

## Supporting Information

# Efficient Syntheses of Macrocycles Ranging from 22-28 Atoms through Spontaneous Dimerization to Yield Bis-hydrazones

Vishal R. Sharma, Arshad Mehmood, Benjamin G. Janesko, and Eric Simanek\*

Department of Chemistry and Biochemistry, Texas Christian University, Fort Worth, Texas 76129.

### Table of Contents

<b>Synthesis of 7</b> .....	<b>9</b>
Figure S1: <sup>1</sup> H-NMR of 7 .....	10
Figure S2: <sup>13</sup> C-NMR of 7 .....	10
<b>Synthesis of 8</b> .....	<b>11</b>
Figure S3: <sup>1</sup> H-NMR of 8 .....	11
<b>Synthesis of 1</b> .....	<b>12</b>
Figure S4: <sup>1</sup> H-NMR of 1 .....	13
Figure S5: <sup>13</sup> C-NMR of 1 .....	13
Figure S6: Mass spectrum of 1 .....	14
<b>Synthesis of 2</b> .....	<b>15</b>
Figure S7: <sup>1</sup> H-NMR of 2 .....	16
Figure S8: <sup>13</sup> C-NMR of 2 .....	16
Figure S9: Mass spectrum of 2 .....	17
<b>Synthesis of 4</b> .....	<b>18</b>

Figure S10: $^1\text{H}$ -NMR of 4 .....	19
Figure S11: $^{13}\text{C}$ -NMR of 4 .....	20
Figure S12: Mass spectrum of 4 .....	20
<b>Synthesis of 11 .....</b>	<b>21</b>
Figure S13: $^1\text{H}$ -NMR of 11 .....	22
Figure S14: $^{13}\text{C}$ -NMR of 11 .....	22
<b>Synthesis of 12 .....</b>	<b>23</b>
Figure S15: $^1\text{H}$ -NMR of 12 .....	24
Figure S16: $^{13}\text{C}$ -NMR of 12 .....	24
<b>Synthesis of 3 .....</b>	<b>25</b>
Figure S17: $^1\text{H}$ -NMR of 3 .....	26
Figure S18: $^{13}\text{C}$ -NMR of 3 .....	27
Figure S19: Mass spectrum of 3 .....	27
<b>Synthesis of 5 .....</b>	<b>28</b>
Figure S20: $^1\text{H}$ -NMR of 5 .....	29
Figure S21: $^{13}\text{C}$ -NMR of 5 .....	30
Figure S22: Mass spectrum of 5 .....	30
<b>Synthesis of 6 .....</b>	<b>31</b>
Figure S23: $^1\text{H}$ -NMR of 6 .....	32
Figure S24: $^{13}\text{C}$ -NMR of 6 .....	33
Figure S25: Mass spectrum of 6 .....	33
<b>Synthesis of 13 .....</b>	<b>34</b>
Figure S26: $^1\text{H}$ -NMR of 13 .....	35
Figure S27: $^{13}\text{C}$ -NMR of 13 .....	35

<b>Synthesis of 14 .....</b>	<b>36</b>
Figure S28: $^1\text{H}$ -NMR of 14.....	37
Figure S29: $^{13}\text{C}$ -NMR of 14.....	37
<b>Synthesis of 15 .....</b>	<b>38</b>
Figure S30: $^1\text{H}$ -NMR of 15 .....	39
Figure S31: $^{13}\text{C}$ -NMR of 15 .....	39
<b>Synthesis of 16 .....</b>	<b>40</b>
<b>Synthesis of model compound 17.....</b>	<b>41</b>
Figure S32: $^1\text{H}$ -NMR of 17 .....	42
<b>Synthesis of model compound 18.....</b>	<b>43</b>
Figure S33: $^1\text{H}$ -NMR of 18 .....	44
Figure S34: $^{13}\text{C}$ -NMR of 18 .....	44
<b>Synthesis of 1*1 .....</b>	<b>45</b>
Figure S35: $^1\text{H}$ -NMR of 1 in DMSO- $d_6$ .....	46
Figure S36: $^1\text{H}$ -NMR of 1*1 in DMSO- $d_6$ with drop of MeOD.....	47
Figure S37: $^1\text{H}$ -NMR of 1*1 in $\text{CD}_3\text{OD}$ .....	47
Figure S38: $^{13}\text{C}$ -NMR of 1*1 in DMSO- $d_6$ .....	48
Figure S39: $^1\text{H}$ -COSY of 1*1 in DMSO- $d_6$ .....	49
Figure S40: $^1\text{H}$ -COSY of 1*1 in DMSO- $d_6$ (Zoomed).....	50
Figure S41: $^1\text{H}$ -NOESY of 1*1 in DMSO- $d_6$ .....	51
Figure S42: X-ray crystal structure of 1*1 .....	52
Table S1: Selected H-bond parameters of 1*1 .....	52
Figure S43: Mass spectrum of 1*1 .....	53
Figure S44: HPLC chromatogram of 1*1.....	53

<b>Synthesis of 2*2 .....</b>	<b>54</b>
Figure S45: <sup>1</sup> H-NMR of 2*2 in DMSO- <i>d</i> <sub>6</sub> .....	55
Figure S46: <sup>1</sup> H-NMR of 2*2 in DMSO- <i>d</i> <sub>6</sub> with drop of MeOD.....	55
Figure S47: <sup>1</sup> H-NMR of 2*2 in CD <sub>3</sub> OD .....	56
Figure S48: <sup>13</sup> C-NMR of 2*2 in CD <sub>3</sub> OD .....	56
Figure S49: <sup>1</sup> H-COSY of 2*2 in DMSO- <i>d</i> <sub>6</sub> .....	57
Figure S50: <sup>1</sup> H-COSY of 2*2 in DMSO- <i>d</i> <sub>6</sub> (Zoomed).....	58
Figure S51: <sup>1</sup> H-NOESY of 2*2 in DMSO- <i>d</i> <sub>6</sub> .....	59
Figure S52: X-ray crystal structure of 2*2 .....	60
Table S2: Selected H-bond parameters of 2*2 .....	60
Figure S53: Mass spectrum of 2*2 .....	61
Figure S54: HPLC chromatogram of 2*2.....	61
<b>Synthesis of mixture of 1*1, 1*2, and 2*2 .....</b>	<b>62</b>
Figure S55: Mass spectrum of 1*1, 1*2, and 2*2.....	63
Figure S56: HPLC chromatogram of mixture of 1*1, 1*2, and 2*2 .....	63
<b>Synthesis of 3*3 .....</b>	<b>64</b>
Figure S57: <sup>1</sup> H-NMR of 3*3 in DMSO- <i>d</i> <sub>6</sub> .....	65
Figure S58: <sup>1</sup> H-NMR of 3*3 in CD <sub>3</sub> OD .....	65
Figure S59: <sup>13</sup> C-NMR of 3*3 in CD <sub>3</sub> OD .....	66
Figure S60: <sup>1</sup> H-COSY of 3*3 in DMSO- <i>d</i> <sub>6</sub> .....	67
Figure S61: <sup>1</sup> H-NOESY of 3*3 in DMSO- <i>d</i> <sub>6</sub> .....	68
Figure S62: X-ray crystal structure of 3*3 .....	69
Table S3: Selected H-bond parameters of 3*3 .....	69
Figure S63: Mass spectrum of 3*3 .....	70
Figure S64: HPLC chromatogram of 3*3.....	70



<b>Synthesis of 4*4 .....</b>	<b>71</b>
Figure S65: $^1\text{H}$ -NMR of 4*4 in $\text{DMSO}-d_6$ .....	72
Figure S66: $^1\text{H}$ -NMR of 4*4 in $\text{CD}_3\text{OD}$ .....	72
Figure S67: $^{13}\text{C}$ -NMR of 4*4 in $\text{CD}_3\text{OD}$ .....	73
Figure S68: $^1\text{H}$ -COSY of 4*4 in $\text{DMSO}-d_6$ .....	74
Figure S69: $^1\text{H}$ -COSY of 4*4 in $\text{DMSO}-d_6$ (Zoomed).....	75
Figure S70: $^1\text{H}$ -NOESY of 4*4 in $\text{DMSO}-d_6$ .....	76
Figure S71: X-ray crystal structure of 4*4 .....	77
Table S4: Selected H-bond parameters of 4*4 .....	77
Figure S72: Mass spectrum of 4*4 .....	78
Figure S73: HPLC chromatogram of 4*4.....	78
<b>Synthesis of 5*5 .....</b>	<b>79</b>
Figure S74: $^1\text{H}$ -NMR of 5*5 in $\text{DMSO}-d_6$ .....	80
Figure S75: $^1\text{H}$ -NMR of 5*5 in $\text{DMSO}-d_6$ with drop of $\text{CD}_3\text{OD}$ .....	80
Figure S76: $^1\text{H}$ -NMR of 5*5 in $\text{CD}_3\text{OD}$ .....	81
Figure S77: $^{13}\text{C}$ -NMR of 5*5 in $\text{DMSO}-d_6$ .....	81
Figure S78: $^1\text{H}$ -COSY of 5*5 in $\text{DMSO}-d_6$ .....	82
Figure S79: $^1\text{H}$ -COSY of 5*5 in $\text{DMSO}-d_6$ (Zoomed).....	83
Figure S80: $^1\text{H}$ -NOESY of 5*5 in $\text{DMSO}-d_6$ .....	84
Figure S81: $^{13}\text{C}$ - $^1\text{H}$ -HMQC of 5*5 in $\text{DMSO}-d_6$ .....	85
Figure S82: $^{13}\text{C}$ - $^1\text{H}$ -HMQC of 5*5 in $\text{DMSO}-d_6$ (Zoomed 1) .....	86
Figure S83: $^{13}\text{C}$ - $^1\text{H}$ -HMQC of 5*5 in $\text{DMSO}-d_6$ (Zoomed 2) .....	86
Figure S84: Mass spectrum of 5*5 .....	87
Figure S85: HPLC chromatogram of 5*5.....	87

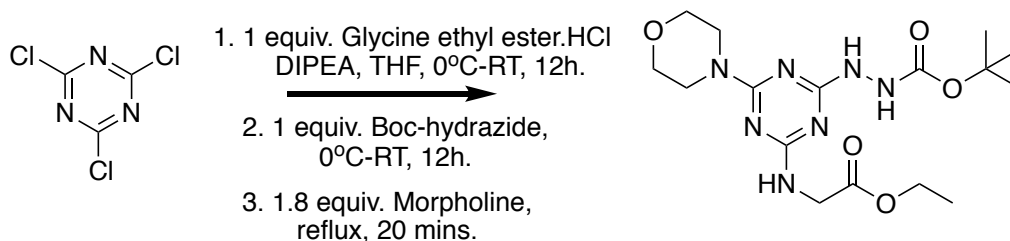
<b>Synthesis of mixture of 3*3, 3*5, and 5*5</b>	<b>88</b>
Figure S86: HPLC chromatogram of a mixture of 3*3, 3*5, and 5*5	88
<b>Synthesis of 6*6</b>	<b>89</b>
Figure S87: <sup>1</sup> H-NMR of 6*6 in DMSO- <i>d</i> <sub>6</sub>	90
Figure S88: <sup>1</sup> H-NMR of 6*6 in CD <sub>3</sub> OD	90
Figure S89: <sup>13</sup> C-NMR of 6*6 in CD <sub>3</sub> OD	91
Figure S90: <sup>1</sup> H-COSY of 6*6 in DMSO- <i>d</i> <sub>6</sub>	92
Figure S91: <sup>1</sup> H-COSY of 6*6 in DMSO- <i>d</i> <sub>6</sub> (Zoomed)	93
Figure S92: <sup>1</sup> H-NOESY of 6*6 in DMSO- <i>d</i> <sub>6</sub>	94
Figure S93: X-ray crystal structure of 6*6	95
Table S5: Selected H-bond parameters of 6*6	95
Figure S94: Mass spectrum of 6*6	96
Figure S95: HPLC chromatogram of 6*6	96
<b>Synthesis of macrocycle 10</b>	<b>97</b>
Figure S96: <sup>1</sup> H-NMR of 10 in DMSO- <i>d</i> <sub>6</sub>	98
Figure S97: <sup>13</sup> C-NMR of 10 in DMSO- <i>d</i> <sub>6</sub>	98
Figure S98: <sup>1</sup> H-COSY of 10 in DMSO- <i>d</i> <sub>6</sub>	99
Figure S99: <sup>1</sup> H-NOESY of 10 in DMSO- <i>d</i> <sub>6</sub>	100
<b>Synthesis of model system 9</b>	<b>101</b>
Figure S100: <sup>1</sup> H-NMR of 9 in DMSO- <i>d</i> <sub>6</sub>	102
Figure S101: <sup>13</sup> C-NMR of 9 in DMSO- <i>d</i> <sub>6</sub>	102
Figure S102: <sup>1</sup> H-COSY of 9 in DMSO- <i>d</i> <sub>6</sub>	103
Figure S103: <sup>1</sup> H-NOESY of 9 in DMSO- <i>d</i> <sub>6</sub>	104
<b>Synthesis of mixture of 14-21 macrocycles</b>	<b>105</b>

Figure S92: HPLC chromatogram of mixture of macrocycles .....	105
<b>X-ray diffraction analysis.....</b>	<b>106</b>
<b>References:.....</b>	<b>107</b>

### **“Author Contribution Statement”**

**Vishal Sharma was responsible for the synthesis, non-crystallographic characterization, and manuscript preparation. Arshad Mehmood was responsible for the crystallography and manuscript preparation. Benjamin Janesko and Eric Simanek oversaw the effort.**

### Synthesis of compound 7 from Cyanuric Chloride:



Cyanuric chloride (5.00 g, 0.027 moles) was dissolved in 40 ml dry THF in a three neck round bottom flask equipped with stirring bar and purged with Ar. Then, glycine ethyl ester hydrochloride salt (3.8 g, 0.027 moles) and DIPEA (14.0 g, 0.104 moles) were dissolved in a mixture of 2 ml MeOH and 5 ml THF was then added to three neck flask dropwise at -20°C. Temperature of the reaction mixture was raised slowly to 25°C and left to stir overnight. The reaction progress was monitored via TLC. Boc-hydrazide (3.6 g, 0.027 moles) was dissolved in 5 ml THF and then added dropwise to the reaction mixture at 0°C. Resulting solution was warmed to room temperature slowly and left to stir over night. The reaction progress was monitored via TLC. Third, morpholine (4.3 g, 0.05 moles) was added to the reaction mixture and then refluxed for 20 minutes. Excess solvent was removed under vacuum and crude product was extracted with DCM/H<sub>2</sub>O, collected in DCM (x3). Column chromatography was performed using 1:1 mixture of Hexane and ethyl acetate to afford 7.7 g (72%) of compound 7 as white solid.

Figure S1: <sup>1</sup>H-NMR, CDCl<sub>3</sub>, δ 4.21 (2H, 2), 4.12-4.06 (2H, q), 3.73-3.65 (8H, m), 1.44 (9H, s), 1.27-1.23 (3H, t).

Figure S2: <sup>13</sup>C-NMR, CDCl<sub>3</sub>, δ 170.8, 167.8, 165.8, 164.9, 156.5, 81.0, 66.8, 61.1, 43.5, 43.0, 28.2, 14.2.

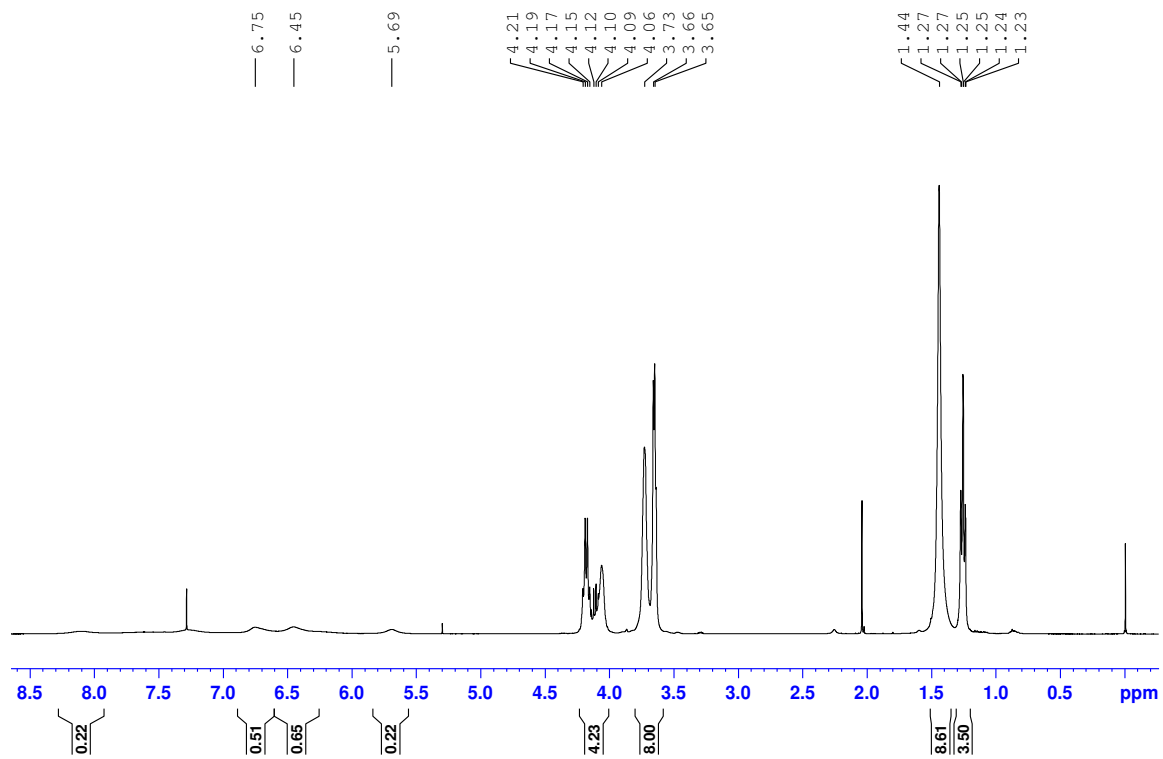


Figure S1: <sup>1</sup>H-NMR of compound 7

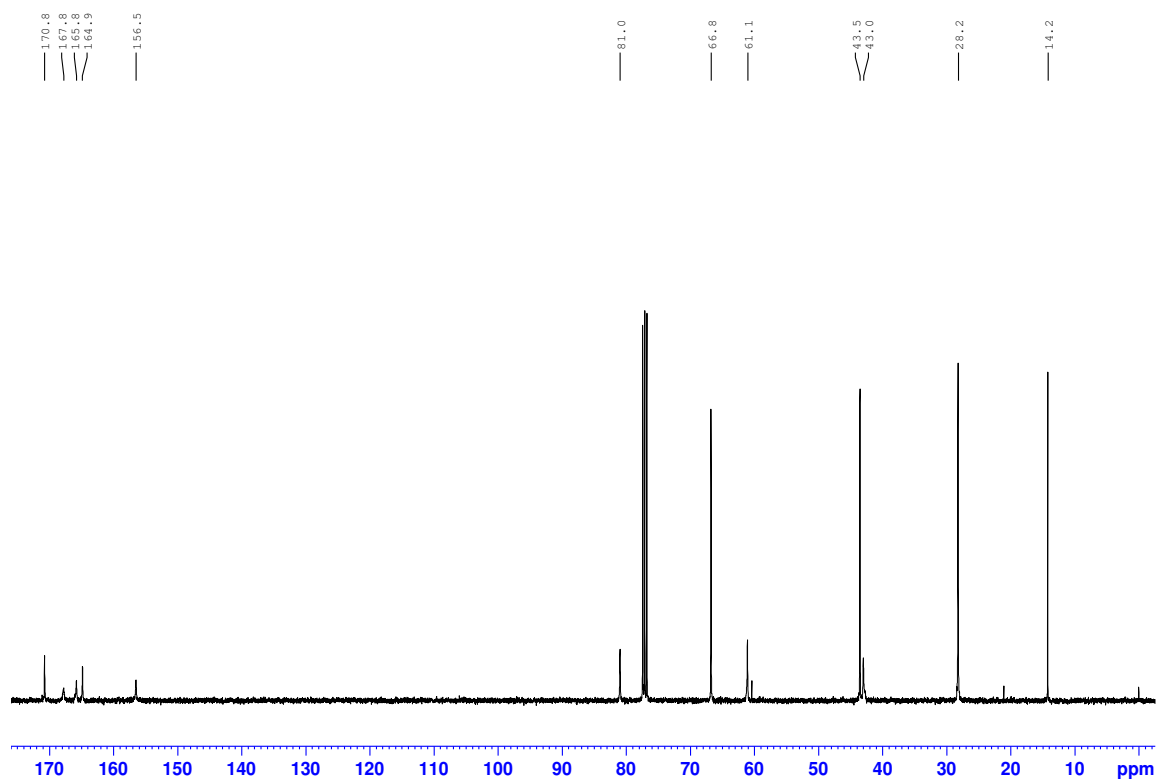
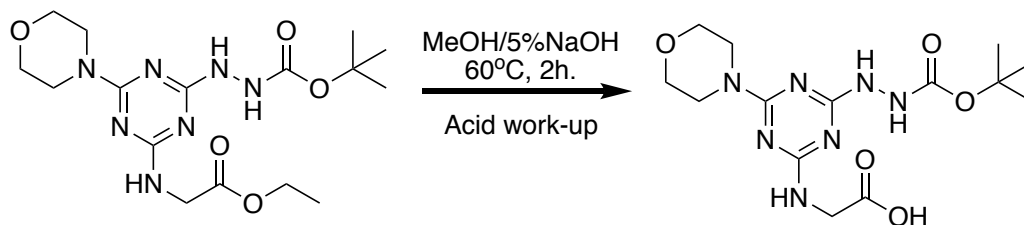


Figure S2: <sup>13</sup>C-NMR of compound 7

### Synthesis of compound **8** (Ester hydrolysis):



Compound **7** (6.5 g, 0.0164 moles) was dissolved in 20 ml MeOH and 10 ml 5% NaOH. The resulting mixture was left to stir at 50°C for 2 hours. MeOH was removed under vacuum and basic extraction was performed to retrieve any impurities and unreacted ester in organic layer. Aq. Layer was brought to acidic pH (5.0) using 1 M HCl was added to precipitate the acid in aq. layer. Solution was filtered to retrieve 5.9 g (98%) of compound **8** as white solid.

Figure S3:  $^1\text{H-NMR}$ ,  $\text{CDCl}_3$ ,  $\delta$  4.15 (2H, 2), 3.85-3.72 (8H, m), 1.47 (9H, s).

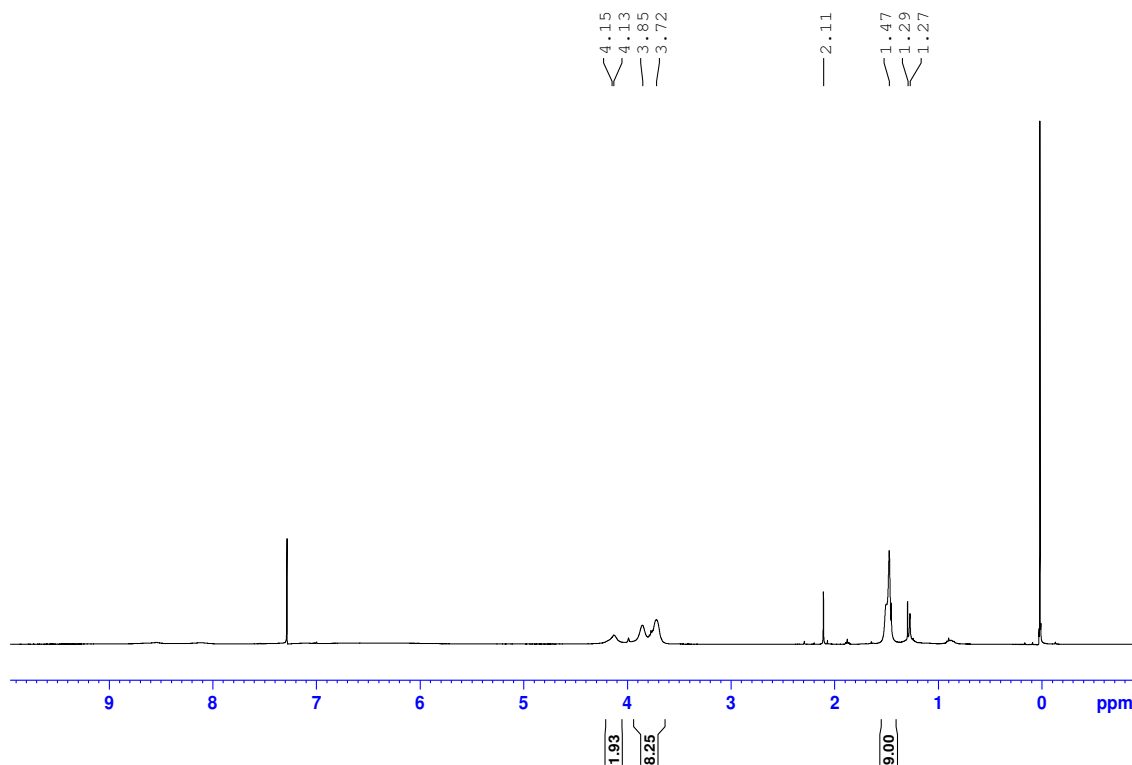
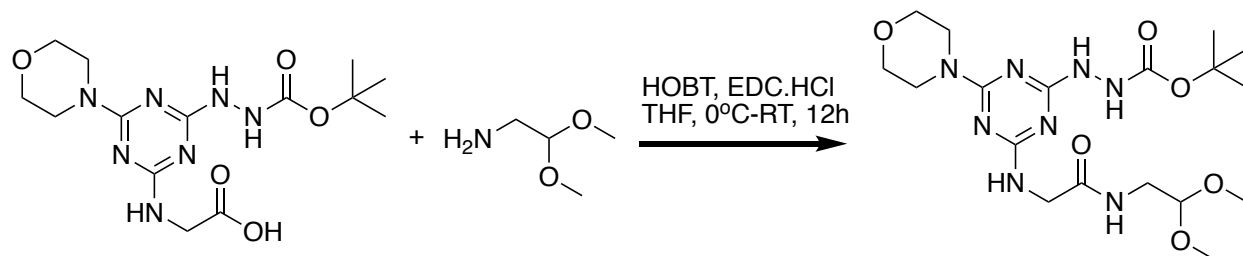


Figure S3:  $^1\text{H-NMR}$  of compound **8**.

### Coupling of **8** with amino acetaldehyde dimethyl acetal, synthesis of **1**:



Compound **8** (1.0 g, 2.7 mmol) was dissolved in a mixture of 10 ml DCM and 5 ml dry DMF in a two-neck flask equipped with stirring bar and purged with Argon. HOBT (0.54 g, 3.5 mmol) and EDC.HCl (0.65 g, 3.6 mmol) were dissolved in 5 ml DMF and added to flask dropwise at 0°C. After stirring the reaction mixture for 10 minutes, 0.35 g of DIPEA was added slowly. After another 10 minutes, amino acetaldehyde dimethyl acetal (3.5 mmol, 0.37 g) was added dropwise to flask. Resulting solution was brought to room temperature and left to stir for 48 hours at room temperature. Excess solvent was removed under vacuum and reaction mixture was washed with water and crude product collected in DCM (x3). Crude product was further purified using column chromatography with 1:1 solvent ratio of Hexane: ethyl acetate to remove the side product of HOBT and then 25:1 solvent ratio of DCM:MeOH to retrieve 610 mg (51%) of **1** as white solid.

Figure S4:  $^1\text{H-NMR}$ ,  $\text{CDCl}_3$ ;  $\delta$  4.15 (2H, 2), 3.98 (2H, s), 3.85-3.72 (8H, m), 3.35 (6H, s), 1.47 (9H, s).

Figure S5:  $^{13}\text{C-NMR}$ ,  $\text{CDCl}_3$ ; 170.7, 167.8, 166.2, 164.9, 156.5, 102.5, 81.3, 66.7, 54.3, 45.1, 43.6, 40.8, 28.2.

Figure S6: MS (ESI/Q-TOF)  $m/z$ :  $[\text{M}+\text{H}]^+$  Calcd for  $\text{C}_{18}\text{H}_{32}\text{N}_8\text{O}_6$  456.24; Found 457.15.



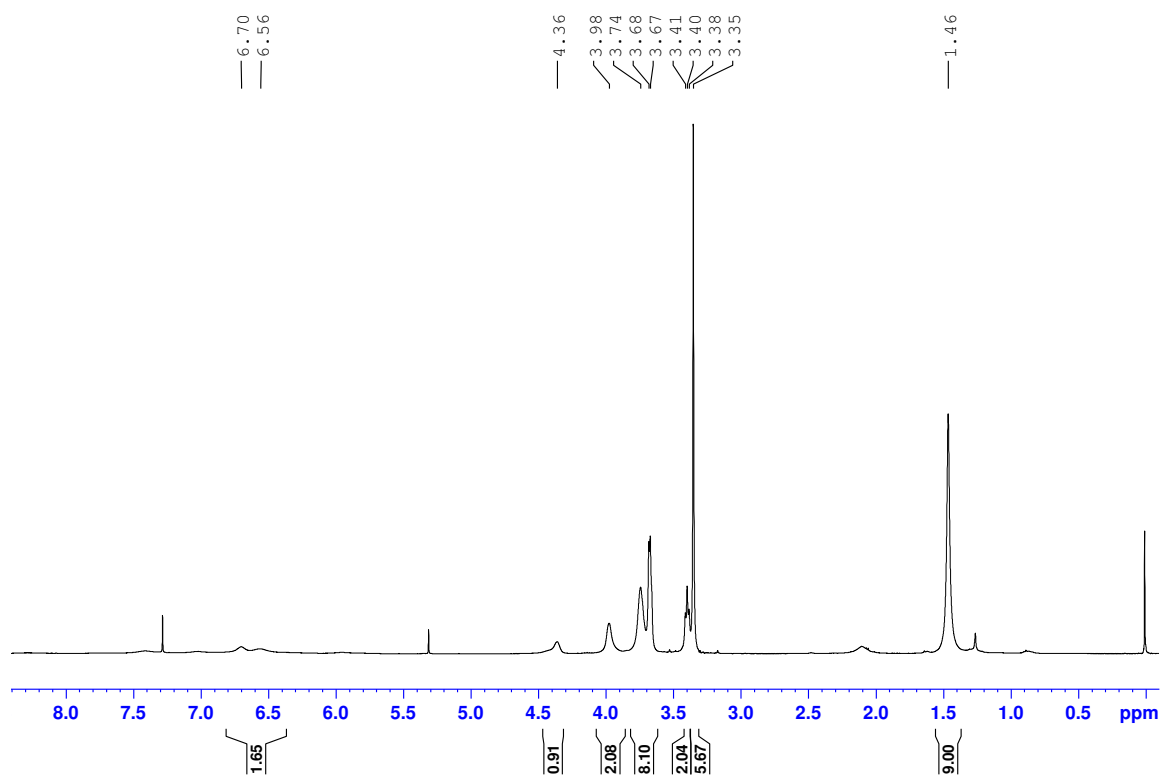


Figure S4:  $^1\text{H}$ -NMR of compound 1.

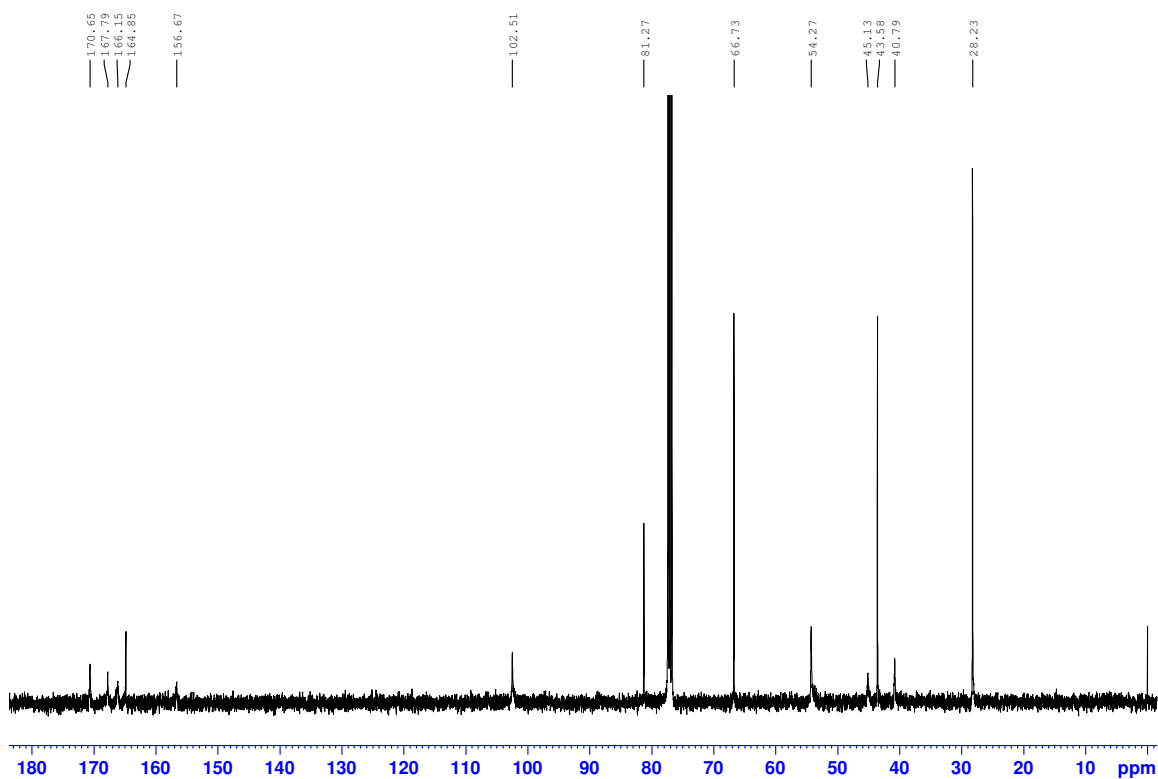


Figure S5:  $^{13}\text{C}$ -NMR of compound **1**.

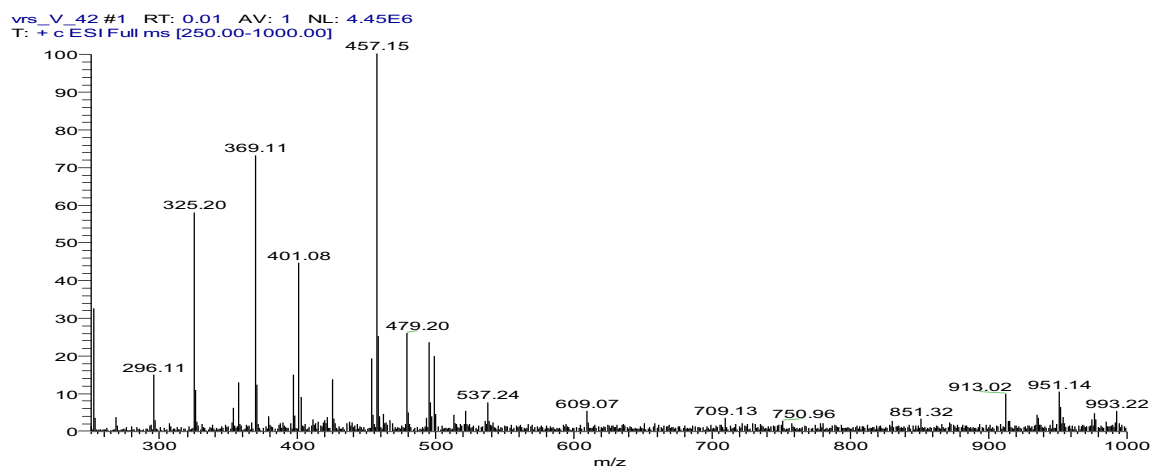
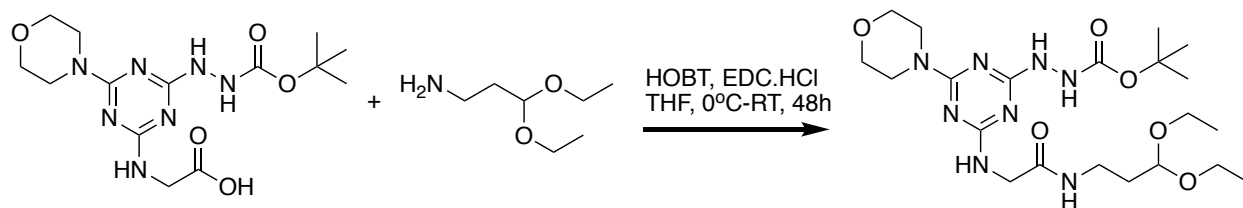


Figure S6: Mass spectrum of compound **1**.

### Coupling of **8** with amino propionaldehyde diethyl acetal, synthesis of **2**:



Compound **8** (1.0 g, 2.7 mmol) was dissolved in a mixture of 10 ml DCM and 5 ml dry DMF in a two-neck flask equipped with stirring bar and purged with Argon. HOBT (0.54 g, 3.5 mmol) and EDC.HCl (0.65 g, 3.6 mmol) dissolved in 5 ml DMF were added to flask dropwise at 0°C. Reaction mixture was left to stir for 10 minutes and 0.35 g of DIPEA was added slowly. After 5 minutes, amino propionaldehyde diethyl acetal (0.52 g, 3.5 mmol) was added dropwise to flask. Resulting solution was brought to room temperature and left to stir for 48 hours at room temperature. Excess solvent was removed under vacuum and reaction mixture was washed with water and crude product collected in DCM (x3). Crude product was further purified using column chromatography with 1:1 solvent ratio of Hexane: ethyl acetate to remove the side product made by HOBT and then 25:1 solvent ratio of DCM:MeOH to retrieve 650 mg (50%) of **2** as white solid.

Figure S7:  $^1\text{H-NMR}$ ,  $\text{CDCl}_3$ ;  $\delta$  4.48 (2H, s), 3.92-3.91 (2H, d), 3.70-3.41 (10H, m), 3.35 (6H, s), 3.39-3.32 (2H, q), 3.32-3.27 (2H, q), 1.78-1.75 (2H, m), 1.42 (9H, s), 1.16-1.12 (3H, t).

Figure S8:  $^{13}\text{C-NMR}$ ,  $\text{CDCl}_3$ ;  $\delta$  170.2, 167.6, 165.9, 164.7, 156.5, 101.7, 81.3, 66.7, 61.6, 45.0, 43.5, 35.3, 33.3, 28.2, 15.2.

Figure S9: MS (ESI/Q-TOF)  $m/z$ :  $[\text{M}+\text{H}]^+$  Calcd for  $\text{C}_{21}\text{H}_{34}\text{N}_8\text{O}_6$  498.29; Found 499.11.

vrs\_V11\_topspot\_100218  
3-6 fractions

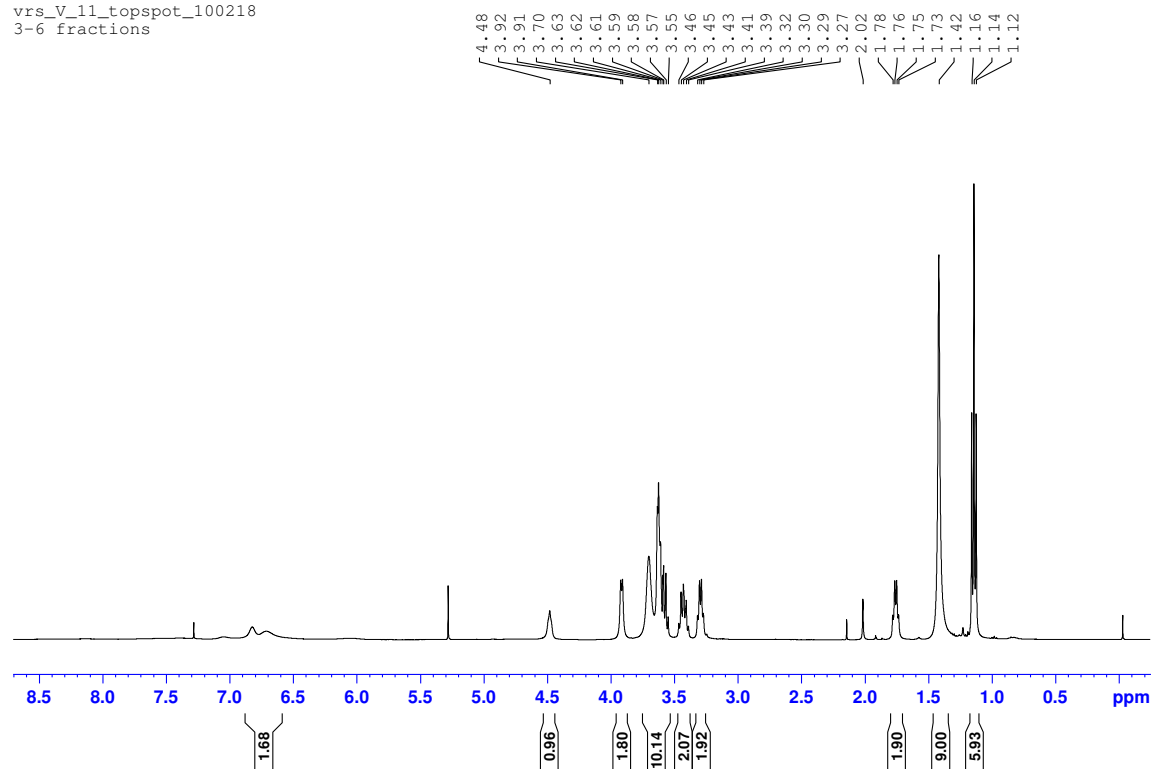


Figure S7:  $^1\text{H}$ -NMR of compound **2**.

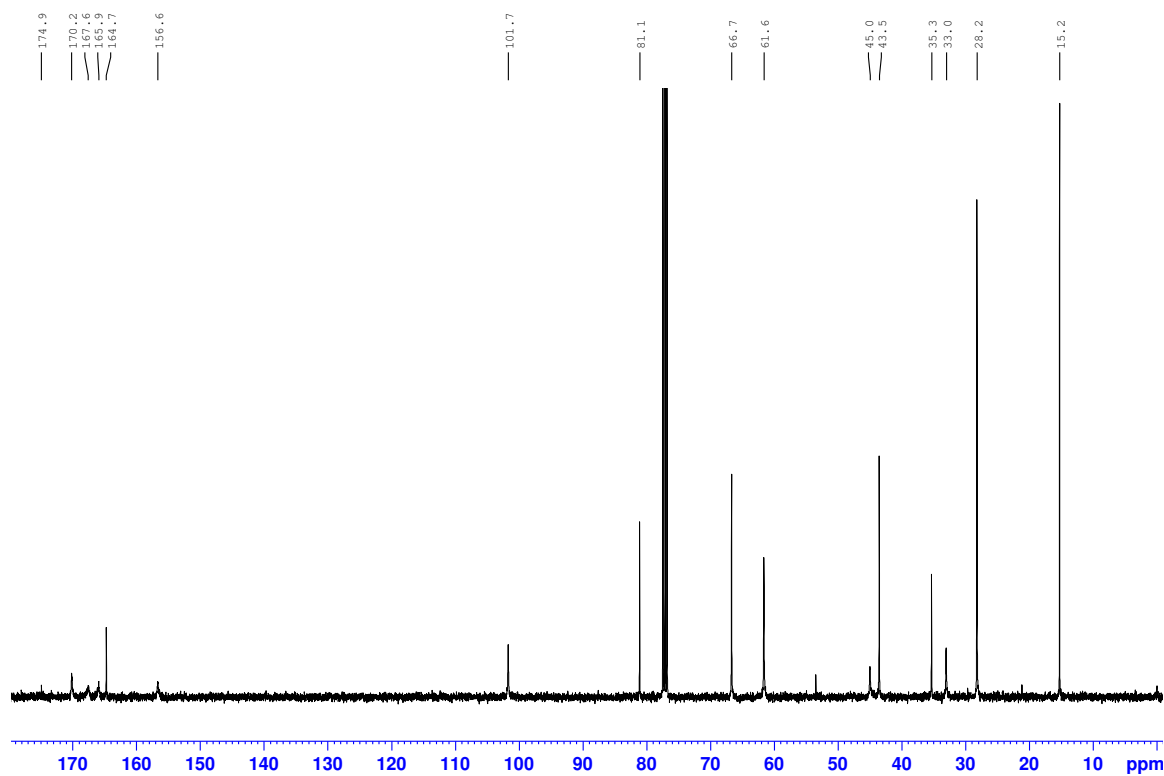


Figure S8: <sup>13</sup>C-NMR of compound 2

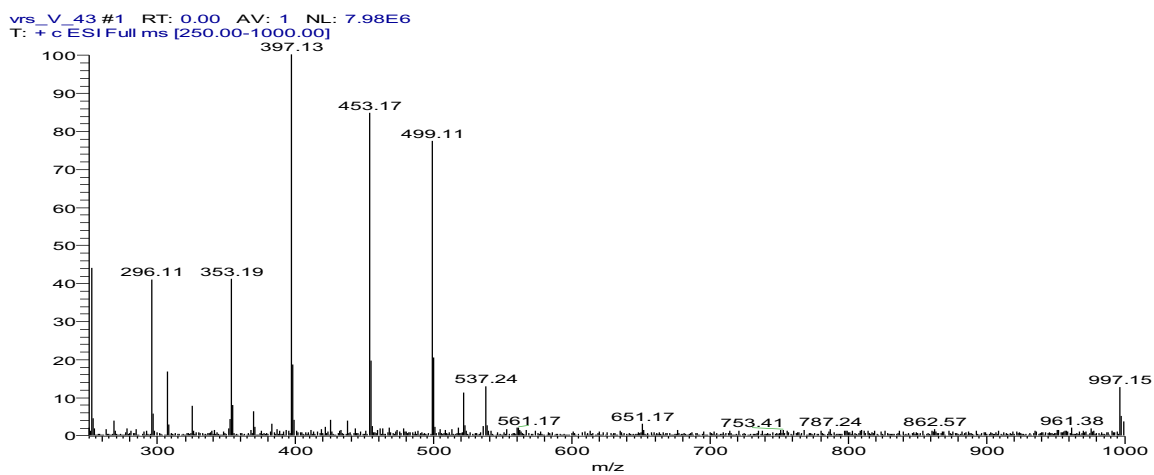
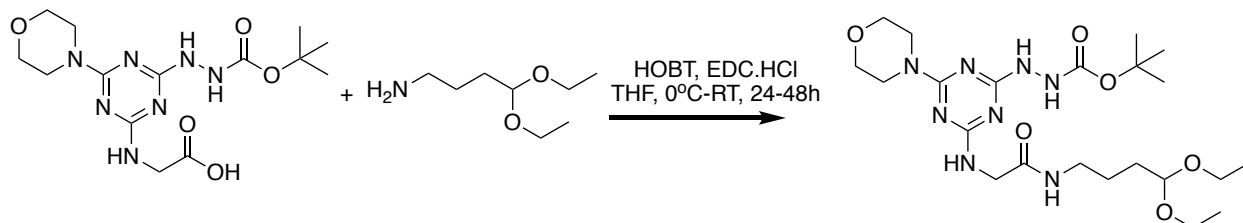


Figure S9: Mass spectrum of compound 2

### Coupling of 8, with 1-amino-4,4-diethoxybutane, synthesis of 4:



Compound **8** (1.0 g, 2.7 mmol) was dissolved in a mixture of 10 ml DCM and 5 ml dry DMF in a two-neck flask equipped with stirring bar and purged with Argon. HOBT (0.54 g, 3.5 mmol) and EDC.HCl (0.65 g, 3.6 mmol) were dissolved in 5 ml DMF and added to flask dropwise at 0°C. Reaction mixture was left to stir for 10 minutes and 0.35 g of DIPEA was added slowly. After stirring the mixture for 10 minutes, amino propionaldehyde diethyl acetal (0.52 g, 3.5 mmol) was added dropwise. Resulting solution was brought to room temperature and left to stir for 30 hours at room temperature. Excess solvent was removed under vacuum and reaction mixture was washed with water and crude product collected in DCM (x3). Crude product was further purified using column chromatography with 1:1 solvent ratio of Hexane: ethyl acetate to remove the side product made by HOBT and then 25:1 solvent ratio of DCM:MeOH to retrieve 550 mg (40%) of **4** as white solid.

Figure S10: <sup>1</sup>H-NMR, CDCl<sub>3</sub>; δ 4.46-4.43 (1H, t), 3.72-3.56 (10H, m), 3.59-3.43 (2H, m), 3.24-3.21 (2H, q), 1.57-1.53 (4H, m), 1.43 (9H, s), 1.18-1.15 (3H, t).

Figure S11: <sup>13</sup>C-NMR, CDCl<sub>3</sub>; δ 170.2, 167.6, 166.1, 164.8, 156.8, 102.5, 81.2, 66.7, 61.2, 46.0, 45.0, 39.0, 30.9, 28.2, 24.7, 24.7, 15.3.

Figure S12: MS (ESI/Q-TOF) m/z: [M+H]<sup>+</sup> Calcd for C<sub>22</sub>H<sub>40</sub>N<sub>8</sub>O<sub>6</sub> 512.31; Found 513.15.

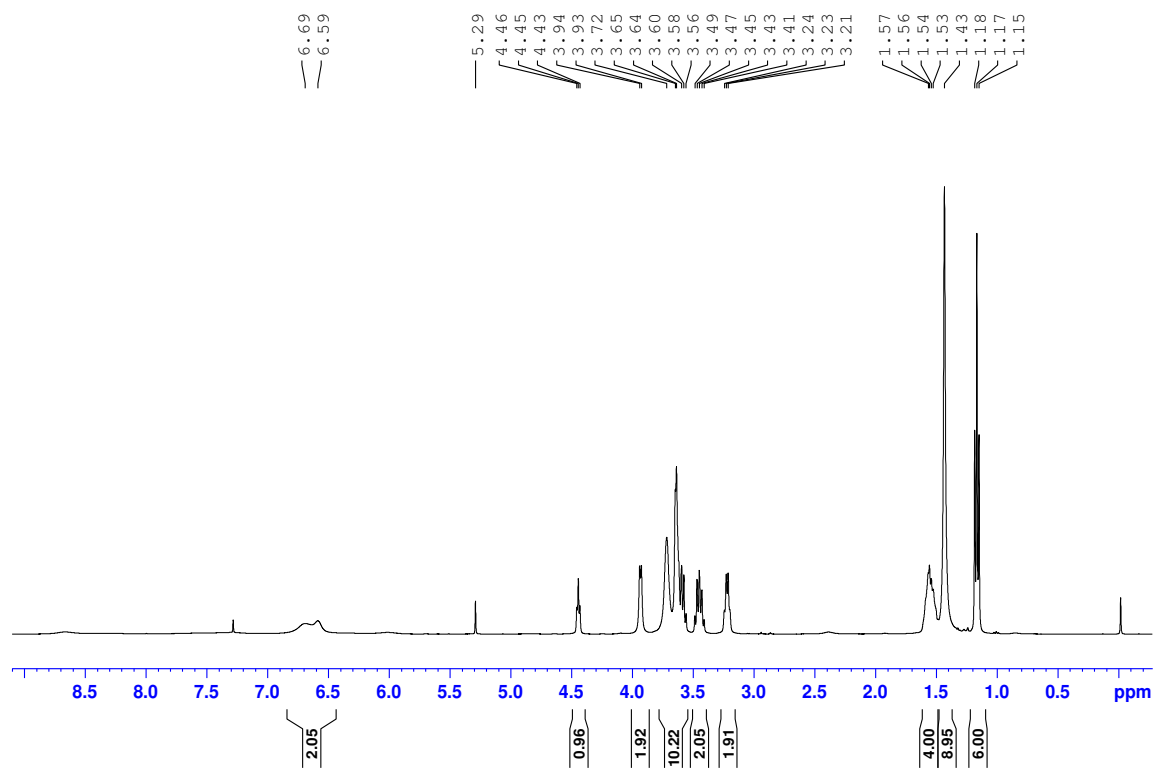


Figure S10:  $^1\text{H}$ -NMR of compound **4**

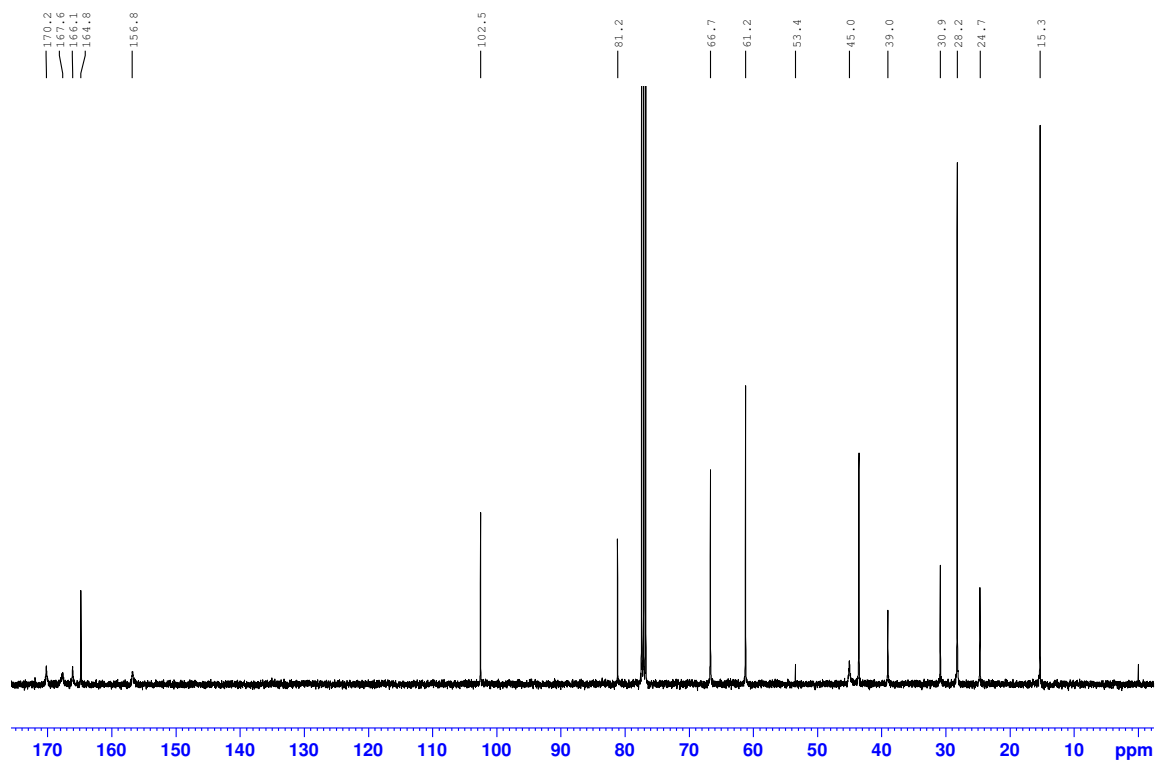


Figure S11:  $^{13}\text{C}$ -NMR of compound 4

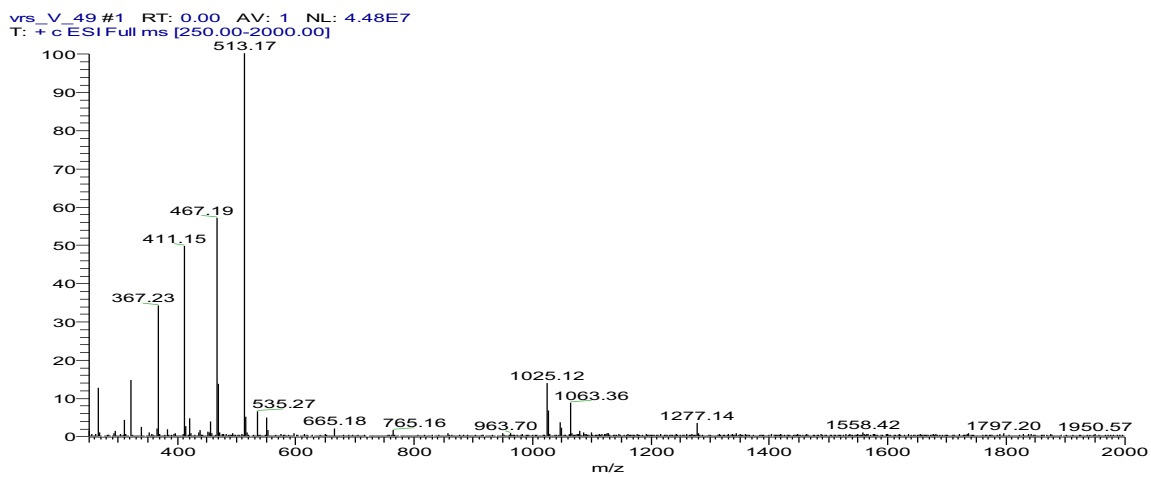
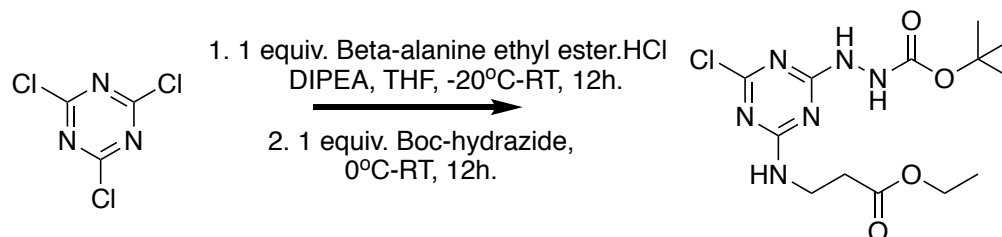


Figure S12: Mass spectrum of compound 4



### Synthesis of **11** from Triazine Chloride:



In a three neck round bottom flask equipped with stirring bar and purged with argon, Cyanuric chloride (3.00 g, 0.016 moles) was dissolved in 40 ml dry THF. Beta-alanine ethyl ester hydrochloride salt (2.45 g, 0.016 moles) and DIPEA (4.3 g, 0.032 moles) were dissolved in a mixture of 2 ml MeOH and 5 ml THF and then added to flask at -20°C. Reaction mixture was brought to room temperature slowly and left to stir overnight. The reaction progress was monitored via TLC. Boc-hydrazide (2.11 g, 0.016 moles) was dissolved in 5 ml THF and then added dropwise to the reaction mixture at 0°C. Temperature of the resulting solution was raised slowly to room temperature and left to stir over night. After confirming the completion of reaction via TLC, excess solvent was removed under vacuum and crude product was extracted with DCM/H<sub>2</sub>O and collected in DCM (x3). Column chromatography was performed using 1:1 mixture of Hexane and ethyl acetate to afford 5.4 g (94%) of **11** as white solid.

Figure S13: <sup>1</sup>H-NMR, CDCl<sub>3</sub>, δ 4.16-4.11 (2H, q), 3.69-3.68 (2H, m), 2.62-2.60 (2H, t), 1.45 (9H, s), 1.27-1.22 (3H, t).

Figure S14: <sup>13</sup>C-NMR, CDCl<sub>3</sub>, δ 172.0, 168.4, 167.5, 165.7, 159.5, 81.7, 60.8, 36.6, 33.6, 28.1, 14.2.

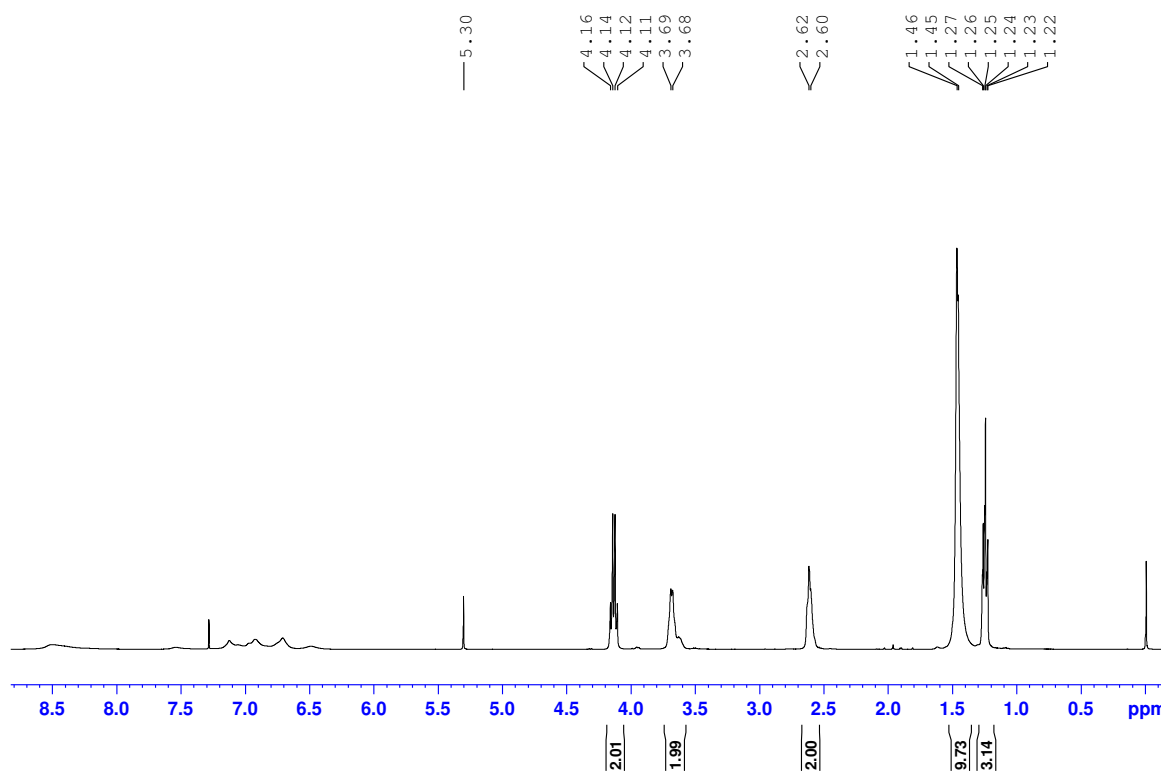


Figure S13: <sup>1</sup>H-NMR of compound **11**

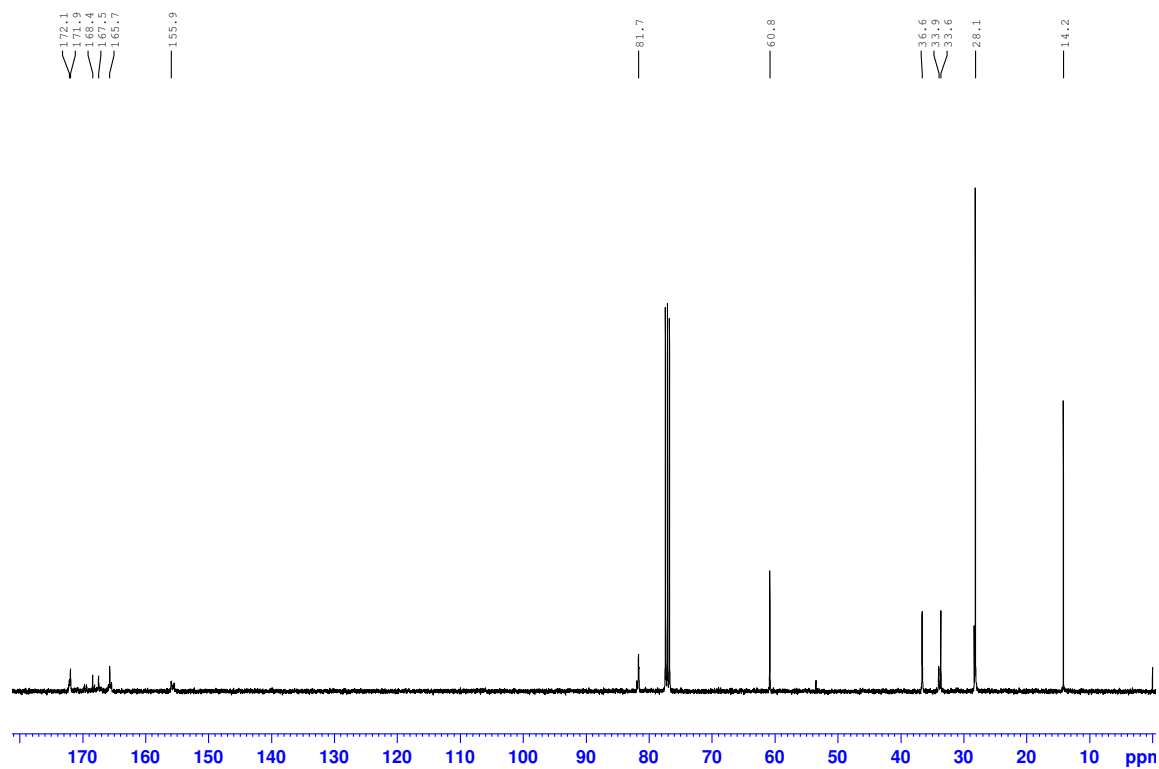
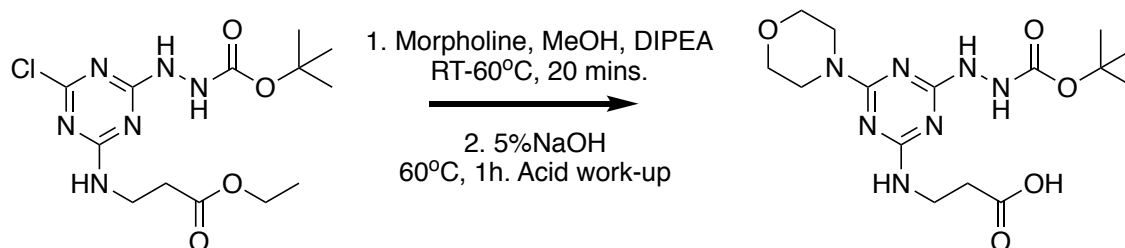


Figure S14: <sup>13</sup>C-NMR of compound **11**

### Synthesis of **12** from **11**:



Compound **11** (2.8 g, 0.0078 moles) was dissolved in methanol in a small flask equipped with stirring bar. To this, morpholine (810 mg, 0.00934 moles) and DIPEA (1.0 g) were added to flask. Reaction mixture was heated to 60°C and left to stir for 15 minutes. Reaction progress was monitored via TLC. After the completion of reaction 5 ml of 5% NaOH was added to reaction mixture and left to stir for one hour. Methanol was removed under vacuum and crude was extracted to remove any impurities in DCM solvent. Aqueous layer was acidified by adding acetic acid until product (acid) precipitated in the solution. Solution was filtered, washed with cold water, and dried under vacuum to retrieve 2.75 g (92%) of **12** as white solid.

Figure S15:  $^1\text{H-NMR}$ ,  $\text{CDCl}_3/\text{CD}_3\text{OD}$ ,  $\delta$  3.77-3.55 (10H, m), 2.44 (2H, m), 1.39 (9H, s).

Figure S16:  $^{13}\text{C-NMR}$ ,  $\text{CDCl}_3/\text{CD}_3\text{OD}$ ,  $\delta$  179.3, 163.0, 158.0, 155.6, 81.3, 66.5, 43.9, 36.5, 34.7, 28.0.

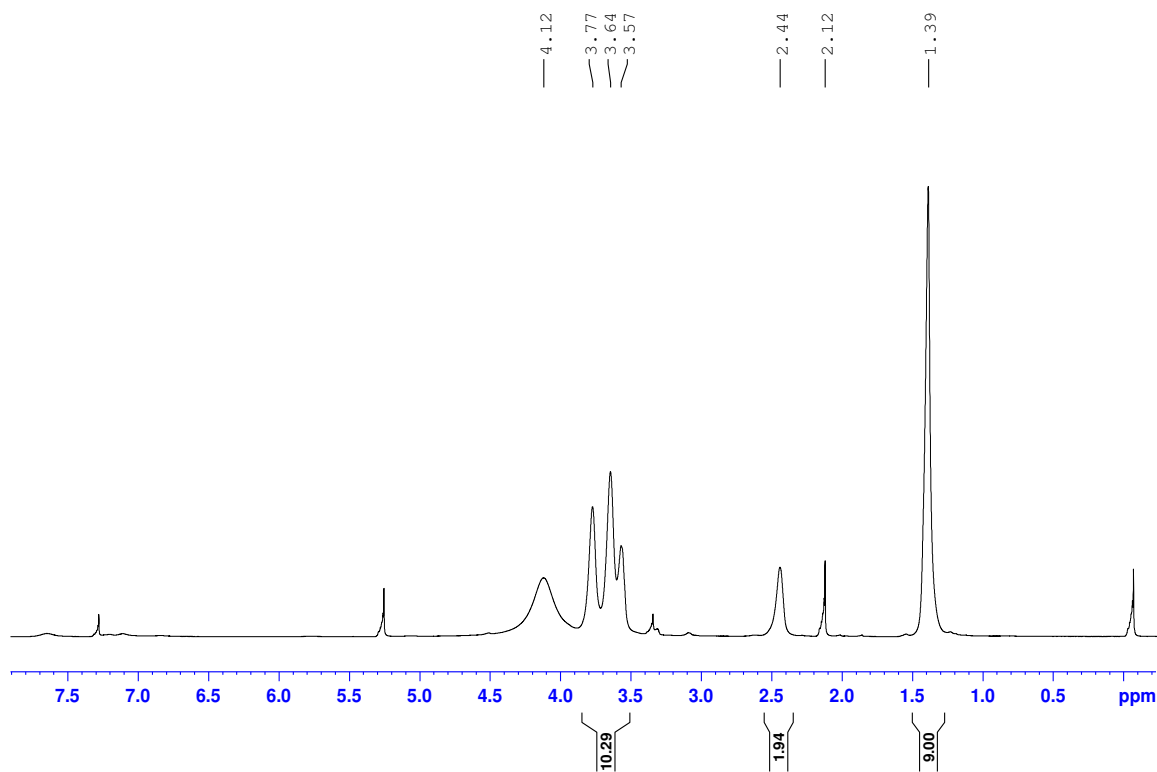


Figure S15:  $^1\text{H}$ -NMR of compound **12**

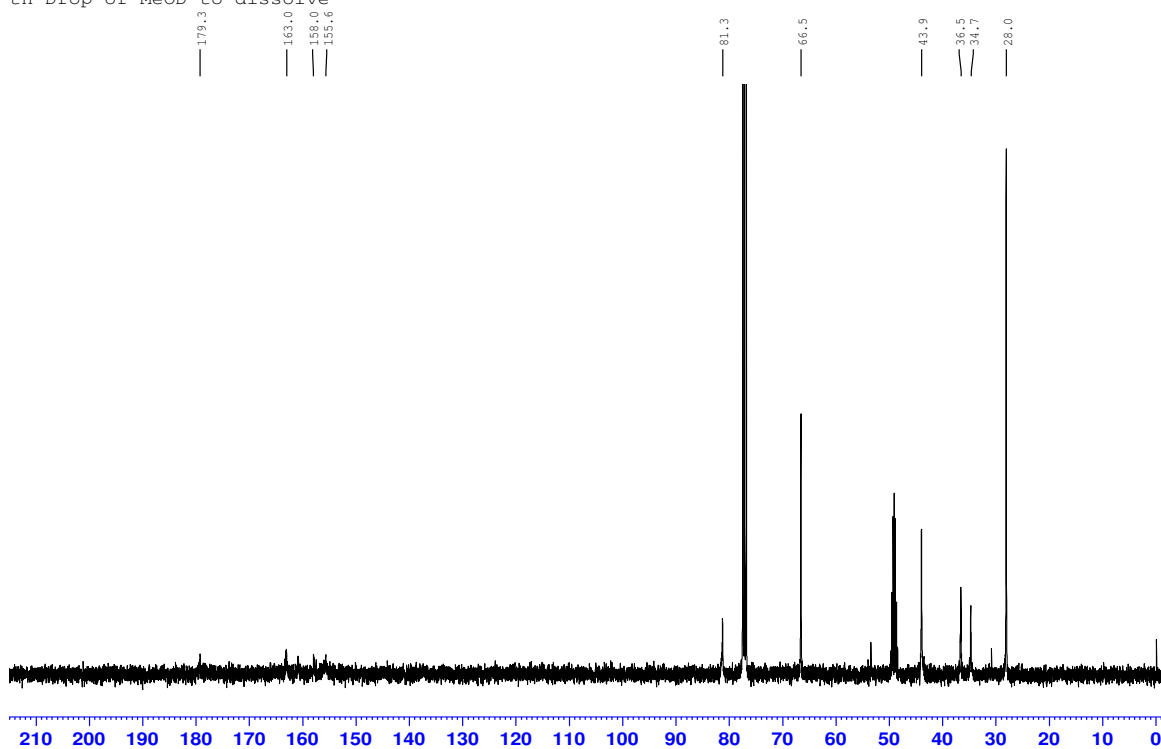
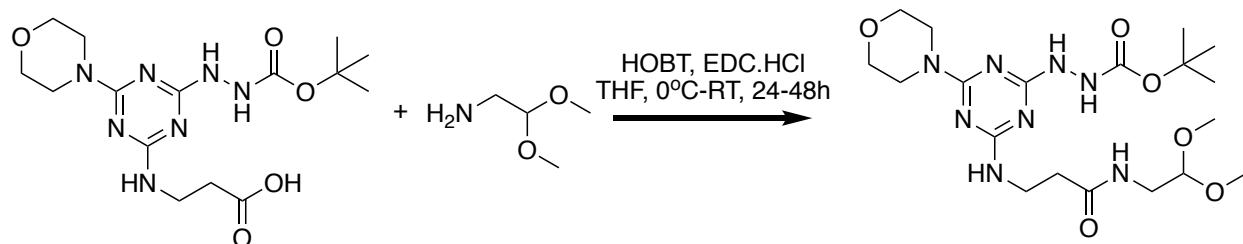


Figure S16:  $^{13}\text{C}$ -NMR of compound **12**

**Coupling of acid, 12, with amino acetaldehyde dimethyl acetal, synthesis of 3:**



Compound **12** (1.0 g, 2.6 mmol) was dissolved in a mixture of 15 ml DCM and 3 ml dry DMF in a two-neck flask equipped with stirring bar and purged with Argon. HOBT (0.52 g, 3.4 mmol) was added to flask dropwise at 0°C. Reaction mixture was left to stir for 10 minutes and then EDC.HCL (0.65, 3.4 mmol) was added. After stirring for 10 minutes, amino acetaldehyde dimethyl acetal (0.33 g, 3.1 mmol) was added dropwise to flask at 0°C. Resulting solution was brought to room temperature slowly and left to stir for 36 hours. Excess solvent was removed under vacuum and reaction mixture was washed with 0.5 M acetic acid and crude product collected in DCM (x3). Crude product was then further washed with saturated NaHCO<sub>3</sub> to remove any unreacted acid intermediate. Crude product was further purified using column chromatography with 1:1 solvent ratio of Hexane: ethyl acetate to remove the side product made by HOBT and then 25:1 solvent ratio of DCM:MeOH to retrieve **3** (580 mg, 56%) as white solid.

Figure S17: <sup>1</sup>H-NMR, CDCl<sub>3</sub>; δ 4.38 (1H, t), 3.72-3.68 (10H, m), 3.39 (8H, m), 2.50-2.47 (2H, t), 1.47 (9H, s).

Figure S18: <sup>13</sup>C-NMR, CDCl<sub>3</sub>; 171.8, 165.0, 156.5, 102.7, 81.1, 66.8, 54.4, 43.5, 40.9, 36.9, 28.2.

Figure S19: MS (ESI/Q-TOF) m/z: [M+H]<sup>+</sup> Calcd for C<sub>19</sub>H<sub>34</sub>N<sub>8</sub>O<sub>6</sub> 470.26; Found 471.18.

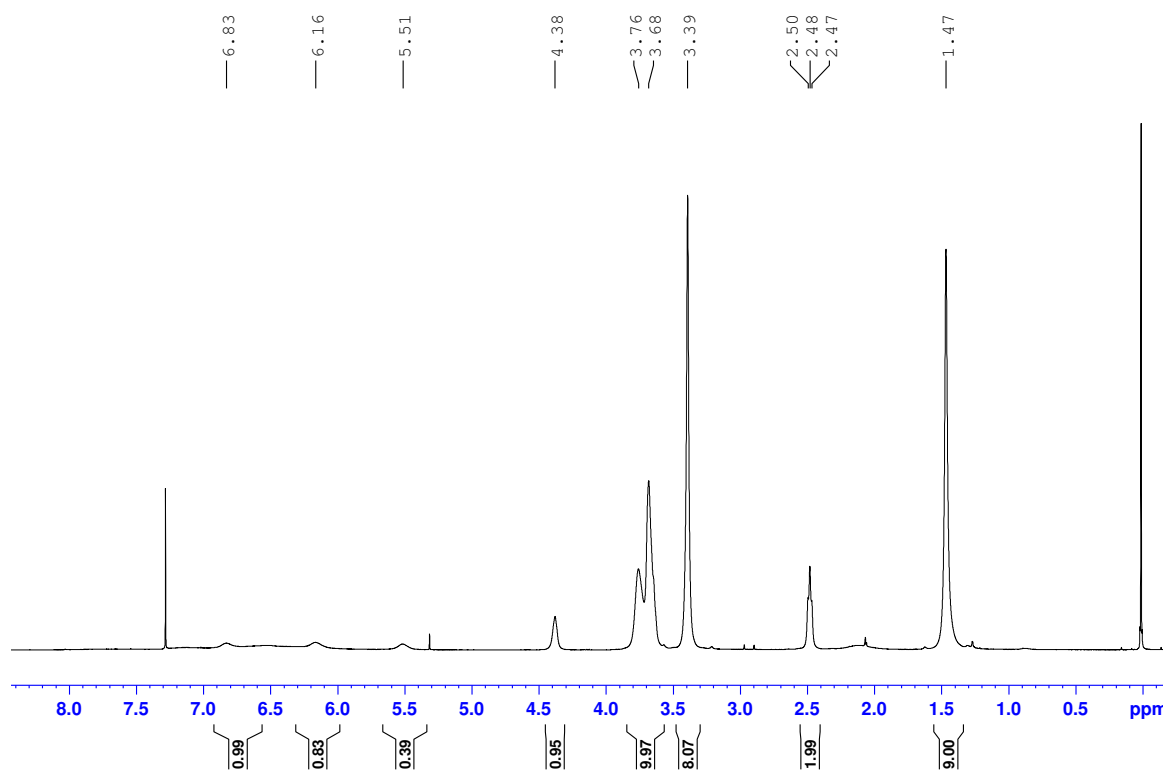


Figure S17: <sup>1</sup>H-NMR of compound **3**.

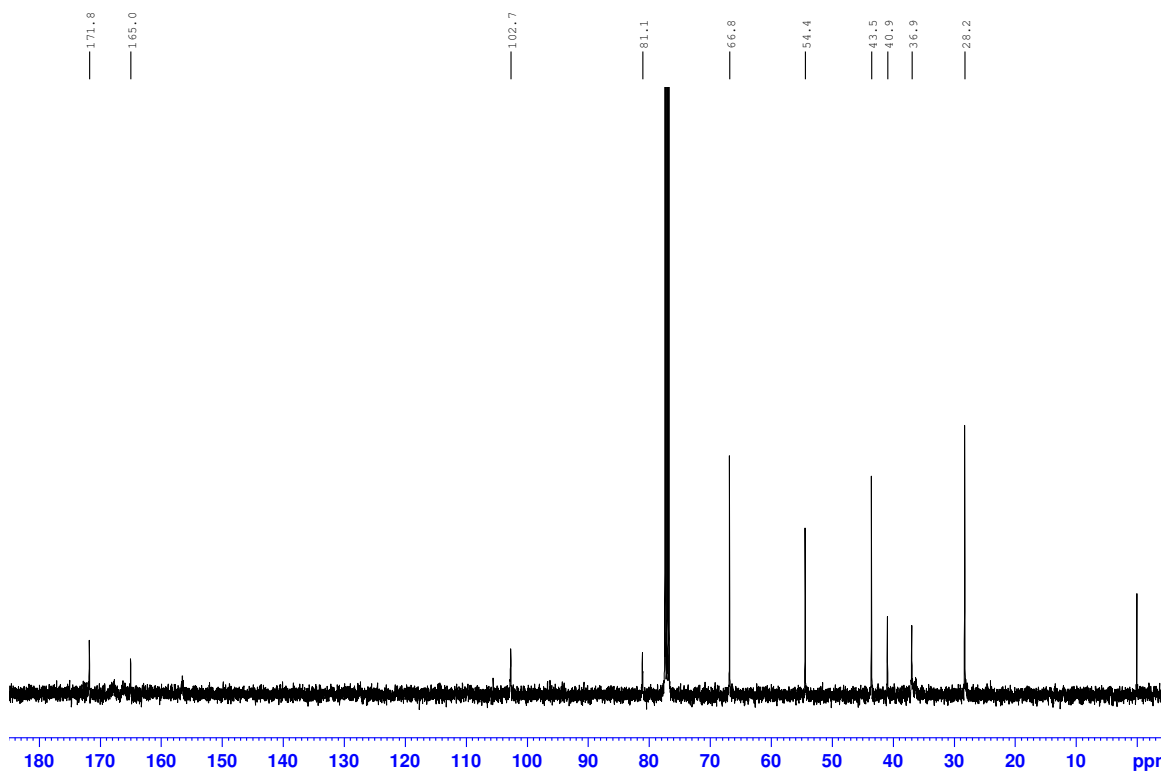


Figure S18: <sup>13</sup>C-NMR of compound **3**

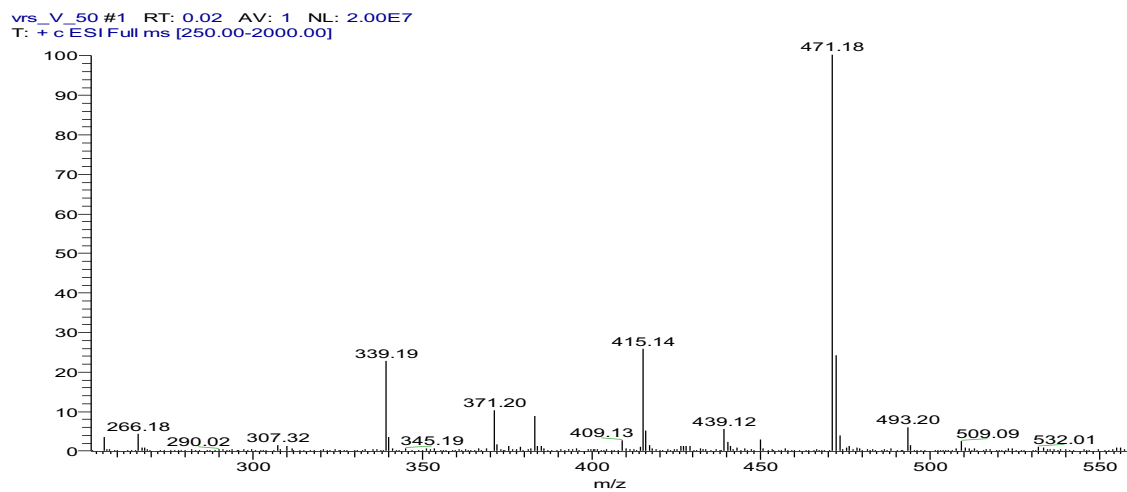
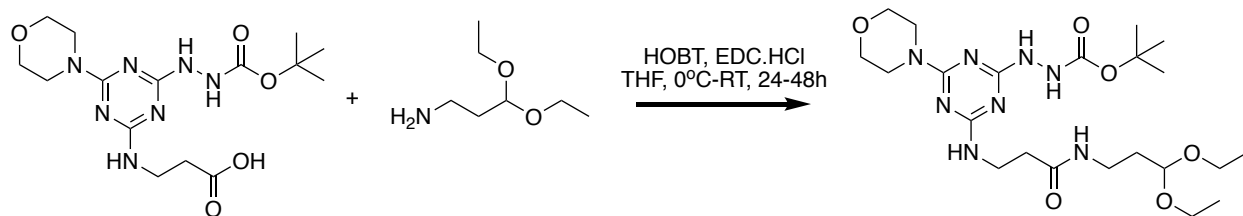


Figure S19: Mass spectrum of compound **3**

**Coupling of acid, 12, with amino propionaldehyde diethyl acetal, synthesis of 5:**



Compound **12** (700 mg, 1.8 mmoles) was dissolved in a mixture of 12 ml DCM and 3 ml dry DMF in a two-neck flask equipped with stirring bar and purged with Argon. HOBT (0.29 g, 1.9 mmoles) was added to flask dropwise at  $0^\circ\text{C}$ . Reaction mixture was left to stir for 10 minutes and then EDC.HCl (0.36 g, 1.9 mmoles) was added. After another 10 minutes, amino propionaldehyde diethyl acetal (0.18g, 1.9 mmoles) was added dropwise to flask at  $0^\circ\text{C}$ . Resulting solution was brought to room temperature slowly and left to stir for 24 hours at room temperature. Excess solvent was removed under vacuum and reaction mixture was washed with 0.5 M acetic acid and crude product collected in DCM (x3). Crude product was then further washed with saturated  $\text{NaHCO}_3$  to remove any unreacted acid intermediate. Crude product was further purified using column chromatography with 1:1 solvent ratio of Hexane: ethyl acetate to remove the side product made by HOBT and then 25:1 solvent ratio of DCM:MeOH to retrieve **5** (510 mg, 55%) as white solid.

Figure S20:  $^1\text{H}$ -NMR,  $\text{CDCl}_3$ ;  $\delta$  4.55 (1H, t), 3.76-3.63 (12H, m), 3.51-3.48 (2H, q), 3.34-3.31 (2H, q), 2.45-2.42 (2H, t), 1.81-1.80 (2H, q), 1.46 (9H, s), 1.22-1.19 (3H, t).

Figure S21:  $^{13}\text{C}$ -NMR,  $\text{CDCl}_3$ ; 171.3, 165.0, 156.6, 102.2, 81.0, 66.8, 61.8, 43.5, 37.0, 36.3, 35.5, 32.8, 28.2, 15.3.

Figure S22: MS (ESI/Q-TOF) m/z:  $[\text{M}+\text{H}]^+$  Calcd for  $\text{C}_{22}\text{H}_{40}\text{N}_8\text{O}_6$  512.31; Found 513.17.



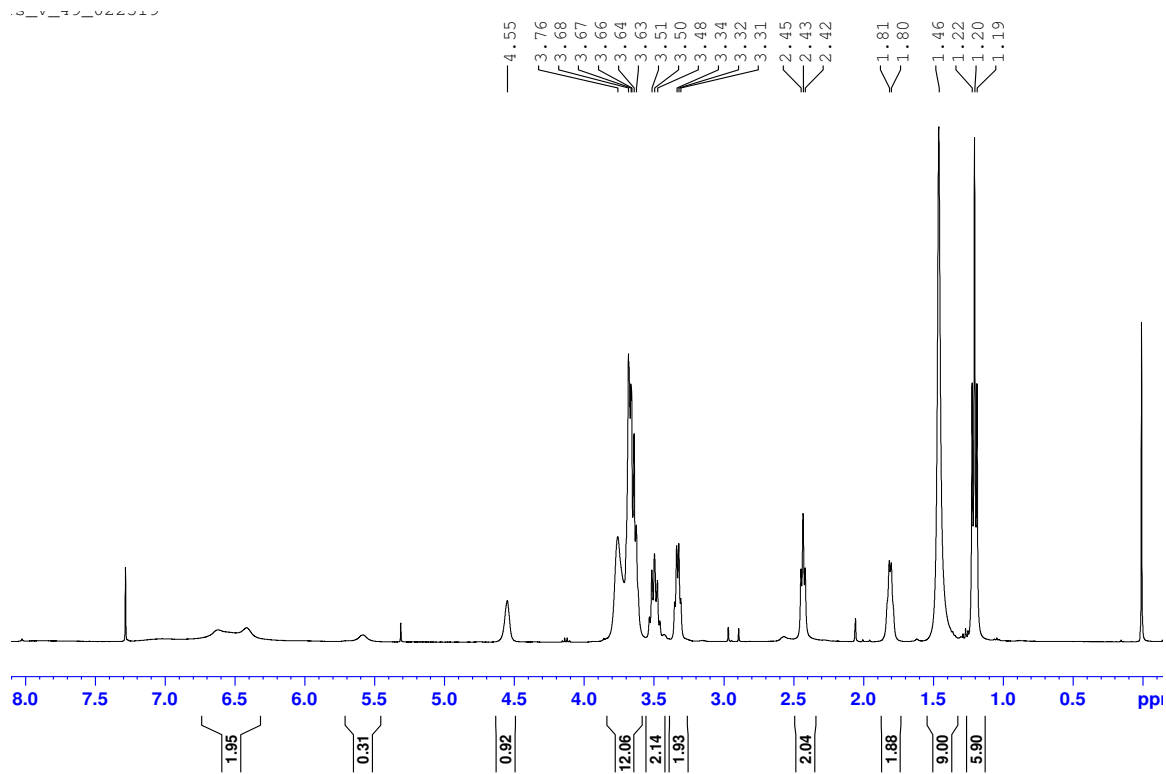


Figure S20:  $^1\text{H}$ -NMR of compound **5**

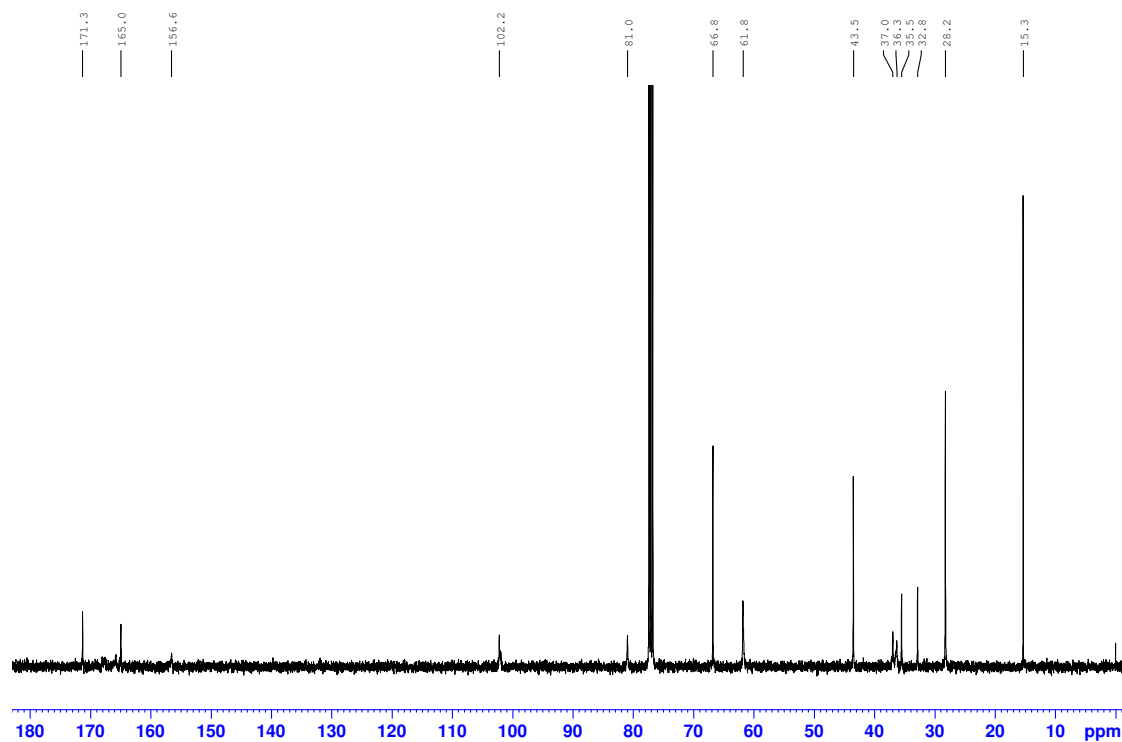


Figure S21:  $^{13}\text{C}$ -NMR of compound **5**

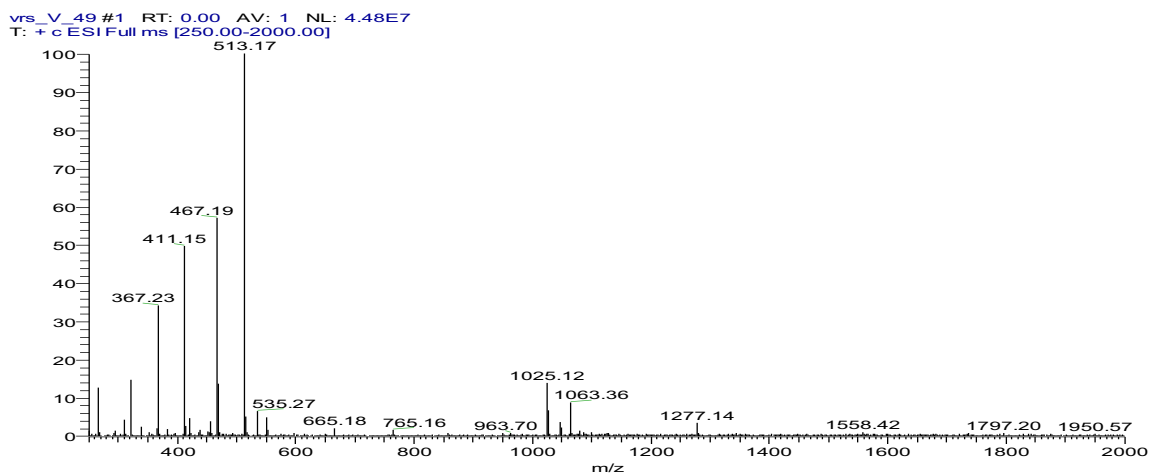
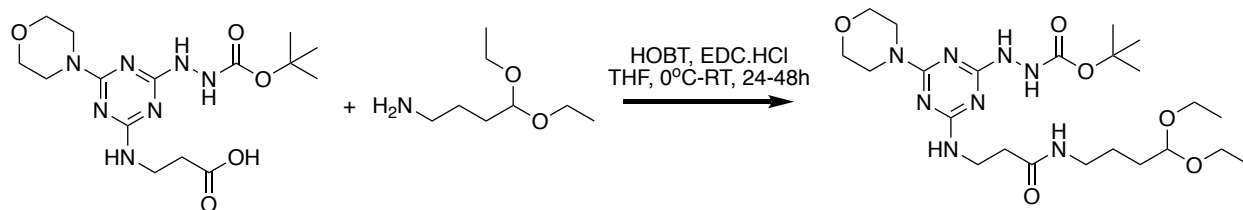


Figure S22: Mass spectrum of compound **5**

**Coupling of acid, 12, with 1-amino-4,4-diethoxybutane, synthesis of 6:**



Compound **12** (920 mg, 2.4 mmol) was dissolved in a mixture of 15 ml DCM and 3 ml dry DMF in a two-neck flask equipped with stirring bar and purged with Argon. HOBT (0.44 g, 2.8 mmol) was added to flask dropwise at 0°C and resulting reaction mixture was left to stir for 10 minutes. Then, EDC.HCl (2.8 mmol, 0.55 g) was added. After 10 minutes, amino butanaldehyde diethyl acetal (2.8 mmol, 0.47 g) was added dropwise to flask at 0°C. Resulting solution was brought to room temperature slowly and left to stir for 24 hours at room temperature. Excess solvent was removed under vacuum and reaction mixture was washed with 0.5 M acetic acid and crude product collected in DCM (x3). Crude product was then further washed with saturated NaHCO<sub>3</sub> to remove any unreacted acid intermediate. Crude product was further purified using column chromatography with 1:1 solvent ratio of Hexane: ethyl acetate to remove the side product made by HOBT and then 25:1 solvent ratio of DCM:MeOH to retrieve **6** (520 mg, 42%) as white solid.

Figure S23: <sup>1</sup>H-NMR, CDCl<sub>3</sub>; δ 4.48-4.45 (1H, t), 3.76-3.60 (12H, m), 3.50-3.44 (2H, m), 3.21-3.20 (2H, q), 2.44-2.41 (2H, t), 1.61-1.59 (2H, m), 1.56-1.54 (2H, m), 1.45 (9H, s), 1.21-1.18 (3H, t).

Figure S24: <sup>13</sup>C-NMR, CDCl<sub>3</sub>; 171.7, 167.6, 166.6, 165.7, 164.9, 156.7, 102.6, 80.8, 66.8, 61.2, 43.5, 39.2, 37.0, 36.3, 31.0, 28.2, 24.5, 15.3.

Figure S25: MS (ESI/Q-TOF) m/z: [M+H]<sup>+</sup> Calcd for C<sub>23</sub>H<sub>42</sub>N<sub>8</sub>O<sub>6</sub> 526.31; Found 527.17.

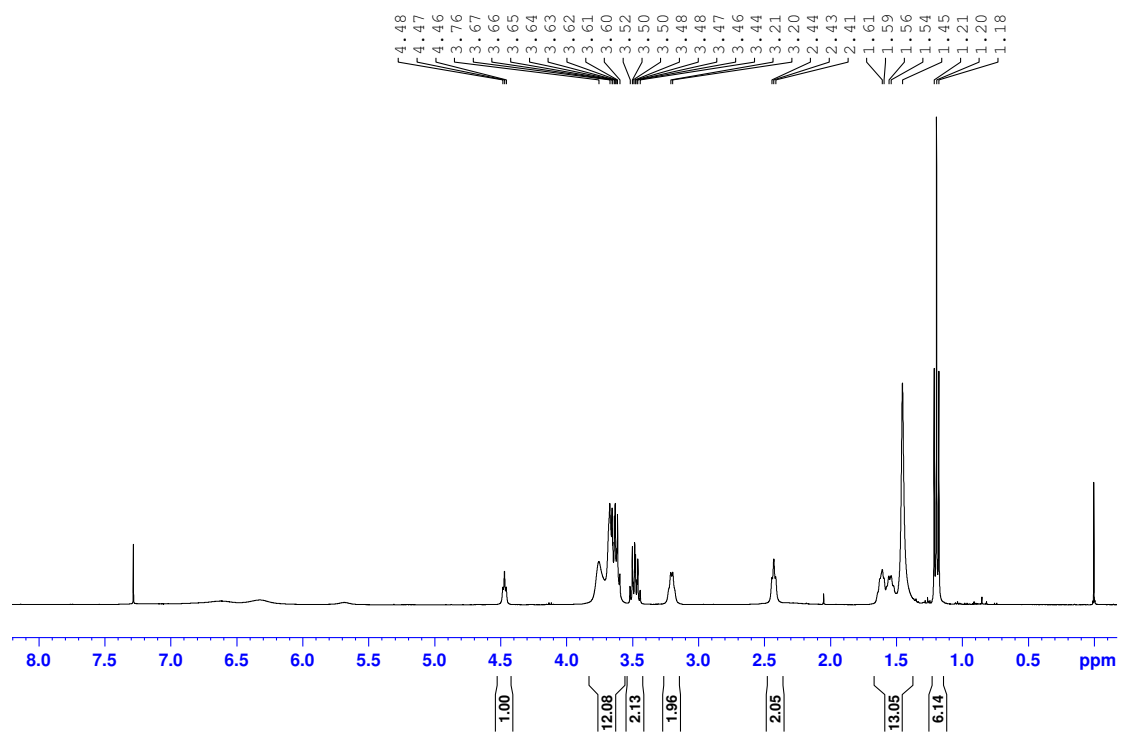


Figure S23:  $^1\text{H}$ -NMR of compound **6**.

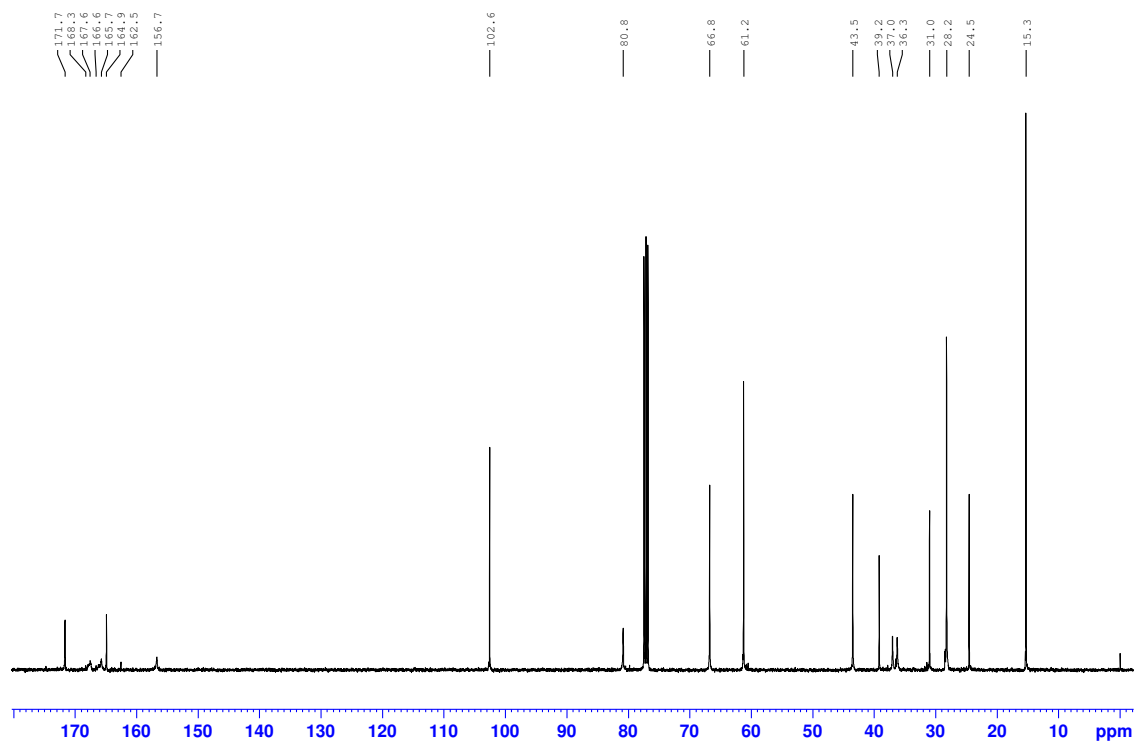


Figure S24:  $^{13}\text{C}$ -NMR of compound **6**.

vrs\_V\_57 #1 RT: 0.01 AV: 1 NL: 9.72E7  
T: + c ESI Full ms [150.00-2000.00]

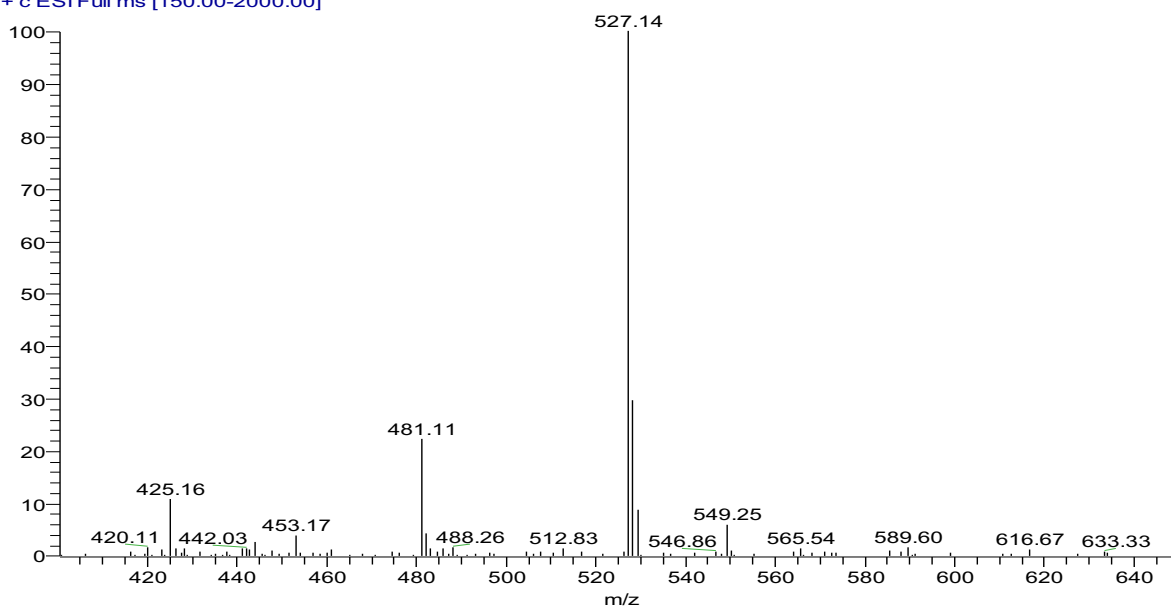
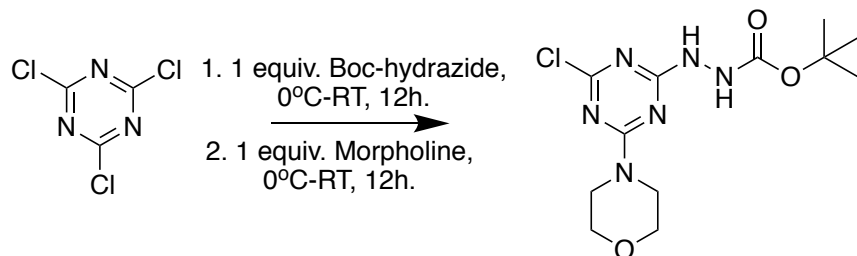


Figure S25: Mass spectrum of compound **6**.

### Synthesis of **13** from Triazine Chloride



In a small three neck flask equipped with stirring bar and purged with argon, 1.0 g (5.4 mmoles) of cyanuric chloride was dissolved in 30 ml THF. Two equivalent DIPEA (1.40 g, 10.8 mmoles) was added to the solution. One equivalent *tert*-butyl carbazate (0.72 g, 5.4 mmoles) dissolved in 10 ml THF was added to reaction mixture at 0°C and resulting solution was brought to room temperature slowly and left to stir overnight at room temperature. Reaction progress was monitored by TLC. Once the reaction was complete, one equivalent morpholine (0.473 g, 5.4 mmoles) was added dropwise to reaction solution at -30°C. Resulting solution was brought to room temperature and left to stir overnight. Excess solvent was removed under vacuum, re-dissolved in DCM, washed with water, dried over brine, and finally chromatographic purifications were done using 2:1 hexane: ethyl acetate solution to afford **13** (1.61 g, 91%) as white solid.

Figure S26: <sup>1</sup>H-NMR, CDCl<sub>3</sub>; δ 7.97 (1H, s), 6.55 (1H, s), 3.82-3.71 (8H, m), 1.47 (9H, s).

Figure S27: <sup>13</sup>C-NMR, CDCl<sub>3</sub>; 169.3, 167.3, 155.4, 81.6, 66.5, 44.0, 39.2, 28.2.

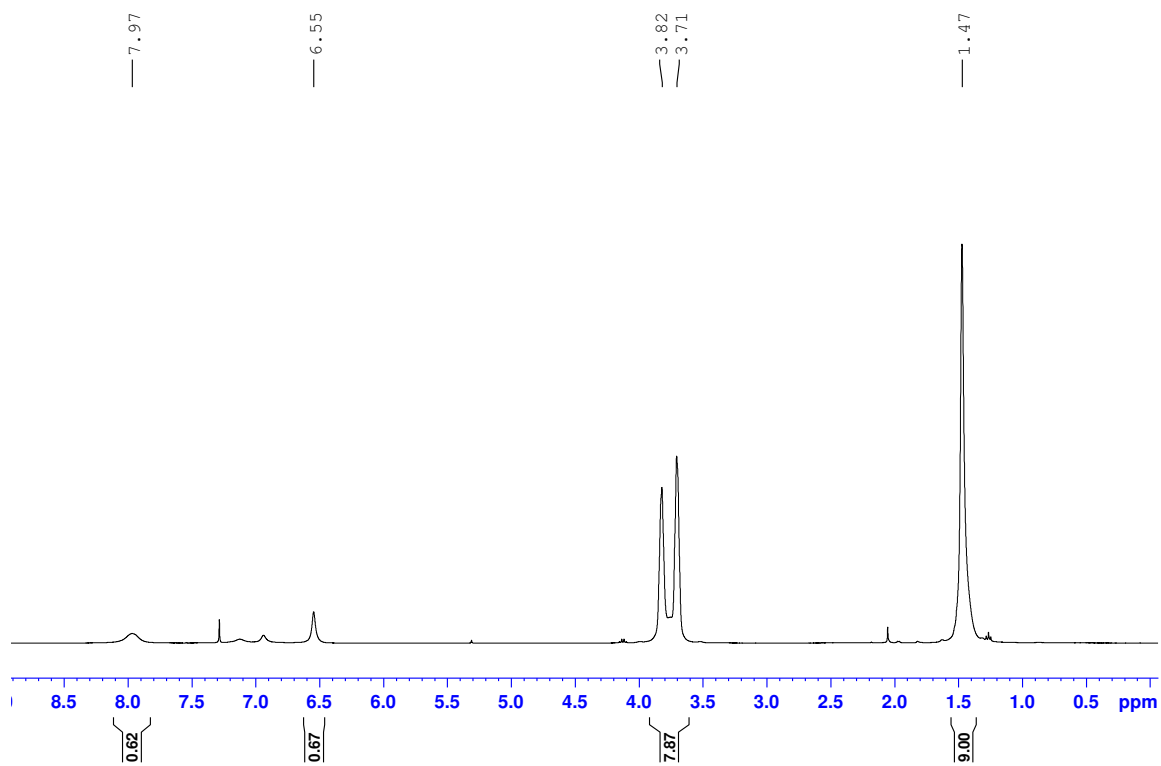


Figure S26: <sup>1</sup>H-NMR of **13**

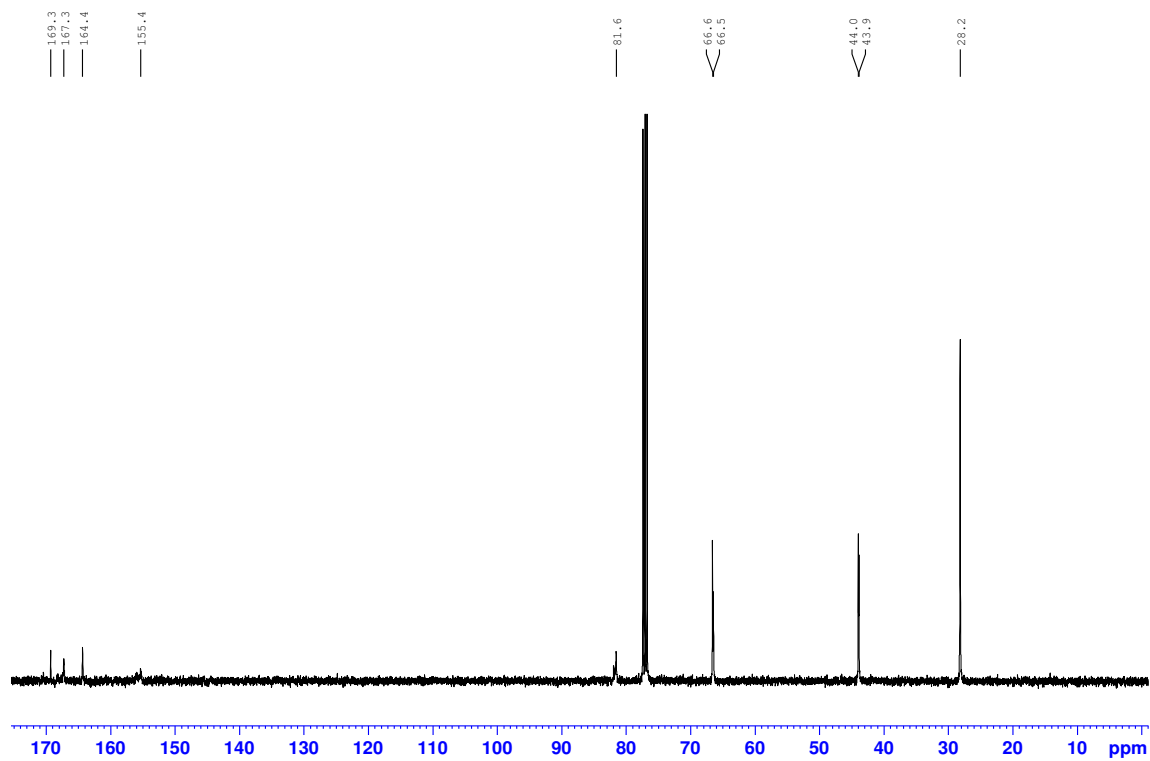
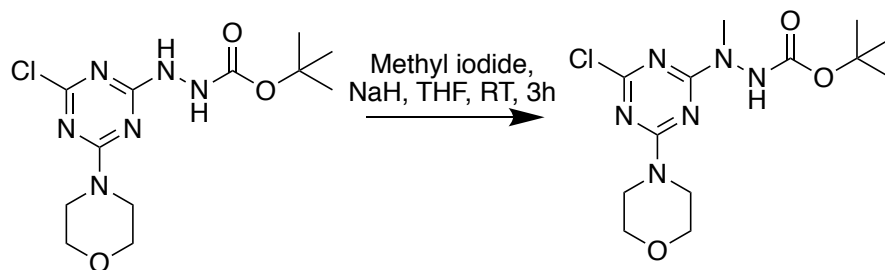


Figure S27: <sup>13</sup>C-NMR of **13**

### Synthesis of 14 from 13



In a small round bottom flask purged with nitrogen, compound **13** (50 mg, 0.15 mmoles) was dissolved in dry THF. One equivalent sodium hydride (3.7 mg, 0.15 mmoles) was added to flask at 0°C. After about 10 minutes, methyl iodide (43 mg, 0.30 mmoles) was added to the solution. Reaction mixture was brought to room temperature and left to stir for 2 hours. Excess solvent removed, re-dissolved in DCM, washed with water and further purified by column chromatography using 2:1 hexane: ethyl acetate solvent system to afford **14** (31 mg, 60%) as white solid.

Figure S28:  $^1\text{H}$ -NMR,  $\text{CDCl}_3$ ;  $\delta$  3.82-3.71 (8H, m), 3.40-3.36 (3H, s), 1.47 (9H, s).

Figure S29:  $^{13}\text{C}$ -NMR,  $\text{CDCl}_3$ ; 164.5, 81.9, 66.6, 43.9, 38.1, 28.2.



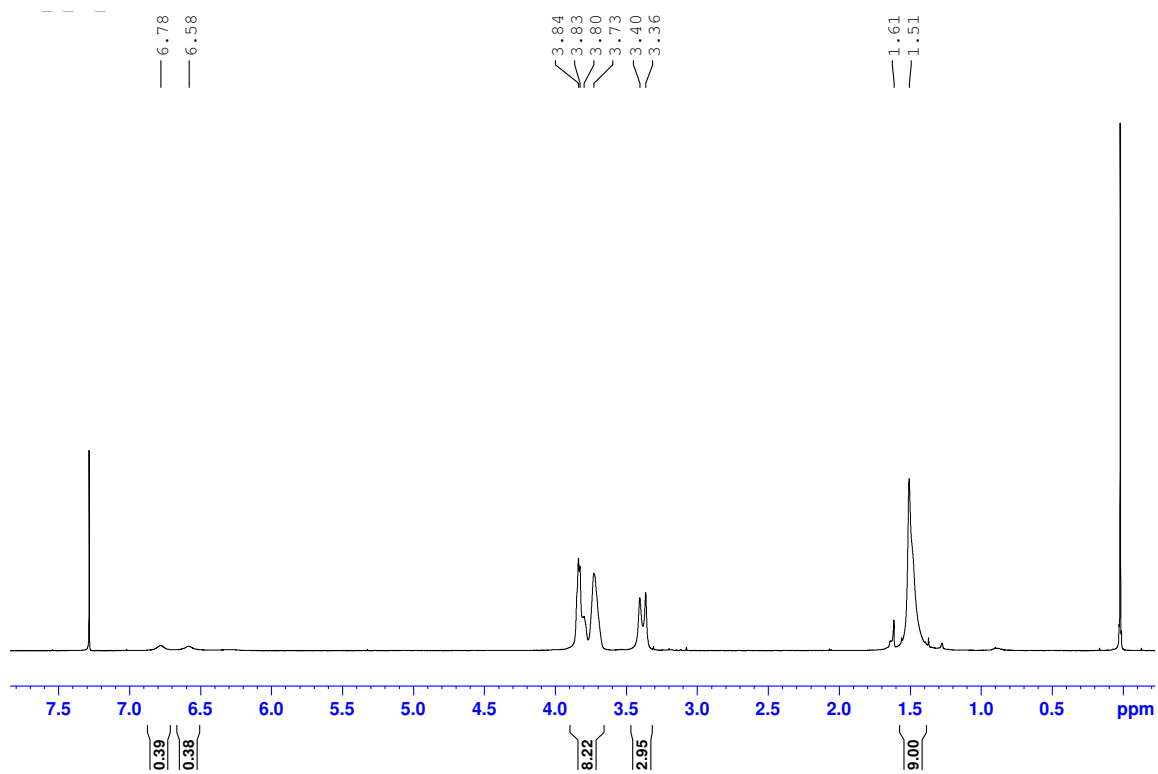


Figure S28: <sup>1</sup>H-NMR of **14**

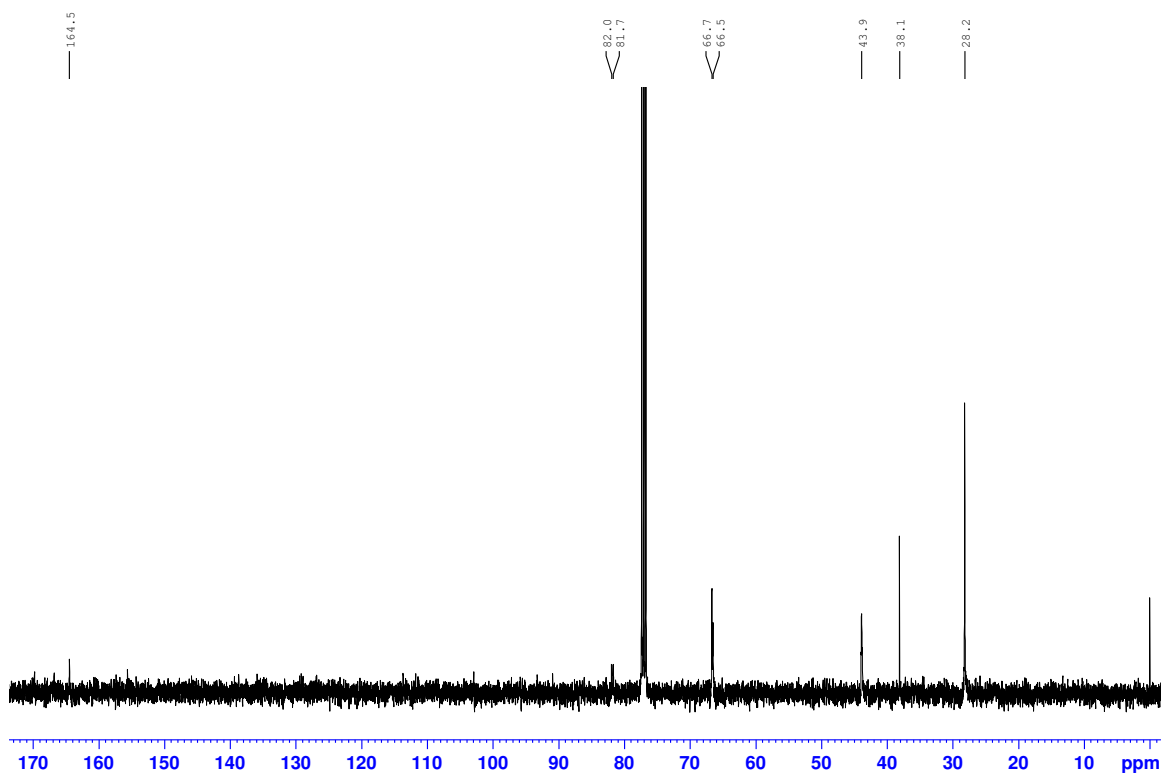
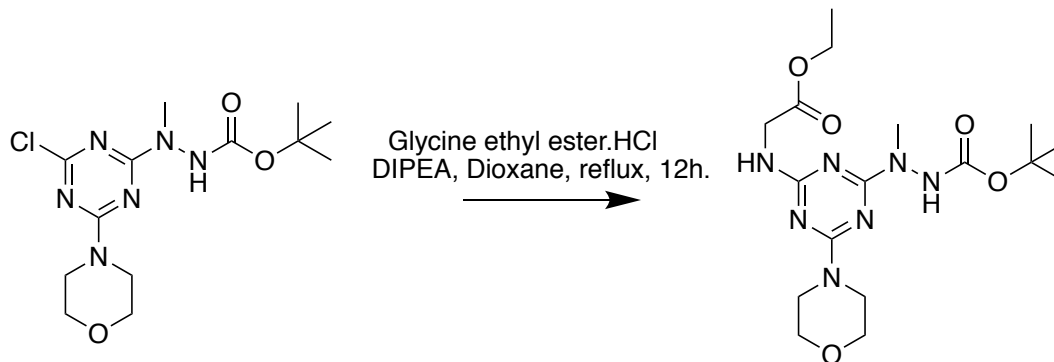


Figure S29: <sup>13</sup>C-NMR of **14**

### Synthesis of 15 from 14



Compound 14 (30 mg, 0.087 mmoles) was dissolved in 2 ml dioxane in a 5 ml round bottom flask. DIPEA (0.5 ml) and glycine ethyl ester (24 mg, 0.17 mmoles) was added to reaction mixture and resulting solution was refluxed for 12 hours. Excess solvent was removed under vacuum and purified by column chromatography using 1:1 hexane: ethyl acetate solution mixture to yield **15** (26 mg, 74%) as white solid.

Figure S30:  $^1\text{H-NMR}$ ,  $\text{CDCl}_3$ ;  $\delta$  5.51 (1H, m), 4.23-4.18 (2H, q), 4.13-4.10 (2H, d), 3.74-3.67 (8H, m), 3.30 (3H, s), 1.47 (9H, s), 1.29-1.26 (3H, t).

Figure S31:  $^{13}\text{C-NMR}$ ,  $\text{CDCl}_3$ ; 170.7, 167.2, 166.0, 165.0, 156.2, 81.0, 66.8, 61.1, 43.5, 43.0, 37.5, 28.2, 14.2.

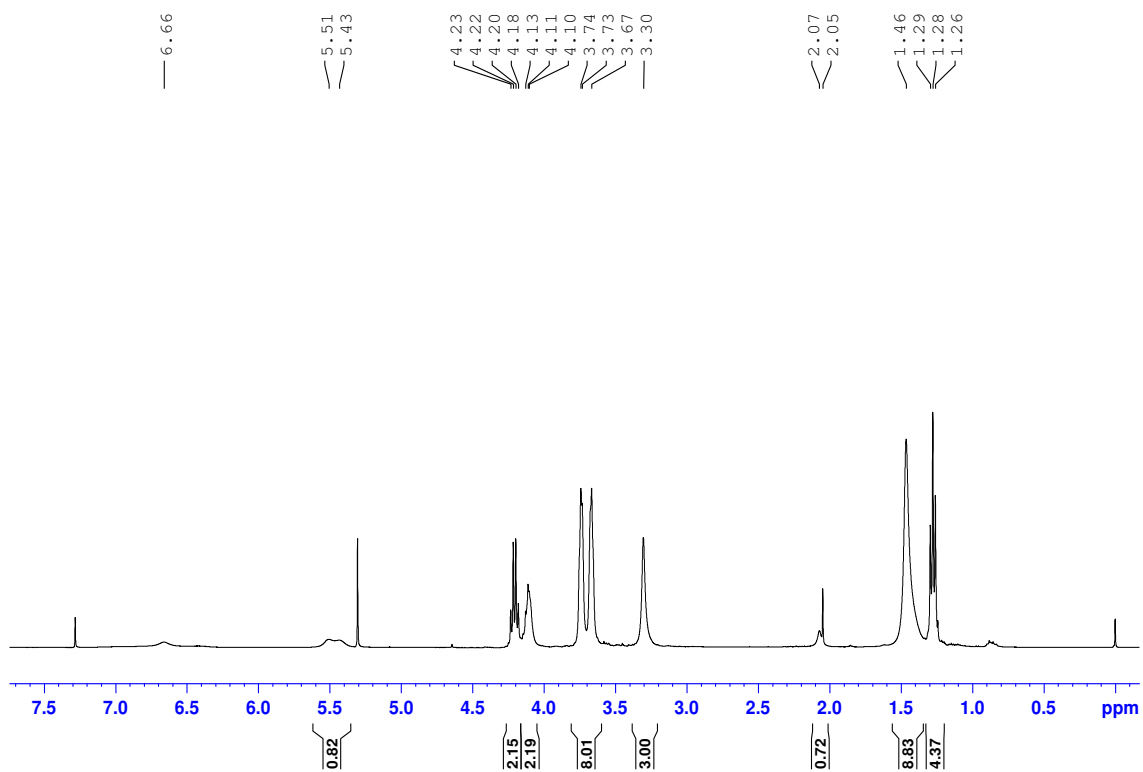


Figure S30: <sup>1</sup>H-NMR of **15**

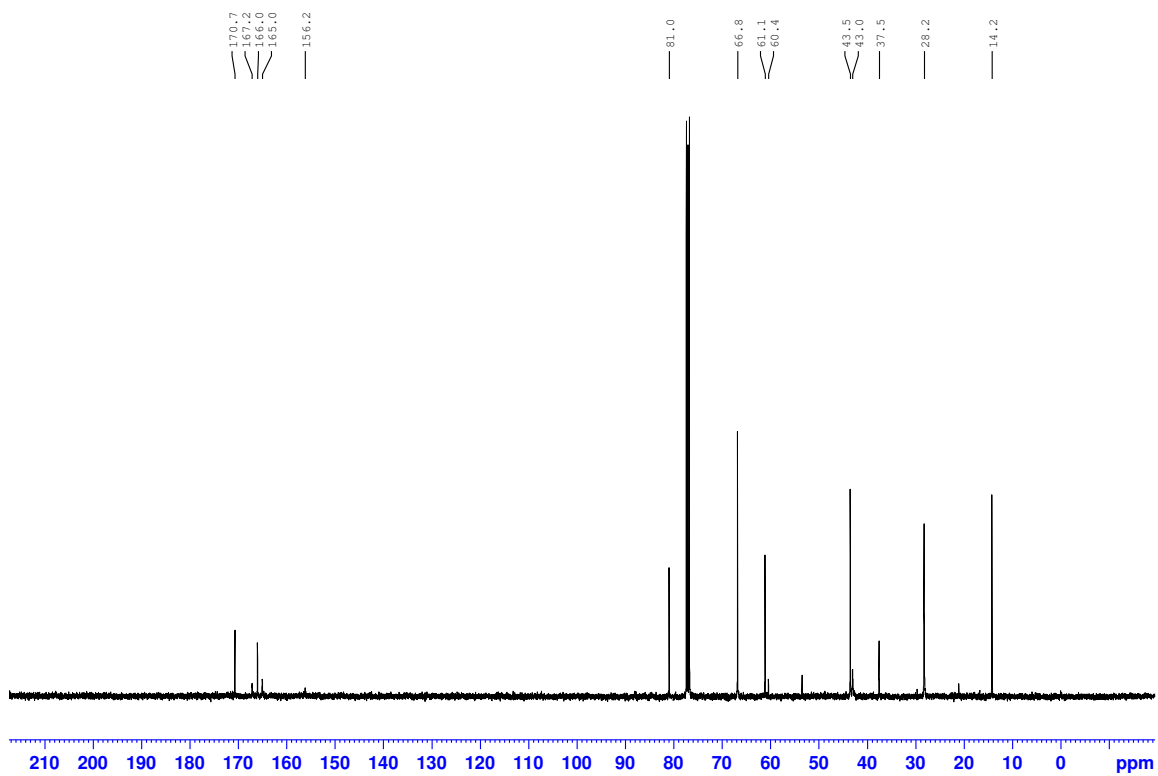
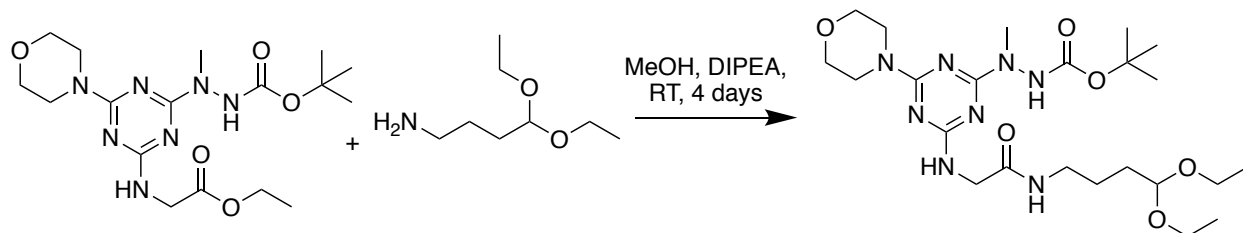


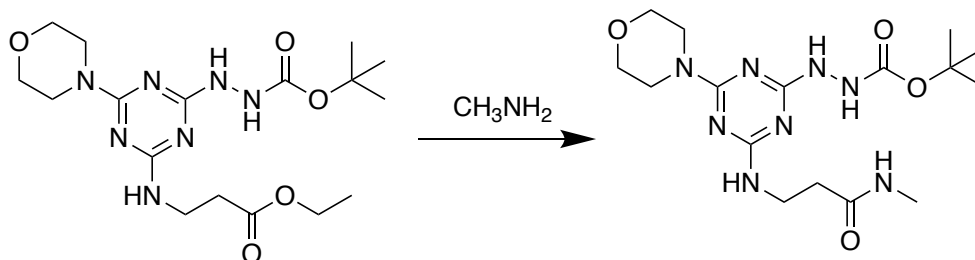
Figure S31: <sup>13</sup>C-NMR of **15**

### Synthesis of **16** from **15** by direct amidation of ester



In a small flask equipped with stirring bar and purged with argon, 21 mg (0.051 mmoles) of **14** was dissolved in 2 ml methanol. DIPEA (0.5 ml) was added followed by the addition of amino butanaldehyde diethyl acetal (17 mg, 0.12 mmoles). Resulting solution was left to stir for 4 days at room temperature. Product formation was determined by the TLC. After completion of the reaction, excess solvent was removed and crude product was re-dissolved in DCM and washed with water. After removing excess DCM, crude product was further purified by column chromatography using 25:1 solvent ratio of DCM:MeOH to retrieve **7** (20 mg, 74%) as white solid. Compound **16** was used to make macrocycle **10** without further characterization. R<sub>f</sub> value of **16** was lower than that of **15** and taken as the evidence of product formation.

### Synthesis of model compound **17**



Compound **16** (200 mg, 0.52 mmoles) was dissolved in 10 ml methanol in small three neck flask equipped with stirring bar and purged with argon. Methyl amine (40 mg, 1.3 mmoles) was added to the reaction flask. Resulting solution was stirred overnight at room temperature for 4 days. Excess solvent was removed and solution was extracted with 0.5 M acetic acid and crude product was recovered in DCM. Crude product was further purified by column chromatography using dichloromethane and methanol (25:1) to recover compound **17** (128 mg, 62%) as white solid.

Figure S32:  $^1\text{H}$ -NMR,  $\text{CDCl}_3$ ;  $\delta$  3.85-3.69 (10H, m), 3.33 (3H, s), 2.55 (2H, m), 1.46 (9H, s).

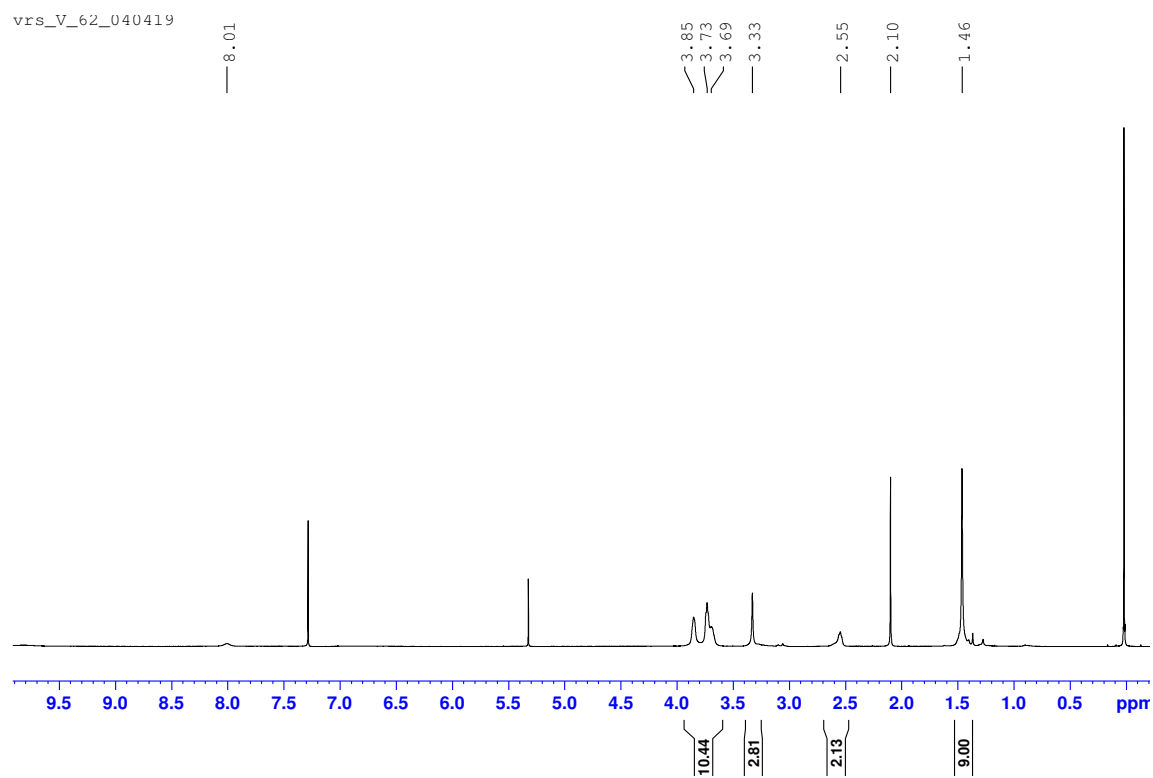
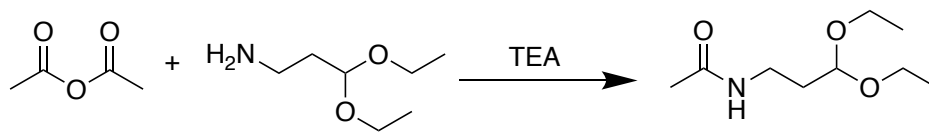


Figure S32:  $^1\text{H}$ -NMR of **17**

### Synthesis of model compound **18**



Acetic anhydride (10 ml) and amino propionaldehyde diethyl acetal (1.1 g, 7.4 mmol) was mixed in a small flask equipped with stirring bar. To the reaction solution, 0.5 ml triethyl amine (TEA) was added and solution was left to stir at room temperature for 12 hours. Reaction progress was measured by TLC. 5 ml of 10% NaOH was added to the reaction mixture and refluxed for 30 minutes. Reaction mixture was extracted using ethyl acetate and water, product was recovered in ethyl acetate and purified by column chromatography using 2:1 hexane: ethyl acetate as solvent system to afford **18** (1.26 g, 89%) as slightly yellow liquid.

Figure S33:  $^1\text{H}$ -NMR,  $\text{CDCl}_3$ ;  $\delta$  6.23 (1H, s), 4.59-4.57 (2H, q), 3.73-3.67 (2H, d), 3.66-3.50 (2H, m), 3.38-3.35 (2H, t), 2.08 (3H, s), 1.97-1.85 (2H, q), 1.25-1.22 (6H, t).

Figure S34:  $^{13}\text{C}$ -NMR,  $\text{CDCl}_3$ ; 170.0, 102.6, 62.0, 35.7, 32.7, 23.3, 20.8, 15.4

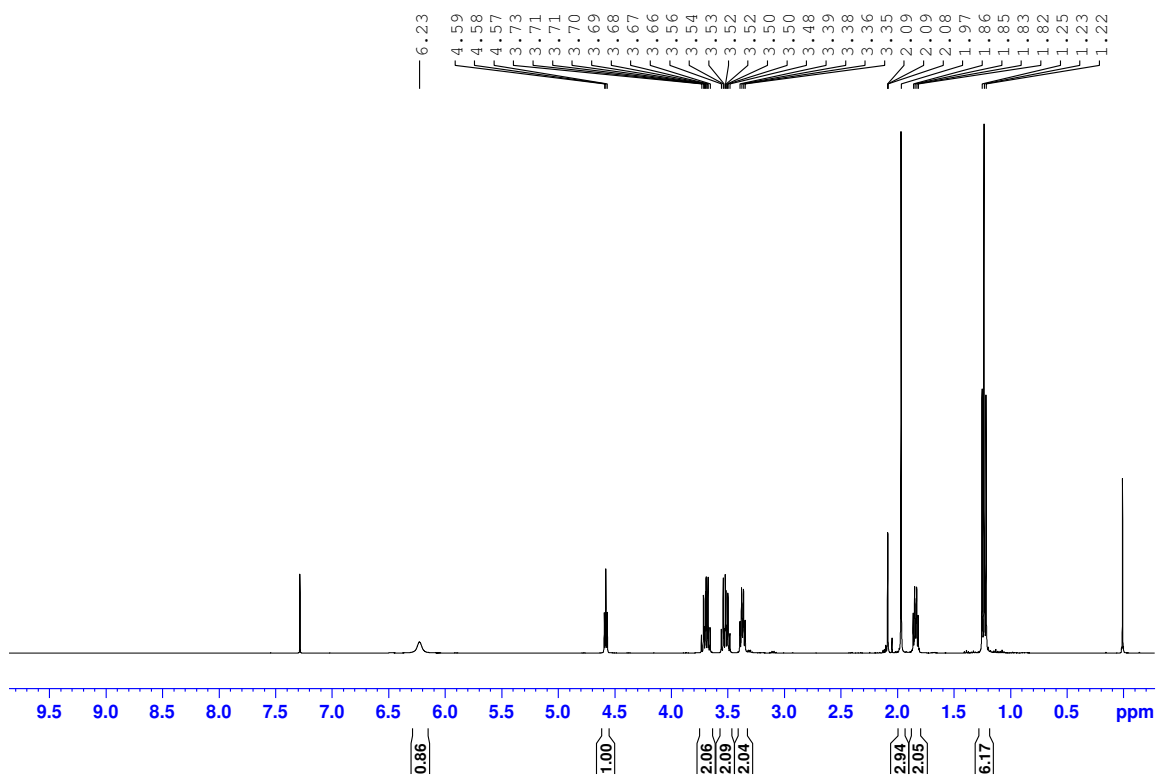


Figure S33: <sup>1</sup>H-NMR of **18**

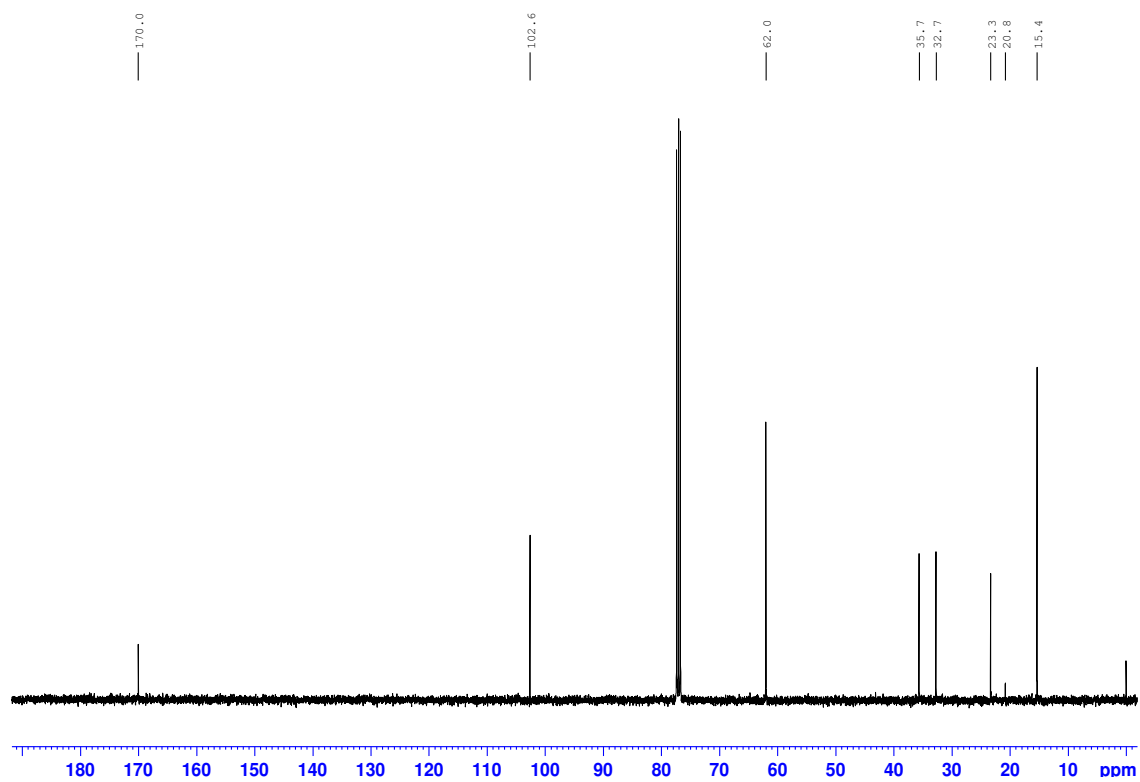
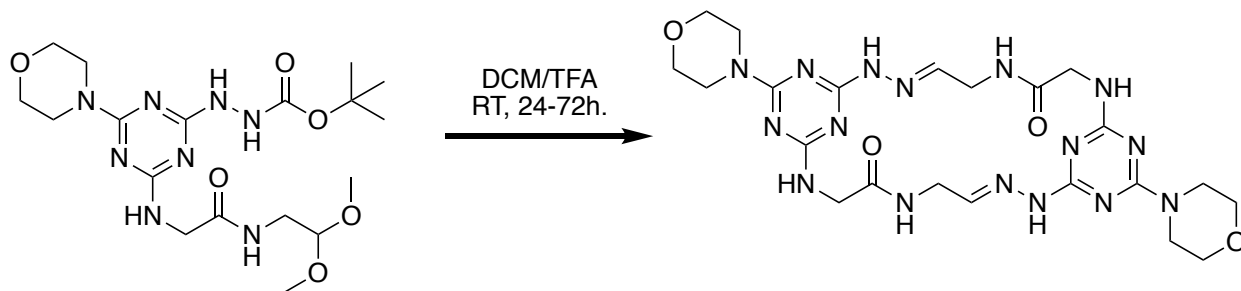


Figure S34: <sup>13</sup>C-NMR of **18**



### Synthesis of Macrocycle-1\*1 from 1:



Compound **1** (220 mg, 0.35 mmol) was dissolved in a 3 ml DCM in a small flask equipped with stirring bar. 3 ml of TFA was added to the reaction mixture and resulting solution was left to stir at room temperature for 48 hours or until all solvents (DCM & TFA) had evaporated. Crude product was then washed with 3 ml DCM (x2) and filtered. The precipitates were then washed with 0.5 ml MeOH (x2). Excess MeOH should be avoided as macrocycle dissolve sparingly in MeOH at room temperature. Precipitates were dried under vacuum to afford macrocycle **1\*1** (130 mg, 93%) as white solid.

Figure S35:  $^1\text{H-NMR}$ ,  $\text{DMSO-}d_6$ ;  $\delta$  12.62 (1H, s), 8.44-8.42 (1H, t), 8.24-8.22 (1H, t), 7.63 (1H, s), 4.21-4.20 (H, d), 4.13-4.12 (2H, d), 3.84-3.66 (8H, m).

Figure S38: C NMR  $\text{DMSO-}d_6$ ; 168.3, 161.7, 155.0, 154.0, 147.5, 66.2, 44.6, 44.5, 41.0.

Figure S43: MS (ESI/Q-TOF)  $m/z$ :  $[\text{M}+\text{H}]^+$  Calcd for  $\text{C}_{22}\text{H}_{32}\text{N}_{16}\text{O}_4$  584.28; Found 585.29.

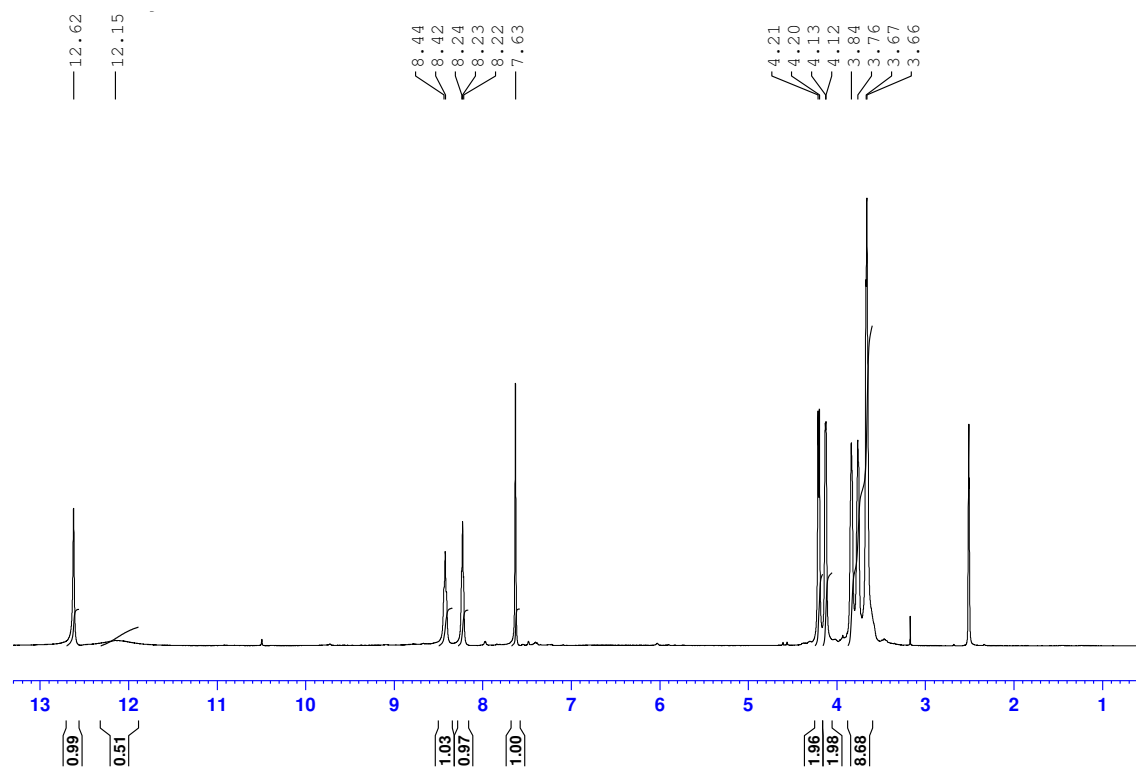


Figure S35:  $^1\text{H}$ -NMR of macrocycle **1\*1** in  $\text{DMSO-}d_6$

All the NH proton disappeared quickly when a drop of  $\text{CD}_3\text{OD}$  was added to NMR tube. Protons of Protonated nitrogen of triazine ring and the hydrazine disappeared faster than the protons of nitrogen of glycine and amide linker.

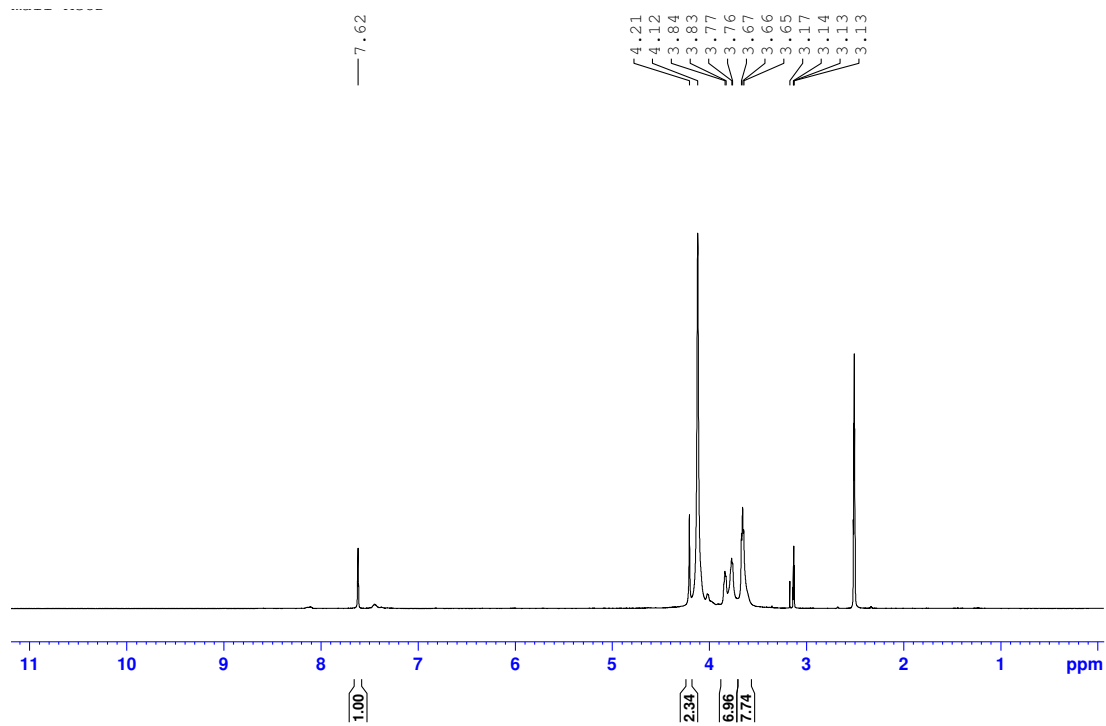


Figure S36:  $^1\text{H}$ -NMR of macrocycle **1\*1** in  $\text{DMSO-}d_6$  with a drop of  $\text{CD}_3\text{OD}$ .

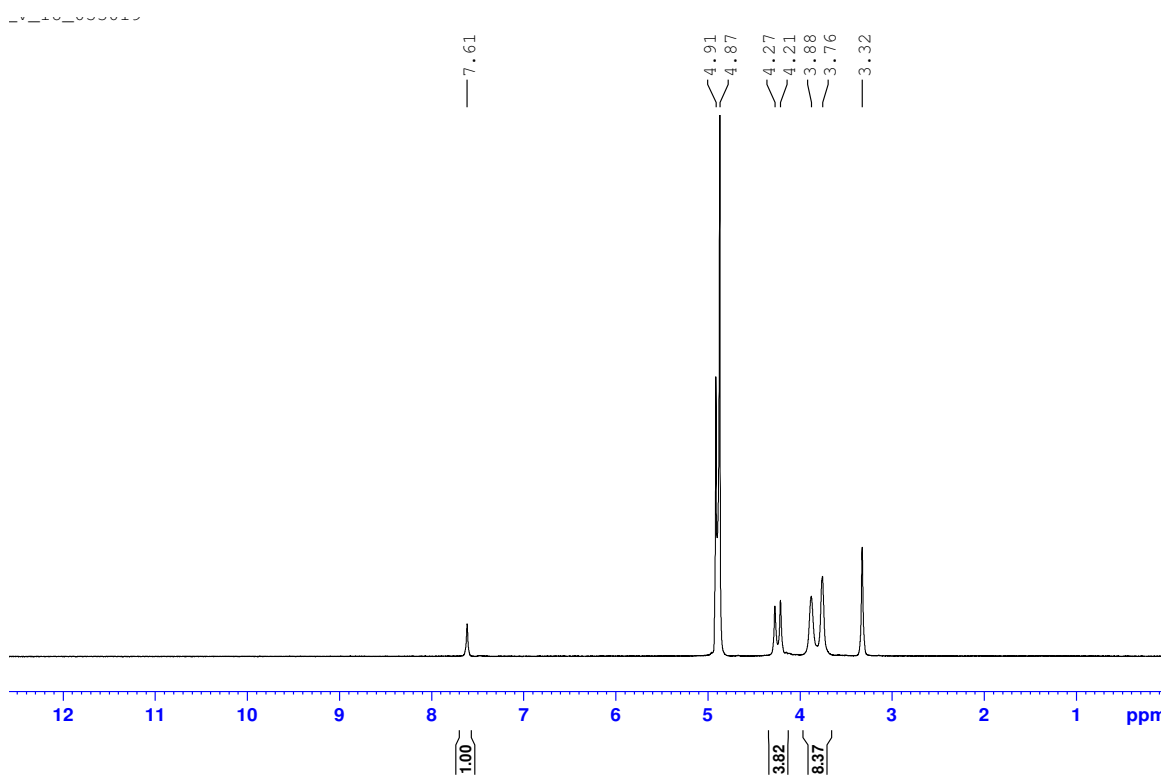


Figure S37:  $^1\text{H}$ -NMR of macrocycle **1\*1** in  $\text{CD}_3\text{OD}$ .

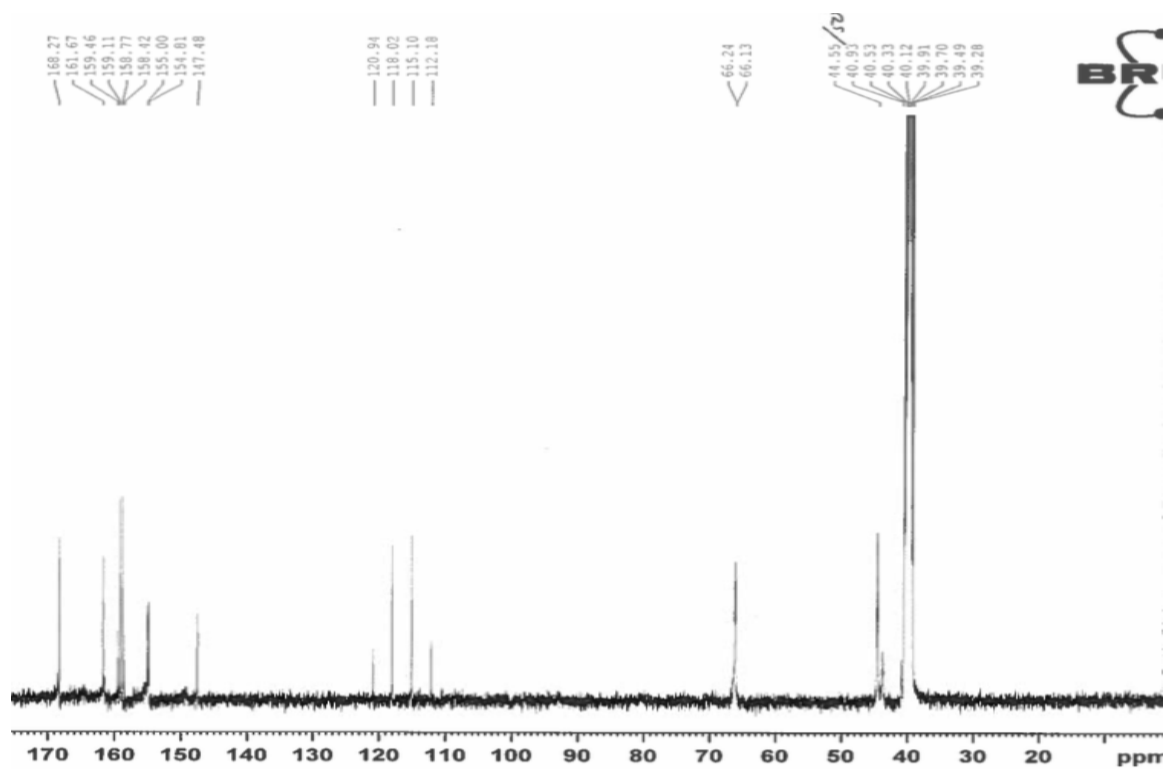


Figure S38: <sup>13</sup>C-NMR of macrocycle **1\*1** in DMSO-*d*<sub>6</sub>

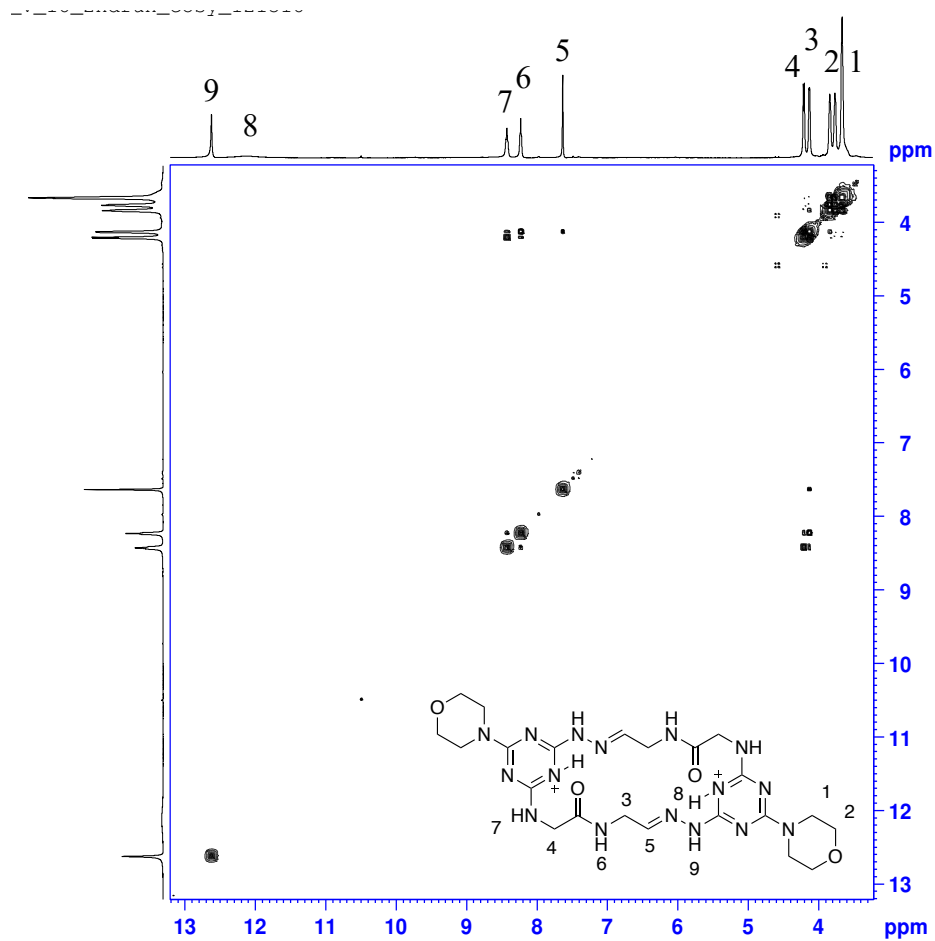


Figure S39: COSY-NMR of macrocycle **1\*1** in DMSO- $d_6$

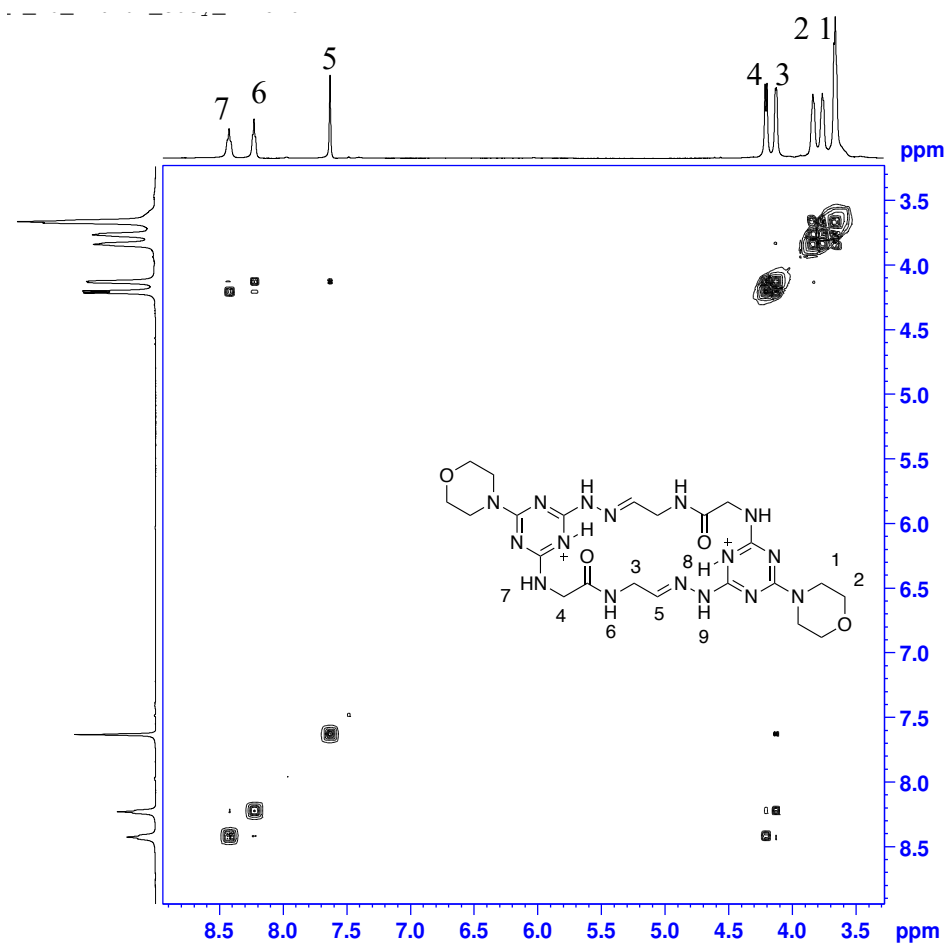


Figure S40: COSY-NMR zoomed of macrocycle **1\*1** in DMSO-*d*<sub>6</sub>

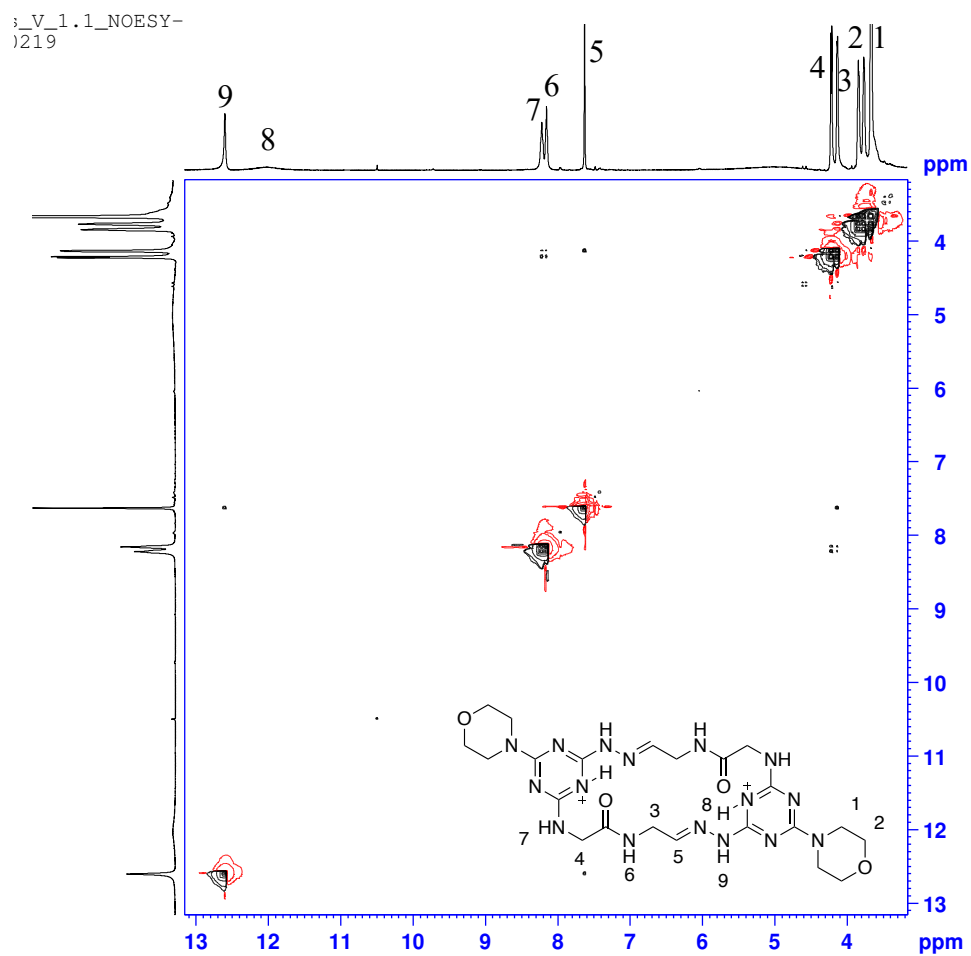


Figure S41: NOESY-NMR of macrocycle **1\*1** in DMSO- $d_6$

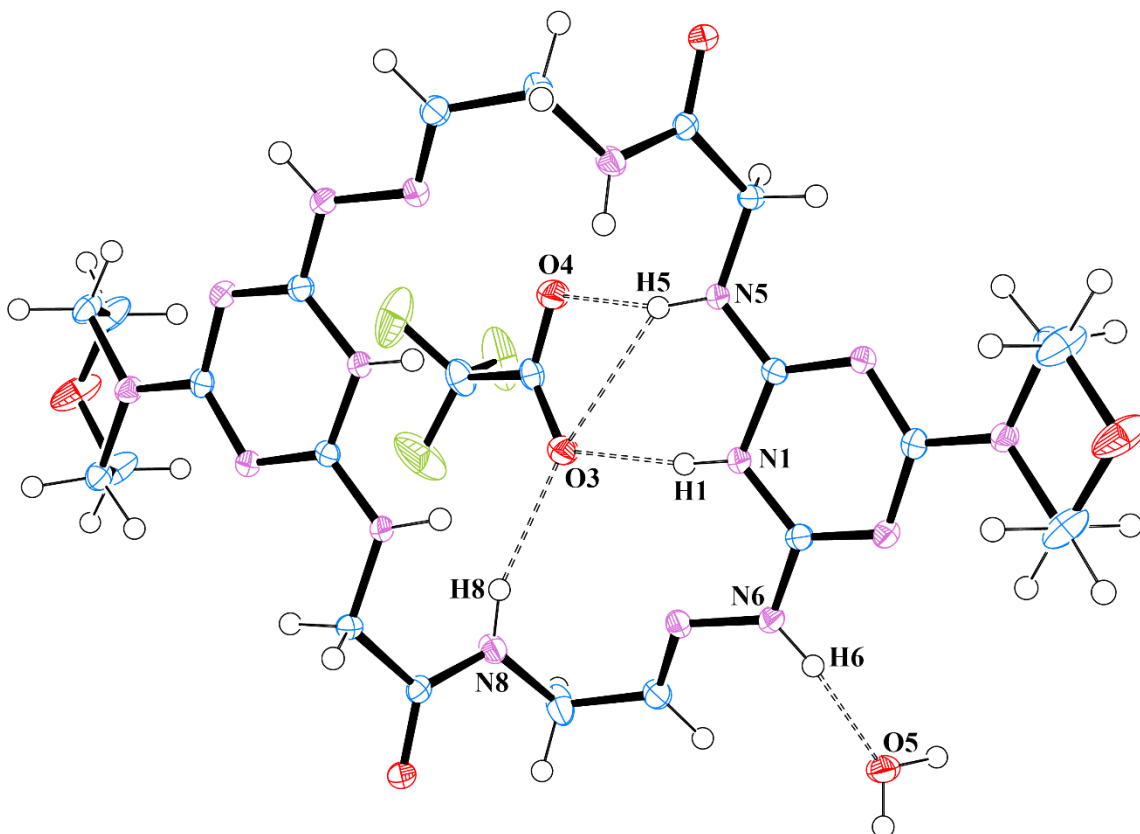


Figure S42: X-ray crystal structure thermal ellipsoid diagram of **1\*1** with 50% probability of at 100K temperature.

Table S1: Selected hydrogen bond parameters of **1\*1** with important labels mentioned in above figure

<i>D</i> —H··· <i>A</i>	<i>D</i> —H (Å)	H··· <i>A</i> (Å)	<i>D</i> ··· <i>A</i> (Å)	<i>D</i> —H··· <i>A</i> (°)
N5—H5···O4	0.88	1.97	2.7820 (18)	153
O5—H5C···O4 <sup>i</sup>	0.82 (3)	2.07 (3)	2.8560 (19)	161 (3)
O5—H5D···O2 <sup>ii</sup>	0.92 (3)	1.94 (3)	2.8544 (18)	175 (3)
N1—H1···O3	0.85 (2)	2.04 (3)	2.8779 (19)	170 (2)
N6—H6···O5	0.89 (2)	1.96 (2)	2.8240 (20)	165 (2)
N8—H8···O3	0.85 (2)	2.20 (2)	3.0227 (19)	161 (1)

Symmetry codes: (i)  $x, y, z-1$ ; (ii)  $x+1, y, z$ .



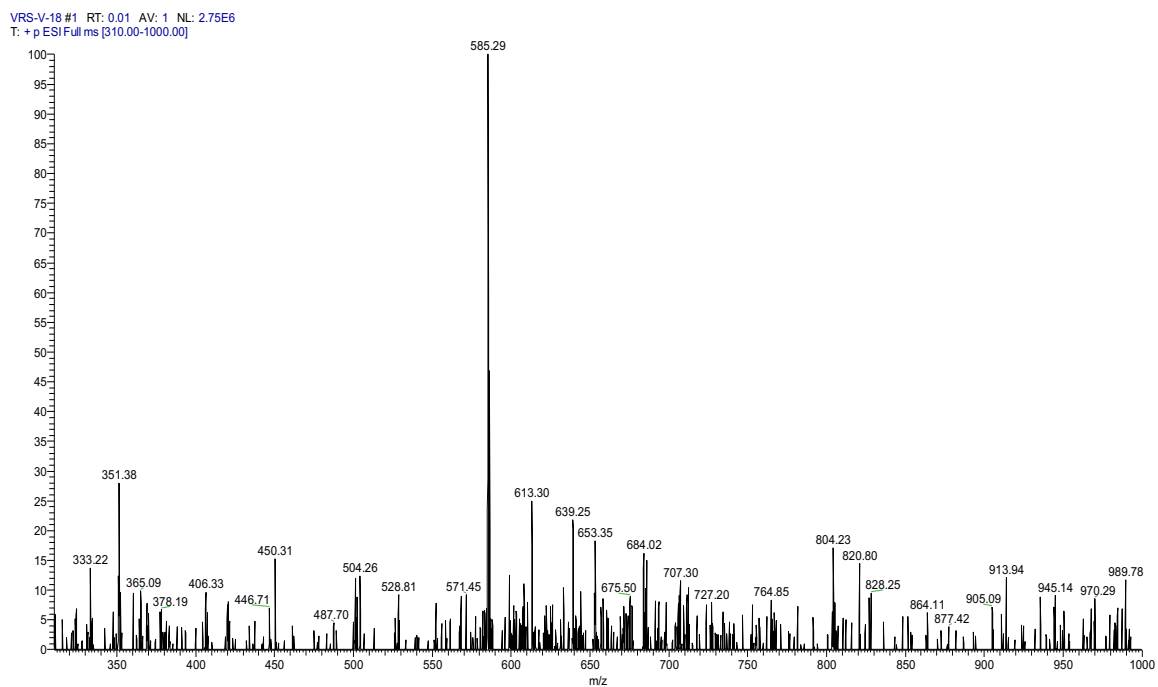


Figure S43: Mass spectrum of macrocycle **1\*1**.

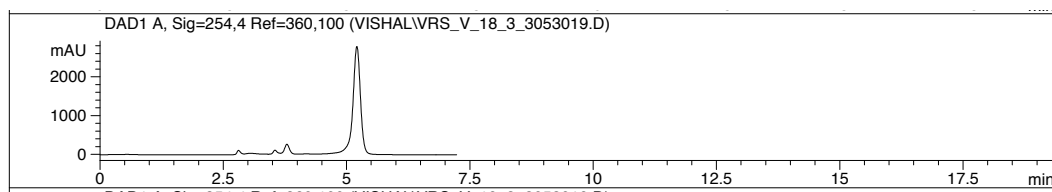
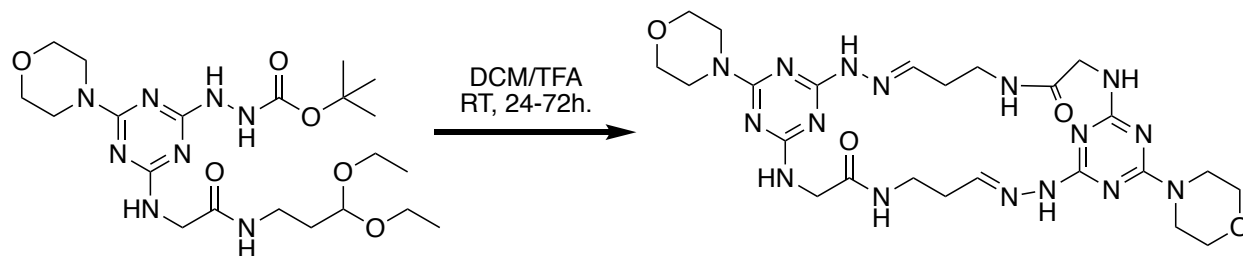


Figure S44: HPLC chromatogram of **1\*1**.

### Synthesis of **2\*2** from **2**:



In a small flask equipped with stirring bar, compound **2** (350 mg, 0.70 mmoles) was dissolved in a 3 ml DCM and 3 ml TFA. Resulting solution was left to stir at room temperature for 48 hours or until all solvents (DCM & TFA) had evaporated. Crude product was then washed with 3 ml DCM (x2) and filtered. The precipitates were then washed with 0.5 ml MeOH (x2). Excess MeOH should be avoided as macrocycle dissolve sparingly in MeOH at room temperature. Precipitates were dried under vacuum to afford **2\*2** (201 mg, 93%) as white solid.

Figure S45:  $^1\text{H}$ -NMR,  $\text{DMSO-}d_6$ ;  $\delta$  12.37 (1H, s), 9.04 (1H, s), 8.91 (1H, s), 7.51 (1H, s), 3.89-3.88 (2H, d), 3.71-3.49 (10H, m), 2.56 (2H, m).

Figure S48:  $^{13}\text{C}$ -NMR,  $\text{CD}_3\text{OD}$ ;  $\delta$  171.8, 161.3, 154.6, 154.0, 147.9, 66.2, 44.7, 43.8, 33.6, 31.6.

Figure S53: MS (ESI/Q-TOF)  $m/z$ :  $[\text{M}+\text{H}]^+$  Calcd for  $\text{C}_{24}\text{H}_{36}\text{N}_{16}\text{O}_4$  612.31; Found 613.32.

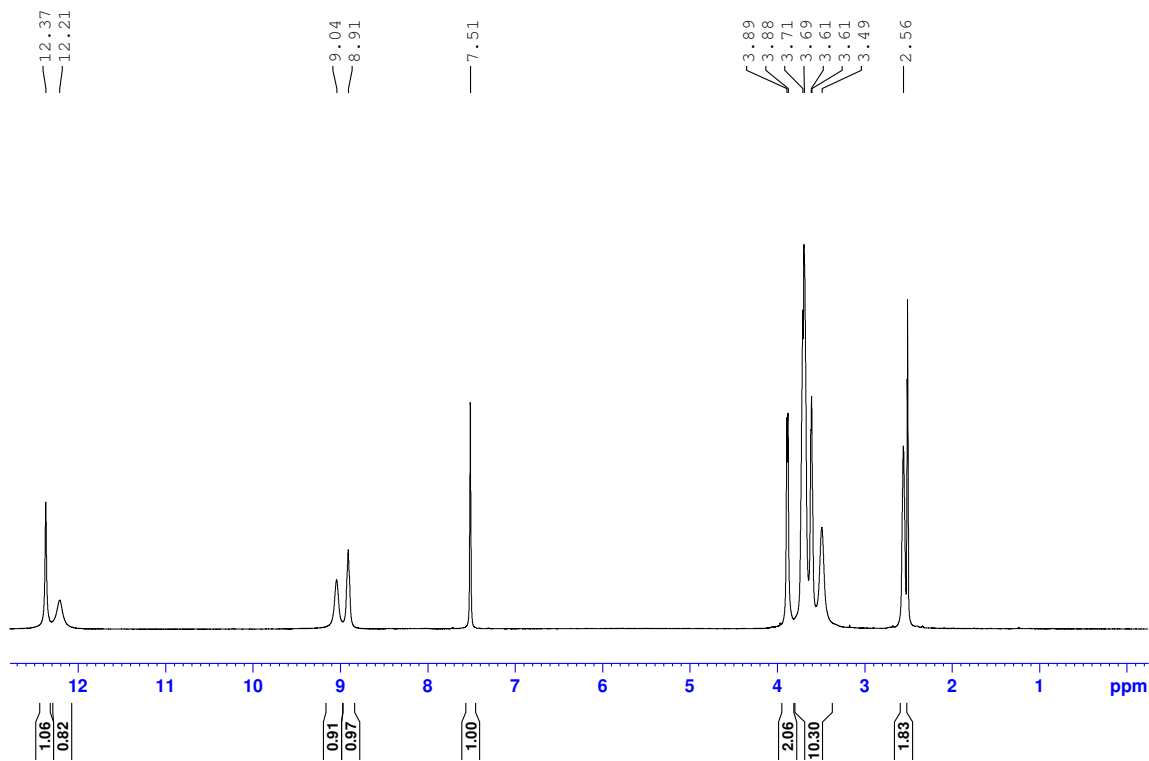


Figure S45: <sup>1</sup>H-NMR of macrocycle **2\*2** in DMSO-*d*<sub>6</sub>

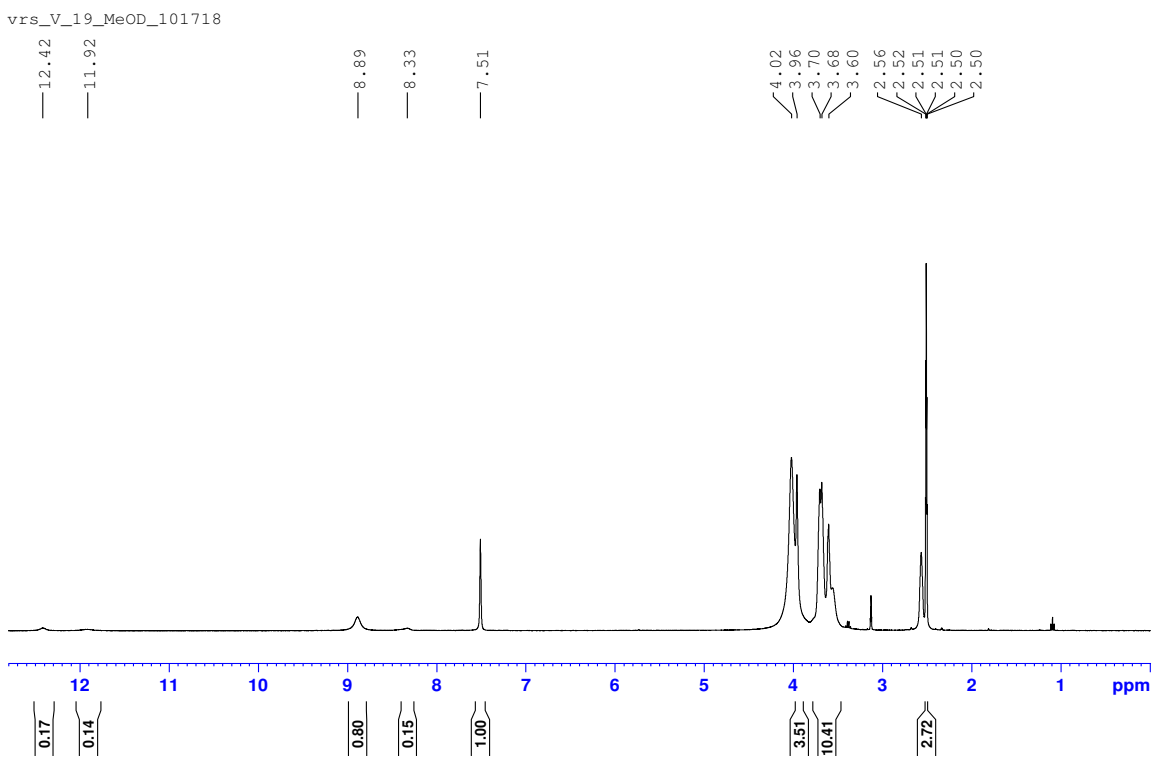


Figure S46: <sup>1</sup>H-NMR of macrocycle **2\*2** in DMSO-*d*<sub>6</sub> with a drop of CD<sub>3</sub>OD

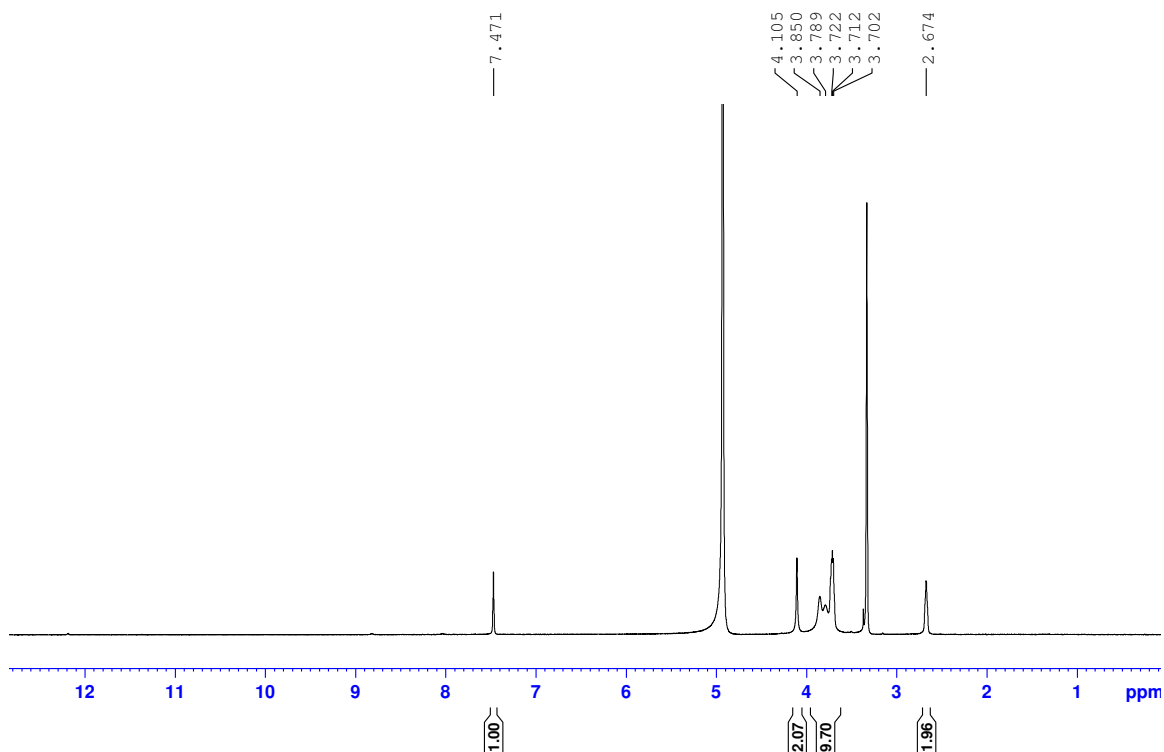


Figure S47:  $^1\text{H}$ -NMR of macrocycle **2\*2** in  $\text{CD}_3\text{OD}$ .

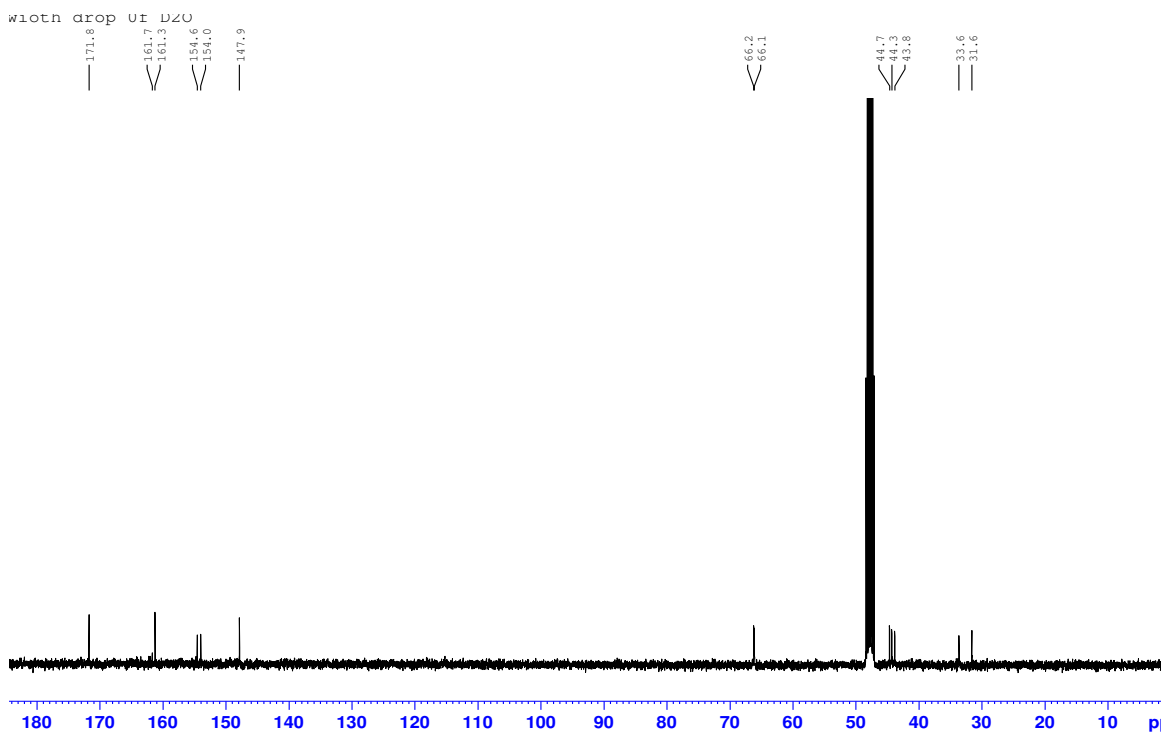


Figure S48:  $^{13}\text{C}$ -NMR of macrocycle **2\*2** in  $\text{CD}_3\text{OD}$ .

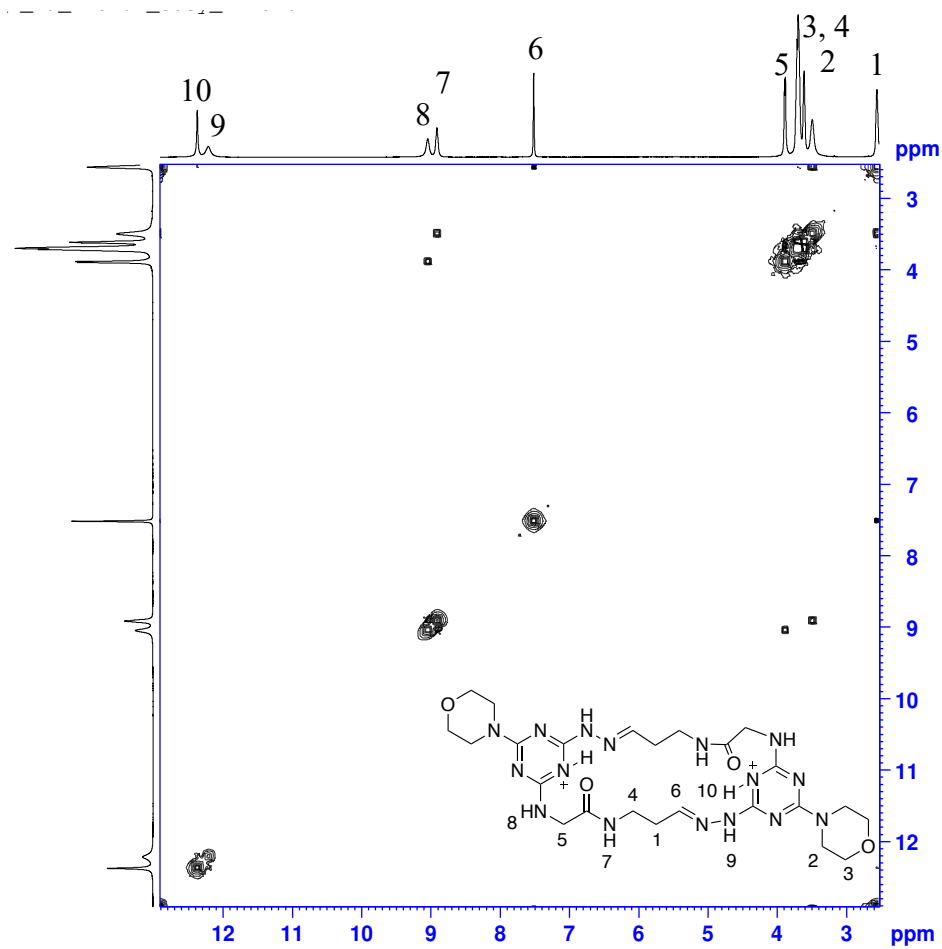


Figure S49: COSY-NMR of macrocycle **2\*2** in DMSO-*d*<sub>6</sub>

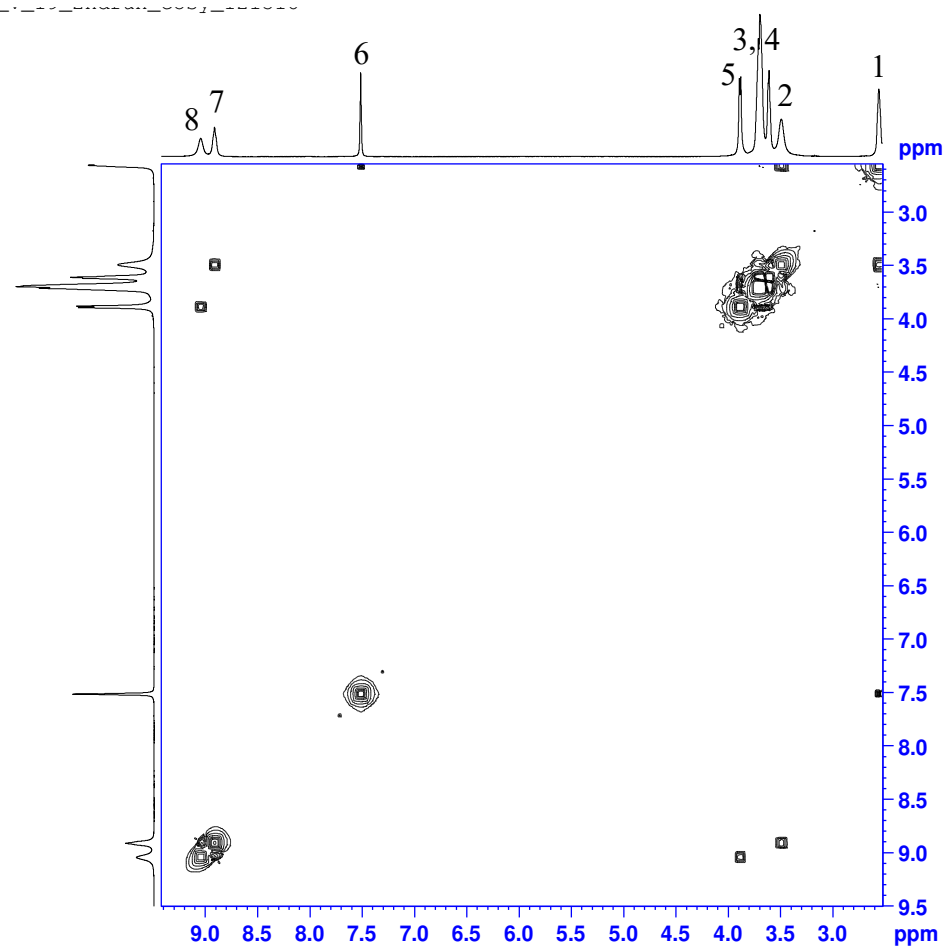


Figure S50: COSY-NMR zoomed of macrocycle **2\*2** in DMSO- $d_6$

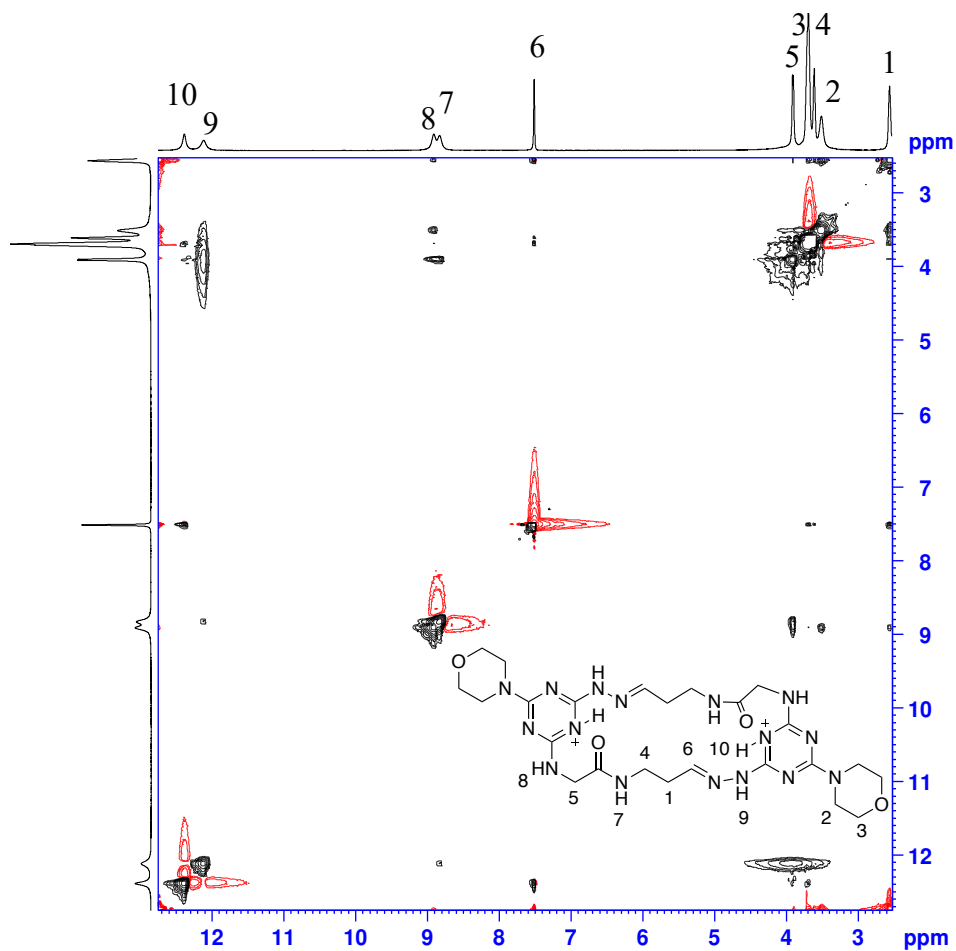


Figure S51: NOESY-NMR of macrocycle **2\*2** in DMSO- $d_6$

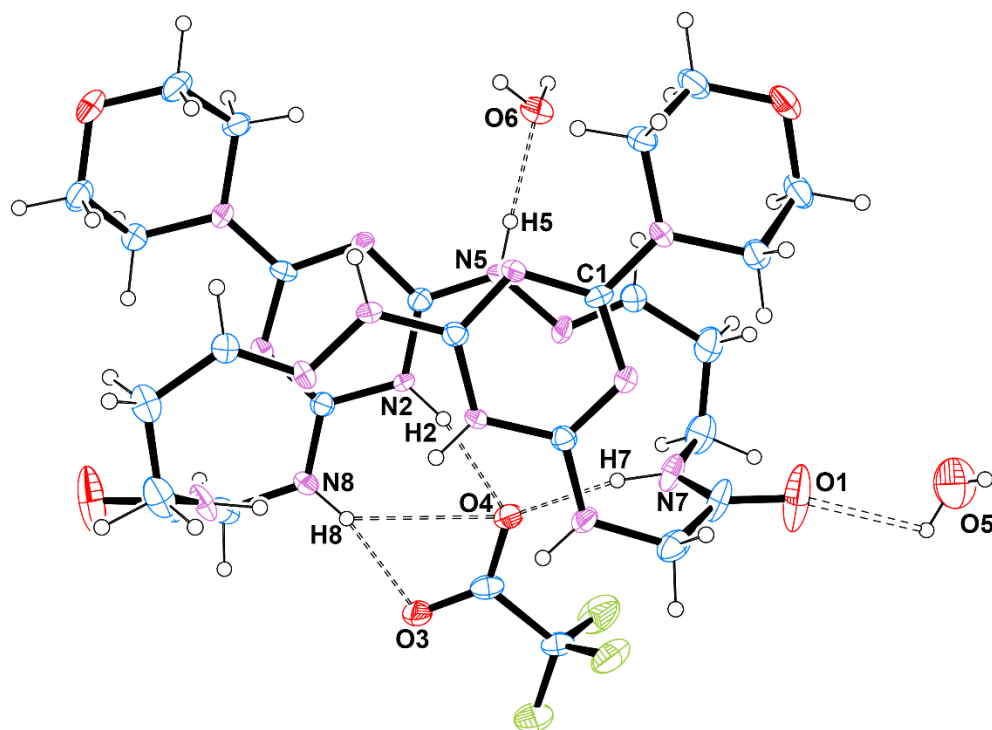


Figure S52: X-ray crystal structure thermal ellipsoid diagram of **2\*2** with 50% probability of at 100K temperature.

Table S2: Selected hydrogen bond parameters of **2\*2** with important labels mentioned in above figure

<i>D</i> —H··· <i>A</i>	<i>D</i> —H (Å)	H··· <i>A</i> (Å)	<i>D</i> ··· <i>A</i> (Å)	<i>D</i> —H··· <i>A</i> (°)
N2—H2···O4	0.88	1.92	2.783 (6)	167
N5—H5···O6	0.88	1.85	2.728 (6)	175
N7—H7···O4	0.88	2.01	2.821 (7)	154
N8—H8···O3	0.88	1.97	2.832 (6)	167
N8—H8···O4	0.88	2.64	3.341 (6)	137



VRS-V-19 #1 RT: 0.00 AV: 1 NL: 7.54E6  
T: +c ESI Full ms [310.00-1000.00]

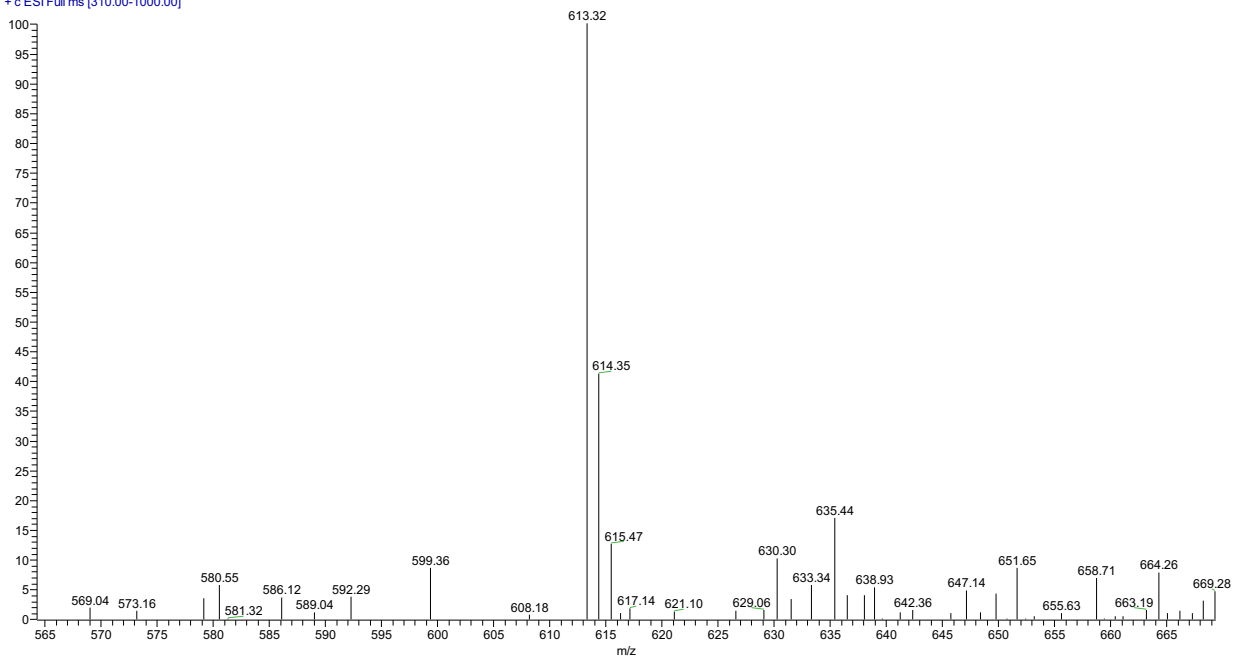


Figure S53: Mass Spectrum of macrocycle **2\*2** in DMSO- $d_6$

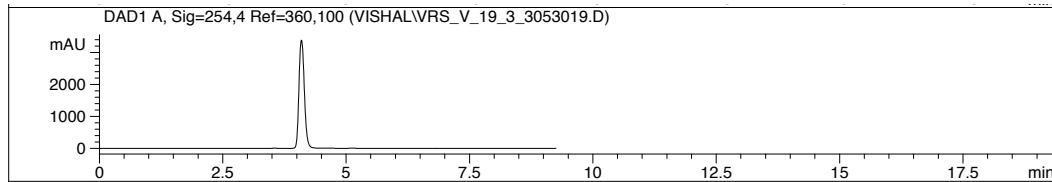
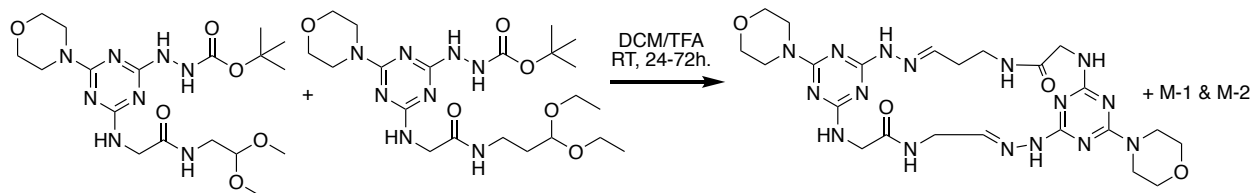


Figure S54: HPLC chromatogram of **2\*2**.

**Mixing one to one mole ratio of 1 and 2 to make mixture of 1\*1, 2\*2, and 1\*2:**



Compound **2** (95 mg, 0.19 mmoles) and compound **1** (87 mg, 0.19 mmoles) were dissolved in a 3 ml DCM and 3 ml TFA in a small flask equipped with stirring bar. Resulting solution was left to stir at room temperature for 48 hours or until all the solvents (DCM & TFA) had evaporated. Crude product was then washed with 3 ml DCM (x2) and filtered. The precipitates were then washed with 0.5 ml MeOH (x2). Excess MeOH should be avoided as macrocycle dissolve sparingly in MeOH at room temperature. Precipitates were dried under vacuum to afford 121 mg of mixture of **1\*1**, **1\*2**, and **2\*2** as white solid.

Figure S55: MS (ESI/Q-TOF) m/z:  $[M+H]^+$  Calcd for (**1\*2**)  $C_{23}H_{34}N_{16}O_4$  612.31; expected; 598.29, observed; 599.33.

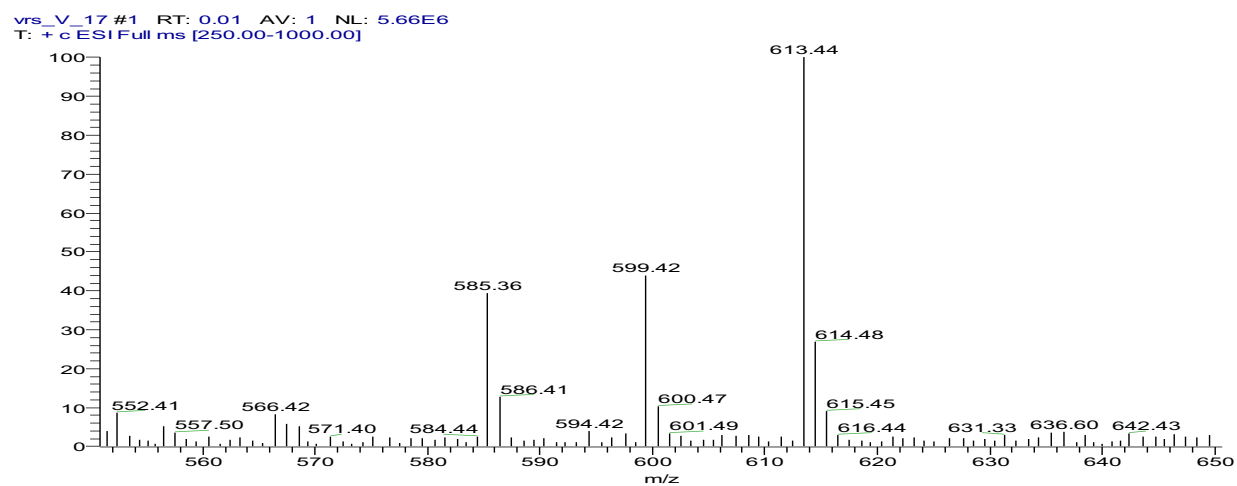


Figure S55: Mass Spectrum of macrocycles **1\*1**, **1\*2**, and **2\*2**.

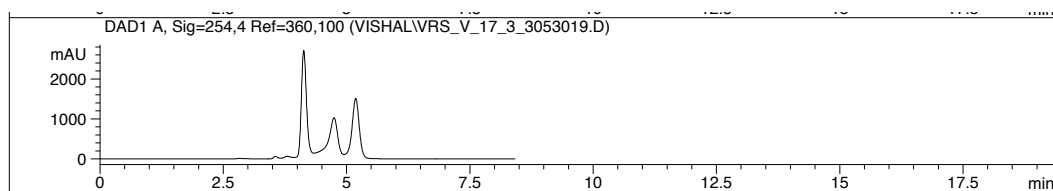
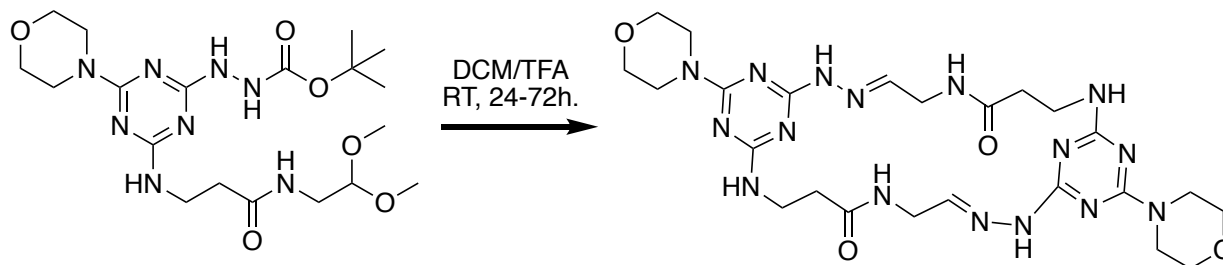


Figure S56: HPLC chromatogram of mixture of **1\*1**, **1\*2**, and **2\*2**.

### Synthesis of 3\*3 from 3:



Compound **3** (90 mg, 0.19 mmol) was dissolved in a mixture of 2 ml DCM and 2 ml TFA in a small flask equipped with stirring bar. Reaction mixture and resulting solution was left to stir at room temperature for 48 hours or until completely dried out. Crude product was then washed with 3 ml DCM (x2) and filtered. The precipitates were then washed with 0.5 ml MeOH (x2). Excess MeOH should be avoided as macrocycle dissolve sparingly in MeOH at room temperature. Precipitates were dried under vacuum to afford **3\*3** (55 mg, 95%) as white solid.

Figure S57:  $^1\text{H-NMR}$ ,  $\text{DMSO-}d_6$ ;  $\delta$  12.62 (1H, s), 11.45 (1H, s), 9.09 (1H, t), 8.35-8.32 (1H, t), 7.62-7.61 (1H, t), 4.30-4.28 (2H, d), 3.86-3.68 (8H, m), 3.28-3.26 (2H, m), 2.39 (2H, m), 1.73-1.72 (2H, m).

Figure S59:  $^{13}\text{C-NMR}$ ,  $\text{CD}_3\text{OD}$ ;  $\delta$  172.6, 161.3, 155.3, 154.3, 146.6, 66.4, 44.3, 40.0, 37.7, 37.3

Figure S63: M/Z: MS (ESI/Q-TOF) m/z:  $[\text{M}+\text{H}]^+$  Calcd for  $\text{C}_{24}\text{H}_{36}\text{N}_{16}\text{O}_4$  612.31; found 613.37. observed; 613.37.

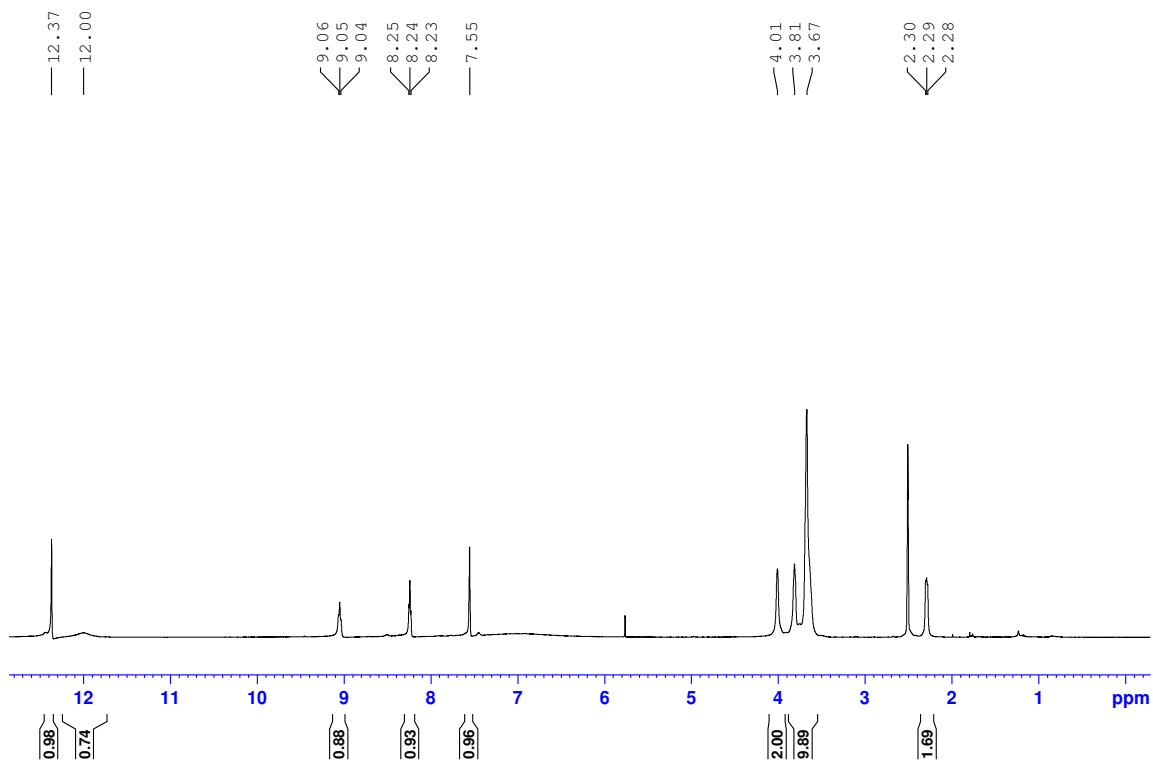


Figure S57:  $^1\text{H}$ -NMR of macrocycle **3\*3** in  $\text{DMSO-}d_6$

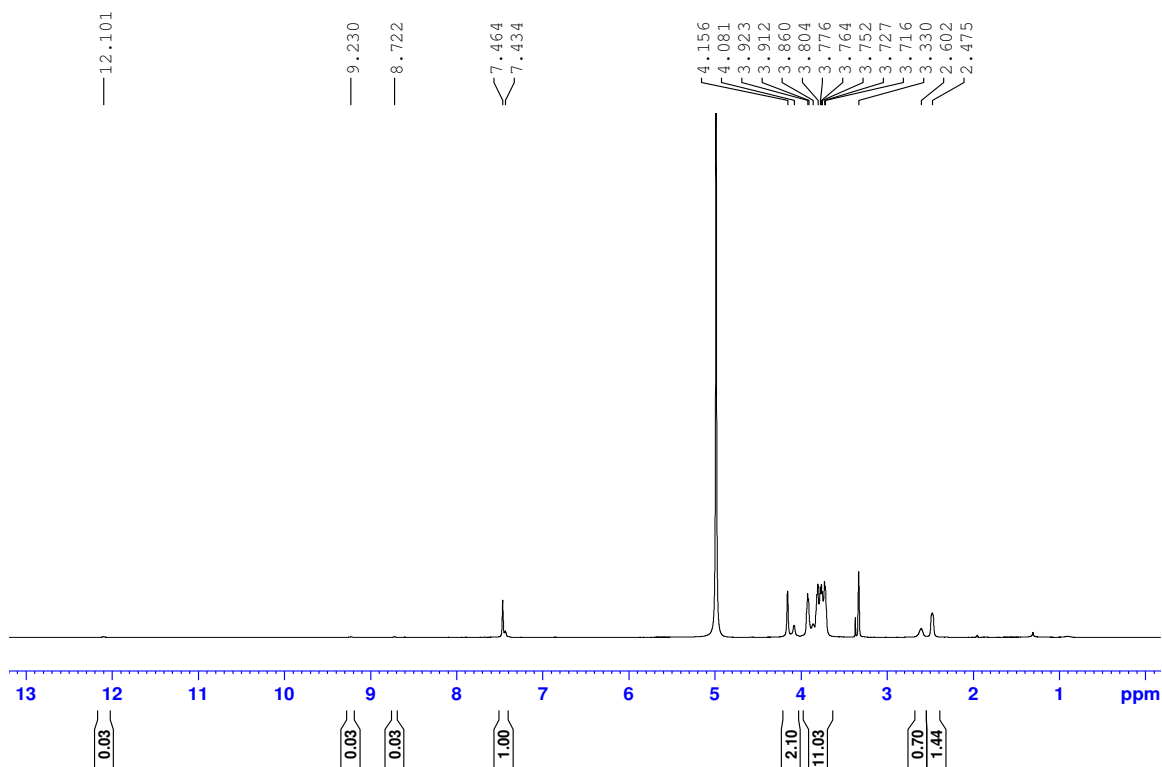


Figure S58:  $^1\text{H}$ -NMR of macrocycle **3\*3** in  $\text{CD}_3\text{OD}$

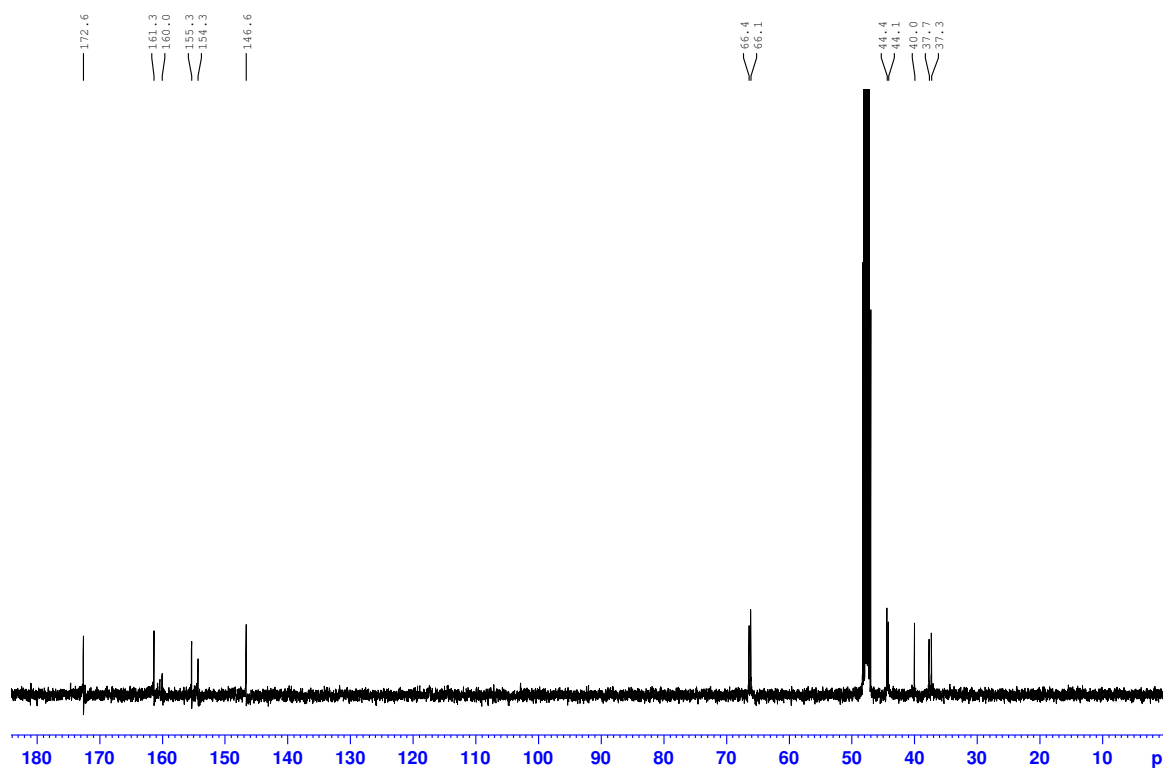


Figure S59: <sup>13</sup>H-NMR of macrocycle **3\*3** in CD<sub>3</sub>OD

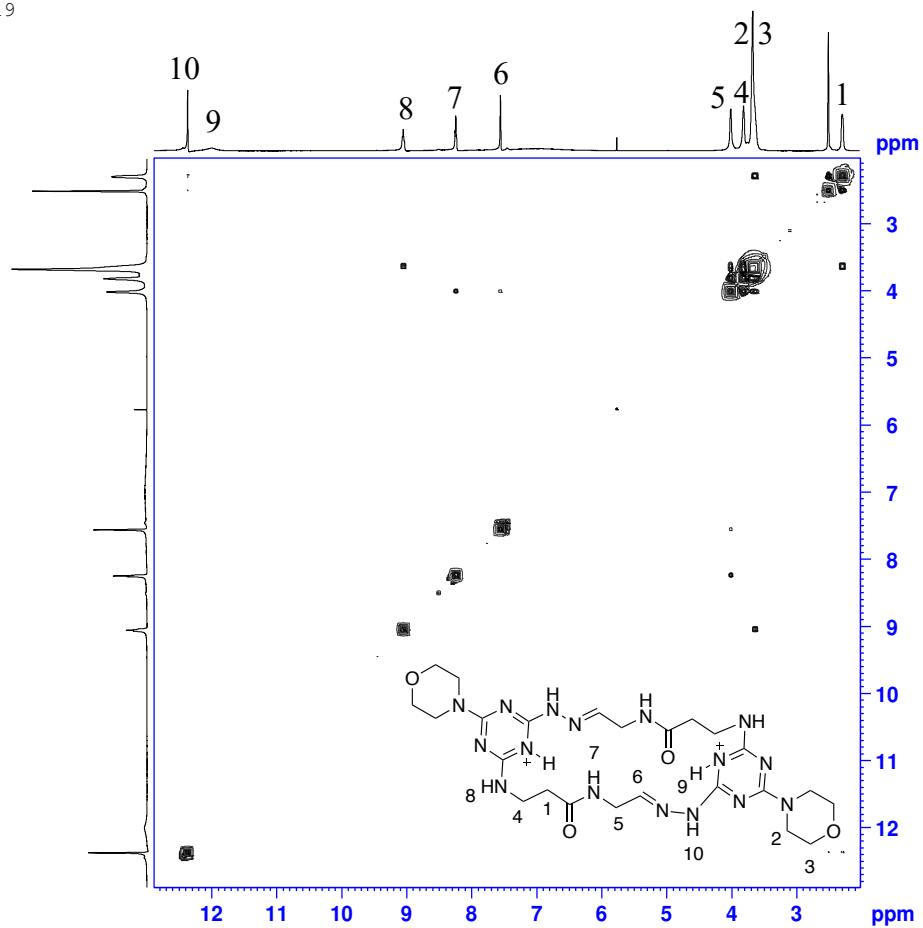


Figure S60:  $^1\text{H}$ - $^{13}\text{C}$  COSY-NMR of macrocycle **3\*3** in  $\text{DMSO}-d_6$

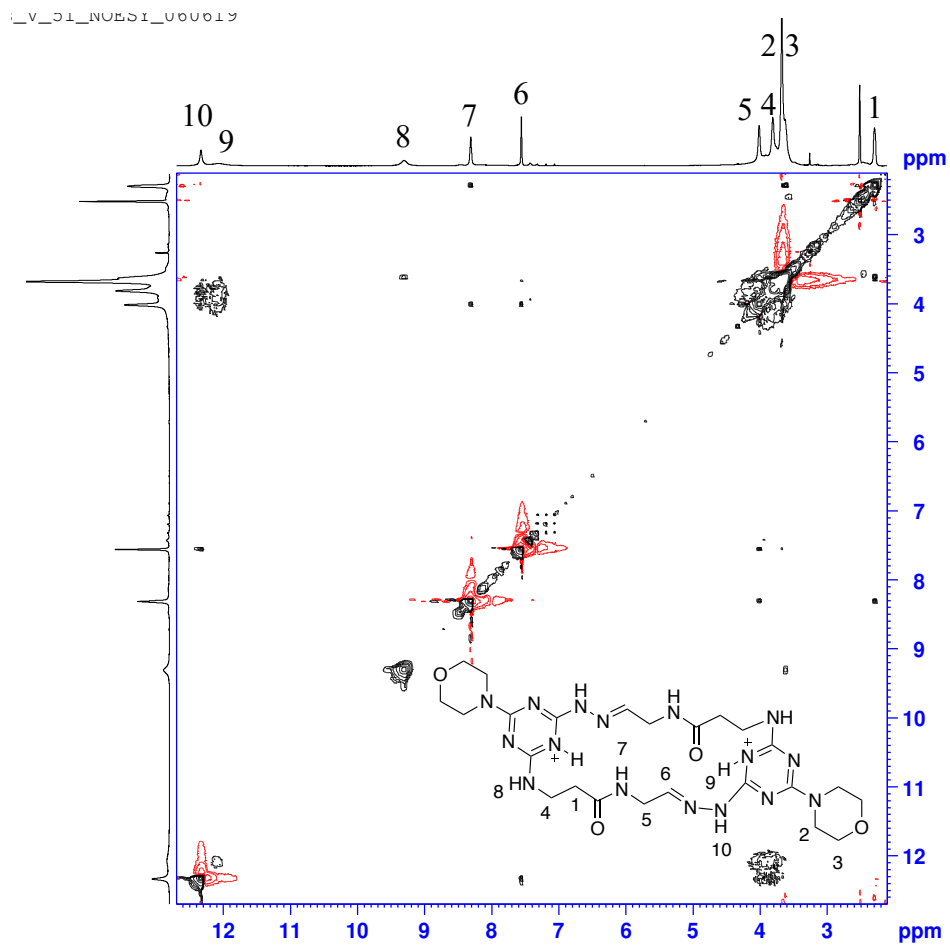


Figure S61:  $^1\text{H}$ -NOESY-NMR of macrocycle **3\*3** in  $\text{DMSO-}d_6$



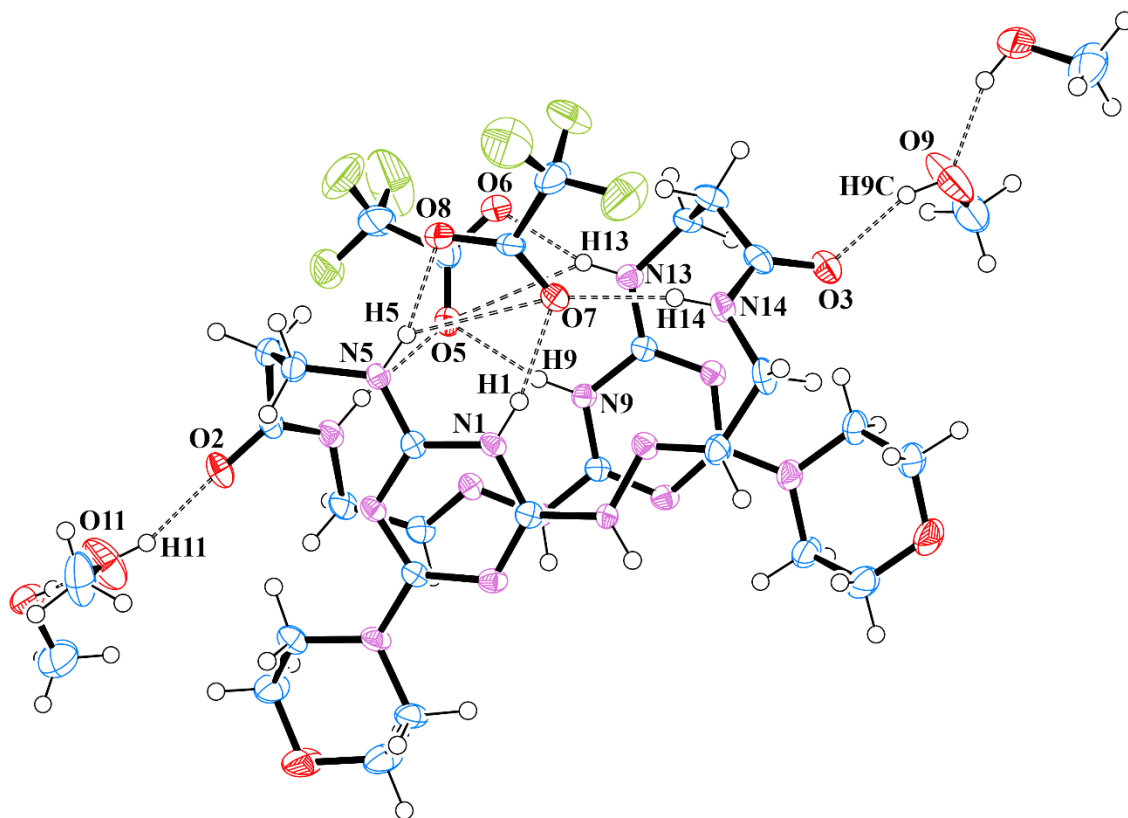


Figure S62: X-ray crystal structure thermal ellipsoid diagram of **3\*3** with 50% probability of at 100K temperature.

Table S3: Selected hydrogen bond parameters of **3\*3** with important labels mentioned in above figure

<i>D</i> —H··· <i>A</i>	<i>D</i> —H (Å)	H··· <i>A</i> (Å)	<i>D</i> ··· <i>A</i> (Å)	<i>D</i> —H··· <i>A</i> (°)
N1—H1···O7	0.88	1.97	2.818 (5)	163
N14—H14···O7	0.88	2.07	2.937 (5)	167
N9—H9···O5	0.88	1.97	2.836 (5)	166
N5—H5···O8	0.88	1.96	2.781 (5)	155
N8—H8···O10 <sup>i</sup>	0.88	1.85	2.730 (5)	175
N6—H6···O5	0.88	2.10	2.957 (5)	166
N13—H13···O6	0.88	1.90	2.750 (5)	163
O9—H9C···O3	0.84	1.88	2.677 (5)	158
O11—H11···O2	0.84	1.88	2.677 (5)	158

Symmetry codes: (i)  $x+1/2, -y+1/2, z+1/2$

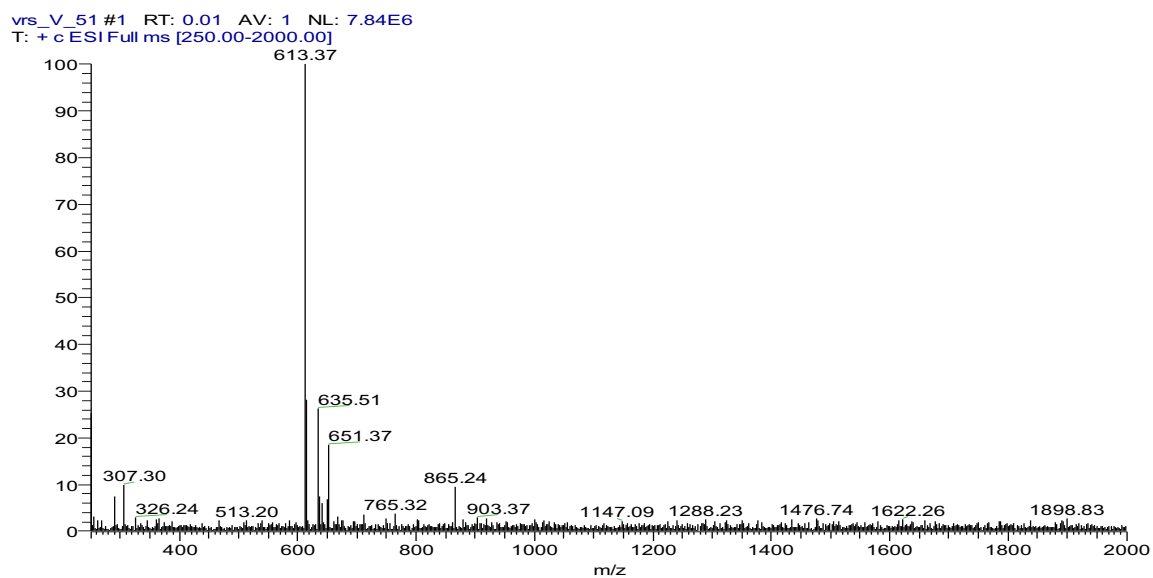


Figure S63: Mass spectrum of macrocycle **3\*3**

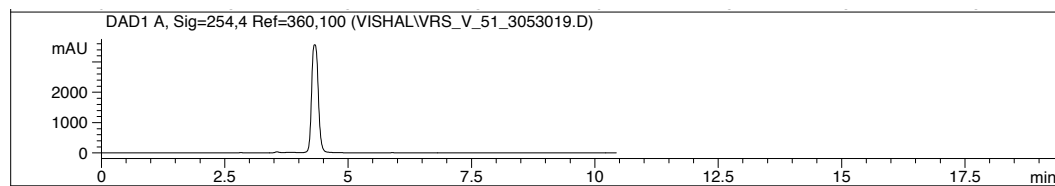
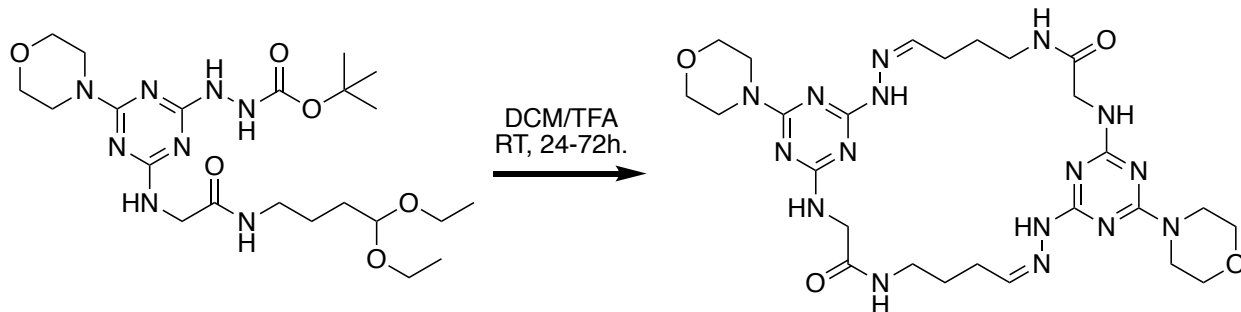


Figure S64: HPLC chromatogram of **3\*3**

### Synthesis of macrocycle **4\*4** from **4**:



Compound **4** (220 mg, 0.39 mmoles) was dissolved in a mixture of 3 ml DCM and 3 ml TFA in a small flask equipped with stirring bar. Produced solution was left to stir at room temperature for 48 hours or until all the solvents (DCM & TFA) had evaporated. Crude product was then washed with 3 ml DCM (x2) and filtered. The precipitates were then washed with 0.5 ml MeOH (x2). Excess MeOH should be avoided as macrocycle dissolve sparingly in MeOH at room temperature. Precipitates were dried under vacuum to afford 103 mg (83%) of Macrocycle **4\*4** as white solid.

Figure S65:  $^1\text{H}$ -NMR, DMSO- $d_6$ ;  $\delta$  12.62 (1H, s), 11.45 (1H, s), 9.09 (1H, t), 8.35-8.32 (1H, t), 7.62-7.61 (1H, t), 4.30-4.28 (2H, d), 3.86-3.68 (8H, m), 3.28-3.26 (2H, m), 2.39 (2H, m), 1.73-1.72 (2H, m).

Figure S67:  $^{13}\text{C}$ -NMR,  $\text{CD}_3\text{OD}$ ;  $\delta$  169.2, 161.6, 154.4, 154.1, 150.5, 66.1, 44.5, 42.6, 37.8, 27.7, 24.5

Figure S72: M/Z: MS (ESI/Q-TOF)  $m/z$ :  $[\text{M}+\text{H}]^+$  Calcd for  $\text{C}_{26}\text{H}_{42}\text{N}_{16}\text{O}_4$  640.31; Found 641.40.

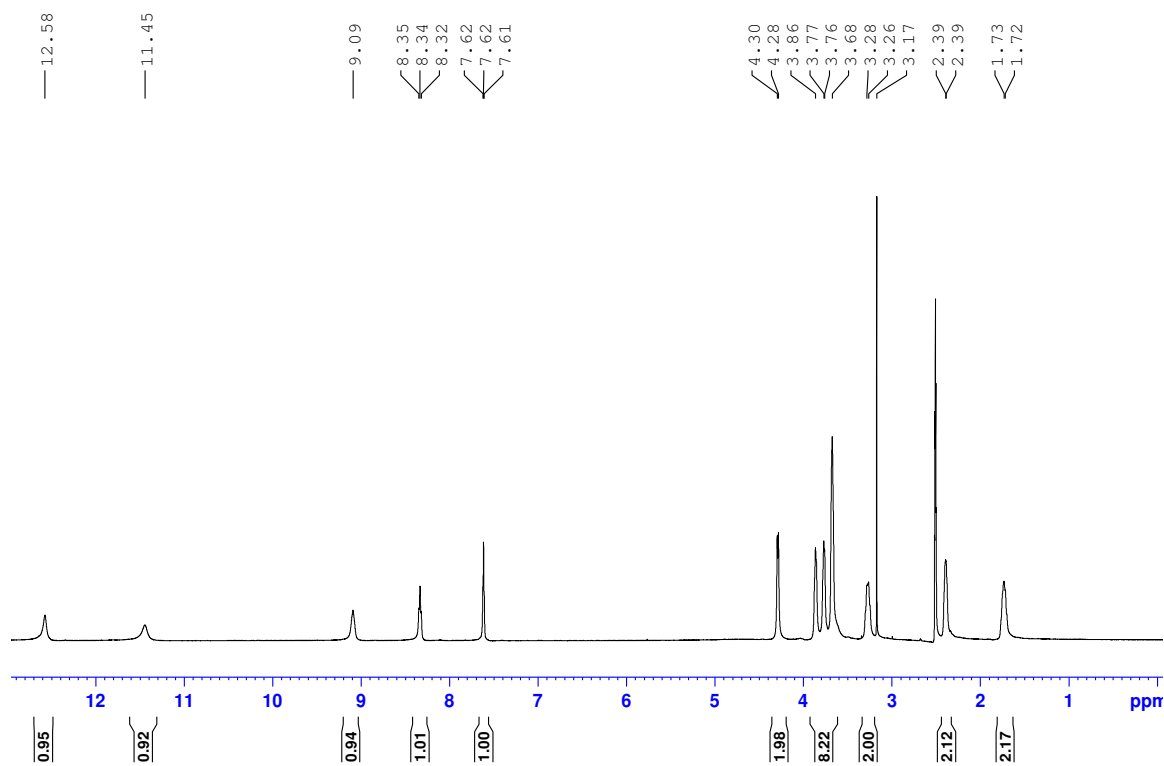


Figure S65:  $^1\text{H}$ -NMR of macrocycle **4\*4** in  $\text{DMSO-}d_6$

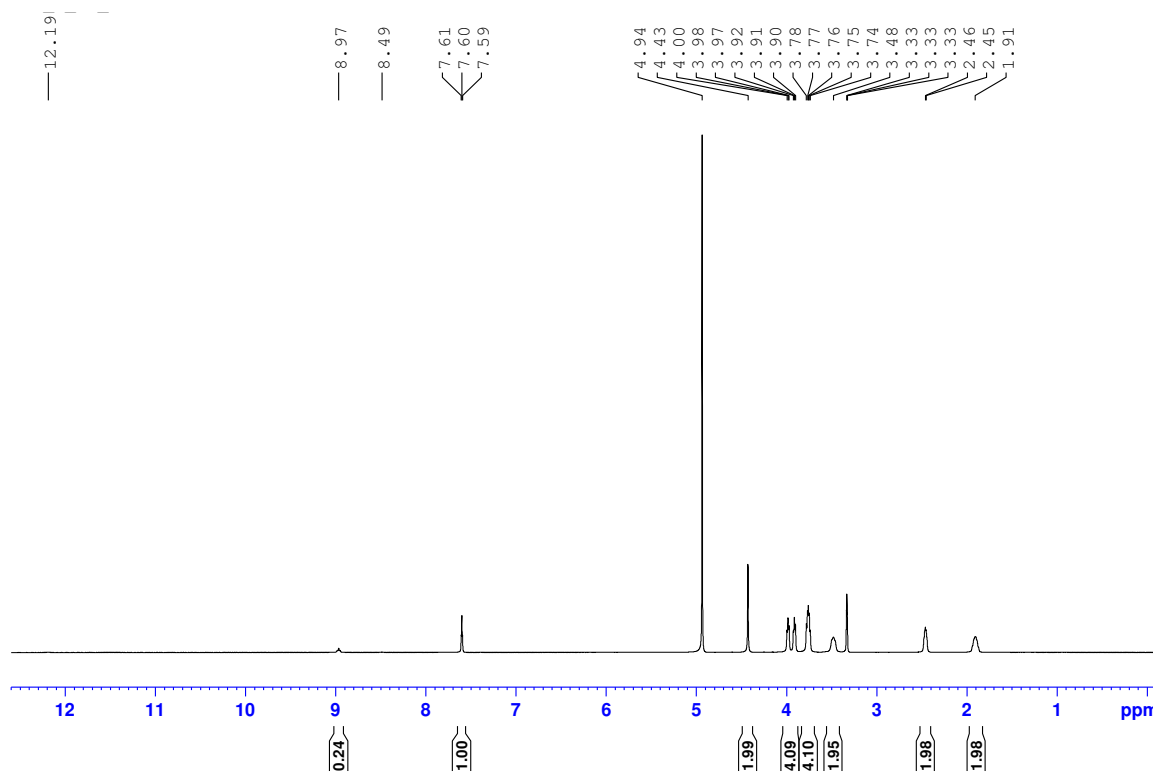


Figure S66:  $^1\text{H}$ -NMR of macrocycle **4\*4** in  $\text{CD}_3\text{OD}$

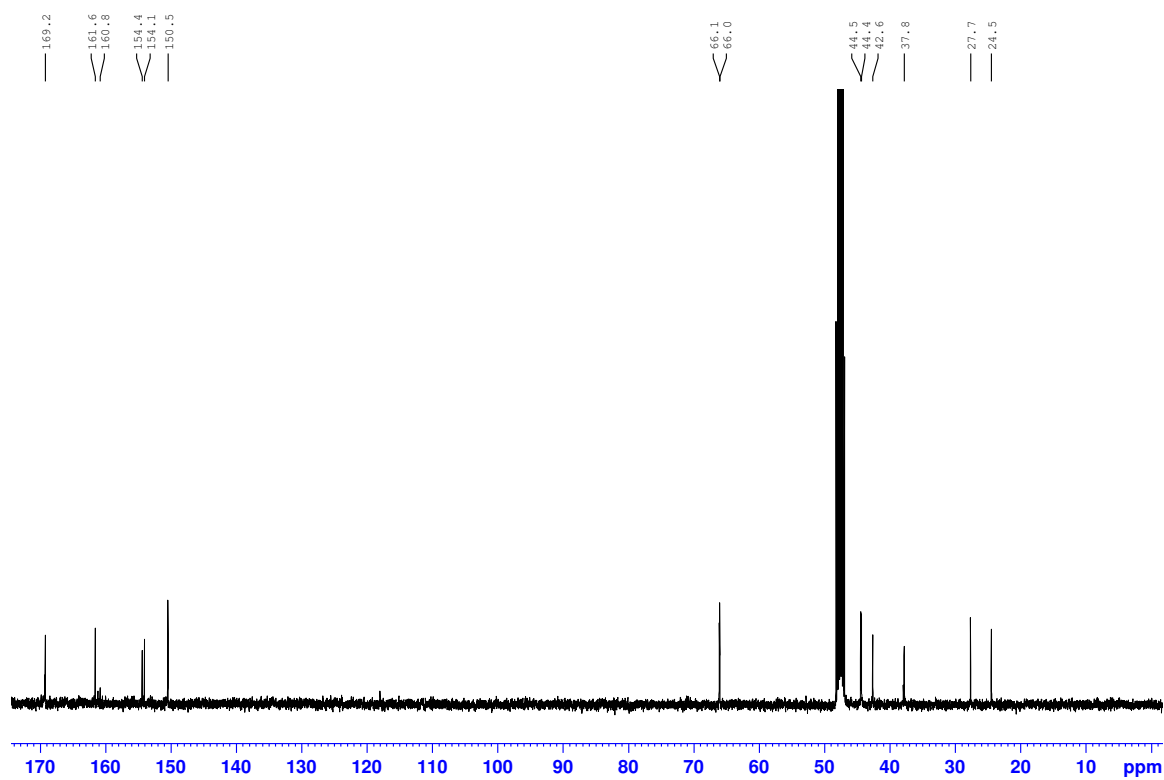


Figure S67: <sup>13</sup>C-NMR of macrocycle **4\*4** in CD<sub>3</sub>OD

.V\_56\_cosy\_030419

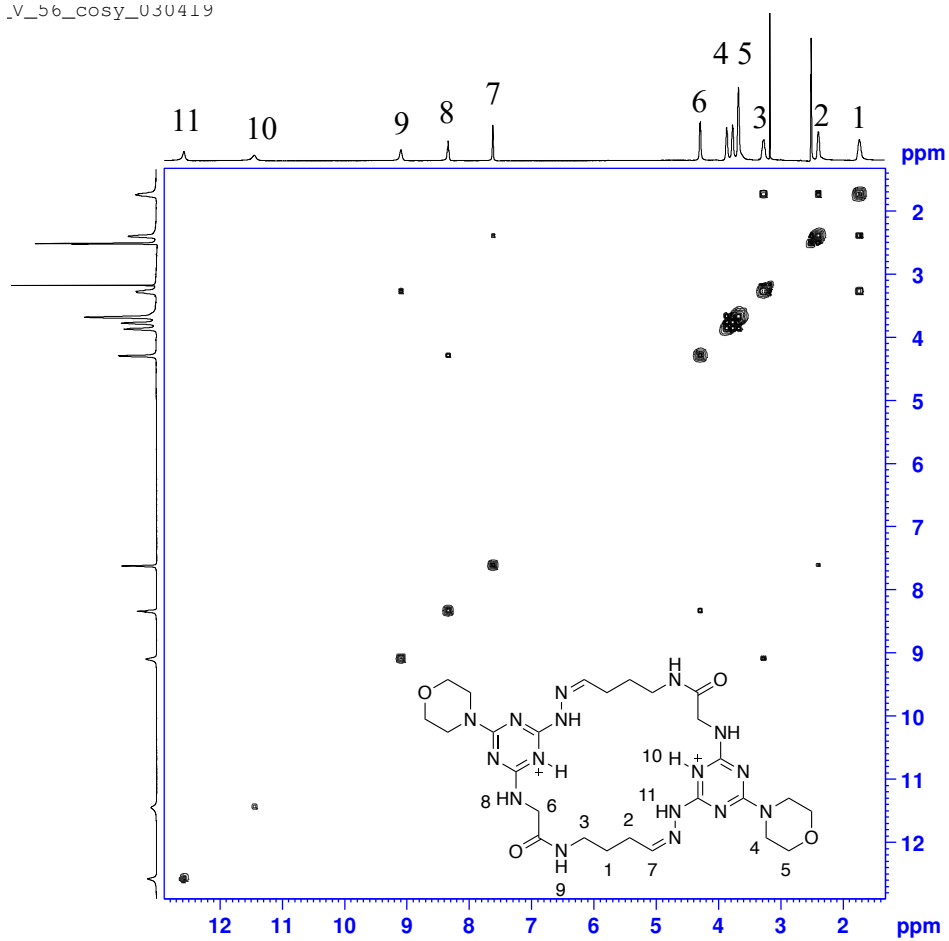


Figure S68: COSY-NMR of macrocycle **4\*4** in DMSO- $d_6$

vrs\_V\_56\_cosy\_030419

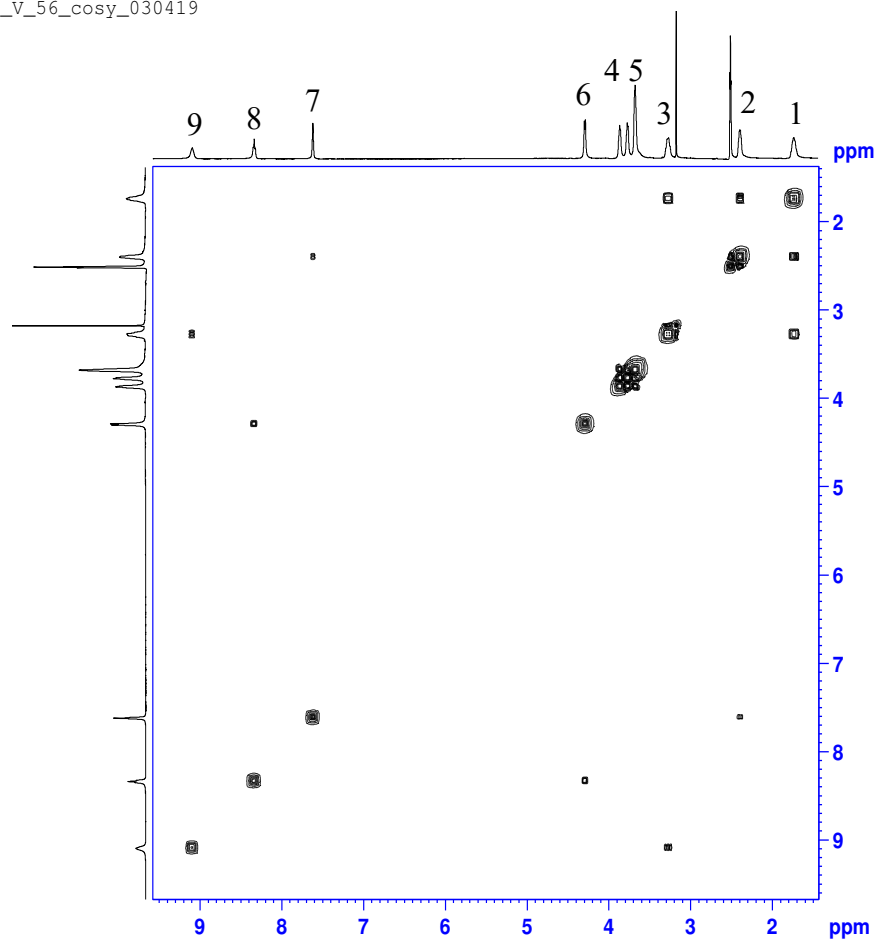


Figure S69: COSY-NMR zoomed of macrocycle **4\*4** in DMSO- $d_6$

\_V\_56\_NOESY\_060519

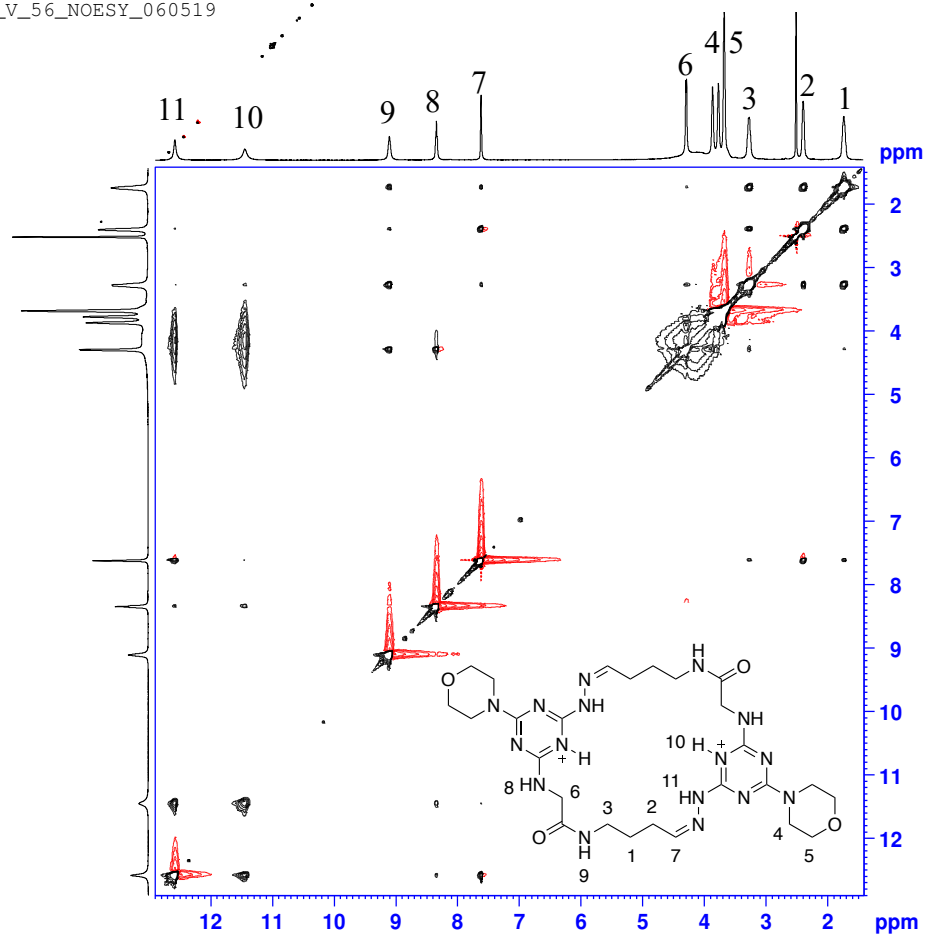


Figure S70: NOESY-NMR of macrocycle 4\*4 in DMSO- $d_6$



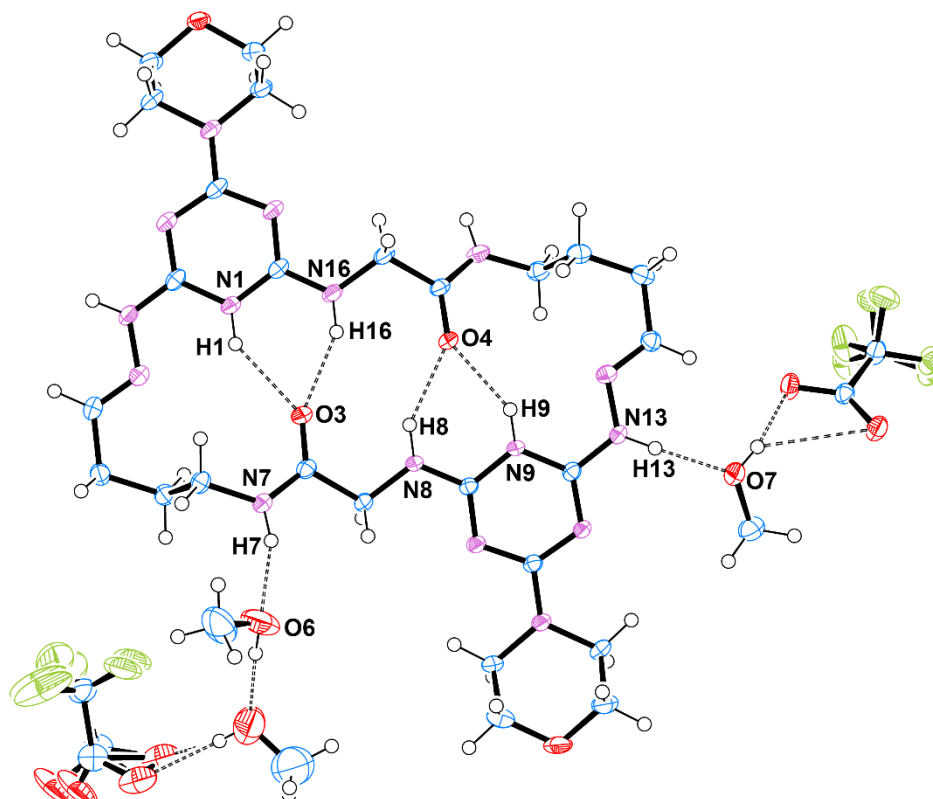


Figure S71: X-ray crystal structure thermal ellipsoid diagram of **4\*4** with 50% probability of at 100K temperature.

Table S4: Selected hydrogen bond parameters of **4\*4** with important labels mentioned in above figure

<i>D</i> —H··· <i>A</i>	<i>D</i> —H (Å)	H··· <i>A</i> (Å)	<i>D</i> ··· <i>A</i> (Å)	<i>D</i> —H··· <i>A</i> (°)
N1—H1···O3	0.88	2.20	2.952 (3)	143
N5—H5···O9 <sup>i</sup>	0.88	1.88	2.735 (3)	164
N7—H7···O6	0.88	1.91	2.753 (4)	160
N8—H8···O4	0.88	2.09	2.875 (3)	148
N9—H9···O4	0.88	2.09	2.862 (3)	146
N13—H13···O7	0.88	1.86	2.740 (3)	178
N16—H16···O3	0.88	2.04	2.840 (3)	151

Symmetry codes: (i)  $x-1, y, z+1$ ;

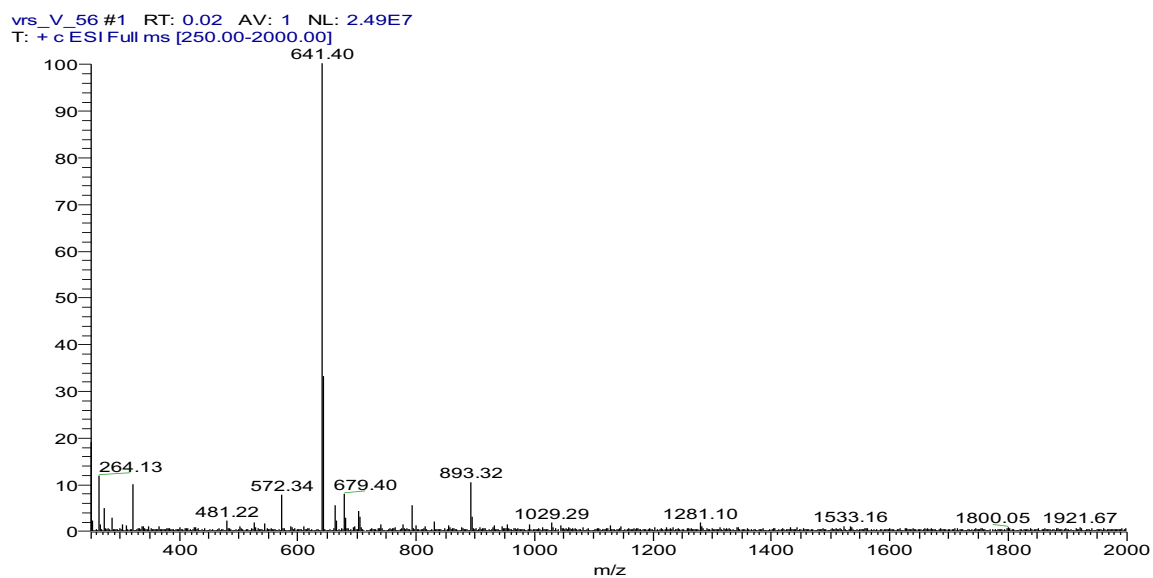


Figure S72: Mass spectrum of macrocycle **4\*4**.

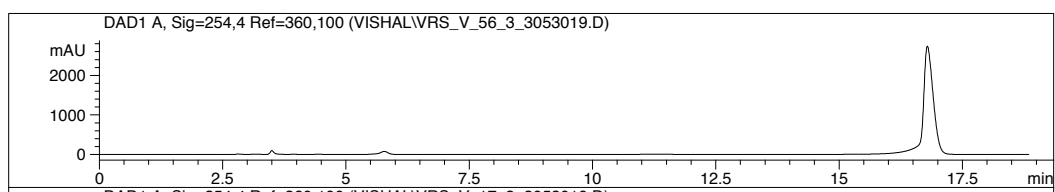
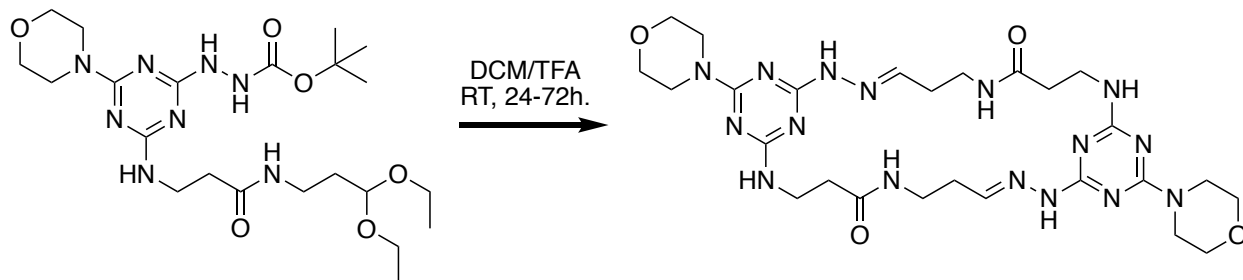


Figure S73: HPLC chromatogram of **4\*4**.

### Synthesis of 5\*5 from 5:



Compound **5** (20 mg, 0.039 mmoles) was dissolved in a mixture of 1 ml DCM and 1 ml TFA in a small flask equipped with stirring bar. Reaction mixture and resulting solution was left to stir at room temperature for 48 hours. Crude product was not washed with DCM or MeOH and dried as it to yield **5\*5** (12 mg, 97%) as white solid. NMR of crude product was taken as it without further purifications.

Figure S74:  $^1\text{H}$ -NMR,  $\text{DMSO-}d_6$ ;  $\delta$  12.62 (1H, s), 11.45 (1H, s), 9.09 (1H, t), 8.35-8.32 (1H, t), 7.62-7.61 (1H, t), 4.30-4.28 (2H, d), 3.86-3.68 (8H, m), 3.28-3.26 (2H, m), 2.39 (2H, m), 1.73-1.72 (2H, m).

Figure S77:  $^{13}\text{C}$ -NMR,  $\text{DMSO-}d_6$ ;  $\delta$  171.5, 161.7, 154.7, 154.6, 150.7, 66.2, 44.6, 36.6, 35.9, 34.4, 33.0

Figure S83: M/Z: MS (ESI/Q-TOF) m/z:  $[\text{M}+\text{H}]^+$  Calcd for  $\text{C}_{26}\text{H}_{40}\text{N}_{16}\text{O}_4$  640.34; Found 641.37.

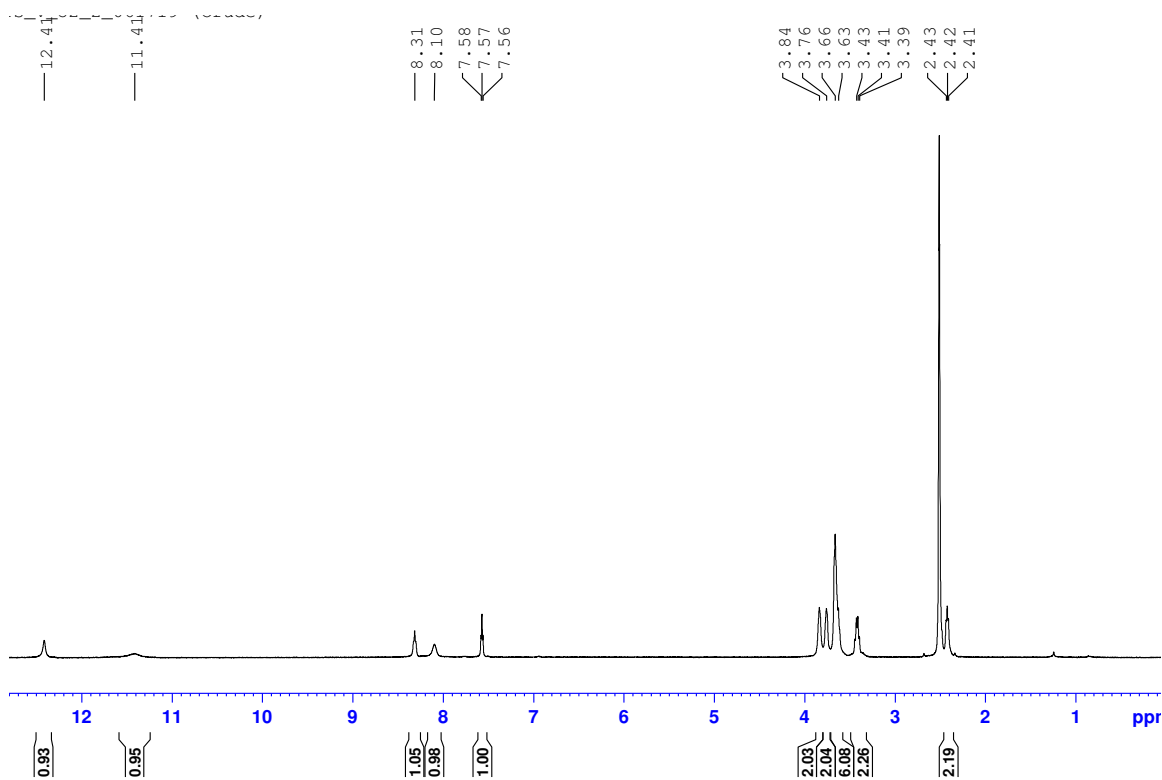


Figure S74:  $^1\text{H}$ -NMR of macrocycle **5\*5** in  $\text{DMSO-}d_6$

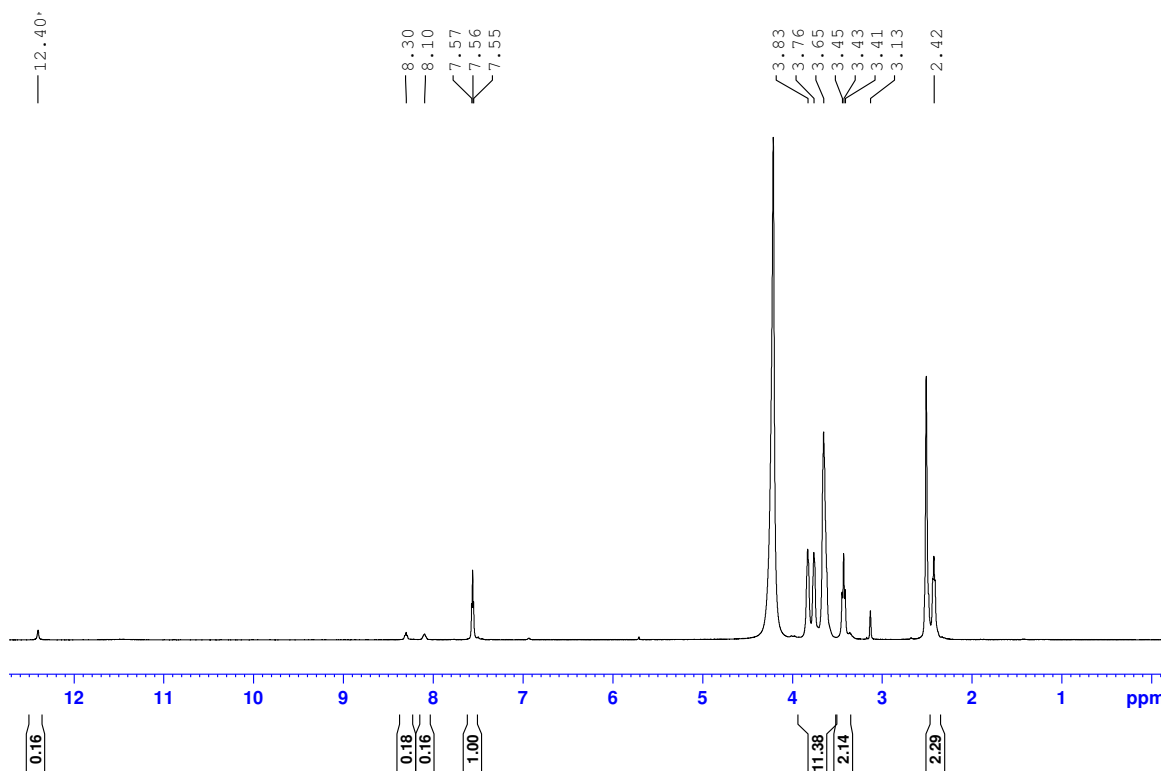


Figure S75:  $^1\text{H}$ -NMR of macrocycle **5\*5** in  $\text{DMSO-}d_6$  with a drop of  $\text{CD}_3\text{OD}$ .

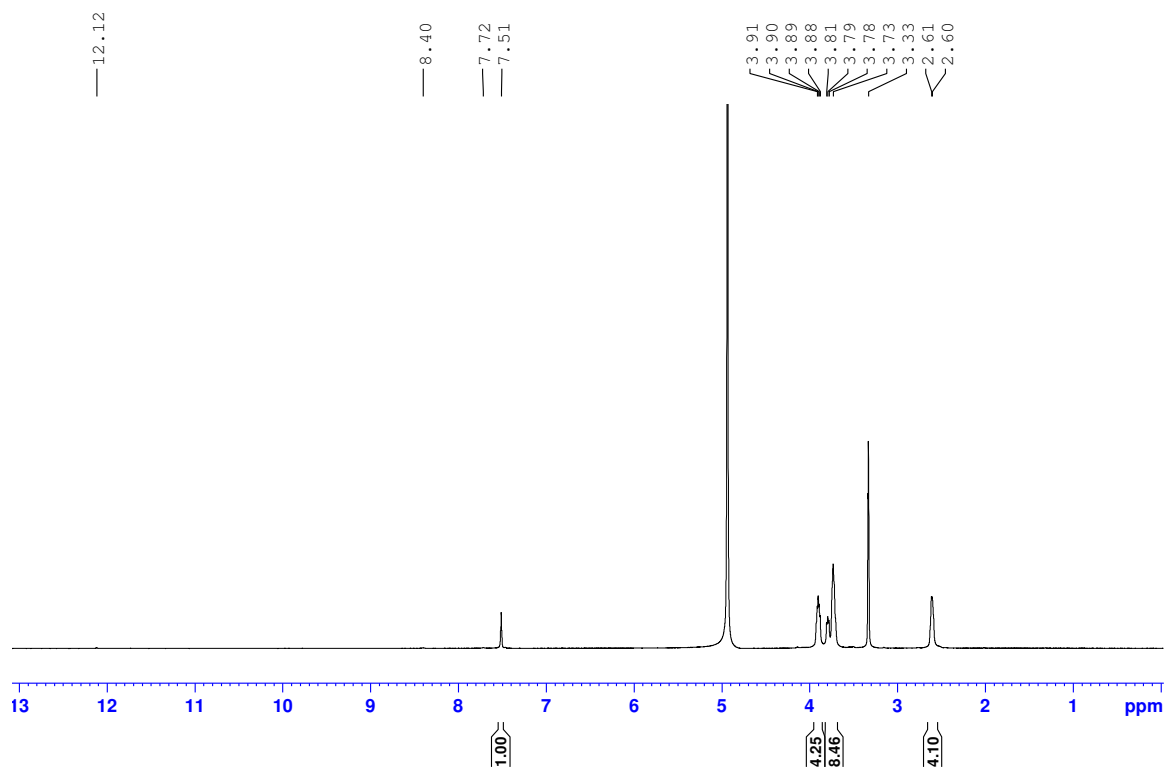


Figure S76: <sup>1</sup>H-NMR of macrocycle **5\*5** in CD<sub>3</sub>OD.

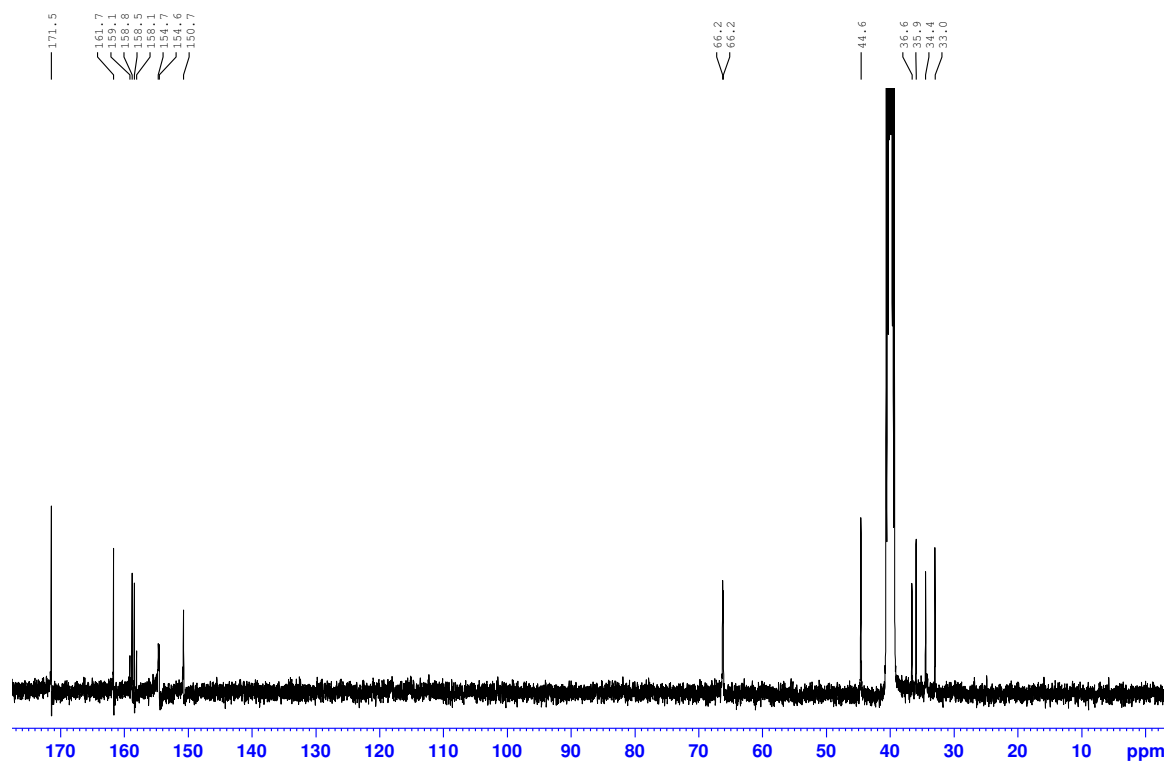


Figure S77: <sup>13</sup>C-NMR of macrocycle **5\*5** in DMSO-*d*<sub>6</sub>

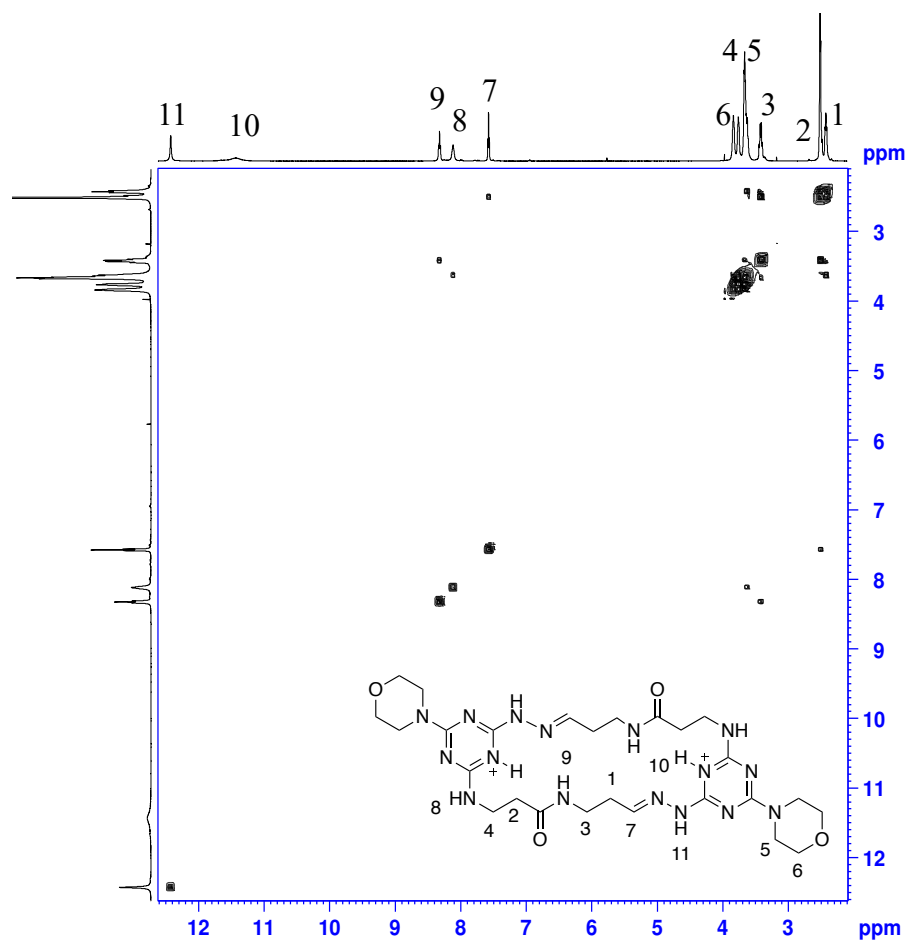


Figure S78: COSY-NMR of macrocycle **5\*5** in DMSO-*d*<sub>6</sub>

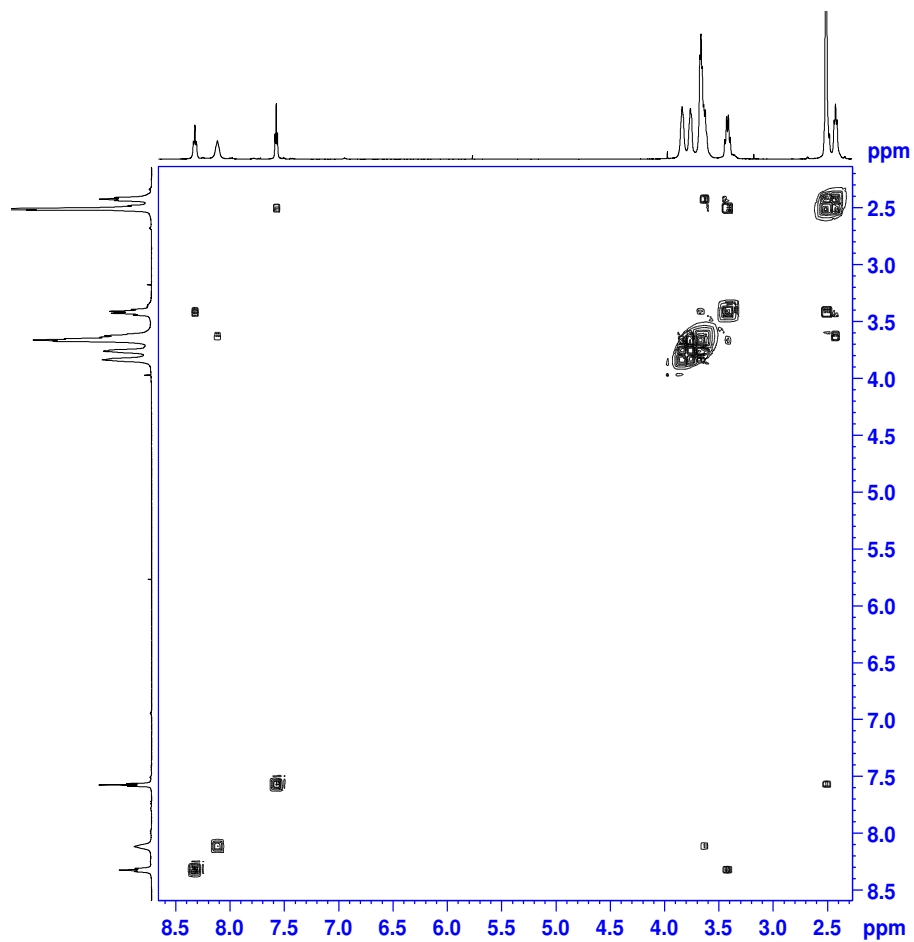


Figure S79: COSY-NMR (zoomed) of macrocycle **5\*5** in DMSO-*d*<sub>6</sub>

\_V\_52\_2\_NOESY\_061719

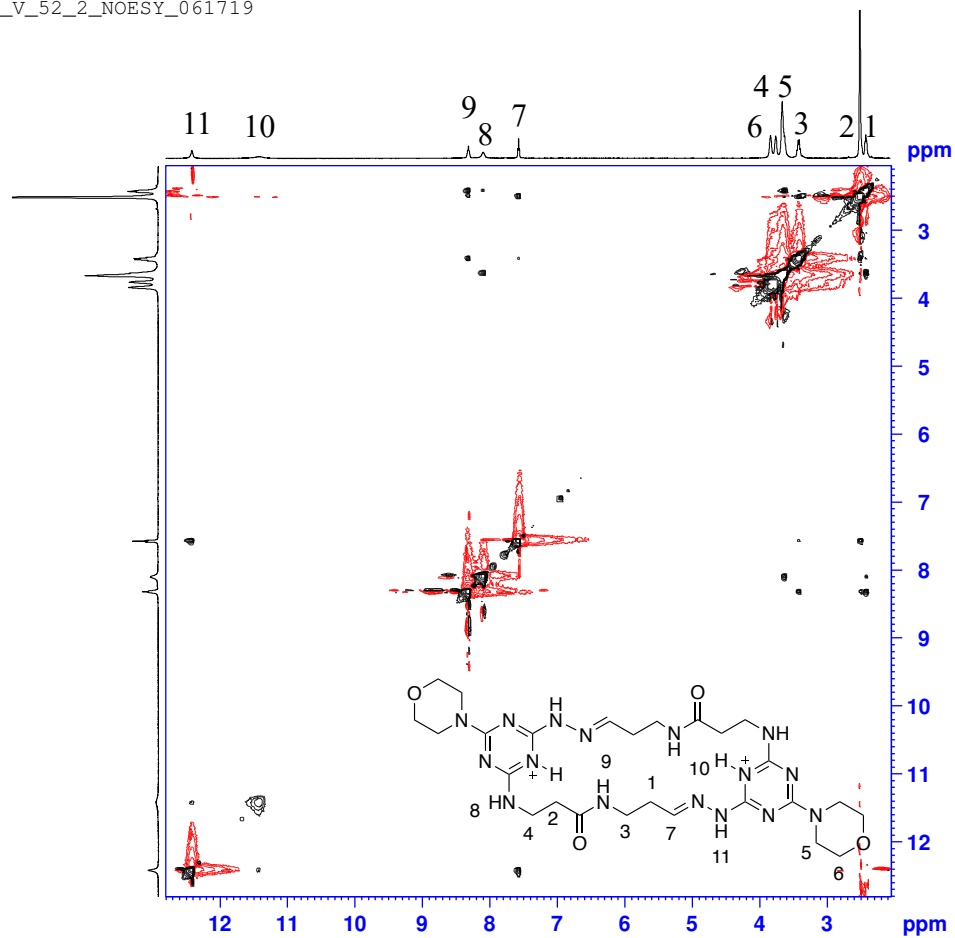


Figure S80: NOESY-NMR of macrocycle **5\*5** in DMSO-*d*<sub>6</sub>



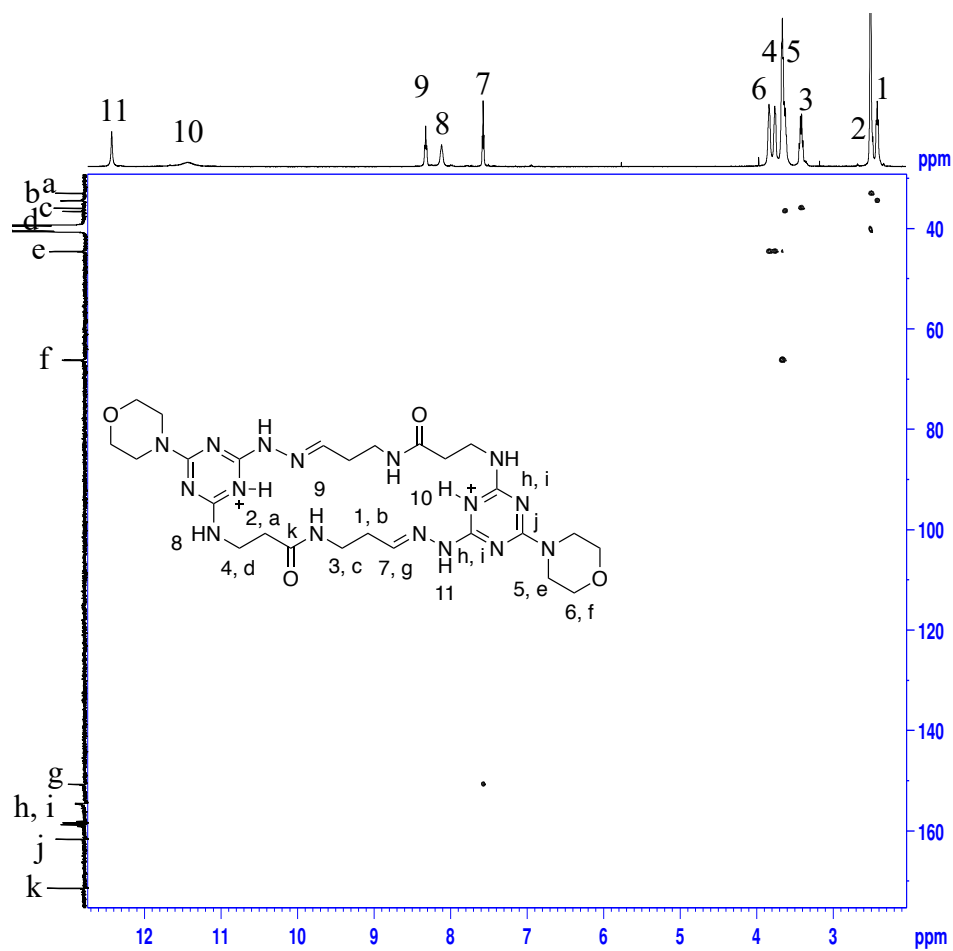


Figure S81:  $^{13}\text{C}$ - $^1\text{H}$ -HMOC-NMR of macrocycle **5\*5** in  $\text{DMSO-}d_6$

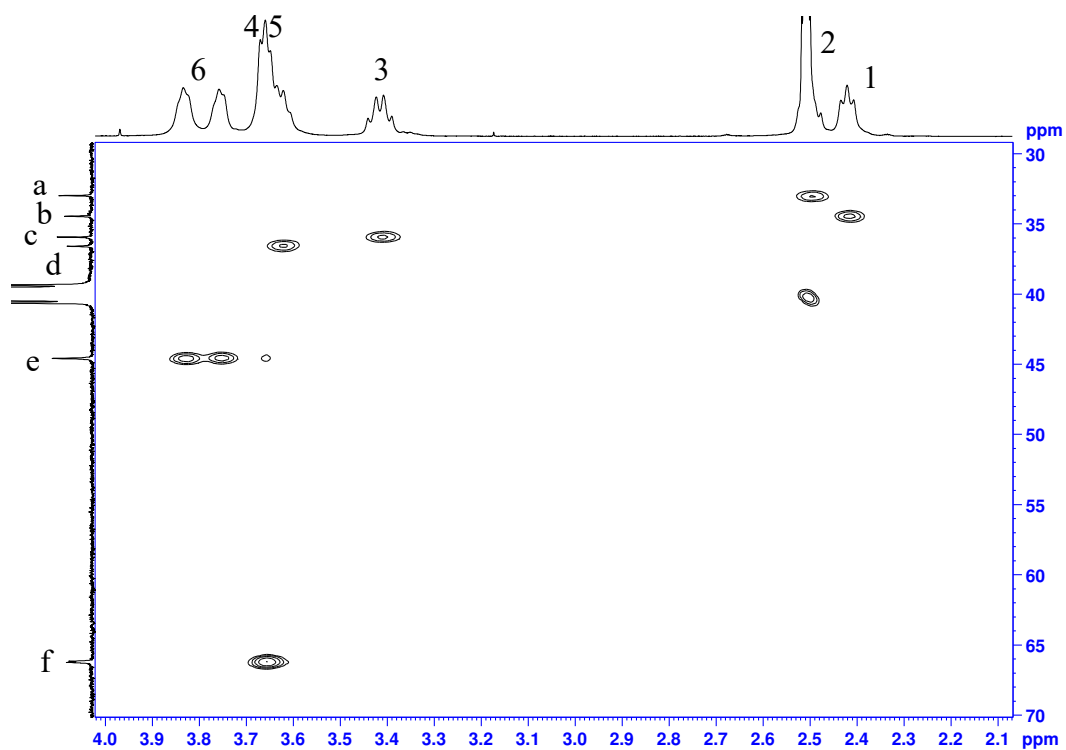


Figure S82:  $^{13}\text{C}$ - $^1\text{H}$ -HMQC-NMR(zoomed 1) of macrocycle **5\*5** in  $\text{DMSO}-d_6$

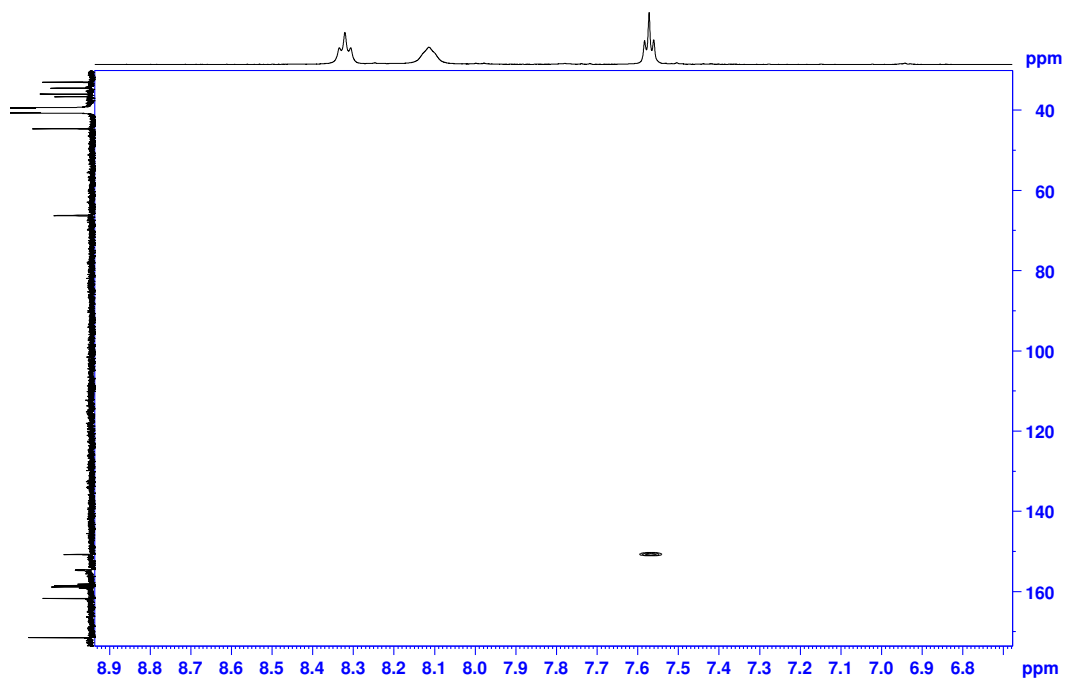


Figure S83:  $^{13}\text{C}$ - $^1\text{H}$ -HMQC-NMR(zoomed 2) of macrocycle **5\*5** in  $\text{DMSO}-d_6$

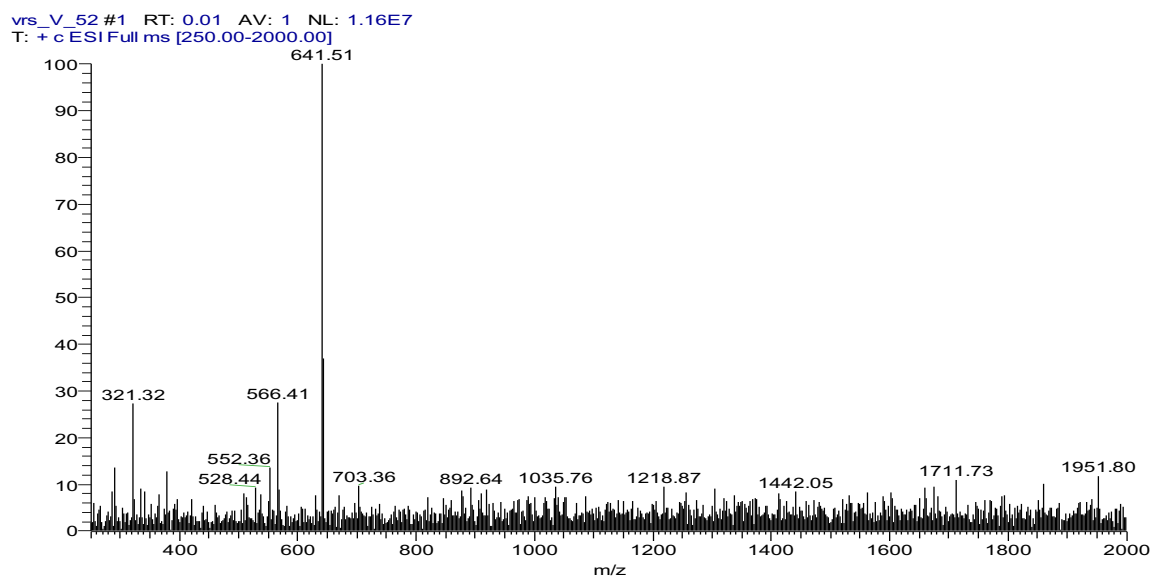


Figure S84: Mass spectrum of macrocycle **5\*5** in DMSO- $d_6$

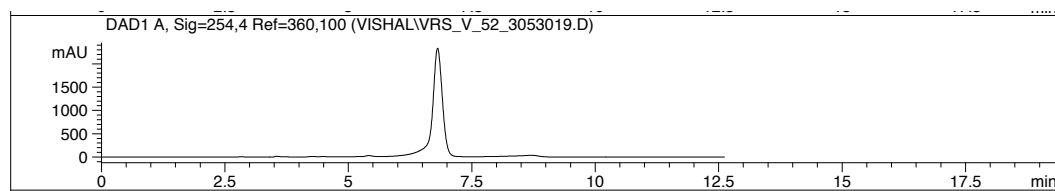


Figure S85: HPLC chromatogram of **5\*5**.

### Synthesis of **3\*3**, **3\*5**, and **5\*5** from one to one mole ratio of **3** and **5**:

Compound **3** (90 mg, 0.192 mmol) and compound **5** (98 mg, 0.192 mmol) were dissolved in equal mixture of 2 ml of DCM and 2 ml of TFA in a small flask. Solution was left to stir for 24 hours at room temperature. Mass spectrum of crude product indicate the formation of **3\*3**, **3\*5**, and **5\*5**. In addition, their formation was confirmed by HPLC analysis.

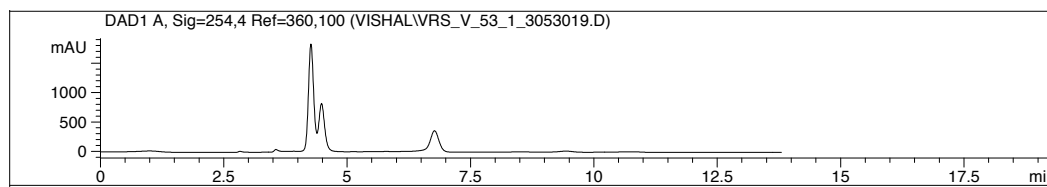
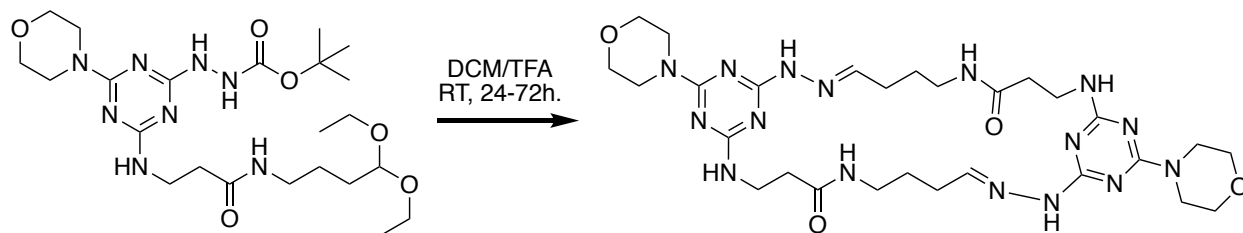


Figure S86: HPLC chromatogram of a mixture of **3\*3**, **3\*5**, and **5\*5**.

### Synthesis of 6\*6 from 6:



Compound **6** (150 mg, 0.29 mmoles) was dissolved in a 3 ml DCM and 3 ml TFA in a small flask equipped with stirring bar. Resulting solution was left to stir at room temperature for 48 hours or until it was completely dried. Crude product was then washed with 1 ml DCM (x2) and filtered. The precipitates were then washed with 0.3 ml MeOH (x2). Excess MeOH should be avoided as macrocycle dissolve sparingly in MeOH at room temperature. Precipitates were air dried to afford of **6\*6** (85 mg, 86%) as white solid.

Figure S87:  $^1\text{H}$ -NMR,  $\text{DMSO}-d_6$ ;  $\delta$  12.62 (1H, s), 11.45 (1H, s), 9.09 (1H, t), 8.35-8.32 (1H, t), 7.62-7.61 (1H, t), 4.30-4.28 (2H, d), 3.86-3.68 (8H, m), 3.28-3.26 (2H, m), 2.39 (2H, m), 1.73-1.72 (2H, m).

Figure S89:  $^{13}\text{C}$ -NMR,  $\text{CD}_3\text{OD}$ ; 172.5, 161.7, 154.7, 154.3, 150.6, 66.1, 44.4, 37.7, 36.4, 33.3, 27.6, 24.5

Figure S94: M/Z: MS (ESI/Q-TOF) m/z:  $[\text{M}+\text{H}]^+$  Calcd for  $\text{C}_{28}\text{H}_{46}\text{N}_{16}\text{O}_4$  668.37; Found 669.46.

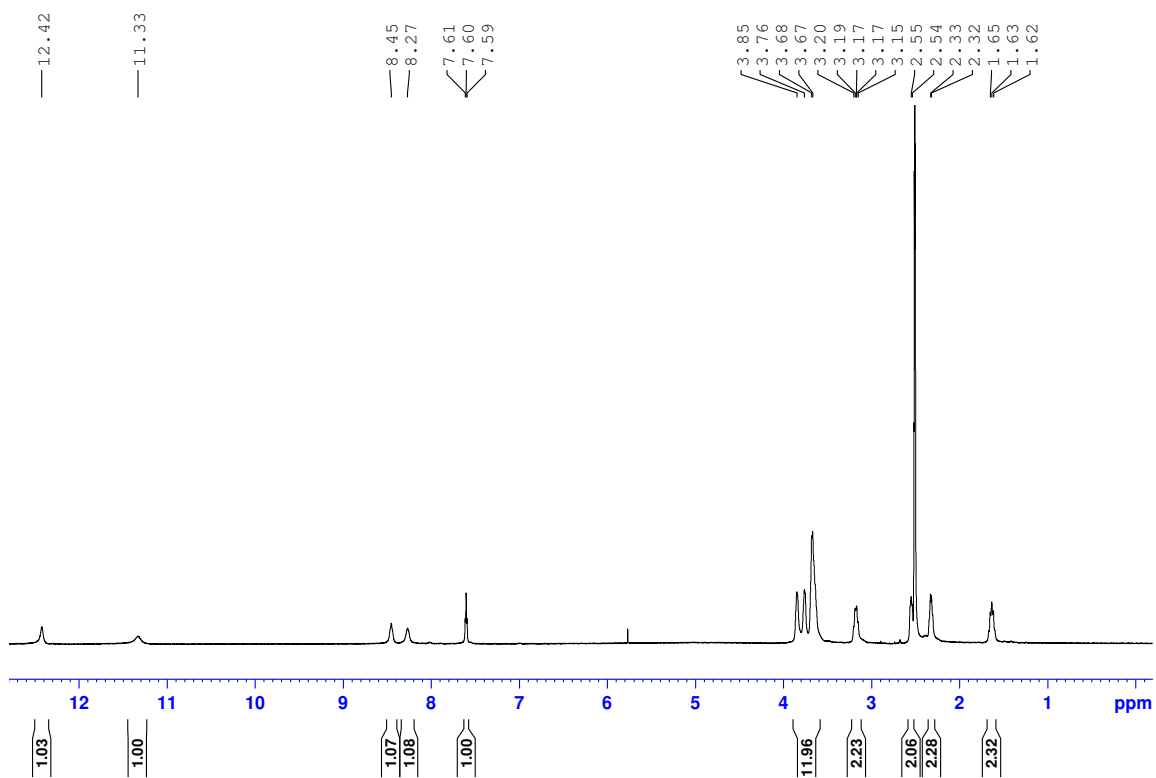


Figure S87:  $^1\text{H}$ -NMR of macrocycle **6\*6** in  $\text{DMSO-}d_6$

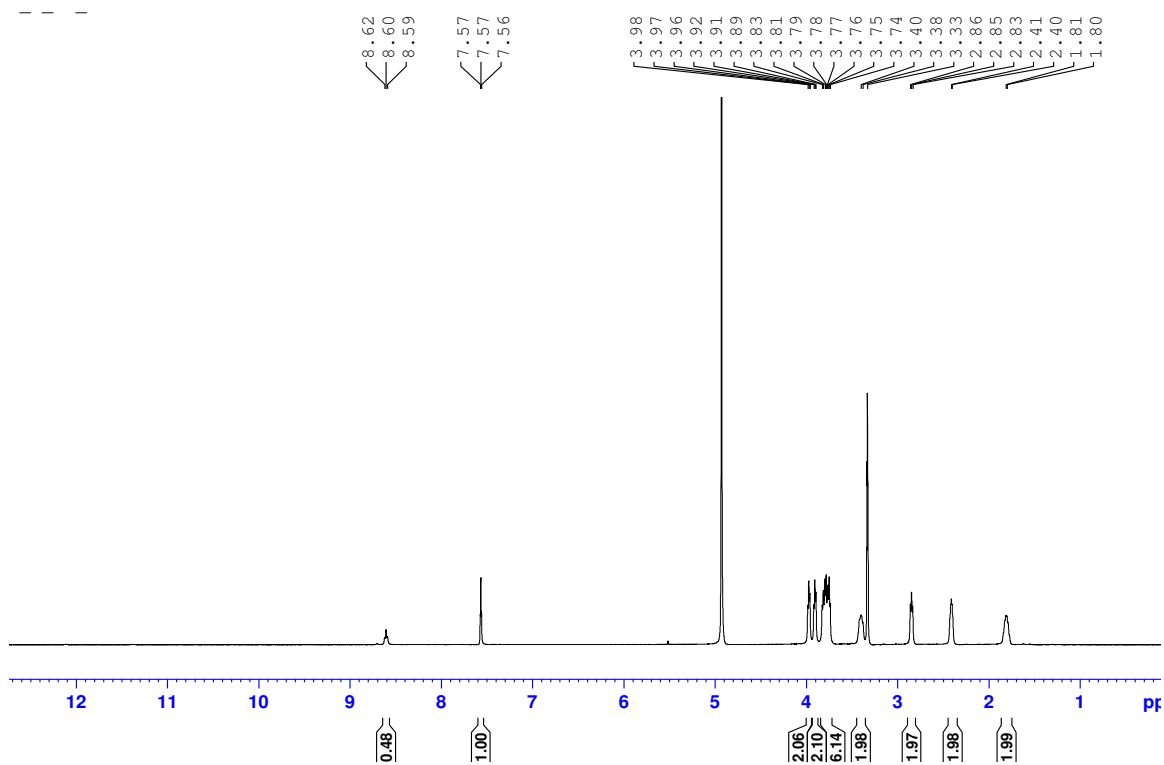


Figure S88:  $^1\text{H}$ -NMR of macrocycle **6\*6** in  $\text{CD}_3\text{OD}$

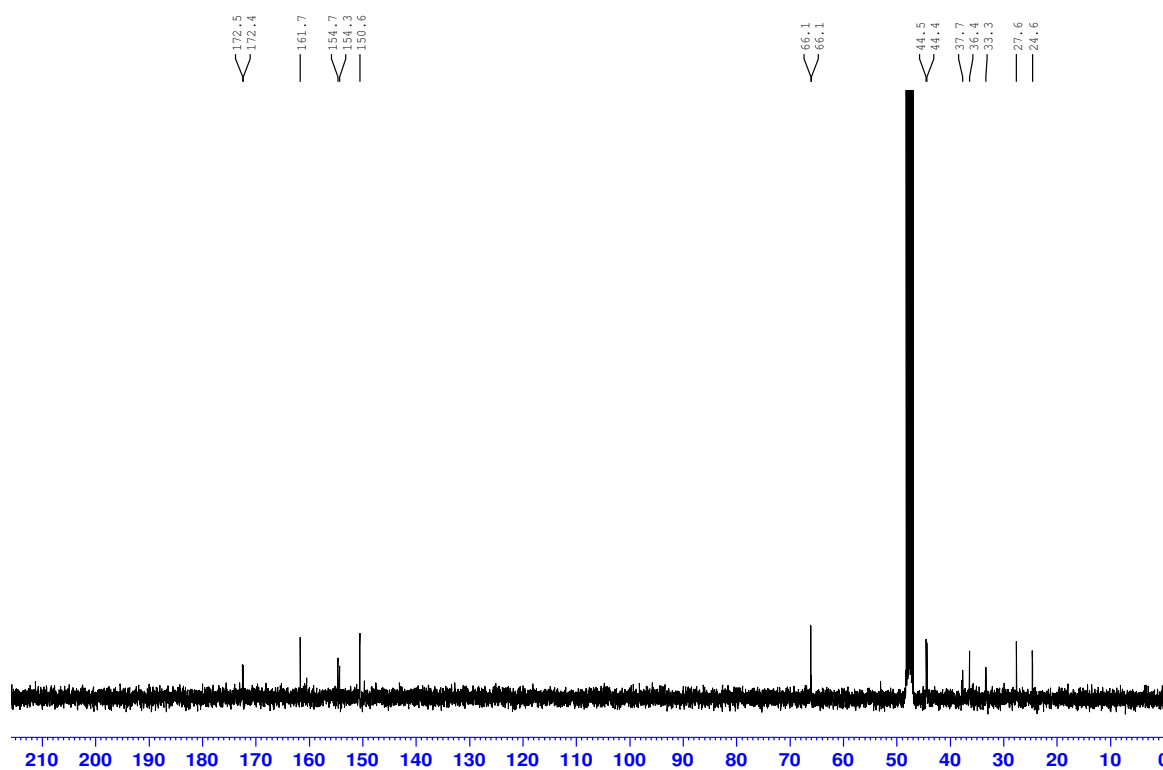


Figure S89:  $^{13}\text{C}$ -NMR of macrocycle **6\*6** in  $\text{CD}_3\text{OD}$

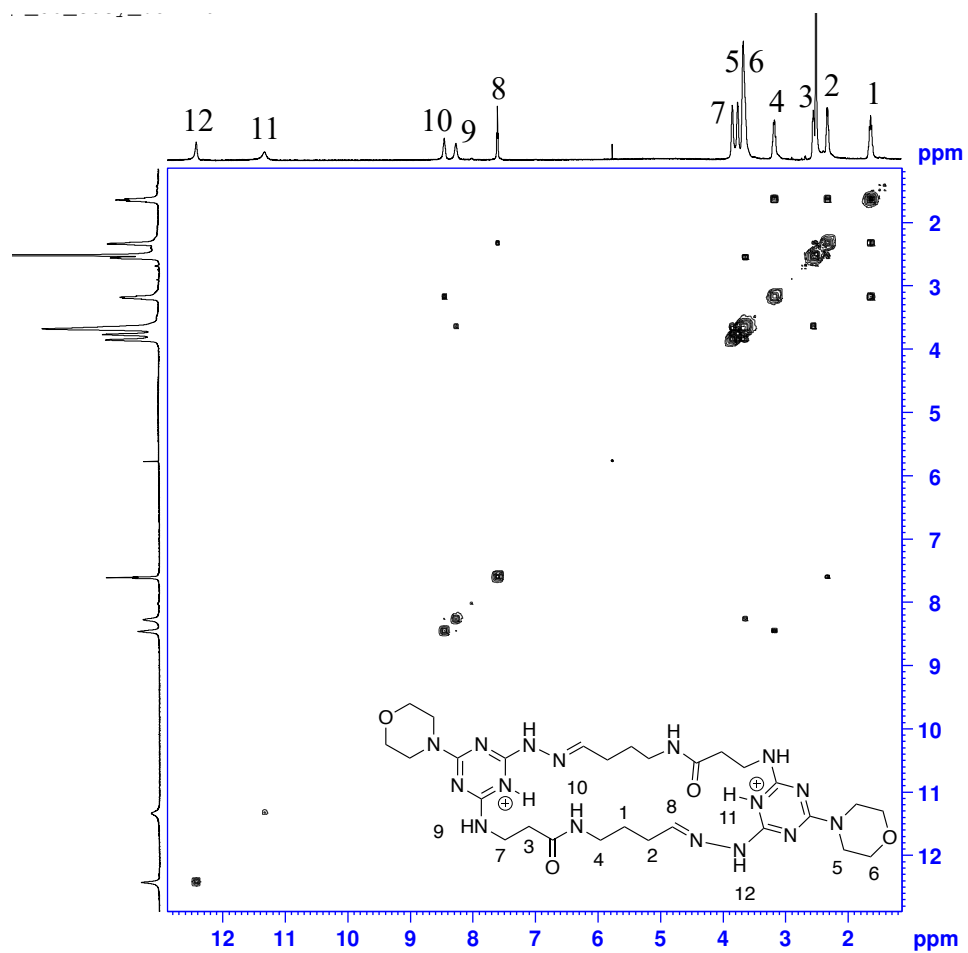


Figure S90:  $^1\text{H}$ -COSY-NMR of macrocycle **6\*6** in  $\text{DMSO-}d_6$



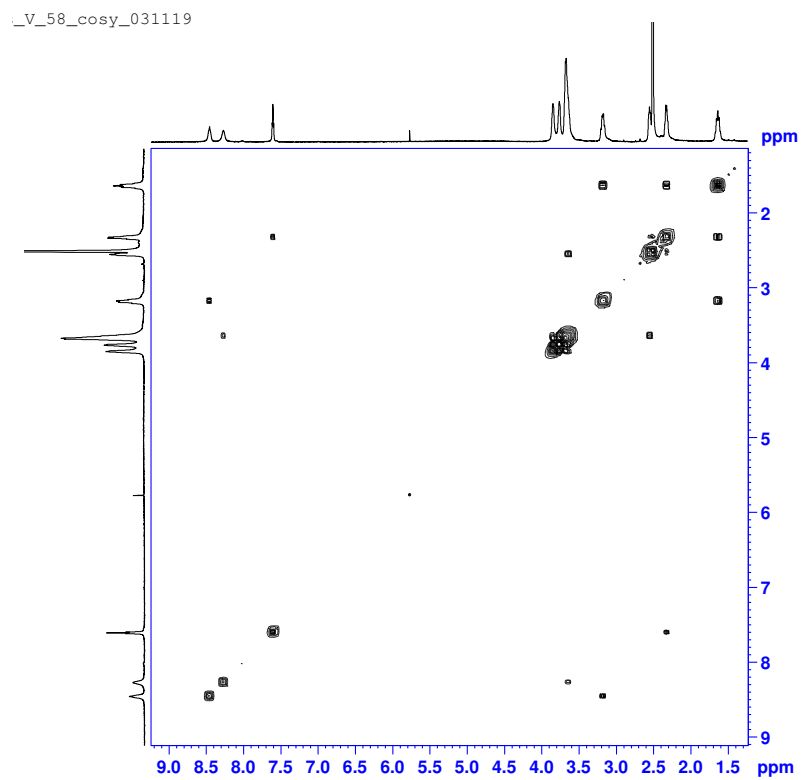


Figure S91:  $^1\text{H}$ -COSY-NMR zoomed of macrocycle **6\*6** in  $\text{DMSO}-d_6$

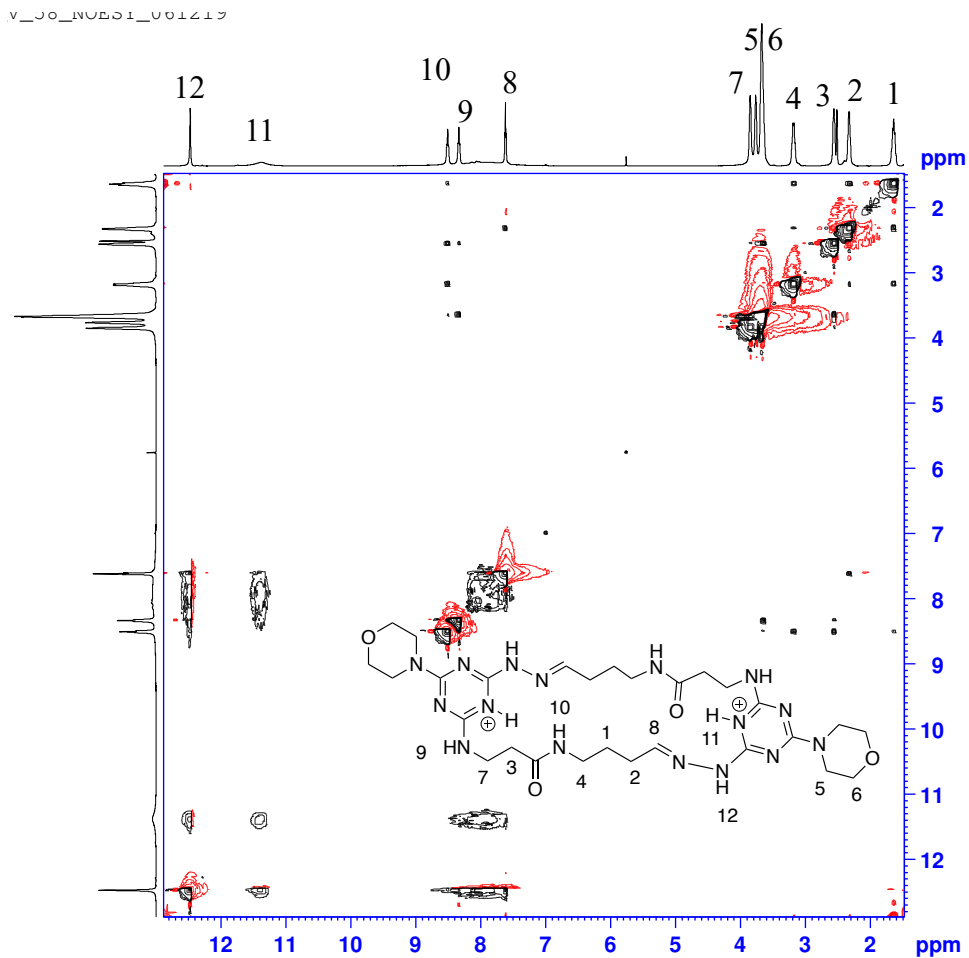


Figure S92:  $^1\text{H}$ -NOESY-NMR of macrocycle **6\*6** in  $\text{DMSO}-d_6$

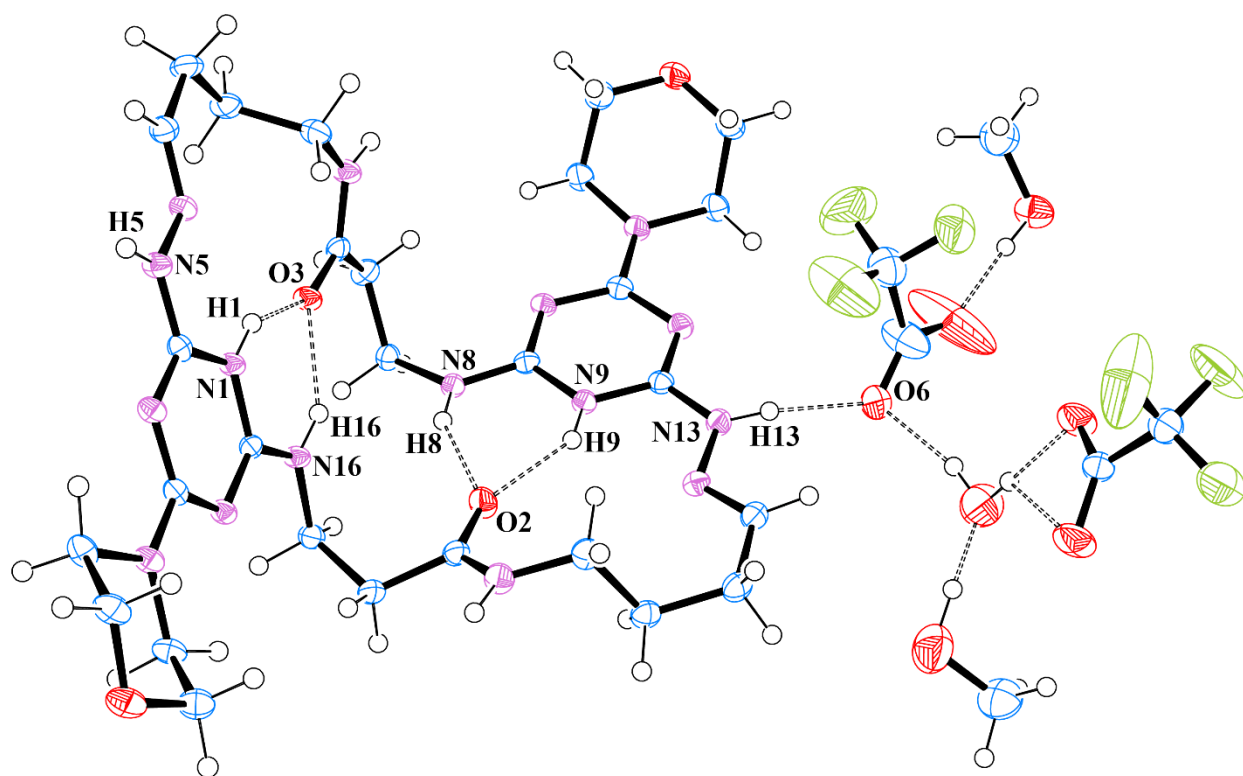


Figure S93: X-ray crystal structure thermal ellipsoid diagram of **6\*6** with 50% probability of at 100K temperature.

Table S5: Selected hydrogen bond parameters of **6\*6** with important labels mentioned in above figure

<i>D</i> —H··· <i>A</i>	<i>D</i> —H (Å)	H··· <i>A</i> (Å)	<i>D</i> ··· <i>A</i> (Å)	<i>D</i> —H··· <i>A</i> (°)
N9—H9···O2	0.88	2.01	2.789 (3)	147
N1—H1···O3	0.88	2.13	2.877 (3)	143
N5—H5···O9 <sup>i</sup>	0.88	1.95	2.814 (3)	167
N8—H8···O2	0.88	2.03	2.828 (3)	150
N16—H16···O3	0.88	1.98	2.771 (3)	149
N15—H15···O7 <sup>ii</sup>	0.88	2.16	2.989 (3)	157
N13—H13···O6	0.88	1.89	2.764 (3)	170
N7—H7···O8 <sup>iii</sup>	0.88	1.97	2.842 (3)	172

Symmetry codes: (i)  $x, y, z+1$ ; (ii)  $-x+1, -y, -z+1$ ; (iii)  $-x+2, -y+1, -z+1$

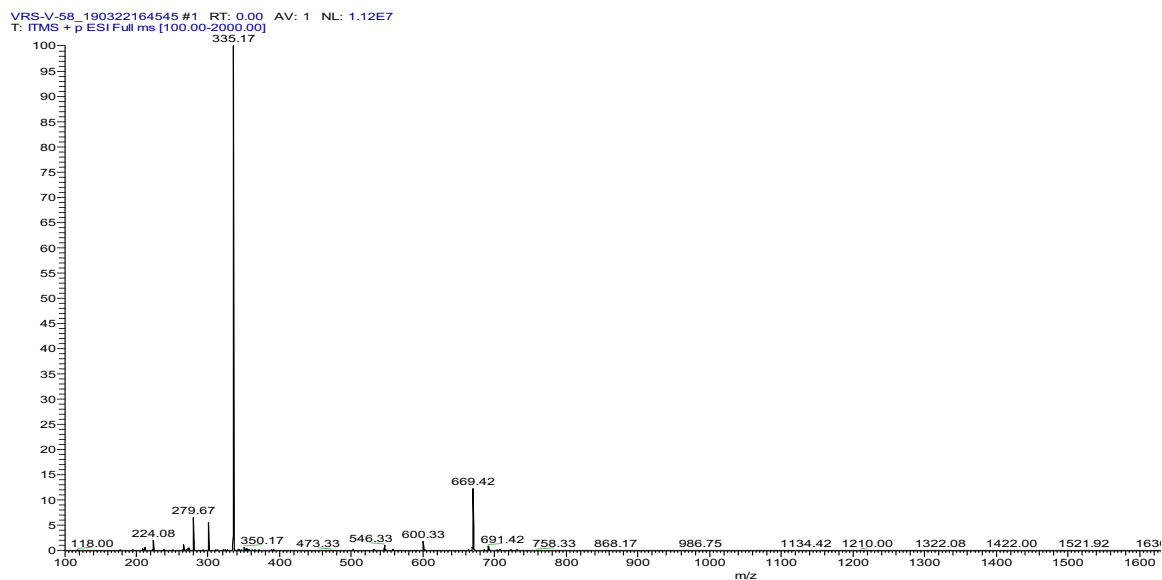


Figure S94: Mass spectrum of macrocycle **6\*6** in DMSO- $d_6$

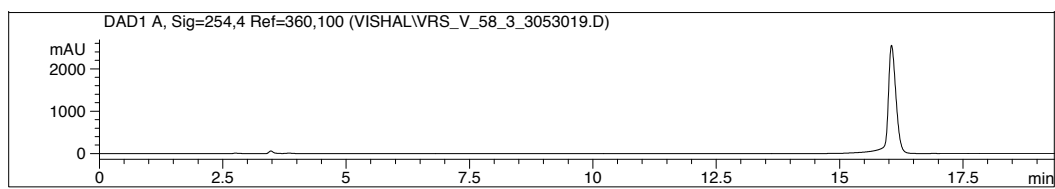
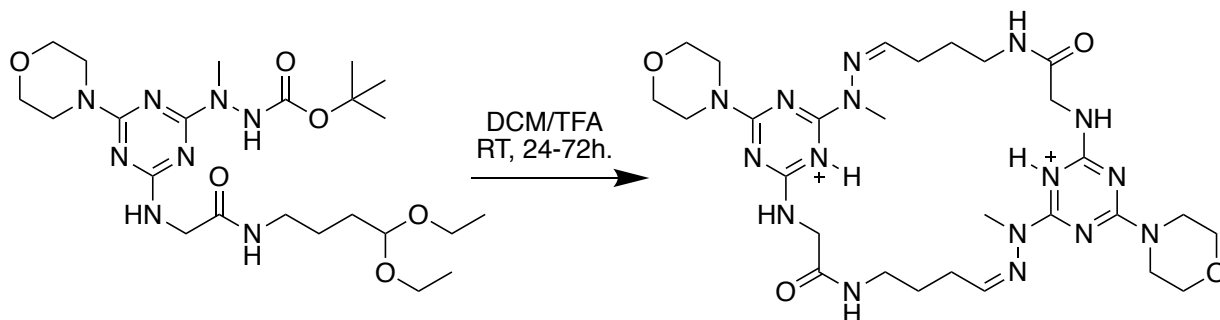


Figure S95: HPLC chromatogram of **6\*6**

### Synthesis of macrocycle **10** from **16**



Compound **16** (20 mg, 0.038 mmoles) was dissolved in a 2 ml DCM and 2 ml TFA in a small flask equipped with stirring bar. Resulting solution was left to stir at room temperature for 48 hours or until it was completely dried. Crude product was then washed with 1 ml DCM (x2) and filtered. Precipitates were dried under vacuum to afford macrocycle **10** (11 mg, 87%) as white solid.

Figure S96:  $^1\text{H-NMR}$ ,  $\text{DMSO-}d_6$ ;  $\delta$  11.53 (1H, s), 9.11-9.08 (1H, t), 8.70-8.67 (1H, t), 7.60-7.59 (1H, t), 4.30-4.29 (2H, d), 3.87-3.86 (4H, m), 3.69-3.67 (4H, m), 3.45 (3H, s), 3.27-3.24 (2H, m), 2.50-2.48 (2H, m), 1.79 (2H, m).

Figure S97:  $^{13}\text{C-NMR}$ ,  $\text{DMSO-}d_6$ ;  $\delta$  169.6, 161.0, 154.7, 154.2, 149.1, 66.2, 44.8, 43.4, 37.7, 29.9, 28.4, 24.5.

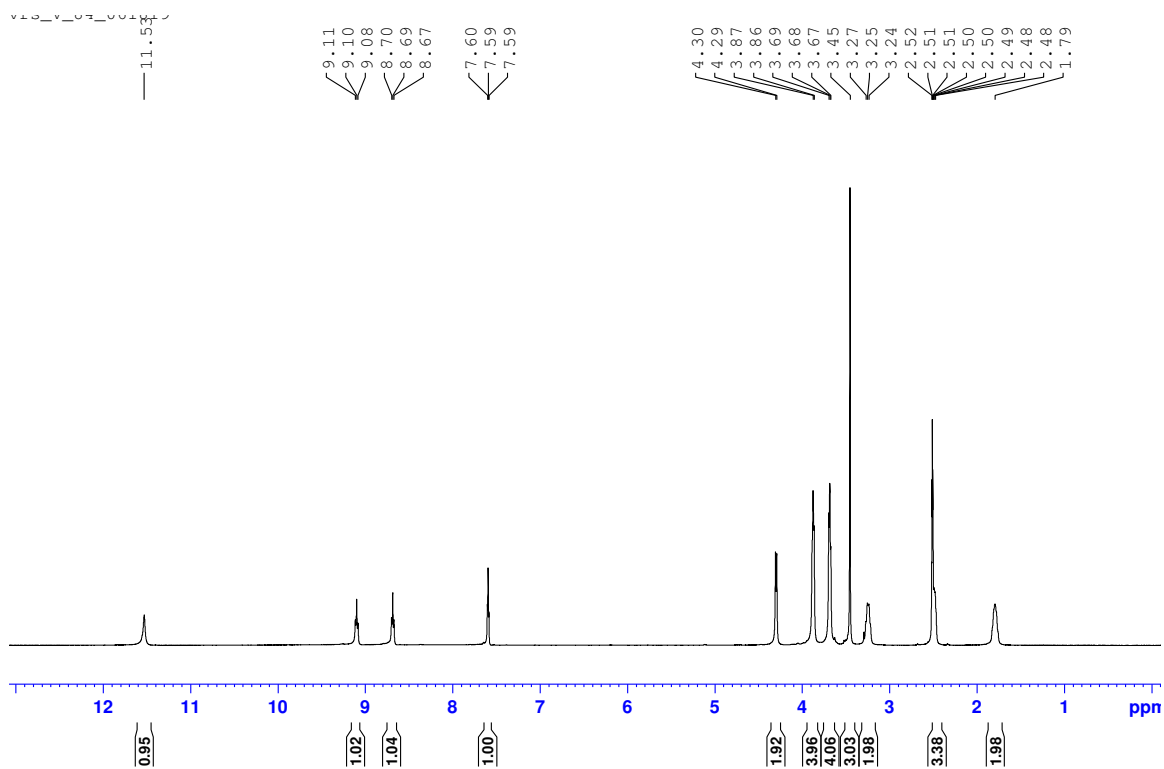


Figure S96: <sup>1</sup>H-NMR of macrocycle **10** in DMSO-*d*<sub>6</sub>

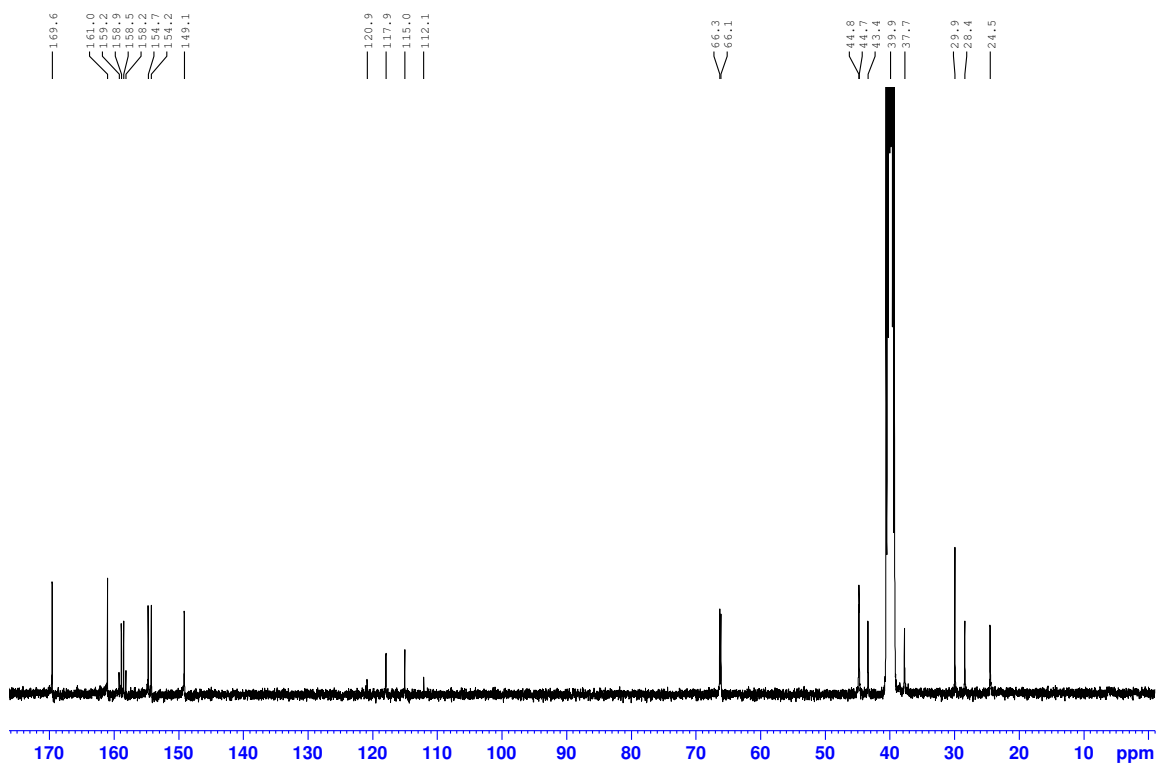


Figure S97: <sup>13</sup>C-NMR of macrocycle **10** in DMSO-*d*<sub>6</sub>

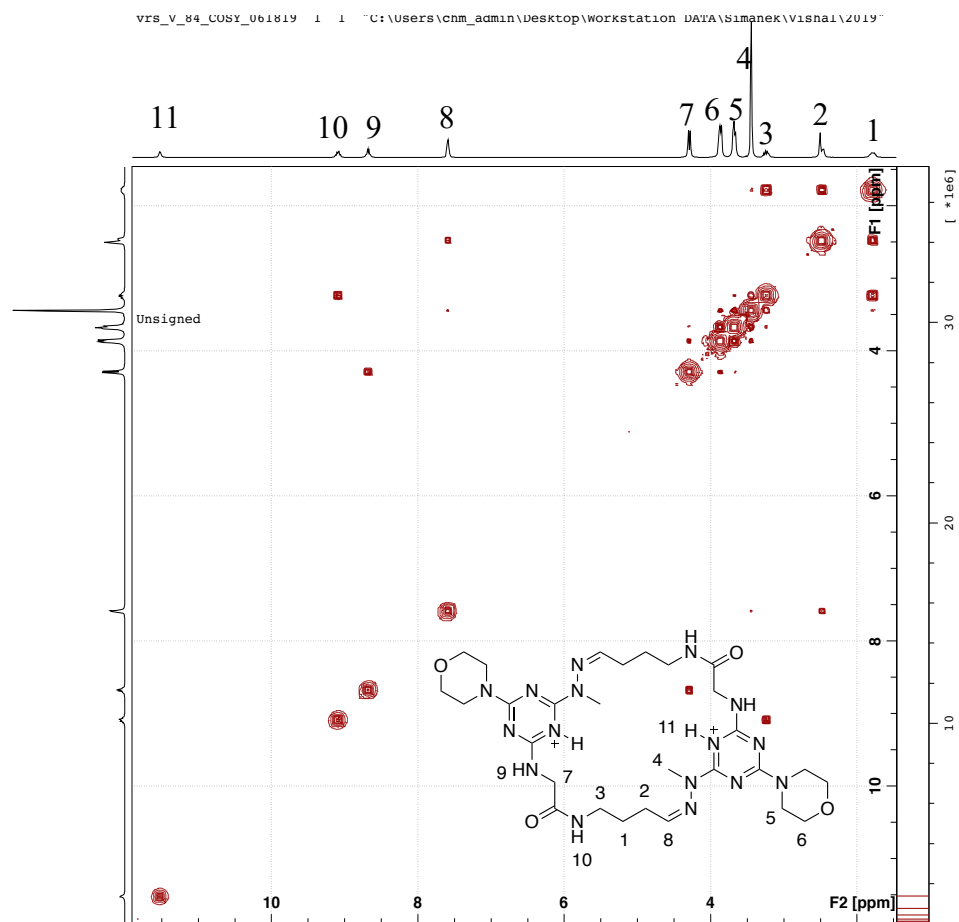
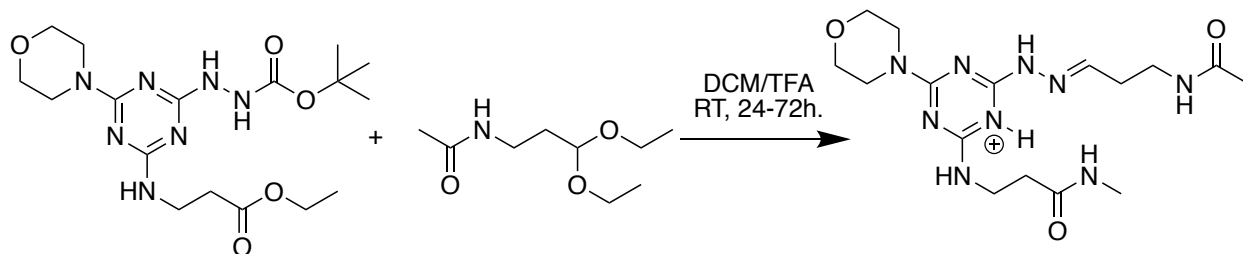


Figure S98: COSY-NMR of macrocycle **10** in DMSO- $d_6$





### Synthesis of model imine 9 from 17 and N-acetyl propionaldehyde acetal (18)



Compound 17 (20 mg, 0.049 mmol) and 18 (9.3 mg, 0.049 mmol) were dissolved in 2 ml DCM and 2 ml TFA. Resulting reaction mixture was left to stir at room temperature for more than 24 hours until the solvents were evaporated. No purification was required and the NMR of the crude product was taken.

Figure S100:  $^1\text{H}$ -NMR,  $\text{DMSO-}d_6$ ;  $\delta$  12.36 (1H, s), 11.55 (1H, s), 8.14-8.13 (1H, t), 8.07-8.04 (1H, t), 7.56-7.55 (1H, t), 3.83-3.57 (10H, m), 3.31-3.26 (2H, q), 2.61-2.59 (3H, d), 2.51-2.41 (4H, m), 1.80 (3H, s)

Figure S101:  $^{13}\text{C}$ -NMR,  $\text{DMSO-}d_6$ ;  $\delta$  171.1, 170.0, 161.6, 154.7, 154.3, 151.8, 66.2, 44.6, 37.0, 36.0, 34.4, 33.2, 25.9, 23.0

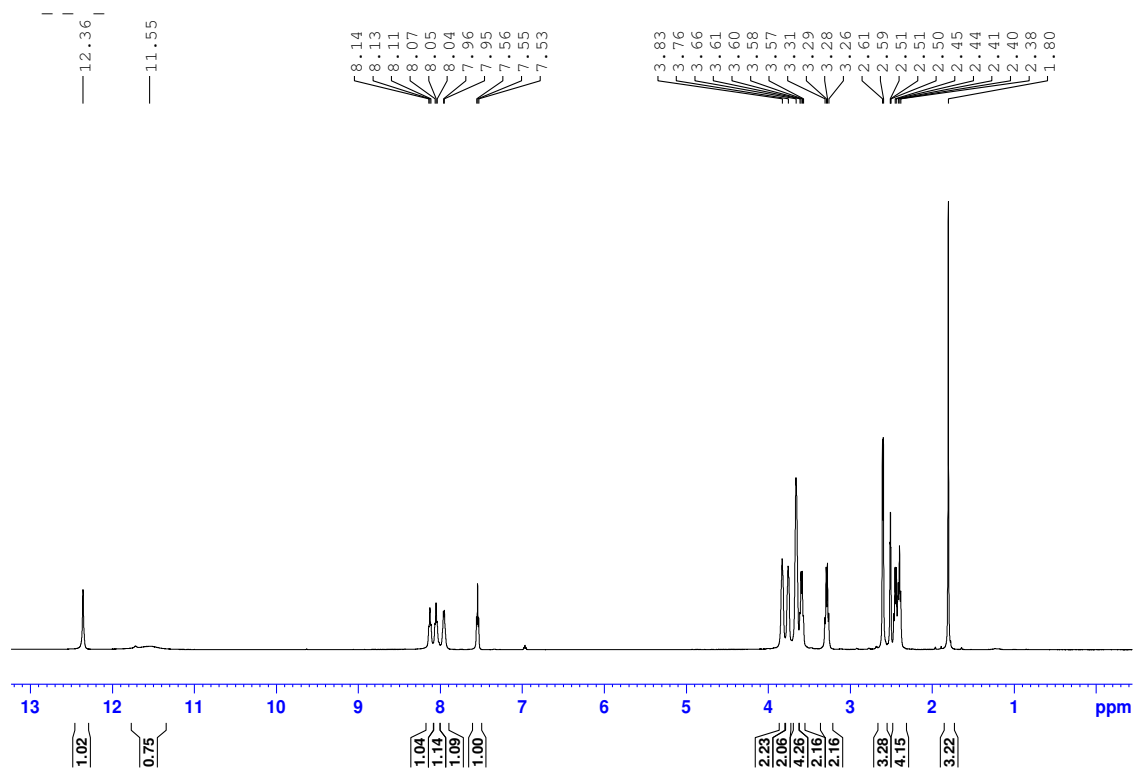


Figure S100: <sup>1</sup>H-NMR of macrocycle **9** in DMSO-*d*<sub>6</sub>

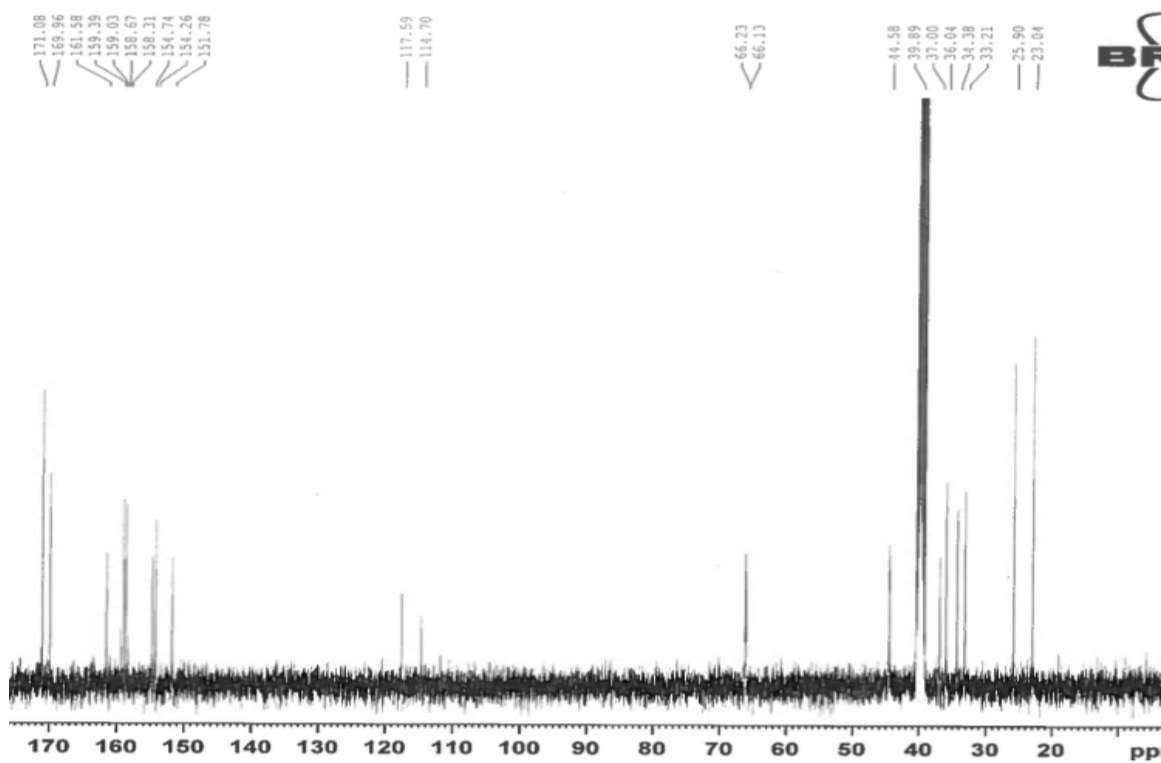


Figure S101: <sup>13</sup>C-NMR of macrocycle **9** in DMSO-*d*<sub>6</sub>

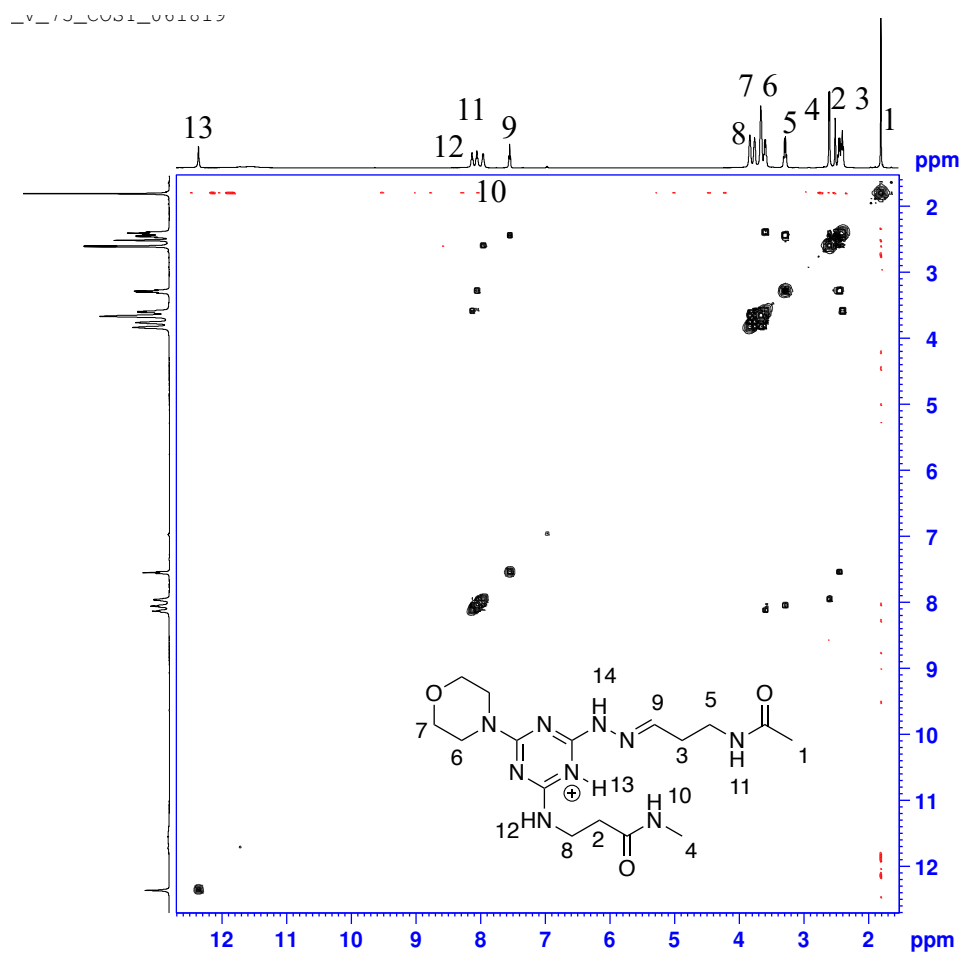


Figure S102: COSY-NMR of macrocycle **9** in DMSO- $d_6$

\_V\_75\_NOESY\_061819

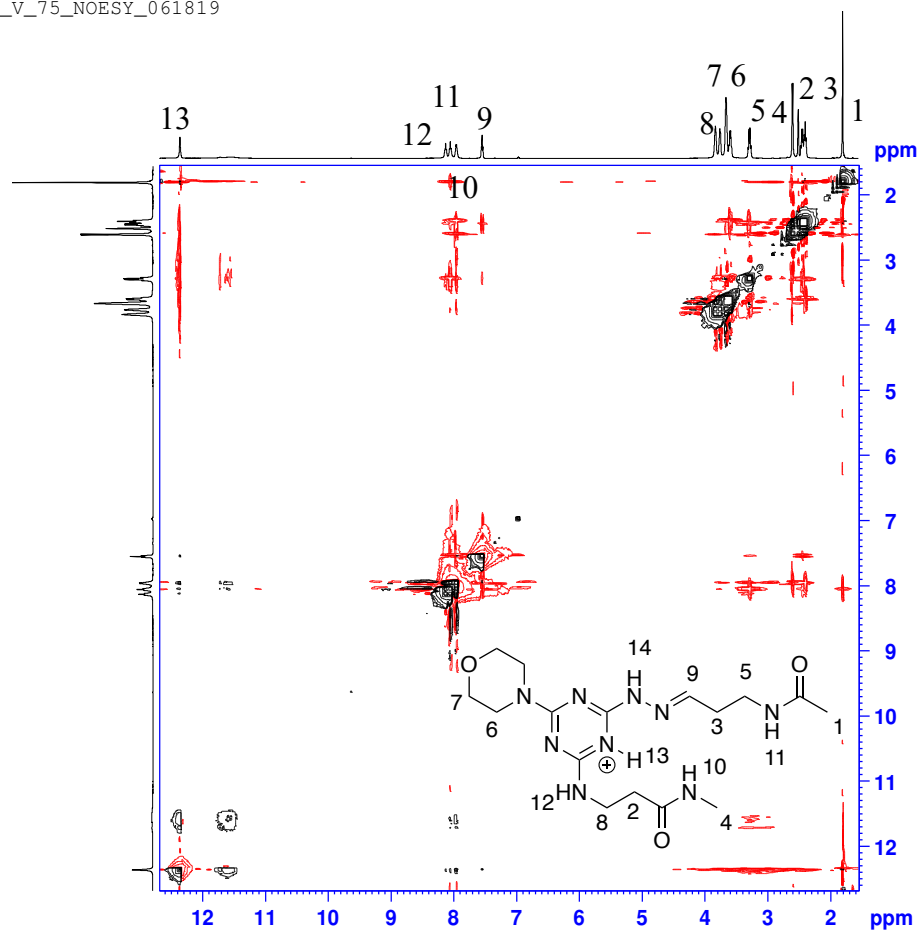


Figure S103: NOESY-NMR of macrocycle **9** in DMSO- $d_6$

### Synthesis of mixture of 14-21 macrocycles:

Compounds **1-6** in equal moles (**1** (40 mg, 0.09 mmol), **2** (43.7 mg), **3** (41.2 mg), **4** (45 mg), **5** (45 mg), and **6** (46.1 mg)) were dissolved in 5 ml DCM and 5 ml TFA. The resulting solution was left to stir at room temperature for more than 48 hours. No purification was done and HPLC was run of the crude sample. HPLC chromatogram shows the formation of at least 14 products. However, identification of the peaks is an ongoing investigation.

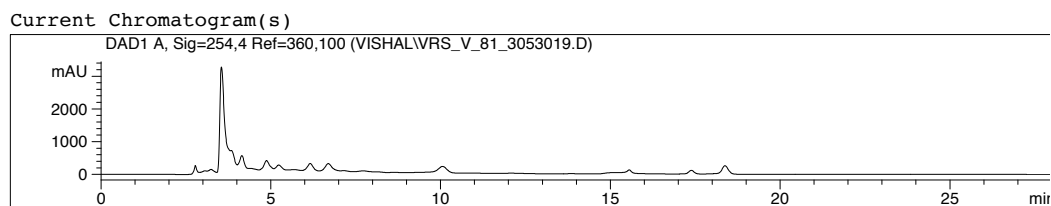


Figure S104: HPLC chromatogram of mixture of macrocycles.

### **X-ray diffraction analysis**

The X-ray diffraction data sets of macrocycles 1\*1, 2\*2, 3\*3, 4\*4 and 6\*6 were collected on a Bruker D8 Quest diffractometer equipped with a Photon 100 CMOS detector with  $K\alpha$  radiation ( $\lambda = 0.71073 \text{ \AA}$ ). The suitable crystal of each macrocycle was mounted on the goniometer head using Paratone-N oil on the tip of MiTeGen MicroLoops LD<sup>TM</sup>, at appropriate distance from the detector. The exposure time of 15 second was selected in shutter-less mode with a scan angle of  $0.75^\circ$  per frame while generator operating at 50 kV and 30 mA. The Bragg intensities of data sets consisting of  $\omega$  and  $\phi$  scans were indexed using APEX3 package<sup>1</sup> whereas the data reduction and absorption corrections were carried out with the SAINT<sup>2</sup> and SADABS<sup>3</sup> packages respectively. The space group was determined using XPREP<sup>1</sup> through analysis of the Laue symmetry and systematic absences. Structures were solved by the intrinsic phasing method using the SHELXT<sup>4</sup> software. The solution of each compound with the best figure of merit revealed the coordinates of all non-hydrogen atoms which were refined using SHELXL<sup>5</sup> program embedded in the OLEX2 package<sup>6</sup>. The hydrogen atoms were located by the difference Fourier analysis and during the structure refinement, the atomic displacement parameters of hydrogen atoms were treated isotropically and non-hydrogen atoms were anisotropically refined using the full-matrix least squares procedure on  $F^2$  (using all data). The hydrogen atoms attached to the carbon atoms were allowed to ride on their carrying atoms, whereas those attached to heteroatoms were refined freely. During the refinement of disorders, the sum of the occupancies of the two disordered parts were fixed to unity. The correlation of the thermal parameters was avoided by constraining the atomic displacement parameters (ADPs) of both parts to be equal during refinement.

## References

1. APEX3 *APEX3*, Version Ver. 2016.9-0; Bruker-AXS: Madison, Wisconsin, USA., 2016.
2. *Software packages SMART and SAINT*, Siemens Analytical X-ray Instrument Inc.: Madison, WI, 1996.
3. *SADABS: Area-Detector Absorption Correction*, SADABS: Area-Detector Absorption Correction, Siemens Industrial Automation, Inc.: Madison, WI, 1996.
4. Sheldrick, G., SHELXT - Integrated space-group and crystal-structure determination. *Acta Crystallographica Section A* **2015**, *71* (1), 3-8.
5. Sheldrick, G., Crystal structure refinement with SHELXL. *Acta Crystallographica Section C* **2015**, *71* (1), 3-8.
6. Dolomanov, O. V.; Bourhis, L. J.; Gildea, R. J.; Howard, J. A. K.; Puschmann, H., OLEX2: a complete structure solution, refinement and analysis program. *J. Appl. Crystallogr.* **2009**, *42* (2), 339-341.