

## ELECTRONIC SUPPORTING INFORMATION

### Low generation Polyamine Dendrimers Bearing Flexible Tetraethylene Glycol as nanocarriers for plasmids and siRNA

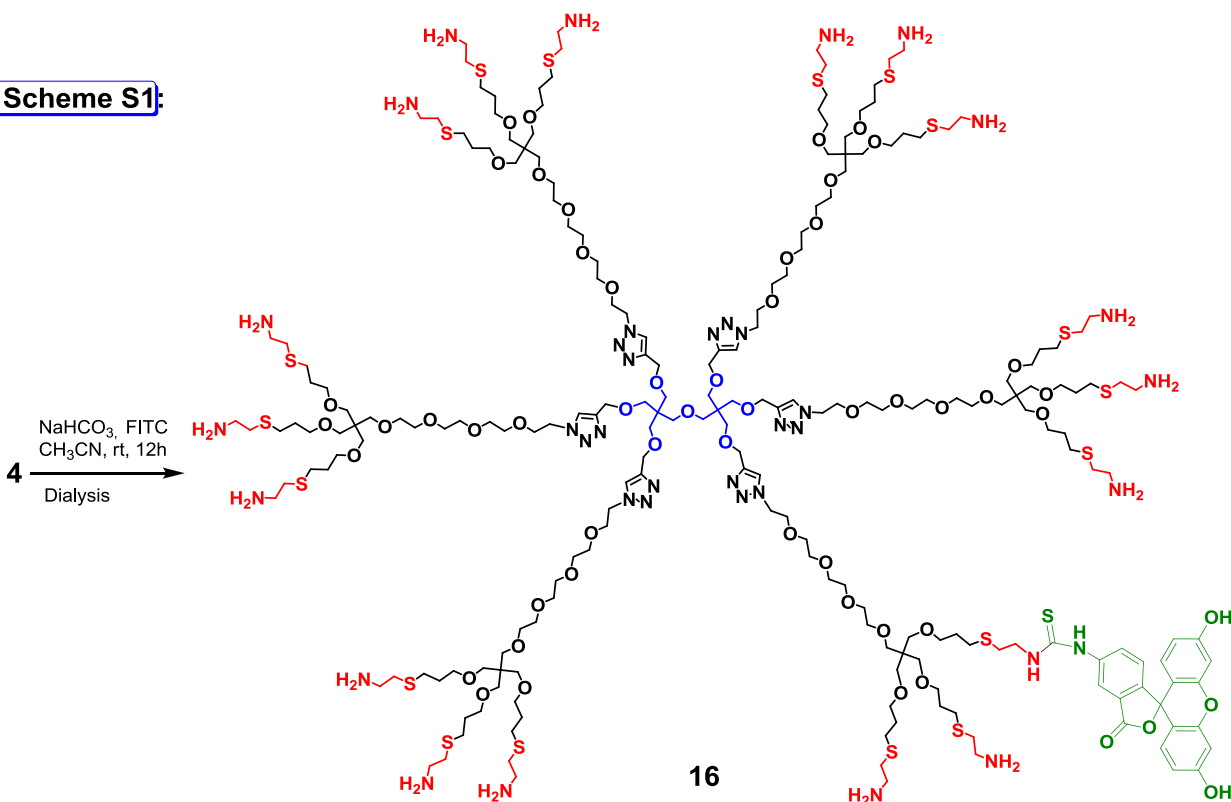
Rishi Sharma,<sup>a</sup> Issan Zhang,<sup>b</sup> Tze Chieh Shiao,<sup>a</sup> Giovanni M. Pavan,<sup>c</sup> Dusica Maysinger,<sup>b,\*</sup> and René Roy<sup>a,\*</sup>

<sup>a</sup>Pharmaqam, Department of Chemistry, Université du Québec à Montréal, P.O. Box 8888, Succ. Centre-ville, Montréal, Canada H3C 3P8. **E-mail:** [roy.rene@uqam.ca](mailto:roy.rene@uqam.ca); **Fax:** +1-514-987-4054; **Tel:** +1-514-987-3000 ext 2546

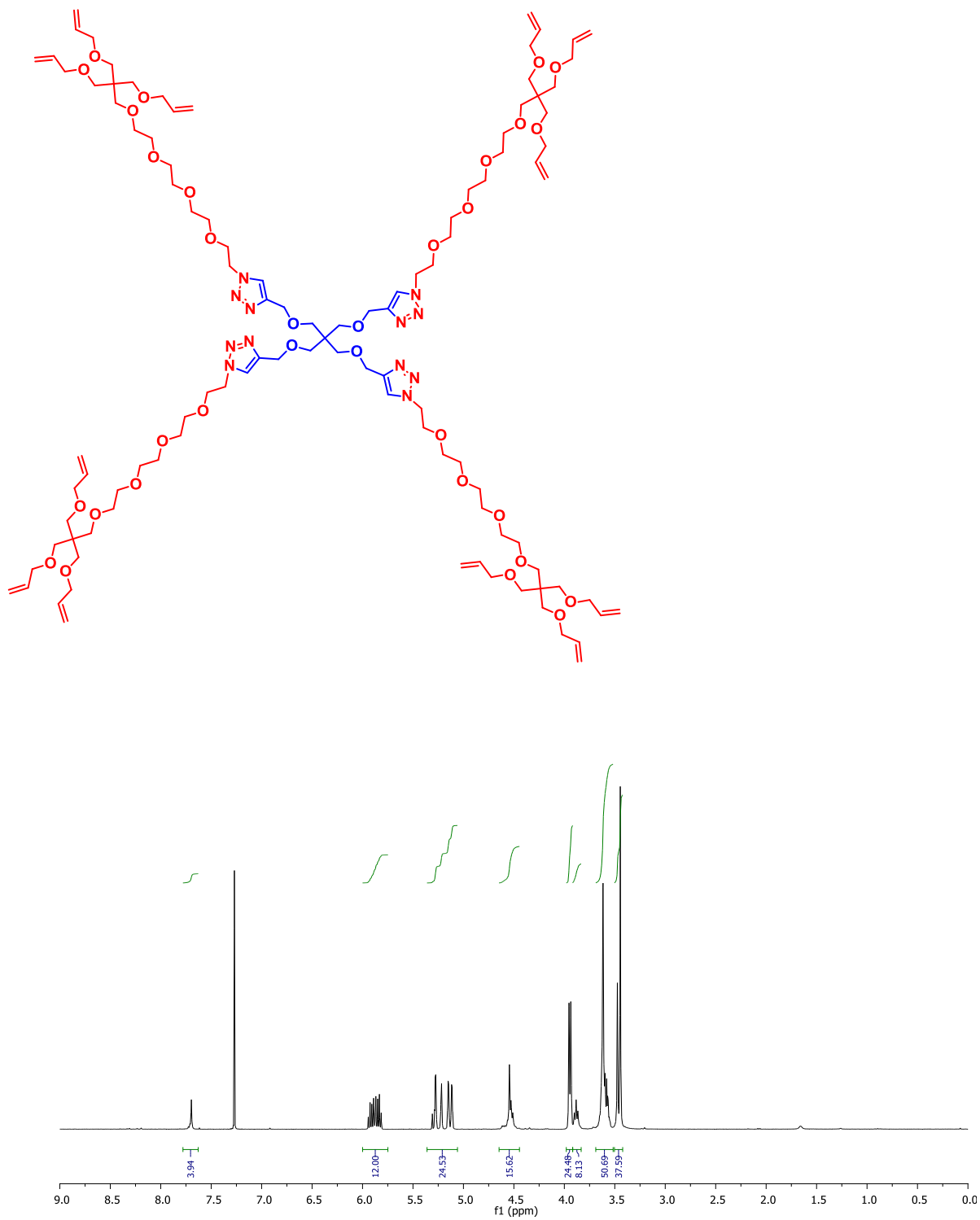
<sup>b</sup>Department of Pharmacology and Therapeutics, McGill University, 3655 Promenade Sir-William-Osler, Montreal, Quebec, H3G 1Y6, Canada. **E-mail:** [dusica.maysinger@mcgill.ca](mailto:dusica.maysinger@mcgill.ca); **Fax:** +514-398-6690; **Tel:** +514-398-1264

<sup>c</sup>Department of Innovative Technologies, University of Applied Sciences and Arts of Southern Switzerland, Galleria 2, 6928 Manno, Switzerland

**Scheme S1:**



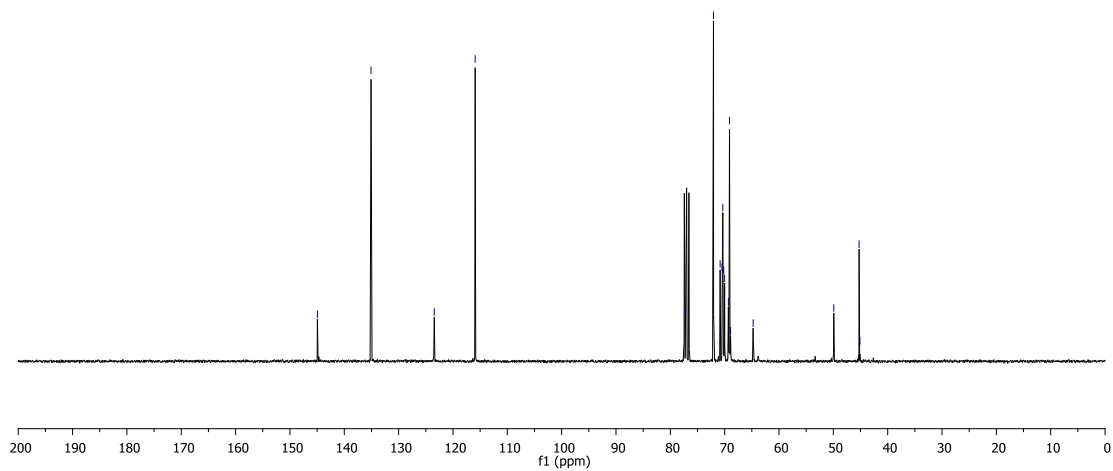
## Compound 9



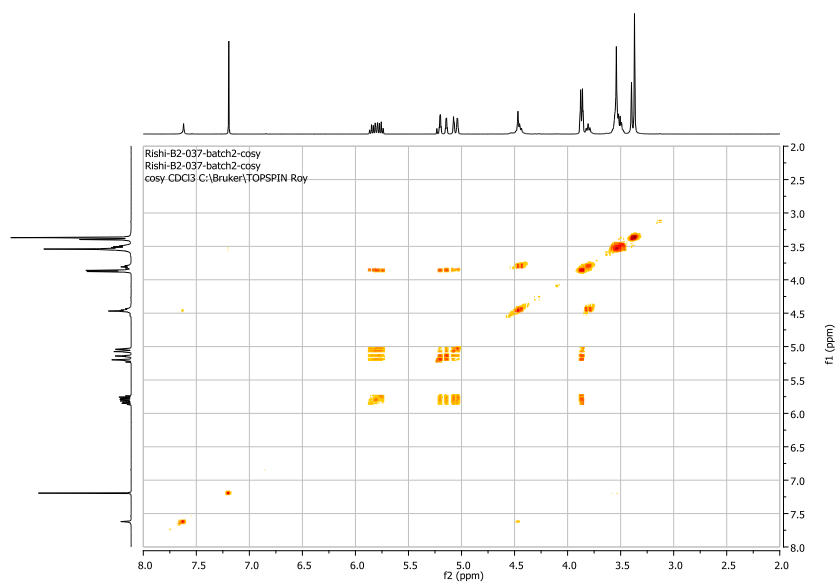
**Figure S1.** <sup>1</sup>H NMR spectrum of compound **9** (CDCl<sub>3</sub>, 300 MHz).

Rishi-B2-037-c13  
Rishi-B2-037-c13  
carbone CDCl3 C:\Bruker\TOPSPIN Roy

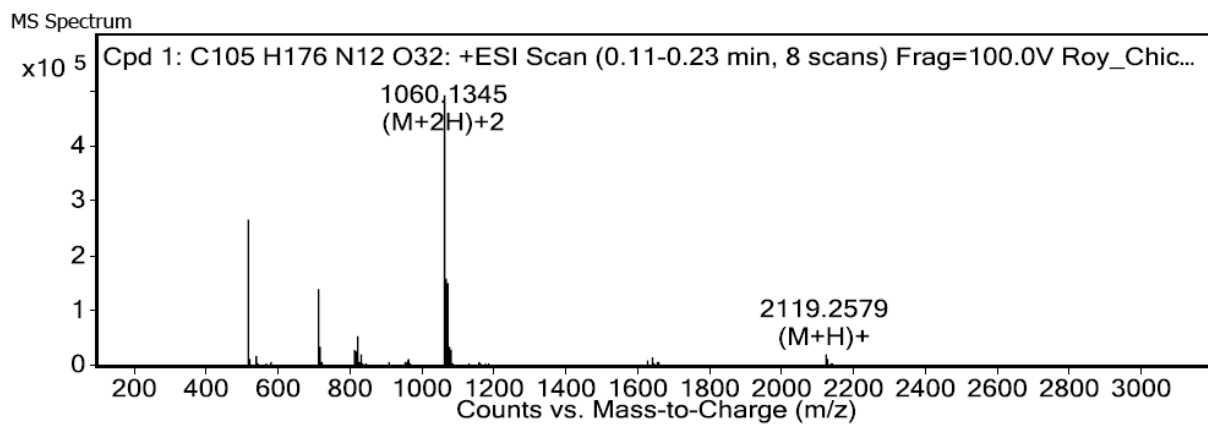
144.95  
135.08  
123.44  
115.91  
77.20  
72.06  
70.96  
70.88  
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70.17  
70.01  
69.85  
69.75  
45.26  
45.11



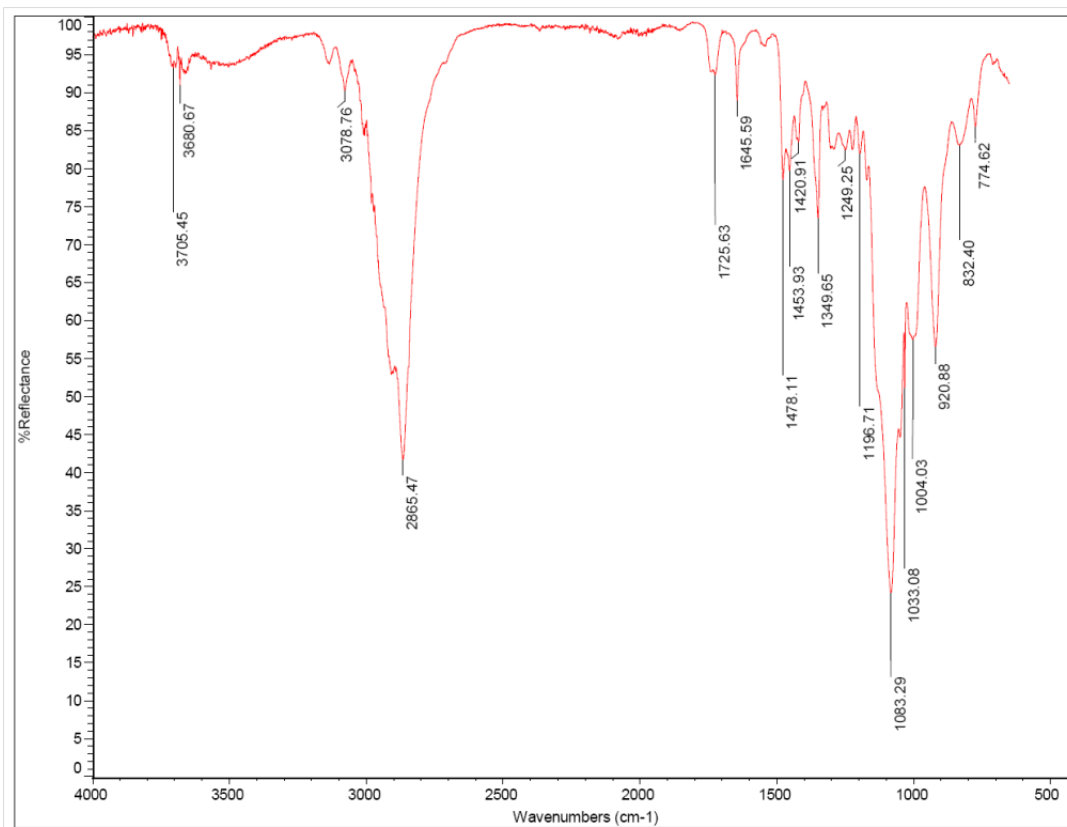
**Figure S2.**  $^{13}\text{C}$  NMR spectrum of compound **9** ( $\text{CDCl}_3$ , 75 MHz).



**Figure S3.** COSY spectrum of compound **9**.



**Figure S4.** HRMS analysis (ESI<sup>+</sup>) for compound **9**.



**Figure S5.** IR spectrum of compound **9**.

## Compound 3

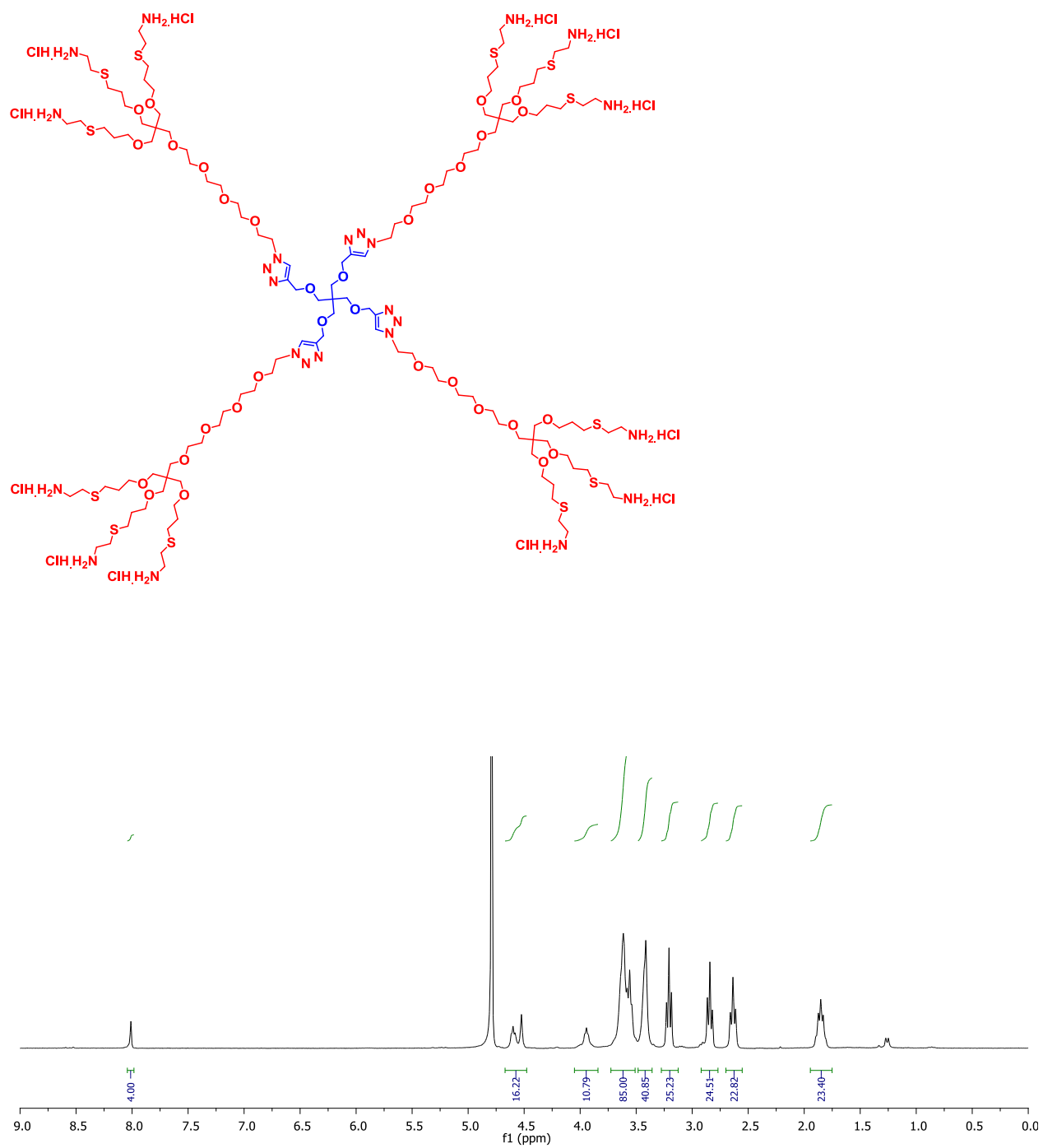
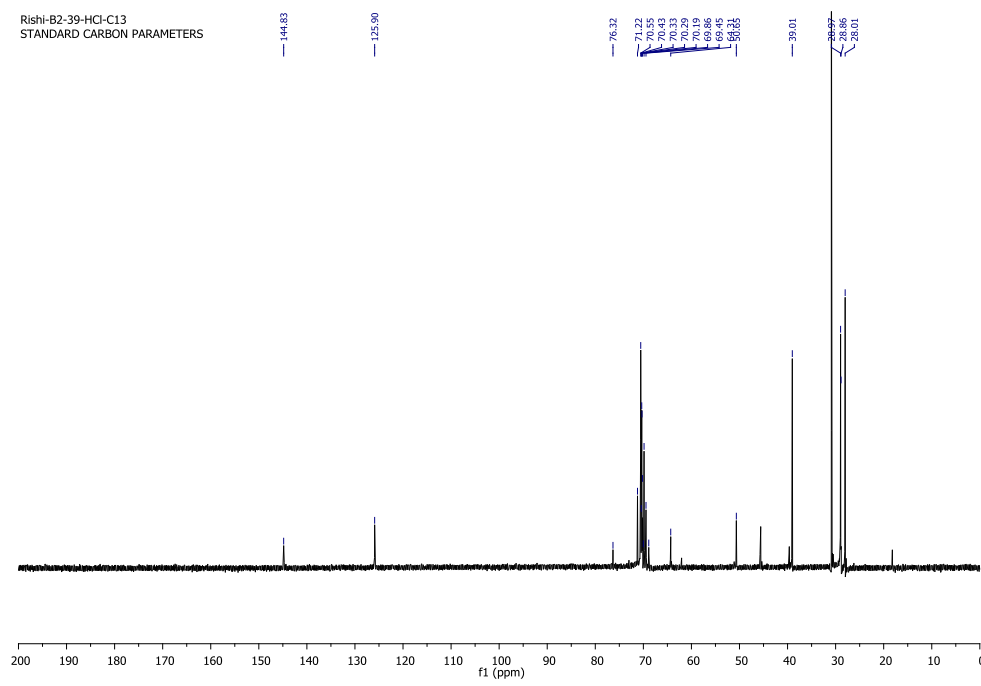
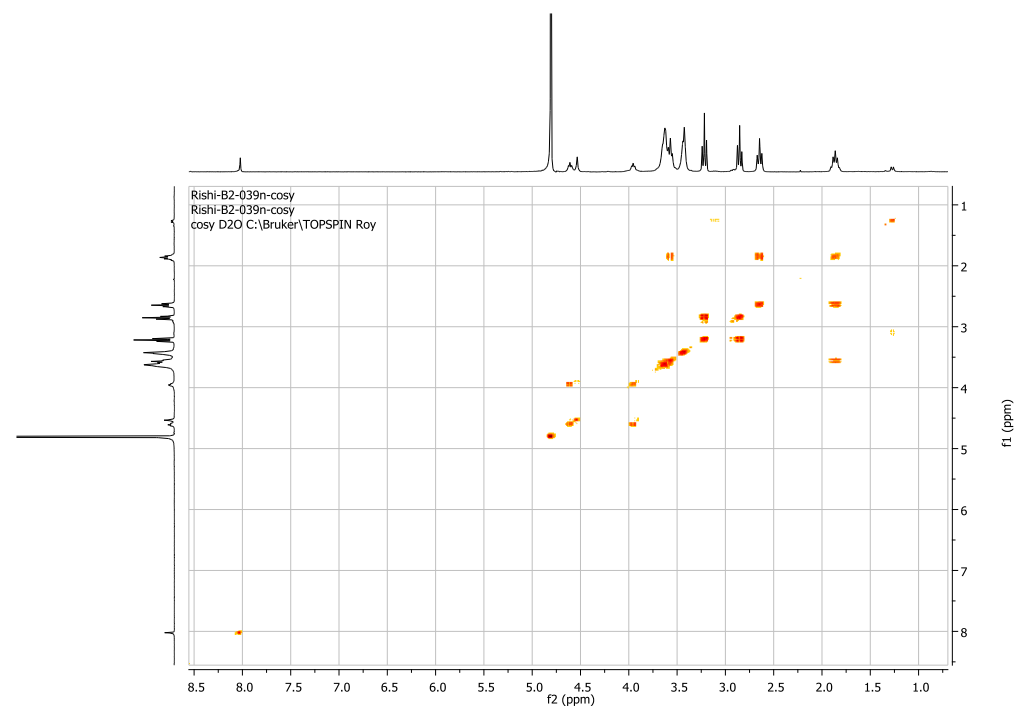


Figure S6. <sup>1</sup>H NMR spectrum of compound 3 (D<sub>2</sub>O, 300 MHz).



**Figure S7.**  $^{13}\text{C}$  NMR spectrum of compound **3** ( $\text{D}_2\text{O}$ , 151 MHz).



**Figure S8.** COSY spectrum of compound **3**

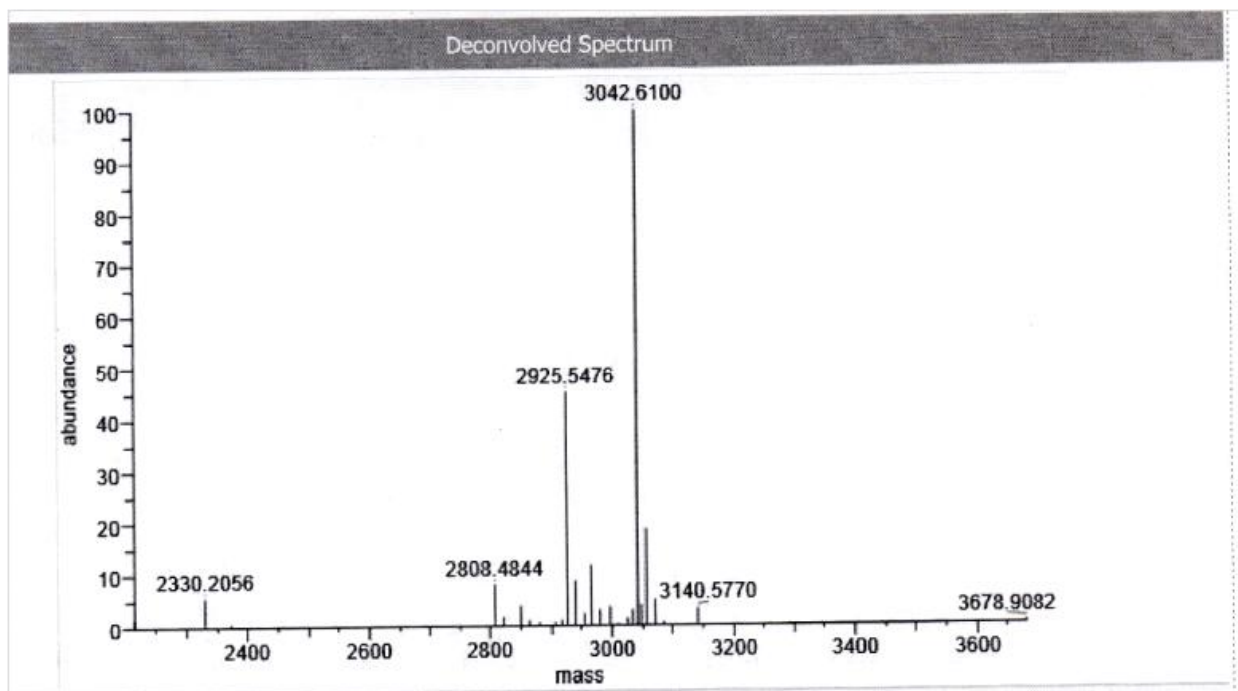


Figure S9. MALDI-TOF trace for compound 3.

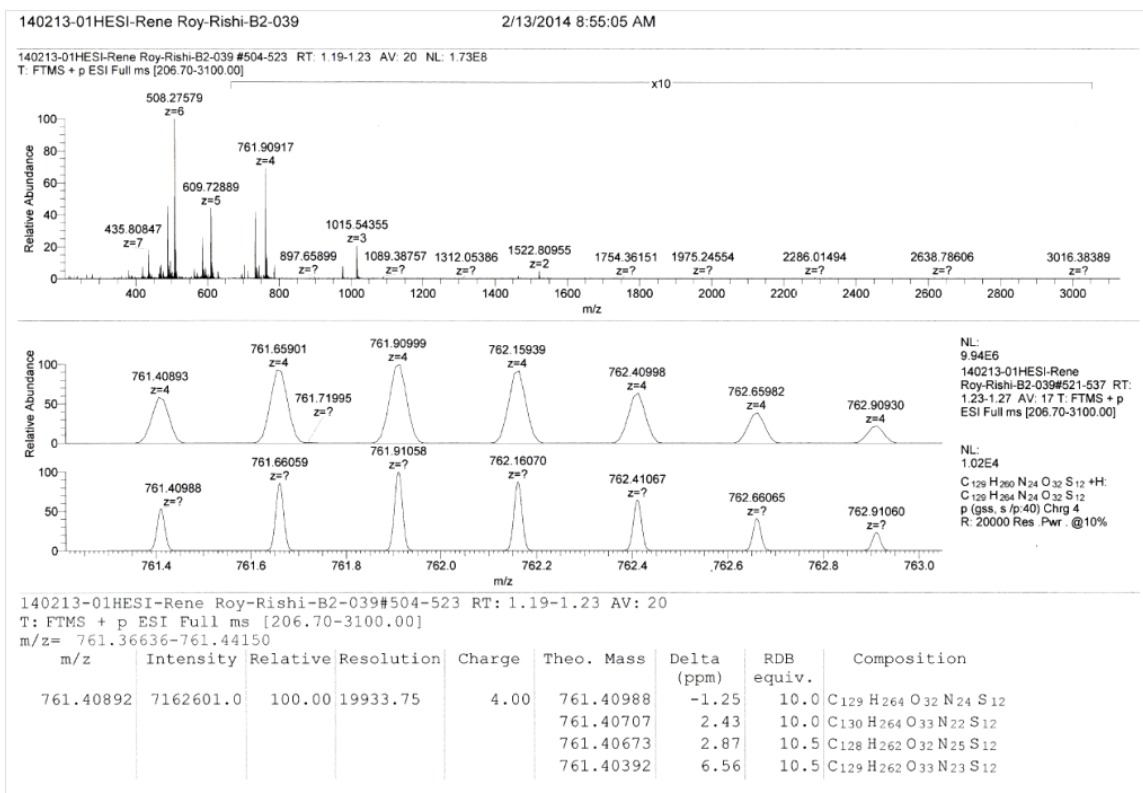
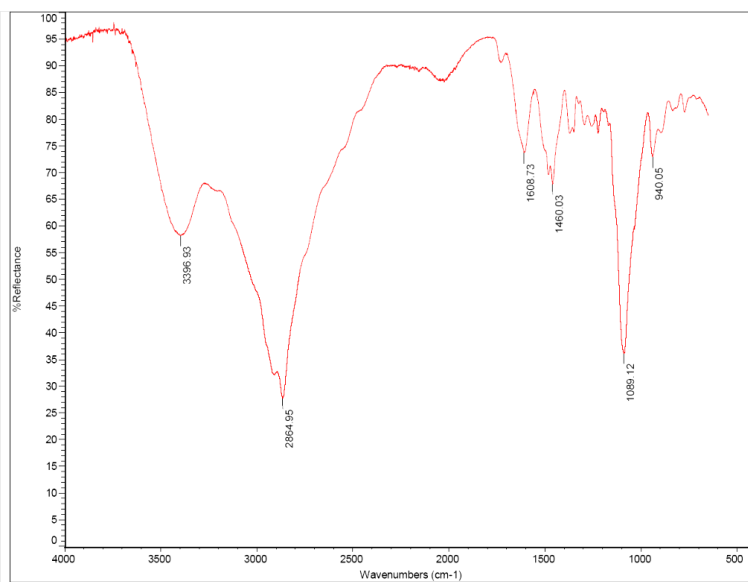


Figure S10. HRMS analysis (ESI<sup>+</sup>) for compound 3.



**Figure S11.** IR spectrum of compound **3**.



# Compound 11

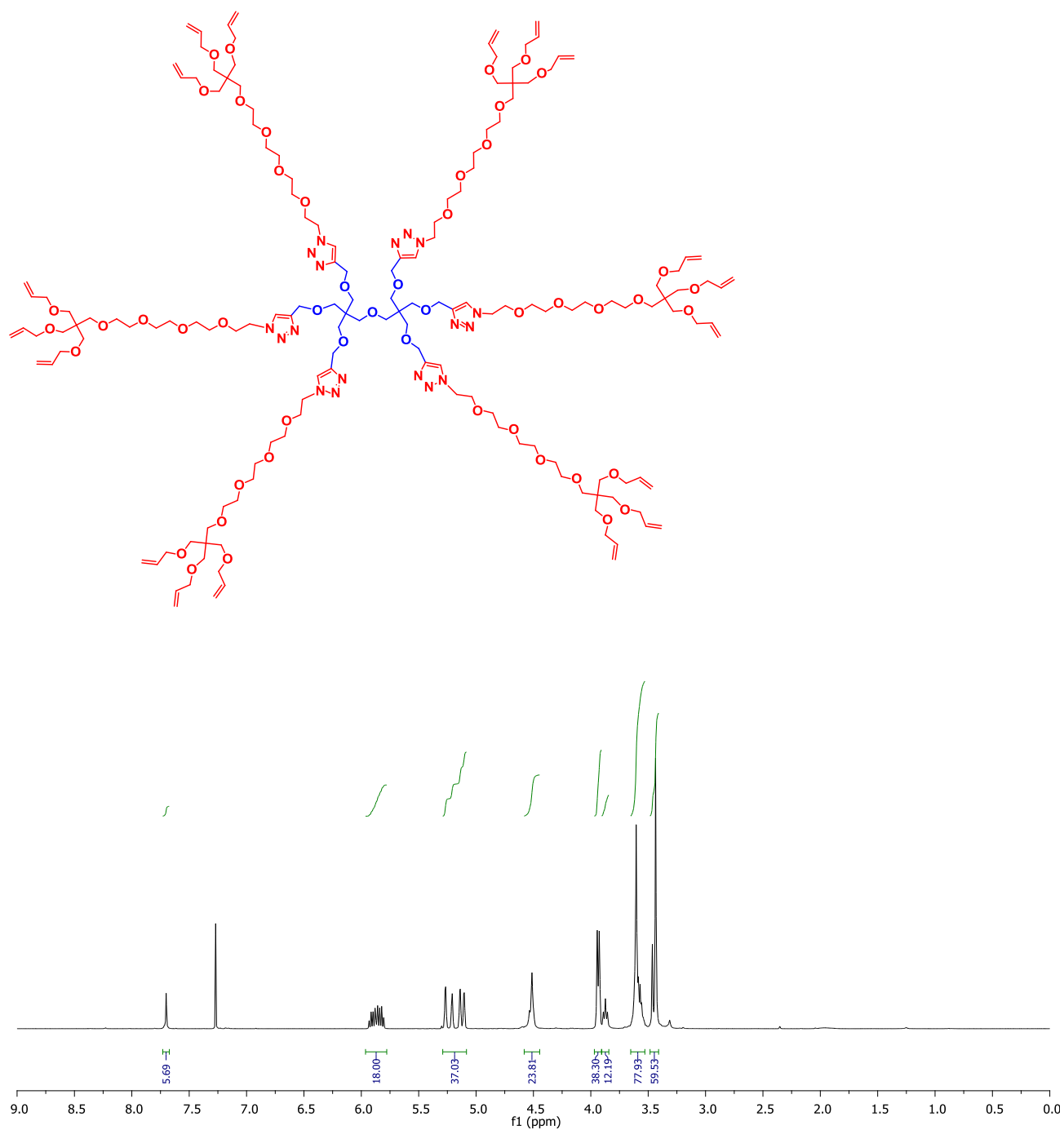
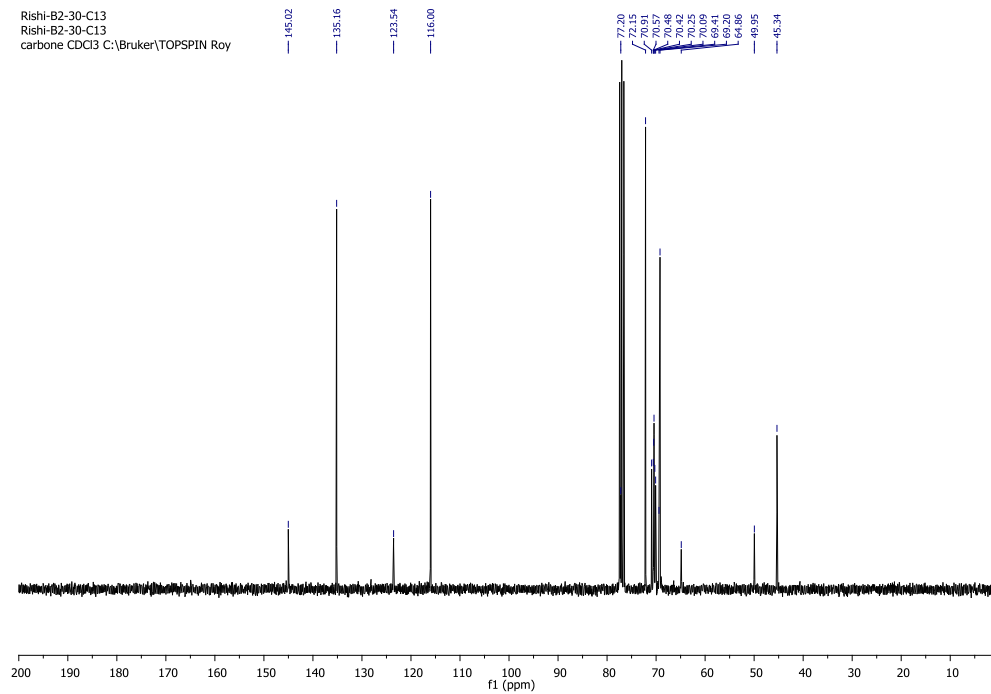
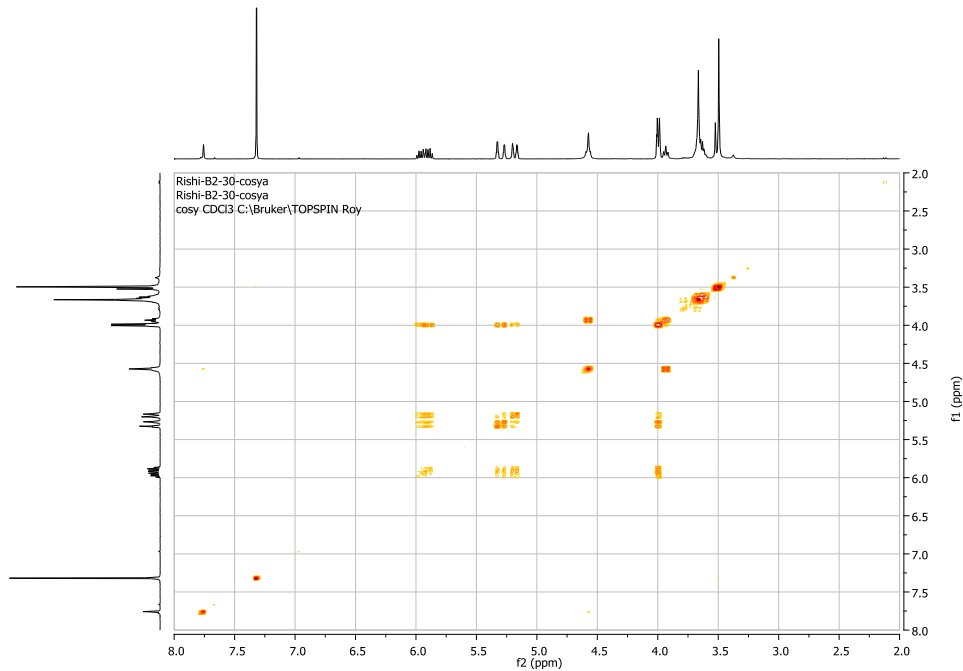


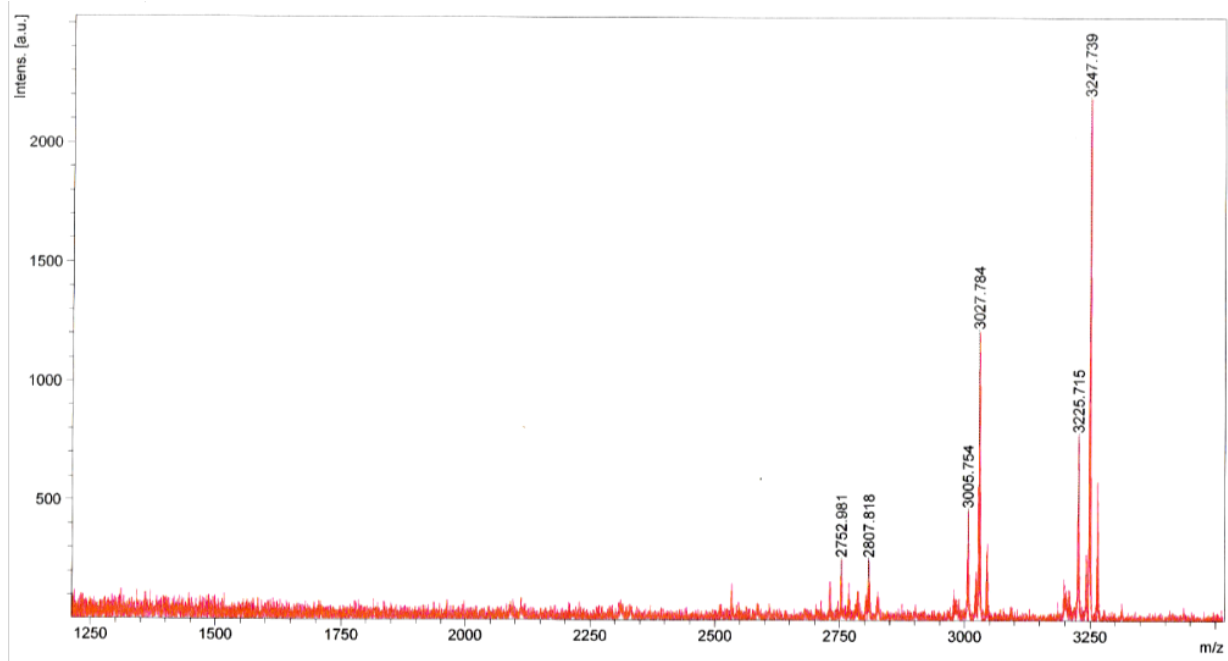
Figure S12. <sup>1</sup>H NMR spectrum of compound 11 (CDCl<sub>3</sub>, 300 MHz).



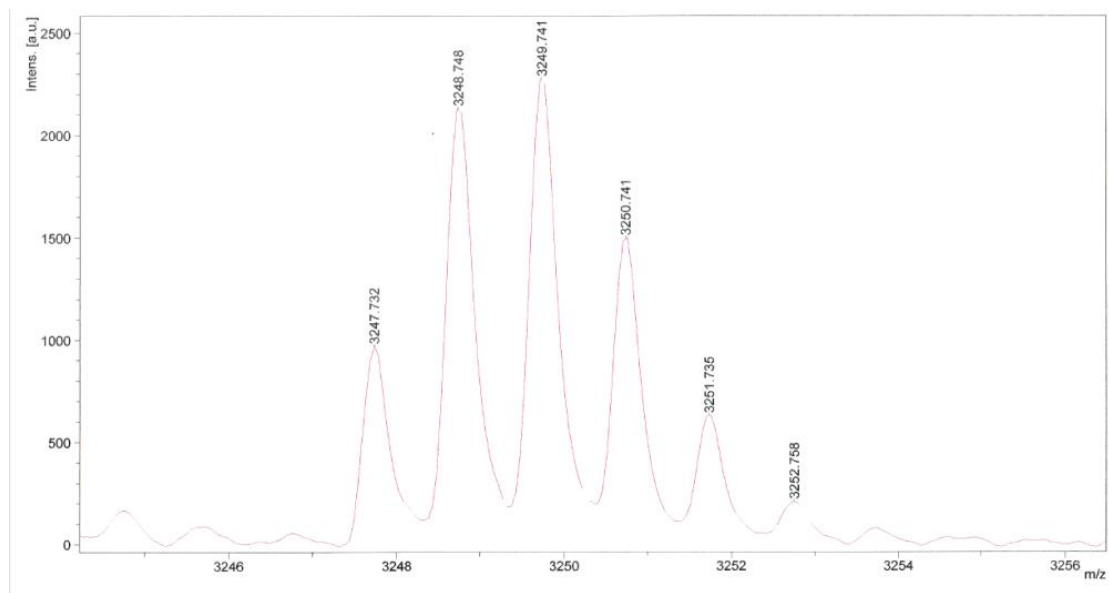
**Figure S13.**  $^{13}\text{C}$  NMR spectrum of compound **11** ( $\text{CDCl}_3$ , 75 MHz).



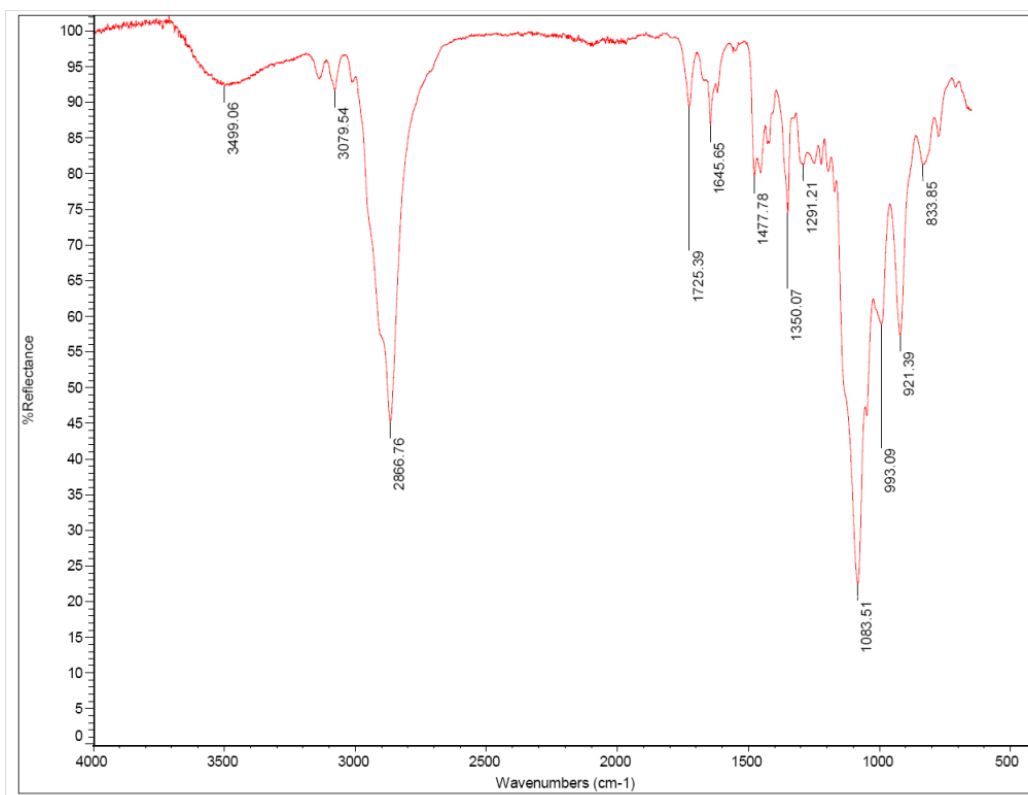
**Figure S14.** COSY spectrum of compound **11**.



**Figure S15.** MALDI-TOF trace for compound **11**.



**Figure S16.** MALDI-TOF trace for compound **11**.



**Figure S17.** IR spectrum of compound **11**

# Compound 4

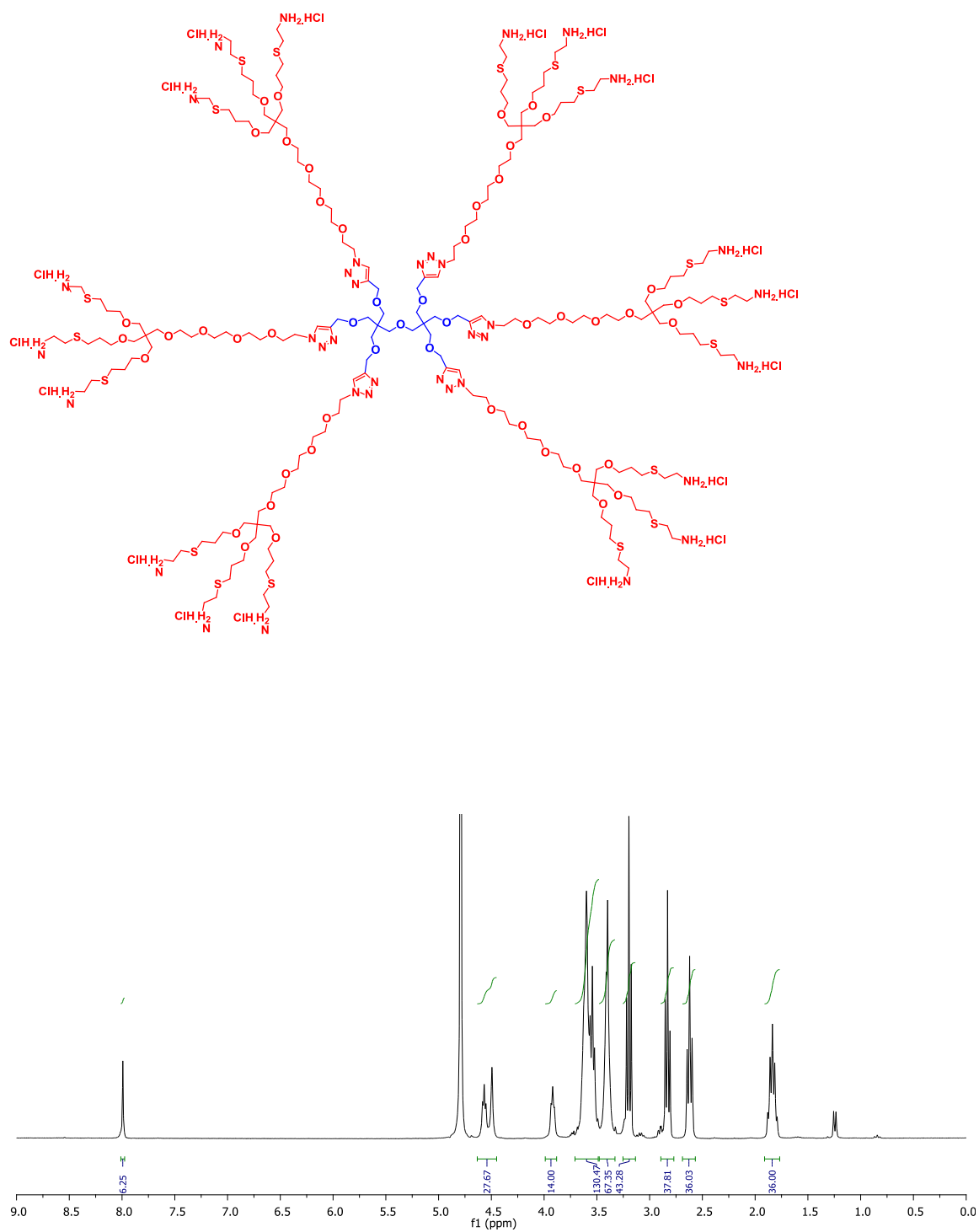
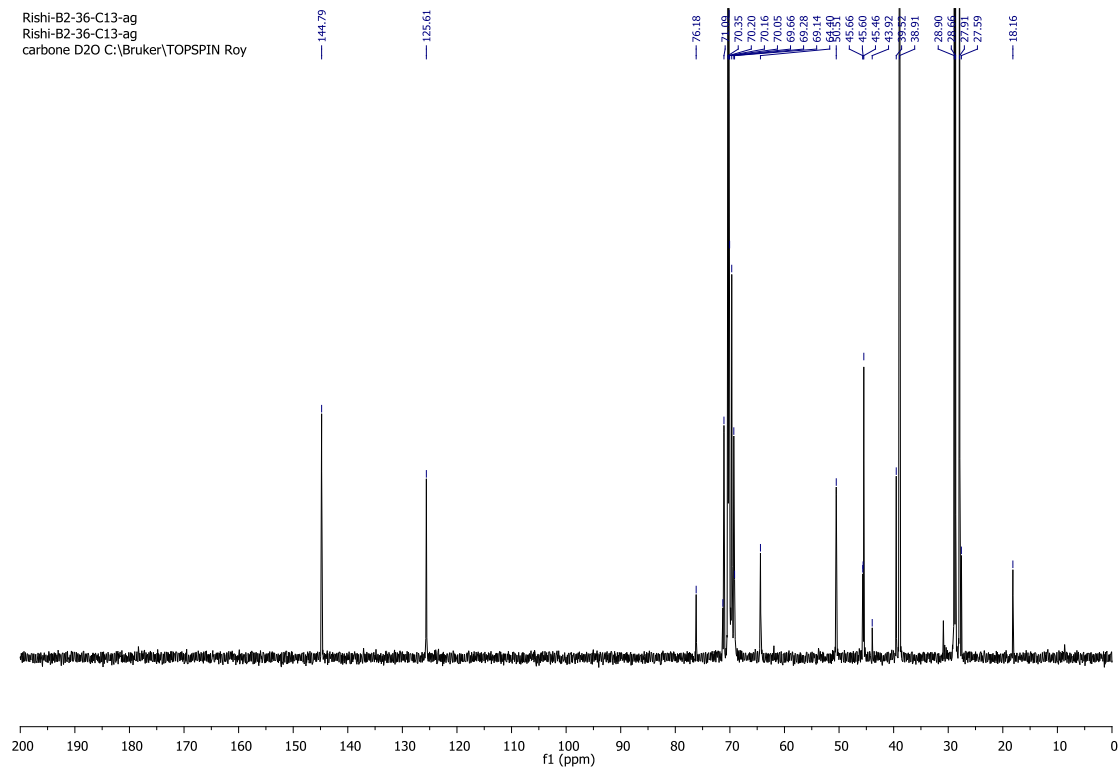
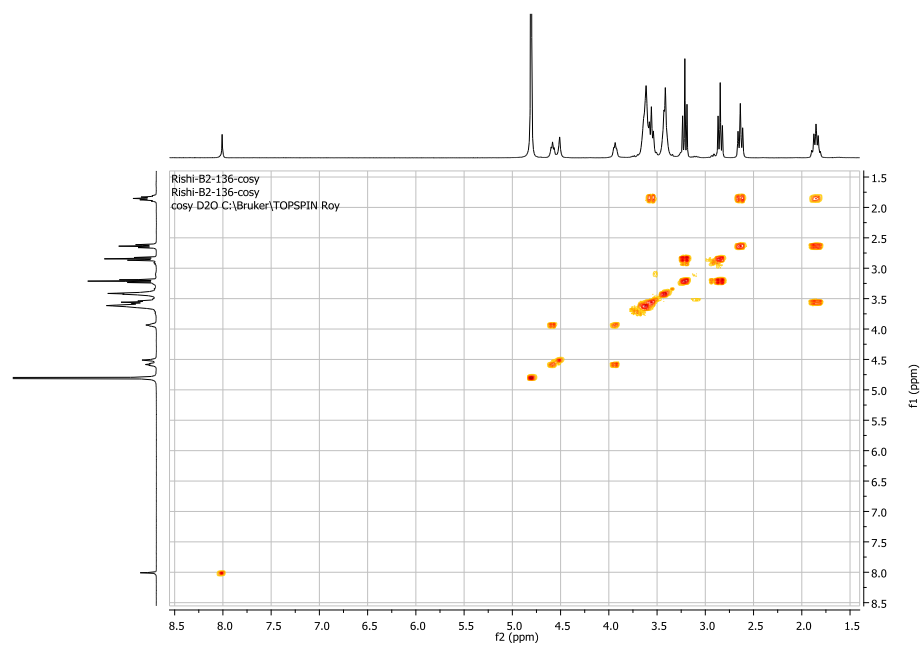


Figure S18. <sup>1</sup>H NMR spectrum of compound 4 (D<sub>2</sub>O, 300 MHz).



**Figure S19.**  $^{13}\text{C}$  NMR spectrum of compound **4** ( $\text{D}_2\text{O}$ , 75 MHz).



**Figure S20.** COSY spectrum of compound **4**.

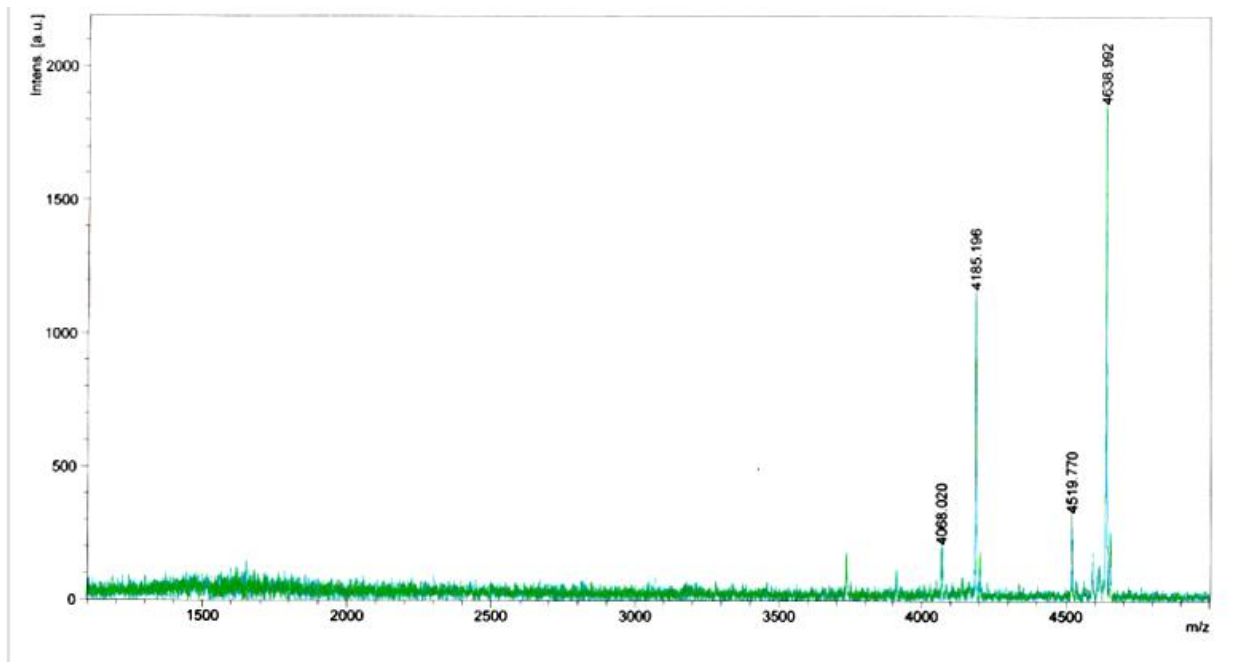


Figure S21. MALDI-TOF trace for compound 4 (DHB matrix).

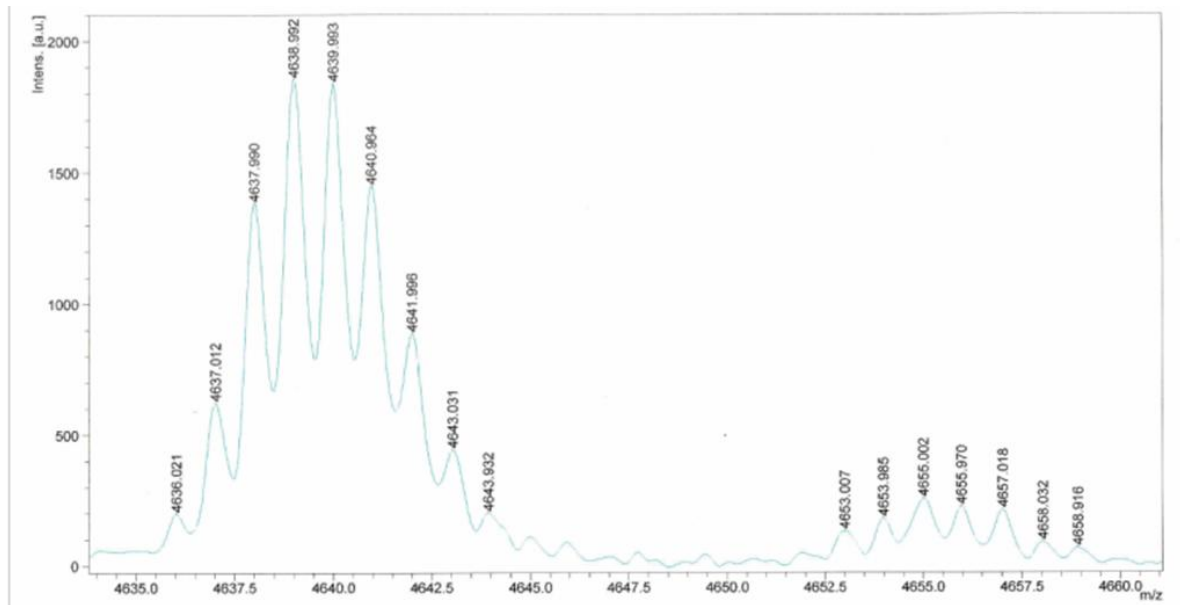
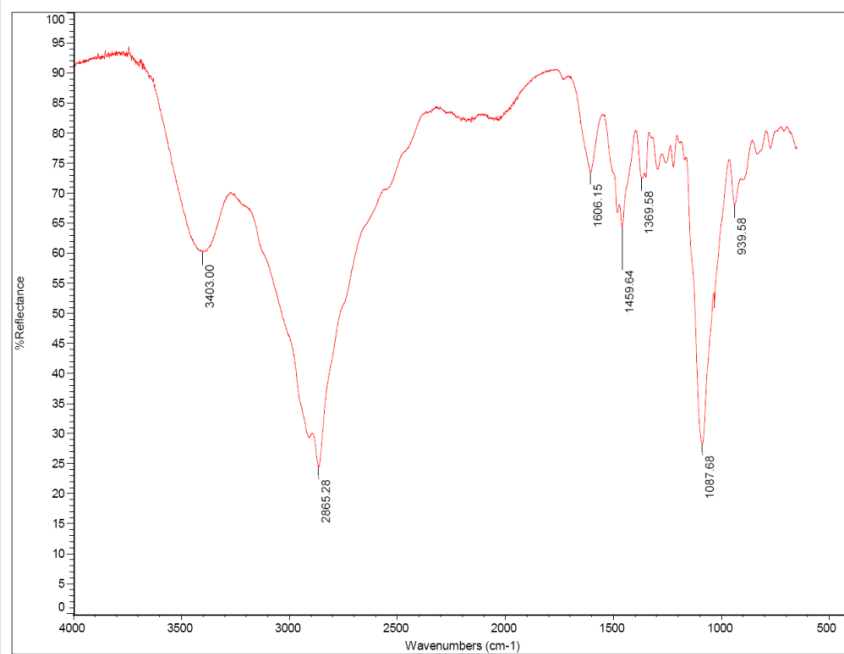


Figure S22. Expanded MALDI-TOF trace for compound 4.



**Figure S23.** IR spectrum of compound **4**.



## Compound 13

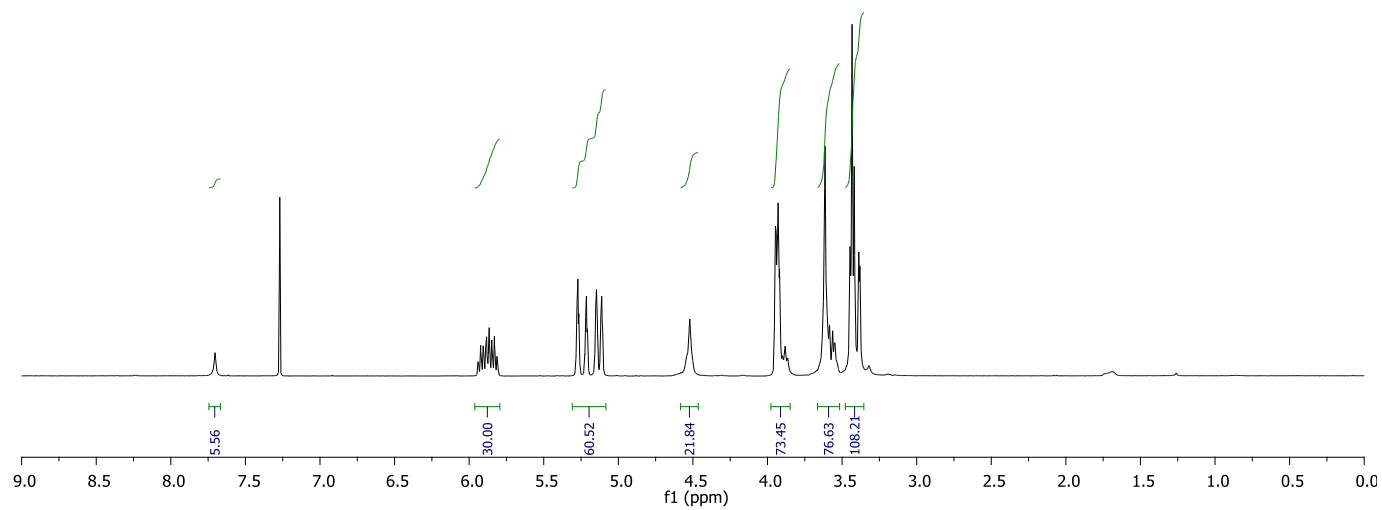
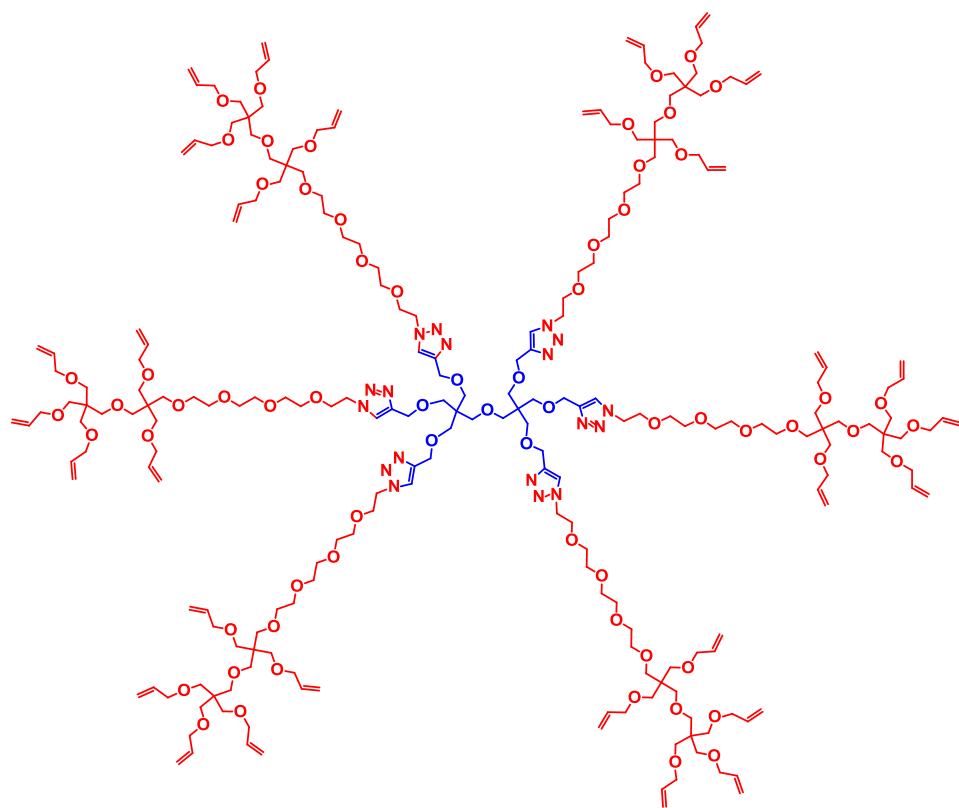
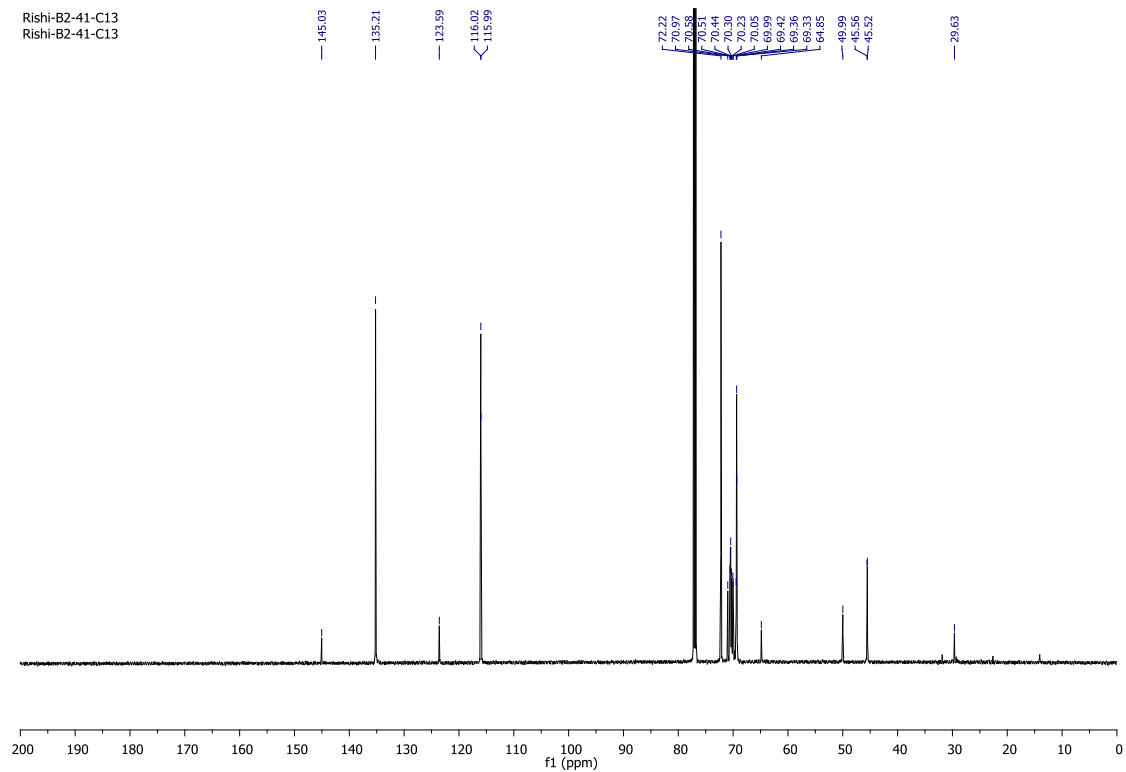
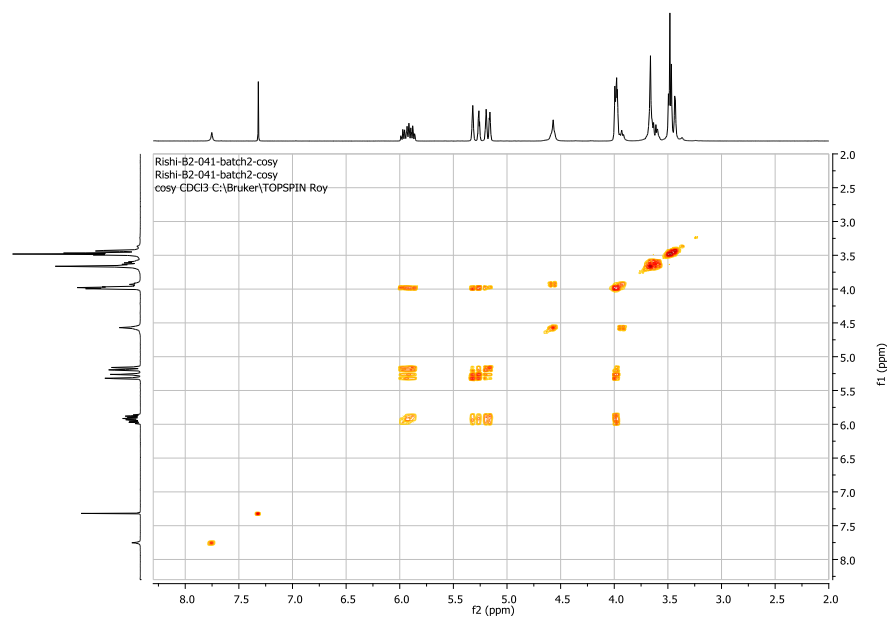


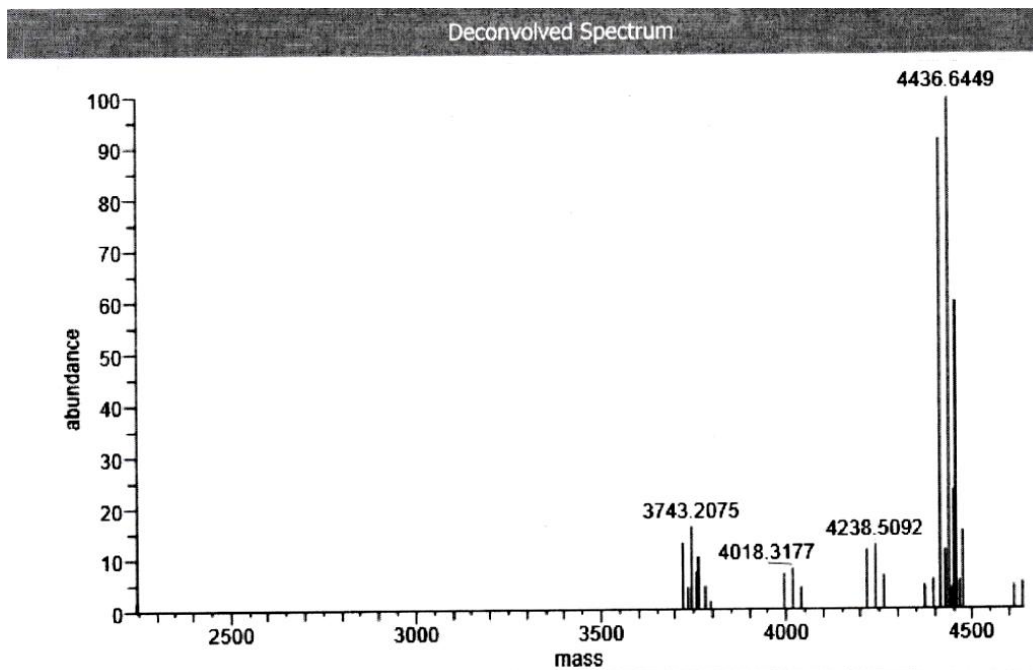
Figure S24. <sup>1</sup>H NMR spectrum of compound 13 (CDCl<sub>3</sub>, 300 MHz).



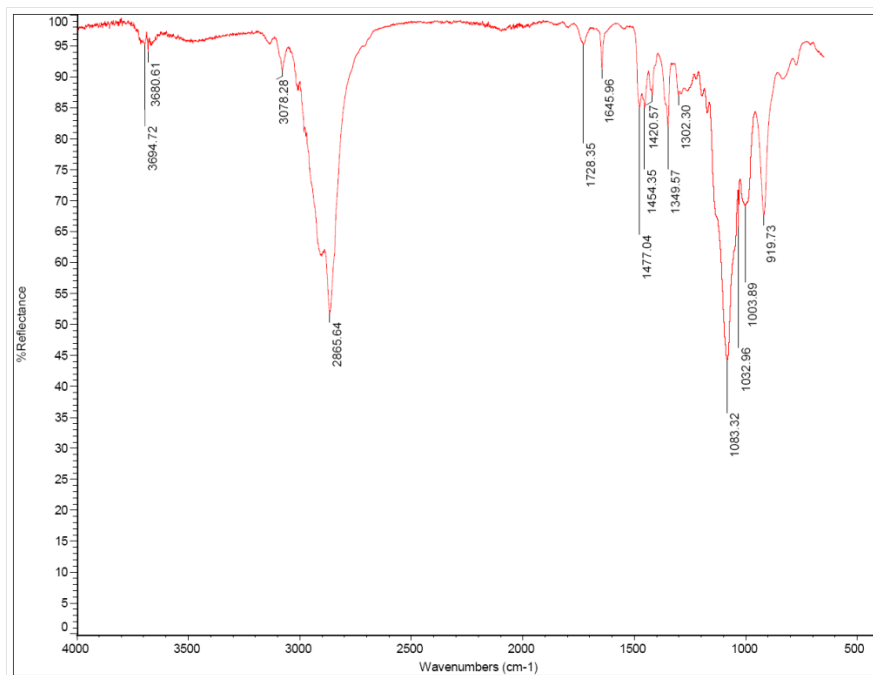
**Figure S25.**  $^{13}\text{C}$  NMR spectrum of compound **13** ( $\text{CDCl}_3$ , 151 MHz).



**Figure S26.** COSY spectrum of compound **13**.



**Figure S27.** MALDI-TOF trace for glycodendrimer **13**.



**Figure S28.** IR spectrum of compound **13**.

# Compound 5

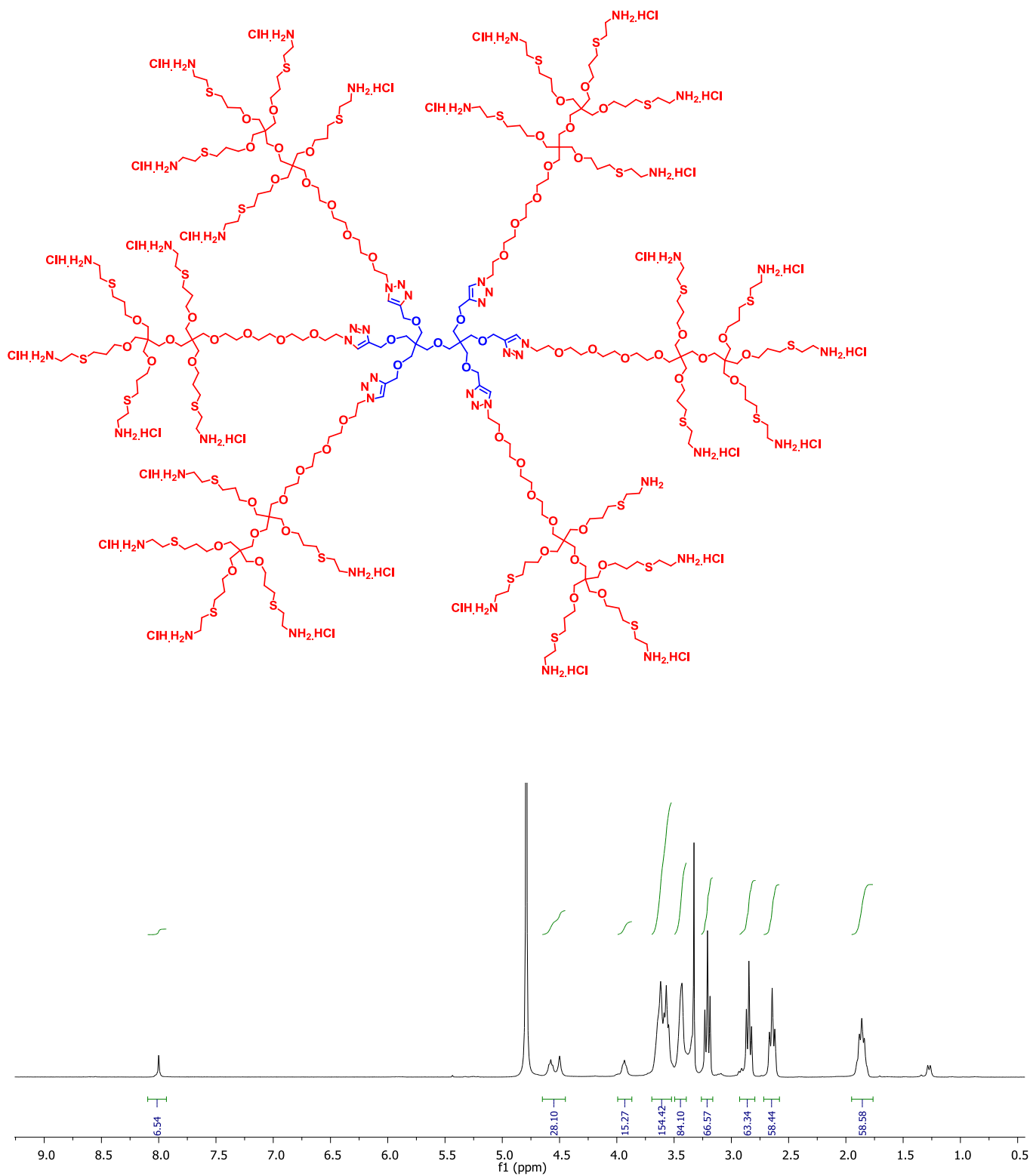
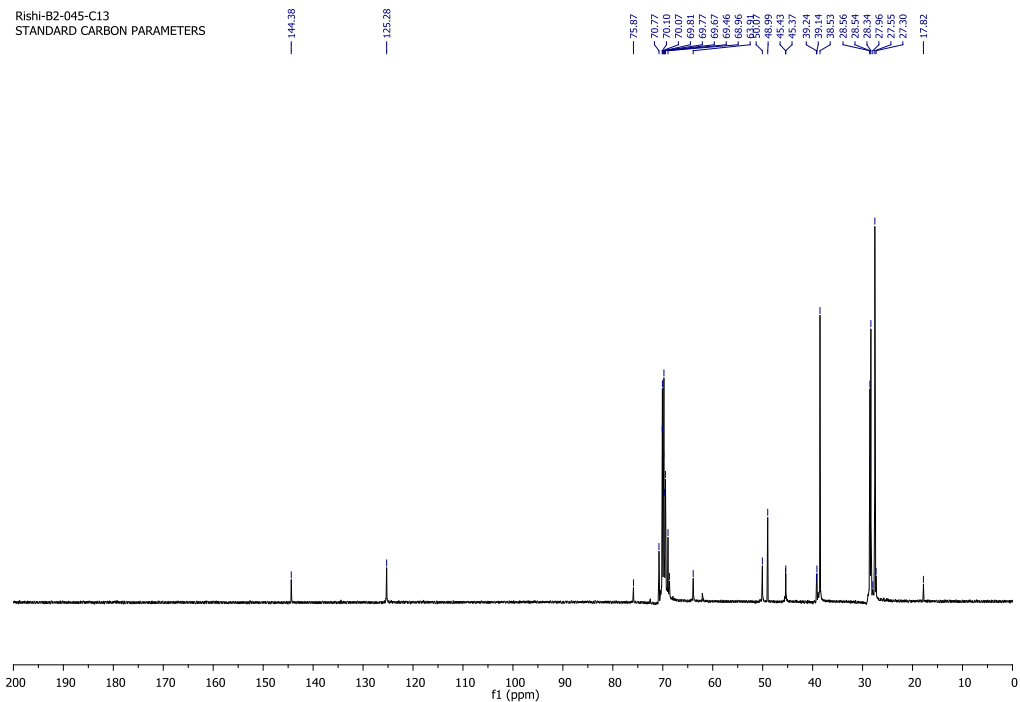
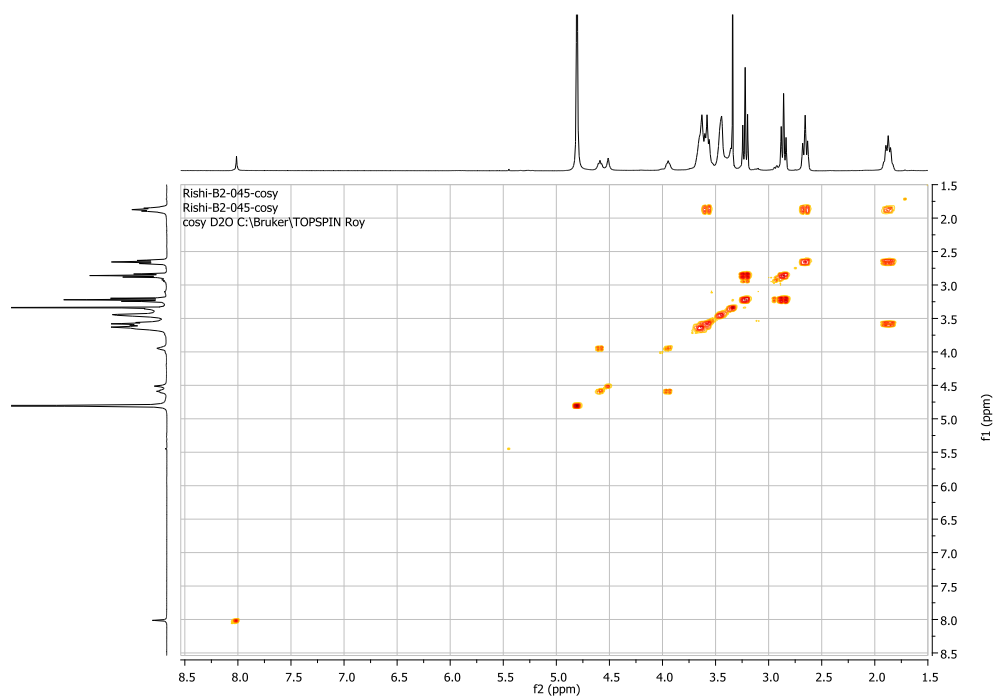


Figure S29. <sup>1</sup>H NMR spectrum of glycodendrimer 5 (D<sub>2</sub>O, 300 MHz).



**Figure S30.**  $^{13}\text{C}$  NMR spectrum of glycodendrimer **5** ( $\text{D}_2\text{O}$ , 151 MHz).



**Figure S31.** COSY spectrum of glycodendrimer **5**.

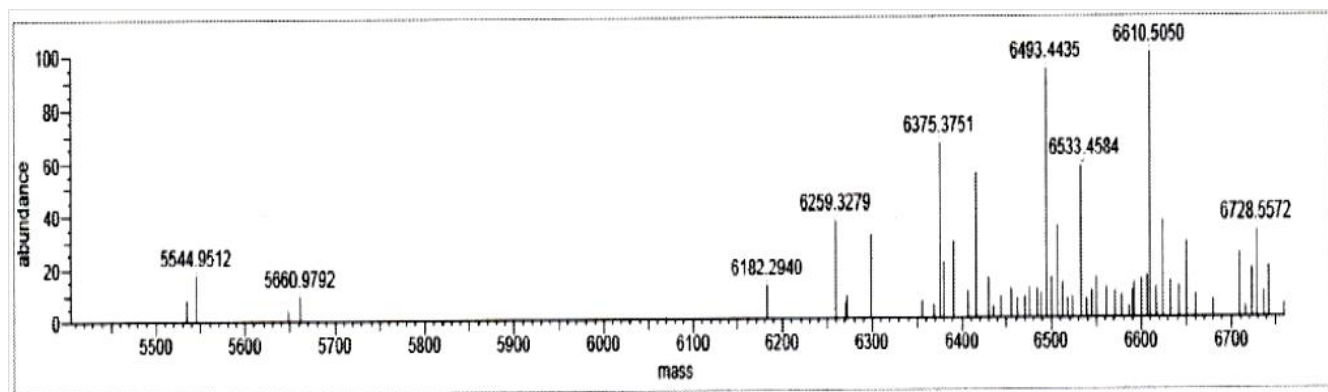


Figure S32. ESI spectrum of compound 5.

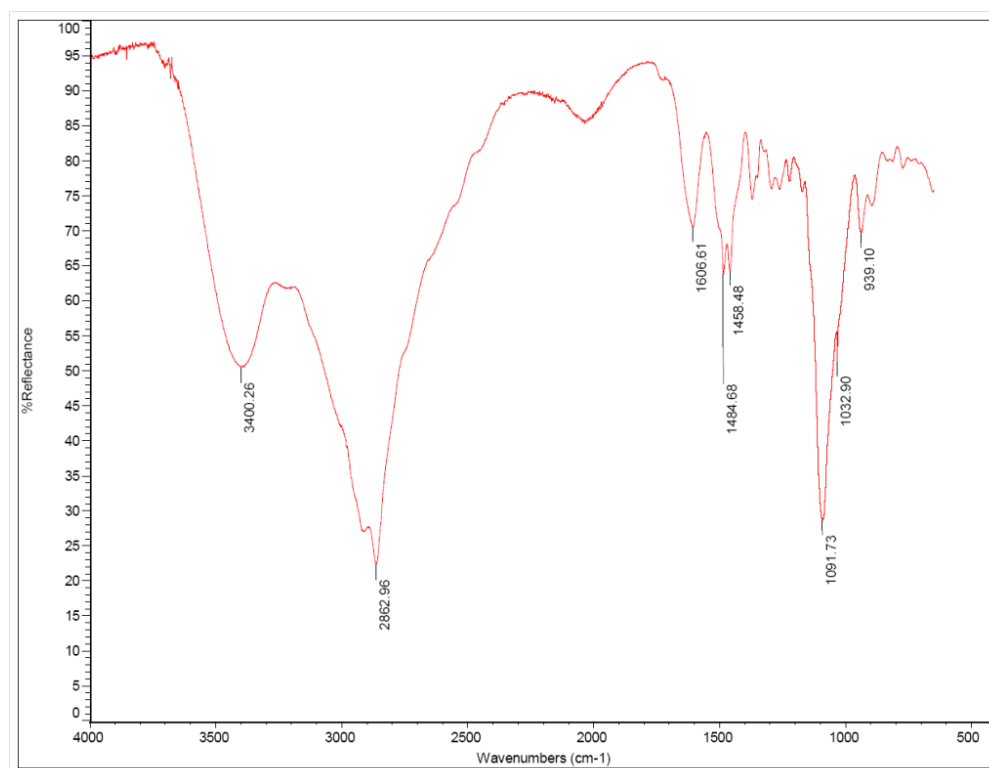
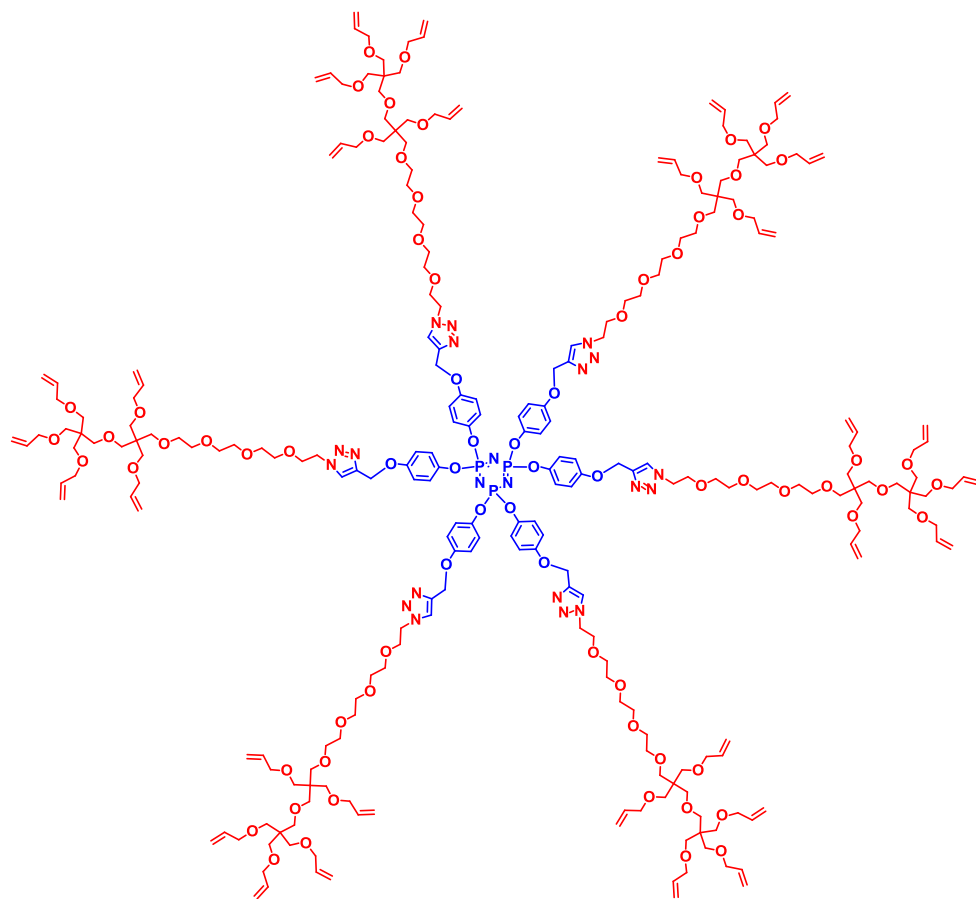


Figure S33. IR spectrum of compound 5.

## Compound 15



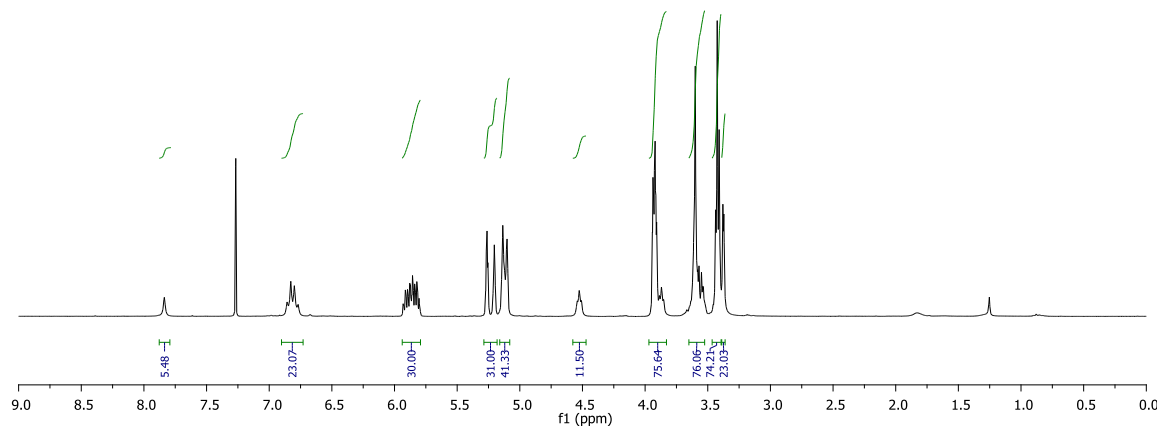


Figure S34.  $^1\text{H}$  NMR spectrum of compound **15** ( $\text{CDCl}_3$ , 300 MHz).

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Rishi-B2-046-P31  
phosphore  $\text{CDCl}_3$  C:\Bruker\TOPSPIN Roy

— 9.76

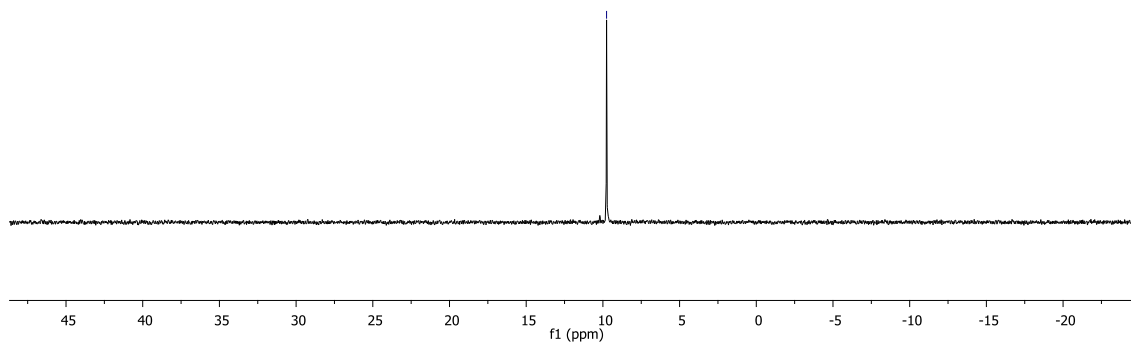
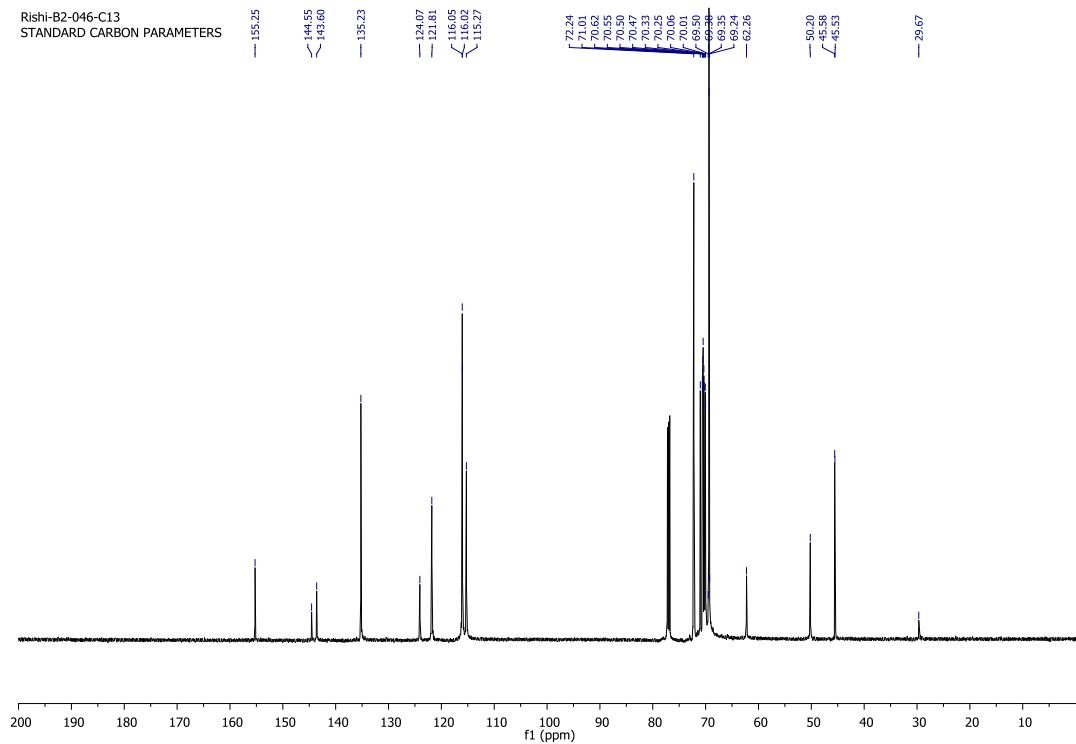
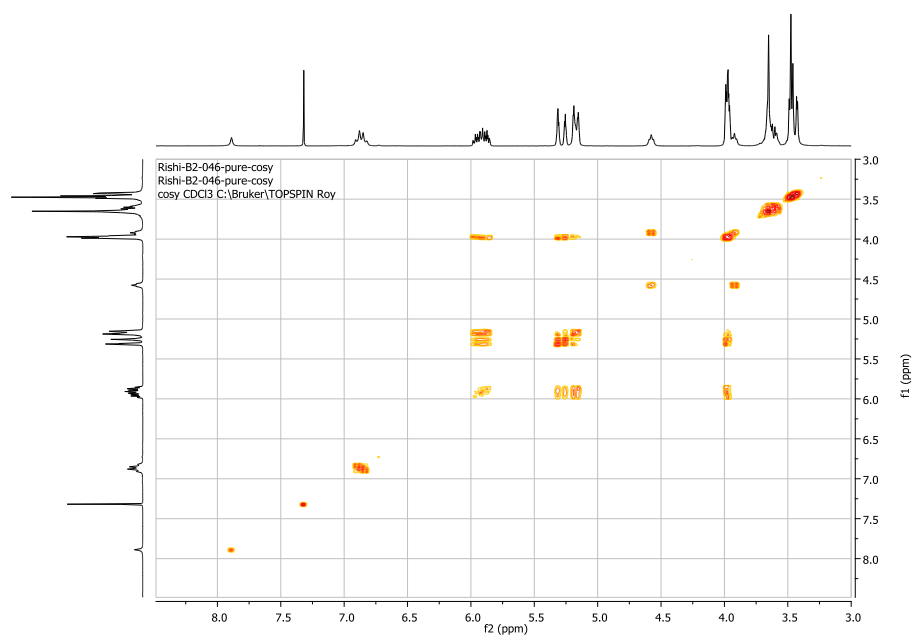


Figure S35.  $^{31}\text{P}$  NMR spectrum of compound **15** (122 MHz,  $\text{CDCl}_3$ ).

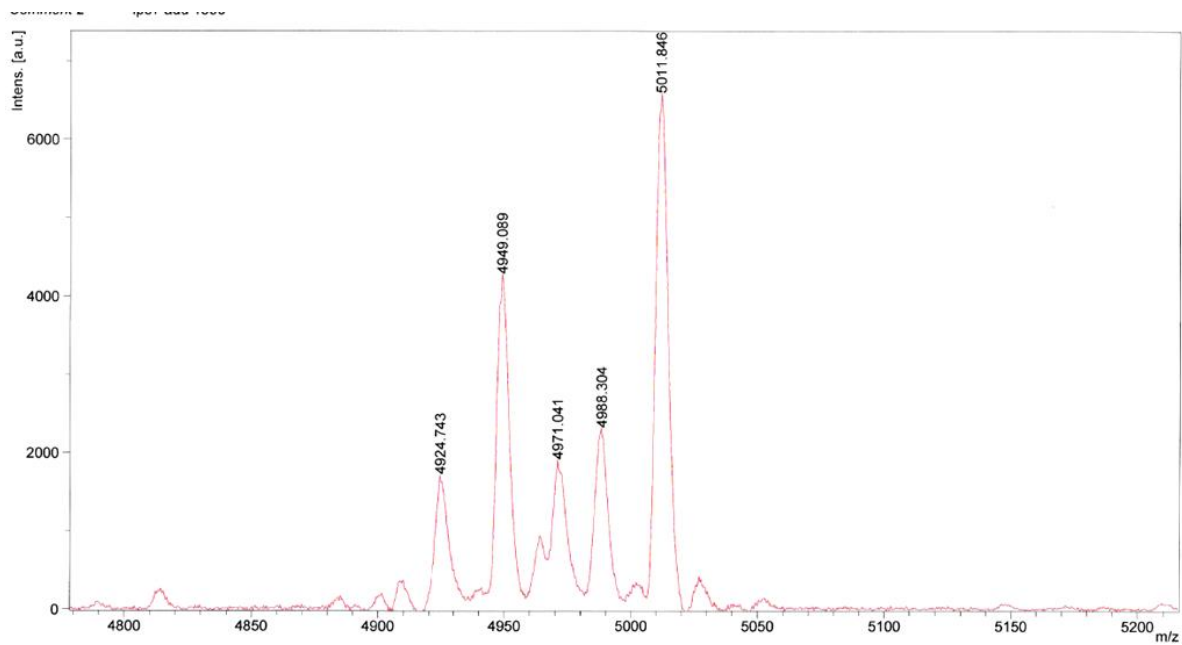
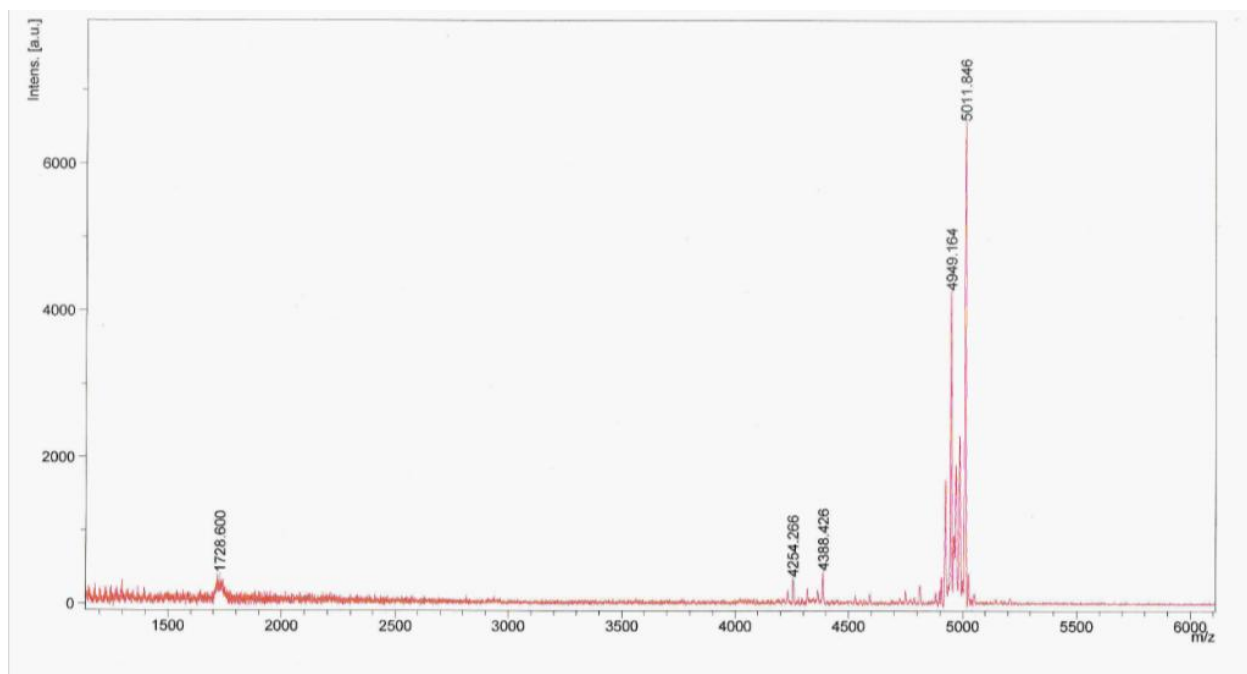




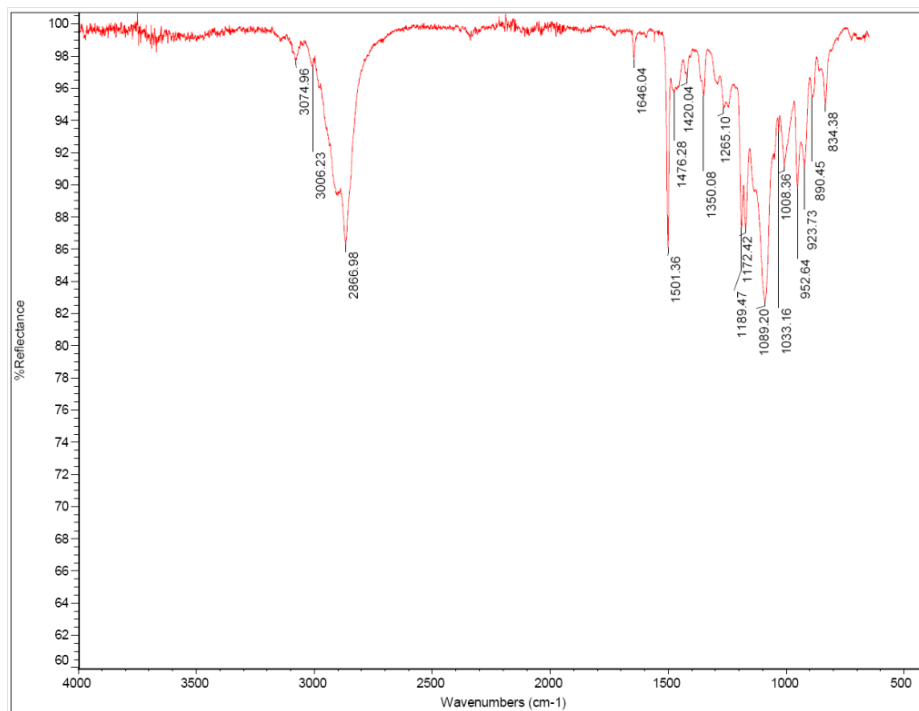
**Figure S36.**  $^{13}\text{C}$  NMR spectrum of compound **15** ( $\text{CDCl}_3$ , 75 MHz).



**Figure S37.** COSY spectrum of compound **15**.

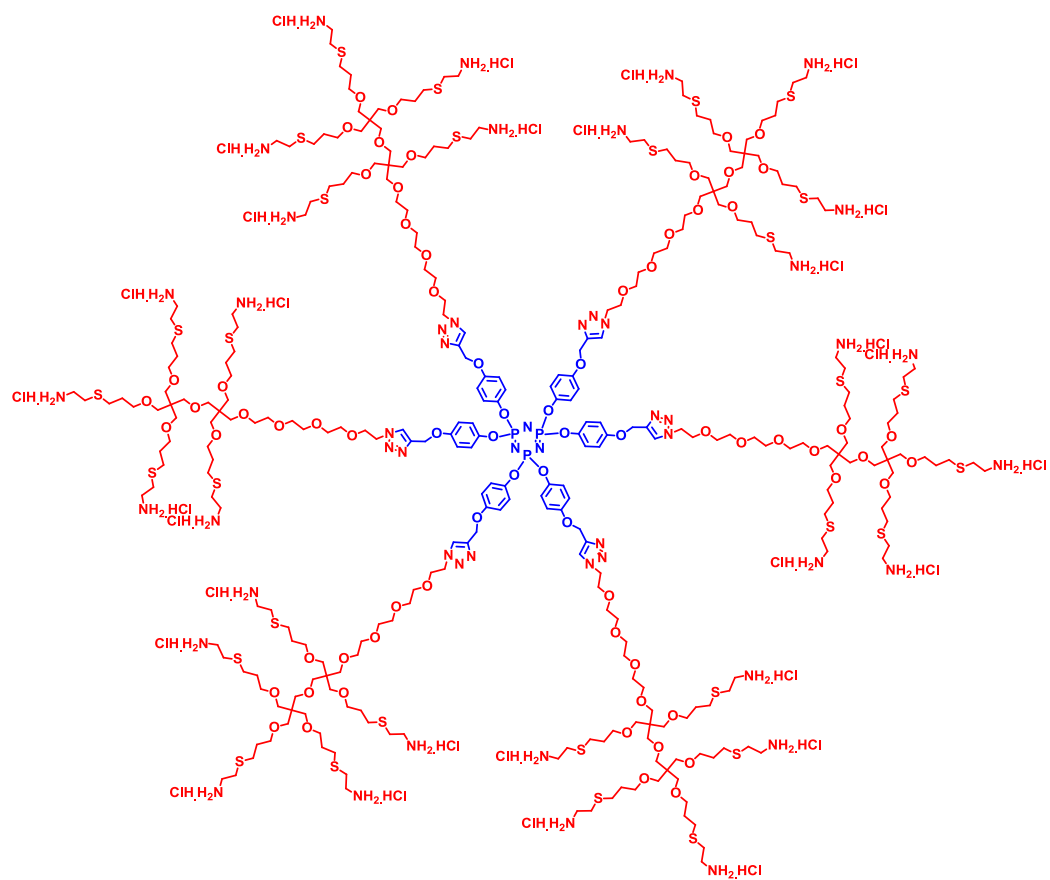


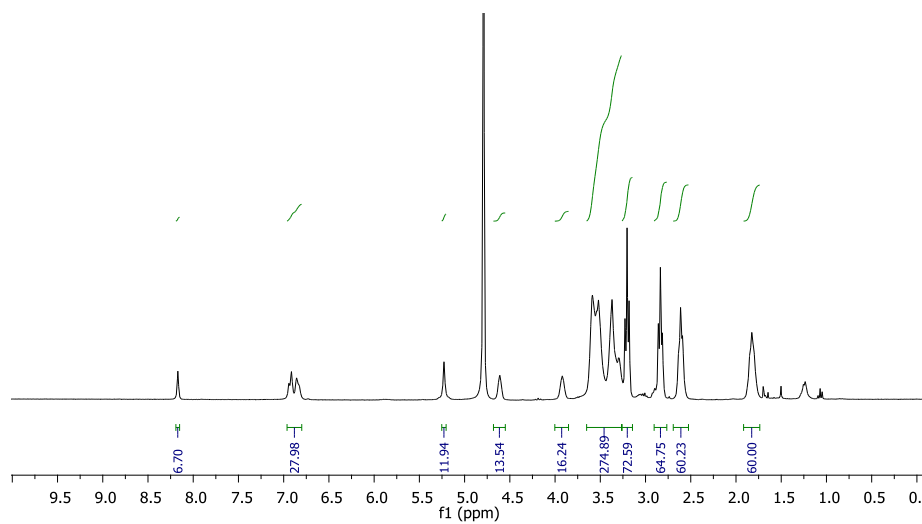
**Figure S38.** MALDI-TOF spectrum of compound **15** (DHB matrix).



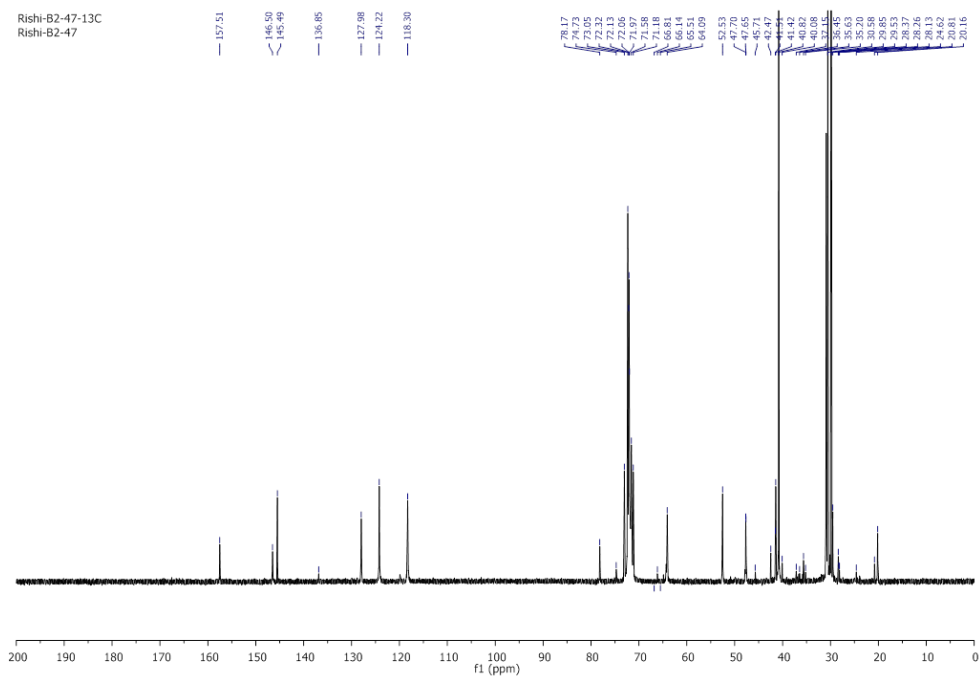
**Figure S39.** IR spectrum of compound **15**.

# Compound 6

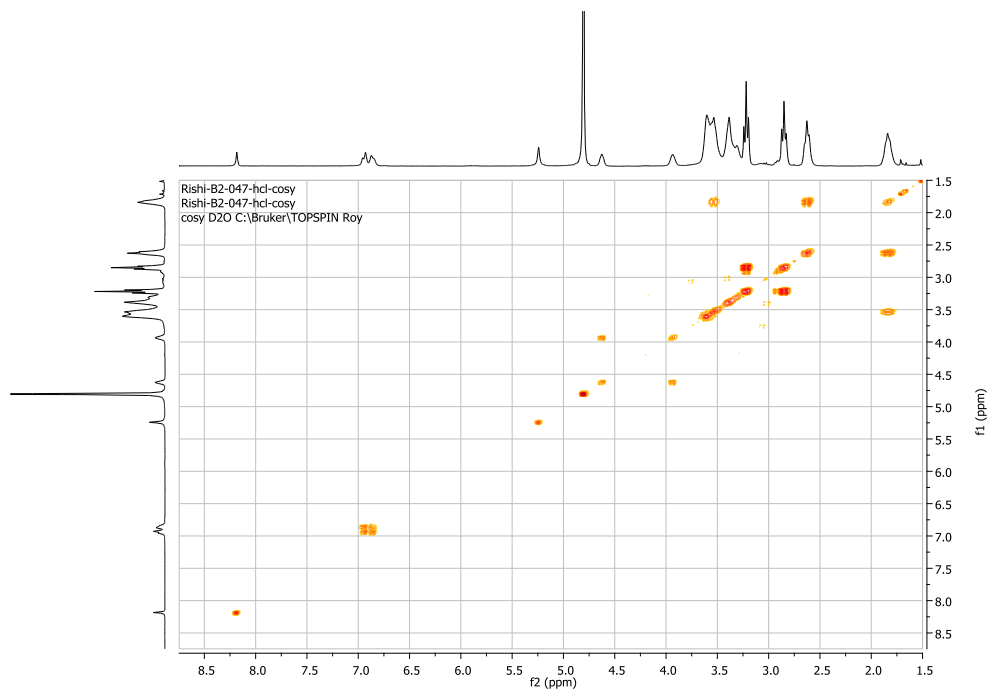




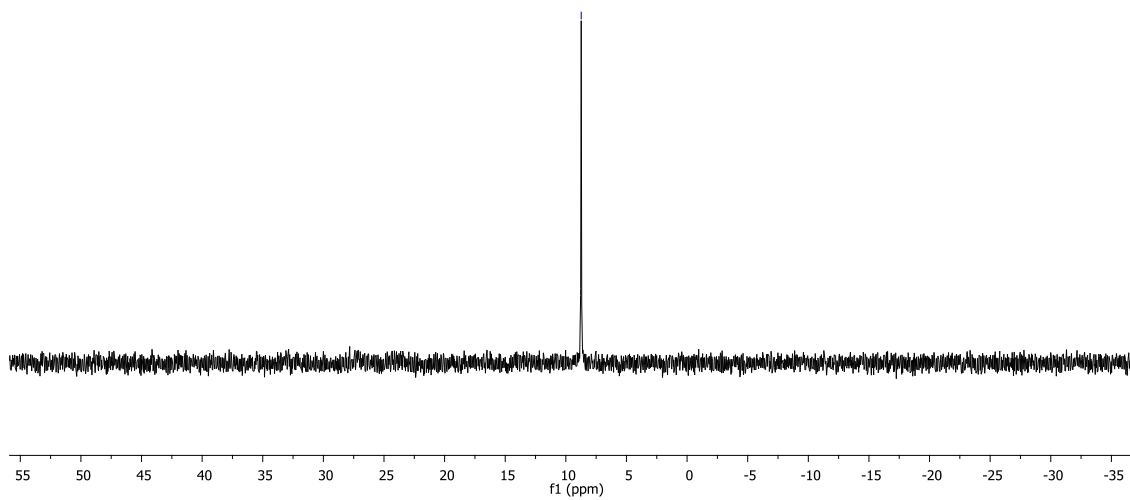
**Figure S40.**  $^1\text{H}$  NMR spectrum of glycodendrimer **6** ( $\text{D}_2\text{O}$ , 300 MHz)



**Figure S41.**  $^{13}\text{C}$  NMR spectrum of glycodendrimer **6** ( $\text{D}_2\text{O}$ , 151 MHz).



**Figure S42.** COSY spectrum of glycodendrimer **6**.



**Figure S43.**  $^{31}\text{P}$  NMR spectrum of compound **6** (122 MHz,  $\text{CD}_3\text{OD}$ ).

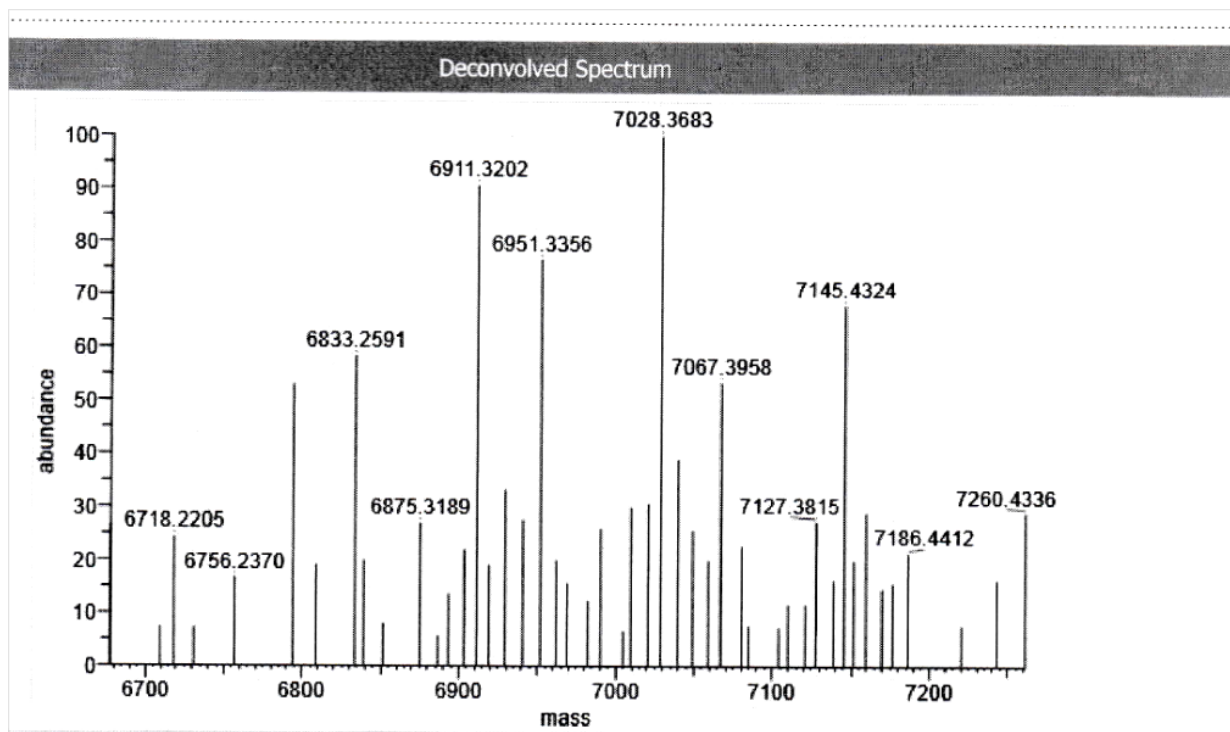


Figure S44. HR ESI spectrum of compound 6

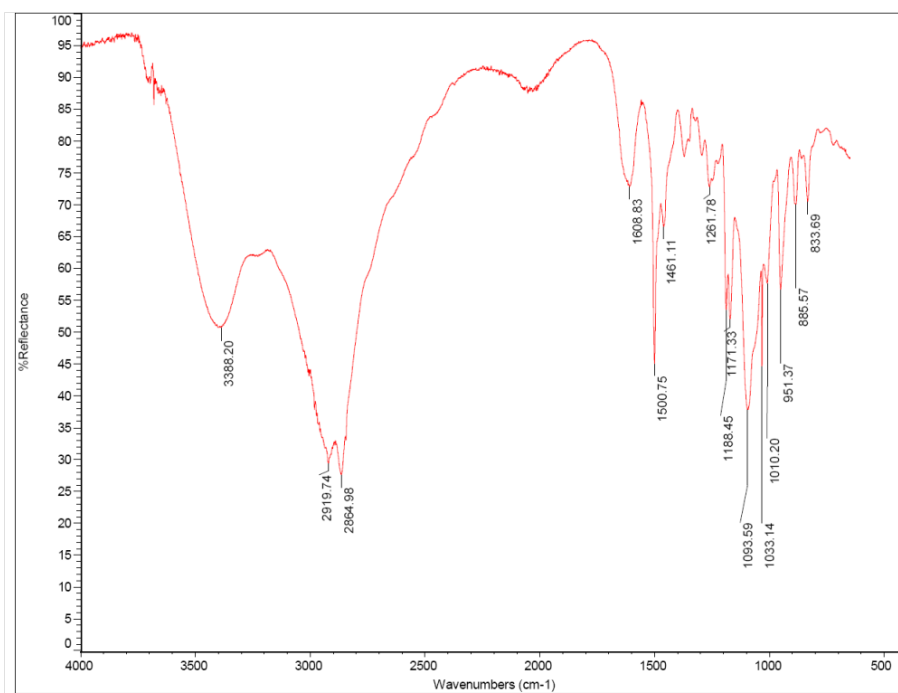


Figure S45. IR spectrum of glycodendrimer 6.

## Structure of compound FITC conjugated dendrimer 16

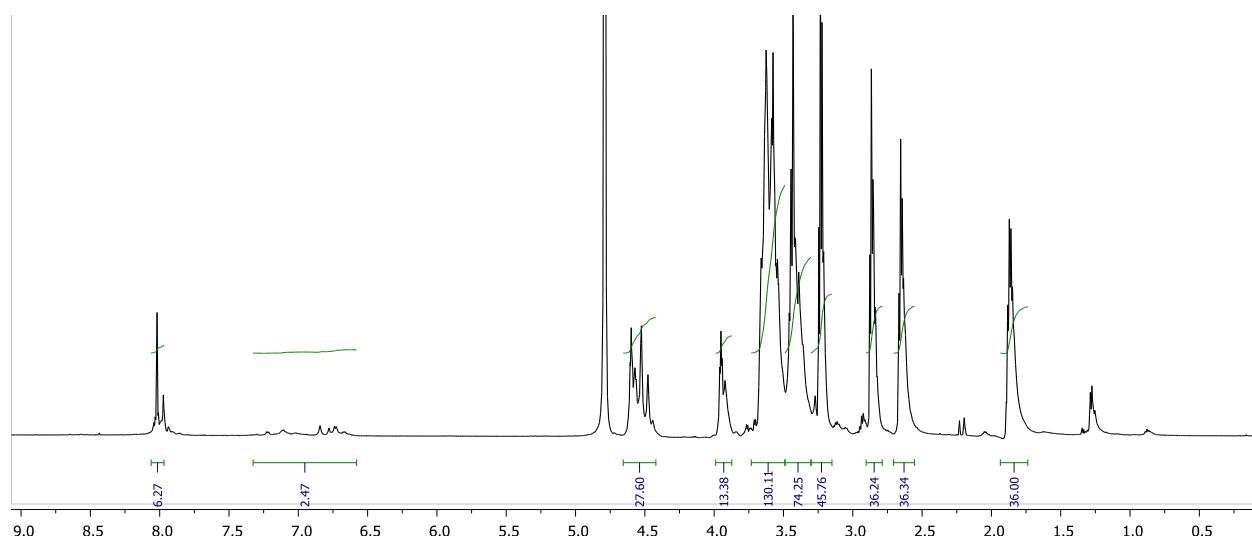
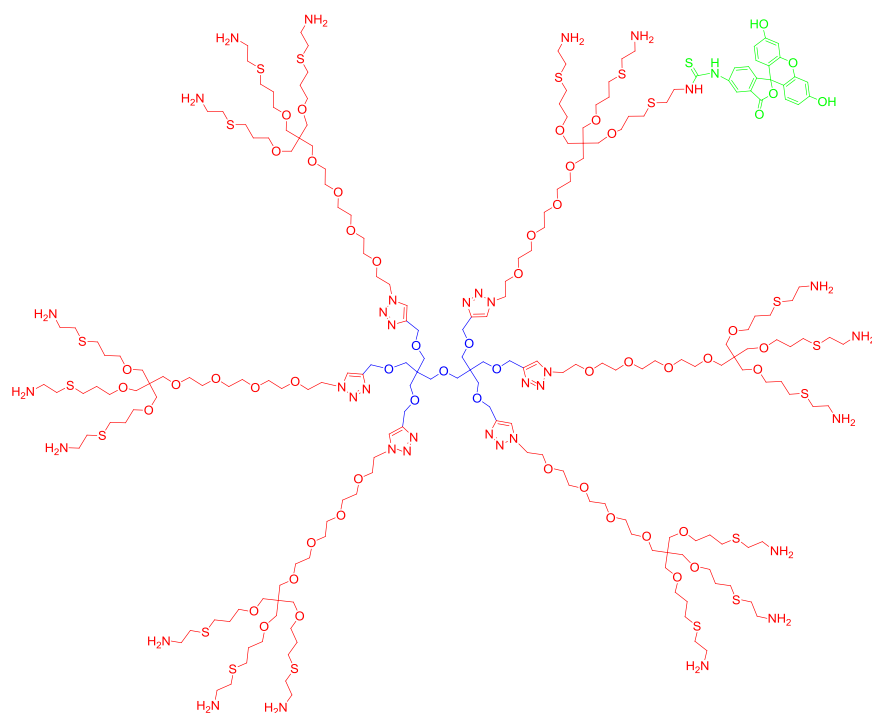
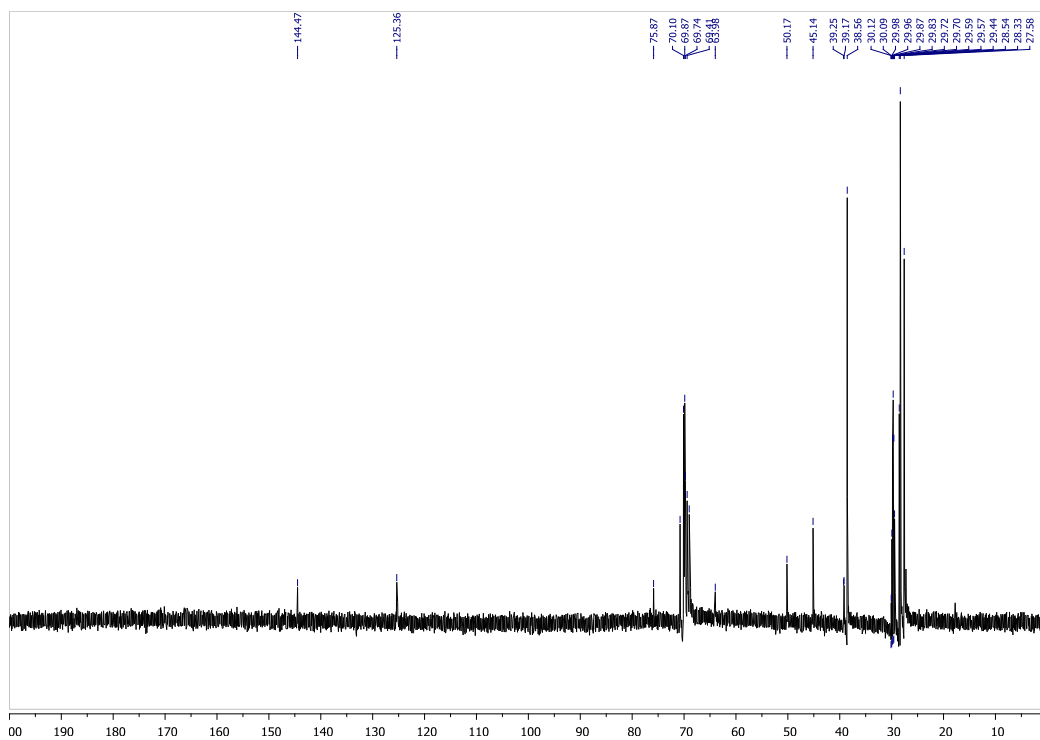
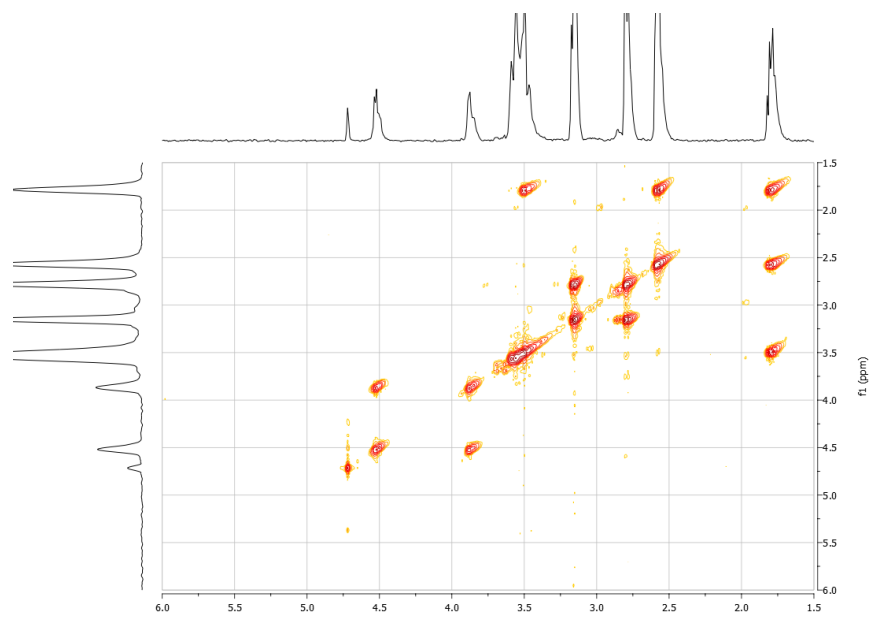


Figure S46. <sup>1</sup>H NMR spectrum of glycodendrimer 6 (D<sub>2</sub>O, 600 MHz).





**Figure S47.**  $^{13}\text{C}$  NMR spectrum of glycodendrimer **16** ( $\text{D}_2\text{O}$ , 150 MHz).



**Figure S48.** IR spectrum of glycodendrimer **16**.

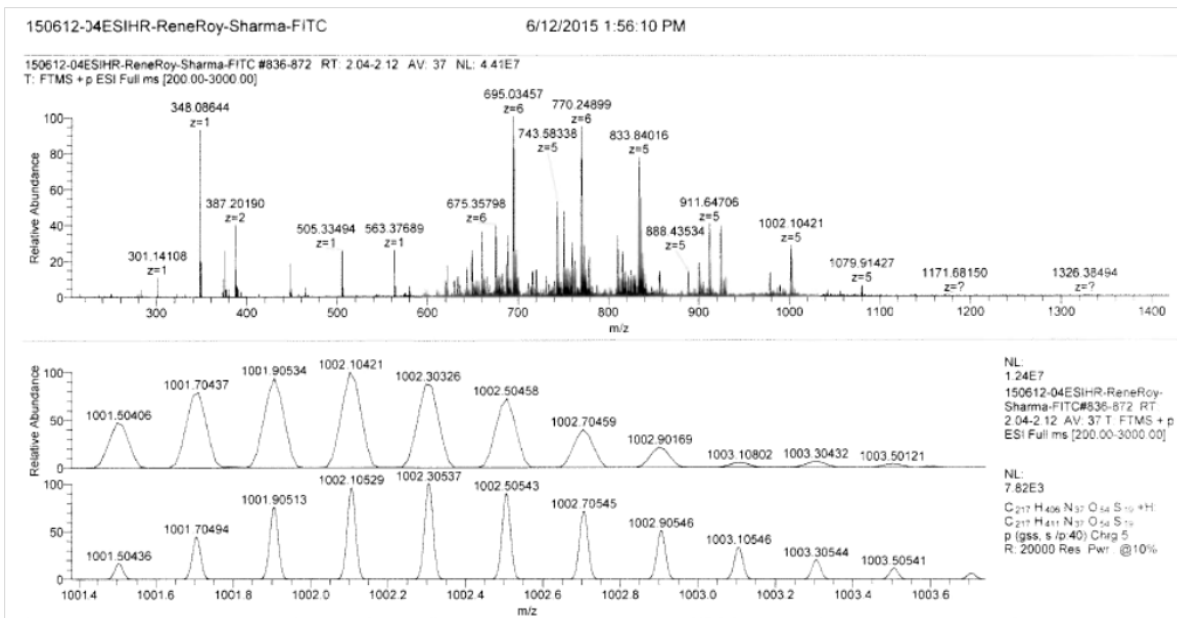


Figure S49. HR ESI spectrum of compound 16

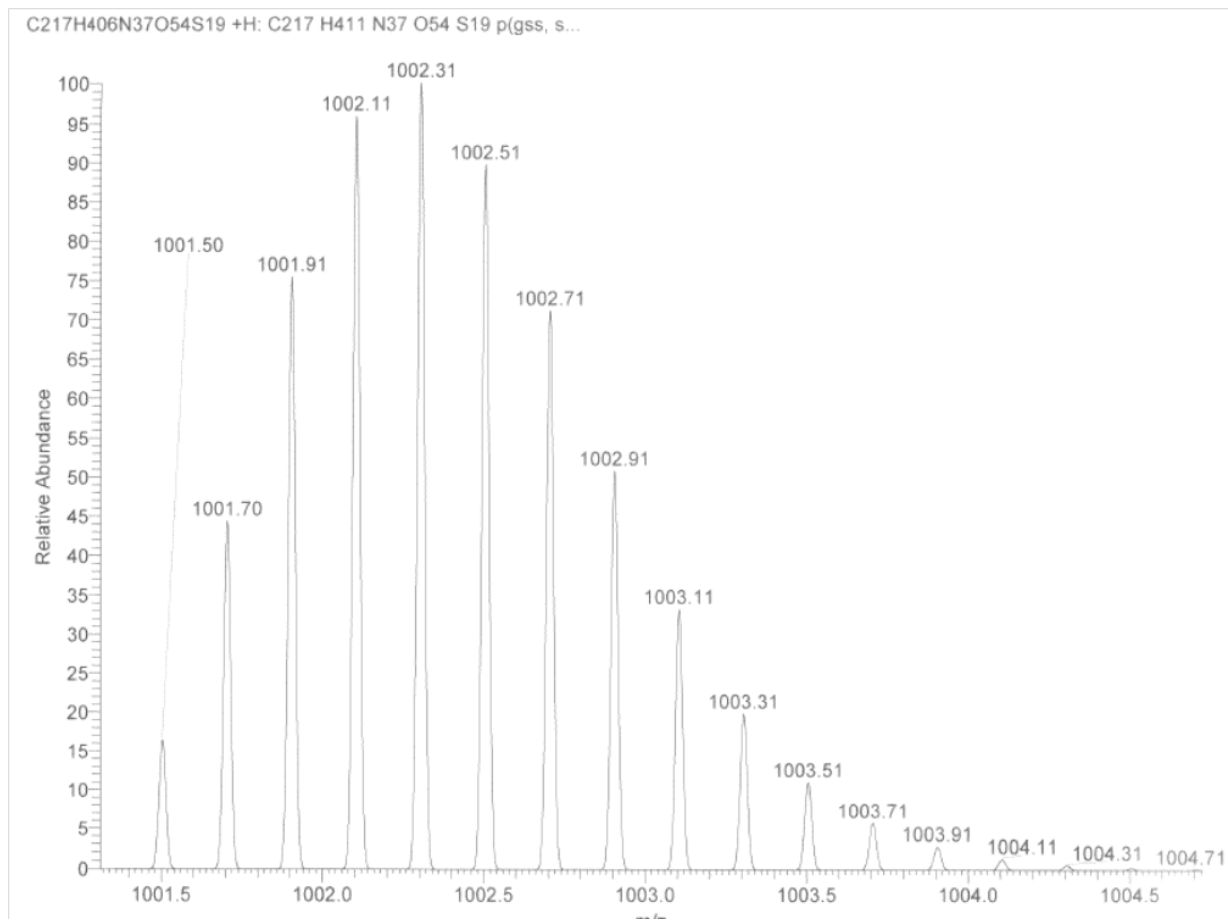
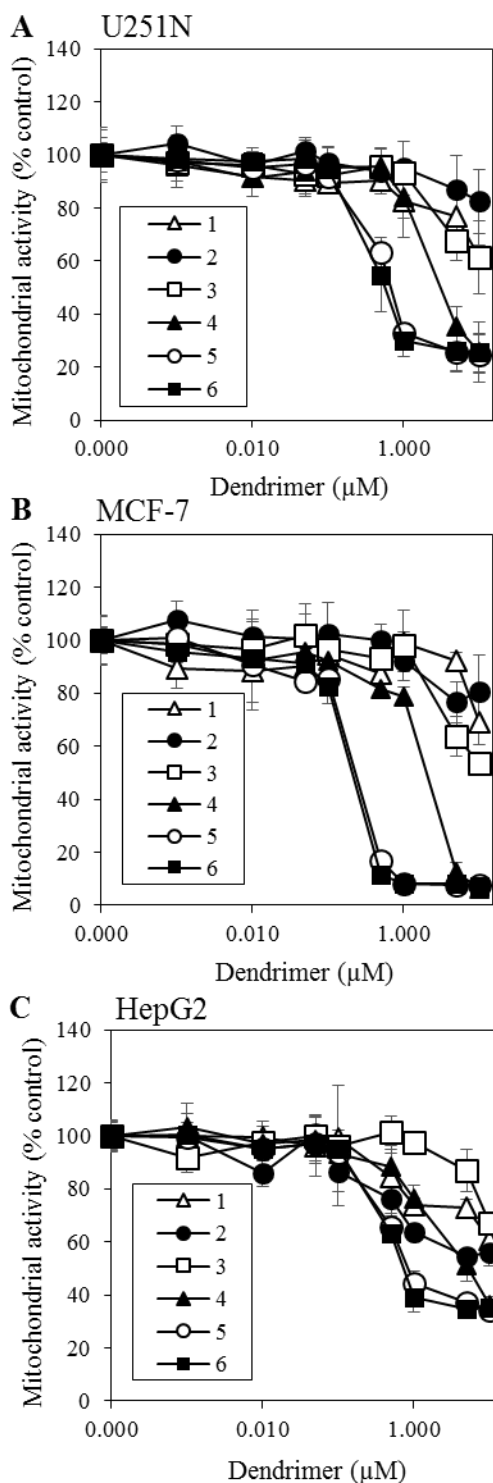
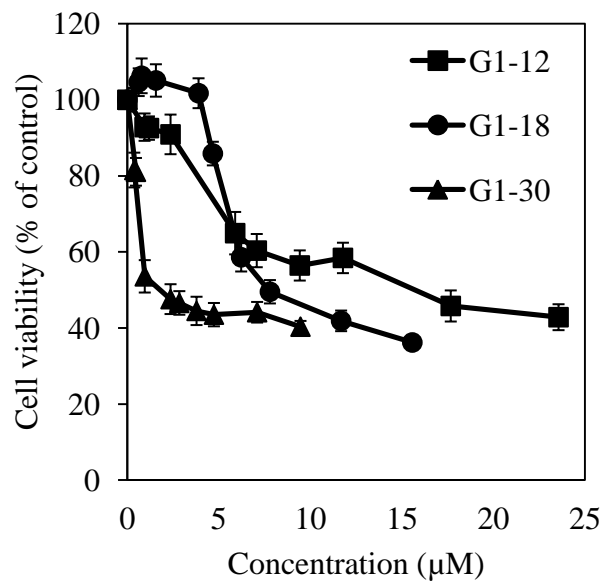


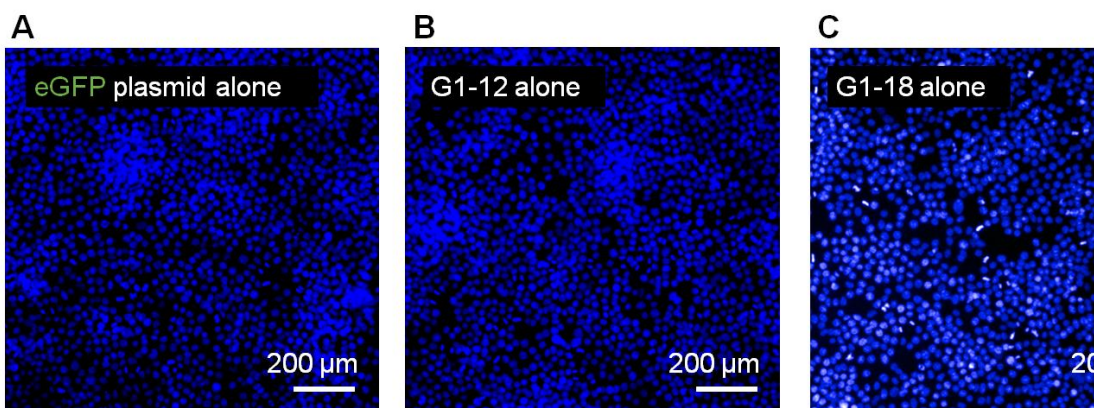
Figure S50. Expanded HR ESI spectrum of compound 16



**Figure S51.** Cytotoxicity of polyamine dendrimers G0-4 (1), G0-6 (2), G1-12 (3), G1-18 (4) and G1-30 (5 and 6, dipentaerythritol and aromatic phosphazene cores) in A) U251N human glioblastoma, B) MCF-7 human breast carcinoma, and C) HepG2 human liver adenocarcinoma cells. Cells were treated with increasing concentrations of dendrimers for 24 hrs, after which the mitochondrial metabolic activity was measured by the MTT assay. Shown are mean percentage values  $\pm$  SEM as compared to the untreated control from three independent experiments.

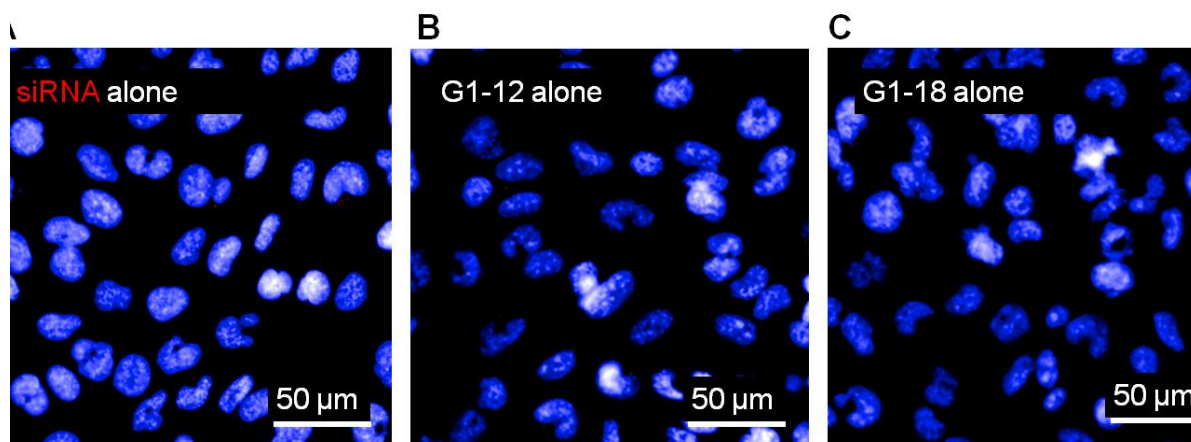


**Figure S52.** Cytotoxicity of polyamine dendrimers G1-12 (3), G1-18 (4) and G1-30 (5, dipentaerythritol core) in U251N human glioblastoma cells. Cells were treated with increasing concentrations of dendrimers for 24 hrs, after which cell nuclei were labeled with Hoechst 33342 and counted. Shown are mean percentage values  $\pm$  SEM as compared to the untreated control from three independent experiments.



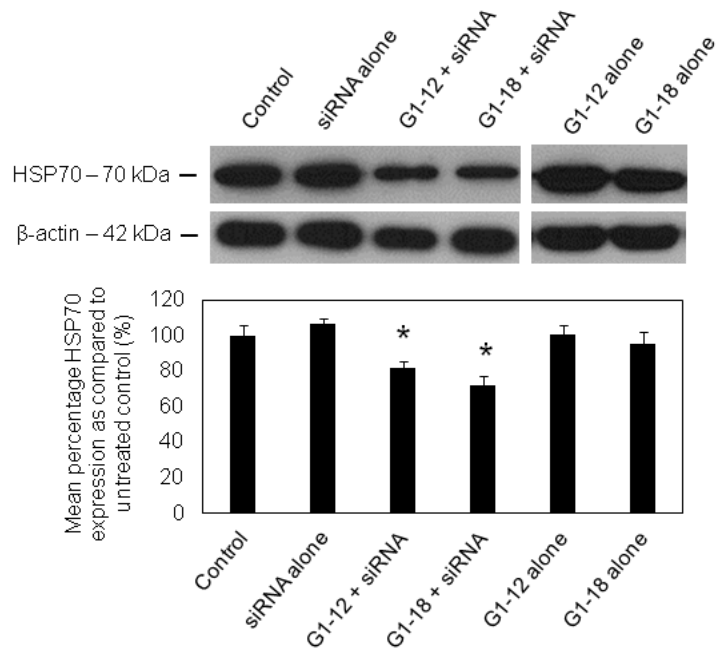
**Figure S53. Transfection negative controls of eGFP plasmid DNA alone and dendrimer vectors alone in HEK 293 human embryonic kidney cells**

Fluorescence micrographs of HEK 293 human embryonic kidney cells incubated with (A) eGFP plasmid DNA (0.5  $\mu\text{g}/\text{well}$ ), (B) G1-12-NH<sub>2</sub> HCl (**3**) (2.9  $\mu\text{M}$ ) or (C) G1-18-NH<sub>2</sub> HCl (**4**) (1.97  $\mu\text{M}$ ) for 24h in serum deprived media. Plasmid DNA and dendrimer concentrations correspond to that of transfections at a charge ratio of 5 (dendrimer to plasmid DNA). No significant eGFP fluorescence was detected. Cell nuclei are labeled with Hoechst 33342 (blue).



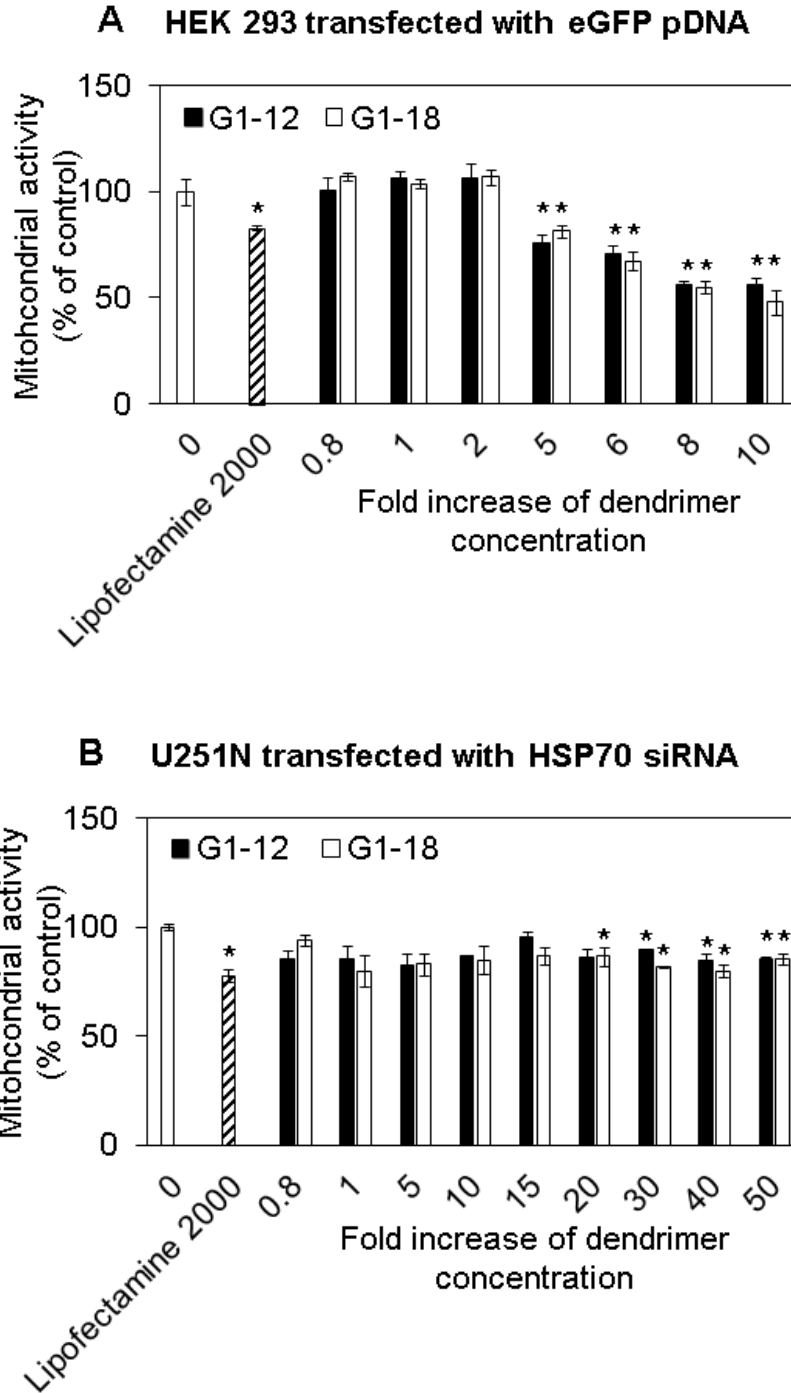
**Figure S54. Transfection negative controls of Cy3-labelled anti-HSP70 siRNA alone and dendrimer vectors alone in U251N glioblastoma cells**

Fluorescence micrographs of human U251N glioblastoma cells incubated with (A) Cy3-labeled anti-HSP70 siRNA (10 nM), (B) G1-12-NH<sub>2</sub> HCl (**3**) (1.18  $\mu\text{M}$ ) or (C) G1-18-NH<sub>2</sub> HCl (**4**) (0.78  $\mu\text{M}$ ) for 24h in serum deprived media. siRNA and dendrimer concentrations correspond to that of transfections at a charge ratio of 1 (dendrimer to siRNA). No significant Cy3 fluorescence was detected. Cell nuclei are labeled with Hoechst 33342 (blue).



**Figure S55. Down-regulation of heat-shock protein 70 (HSP70) protein expression in U251N glioblastoma cells using small interfering RNA (siRNA) delivered with G1-12 and G1-18 dendrimers.**

The expression of HSP70 in control and transfected cells was verified by immunoblotting.  $\beta$ -actin served as loading control. Cells were treated with siRNA (10 nM) alone, siRNA complexed with dendrimers G1-12 (**3**) and G1-18 (**4**) at a charge ratio of 1 to 20 (siRNA to dendrimer), or dendrimers alone for 48h. Shown are representative immunoblots and mean percentage HSP70 expression (normalized to the loading control) as compared to the untreated control  $\pm$  S.D. from two independent experiments. (\* $p < 0.05$ )



**Figure S56. Mitochondria metabolic (MTT) activity of HEK 293 human embryonic kidney cells and U251N human glioblastoma cells in response to transfection of eGFP plasmid DNA (pDNA) and heat-shock protein 70 (HSP70) small interfering RNA (siRNA), respectively, delivered with G1-12 (3) and G1-18 (4) dendrimers for 24h.**

(A) HEK 293 cells were transfected with eGFP plasmid DNA (0.5  $\mu\text{g}/\text{well}$ ) using G1-12 (3) or G1-18 (4) polyamine dendrimers at different charge ratios (dendrimer to pDNA) for 24h.

Lipofectamine 2000© was included as a commercial comparator, and was used as recommended by the manufacturer. Mitochondrial metabolic activity was measured by the MTT assay. Shown are mean percentage values  $\pm$  S.D. as compared to untreated controls. Each treatment was included in triplicate. (\*p<0.01)

(B) U251N cells were transfected with HSP70 siRNA (10 nM) using G1-12 or G1-18 polyamine dendrimers at different charge ratios (dendrimer to siRNA) for 24h. Lipofectamine 2000© was included as a commercial comparator, and was used as recommended by the manufacturer. Mitochondrial metabolic activity was measured by the MTT assay. Shown are mean percentage values  $\pm$  S.D. as compared to untreated controls. Each treatment was included in triplicate. (\*p<0.01)