. Details of single crystal structure of $(1)_{15}$

Single crystals were obtained by slow diffusion of acetone into a water solution of foldamer $(\mathbf{1})_{15}$. Data collection of foldamer was performed at the X-ray diffraction beamline (XRD1) of the Elettra Synchrotron of Trieste (Italy), with a Pilatus 2M image plate detector. Complete dataset was collected at 100 K (nitrogen stream supplied by an Oxford Cryostream 700) with a monochromatic wavelength of 0.700 Å, respectively, through the rotating crystal method. The crystal was dipped in N-paratone and mounted on the goniometer head with a nylon loop. The diffraction data were indexed, integrated and scaled using XDS.[1] The structure was solved by direct methods using SIR2014[2] and subsequent Fourier analysis and refinements with the full-matrix least-squares method based on F^2 were performed with SHELXL.[3] Anisotropic thermal motion was allowed for all non-H atoms, except for the oxygens of lattice water molecules. Hydrogen atoms were placed at calculated positions and no H atoms were assigned to water molecules. The unit cell presents almost 39.7% of void, likely occupied by highly disordered solvent molecules, and the program Squeeze was applied to the data set to take into account this fact. Graphics were drawn with program Diamond.[4]

Crystal data: $C_{270}H_{240}N_{105}O_{60}S_{30}.27.5(H_2O)$, M = 7381.46, monoclinic, space group $P\ 2_12_12$, a = 100.02(2), b = 18.644(4), c = 29.230(6) Å, V = 54507(19) Å^3, Z = 4, D_c = 0.892 g/cm^3, μ(Mo-Kα) = 0.142 mm^{-1}, F(000) = 15108, θ range = 0.69 to 19.55°. Final $R1$ = 0.1336, $wR2$ = 0.3475, S = 1.253 for 4839 parameters and 89211 reflections, 47051 unique [R(int) = 0.0270], of which 22953 with $I > 2\sigma(I)$, max positive and negative peaks in ΔF map 0.449 and -0.457 e. Å^{-3}.

14. Product distribution of the library made from building block 2

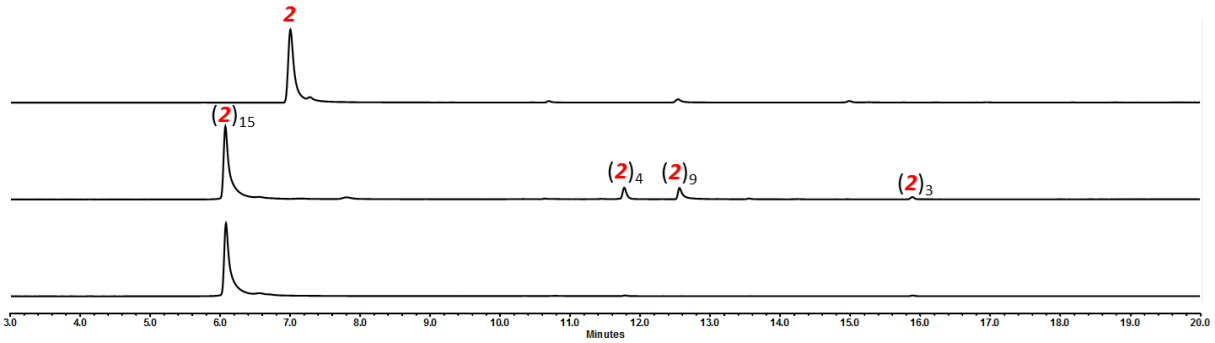


Fig. S18. UPLC analyses (absorption at 254 nm) of the product distribution of a library made from building block **2** in borate buffer (pH=8.2, 50 mM) at 1.0 mM concentration: (**a**) immediately after dissolving building block **2**; (**b**) after stirring for 7days (**c**) after stirring for 7 days in the presence of 1.0 M NaCl.

15. Product distribution of the library made from building block 3

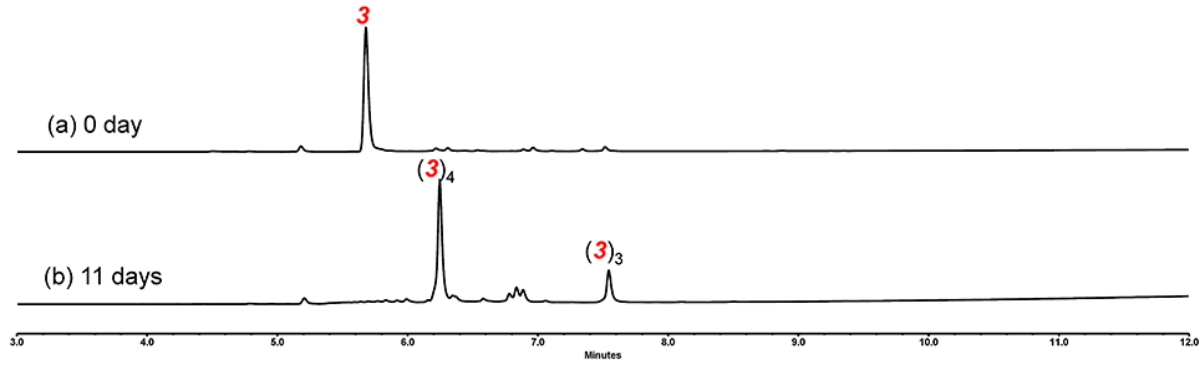


Fig. S19. UPLC analyses (absorption at 254 nm) of the product distribution of a library made from building block **3** in borate buffer (pH=8.2, 50 mM) at 1.0 mM concentration: (**a**) immediately after dissolving building block **3**; (**b**) after stirring for 11 days.

16. Mass spectrometry analysis

16.1 Mass spectrometry analysis of the library made from **1**

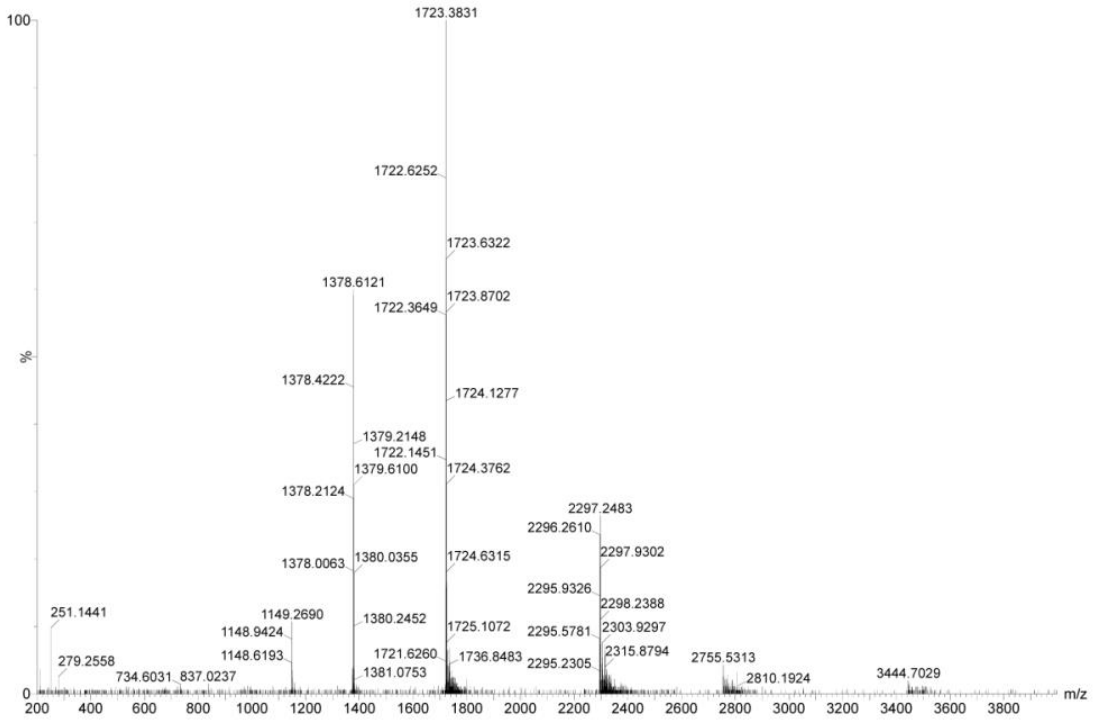


Fig. S20. Mass spectrum of $(\mathbf{1})_{15}$ (retention time 5.6 min in Fig. 1B) from the LC-MS analysis of a DCL made from **1**. m/z calculated: 2297.23 $[M+3H]^{3+}$, 1723.55 $[M+4H]^{4+}$, 1379.04 $[M+5H]^{5+}$, 1149.37 $[M+6H]^{6+}$; m/z observed: 2297.25 $[M+3H]^{3+}$, 1723.38 $[M+4H]^{4+}$, 1378.61 $[M+5H]^{5+}$, 1149.27 $[M+6H]^{6+}$.

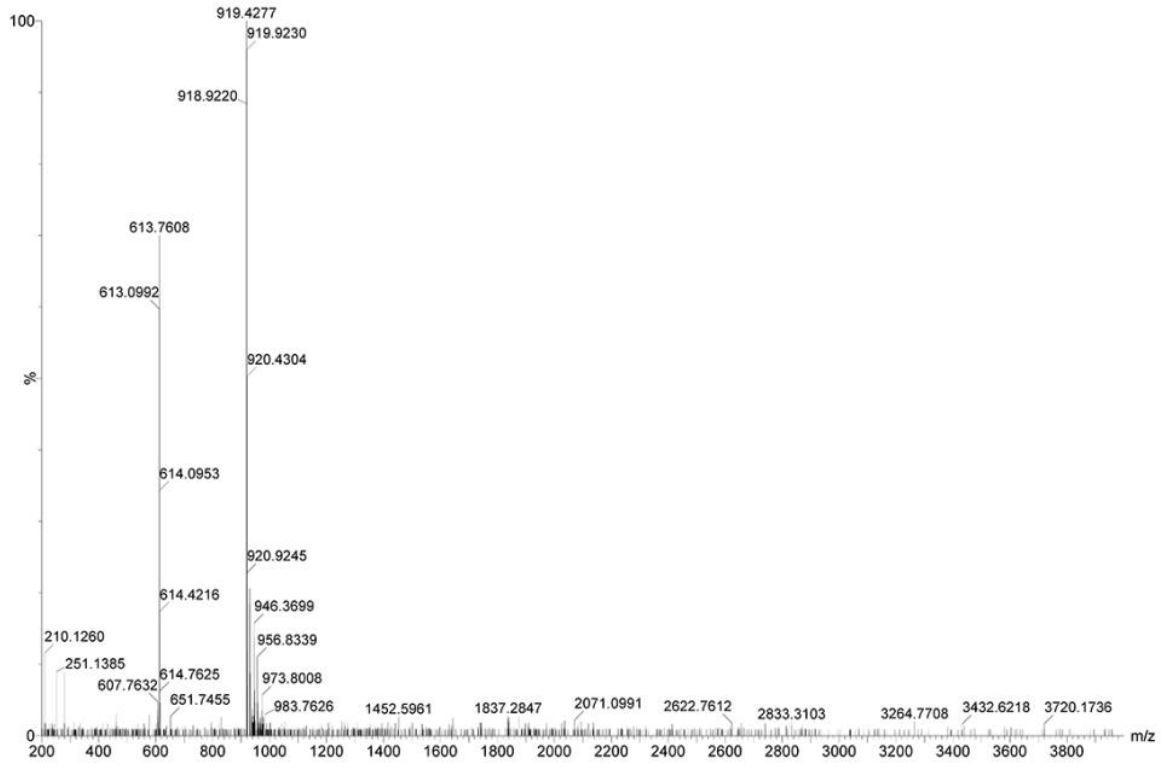


Fig. S21. Mass spectrum of $(\mathbf{1})_4$ (retention time 7.7 min in Fig. 1B) from the LC-MS analysis of a DCL made from **1**. m/z calculated: 919.16 $[M+2H]^{2+}$, 613.11 $[M+3H]^{3+}$; m/z observed: 919.43 $[M+2H]^{2+}$, 613.76 $[M+3H]^{3+}$.

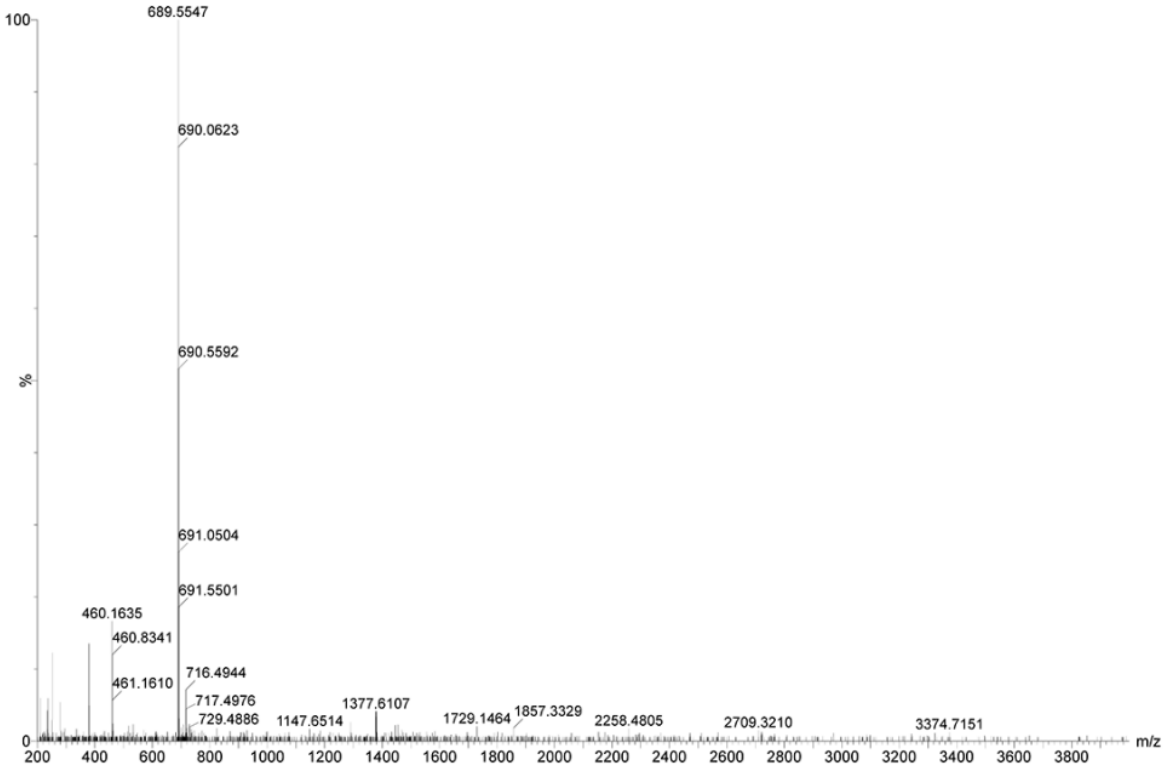


Fig. S22. Mass spectrum of $(\mathbf{1})_3$ (retention time 9.6 min in Fig. 1B) from the LC-MS analysis of a DCL made from **1**. m/z calculated: 689.62 $[M+2H]^{2+}$, 460.09 $[M+3H]^{3+}$; m/z observed: 689.55 $[M+2H]^{2+}$, 460.16 $[M+3H]^{3+}$.

16.2 Mass spectrometry analysis of the library made from **2**

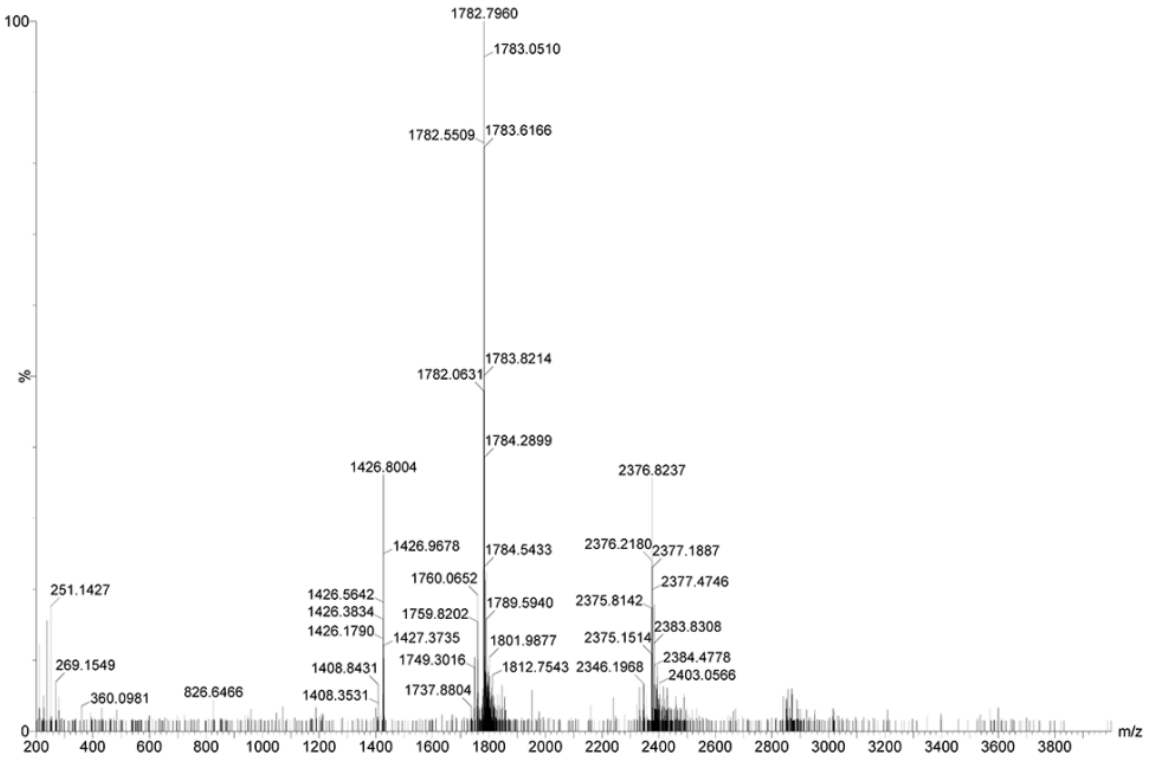


Fig. S23. Mass spectrum of $(\mathbf{2})_{15}$ (retention time 6.1 min in Fig. S18) from the LC-MS analysis of a DCL made from **2**. m/z calculated: 2377.01 $[M+3H]^{3+}$, 1783.53 $[M+4H]^{4+}$, 1427.03 $[M+5H]^{5+}$; m/z observed: 2376.82 $[M+3H]^{3+}$, 1782.80 $[M+4H]^{4+}$, 1426.80 $[M+5H]^{5+}$.

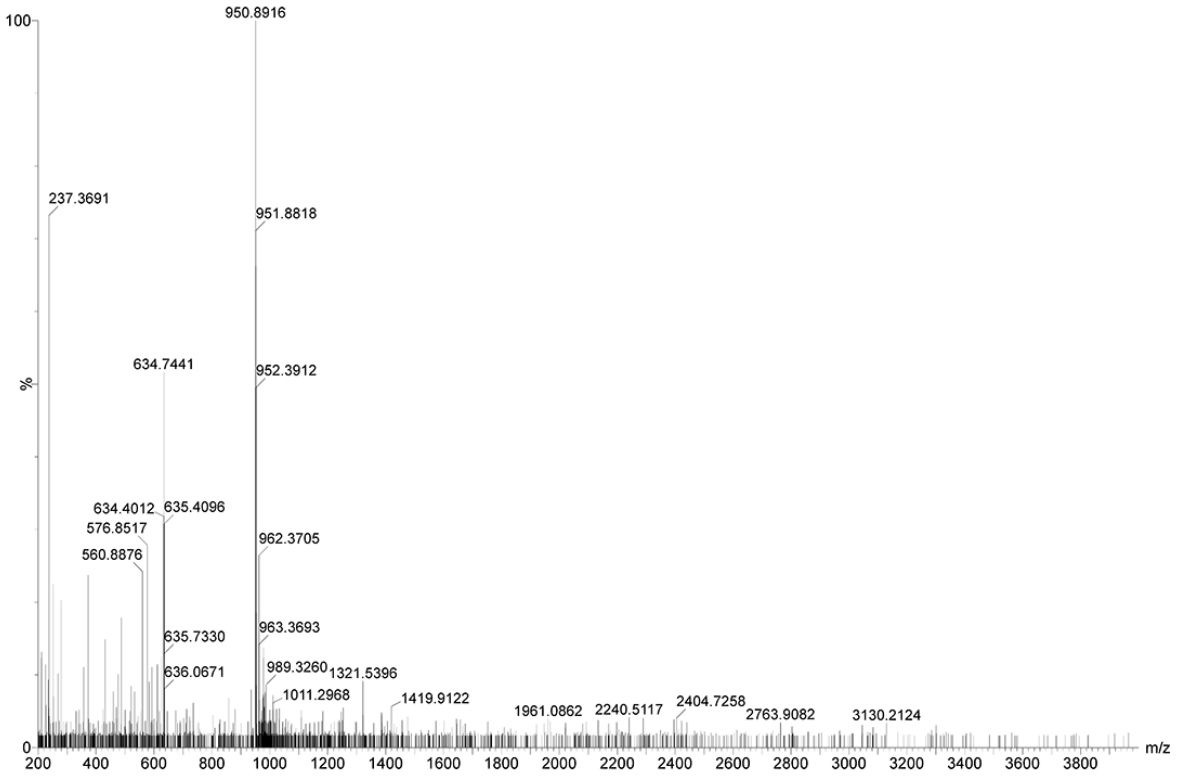


Fig. S24. Mass spectrum of $(\mathbf{2})_4$ (retention time 11.8 min in Fig. S18) from the LC-MS analysis of a DCL made from **2**. m/z calculated: 951.15 $[M+2H]^{2+}$, 634.45 $[M+3H]^{3+}$; m/z observed: 950.89 $[M+2H]^{2+}$, 634.74 $[M+3H]^{3+}$.

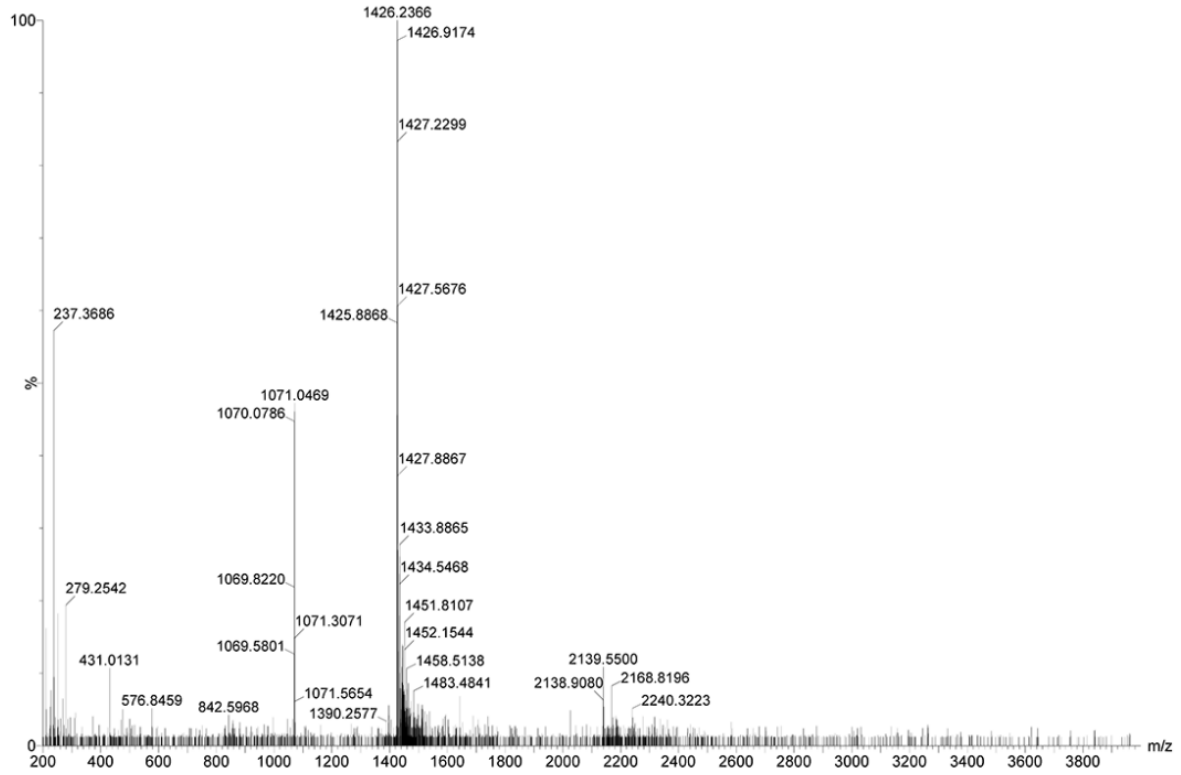


Fig. S25. Mass spectrum of $(\mathbf{2})_9$ (retention time 12.6 min in Fig. S18) from the LC-MS analysis of a DCL made from **2**. m/z calculated: 2139.34 $[M+2H]^{2+}$, 1426.56 $[M+3H]^{3+}$, 1070.17 $[M+4H]^{4+}$; m/z observed: 2139.55 $[M+2H]^{2+}$, 1426.24 $[M+3H]^{3+}$, 1071.05 $[M+4H]^{4+}$.

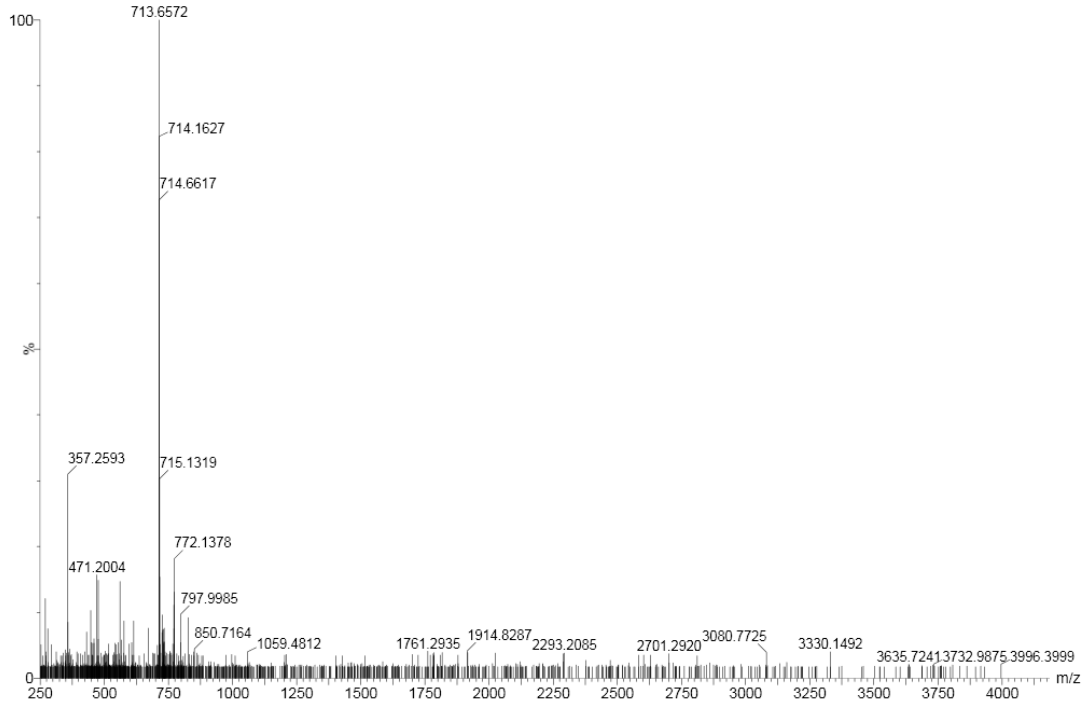


Fig. S26. Mass spectrum of $(\mathbf{2})_3$ (retention time 12.9 min in Fig. S18) from the LC-MS analysis of a DCL made from **2**. m/z calculated: 713.62 $[M+2H]^{2+}$; m/z observed: 713.66 $[M+2H]^{2+}$.

16.3 Mass spectrometry analysis of the library made from **3**

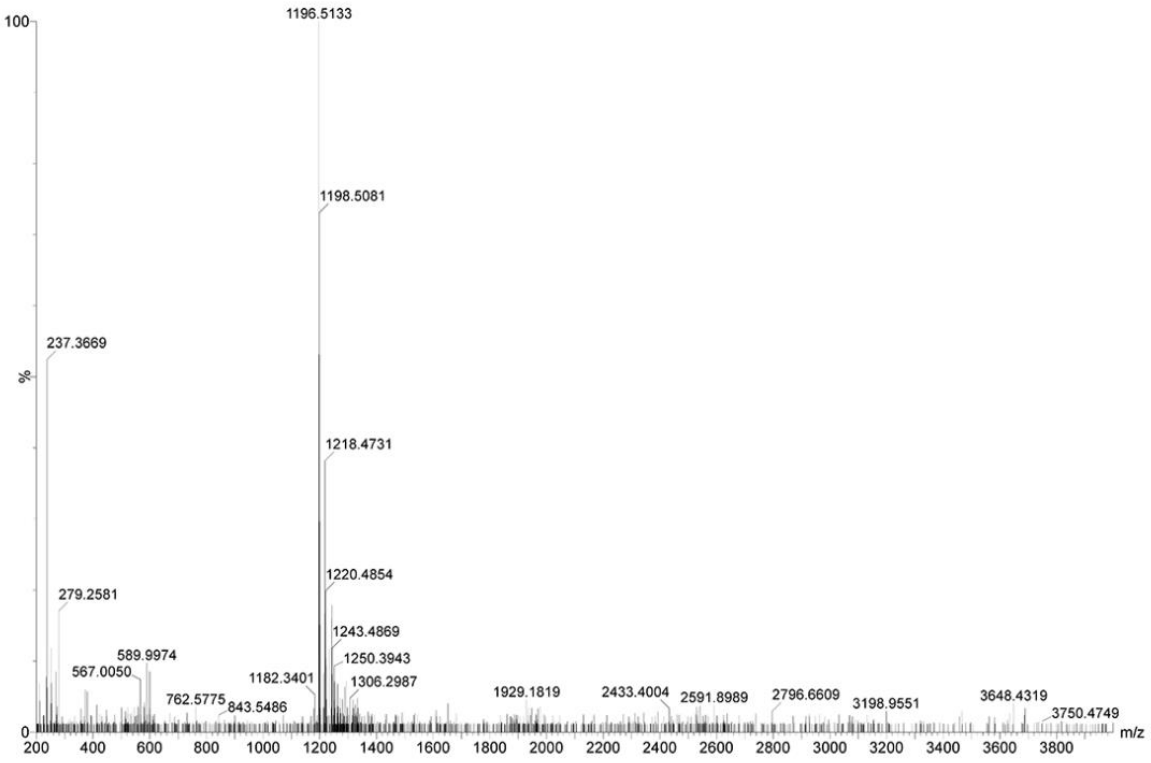


Fig. S27. Mass spectrum of $(\mathbf{3})_4$ (retention time 6.3 min in Fig. S19) from the LC-MS analysis of a DCL made from **3**. m/z calculated: 1196.98 $[M+1H]^+$; m/z observed: 1196.51 $[M+H]^+$.

Fig. S28. Mass spectrum of $(\mathbf{3})_3$ (retention time 7.6 min in Fig. S19) from the LC-MS analysis of a DCL made from **3**. m/z calculated: 897.98 $[M+1H]^+$; m/z observed: 897.77 $[M+1H]^+$.

17. NMR Spectra

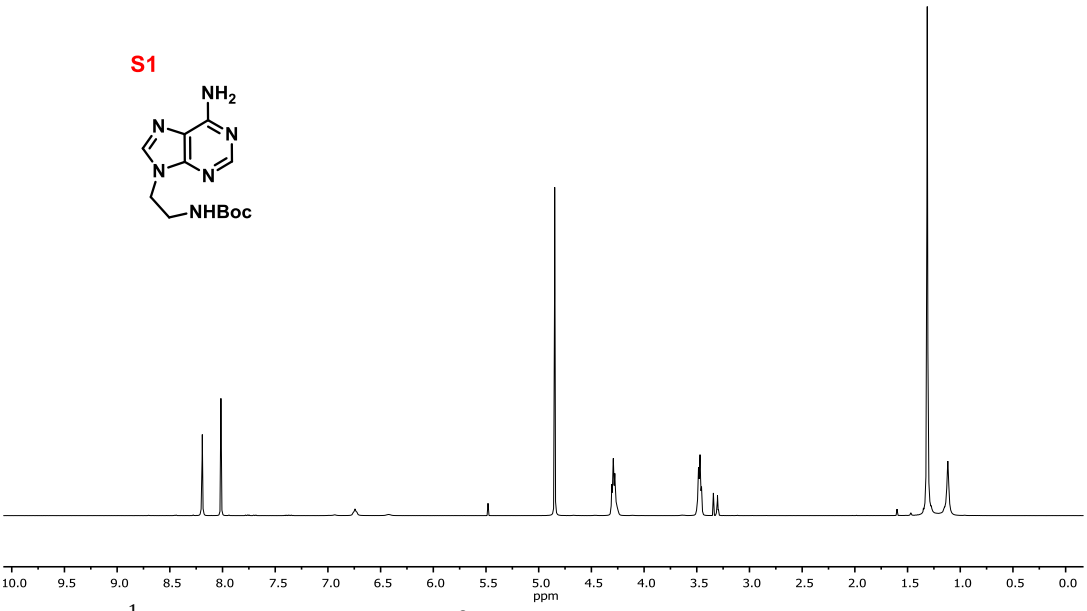


Fig. S29. ^{1}H (400 MHz, CD_3OD) of **S1**

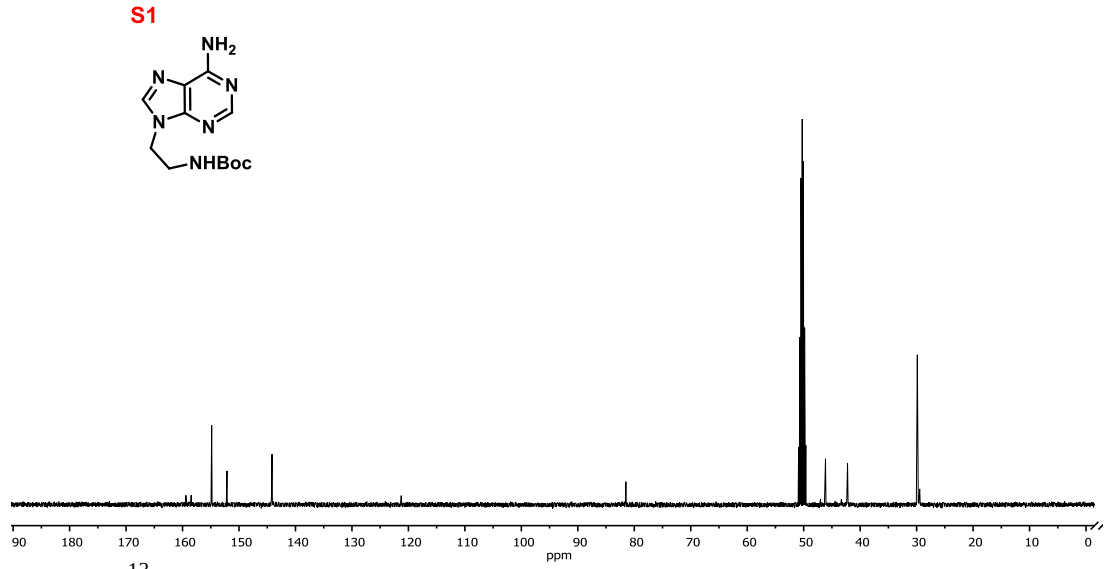


Fig. S30. ^{13}C (101 MHz, CD_3OD) of **S1**

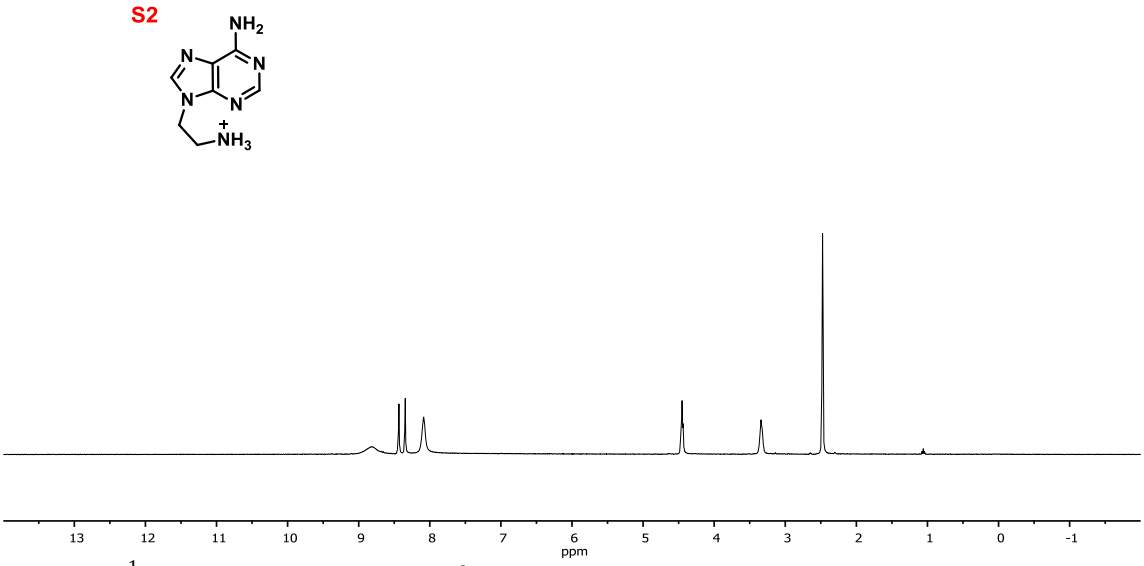


Fig. S31. ^{1}H (400 MHz, CD_3OD) of **S2**

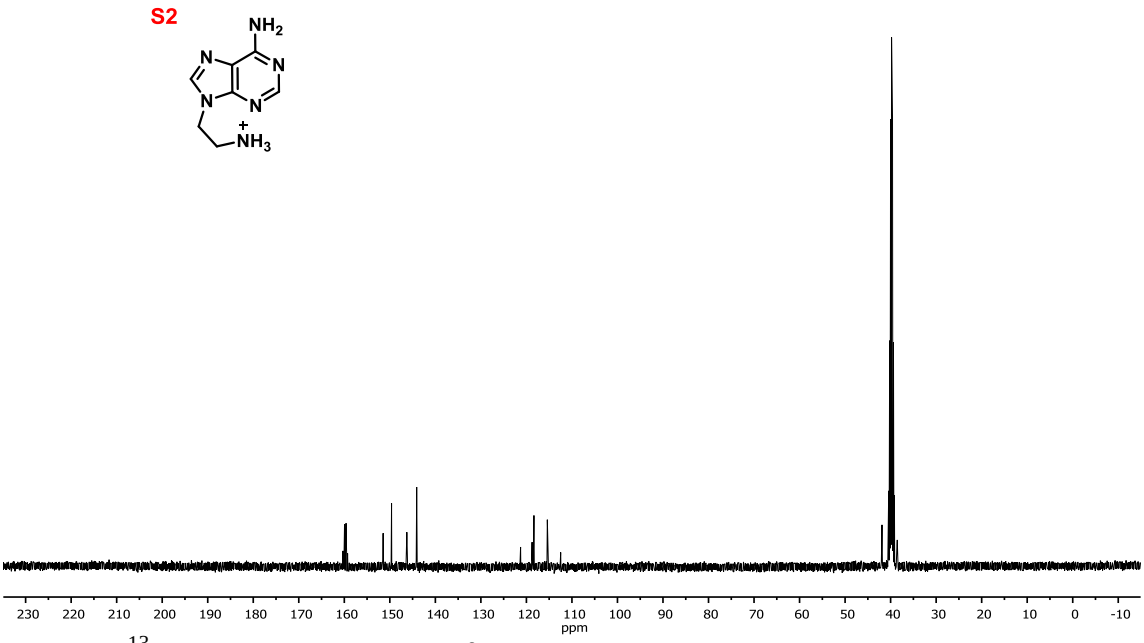


Fig. S32. ^{13}C (101 MHz, CD_3OD) of **S2**

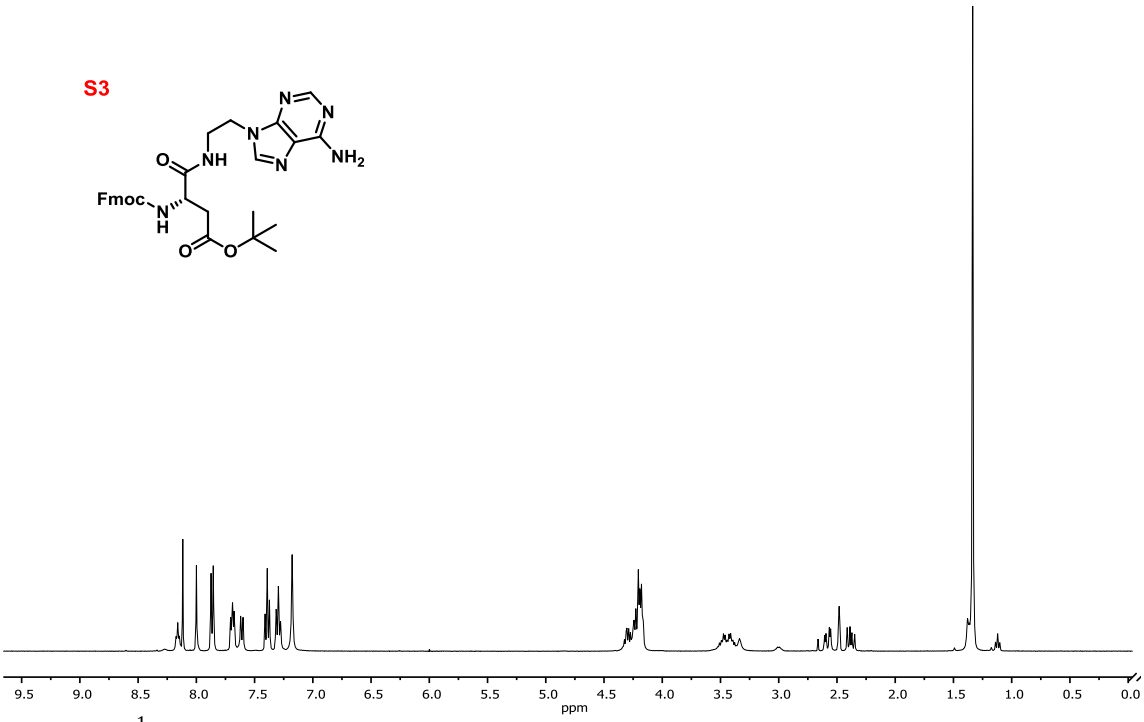


Fig. S33. ^{1}H (400 MHz, DMSO-d6) of **S3**

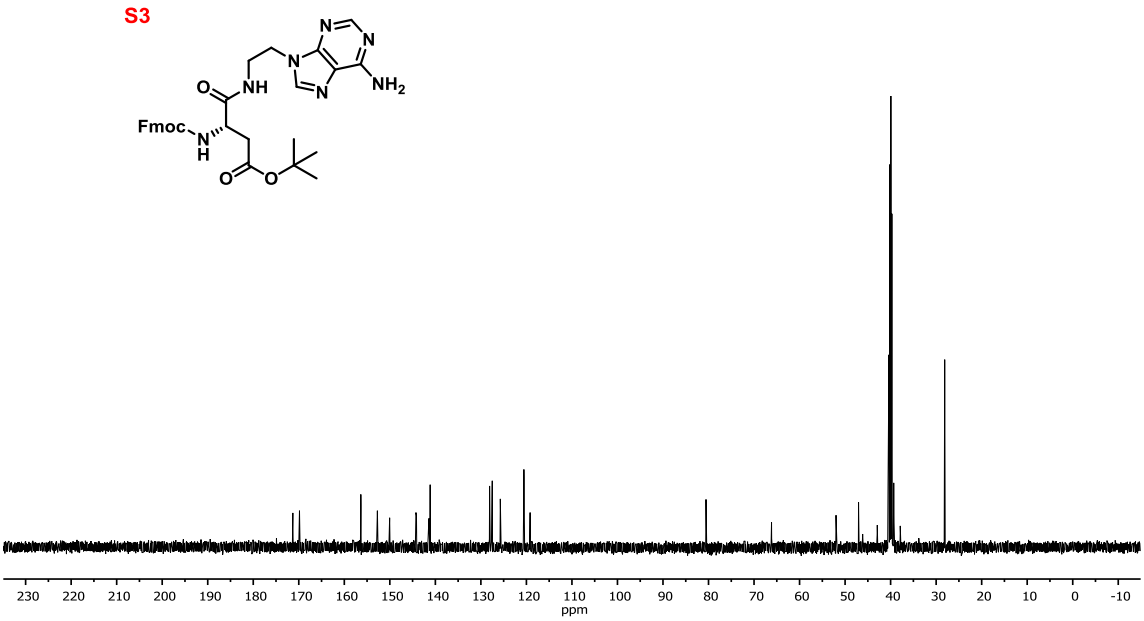


Fig. S34. ^{13}C (101 MHz, DMSO-d6) of **S3**

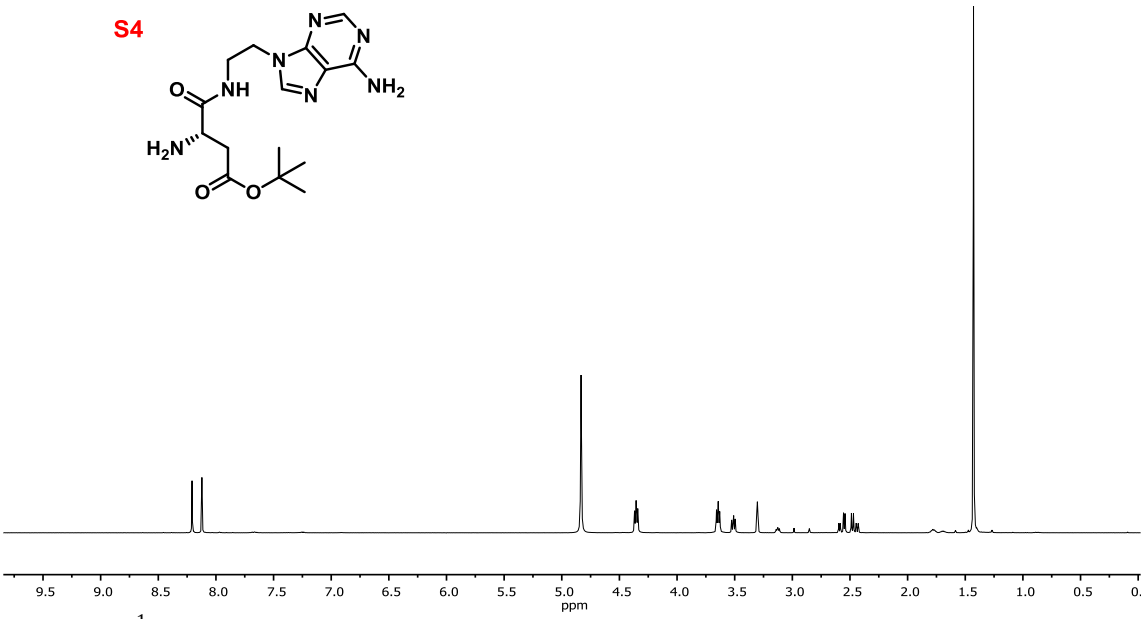


Fig. S35. ^{1}H (400 MHz, CD_3OD) of **S4**

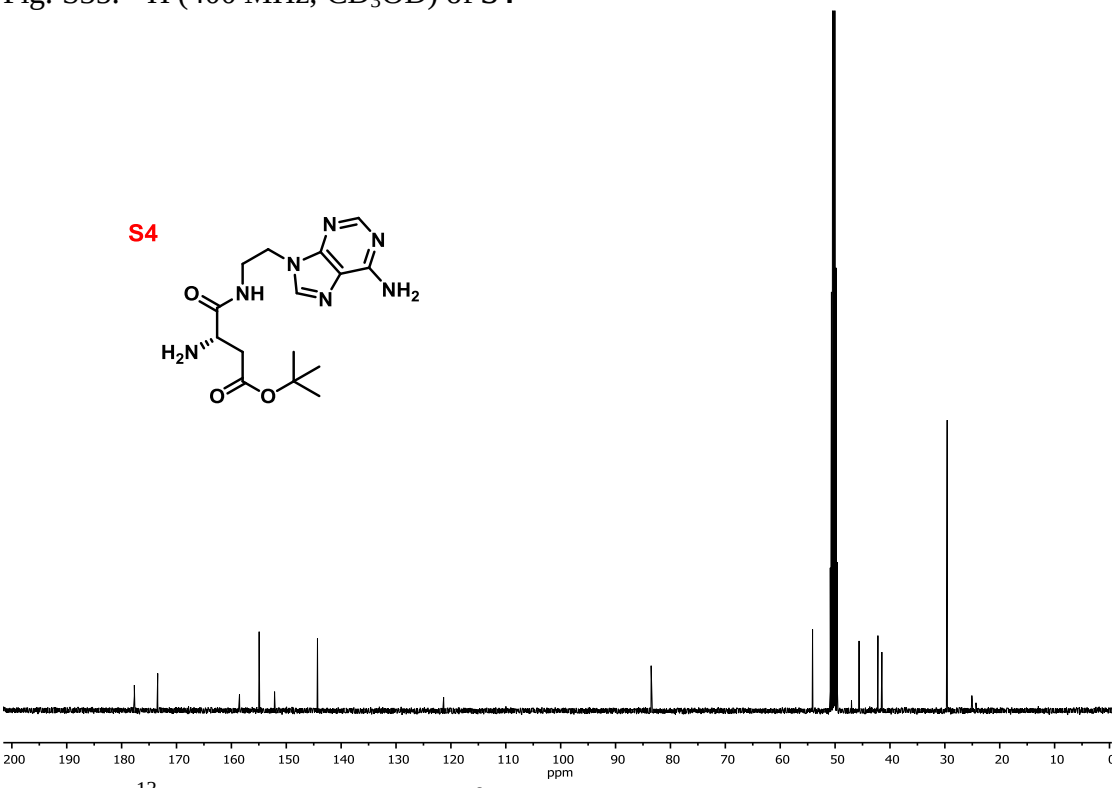


Fig. S36. ^{13}C (101 MHz, CD_3OD) of **S4**

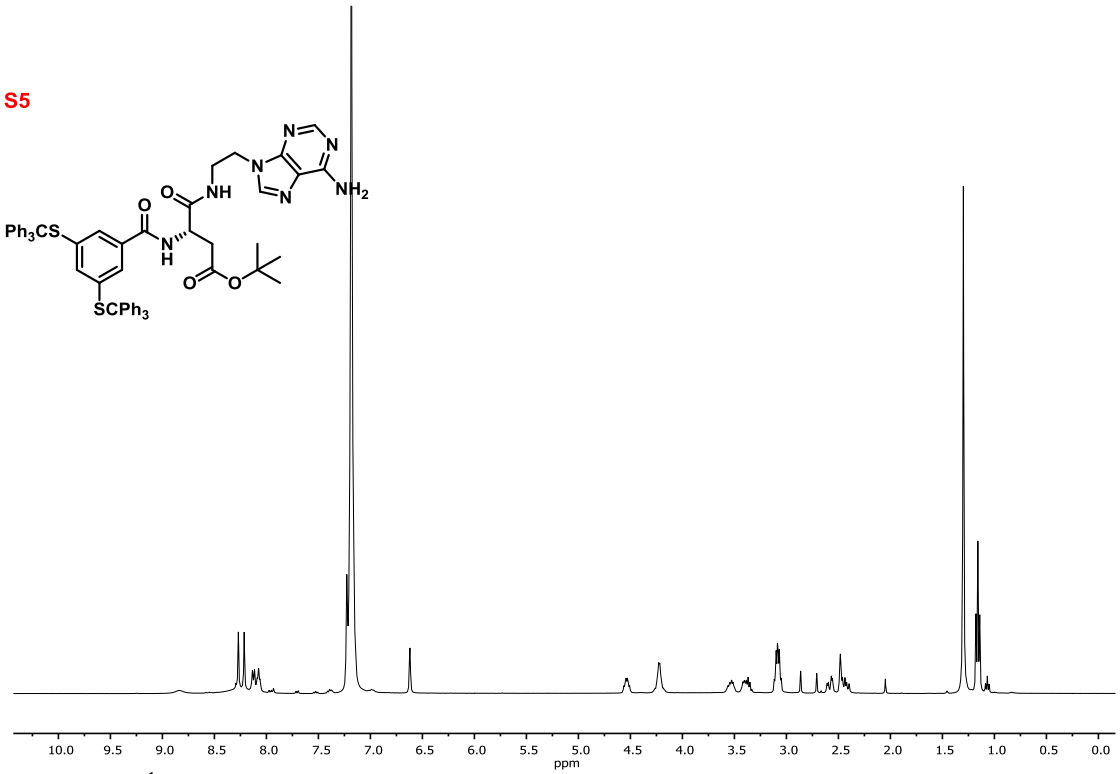


Fig. S37. ^{1}H (400 MHz, DMSO-d6) of **S5**

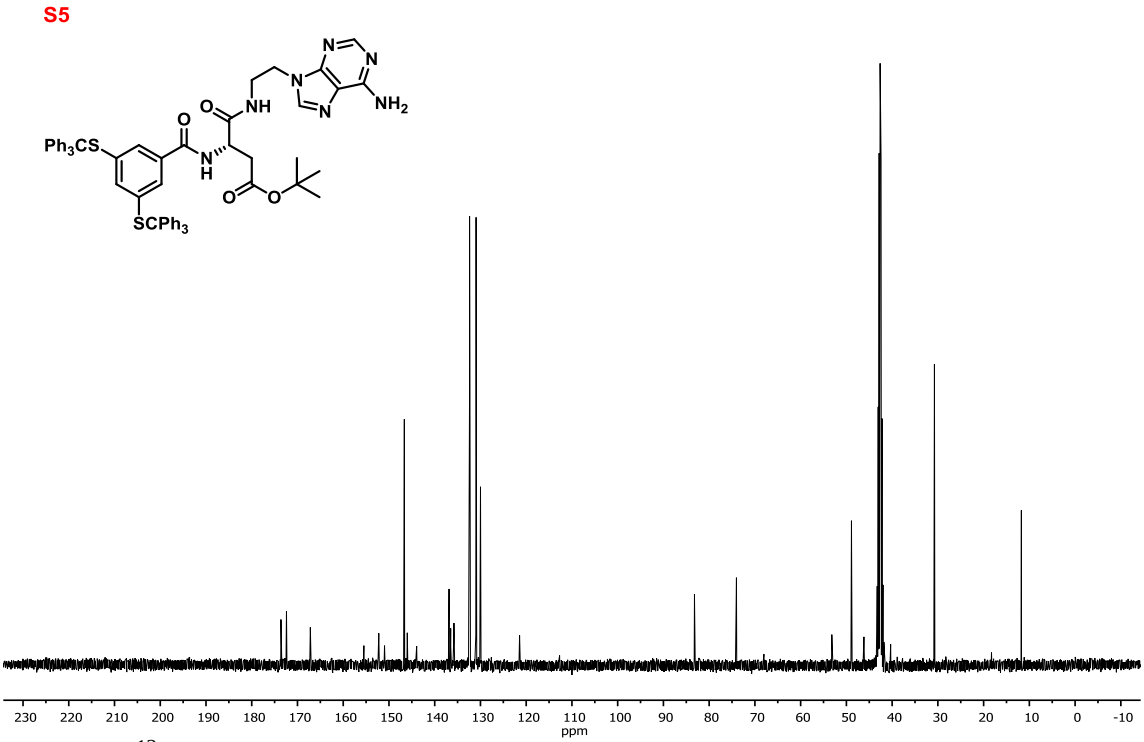


Fig. S38. ^{13}C (101 MHz, DMSO-d6) of **S5**

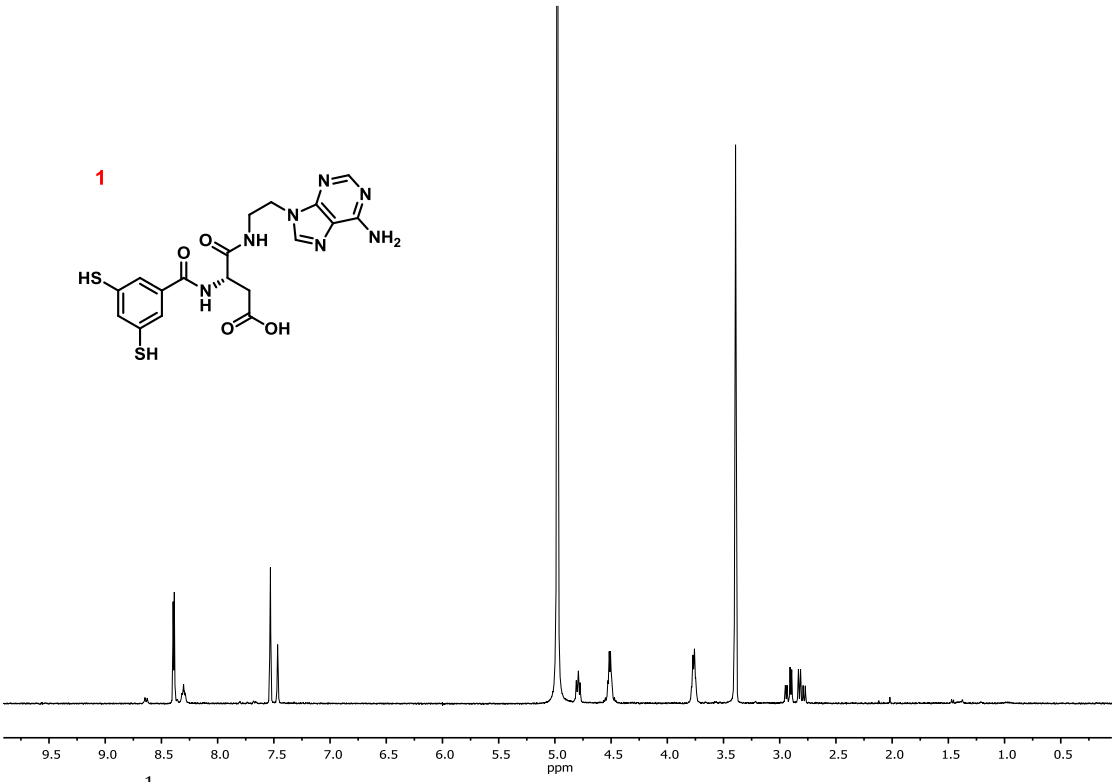


Fig. S39. ^{1}H (400 MHz, CD_3OD) of **1**

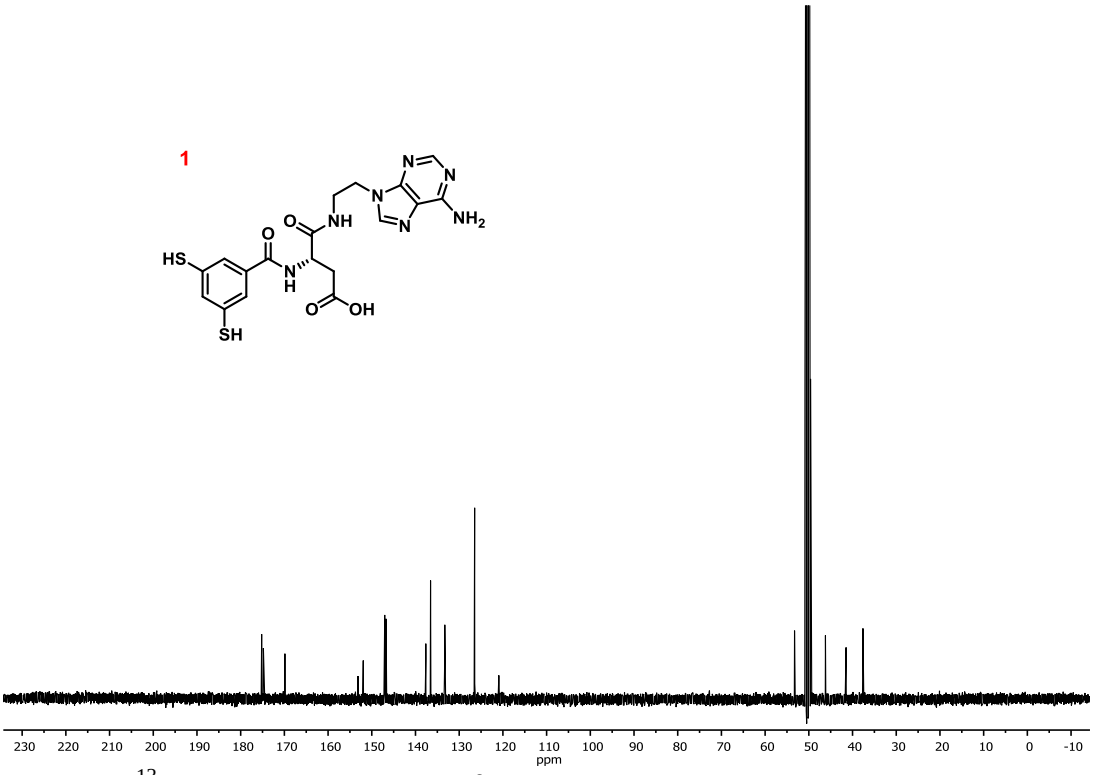


Fig. S40. ^{13}C (101 MHz, CD_3OD) of **1**

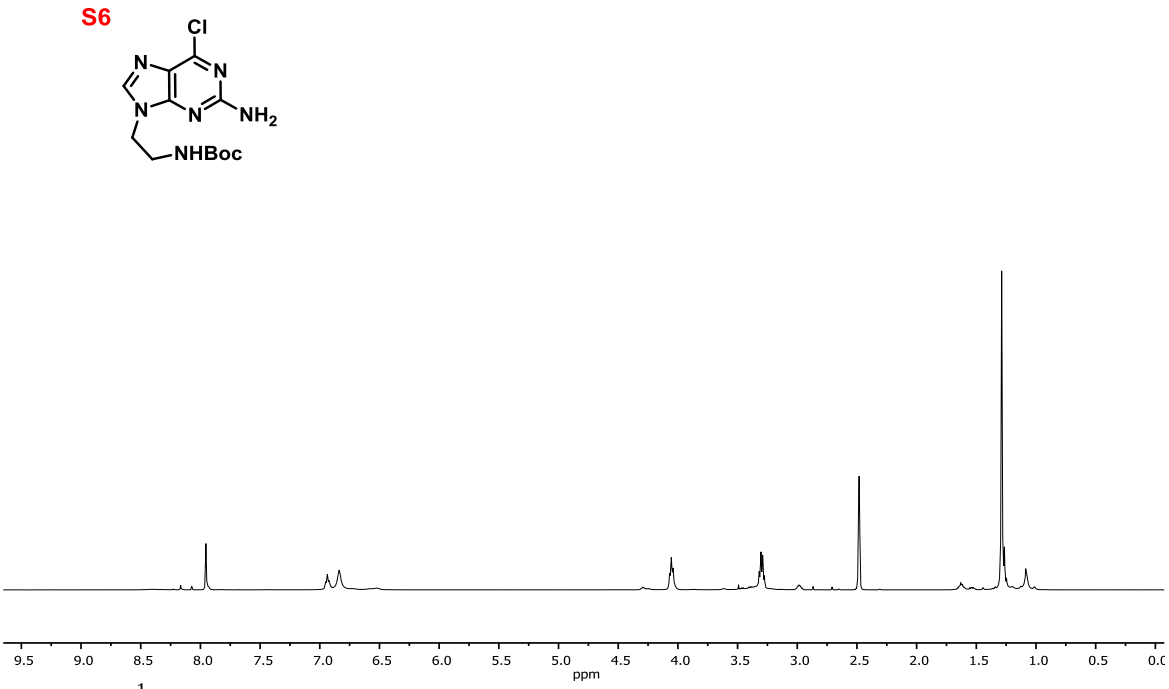


Fig. S41. ^{1}H (400 MHz, DMSO-d6) of **S6**

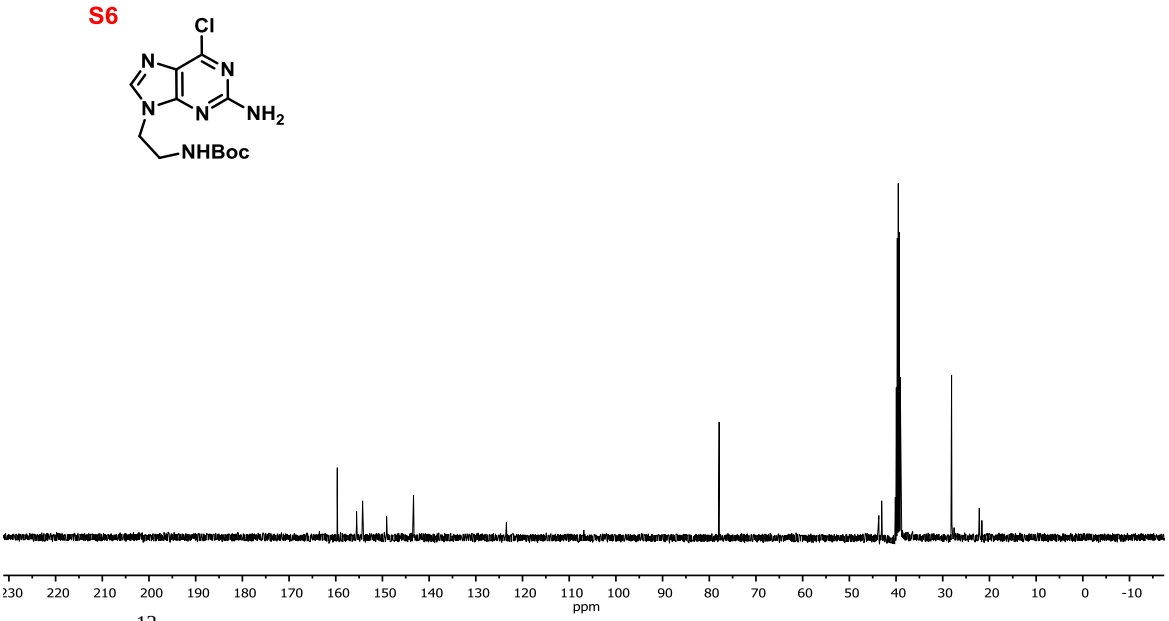


Fig. S42. ^{13}C (101 MHz, DMSO-d6) of **S6**

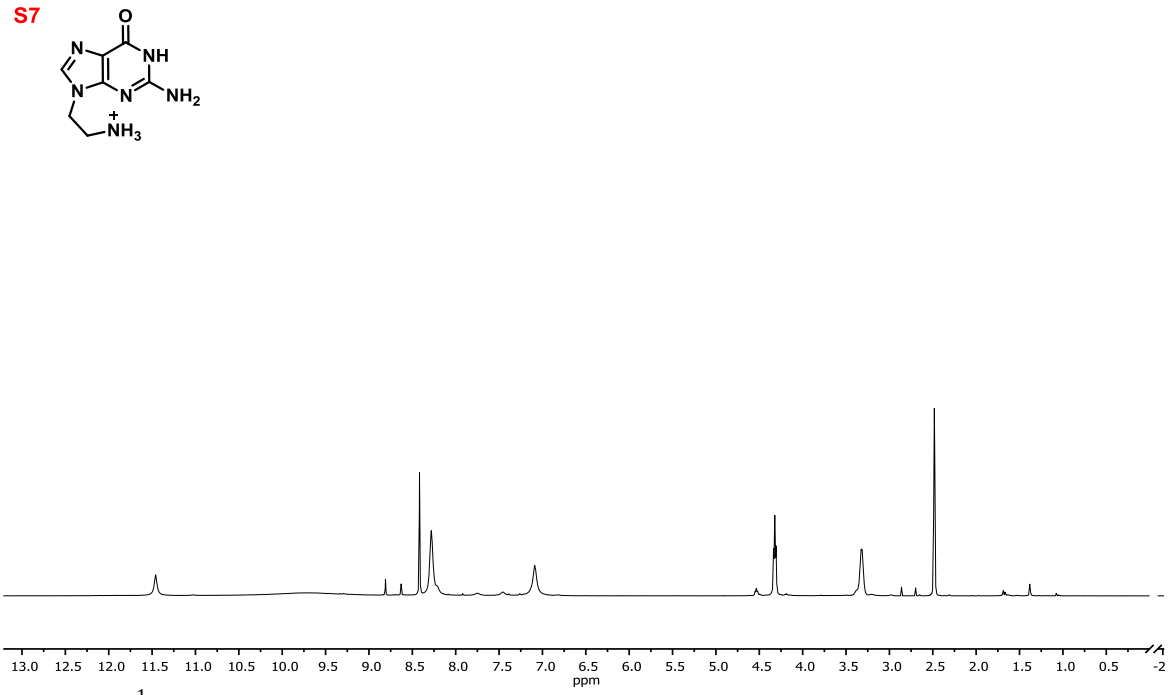


Fig. S43. ^{1}H (400 MHz, DMSO-d6) of **S7**

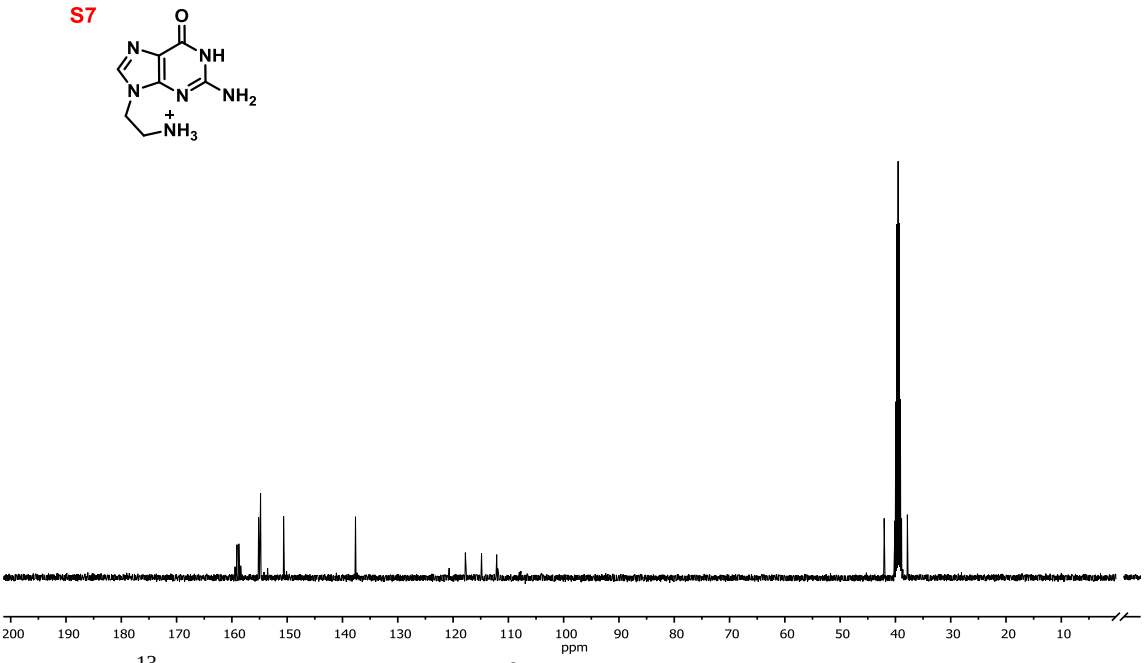


Fig. S44. ^{13}C (101 MHz, DMSO-d6) of **S7**

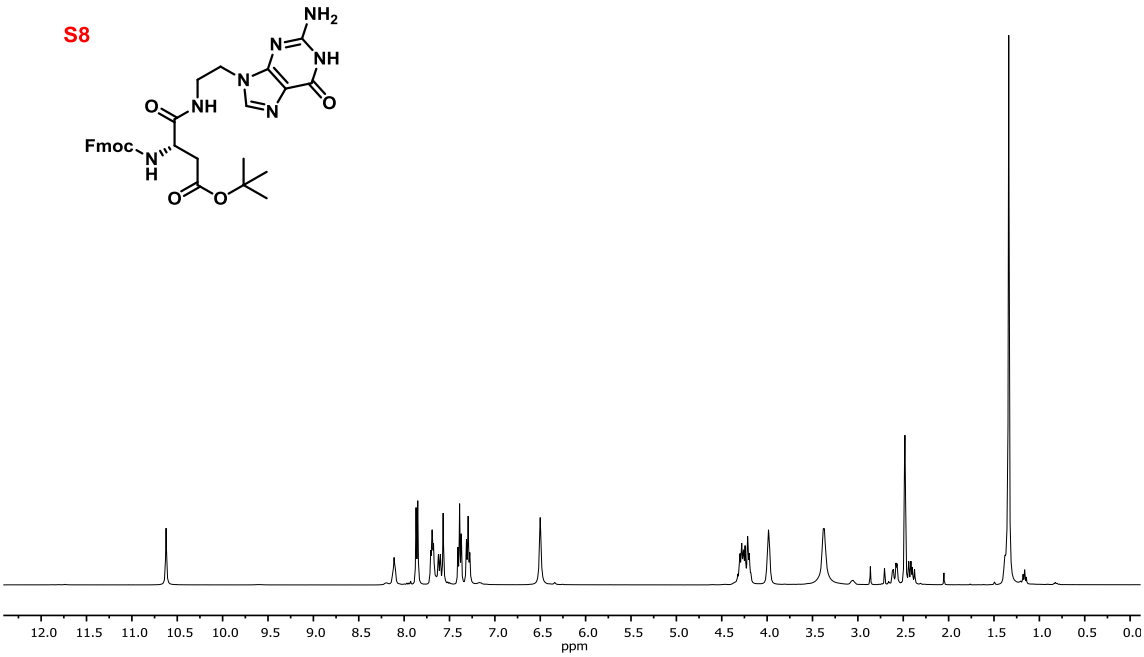


Fig. S45. ^{1}H (400 MHz, DMSO-d6) of **S8**

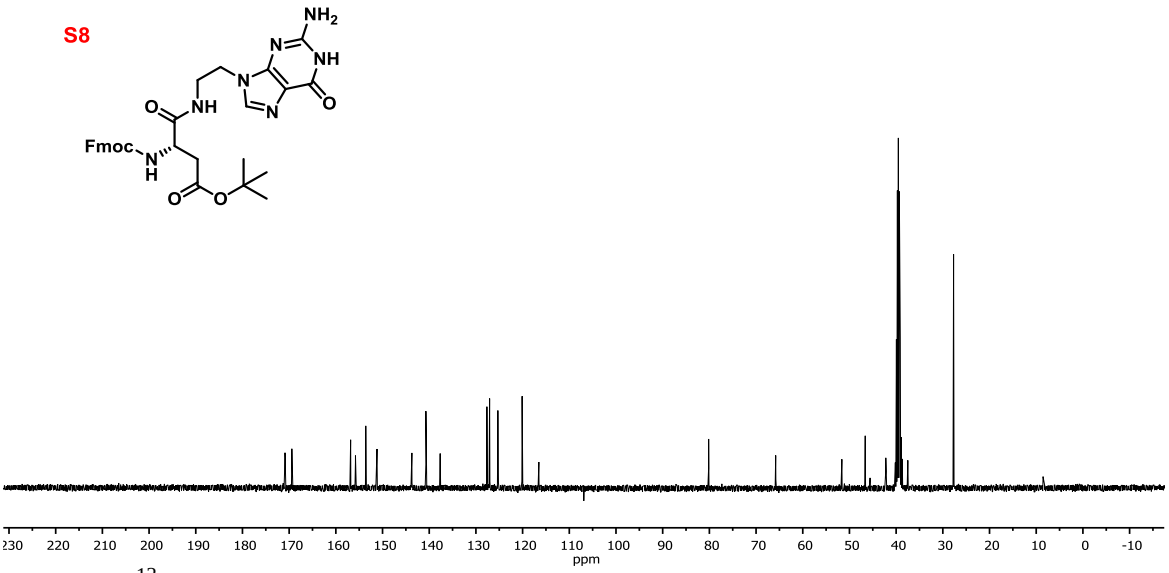


Fig. S46. ^{13}C (101 MHz, DMSO-d6) of **S8**

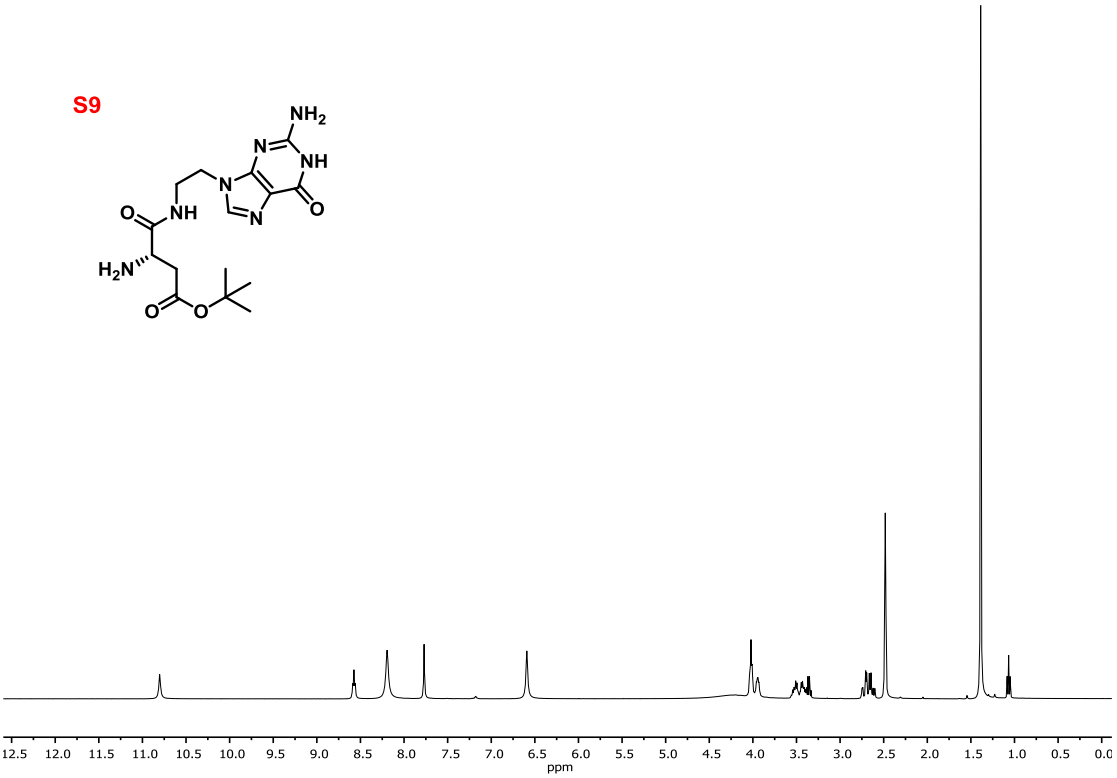


Fig. S47. ^{1}H (400 MHz, DMSO-d6) of **S9**

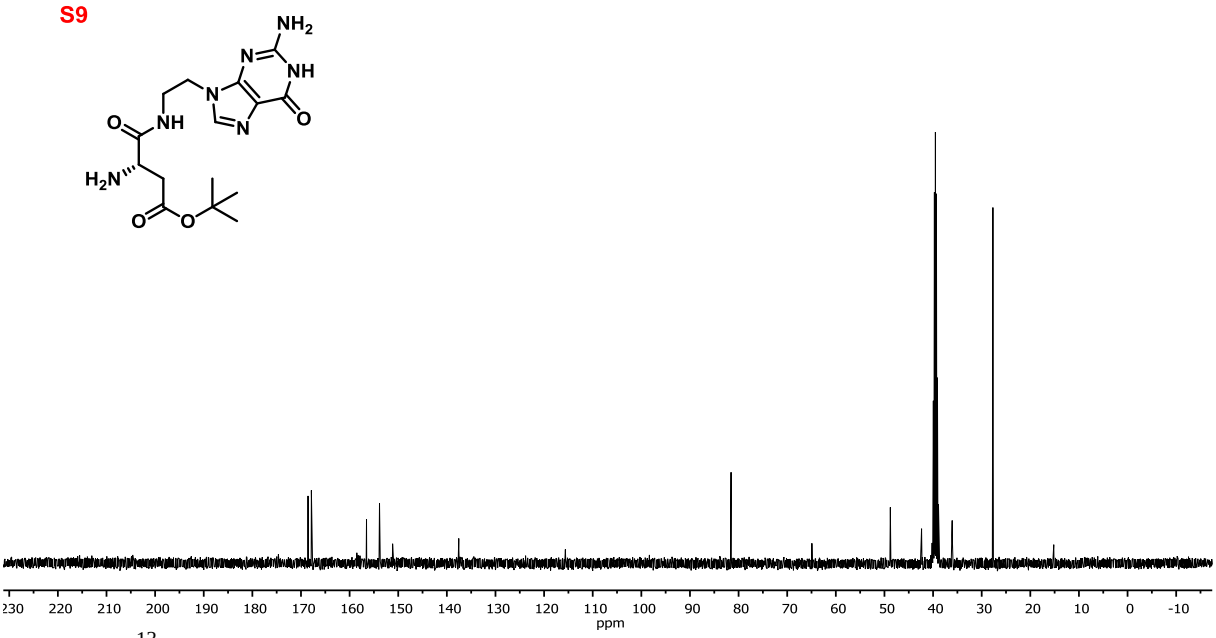


Fig. S48. ^{13}C (101 MHz, DMSO-d6) of **S9**

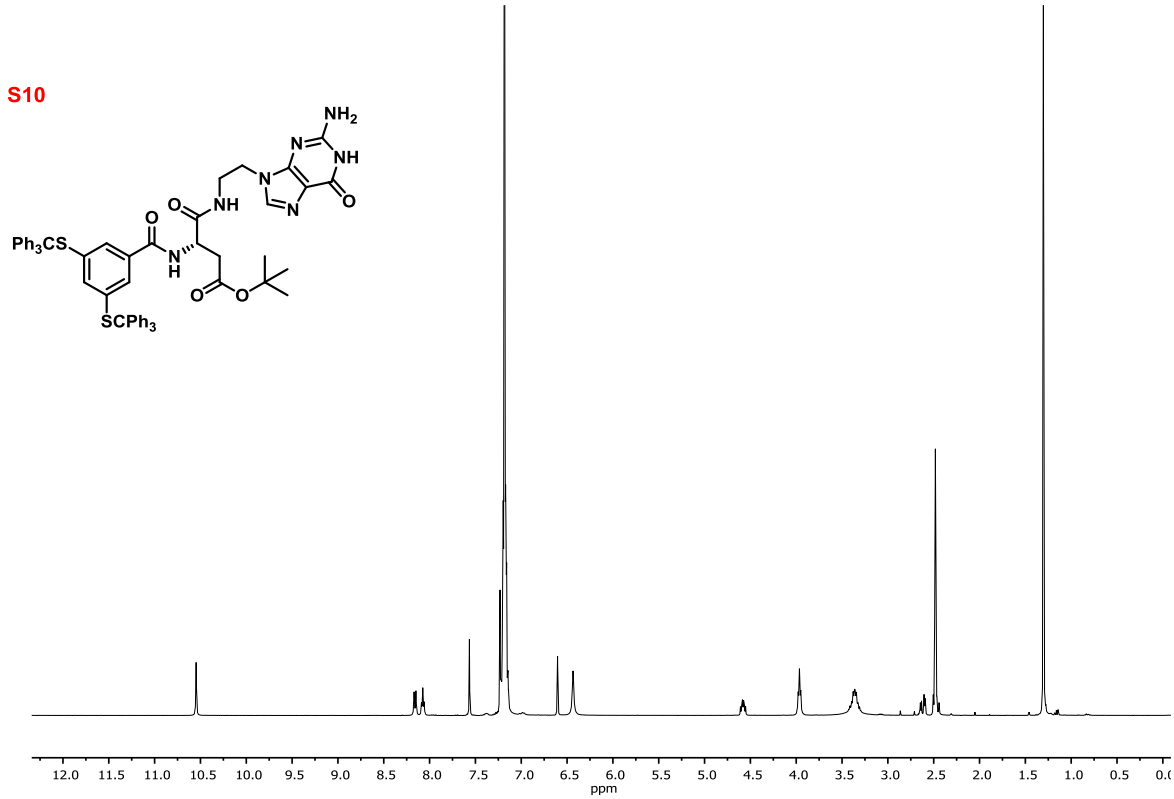


Fig. S49. ^{1}H (400 MHz, DMSO-d6) of **S10**

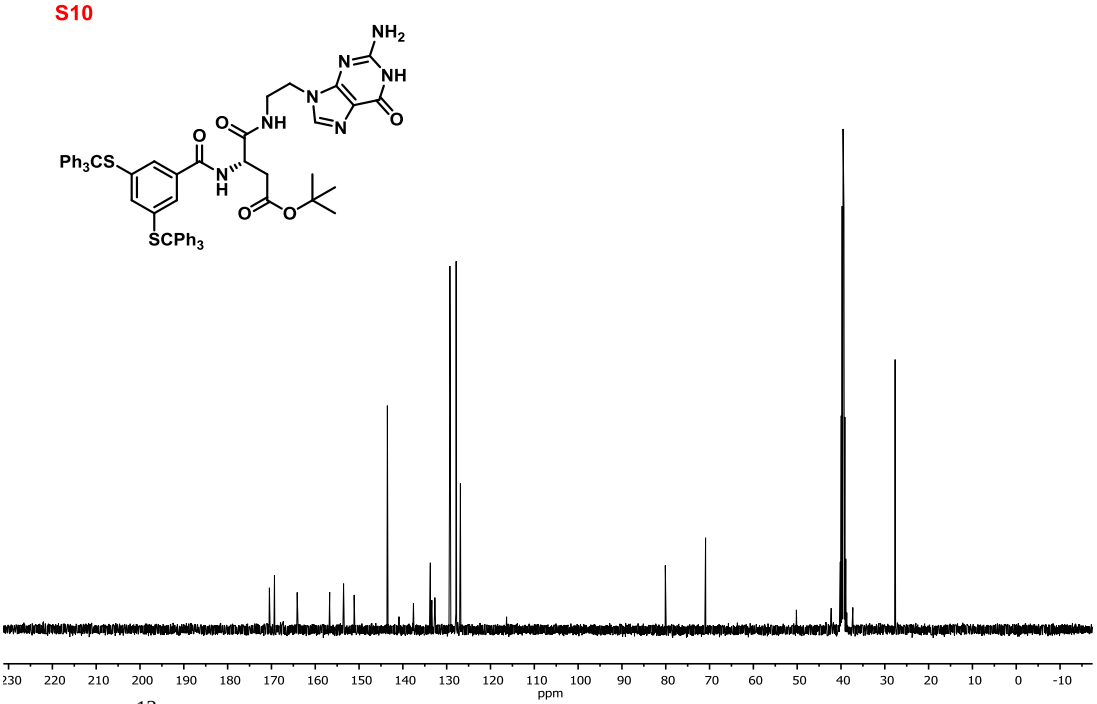


Fig. S50. ^{13}C (101 MHz, DMSO-d6) of **S10**

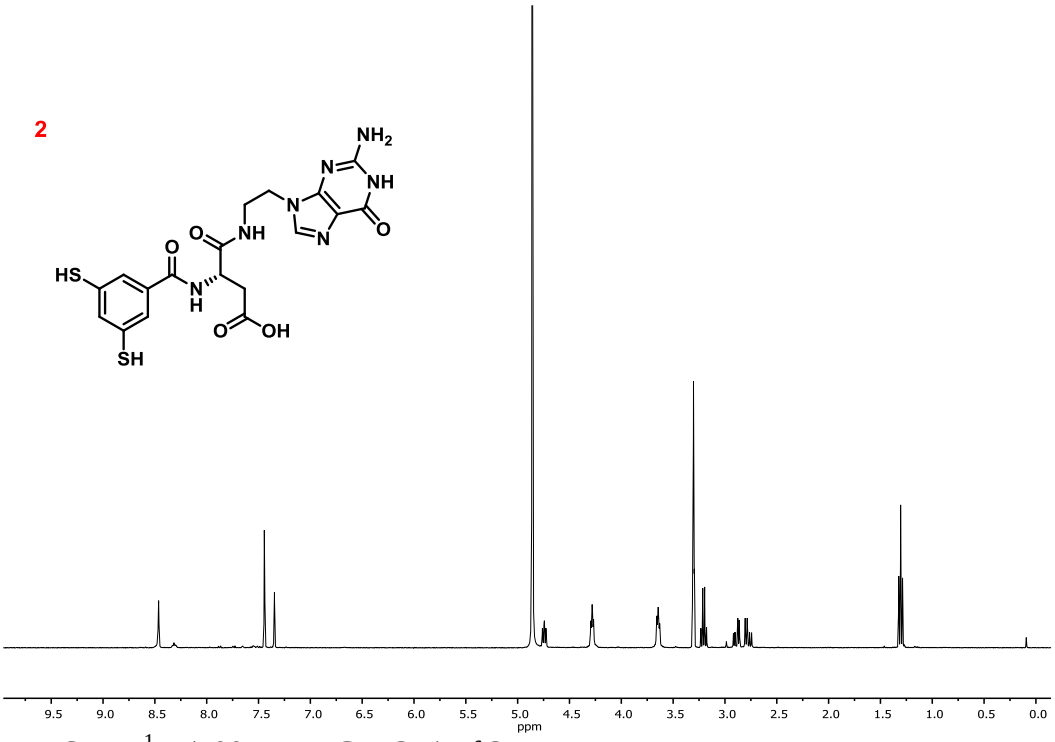


Fig. S51. ^{1}H (400 MHz, CD_3OD) of **2**

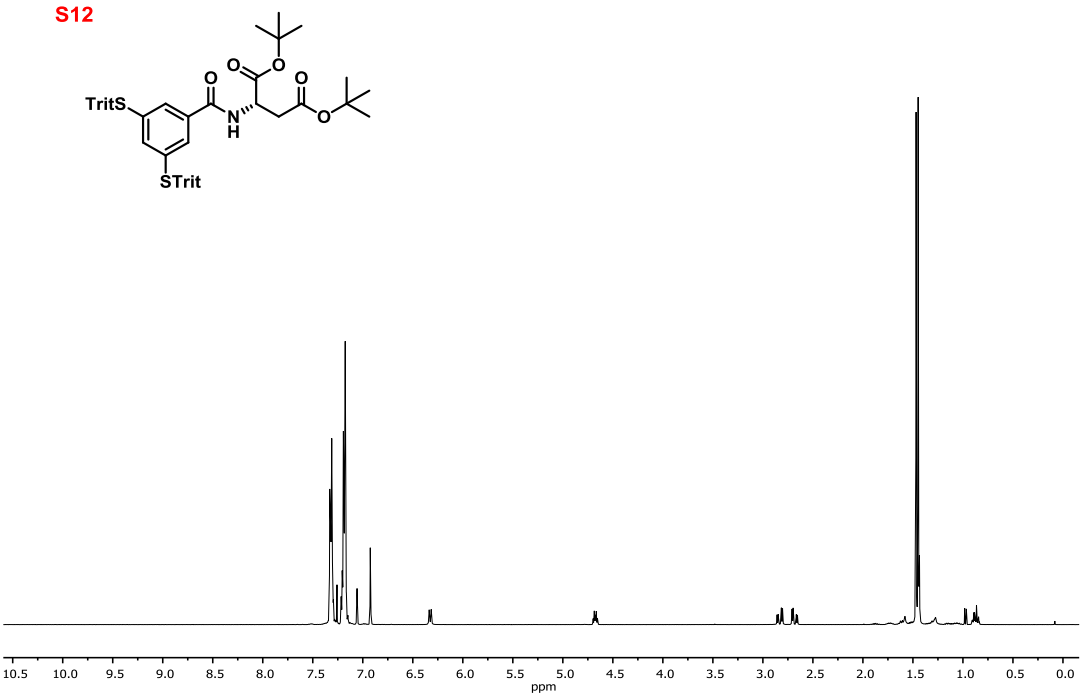


Fig. S52. ^{1}H (400 MHz, $CDCl_3$) of **S12**

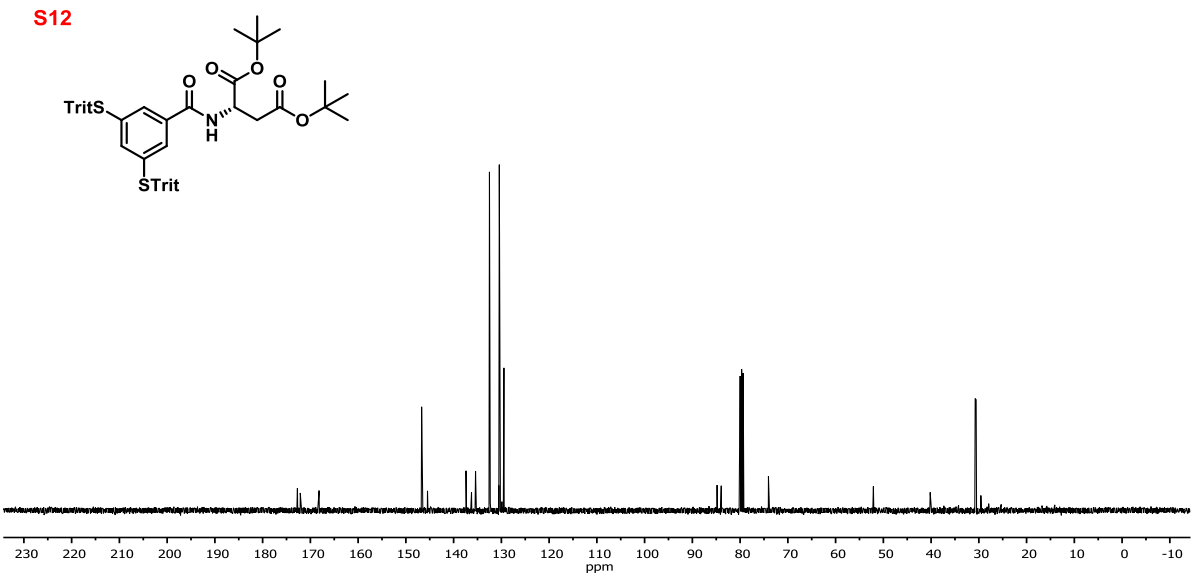


Fig. S53. ^{13}C (101 MHz, $CDCl_3$) of **S12**

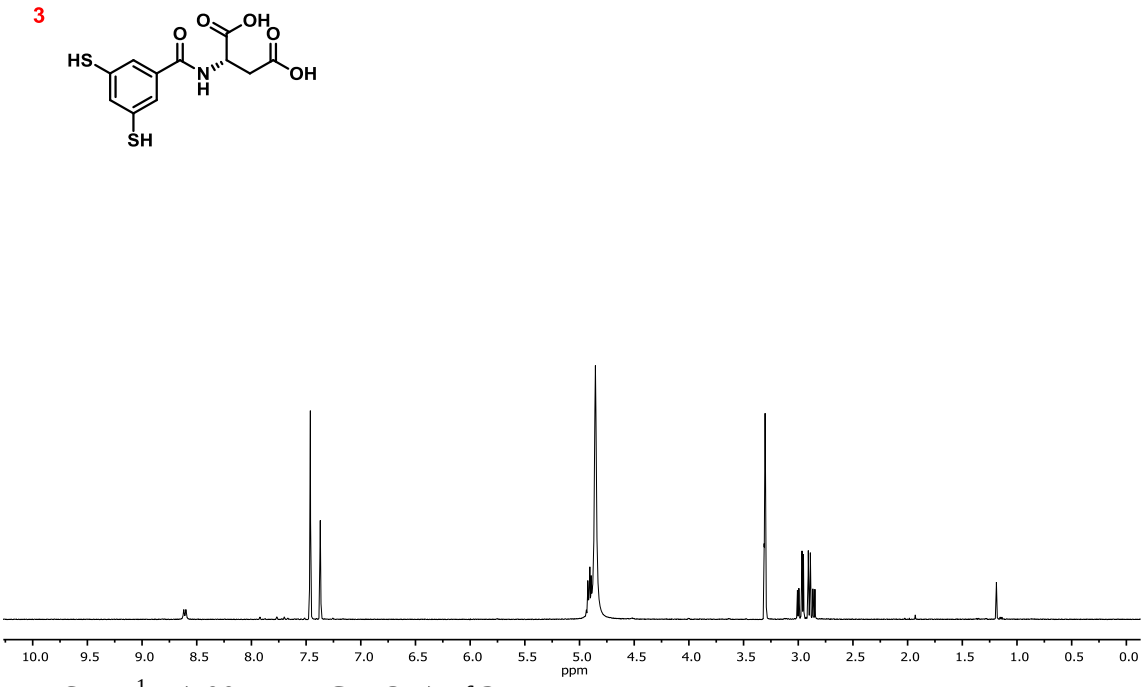


Fig. S54. ^{1}H (400 MHz, CD_3OD) of **3**

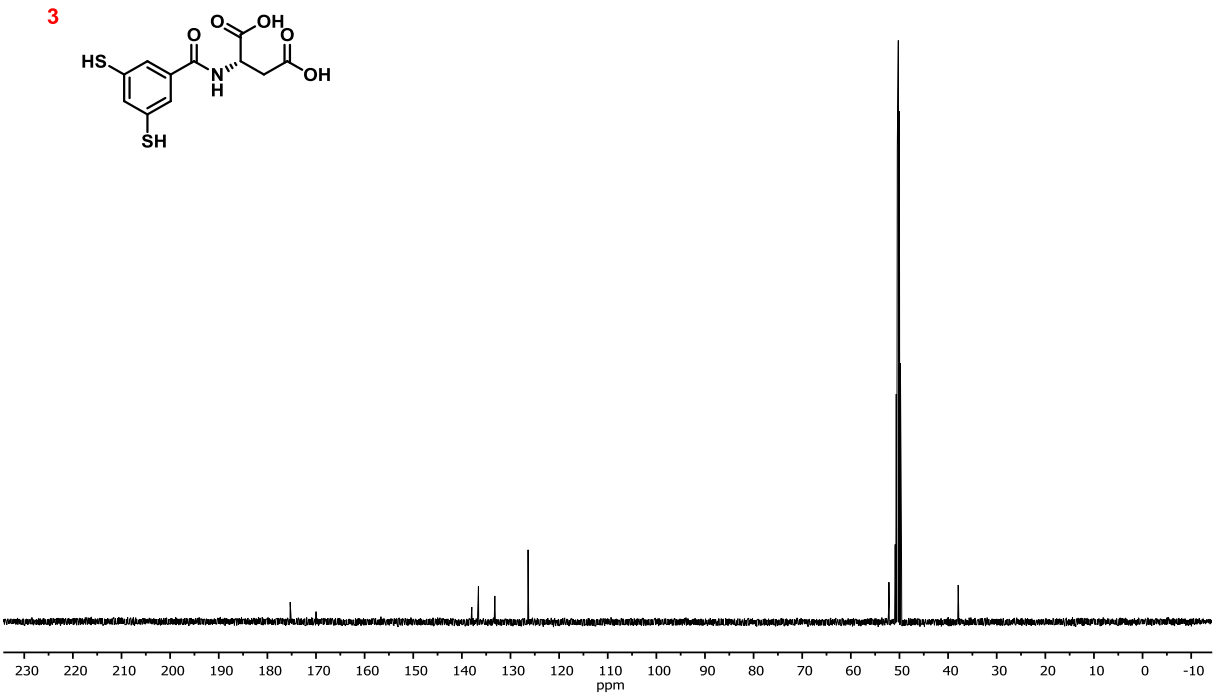


Fig. S55. ^{13}C (101 MHz, CD_3OD) of **3**

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(2) Burla, M. C.; Caliandro, R.; Carrozzini, B.; Cascarano, G. L.; Cuocci, C.; Giacovazzo, C.; Mallamo, M.; Mazzone, A.; Polidori, G. *J. Appl. Crystallogr.* **2015**, *48*, 306.
(3) Sheldrick, G. M. *Acta Crystallogr. Sect. A: Found. Crystallogr.* **2008**, *64*, 112.
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