

Supplementary data

for

Induction of Effective Immune Responses against Lewis Y Tumor-Associated Carbohydrate Antigen
by Its Densely Displaying System of the Self-Assembling Nanocarriers

Yuji Yamazaki^a, Yukiko Nanbu^b, Masashi Ohmae^a, Manabu Sugai^b and Shunsaku Kimura^{a*}

^a Department of Material Chemistry, Graduate School of Engineering, Kyoto University, Kyoto
Daigaku-Katsura, Nishikyo-ku, Kyoto 615-8510, Japan

^b Division of Molecular Genetics, Department of Biochemistry and Bioinformative Sciences, School
of Medicine, University of Fukui, 23-3 Matsuokashimoaizuki, Eiheiji-cho, Yoshida-gun, Fukui 910-
1193, Japan

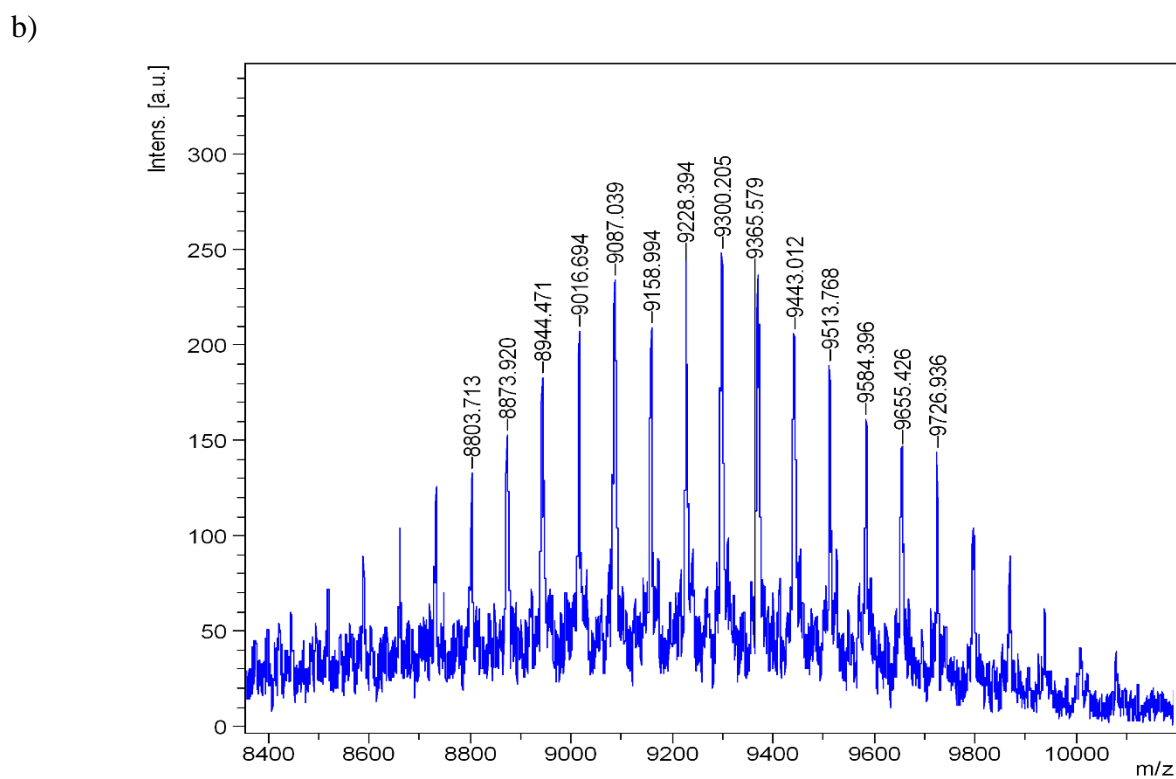
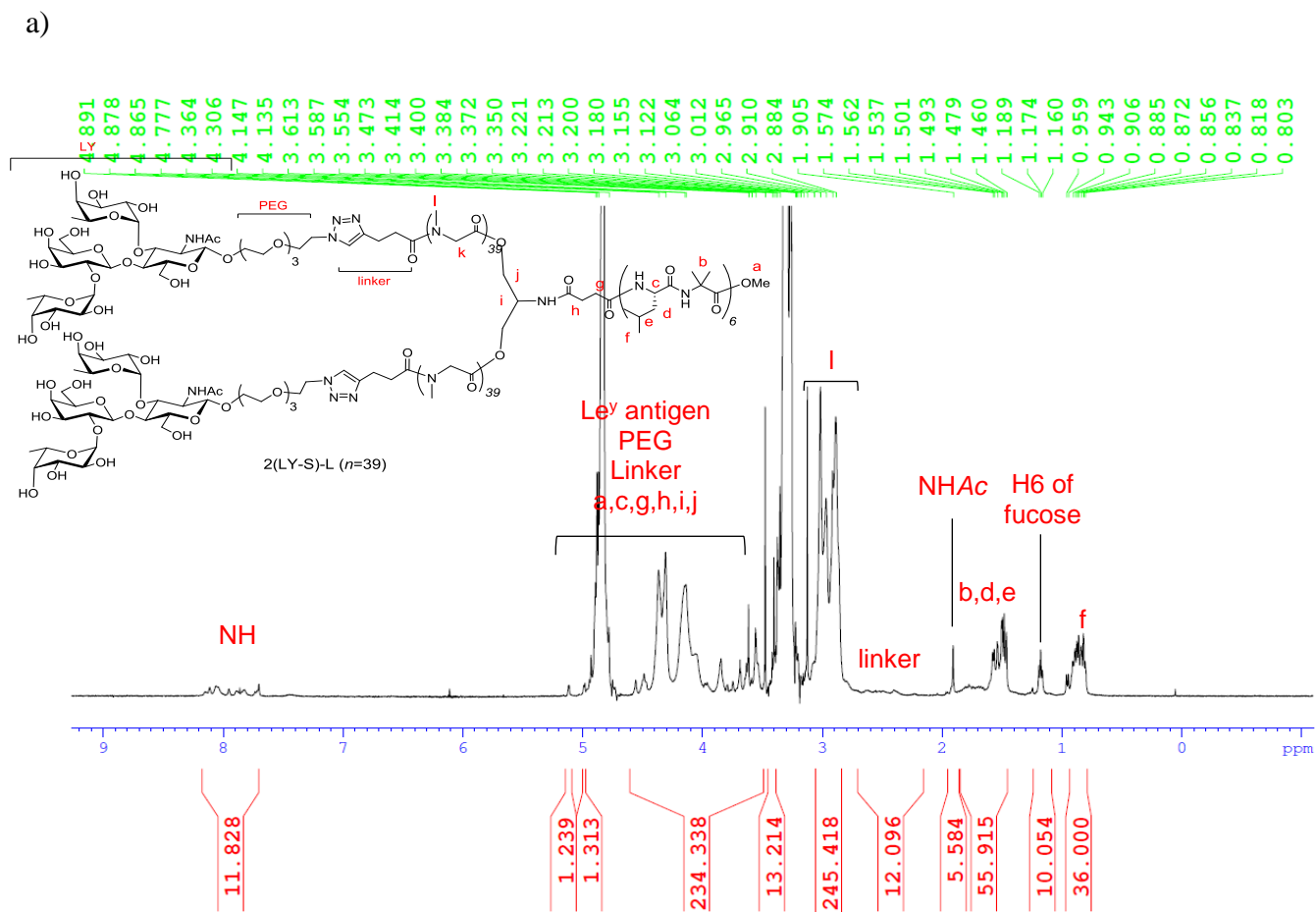


Fig. S1 a) ^1H NMR and b) MALDI-TOF mass spectra of 2(LY-S)-L.

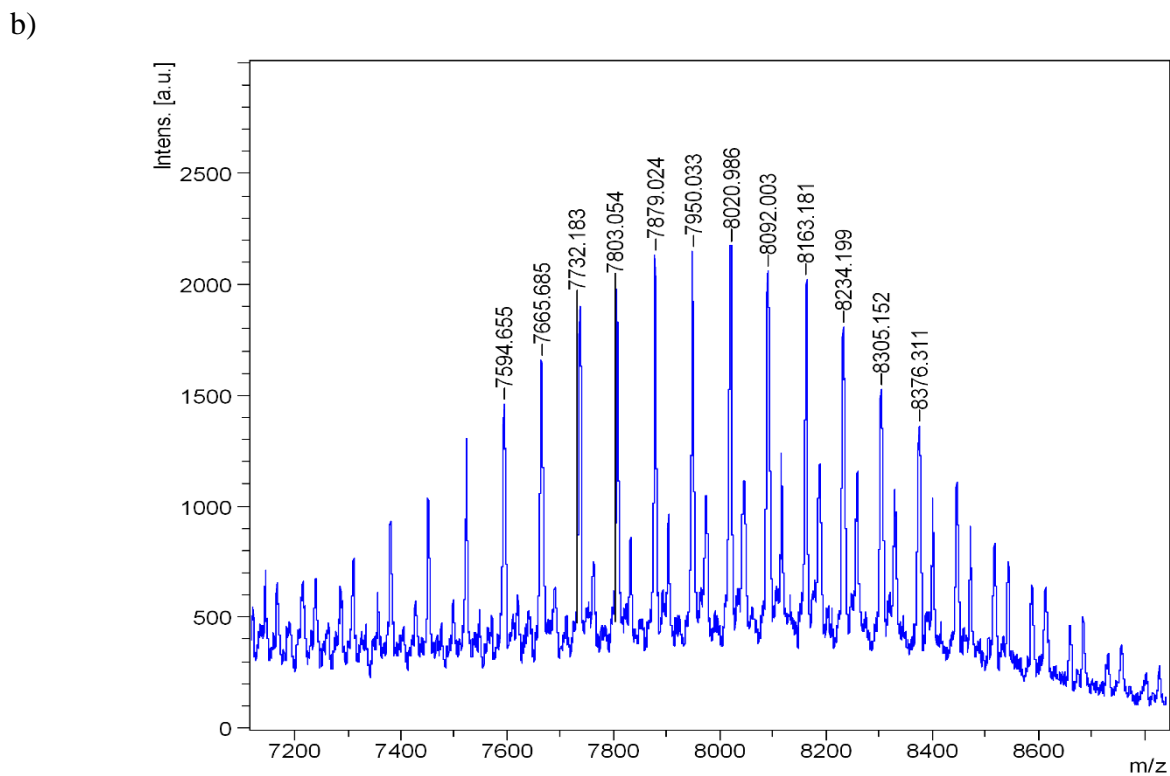
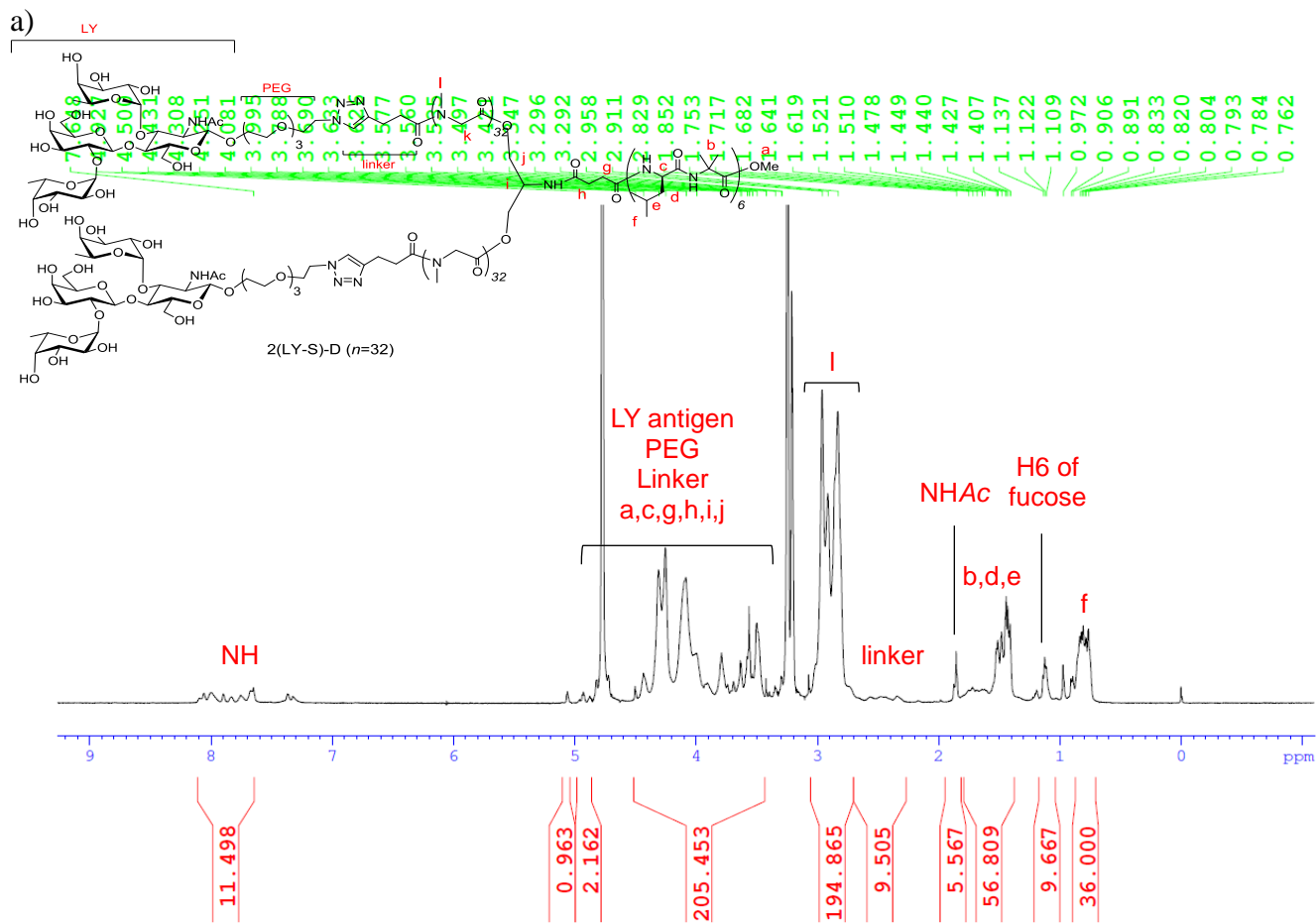
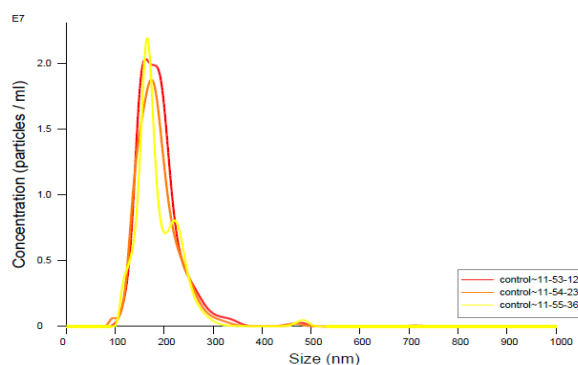


Fig. S2 a) ^1H NMR and b) MALDI-TOF mass spectra of 2(LY-S)-D.

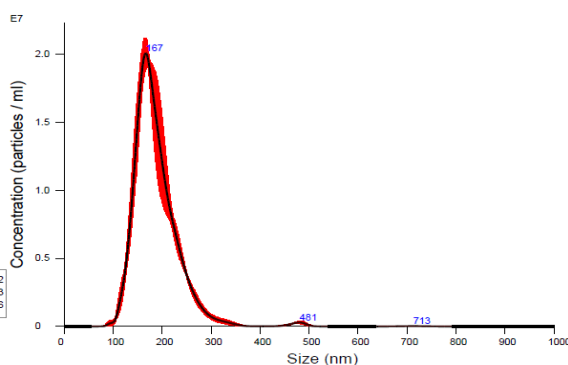
a) G0

NANOSIGHT

control 2018-05-07 11-53-04



FTLA Concentration / Size graph for Experiment:
control 2018-05-07 11-53-04



Averaged FTLA Concentration / Size for Experiment:
control 2018-05-07 11-53-04
Error bars indicate + / - 1 standard error of the mean

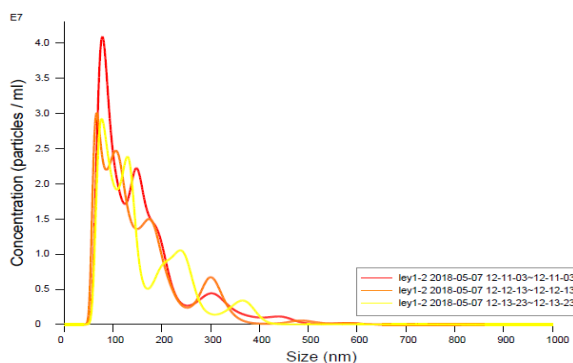
<u>Included Files</u>	<u>Results</u>
control 2018-05-07 11-53-12 control 2018-05-07 11-54-23 control 2018-05-07 11-55-36	Stats: Merged Data Mean: 187.9 nm Mode: 166.5 nm SD: 49.3 nm D10: 140.0 nm D50: 177.8 nm D90: 241.1 nm
<u>Details</u> NTA Version: NTA 3.2 Dev Build 3.2.16 Script Used: SOP Standard Measurement 11-53-04AM 07- Time Captured: 11:53:04 07/05/2018 Operator: yamazaki Pre-treatment: Sample Name: control Diluent: 1/200 Remarks:	Stats: Mean +/- Standard Error Mean: 187.8 +/- 0.7 nm Mode: 167.3 +/- 3.6 nm SD: 49.4 +/- 1.8 nm D10: 139.8 +/- 1.3 nm D50: 177.5 +/- 1.9 nm D90: 241.2 +/- 0.6 nm Concentration: 1.63e+009 +/- 1.22e+008 particles/ml 82.7 +/- 6.2 particles/frame 83.5 +/- 6.0 centres/frame
<u>Capture Settings</u> Camera Type: sCMOS Laser Type: Blue405 Camera Level: 13 Slider Shutter: 1232 Slider Gain: 219 FPS: 25.0 Number of Frames: 1498 Temperature: 22.4 °C Viscosity: (Water) 0.9 cP Dilution factor: Dilution not recorded	
<u>Analysis Settings</u> Detect Threshold: 3 Blur Size: Auto Max Jump Distance: Auto: 8.0 - 8.3 pix	

Fig. S3 The data sheets obtained by nanoparticle tracking analysis (NTA) of a) G0, b) G1, c) G2, d) G3 and e) G4 using NANOSIGHT LM10.

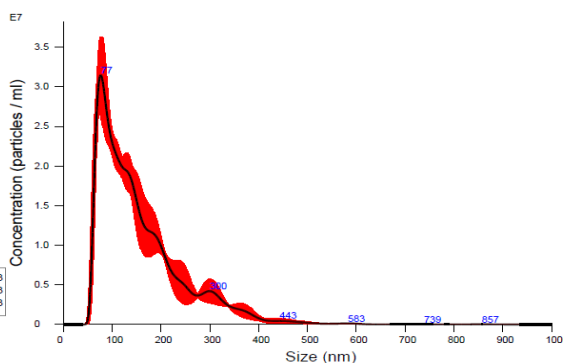
b) G1

NANOSIGHT

ley1-2 2018-05-07 12-07-51



FTLA Concentration / Size graph for Experiment:
ley1-2 2018-05-07 12-07-51



Averaged FTLA Concentration / Size for Experiment:
ley1-2 2018-05-07 12-07-51
Error bars indicate +/- 1 standard error of the mean

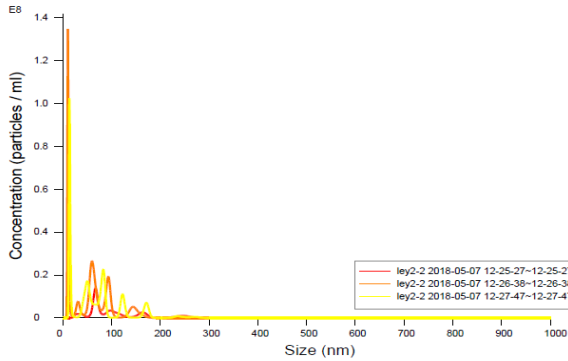
Included Files	Results
<p>ley1-2 2018-05-07 12-11-03 ley1-2 2018-05-07 12-12-13 ley1-2 2018-05-07 12-13-23</p> <p>Details</p> <p>NTA Version: NTA 3.2 Dev Build 3.2.16 Script Used: SOP Standard Measurement 12-07-51PM 07- Time Captured: 12:07:51 07/05/2018 Operator: yamazaki Pre-treatment: Sample Name: ley1-2 Diluent: 1/200 Remarks:</p> <p>Capture Settings</p> <p>Camera Type: sCMOS Laser Type: Blue405 Camera Level: 11 Slider Shutter: 890 Slider Gain: 146 FPS: 25.0 Number of Frames: 1498 Temperature: 22.3 - 22.3 °C Viscosity: (Water) 0.945 - 0.947 cP Dilution factor: Dilution not recorded</p> <p>Analysis Settings</p> <p>Detect Threshold: 7 Blur Size: Auto Max Jump Distance: Auto: 9.7 - 10.1 pix</p>	<p>Stats: Merged Data</p> <p>Mean: 156.3 nm Mode: 76.3 nm SD: 87.1 nm D10: 71.9 nm D50: 130.7 nm D90: 283.5 nm</p> <p>Stats: Mean +/- Standard Error</p> <p>Mean: 156.4 +/- 2.0 nm Mode: 74.1 +/- 3.7 nm SD: 86.9 +/- 1.5 nm D10: 71.7 +/- 2.2 nm D50: 130.6 +/- 1.7 nm D90: 282.0 +/- 6.0 nm</p> <p>Concentration:</p> <p>3.52e+009 +/- 1.81e+008 particles/ml 178.7 +/- 9.2 particles/frame 238.5 +/- 10.3 centres/frame</p>

Fig. S3 continued.

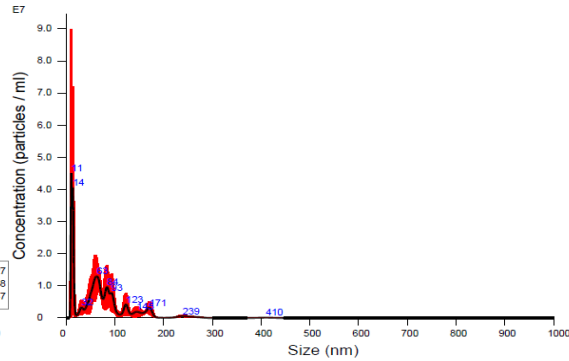
c) G2

NANOSIGHT

ley2-2 2018-05-07 12-23-54



FTLA Concentration / Size graph for Experiment:
ley2-2 2018-05-07 12-23-54



Averaged FTLA Concentration / Size for Experiment:
ley2-2 2018-05-07 12-23-54
Error bars indicate +/- 1 standard error of the mean

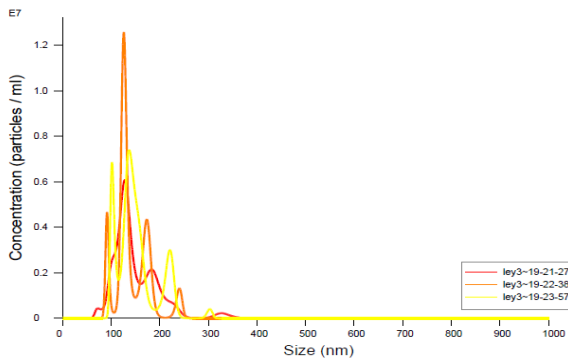
Included Files	Results
<p>ley2-2 2018-05-07 12-25-27 ley2-2 2018-05-07 12-26-38 ley2-2 2018-05-07 12-27-47</p>	<p>Stats: Merged Data Mean: 69.6 nm Mode: 10.6 nm SD: 54.4 nm D10: 10.5 nm D50: 61.9 nm D90: 143.8 nm</p>
<p>Details</p> <p>NTA Version: NTA 3.2 Dev Build 3.2.16 Script Used: SOP Standard Measurement 12-23-54PM 07- Time Captured: 12:23:54 07/05/2018 Operator: yamazaki Pre-treatment: Sample Name: ley2-2 Diluent: 1/200 Remarks:</p>	<p>Stats: Mean +/- Standard Error Mean: 78.1 +/- 15.1 nm Mode: 30.6 +/- 18.4 nm SD: 54.8 +/- 7.4 nm D10: 24.7 +/- 13.9 nm D50: 64.9 +/- 7.6 nm D90: 153.2 +/- 28.3 nm Concentration: 9.82e+008 +/- 2.75e+008 particles/ml 49.9 +/- 14.0 particles/frame 107.3 +/- 33.0 centres/frame</p>
<p>Capture Settings</p> <p>Camera Type: sCMOS Laser Type: Blue405 Camera Level: 16 Slider Shutter: 1300 Slider Gain: 512 FPS: 25.0 Number of Frames: 1498 Temperature: 22.4 °C Viscosity: (Water) 0.942 - 0.943 cP Dilution factor: Dilution not recorded</p>	
<p>Analysis Settings</p> <p>Detect Threshold: 3 Blur Size: Auto Max Jump Distance: Auto: 12.8 - 20.2 pix</p>	

Fig. S3 continued.

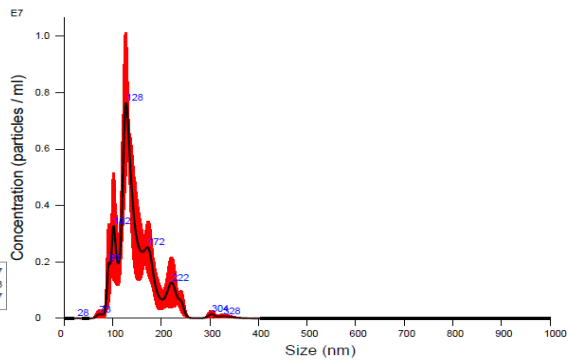
d) G3

NANOSIGHT

ley3 2018-04-10 19-20-32



FTLA Concentration / Size graph for Experiment:
ley3 2018-04-10 19-20-32



Averaged FTLA Concentration / Size for Experiment:
ley3 2018-04-10 19-20-32
Error bars indicate + / - 1 standard error of the mean

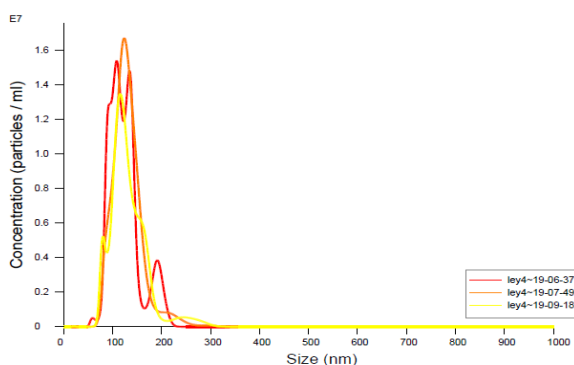
<p>Included Files</p> <p>ley3 2018-04-10 19-21-27 ley3 2018-04-10 19-22-38 ley3 2018-04-10 19-23-57</p> <p>Details</p> <p>NTA Version: NTA 3.2 Dev Build 3.2.16 Script Used: SOP Standard Measurement 07-20-32PM 10A- Time Captured: 19:20:32 10/04/2018 Operator: yamazaki Pre-treatment: Sample Name: ley3 Diluent: 1/200 Remarks:</p> <p>Capture Settings</p> <p>Camera Type: sCMOS Laser Type: Blue405 Camera Level: 13 Slider Shutter: 1232 Slider Gain: 219 FPS: 25.0 Number of Frames: 1498 Temperature: 22.5 - 22.5 °C Viscosity: (Water) 0.941 - 0.942 cP Dilution factor: Dilution not recorded</p> <p>Analysis Settings</p> <p>Detect Threshold: 3 Blur Size: Auto Max Jump Distance: Auto: 9.2 - 10.4 pix</p>	<p>Results</p> <p>Stats: Merged Data</p> <p>Mean: 148.0 nm Mode: 127.5 nm SD: 41.8 nm D10: 101.8 nm D50: 135.7 nm D90: 210.0 nm</p> <p>Stats: Mean +/- Standard Error</p> <p>Mean: 147.8 +/- 2.7 nm Mode: 130.5 +/- 3.4 nm SD: 41.3 +/- 3.4 nm D10: 100.0 +/- 2.3 nm D50: 135.3 +/- 3.5 nm D90: 201.6 +/- 10.9 nm</p> <p>Concentration: 3.85e+008 +/- 2.14e+007 particles/ml 19.5 +/- 1.1 particles/frame 20.8 +/- 1.1 centres/frame</p>
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Fig. S3 continued.

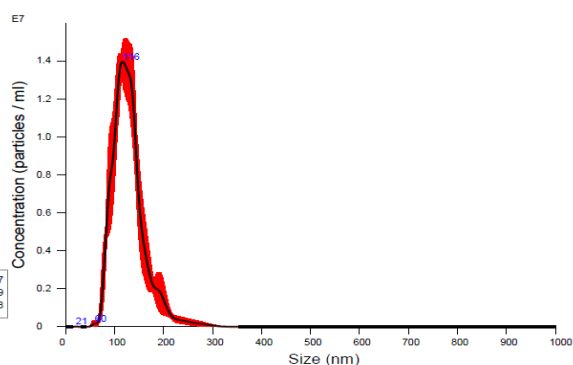
(e) G4

NANOSIGHT

ley4 2018-04-10 19-04-04



FTLA Concentration / Size graph for Experiment:
ley4 2018-04-10 19-04-04



Averaged FTLA Concentration / Size for Experiment:
ley4 2018-04-10 19-04-04
Error bars indicate +/- 1 standard error of the mean

<u>Included Files</u>	<u>Results</u>
ley4 2018-04-10 19-06-37 ley4 2018-04-10 19-07-49 ley4 2018-04-10 19-09-18	Stats: Merged Data Mean: 128.7 nm Mode: 115.9 nm SD: 33.8 nm D10: 90.6 nm D50: 122.9 nm D90: 169.8 nm
<u>Details</u> NTA Version: NTA 3.2 Dev Build 3.2.16 Script Used: SOP Standard Measurement 07-04-04PM 10A~ Time Captured: 19:04:04 10/04/2018 Operator: yamazaki Pre-treatment: Sample Name: ley4 Diluent: 1/200 Remarks:	Stats: Mean +/- Standard Error Mean: 128.9 +/- 2.3 nm Mode: 116.1 +/- 4.5 nm SD: 33.7 +/- 2.3 nm D10: 91.0 +/- 1.5 nm D50: 122.5 +/- 2.2 nm D90: 170.9 +/- 4.2 nm Concentration: 9.60e+008 +/- 4.60e+007 particles/ml 48.7 +/- 2.3 particles/frame 52.5 +/- 2.4 centres/frame
<u>Capture Settings</u> Camera Type: sCMOS Laser Type: Blue405 Camera Level: 13 Slider Shutter: 1232 Slider Gain: 219 FPS: 25.0 Number of Frames: 1498 Temperature: 22.8 °C Viscosity: (Water) 0.934 - 0.935 cP Dilution factor: Dilution not recorded	
<u>Analysis Settings</u> Detect Threshold: 3 Blur Size: Auto Max Jump Distance: Auto: 9.6 - 10.3 pix	

Fig. S3 continued.

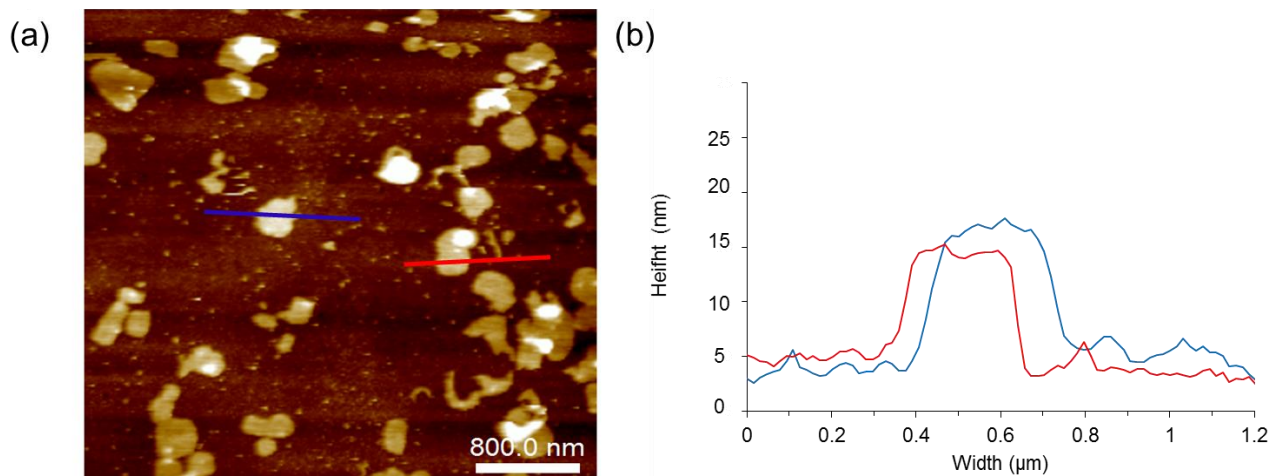
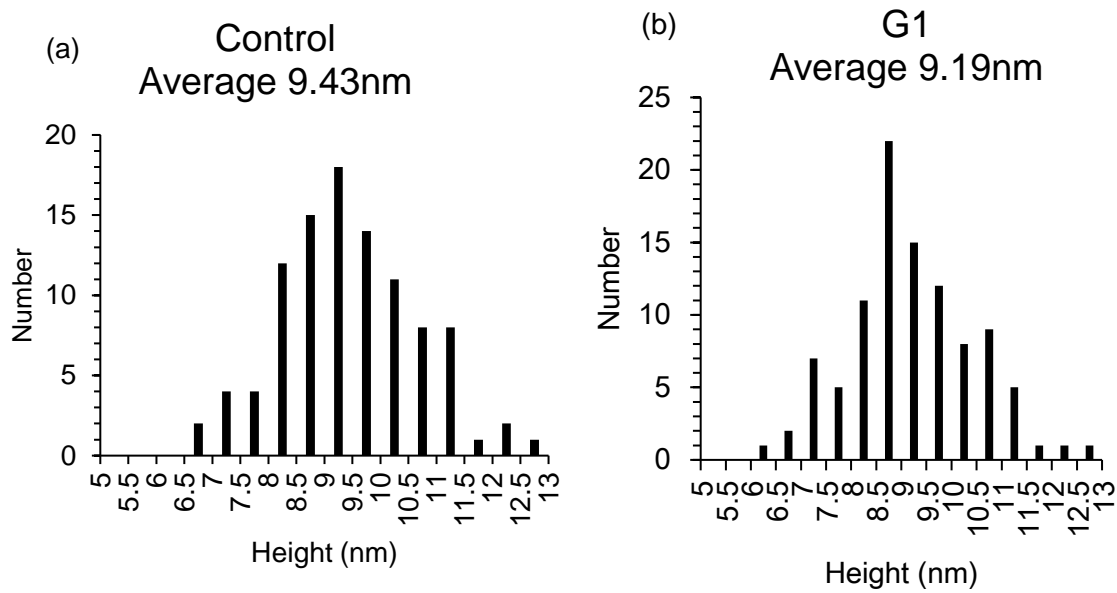


Fig. S4 (a) The AFM image of the G0 assemblies on an APTES-modified Si-wafer in water, and (b) the height traces along the red and blue lines in (a).



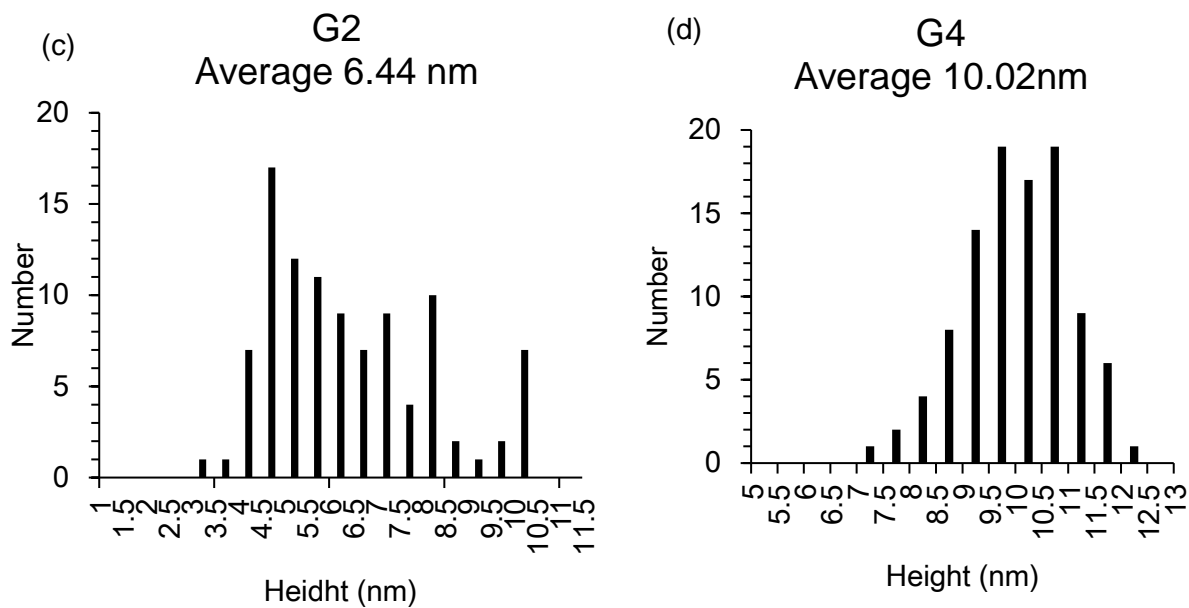
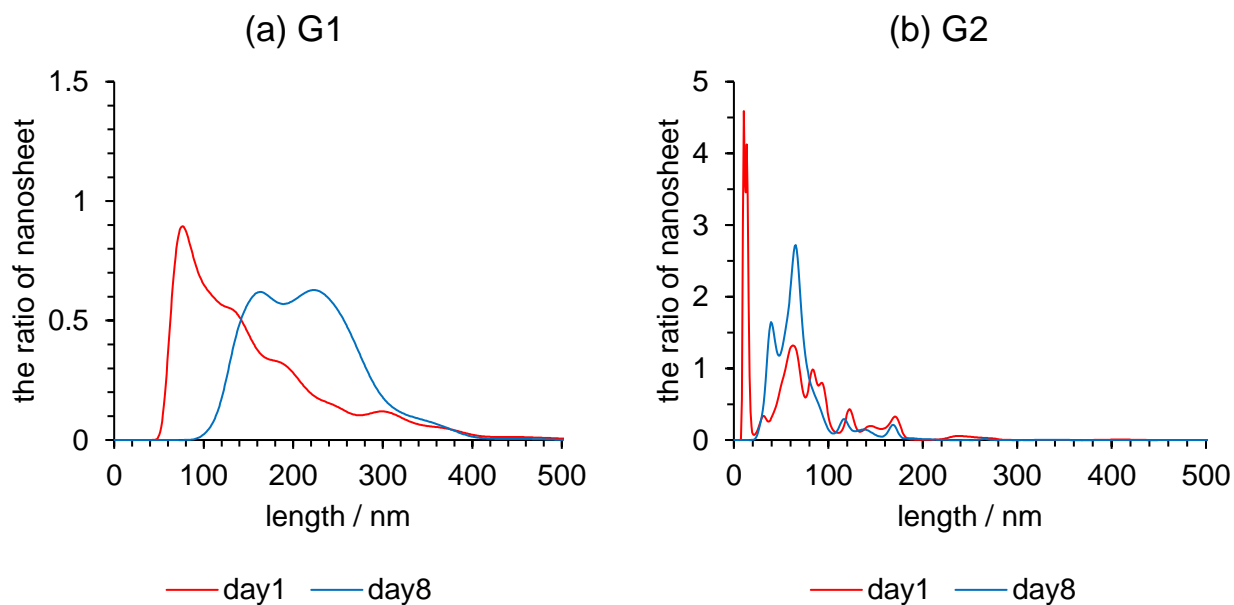


Fig. S5 The height histograms of the assemblies (a) G0, (b) G1, (c) G2, and (d) G4 determined by the AFM measurements on an APTES-modified Si-wafer in water. n=100.



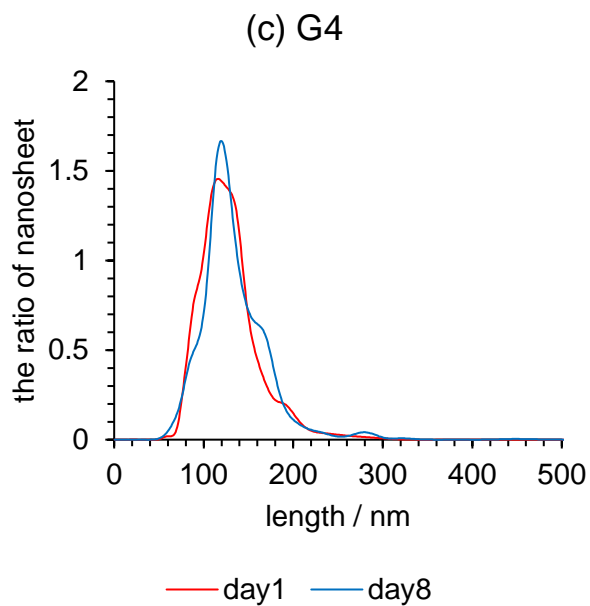


Fig. S6 The size distribution of the assemblies of (a) G1, (b) G2, and (c) G4 at day 1 (red) and day 7 (blue) stored at 4 °C after preparation.

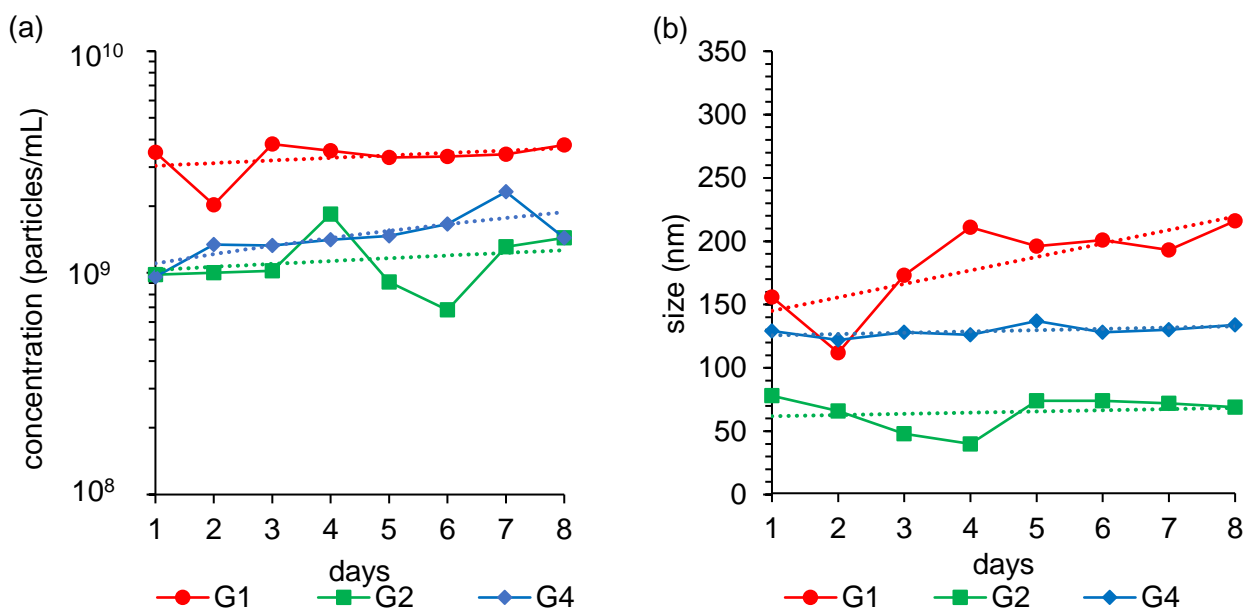


Fig. S7 The time dependence of (a) the concentration and (b) the size of the assemblies in G1 (●), G2 (■), and G4 (▲). The colored dashed lines represent the corresponding linear approximations.

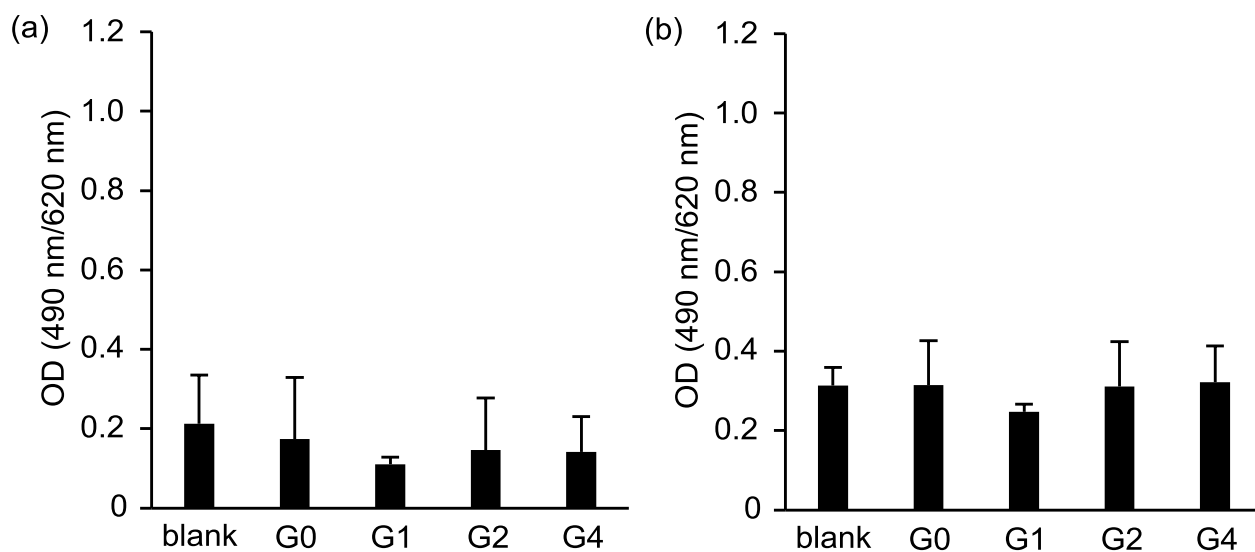


Fig. S8 IgM amounts after the twice administrations of the assemblies (G0–G2, and G4) determined by ELISA on the plates of (a) PEG-(LLeuAib)₆-OMe- and (b) Boc-(LLeuAib)₆-OMe-coatings.

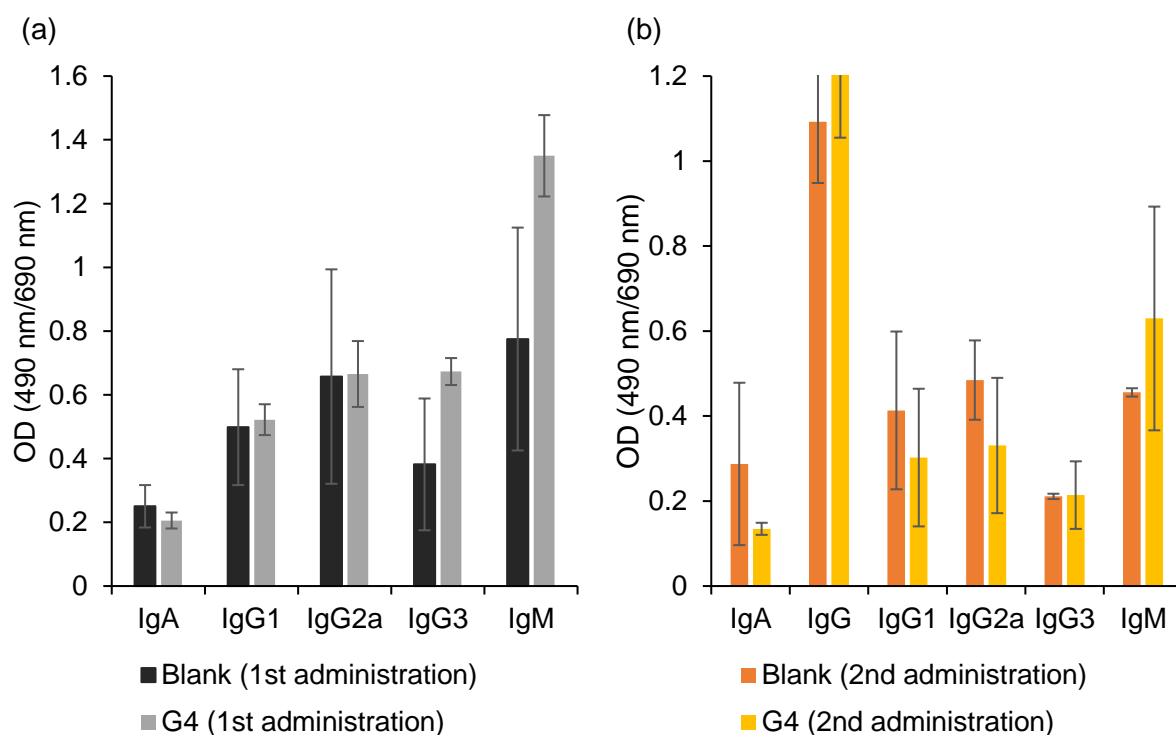


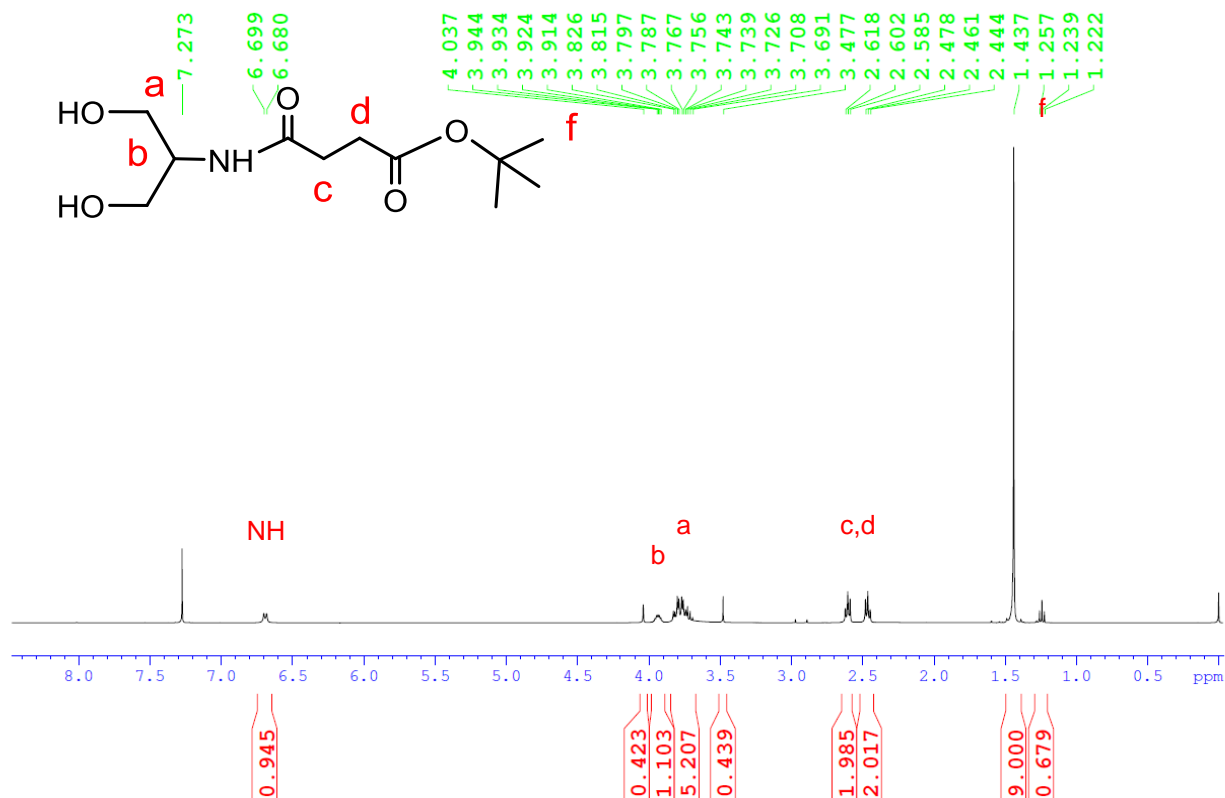
Fig. S9 The amounts of IgA, IgG1, IgG2a and IgG3 against LY antigen determined by ELISA of the sera of G4 at (a) day 7 and (b) day 14. ELISA was carried out on the LY-L plate. Significant difference between blank and G4 in any antibody type was not detected. ■: the sera prepared from blank group after 1st administration, ▒: the sera prepared from G4 after 1st administration, ■: the sera prepared

from blank group after 2nd administration, ■: the sera prepared from G4 after 2nd administration.

NMR and MALDI-MS spectrum

Butanoic acid, 4-[[2-hydroxy-1-hydroxymethylethyl]amino]-4-oxo-tert-butyl ester (3)

a)



b)

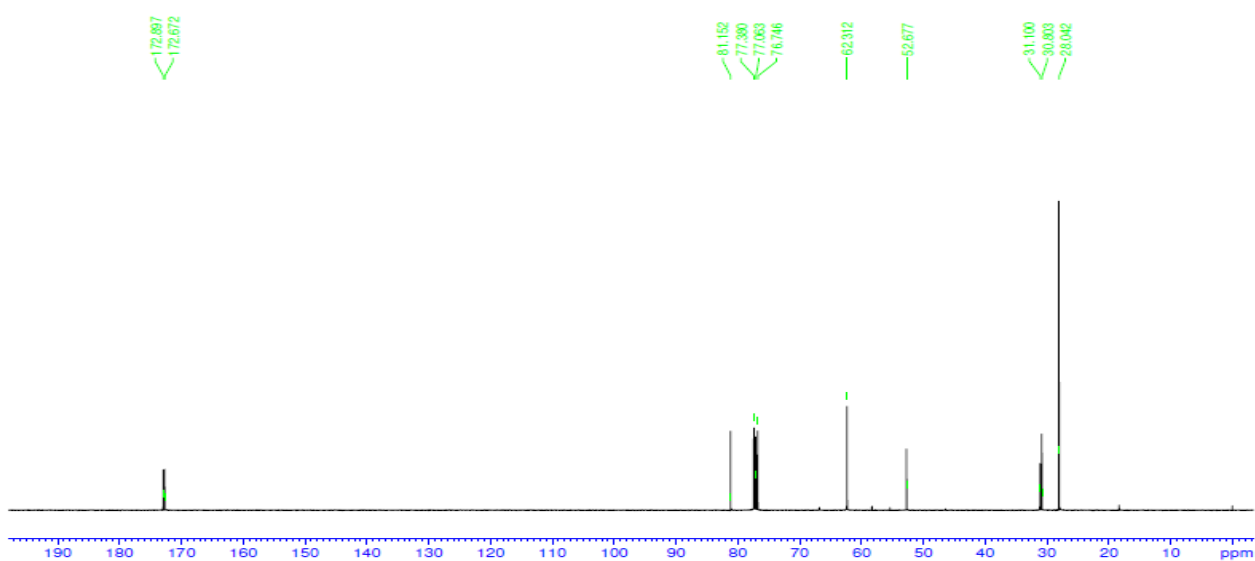
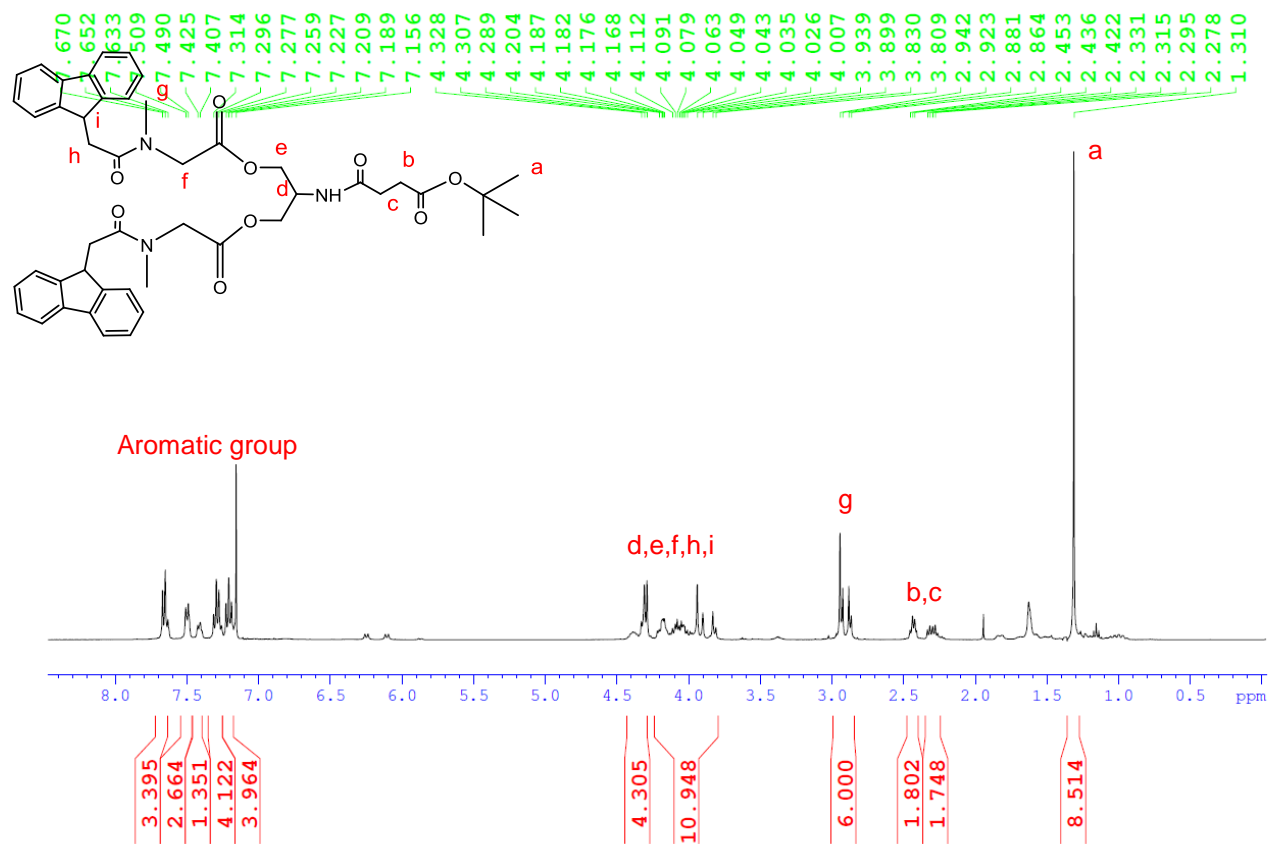


Figure S10. a) ¹H NMR and b) ¹³C NMR spectrum of **3** in CDCl₃.

Compound 5

a)



b)

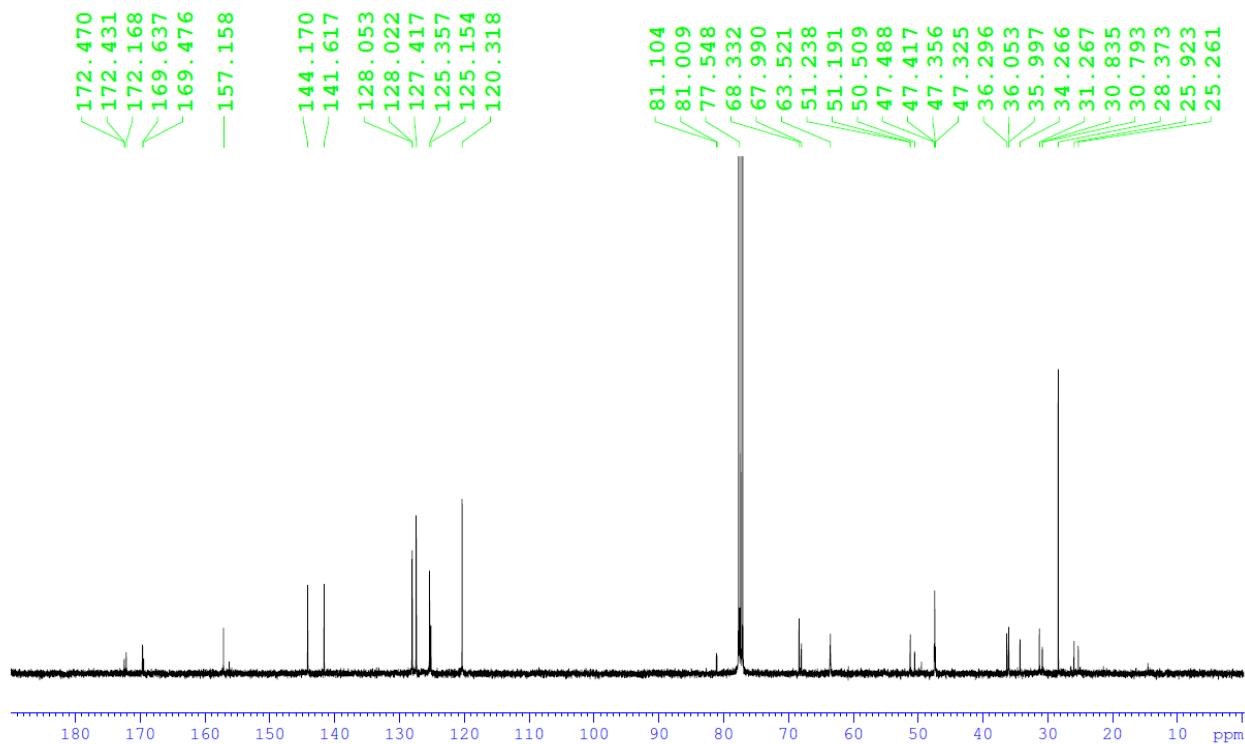


Figure S11. a) ^1H NMR and b) ^{13}C NMR spectrum of **5** in CDCl_3 .

Compound 6

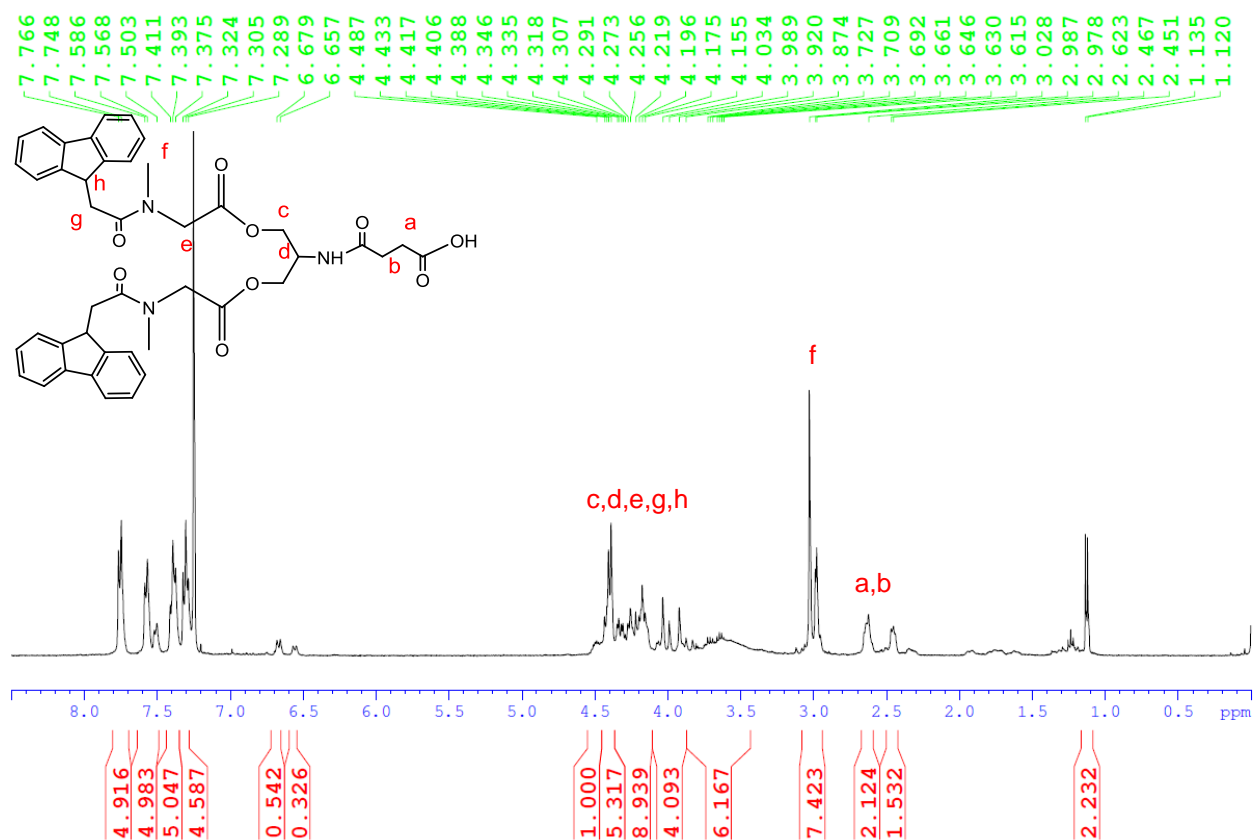


Figure S12. ¹H NMR spectra of **6** in CDCl₃.

Compound 9

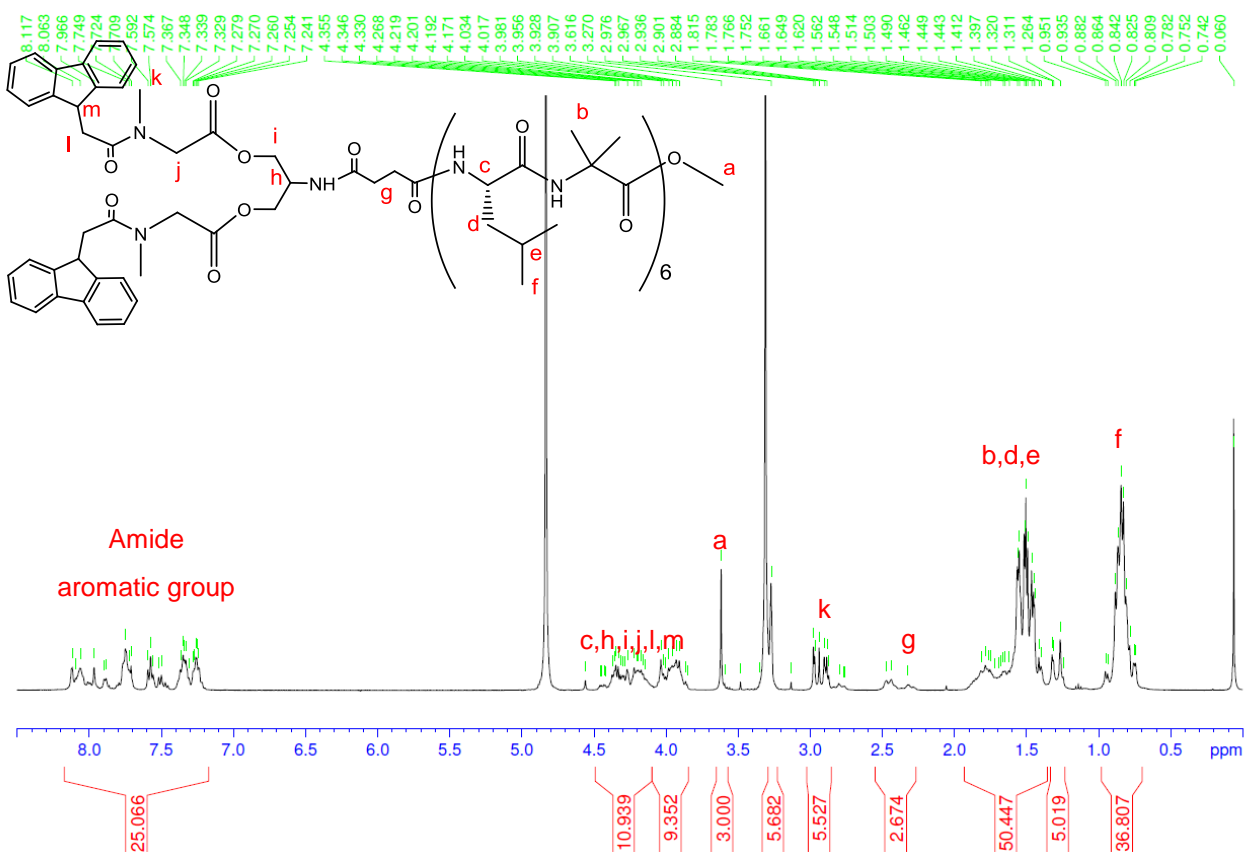


Figure S13. ¹H NMR spectra of 9 in MeOH-d₄.

Compound 10

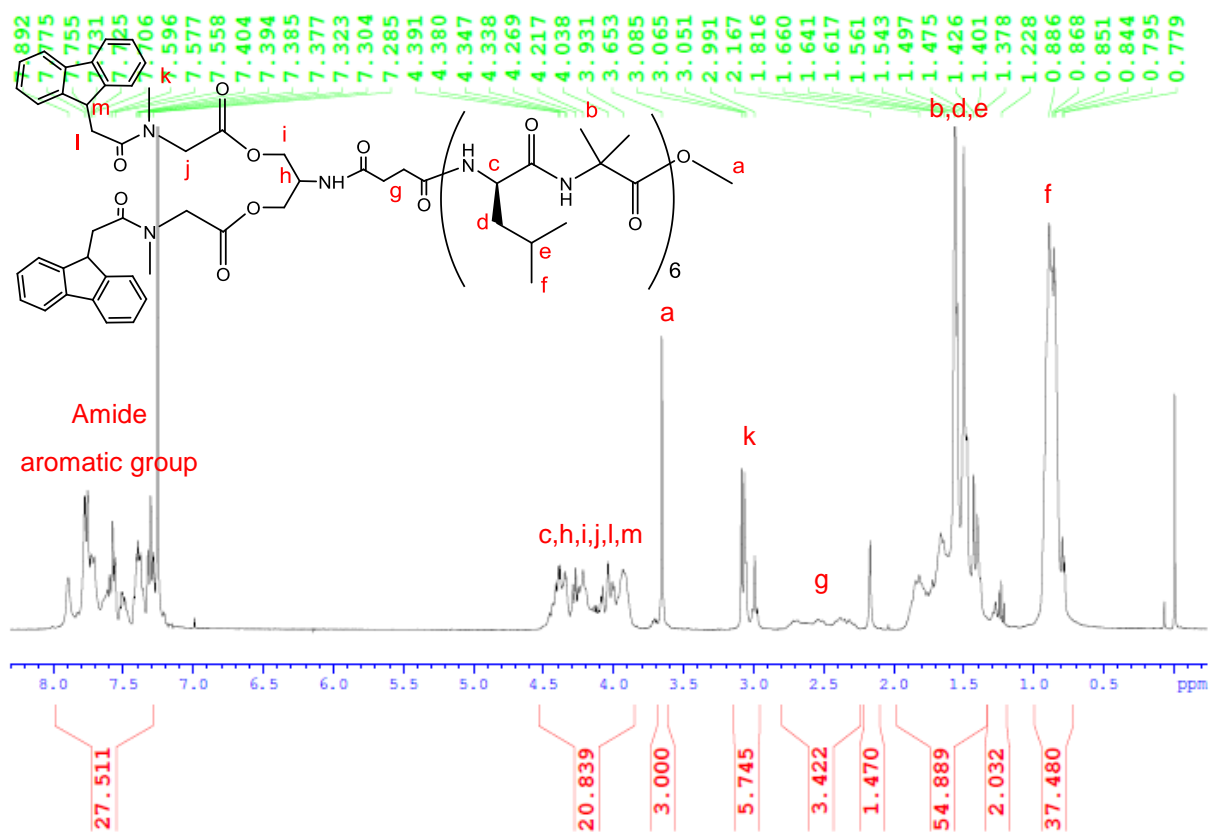
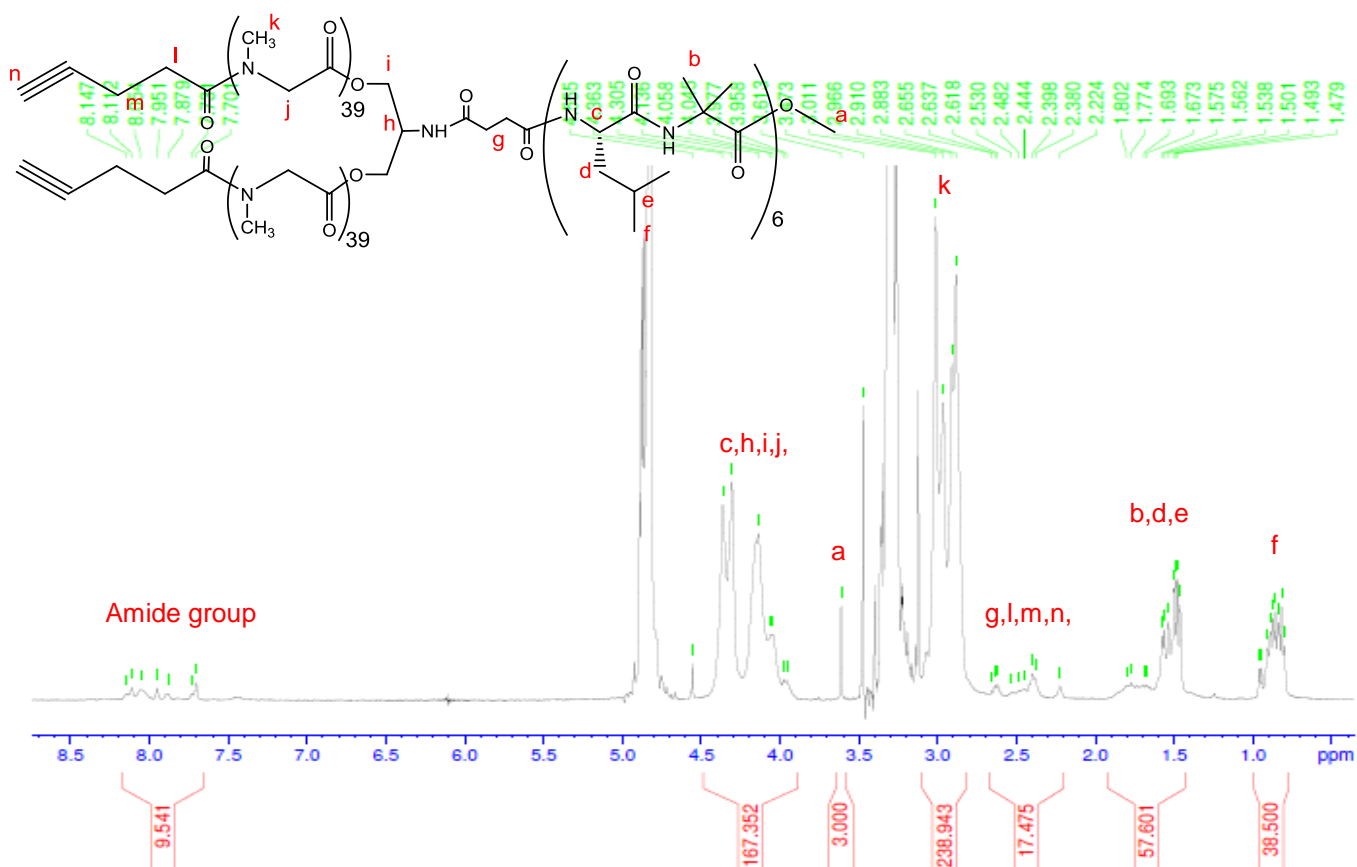


Figure S14. ¹H NMR spectra of **10** in CDCl₃.

Compound 11

a)



b)

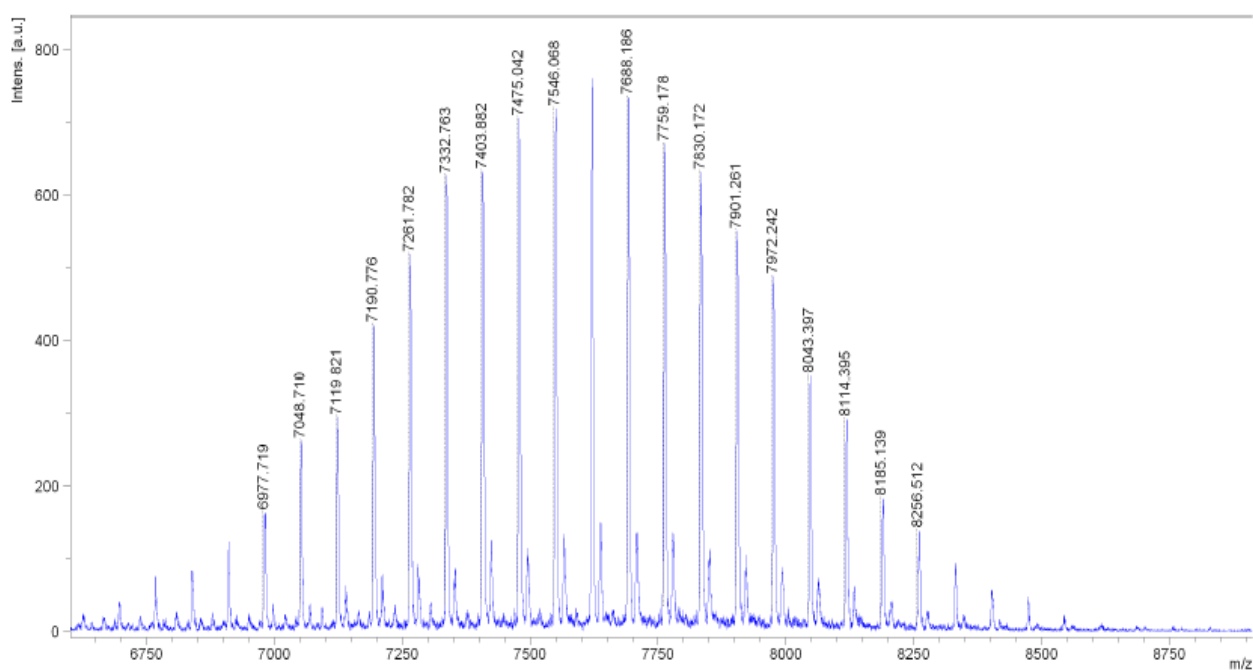
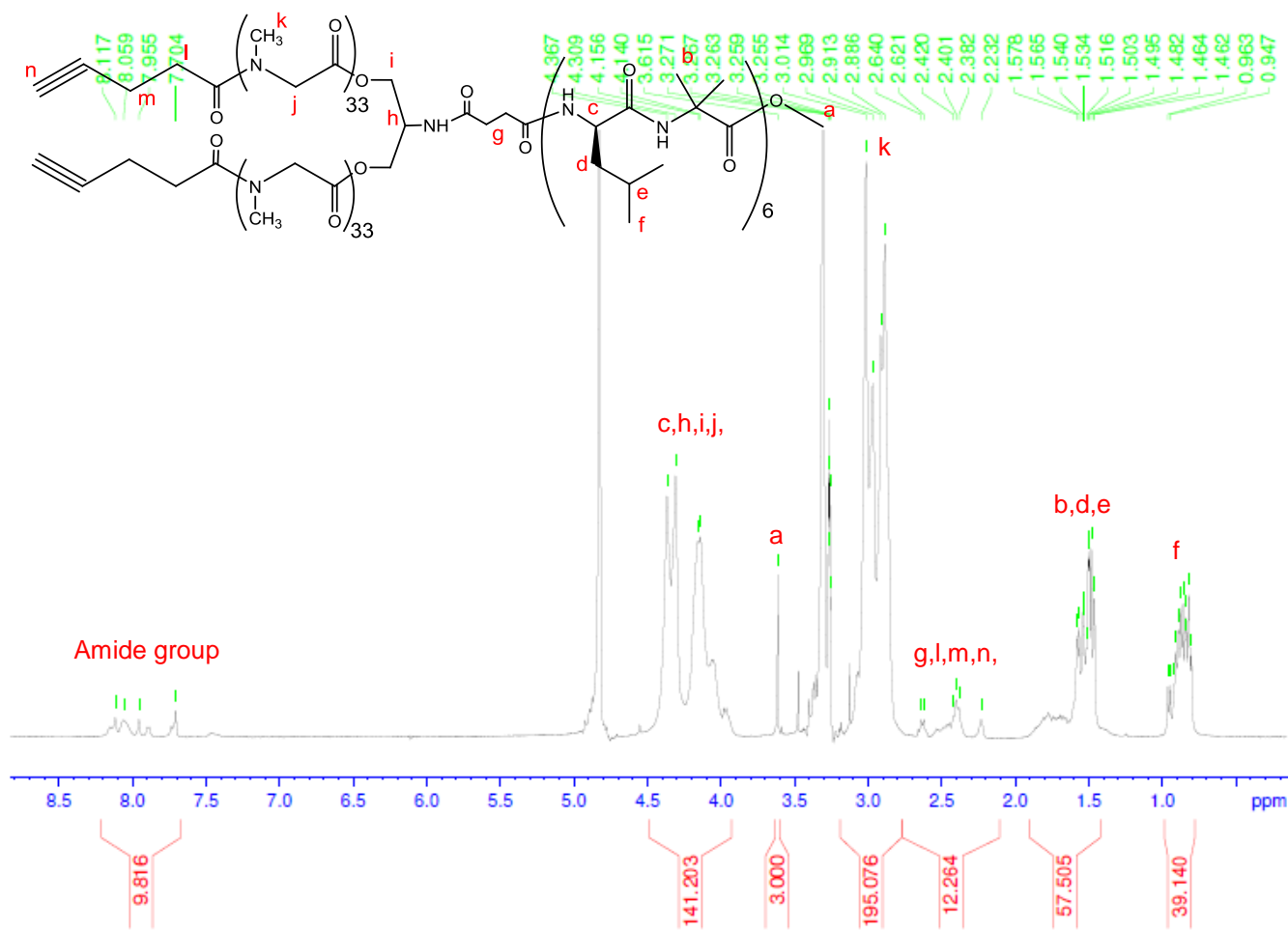


Figure S15. a) ^1H NMR spectra in MeOH-d_4 and b) MALDI-TOF-MS spectra of **11**.

Compound 12

a)



b)

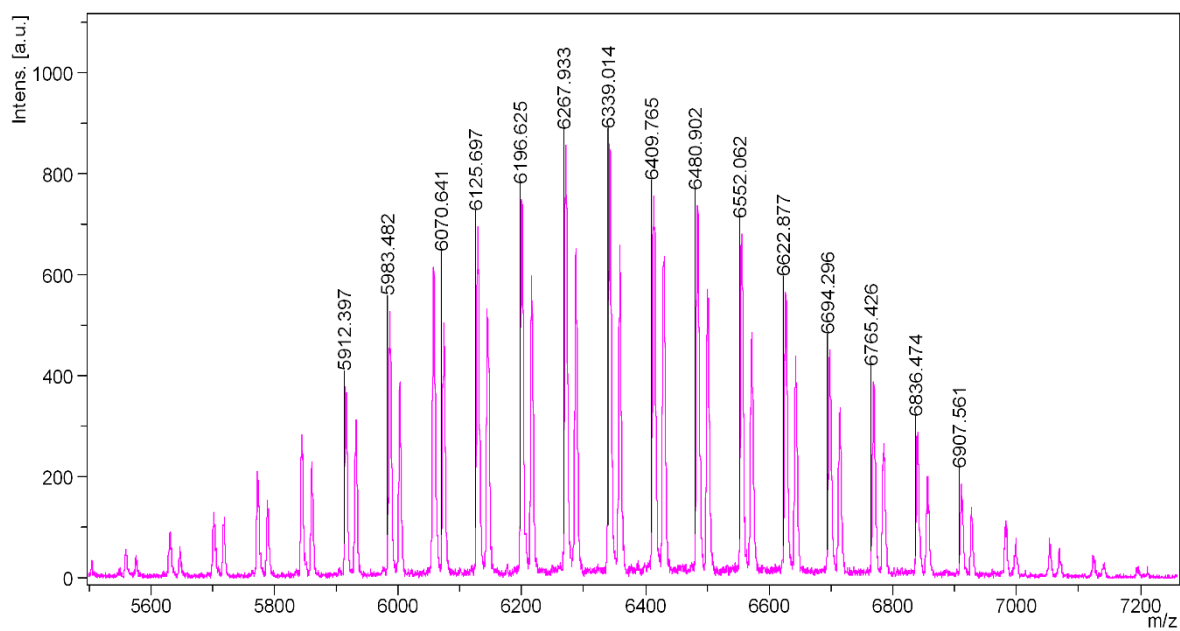
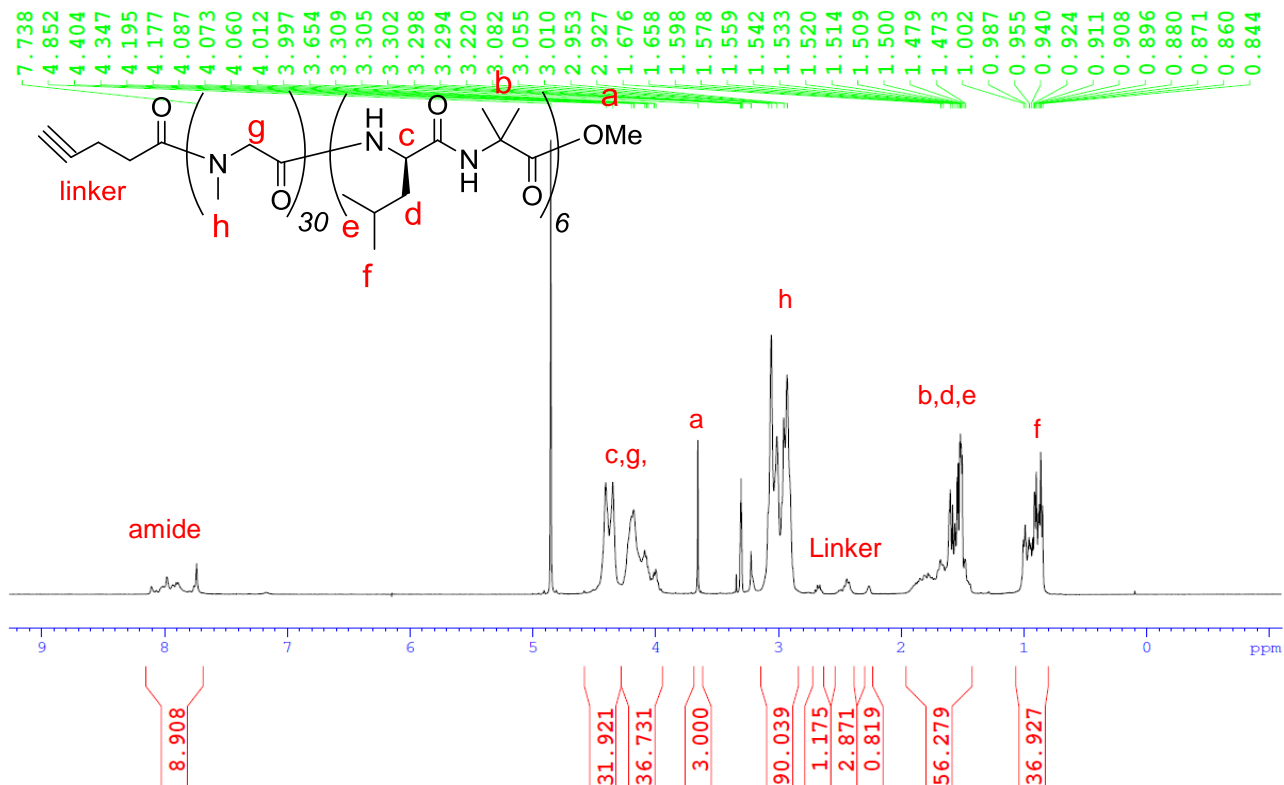


Figure S16. a) ¹H NMR spectra in MeOH-d₄ and b) MALDI-TOF-MS spectra of **12**.

Alkyne-functionalized PSar30-(D-LeuAib)6-OMe (Alkyne-functionalized AB type amphiphile)

a)



b)

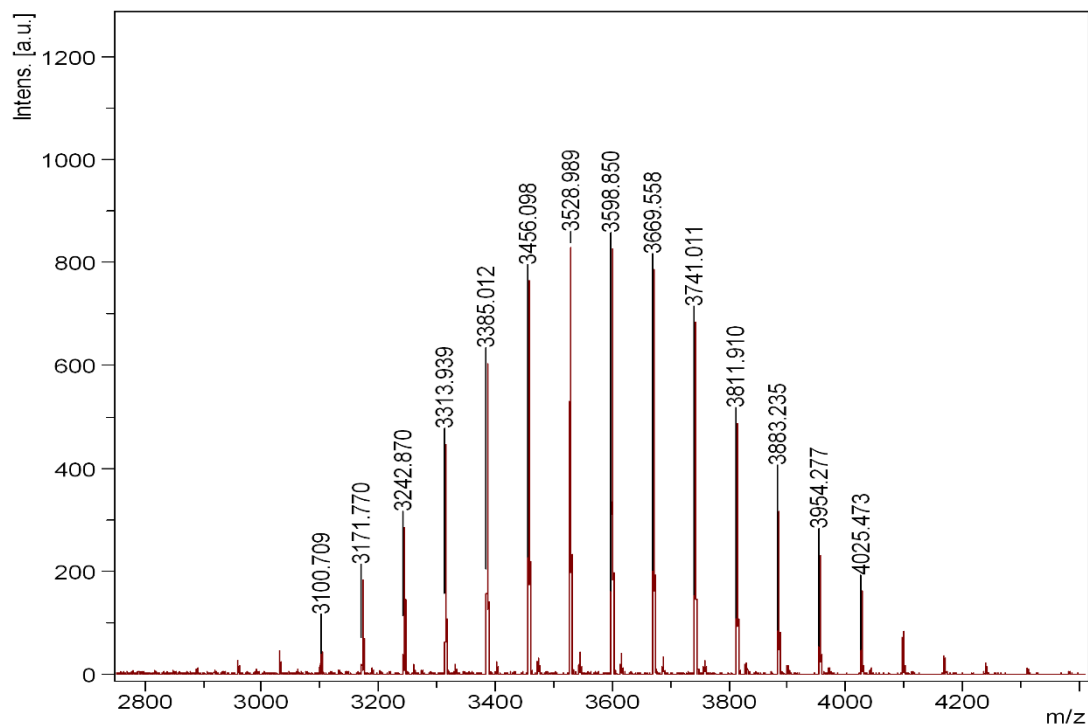


Figure S17. a) ^1H NMR spectra in MeOH- d_4 and b) MALDI-TOF-MS spectra of Alkyne capping PSar30-(D-LeuAib) $_6$ -OMe.

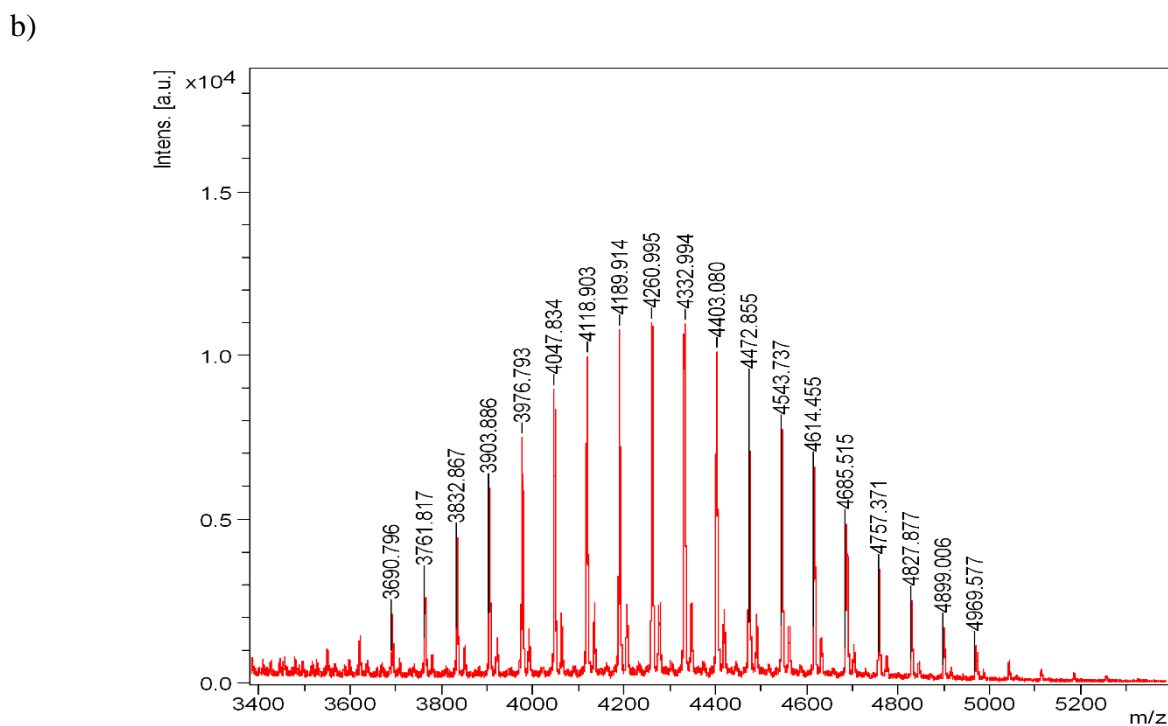
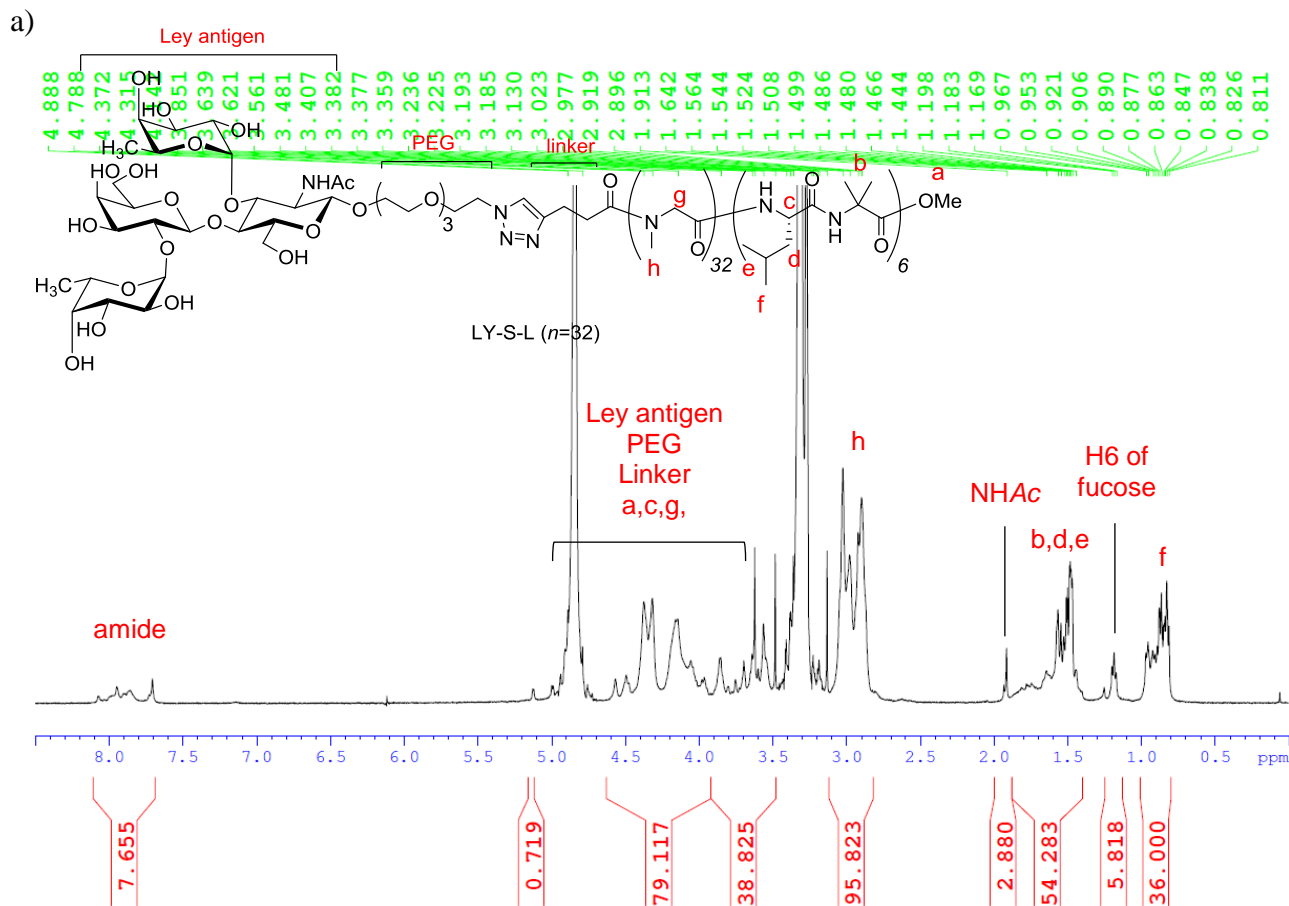


Figure S18. a) ^1H NMR spectra in MeOH- d_4 and b) MALDI-TOF-MS spectra of LY-S-L.

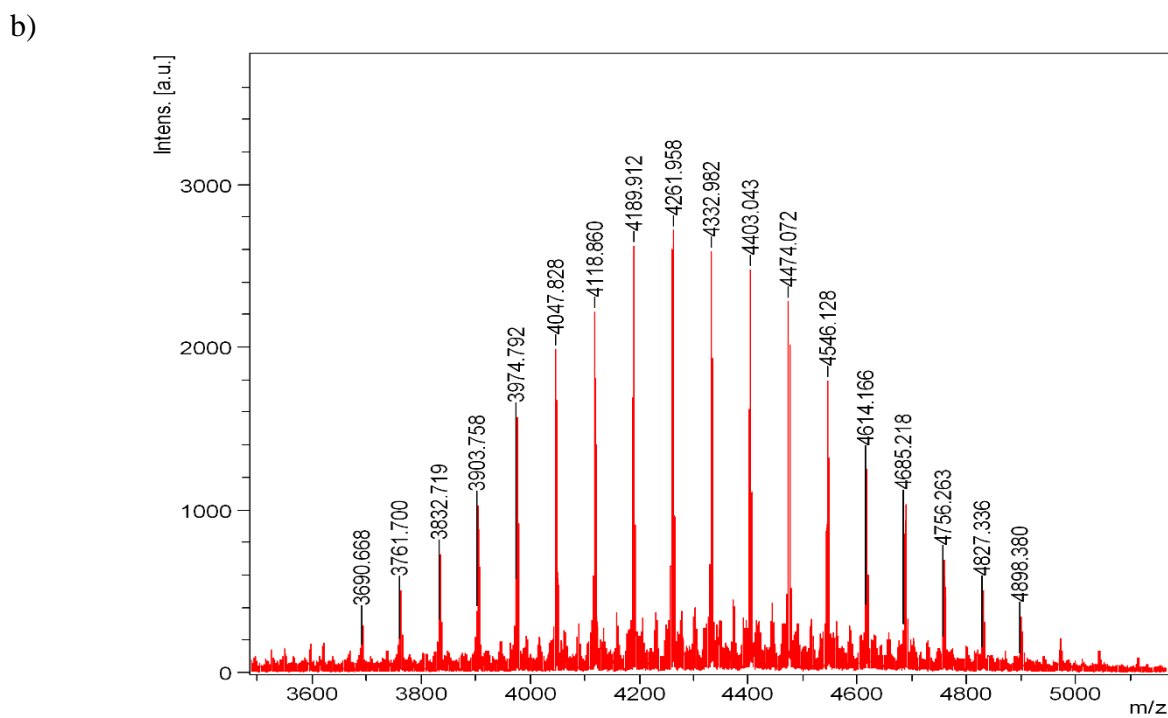
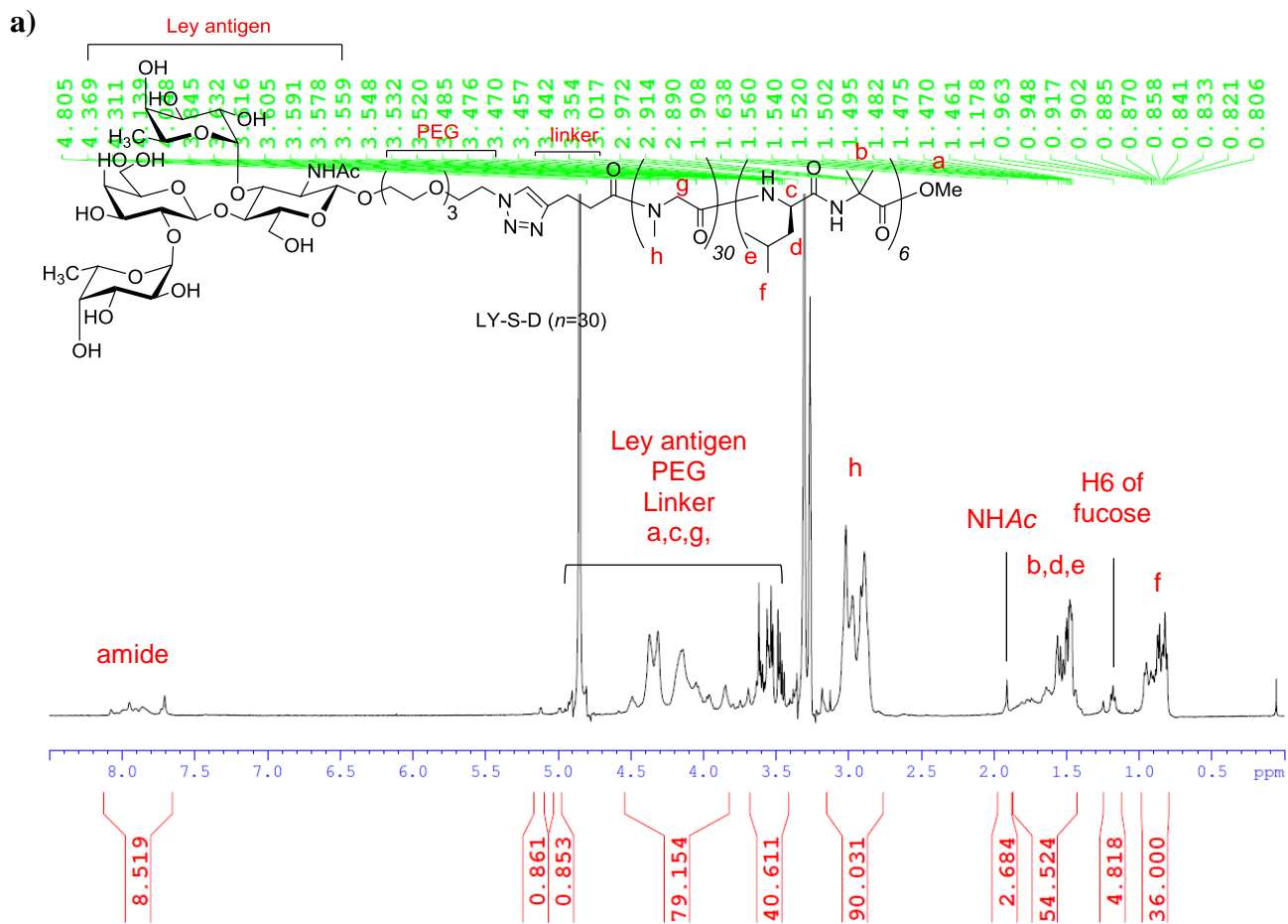


Figure S19. a) ^1H NMR spectra in MeOH- d_4 and b) MALDI-TOF-MS spectra of LY-S-D.