Supporting Information For:

Identification of Diamine Linkers with Differential Reactivity and their Application in the Synthesis of a Melamine Dendrimer

*Karlos X. Moreno and Eric E. Simanek**

Table of Contents

NMR Experimental Procedures S3
Table S1. Measured Product Distributions S3
Dimorpholinotriazine (DMTA)
Synthesis S4
Typical Competition Reaction
General Procedure S4
Bis(3-BOC-3-aminopropyl)amine
Synthesis S4
Intermediate 2
Synthesis S5
^{1}H Spectrum S6
^{13}C Spectrum S6
Intermediate 3
Synthesis S7
^{1}H Spectrum S7
^{13}C Spectra S8
(^{1}H-^{1}H) COSY Spectrum S8
Intermediate 4
Synthesis S9
^{1}H Spectrum S9
^{13}C Spectra S10
(^{1}H-^{1}H) COSY Spectrum S10
Intermediate 5
Synthesis S11
^{1}H Spectrum S11
^{13}C Spectra S12
(^{1}H-^{1}H) COSY Spectrum S12
Intermediate 6
Synthesis S13
^{1}H Spectrum S14
^{13}C Spectrum S14

Table of Contents cont'd.

Intermediate 7
Synthesis S15
^{1}H Spectrum S15
^{13}C Spectrum S16
Dendrimer (1)
Synthesis S17
^{1}H Spectrum S18
^{13}C Spectrum S18
(^{1}H-^{1}H) TOCSY Spectrum S19
References S20

2D COSY NMR. The $^1H–^1H$ COSY[1] spectra were performed using a Varian Inova 500 spectrometer. The data was collected using a $\pi/2$ pulse width of 7.0 μs, a relaxation delay of 10 s, 4.5 kHz spectral window and 0.228 s acquisition time; 16 transients were averaged for each of the 2 x 256 increments using the States method.[2] The data were zero–filled to a 2048 x 2048 data matrix before Fourier transformation.

2D TOCSY NMR. The $^1H–^1H$ TOCSY[3] spectra were performed using the same 500 MHz spectrometer. The data was collected using a $\pi/2$ pulse width of 7.3 μs, a relaxation delay of 10 s, 4.5 kHz spectral window and 0.228 s acquisition time; a spin–lock pulse was applied for a period of 0.046 s with a spin–lock field of 5.4 kHz with MLEV–17 modulation; 16 transients were averaged for each of the 2 x 256 increments using the States method[2] of phase sensitive detection. The data were zero–filled to a 1024 x 1024 data matrix before Fourier transformation.

Chemicals were purchased from Aldrich and Acros and used without further purification. 1-Boc-3-aminoazetidine was purchased from CNH Technologies and used without further purification. All solvents were ACS grade and used without further purification. NMR spectra were recorded on an Inova 500 MHz spectrometer in $CDCl_3$ or DMSO–d_6. All mass spectral analyses were carried out by the Laboratory for Biological Mass Spectrometry at Texas A&M University.

Table S1. Measured Product Distributions

Trial	Amines	Product Ratio
1	**A,G**	16:84
2	**A,H**	23:77
3	**H,I**	84:16
4	**A,I**	62:38
5	**A,K**	92:8
6	**I,J**	69:31
7	**I,K**	86:14
8	**F,I**	2:98[a]
9	**F,J**	4:96[a]
10	**F,K**	12:88

[a]Accurate values for these competitions are not required and would be difficult given the product ratios. Better estimates of relative reactivity are provided by comparisons of amines with similar reactivities.

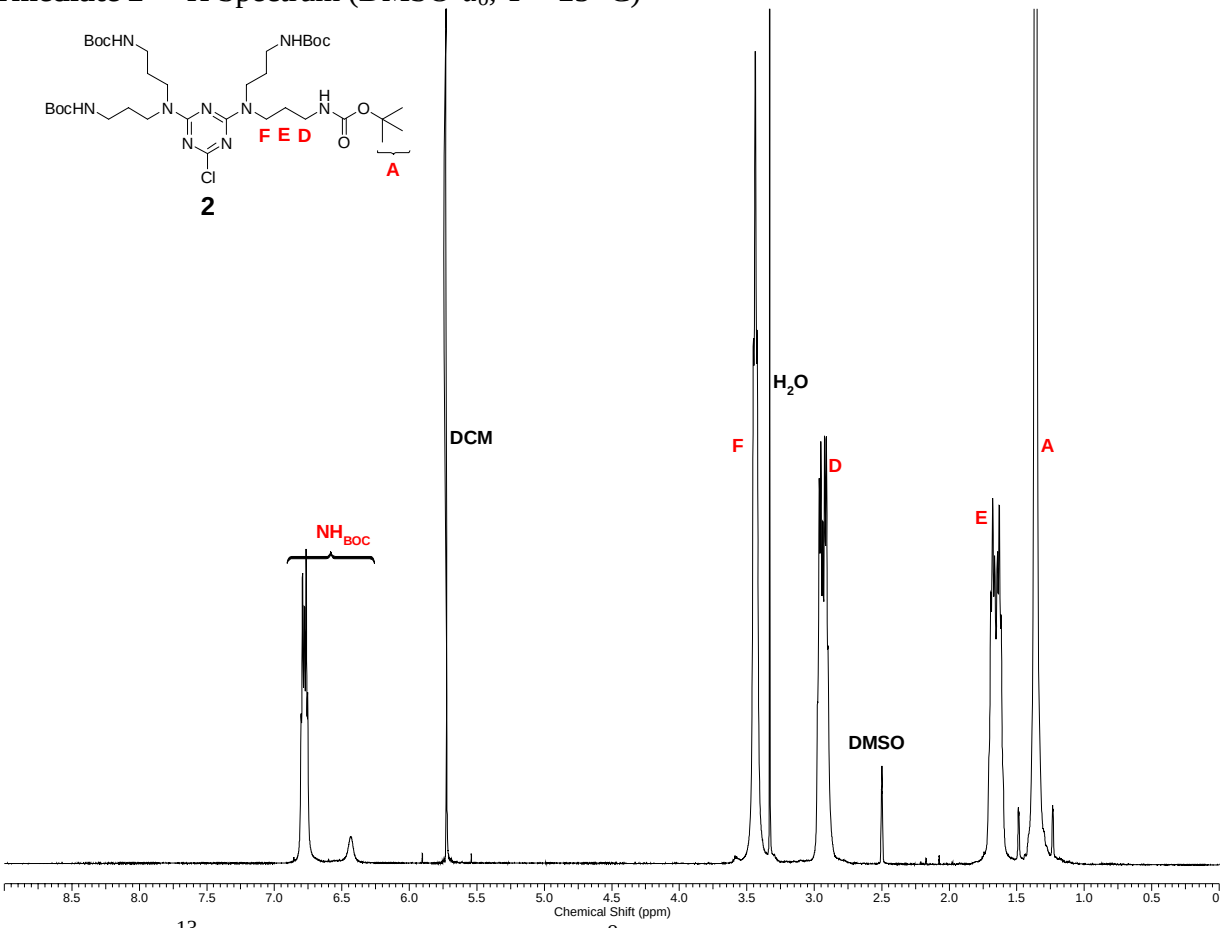
Dimorpholinomonochlorotriazine (DMTA). To a solution of cyanuric chloride (10.3 g, 55.8 mmol) in THF (250 mL) at 0 ^{o}C, morpholine (9.70 mL, 111 mmol) and Hunig's base (20.0 mL, 115 mmol) were added. After six hours, the solution was filtered and the solvent removed. The crude product was dissolved in hot methanol and precipitated by cooling. The product was reprecipitated from methanol again yielding a white solid (12.6 g, 79 %). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 3.78 (br m, 8H), 3.70 (t, *J* = 4.95 Hz, 8H). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 171.4, 164.7, 66.9, 44.1; MS (ESI) mass calc'd for $C_{16}H_{33}N_3O_4$ = 285.73; found 286.3 $[M+H]^+$.

Typical Competition Reaction. Pyrrolidine (107 mg, 1.5 mmol) and piperidine (128 mg, 1.5 mmol) were added to a vial with THF (10 mL).[4] To this solution dimorpholino-monochlorotriazine (DMTA, 143 mg, 0.5 mmol) was added and the reaction was left to stir for 18 hr. TLC confirmed the absence of DMTA for all reactions. The solvent was then removed and the residue was passed through a silica gel column containing DCM:Methanol (9:1) to remove excess amines. Fractions containing UV-active material that were not positive to ninhydrin staining (those excluding benzylic amines) were combined and analyzed using ^{1}H NMR.

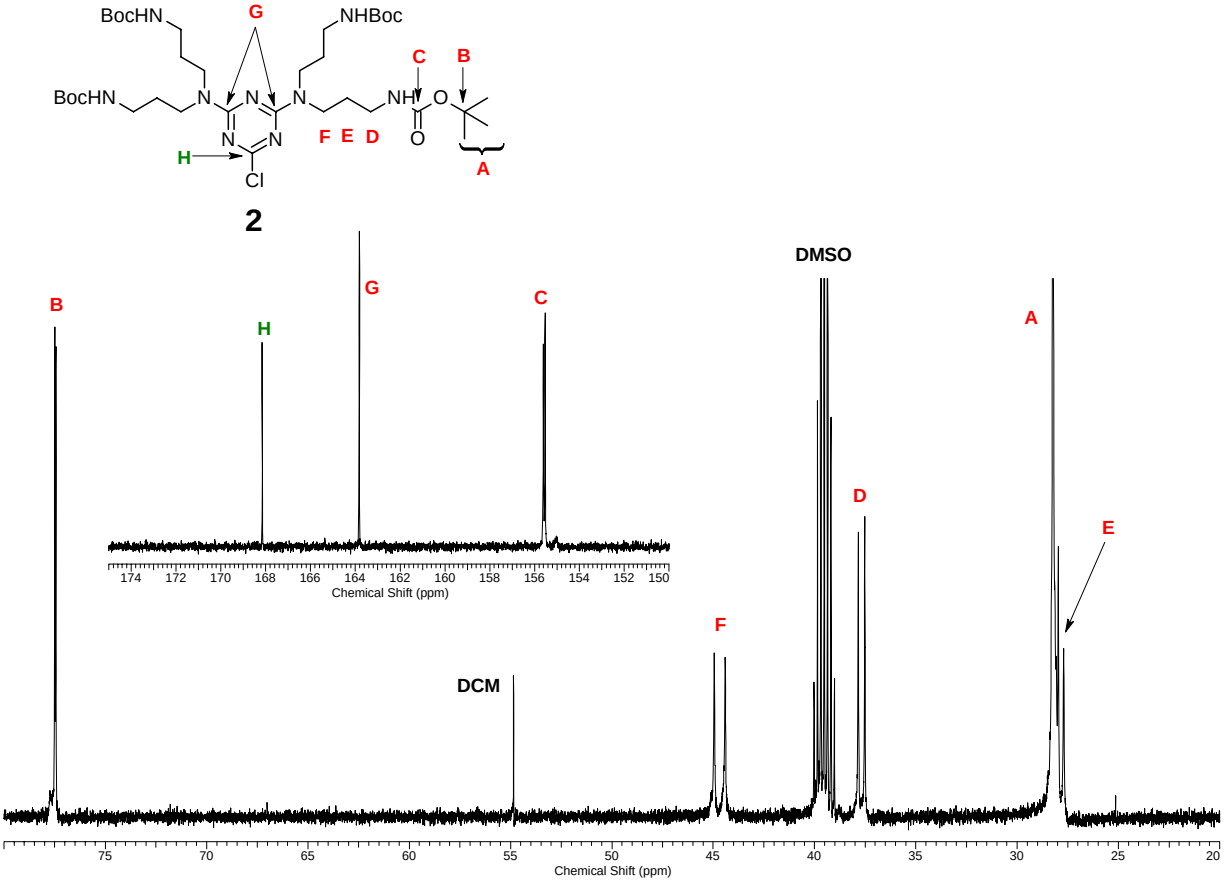
Bis(3-BOC-3-aminopropyl)amine. A solution of BOC-ON (2-(*tert*-butoxycarbonyloxyimino)-2-phenylacetonitrile) (40.11 g, 162.9 mmol) in THF (320 mL) was added drop wise over a period of 2 h to an ice – bath cooled solution of bis(3-aminopropyl)amine (11.62 mL, 81.47 mmol) and Hunig's base (43.0 mL, 247 mmol) in THF (65 mL). The reaction warmed to room temperature over a period of 4 h. The solvent was subsequently removed by reduced – pressure evaporation. The yellow – green liquid residue was dissolved in DCM and washed with three portions of 5% (w/v) NaOH. The organic layer was dried over $MgSO_4$, filtered, and the solvent removed by reduced – pressure evaporation to give thick clear oil. The product was precipitated from petroleum ether to give a white solid (22.85 g, 84.6 %). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 5.3 (brs, NH), 3.16 (br m, 4H), 2.61 (t, *J* = 6.6 Hz, 4H), 1.61 (m, 4H), 1.4 (s, 18H); ^{13}C NMR (75 MHz, $CDCl_3$, δ): 156.4, 79.2, 47.6, 39.0, 29.9, 28.6. MS (ESI) mass calc'd for $C_{16}H_{33}N_3O_4$ = 331.45; found 332.26 $[M+H]^+$.

Intermediate 2. A solution of bis(3-Boc-3-aminopropyl)amine (12.3 g, 37.1 mmol) and Hunig's Base (20 mL, 115 mmol) in THF (100 mL) was added to a solution of cyanuric chloride (3403 mg, 18.45 mmol) in THF (30 mL) at room temperature. After stirring overnight, the solvent was removed by reduced pressure evaporation to give an oil. The oil was dissolved in 70 mL DCM and washed with three 70 mL portions (5 %) HCl solution, four 70 mL portions (5 %) NaOH solution, and three 70 mL portions of brine solution. The organic layer was dried over $MgSO_4$ and filtered. The solvent was removed from the filtrate to give off-white colored foam. The foam was dissolved in DCM and a silica gel column was performed using a DCM:Methanol (50:1) solvent system. Fractions containing product, as determined by TLC, were combined and had their solvent removed by reduced pressure evaporation to give white foam (10083.6 mg, 70.6 %). ^{1}H NMR (500 MHz, DMSO-d_6, δ): 6.8 (m, NH), 3.42 (t, J = 7.0 Hz, 8H), 2.92 (m, 4H), 1.64 (m, 4H), 1.4 (s, 36H). ^{13}C NMR (125 MHz, DMSO-d_6, δ): 169.1, 164.7, 156.5, 156.4, 78.4, 78.4, 55.8, 45.9, 45.3, 38.8, 38.4, 29.2, 28.9, 28.6; MS (ESI) mass calc'd for $C_{35}H_{64}ClN_9O_8$ = 774.39; found 774.48 $[M+H]^+$.

Intermediate 2 – ^{1}H Spectrum (DMSO-d_6, T = 25 ^{o}C)

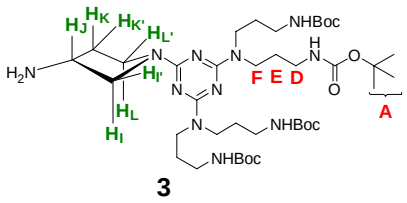


Intermediate 2 – ^{13}C Spectrum (DMSO-d_6, T = 25 ^{o}C)

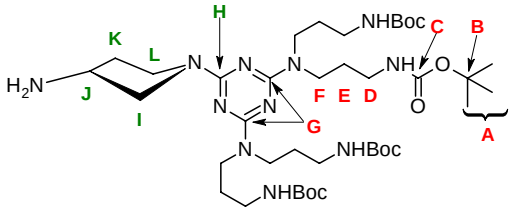


Intermediate 3. A solution of **2** (10.262 g, 13.251 mmol) in THF (50 mL) was added dropwise to a solution of *R*-3-aminopyrrolidine (2.60 mL, 29.7 mmol) in THF (10 mL) at room temperature. After stirring overnight, the solvent was removed by reduced pressure evaporation to give red foam. The foam was dissolved in DCM and passed through a silica gel column using DCM:Methanol (19:1). Fractions containing product, as determined by TLC, were combined and solvent removed yielding pale yellow foam (10.32 g, 94.5 %). ^{1}H NMR (500 MHz, DMSO-d_6, δ): 6.7 (br m, NH), 6.4 (br s, NH), 3.32-3.59 (br m, 12H), 3.15 (m, 1H), 2.92 (m, 8H), 1.96 (m, 1H), 1.64 (m, 9H), 1.4 (s, 36H). ^{13}C NMR (125 MHz, DMSO-d_6, δ): 165.2, 164.1, 156.5, 78.4. 54.2, 51.3, 44.9, 44.7, 44.6, 38.9, 38.7, 38.4, 34.3, 29.3; MS (ESI) mass calc'd for $C_{35}H_{64}ClN_9O_8$ = 823.56; found 824.59 $[M+H]^+$, 312.74 $[M+2H]^{+2}$, 262.71 $[M+2H]^{+2}$, 212.68 $[M+2H]^{+2}$.

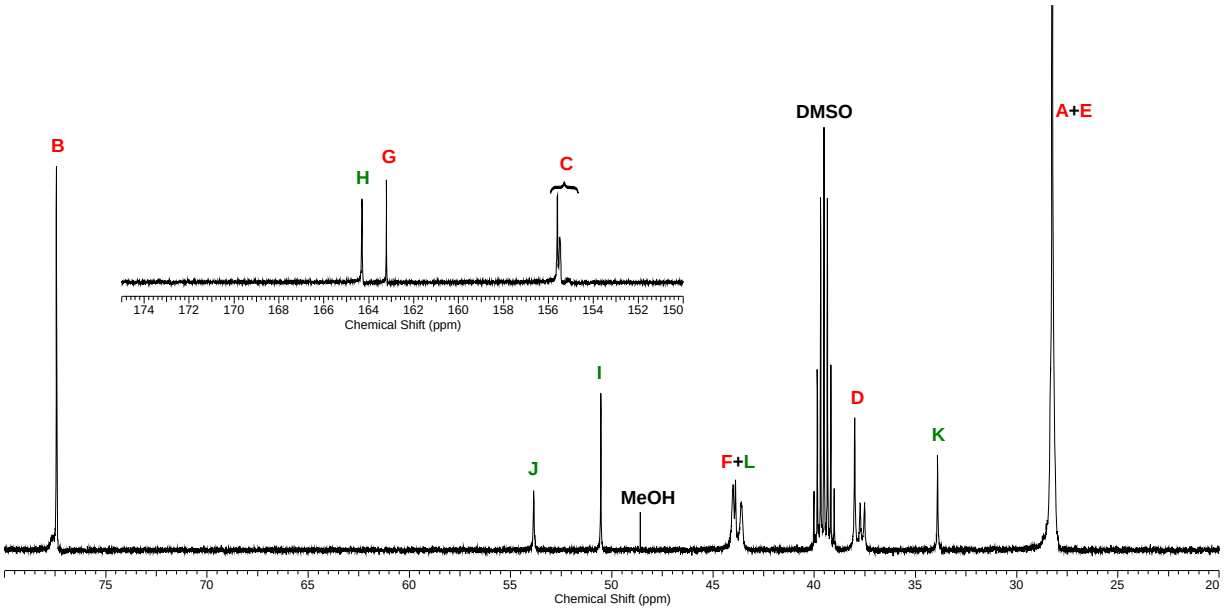
Intermediate 3 – ^{1}H Spectrum (DMSO-d_6, T = 25 ^{o}C)



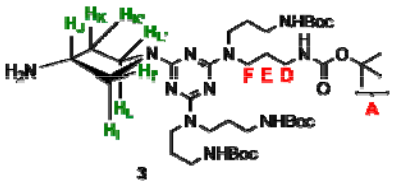
Intermediate 3 – ^{13}C Spectrum (DMSO-d_6, T = 25 ^{o}C)

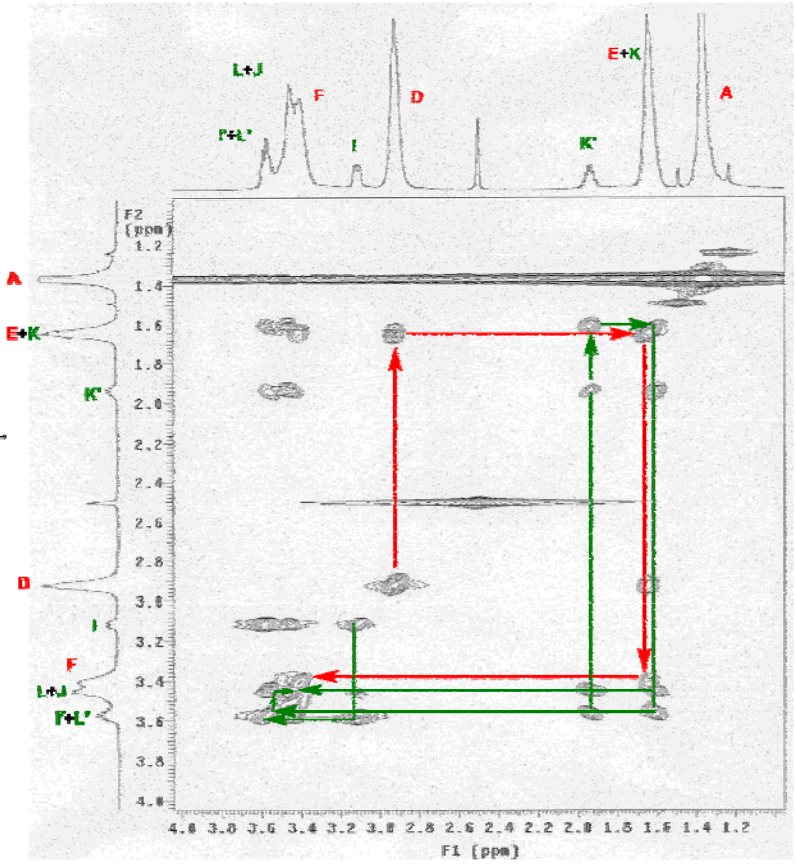


3



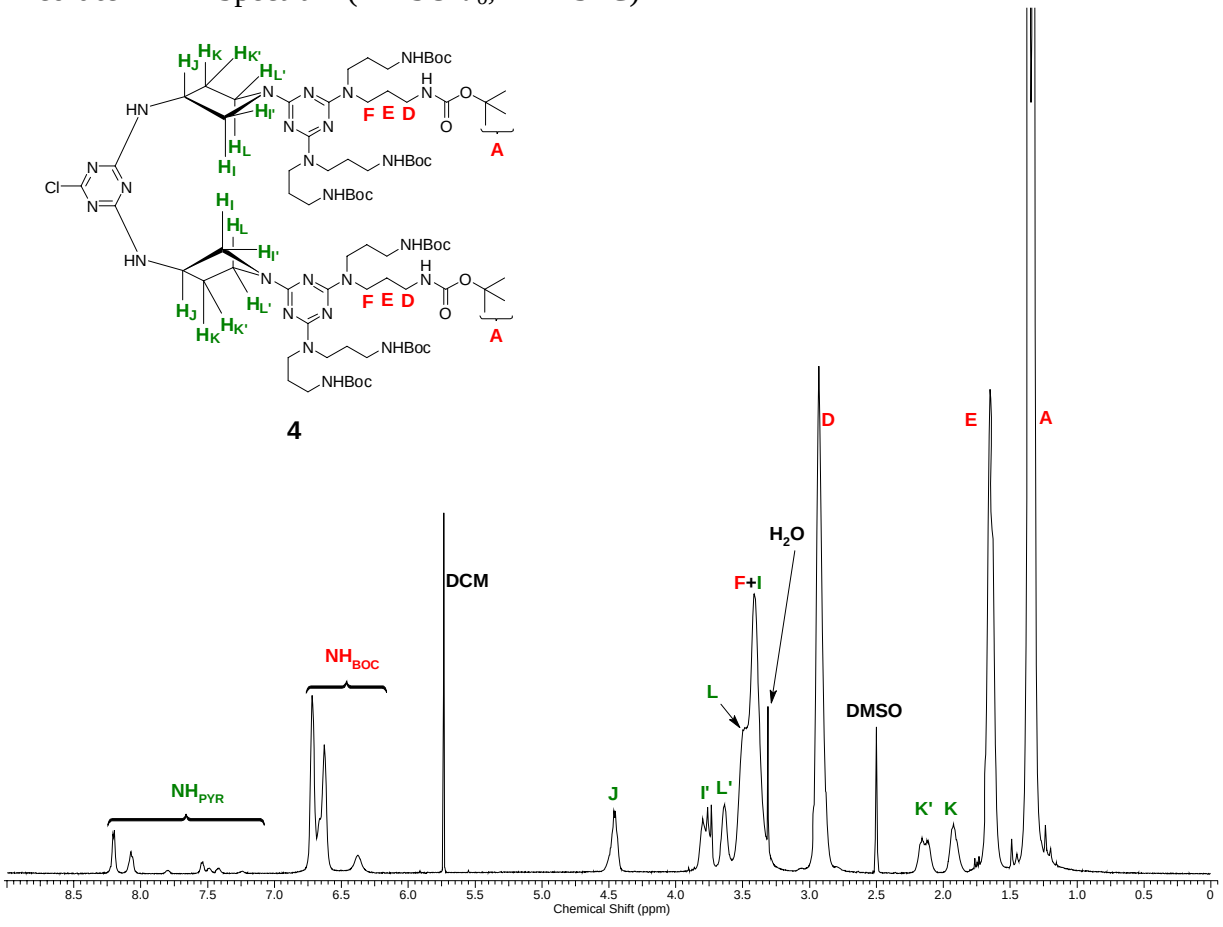
Intermediate 3 – (^{1}H–^{1}H) COSY Spectrum (DMSO-d_6, T = 25 ^{o}C)



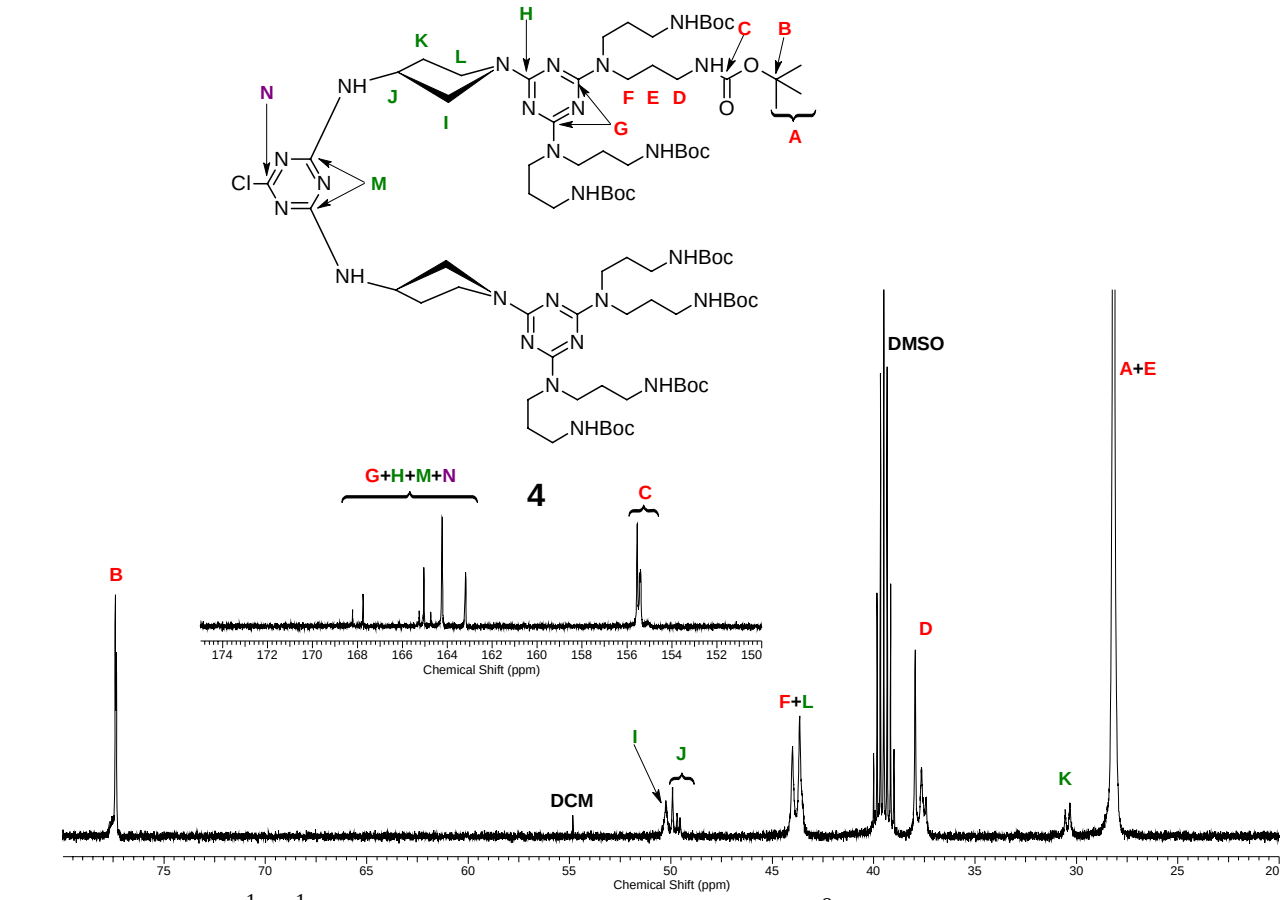


Intermediate 4. A solution of **3** (5.95 g, 7.22 mmol) and Hunig's base (4.0 mL, 23 mmol) in THF (30 mL) was added to a solution of cyanuric chloride (634 mg, 3.44 mmol) in THF (5 mL) at room temperature. The reaction was heated to 40 ^{o}C overnight. The solvent was removed by reduced pressure evaporation to give a pale yellow foam. The foam was dissolved in DCM and passed through a silica gel column using DCM:Methanol (30:1). Fractions containing product, as determined by TLC, were combined and solvent removed yielding a pale yellow foam (5.373 g, 88.8 %). ^{1}H NMR (500 MHz, DMSO-d_6, δ): 6.78 (br s, NH), 6.73 (br s, NH), 6.7 (br s, NH), 4.48 (br m, 2H), 3.80 (br m, 2 H), 3.66 (br m, 2H), 3.4 -3.6 (br m, 24H), 2.96 (br m, 16H), 2.17 (br m, 2H), 1.94 (br m, 2H), 1.68 (br m, 16H), 1.4 (s, 36H), 1.36 (s, 36H). ^{13}C NMR (125 MHz, DMSO-d_6, δ): 169.2, 168.7, 166.0, 165.2, 164.1, 156.5, 156.3, 78.4, 78.3, 51.2, 50.9, 50.6, 50.5, 45.0, 44.6, 38.9, 38.6, 38.4, 31.5, 31.3, 29.2, 29.1; MS (MALDI) mass calcd for $C_{81}H_{144}ClN_{25}O_{16}$ = 1759.62; found 1760.21 $[M+H]^+$, 1783.18 $[M+Na]^+$, 1799.15 $[M+K]^+$, 1659.12 $[M+H\text{-Boc}]^+$, 1560.04 $[M+H\text{-2 Boc}]^+$, 1359.00 $[M+H\text{-4 Boc}]^+$.

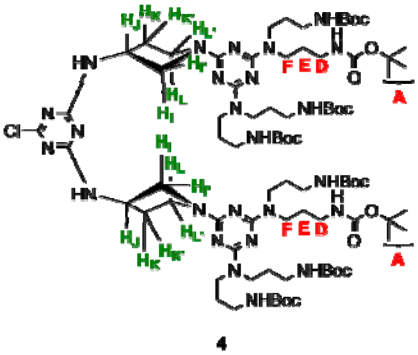
Intermediate 4 – ^{1}H Spectrum (DMSO-d_6, T = 25 ^{o}C)

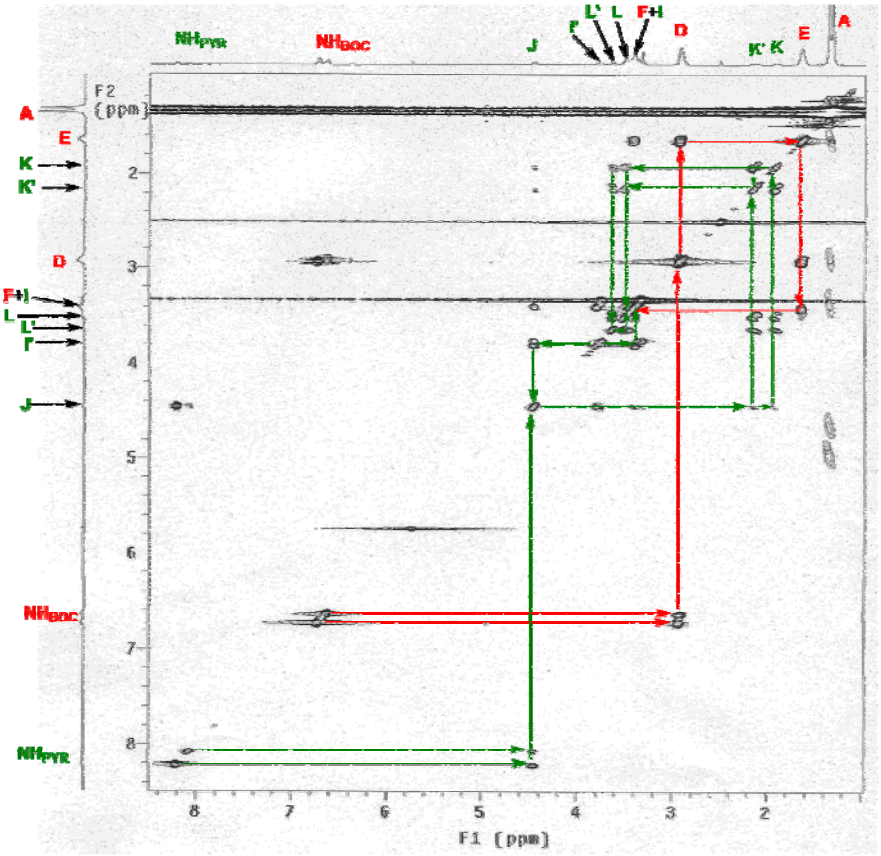


Intermediate 4 – ^{13}C Spectrum (DMSO-d_6, T = 25 ^{o}C)



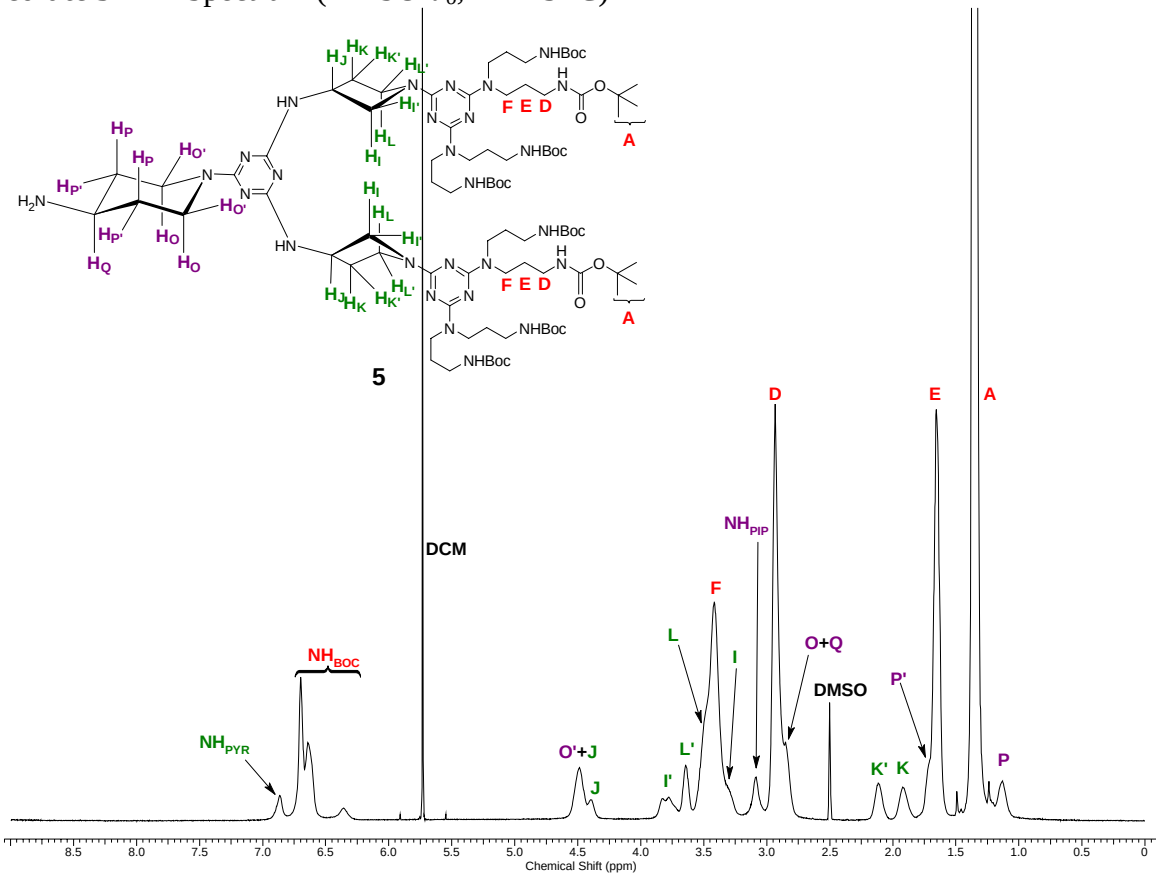
Intermediate 4 – (^{1}H–^{1}H) COSY Spectrum (DMSO-d_6, T = 25 ^{o}C)



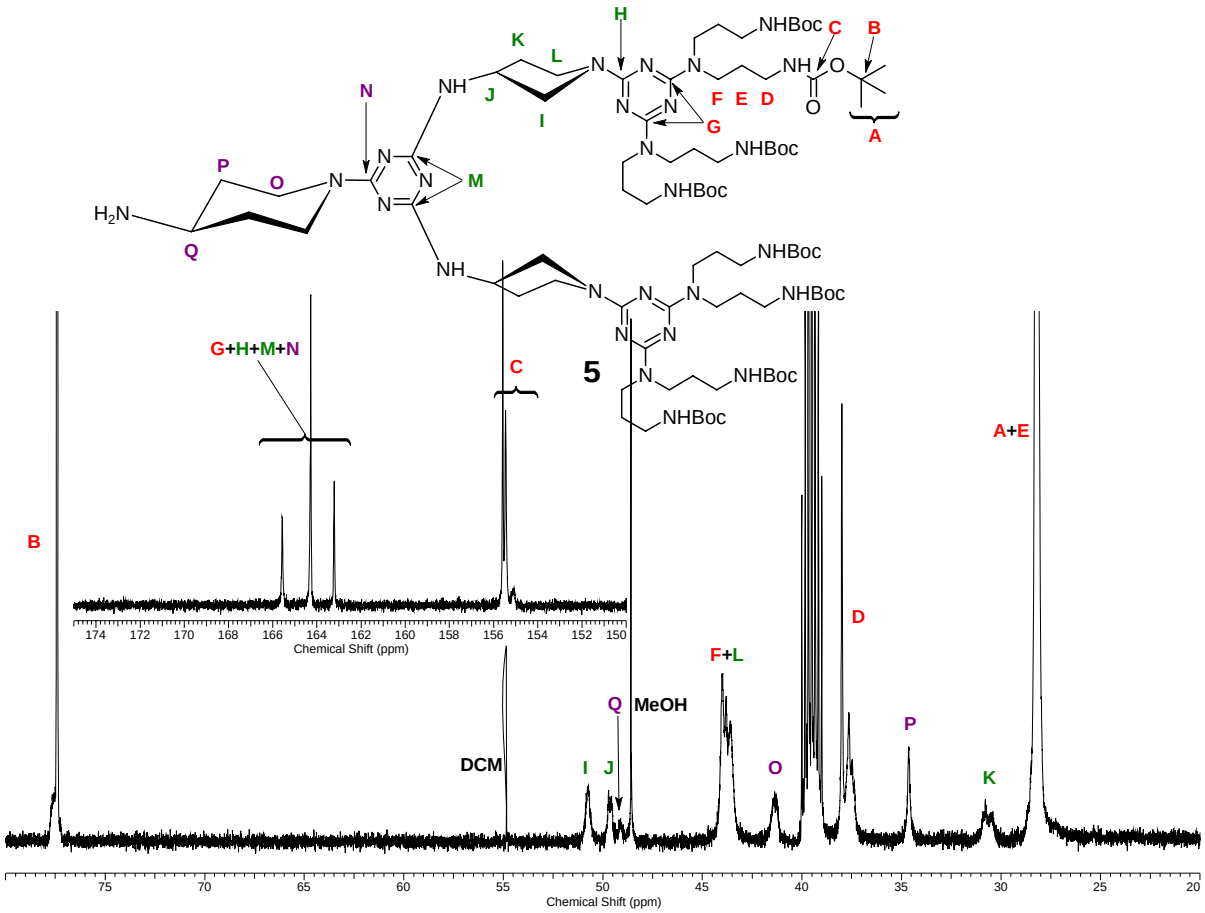


Intermediate 5. A solution of **4** (7.00 g, 3.98 mmol) in THF (15 mL) was added dropwise to a solution of 4-aminopiperidine (1.26 mL, 12.0 mmol) in THF (5 mL) at room temperature. After reacting overnight, the solvent was removed by reduced pressure evaporation and the residue passed through a silica gel column using DCM:Methanol (19:1). Fractions containing product, as determined by TLC, were combined and solvent removed yielding white foam (6474.4 mg, 89.3 %). ^{1}H NMR (500 MHz, DMSO-d_6, δ): 8.20 (br s, NH), 8.08, (br s, NH), 7.80 (br s, NH), 6.72 (br s, NH), 6.63 (br s, NH), 6.38 (br s, NH), 4.45 (br m, 2H), 3.80 (br m, 1H), 3.75 (br m, 1H), 3.63 (br m, 2H), 3.50 (br m, 2H), 3.41, (br m, 16H), 3.36 (br m, 2H), 2.93 (br m, 16H), 2.16 (br m, 1H), 2.11 (br m, 1H), 1.65 (br m, 16H), 1.36 (s, 44H), 1.32 (s, 28H). ^{13}C NMR (125 MHz, DMSO-d_6, δ): 168.2, 167.8, 165.2, 165.0, 164.7, 164.2, 163.2, 155.5, 155.4, 155.3, 155.1, 77.4, 77.3, 50.2, 49.9, 49.7, 49.5, 44.0, 43.6, 37.9, 37.6, 37.4, 30.6, 30.3, 28.2, 28.1; MS (MALDI) mass calcd for $C_{86}H_{155}N_{27}O_{16}$ = 1823.32; found 1824.00 $[M+H]^+$, 1845.97 $[M+Na]^+$, 1861.94 $[M+K]^+$, 1723.96 $[M+H\text{-Boc}]^+$.

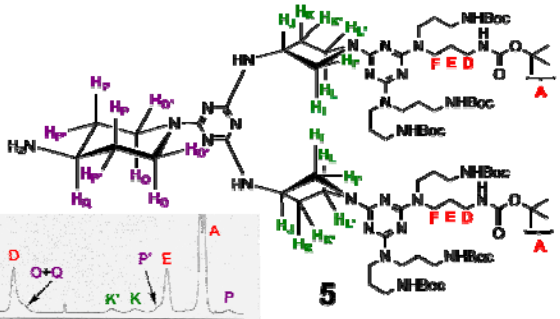
Intermediate 5 – ^{1}H Spectrum (DMSO-d_6, T = 25 ^{o}C)

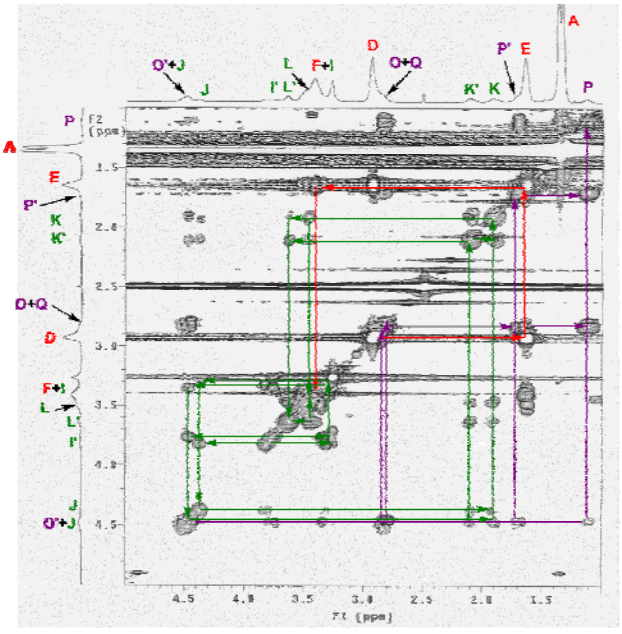


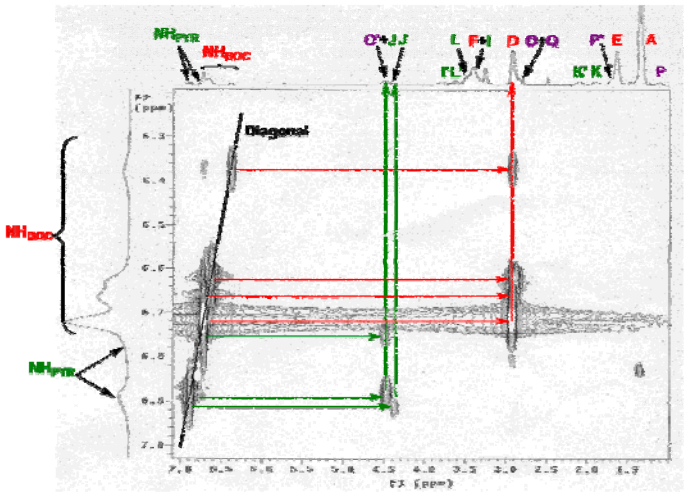
Intermediate 5 – ^{13}C Spectrum (DMSO-d_6, T = 25 ^{o}C)



Intermediate 5 – (^{1}H–^{1}H) COSY Spectrum (DMSO-d_6, T = 25 ^{o}C)

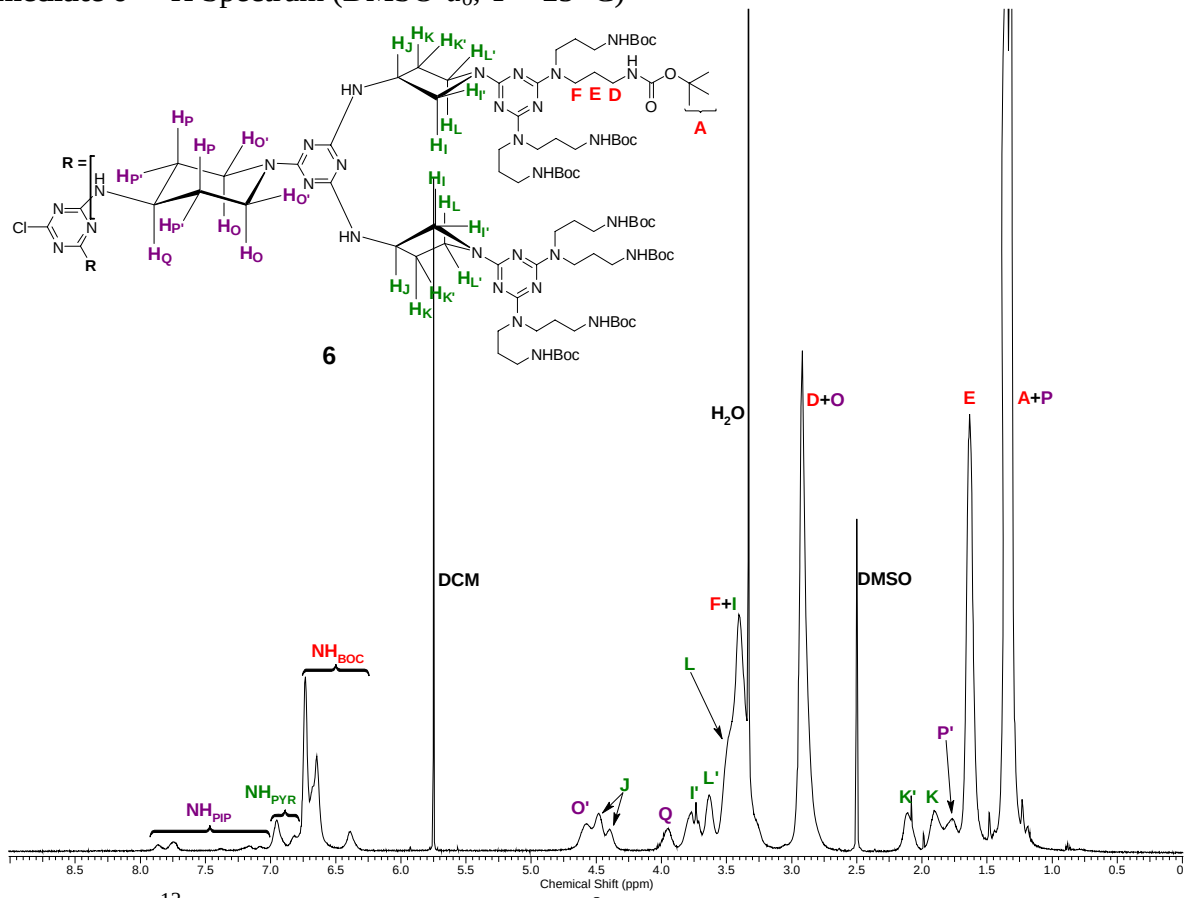




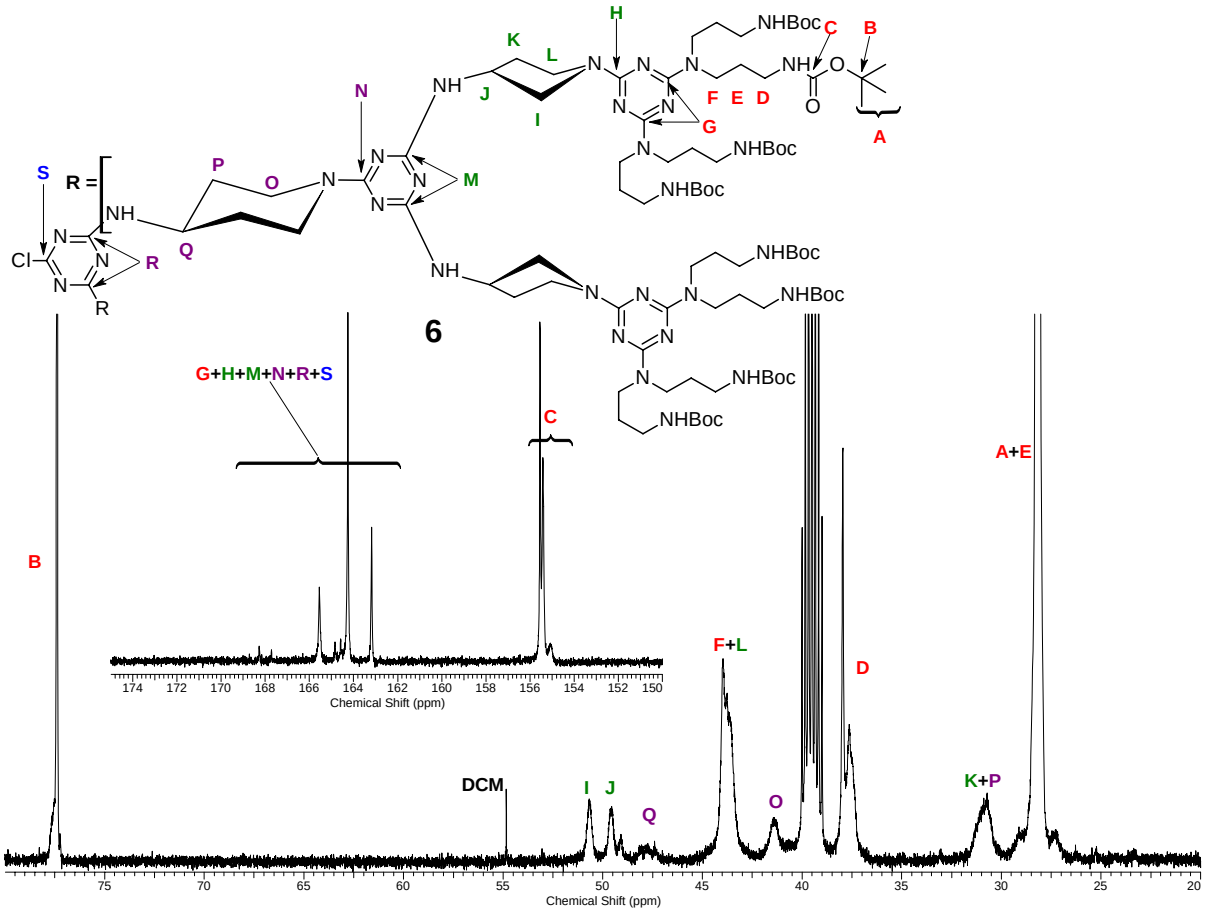


Intermediate 6. A solution of **5** (5.28 g, 2.89 mmol) and Hunig's base (1.5 mL, 8.6 mmol) in THF (5 mL) was added to a solution of cyanuric chloride (243 mg, 1.31 mmol) in THF (5 mL) at room temperature. The reaction was heated to 40 ^{o}C and allowed to react for 2 days. The solvent was removed by reduced – pressure evaporation and the residue was passed through a silica gel column using DCM:MeOH (25:1). Fractions containing product, as determined by TLC, were combined and solvent removed yielding a pale yellow foam (4.31 mg, 87 %). ^{1}H NMR (500 MHz, DMSO-d_6, δ): 7.86 (br s, NH), 7.75 (br s, NH), 6.95 (br s, NH), 6.82 (br s, NH), 6.73 (br s, NH), 6.67 (br s, NH), 6.64 (br s, NH), 6.38 (br s, NH), 4.56 (br m, 2H), 4.47 (br m, 2H), 4.39 (br m, 2H), 3.94 (brs, 1 H), 3.77 (br m, 2 H), 3.71 (br s, 1 H), 3.62 (br m, 4H), 3.4 -3.6 (br m, 40H), 2.91 (br m, 32H), 2.11 (br m, 4H), 1.89 (br m, 2H), 1.76 (br m, 2H), 1.62 (br m, 33H), 1.35 (s, 92H), 1.31 (s, 52H). ^{13}C NMR (125 MHz, DMSO-d_6, δ): 165.6, 164.2, 163.2, 155.6, 155.4, 155.1, 77.4, 50.7, 49.6, 49.1, 48.1, 47.7, 47.4, 44.0, 43.8, 43.6, 41.4, 38.0, 37.6, 37.4, 31.0, 30.7, 28.2; MS (MALDI) mass calcd for $C_{175}H_{308}ClN_{57}O_{32}$ = 3758.13; found 3759.02 $[M+H]^+$, 3780.95 $[M+Na]^+$, 3795.93 $[M+K]^+$, 3657.97 $[M+H\text{-Boc}]^+$, 3557.89 $[M+H\text{-2 Boc}]^+$.

Intermediate 6 – ^{1}H Spectrum (DMSO-d_6, T = 25 ^{o}C)

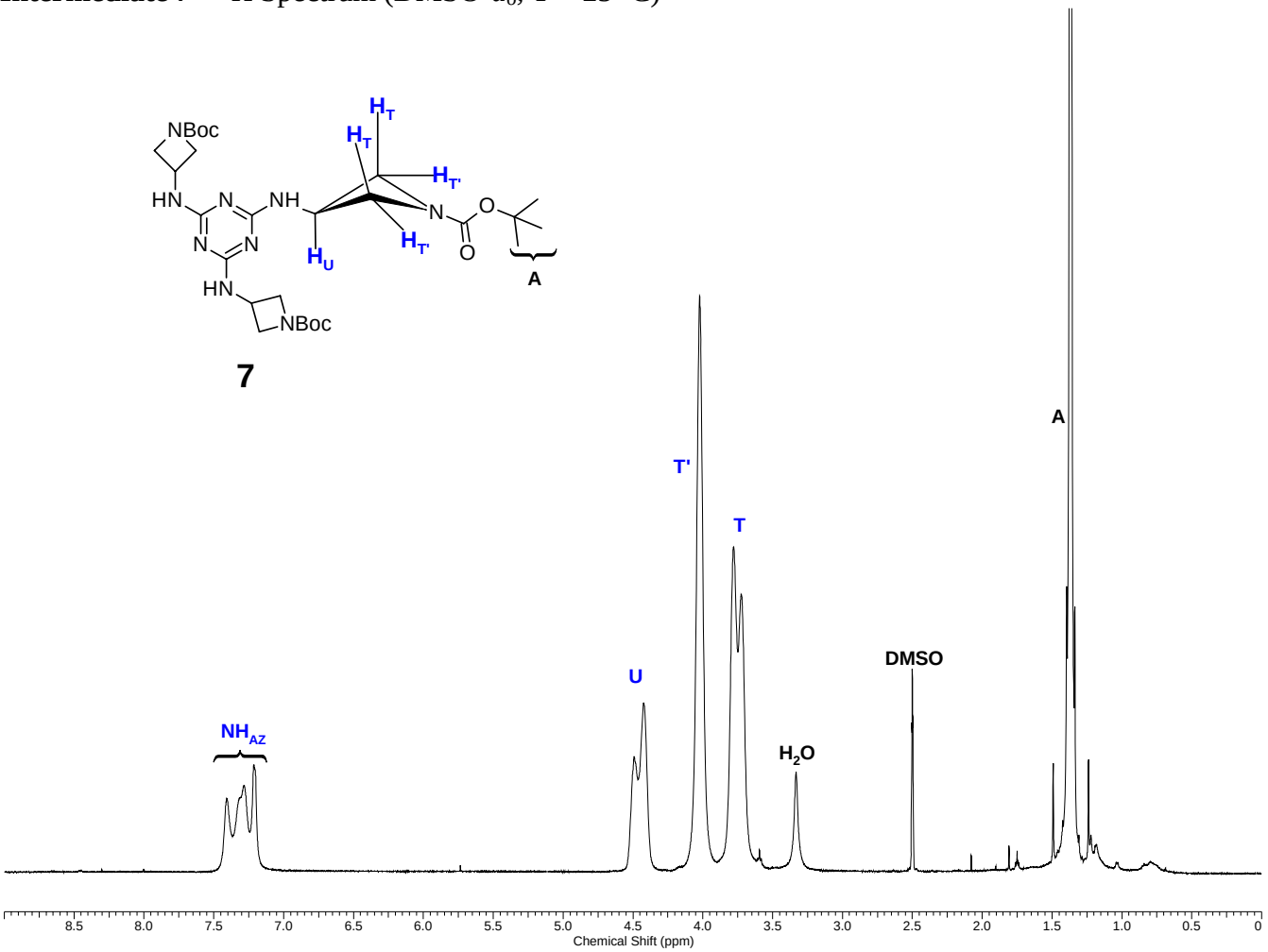


Intermediate 6 – ^{13}C Spectrum (DMSO-d_6, T = 25 ^{o}C)

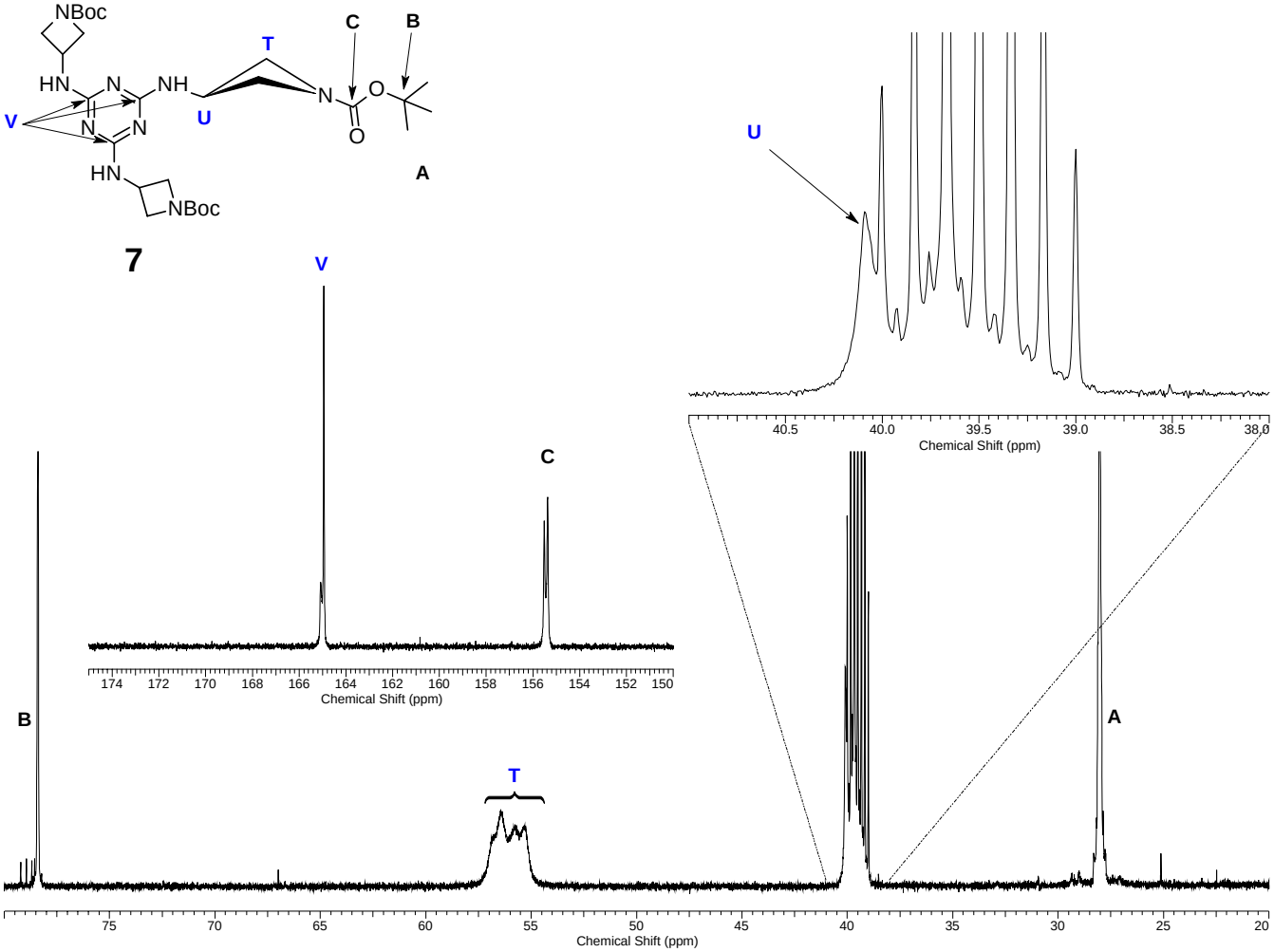


Intermediate 7. 1-Boc-3-amino-azetidine (172 mg, 0.999 mmol) and Hunig's base (DIPEA, diisopropylethylamine) (0.690 mL, 1.33 mmol) was added to a stirred solution of cyanuric chloride (61.4 mg, 0.333 mmol) in THF (2 mL) at room temperature. The reaction was then heated to 70 ^{o}C for seven days. The solvent was removed by reduced pressure evaporation and the residue passed through a silica gel column using (20:1:3) DCM:MeOH:Ethyl Acetate to give a white solid (186.4 mg, 94.6 %). ^{1}H NMR (500 MHz, DMSO-d_6, δ): 7.3 (br m, NH), 4.45 (br m, 3H), 4.02 (s, 6H), 3.74 (br m, 6H), 1.4 (s, 27H); ^{13}C NMR (125 MHz, DMSO-d_6, δ): 166.1, 165.9, 156.5, 156.3, 79.4, 57.4, 56.6, 41.0, 29.0; MS (ESI) mass calc'd for $C_{27}H_{45}N_9O_6$ = 591.7; found 592.37 $[M+H]^+$, 614.33 $[M+Na]^+$.

Intermediate 7 – ^{1}H Spectrum (DMSO-d_6, T = 25 ^{o}C)

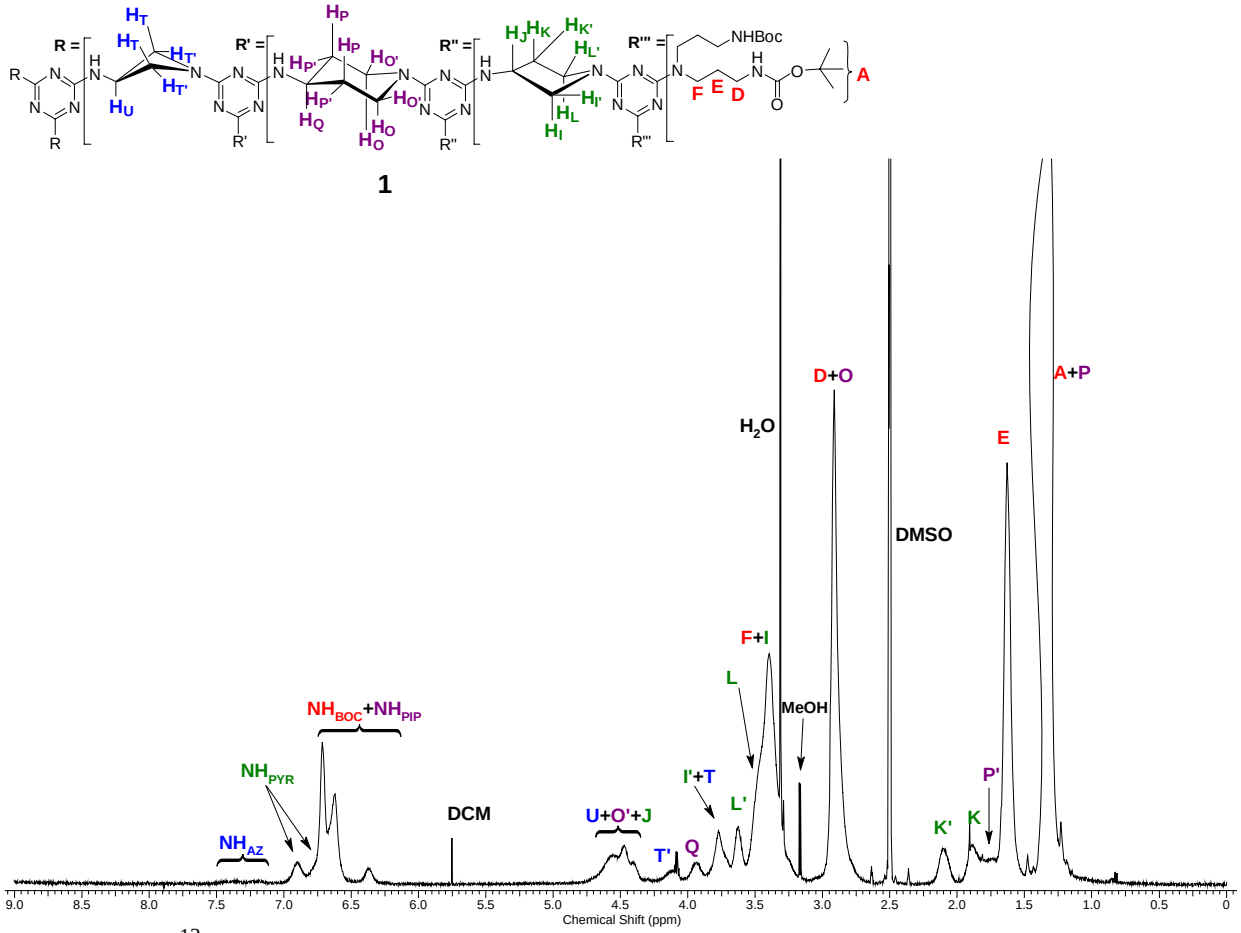


Intermediate 7 – ^{13}C Spectrum (DMSO-d_6, T = 25 ^{o}C)

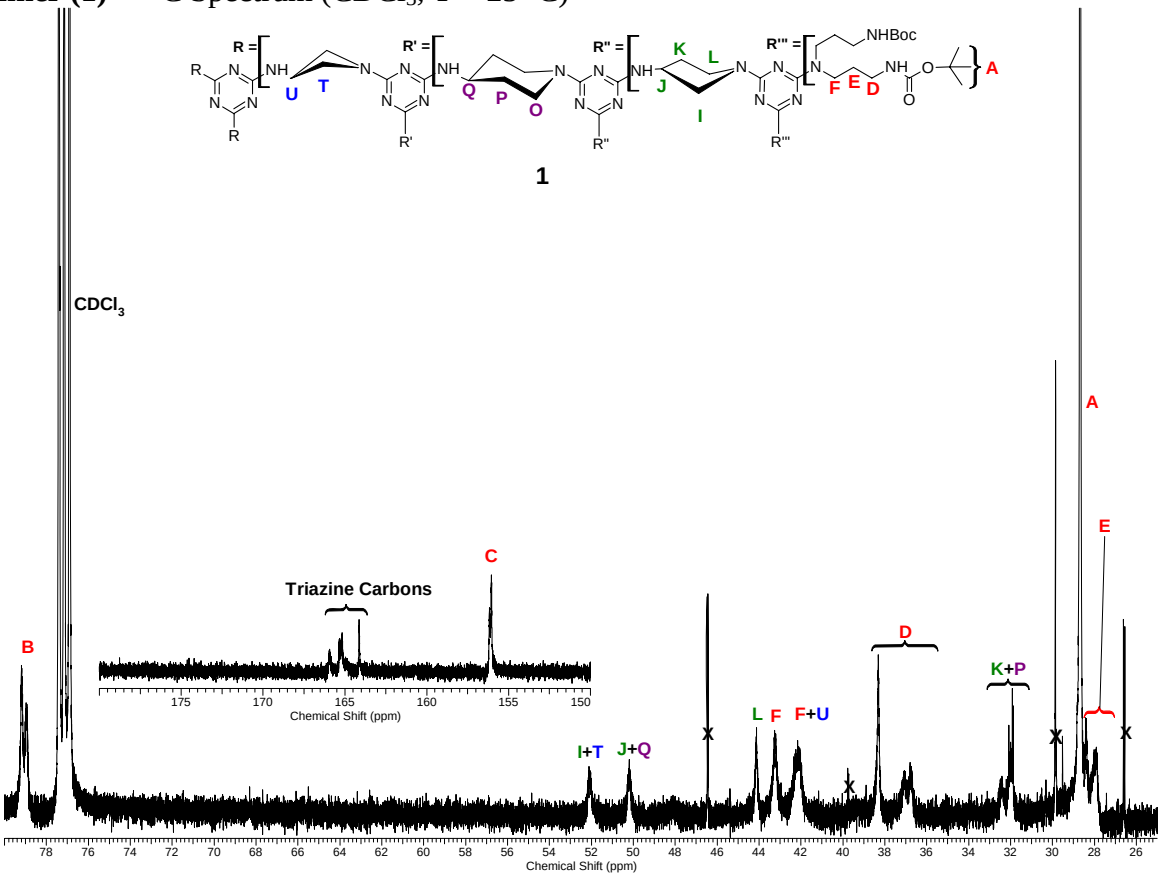


Dendrimer (1). One milliliter of a 1:1 mixture of DCM:TFA was added to a solution of **7** (54.0 mg, 0.0912 mmol) in DCM (1 mL). After an hour, the solvent was removed by reduced pressure evaporation. The residue was redissolved several times in a MeOH/triethylamine (TEA) solution and evaporated under reduced – pressure. The residue was dissolved in THF (1.5 mL) followed by the addition of BEMP resin (0.508 g, ~ 1.118 mmol). A solution of **6** (3.67 g, 0.976 mmol) in THF (8.5 mL) was added. The slurry was heated to 70 ^{o}C and allowed to react for 7 days. After 7 days, the reaction was allowed to cool and then filtered. The filter cake was washed several times with THF to recover as much material as possible. The solvent from the filtrate was removed by reduced – pressure evaporation and residue passed through a silica gel column using (25:1:10) $CHCl_3$:MeOH:Ethyl Acetate (EtOAc). Once the spot for compound **6** passed through, the solvent system was switched to (20:1:1) $CHCl_3$:MeOH:EtOAc to collect product. Fractions containing pure product, as determined by TLC, were combined and solvent removed to give white foam (378 mg, 36.2 %). ^{1}H NMR (500 MHz, DMSO-d_6, δ): 7.37 (br s, NH), 7.18, (br s, NH), 6.89 (br s, NH), 6.71, (br s, NH), 6.62 (br s, NH), 6.37 (br s, NH), 4.56 (br m, 15H), 4.47 (br m, 6H), 4.40 (br m, 6H), 4.11, (br m, 6H), 3.93 (br m, 6H), 3.77 (br m, 18H), 3.63 (br m, 12H), 3.55 – 3.25 (br m, 120H), 2.91 (br m, 108H), 2.10 (br m, 12H), 1.88 (br m, 12H), 1.75 (br m, 12H), 1.63 (br m, 96H), 1.35 (s, 271H), 1.31 (s, 173H). ^{13}C NMR (125 MHz, $CDCl_3$, δ): 165.9 (br), 165.3, 165.1, 164.8, 164.1, 156.2, 156.0, 79.3, 79.1, 78.9, 52.0 (br), 50.1 (br), 48.3, 47.9, 44.1, 43.1 (br), 42.1 (br), 41.9 (br), 39.3, 38.2, 37.0 (br), 36.6 (br), 32.4 (br), 31.9 (br), 28.6, 28.3, 27.9, 27.8; MS (MALDI) mass calcd for $C_{537}H_{942}N_{180}O_{96}$ = 11449.44; found 11479.26.

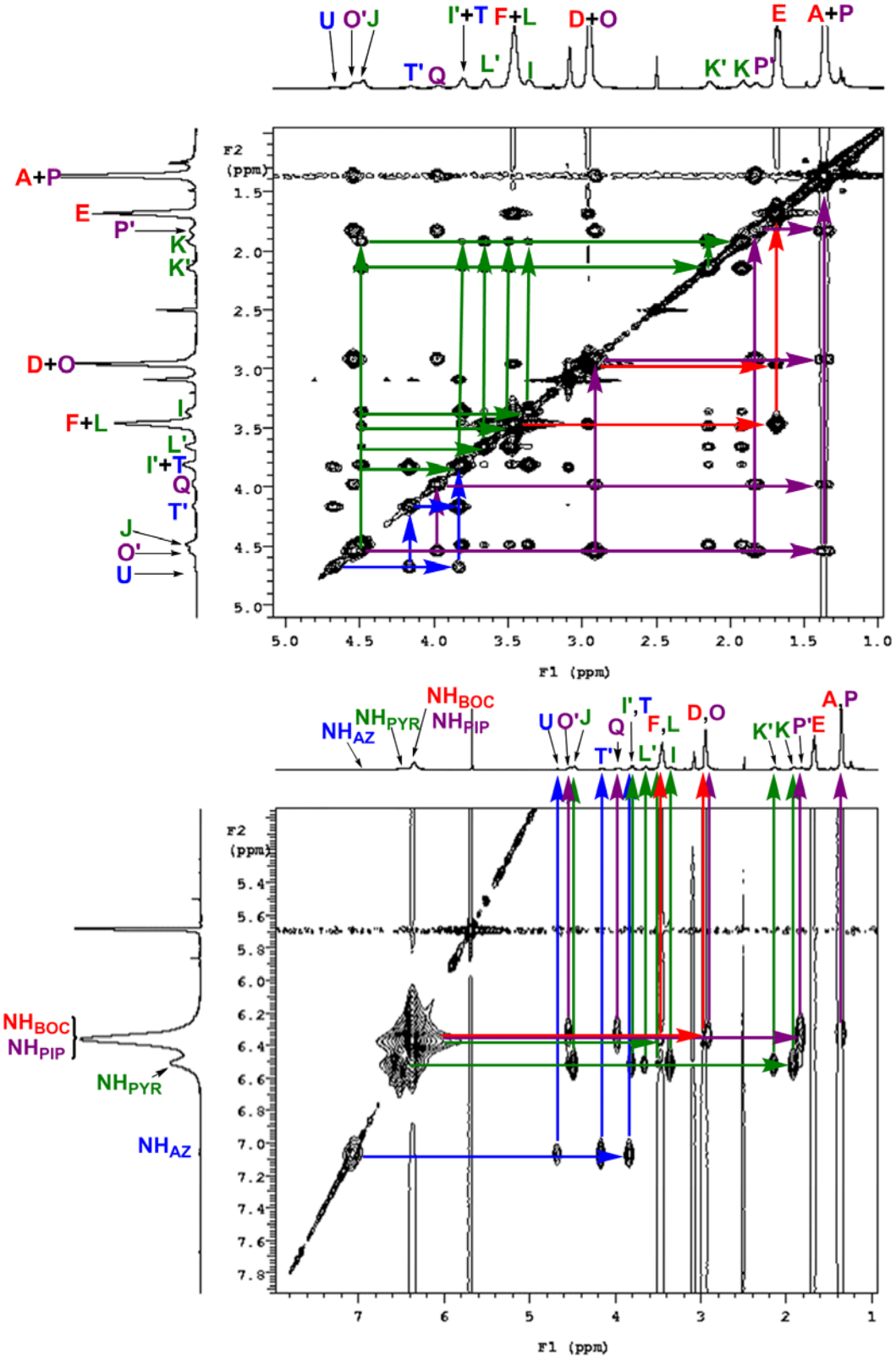
Dendrimer (1) – ^{1}H Spectrum (DMSO-d_6, T = 25 ^{o}C)



Dendrimer (1) – ^{13}C Spectrum ($CDCl_3$, T = 25 ^{o}C)



Dendrimer (1) – (^{1}H-^{1}H) TOCSY Spectrum (DMSO-d_6, T = 75 ^{o}C)



References:

1. Freeman, R.; Morris, G. A. *Bull. Magn. Reson.* **1979**, *1*, 5-26.

2. States, D. J.; Haberkorn, R. A.; Ruben, D. J. *J. Magn. Reson.* **1982**, *48*, 286-292.

3. Bax, A.; Davis, D. G. *J. Magn. Reson.* **1985**, *65*, 355-360.

4. Competition studies between azetidine·HCl and piperidine were allowed to equilibrate for 12 hours before the addition of DMTA.