Supplementary information

Effect of carbohydrate structures on the hydrogelation ability and morphology of self-assembled structures of peptide–carbohydrate conjugates in water

Tomoya Tsuzuki,[a] Marina Kabumoto,[b] Hanae Arakawa[a] and Masato Ikeda[a, b, c] *

[a] *Department of Life Science and Chemistry, Graduate School of Natural Science and Technology, Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan*

Tel: +81-58-293-2639, Fax: +81-58-293-2794 E-mail: m_ikeda@gifu-u.ac.jp

[b] *United Graduate School of Drug Discovery and Medical Information Sciences, Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan*

[c] *Center for Highly Advanced Integration of Nano and Life Sciences, Gifu University (G-CHAIN), 1-1 Yanagido, Gifu 501-1193, Japan*

Contents:

1. Experimental generals

Unless stated otherwise, all commercial reagents were used as received. All water used in the experiments refers to ultra-pure water obtained from an ultrapure water system (Millipore Direct-Q or Advantec aquarius RFU 424CA) having a specific resistance of 18 MΩ•cm. Thin layer chromatography (TLC) was performed on silica gel $60F_{254}$ (Merck). Column chromatography was performed on silica gel 60N (Kanto, 40–50 μm) or silica gel PSQ-100B (Fuji Silysia Chemical, 100 μm). Reverse phase HPLC (RP-HPLC) was conducted with a Shimadzu Prominence instrument LC-20AT and SPD-20A equipped with a YMC Triart C18 column (150 mm × 4.6 mm I. D.) for analysis. ^{1}H and ^{13}C NMR spectra were obtained on a JEOL JNM ECS-400 spectrometer (400 MHz for ^{1}H, 100 MHz for ^{13}C) with tetramethylsilane (TMS) or residual non-deuterated solvents as the internal references. Multiplicities are abbreviated as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = double doublet, br = broad. Electron-splay ionization (ESI) TOF mass spectrometry was performed on a Waters Xevo G1 QTof or a JEOL JMS-T100LP AccuTOF LC-plus mass spectrometer.

2. Characterization of self-assembled structures

Preparation of hydrogel

Gelation ability was evaluated by an inverted tube test. Typically, powder of Z-F_2-Glc (4.0 mg, 1.5 μmol) was suspended in 400 μL of aqueous buffer (100 mM HEPES (pH 7.4)) inside a glass vial (The diameter of glass vial: 5.4 mm). The suspension was heated until a homogeneous solution was obtained. The solution was left to cool down at room temperature without any disturbance. The gel formation was confirmed whether the sample flows or not by inverting the glass vial.

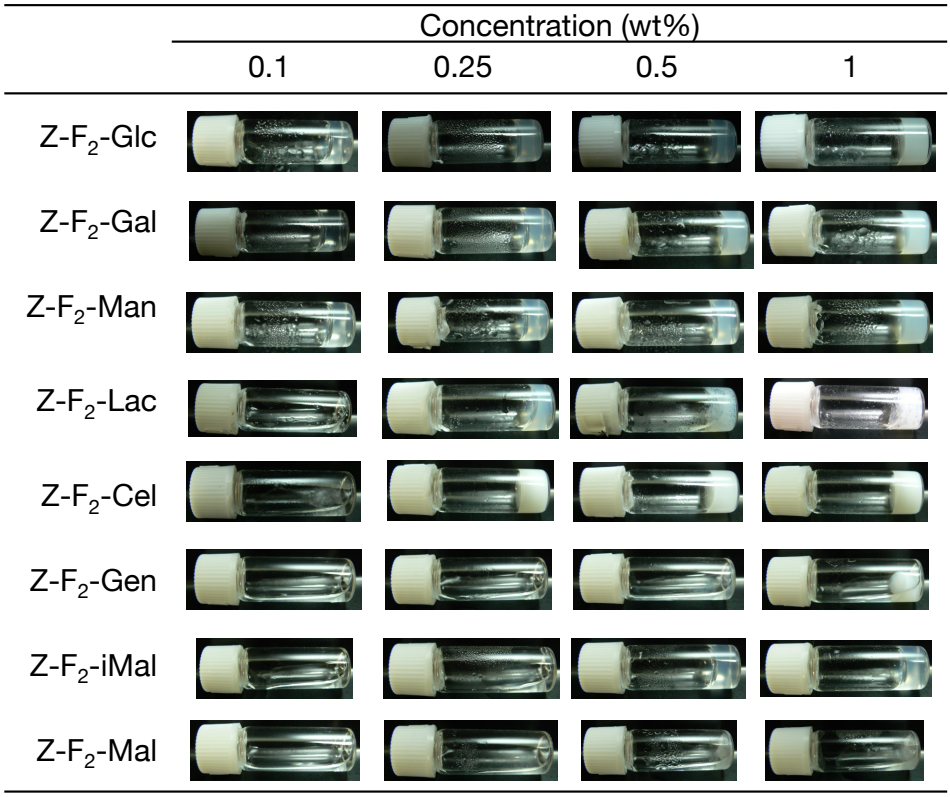


Figure S1. Photographs showing gelation ability of Z-F_2-glycosides at various concentrations.

Circular dichroism

CD spectra were recorded in a 0.1-mm quartz cell unless otherwise noted on a Jasco J-820 spectropolarimeter equipped with a programmable temperature-control unit (Julabo HP-4). The spectra were obtained by using a 2-nm slit width and a scanning step of 0.1 nm from 280 to 200 nm. Each spectrum was an average of 4 scans with the buffer background subtracted.

FTIR

FTIR spectra were acquired using a Shimadzu IRAffinity-1 spectrometer with a spectral resolution of 4 cm^{-1}. The spectra were obtained by averaging 64 scans for each sample with the solvent background subtracted. Hydrogels prepared with D_2O were sandwiched directly between CaF_2 windows (32 × 3 mm) separated by a 50 μm PTFE spacer.

TEM observation

Sample of hydrogel (ca. 10 μL) was dropped on a copper TEM grid covered by an elastic carbon-support film (20–25 nm) with a filter paper underneath and the excess solution was blotted with the filter paper immediately. The TEM grid was dried under a reduced pressure for at least 6 h

prior to TEM observation. TEM images were acquired using a Hitachi H-7000 (accelerating voltage: 100 kV) equipped with a CCD camera and AMTV600 software.

CLSM observation

A freshly prepared homogeneous solution (0.25 wt%, 100 mM HEPES pH 7.4, 400 μL) of Z-F_2-glycosides obtained by heating was mixed with a DMSO solution of Nile-blue or Nile-red (5 mM (final concentration is 25 μM), 1 μL). The solution was left to cool down at room temperature without any disturbance. The gel (ca. 20 μL) was spotted on a glass coverslips (diameter: 25 mm, thickness: 0.13–0.17 mm, Fisher Scientific) placed in Attofluor cell chamber (Thermo Fisher Scientific). For ConA experiments, a suspension of a Z-F_2-glycoside (20 μL) was mixed with an aqueous solution of FITC-ConA (1.0 mg/mL, 10 μL) on a glass coverslips (diameter: 25 mm, thickness: 0.13–0.17 mm, Fisher Scientific) placed in Attofluor cell chamber (Thermo Fisher Scientific). The samples were subjected to observations using a Zeiss LSM 710 confocal microscope (Carl Zeiss) equipped with He-Ne lasers (543 and 633 nm) and an Ar laser (488 nm). A 100× (NA = 1.46) oil objective was employed to obtain images (typically, 1024 × 1024 pixel). The images were obtained analyzed using ZEN software.

Rheological measurement

Dynamic frequency and strain sweep experiments were carried out on a TA instruments AR-G2 rheometer using a 20-mm stainless steel parallel plate (The temperature of the plate was controlled at 25 ºC by peltier system) at the gap of 1000 μm. Hydrogel samples were placed on the plate. All the gels showed almost linear viscoelastic regime up to 1.0% strain (frequency: 1.0 rad/s). Therefore, frequency sweep (0.1–100 rad/s) was performed under 0.2 % strain.

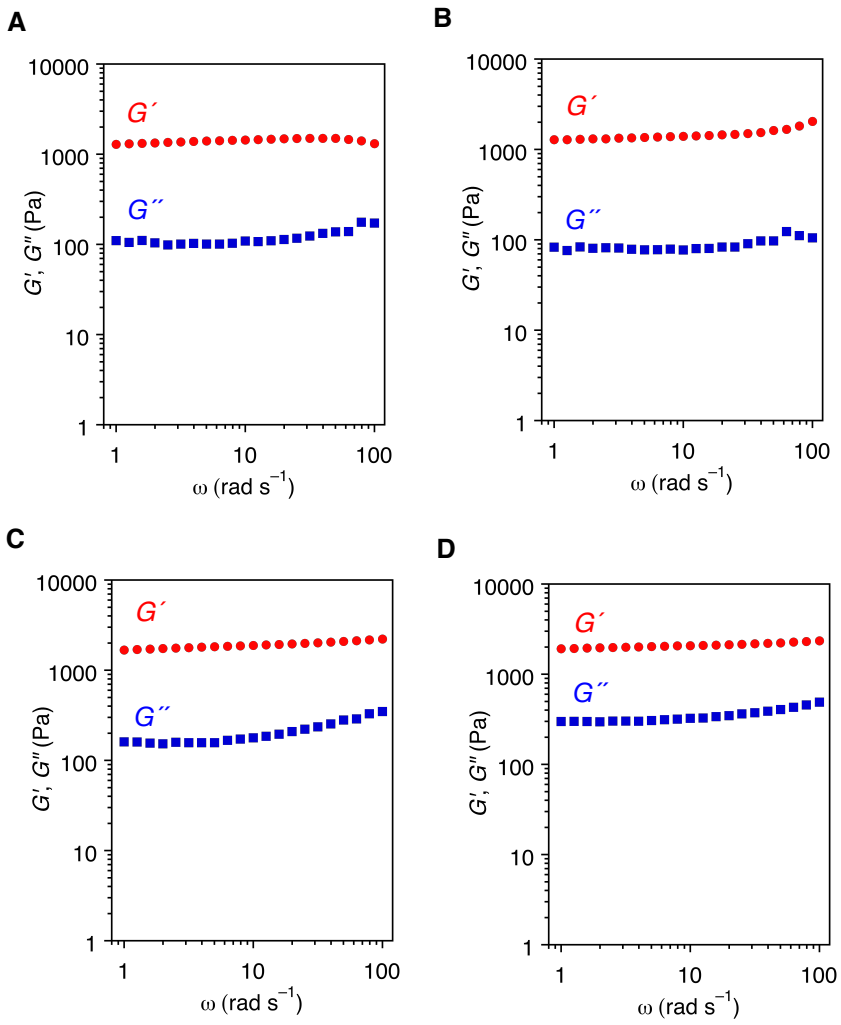
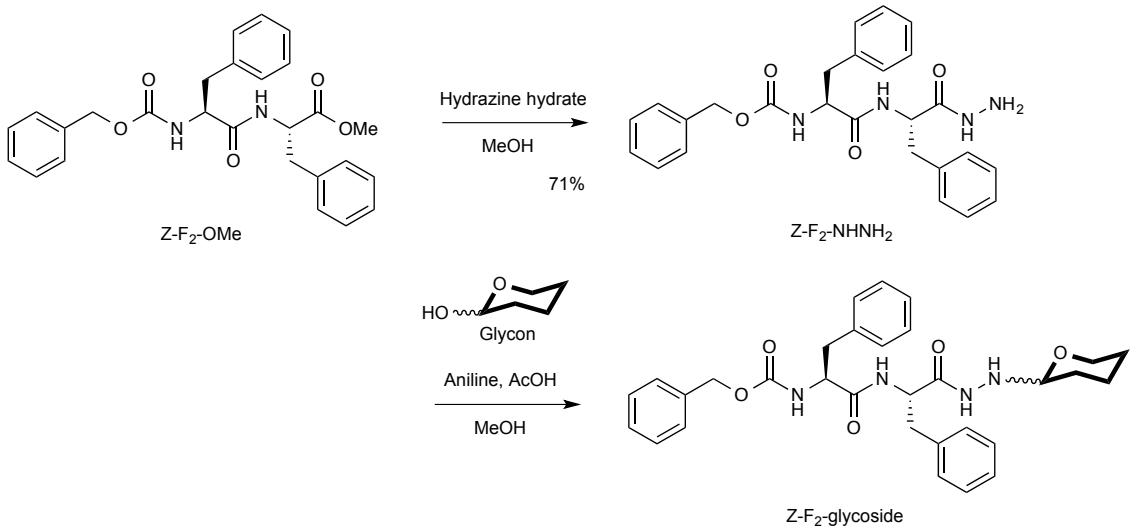


Figure S2. Frequency sweep (0.2% strain, *G´*: storage shear modulus, *G´´*: loss shear modulus) rheological properties of the Z-F_2-glycoside hydrogels (**A**: Z-F_2-Gal, **B**: Z-F_2-Man, **C**: Z-F_2-Lac, **D**: Z-F_2-Cel). Conditions: [Z-F_2-glycoside] = 0.25 wt%, 100 mM HEPES (pH 7.4), 25 °C

3. Synthesis

Synthesis of Z-F_2-glycoside



Scheme S1. Synthesis of Z-F_2-glycoside.

Synthesis of Z-F_2-NHNH_2: Hydrazine hydrate (0.10 mL, 2.0 mmol) was added to a solution of Z-F_2-OMe[S1] (106 mg, 0.23 mmol) in MeOH (5 mL). The reaction mixture was refluxed under Ar atmosphere for 17 h. After the mixture was cooled, H_2O (30 mL) was added and the resultant precipitate was collected by filtration, washed with H_2O, and dried to yield Z-F_2-NHNH_2 (71 mg, 71%) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 2.60–2.96 (m, 4H), 4.22 (m, 3H), 4.49 (m, 1H), 4.93 (s, 2H), 7.08–7.33 (m, 15H), 7.43 (d J = 8.0 Hz, 1H), 8.12 (d, J = 8.0 Hz, 1H), 9.18 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 37.48, 38.16, 52.60, 56.11, 65.18, 126.18, 126.28, 127.39, 127.65, 128.00, 128.06, 128.20, 128.28, 129.14, 129.22, 129.22, 136.98, 137.50, 138.00, 155.65, 169.98, 121.08; HRMS (ESI, positive): Calcd. for [M($C_{26}H_{28}N_4O_4$) + H]$^+$: m/z = 461.2189; Found: 461.1934.

Synthesis of Z-F_2-Glc: To a solution of Z-F_2-NHNH_2 (46 mg, 0.10 mmol) in methanol (4 mL), and D-glucose (20 mg, 0.11 mmol, 1.1 equiv.), acetic acid (0.1 mL) was added to solution. The resultant mixture was stirred at 65 ºC under Ar atmosphere for 3 hours and additionally for 14 hours at room temperature. The mixture was concentrated by evaporation and the crude product was purified by column chromatography (SiO_2, chloroform-methanol = 5:1 (v/v)) to afford Z-F_2-Glc (26 mg, 42%) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 2.62–2.74 (m, 2H), 2.81–3.07 (m, 8H), 3.08–3.21 (m, 2H), 3.36–3.47 (m, 2H), 3.53 (dd, J = 8.6, 2.9 Hz, 0.8H),

3.60－3.71 (m, 2H), 3.85 (t, J = 9.3 Hz, 0.2H), 4.19–4.33 (m, 3H), 4.52–4.63 (m, 1H), 4.90 (d, J = 4.8 Hz, 1H), 4.91–4.97 (m, 3H), 5.03 (d, J = 4.1 Hz, 0.82H), 5,15–5.24 (m, 0.21H), 5.59–5.64 (m, 0.82+0.18H), 7.13–7.36 (m, 15H), 7.43 (d, J = 8.0 Hz, 1H), 7.84 (d, J = 8.0 Hz, 0.18H), 8.19 (d, J = 8.2 Hz, 1H), 9.58 (d, J = 4.4 Hz, 0.83H); ^{13}C NMR (100 MHz, DMSO–d_6) δ (ppm) 38.0, 38.5, 52.8, 56.5, 62.1, 65.7, 71.0, 71.6, 77.3, 78.6, 91.4, 126.7, 126.9, 127.9, 127.9, 128.2, 128.6, 128.6, 128.8, 129.7, 129.9, 137.5, 137.7, 138.6, 156.2, 170.6, 171.8; HRMS (ESI, positive): Calcd. for [M($C_{32}H_{38}N_4O_9$) + Na]$^+$: m/z = 645.2537; Found: 645.2557.

Synthesis of Z-F_2-Gal: The titled compound was prepared from Z-F_2-$NHNH_2$ (46 mg, 0.10 mmol) and D-galactose (20 mg, 0.11 mmol, 1.1 equiv.) in the similar way for Z-F_2-Glc and was obtained in 42% yield (26 mg) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 2.61–2.74 (m, 1H), 2.81–2.99 (m, 3H), 3.25–3.30 (m, 2H (H_2O peak obscuring)), 3.43–3.58 (m, 1+2H), 3.62–3.68 (m, 1H), 3.82 (t, J = 7.6 Hz, 0.14H), 4.21–4.33 (m, 2H), 4.36–4.49 (m, 1H), 4.56–4.64 (m, 1H), 4.89–4.95 (m, 0.86H), 5.13–5.27 (m, 0.18H), 5.57–5.63 (m, 0.14+0.86H), 7.15–7.36 (m, 15H), 7.44 (d, J = 8.9 Hz, 1H), 7.82 (d, J = 8.7 Hz, 0.14H), 8.12 (d, J = 8.2 Hz, 1H), 9.65 (d, J = 4.8 Hz, 0.86H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 37.9, 38.7, 52.8, 56.6, 60.8, 65.7, 68.7, 68.9, 74.1, 77.0, 92.1, 126.7, 126.9, 127.9, 127.9, 128.2, 128.3, 128.6, 128.8, 129.7, 129.9, 137.5, 137.7, 138.6, 156.2, 170.6, 171.7; HRMS (ESI, positive): Calcd. for [M($C_{32}H_{38}N_4O_9$) + Na]$^+$: m/z = 645.2537; Found: 645.2549.

Synthesis of Z-F_2-Man: The titled compound was prepared from Z-F_2-$NHNH_2$ (46 mg, 0.10 mmol) and D-mannose (20 mg, 0.11 mmol, 1.1 equiv.) in the similar way for Z-F_2-Glc and was obtained in 74% yield (46 mg) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 2.61–2.72 (m, 1H), 2.77–2.96 (m, 3H), 3.15–3.24 (m, 1H), 3.56–3.69 (m, 3H), 4.18–4.36 (m, 1H), 4.51–4.61 (m, 1H), 4.66–4,73 (m, 1H), 4,73–4.77 (m, 2H), 4.89 (d, 0.79H), 4.90–4.95 (m, 2H), 5.10–5.29 (m, 1+0.11H), 5.35–5.40 (m, 0.11H), 7.14–7.36 (m, 15H), 7.39–7.50 (m, 1H), 8.01 (d, J = 8.0 Hz, 0.35H), 8.17 (d, J = 8.0 Hz, 1H), 9.23 (d, J = 4.4 Hz, 0.65H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 38.0, 38.8, 52.9, 56.6, 62.7, 65.7, 68.1, 70.2, 74.7, 78.6, 88.2, 126.7, 126.8, 127.9, 127.9, 128.2, 128.5, 128.6, 128.8, 129.7, 129.9, 129.9, 137.5, 137.7, 138.5, 156.2, 171.8, 174.7; HRMS (ESI, positive): Calcd. for [M($C_{32}H_{38}N_4O_9$) + Na]$^+$: m/z = 645.2537; Found: 645.2561.

Synthesis of Z-F_2-Lac: The titled compound was prepared from Z-F_2-$NHNH_2$ (46 mg, 0.10 mmol) and D-lactose monohydrate (40 mg, 0.11 mmol, 1.1 equiv.) in the similar way for Z-F_2-Glc

and was obtained in 68% yield (53 mg) as a white solid: ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ (ppm) 2.58–2.70 (m, 1H), 2.75–2.99 (m, 4H), 3.10–3.31 (m, 2H (H_2O peak obscuring)), 3.35–3.62 (m, 5H), 3.66–3.76 (m, 1H), 4.10–4.16 (m, 1H), 4.18–4.27 (m, 1H), 4.33 (t, *J* = 6.0 Hz, 1H), 4.47 (d, *J* = 4.7 Hz, 1H), 4.50–4.58 (m, 1H), 4.59–4.64 (m, *J* = 4.0 Hz, 1H), 4.64–4.68 (m, 1H), 4.66 (s, 1H), 4.74 (d, *J* = 4.0 Hz, 1H), 4.86–4.91 (m, 2H), 5.09 (d, *J* = 4.0 Hz, 0.88H), 5.11 (d, *J* = 4.0 Hz, 1H), 5.59–5.63 (m, 1H), 5.65 (d, *J* = 9.2 Hz, 0.12H), 7.10–7.31 (m, 15H), 7.39 (d, *J* = 9.0 Hz, 1H), 7.78 (d, *J* = 8.5 Hz, 0.10H), 8.16 (d, *J* = 8.0 Hz, 1H), 9.55 (d, *J* = 4.0 Hz, 0.90H); ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ (ppm) 38.0, 38.6, 52.8, 56.5, 60.9, 61.5, 65.7, 68.7, 71.0, 71.4, 73.8, 75.5, 76.0, 76.5, 81.7, 91.2, 104.4, 126.8, 126.9, 127.9, 128.2, 128.6, 128.6, 128.8, 129.7, 129.9, 137.5, 137.7, 138.5, 156.2, 170.5, 171.8; HRMS (ESI, positive): Calcd. for $[M(C_{38}H_{48}N_4O_{14}) + Na]^+$: m/z = 807.3065; Found: 807.3044.

Synthesis of Z-F$_2$-Cel: The titled compound was prepared from Z-F$_2$-NHNH$_2$ (46 mg, 0.10 mmol) and D-cellobiose (38 mg, 0.11 mmol, 1.1 equiv.) in the similar way for Z-F$_2$-Glc except for the addition of LiCl (1.0 g, 24 mmol) to solubilize D-cellobiose and was obtained in 37% yield (29 mg) as a white solid: ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ (ppm) 2.62–2.74 (m, 1H), 2.85–3.02 (m, 5H), 3.03–3.45 (m, 2H (H_2O peak obscuring)), 3.55–3.63 (m, 2H), 3.64–3.78 (m, 2H), 4.19–4.31 (m, 2H), 4.39 (t, *J* = 5.8 Hz, 1H), 4.53–4.63 (m, 2H), 4.91–4.94 (m, 2H), 4.99 (d, *J* = 4.8 Hz, 1H), 5.02 (d, *J* = 4.8 Hz, 1H), 5.15 (d, *J* = 4.4 Hz, 0.83H), 5.26 (d, *J* = 4.6 Hz, 1H), 5.69 (m, 1H), 5.70 (d, *J* = 9.6 Hz, 0.17H), 7.15–7.35 (m, 15H), 7.43 (d, *J* = 8.0 Hz, 1H), 7.82 (d, *J* = 8.0 Hz, 0.15H), 8.20 (d, *J* = 8.0 Hz, 1H), 9.58 (d, *J* = 4.0 Hz, 0.85H); ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ (ppm) 38.0, 38.5, 52.8, 56.5, 61.4, 61.5, 65.7, 70.5, 71.4, 73.8, 75.6, 76.6, 77.0, 77.3, 81.6, 91.1, 103.8, 126.7, 126.9, 127.9, 128.2, 128.6, 128.6, 128.8, 129.7, 129.9, 137.5, 137.7, 138.5, 156.2, 170.6, 171.8; HRMS (ESI, positive): Calcd. for $[M(C_{38}H_{48}N_4O_{14}) + K]^+$: m/z = 823.2804; Found: 823.2786.

Synthesis of Z-F$_2$-Gen: The titled compound was prepared from Z-F$_2$-NHNH$_2$ (46 mg, 0.10 mmol) and β-gentiobiose (38 mg, 0.11 mmol, 1.1 equiv.) in the similar way for Z-F$_2$-Glc and was obtained in 68% yield (53 mg) as a white solid: ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ (ppm) 2.62–2.72 (m, 1H), 2.88–3.01 (m, 4H), 3.04–3.19 (m, 3H), 3.22–3.28 (m, 1H), 3.40–3.49 (m, 1H), 3.51–3.59 (m, 1H), 3.61–3.70 (m, 2H), 4.01 (d, *J* = 10.5 Hz, 1H), 4.06–4.14 (m, 1H), 4.19 (d, *J* = 7.8 Hz, 1H), 4.22–4.32 (m, 1H), 4.48 (t, *J* = 5.5 Hz, 1H), 4.57–4.64 (m, 1H), 4.87 (d, *J* = 4.8 Hz, 1H), 4.90 (d, *J* = 4.8 Hz, 1H), 4.91–4.94 (m, 2H), 4.97 (d, *J* = 5.0 Hz, 1H), 4.99 (d, *J* = 4.4 Hz, 0.93H), 5.07 (d, *J* = 3.2 Hz, 1H), 5.60 (d, *J* = 8.5 Hz, 0.07H), 5.67–5.71 (m, 1H), 7.14–7.36 (m, 15H), 7.42 (d, *J* = 8.7 Hz, 1H), 7.87 (d, *J* = 7.1 Hz, 0.09H), 8.14 (d, *J* = 8.4 Hz, 1H), 9.74 (d, *J* = 4.4 Hz, 0.91H); ^{13}C NMR

(100 MHz, DMSO-d_6) δ (ppm) 37.9, 38.5, 49.1, 52.8, 56.5, 61.5, 65.7, 69.0, 70.4, 70.5, 71.2, 74.1, 77.1, 77.3, 77.3, 77.4, 91.4, 103.7, 126.7, 126.9, 127.9, 128.2, 128.6, 128.8, 129.7, 130.0, 137.5, 137.7, 138.5, 156.2, 171.6, 171.8. HRMS (ESI, positive): Calcd. for [M($C_{38}H_{48}N_4O_{14}$) + Na]$^+$: m/z = 807.3065; Found: 807.3052.

Synthesis of Z-F_2-iMal: The titled compound was prepared from Z-F_2-$NHNH_2$ (46 mg, 0.10 mmol) and D-isomaltose (38 mg, 0.11 mmol, 1.1 equiv.) in the similar way for Z-F_2-Glc and was obtained in 66% yield (52 mg) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 2.63–2.74 (m, 1H), 2.82–2.98 (m, 3H), 3.04–3.25 (m, 4H), 3.30–3.51 (m, 2H (H_2O peak obscuring)), 3.51–3.57 (m, 1H), 3.57–3.64 (m, 1H), 3.71–3.78 (m, 1H), 4.22–4.30 (m, 1H), 4.47 (t, J = 5.5 Hz, 1H), 4.54–4.61 (m, 1H), 4.62–4.66 (m, 1H), 4.77 (d, J = 4.8 Hz, 1H), 4.83 (d, J = 5.5 Hz, 1H), 4.90–4.94 (m, 2H), 5.02 (d, J = 4.4 Hz, 0.92H), 5.09 (d, J = 3.9 Hz, 1H), 5,17–5.25 (m, 0.11H), 5.61 (d, J = 8.9 Hz, 0.08H), 5.66–5.72 (m, 0.89H), 7.15–7.35 (m, 15H), 7.43 (d, J = 8.7 Hz, 1H), 7.88 (d, J = 8.0 Hz, 0.09H), 8.14 (d, J = 8.0 Hz, 1H), 9.71 (d, J = 4.4 Hz, 0.91H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 37.9, 38.5, 52.8, 56.5, 61.5, 65.7, 67.1, 70.7, 70.7, 71.3, 72.5, 73.1, 73.7, 76.8, 77.3, 91.6, 99.1, 126.8, 126.9, 127.9, 128.2, 128.6, 128.6, 128.8, 129.7, 129.9, 137.5, 137.7, 138.6, 156.2, 171.3, 171.8; HRMS (ESI, positive): Calcd. for [M($C_{38}H_{48}N_4O_{14}$) + Na]$^+$: m/z = 807.3065; Found: 807.3042.

Synthesis of Z-F_2-Mal: The titled compound was prepared from Z-F_2-$NHNH_2$ (46 mg, 0.10 mmol) and D-maltose monohydtate (38 mg, 0.11 mmol, 1.1 equiv.) in the similar way for Z-F_2-Glc and was obtained in 66% yield (52 mg) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 2.63–2.71 (m, 1H), 2.81–3.09 (m, 4H), 3.12–3.26 (m, 2H), 3.31–3.49 (m, 2H (H_2O peak obscuring)), 3.51–3.65 (m, 2H), 3.66–3.74 (m, 1H), 4.22–4.31 (m, 2H), 4.50–4.57 (m, 2H), 4.92 (m, 2H), 4.99 (d, J = 3.8 Hz, 1H) 5.10 (d, J = 4.3 Hz, 0.81H), 5.45 (d, J = 6.1 Hz, 1H), 5.50 (d, J = 2.9 Hz, 1H), 5.65 (m, J = 3.8 Hz, 1H), 5.70 (d, J = 2.9 Hz, 0.19H), 7.13–7.35 (m, 15H), 7.43 (d, J = 8.8 Hz, 1H), 7.82 (d, J = 8.1 Hz, 0.17H), 8.19 (d, J = 8.4 Hz, 1H), 9.60 (d, J = 4.5 Hz, 0.83H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 38.0, 38.5, 52.8, 56.5, 61.4, 61.6, 65.7, 70.5, 71.2, 72.9, 73.8, 74.1, 76.9, 77.0, 80.6, 91.3, 101.4, 126.8, 126.9, 127.9, 128.2, 128.6, 128.6, 128.8, 129.7, 129.8, 137.5, 137.7, 138.5, 156.2, 170.7, 171.8; HRMS (ESI, positive): Calcd. for [M($C_{32}H_{38}N_4O_9$) + K]$^+$: m/z = 823.2804; Found: 823.2830.

Table S1. Isolated yield and isomeric ratio determined by ^{1}H NMR.

compound	glycoside	yield (%)	isomeric ratio (%)	
			β	α
Z-F_2-Glc	glucose	42	82	18
Z-F_2-Gal	galactose	42	86	14
Z-F_2-Man	mannose	74	79	21
Z-F_2-Lac	lactose	68	88	12
Z-F_2-Cel	cellobiose	37	83	17
Z-F_2-Gen	gentiobiose	68	93	7
Z-F_2-iMal	isomaltose	66	92	8
Z-F_2-Mal	maltose	51	81	19

[a] Isolated yield. [b] Isomeric ratio determined by ^{1}H NMR analysis.

4. Characterization of compounds

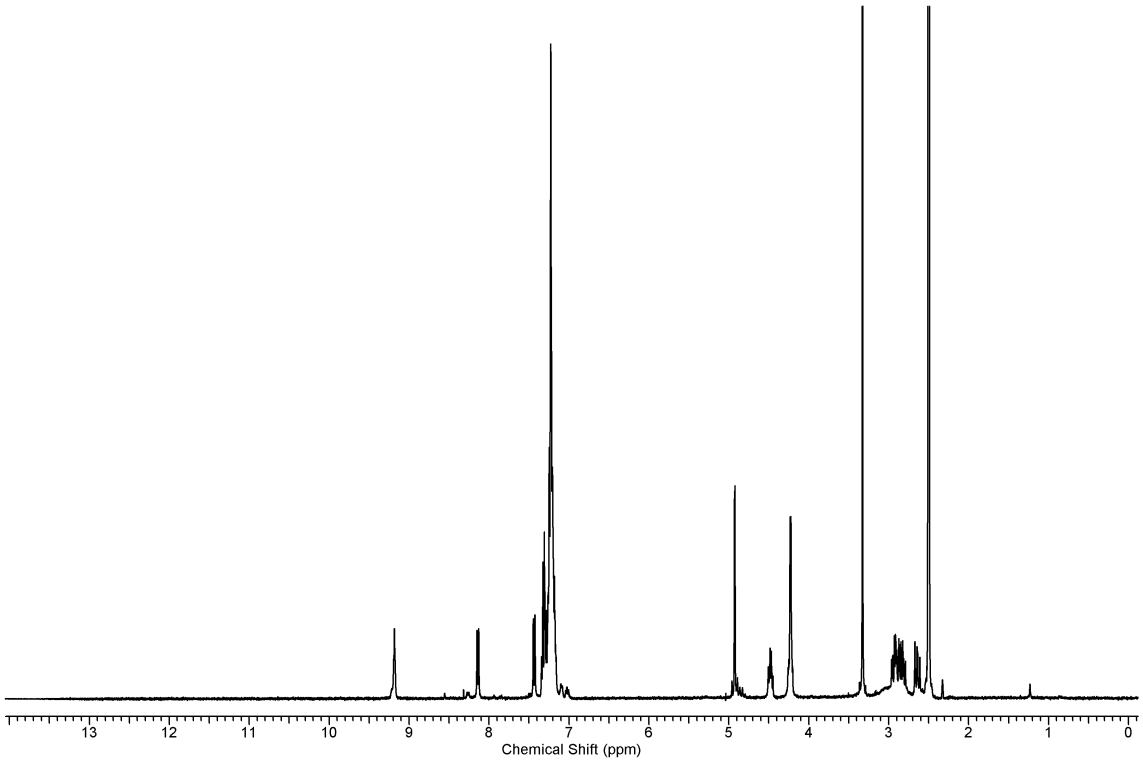


Figure S3. ^{1}H NMR spectrum (400 MHz, DMSO-*d*$_6$) of **Z-F$_2$-NHNH$_2$**.

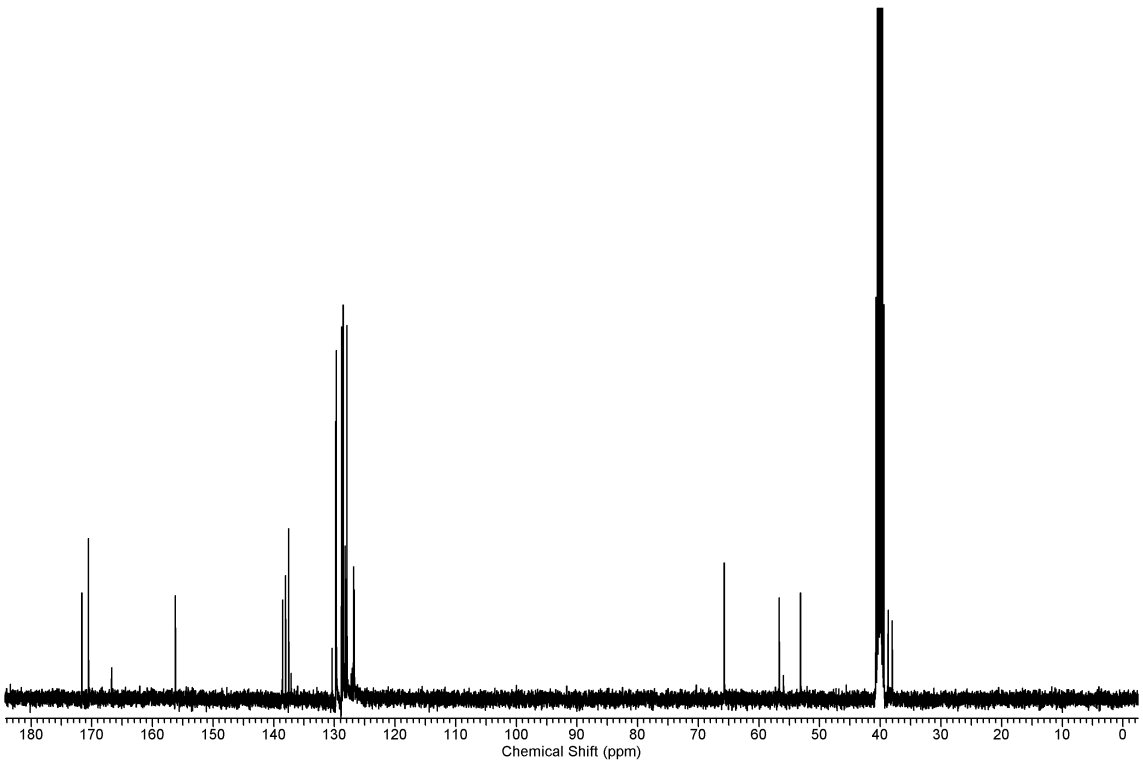


Figure S4. ^{13}C NMR spectrum (100 MHz, DMSO-*d*$_6$) of **Z-F$_2$-NHNH$_2$**.

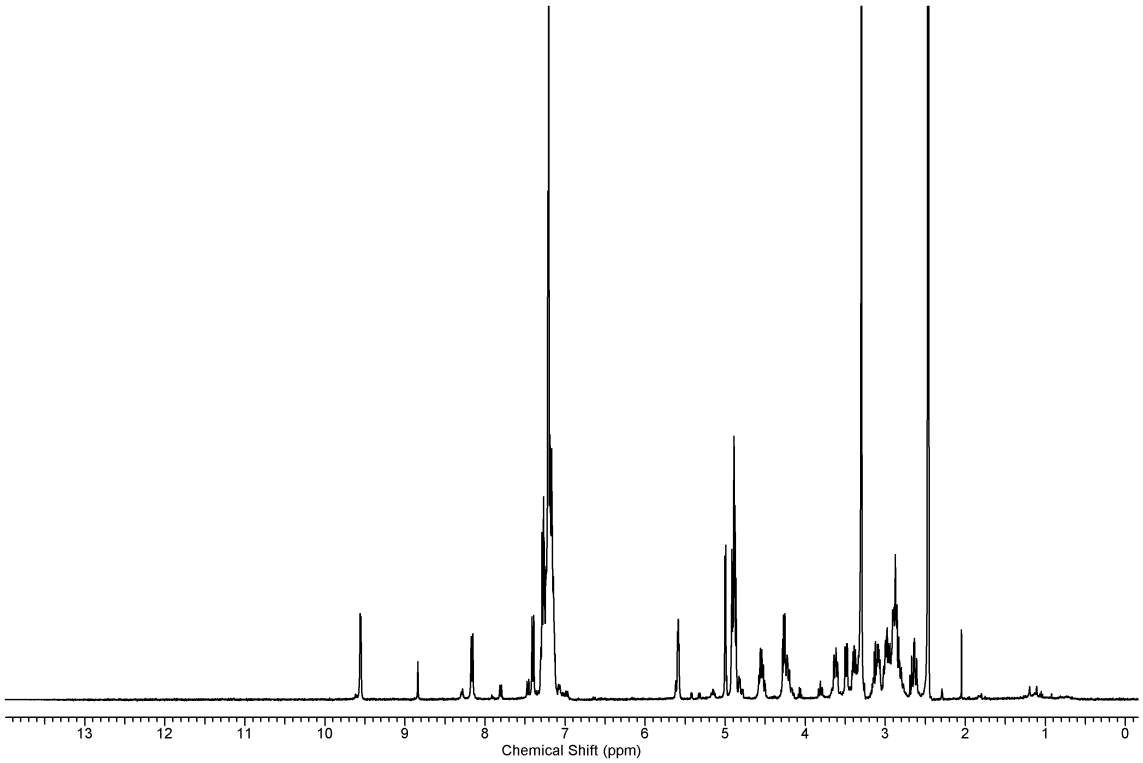


Figure S5. ^{1}H NMR spectrum (400 MHz, DMSO-*d*$_6$) of **Z-F$_2$-Glc**.

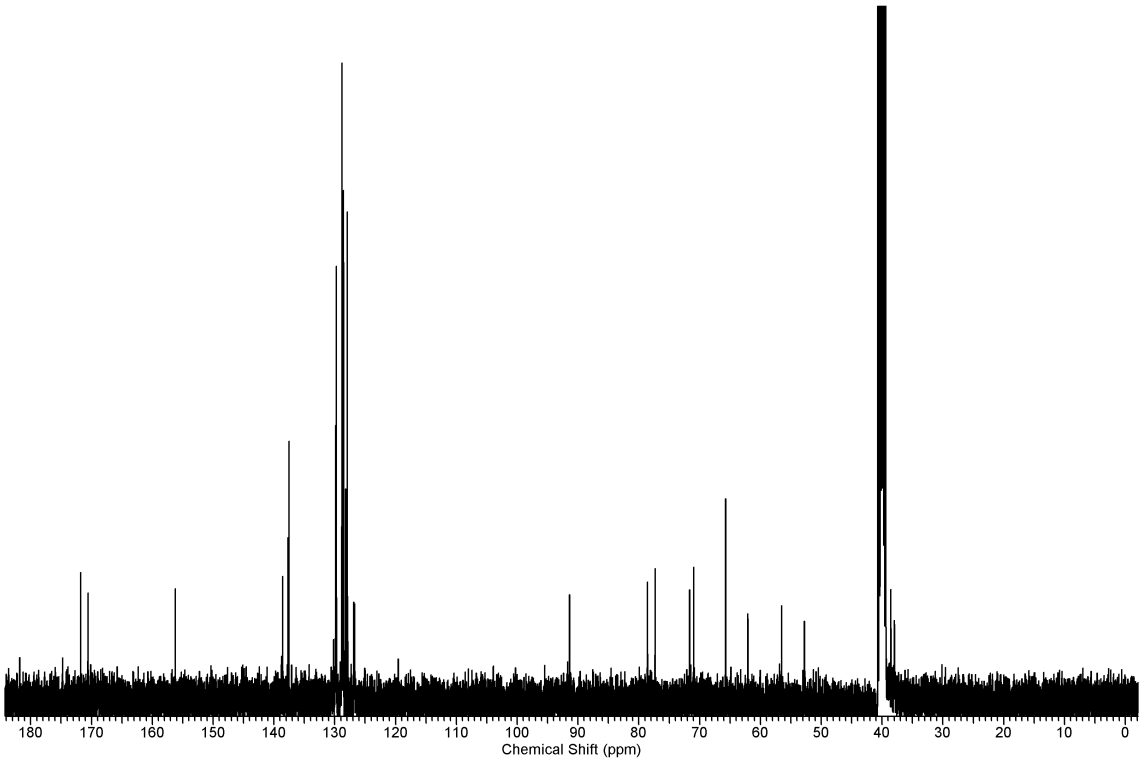


Figure S6. ^{13}C NMR spectrum (100 MHz, DMSO-*d*$_6$) of **Z-F$_2$-Glc**.

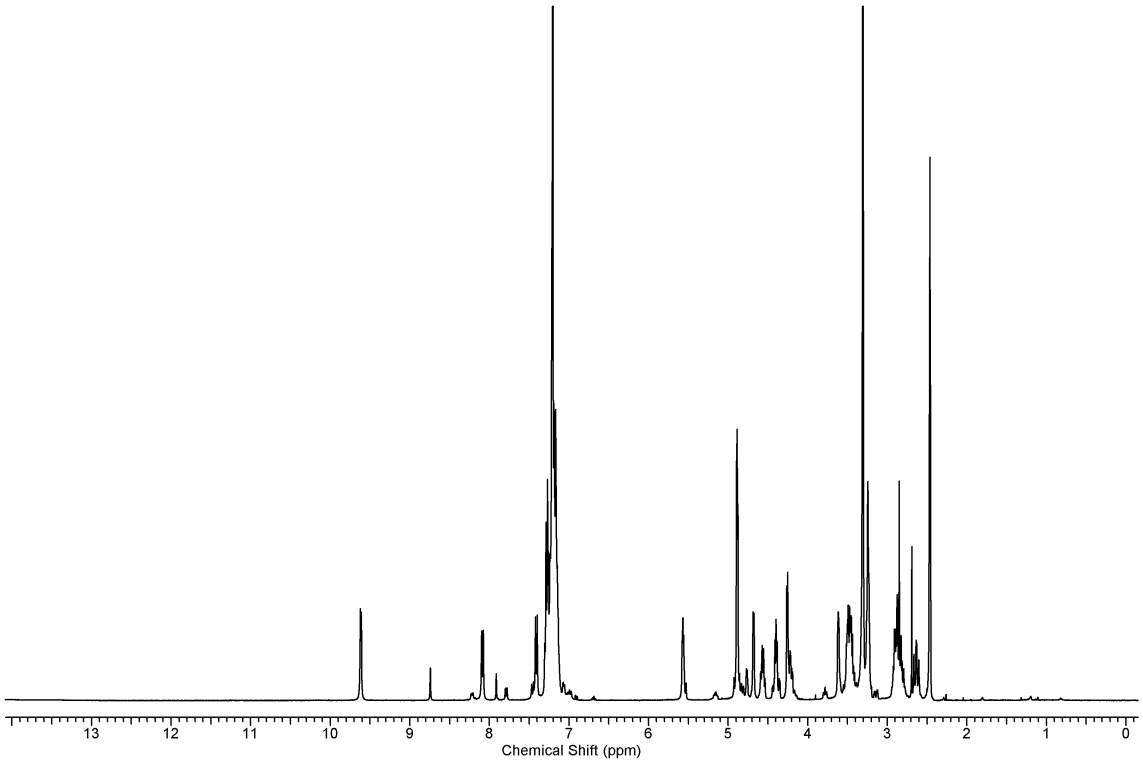


Figure S7.[1]H NMR spectrum (400 MHz, DMSO-d_6) of **Z-F_2-Gal**.

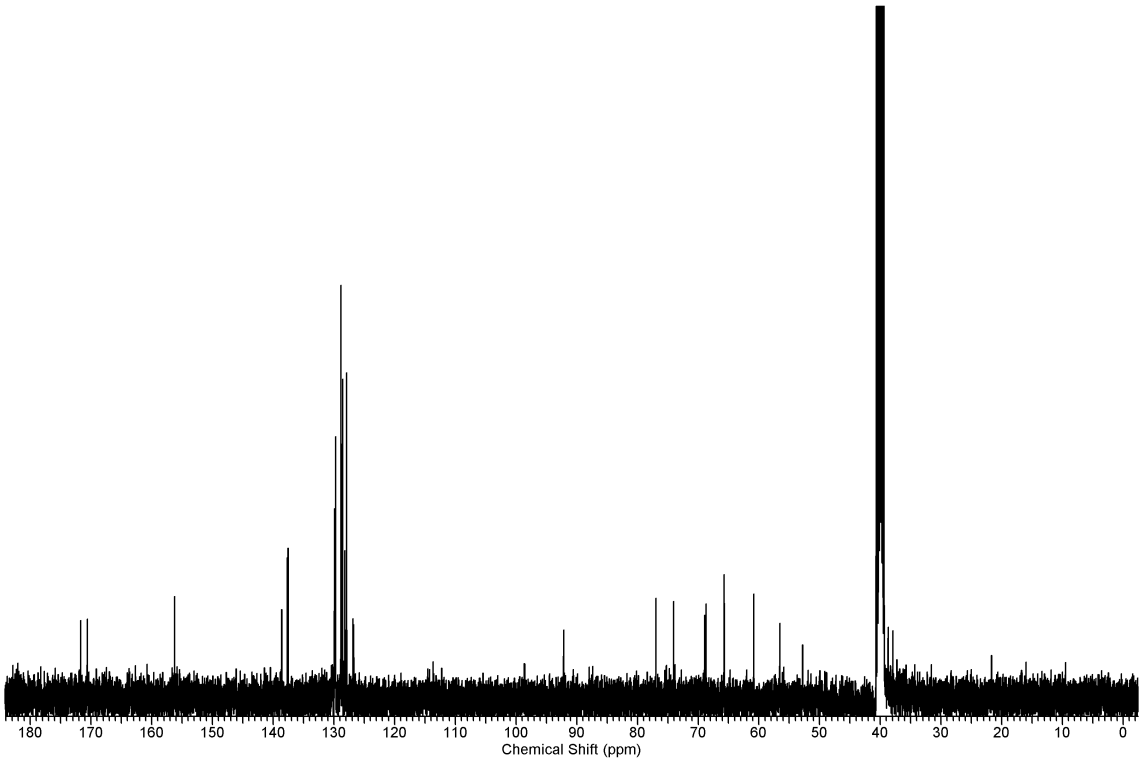


Figure S8. [13]C NMR spectrum (100 MHz, DMSO-d_6) of **Z-F_2-Gal**.

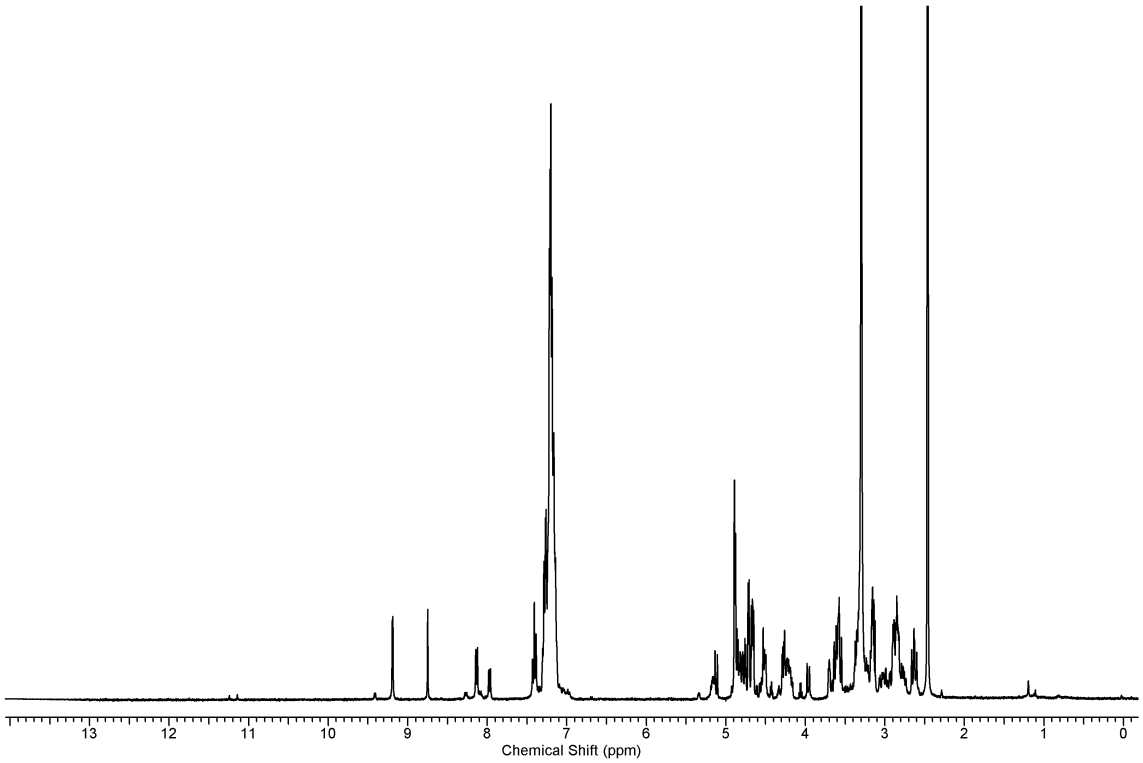


Figure S9. ^{1}H NMR spectrum (400 MHz, DMSO-d_6) of **Z-F$_2$-Man**.

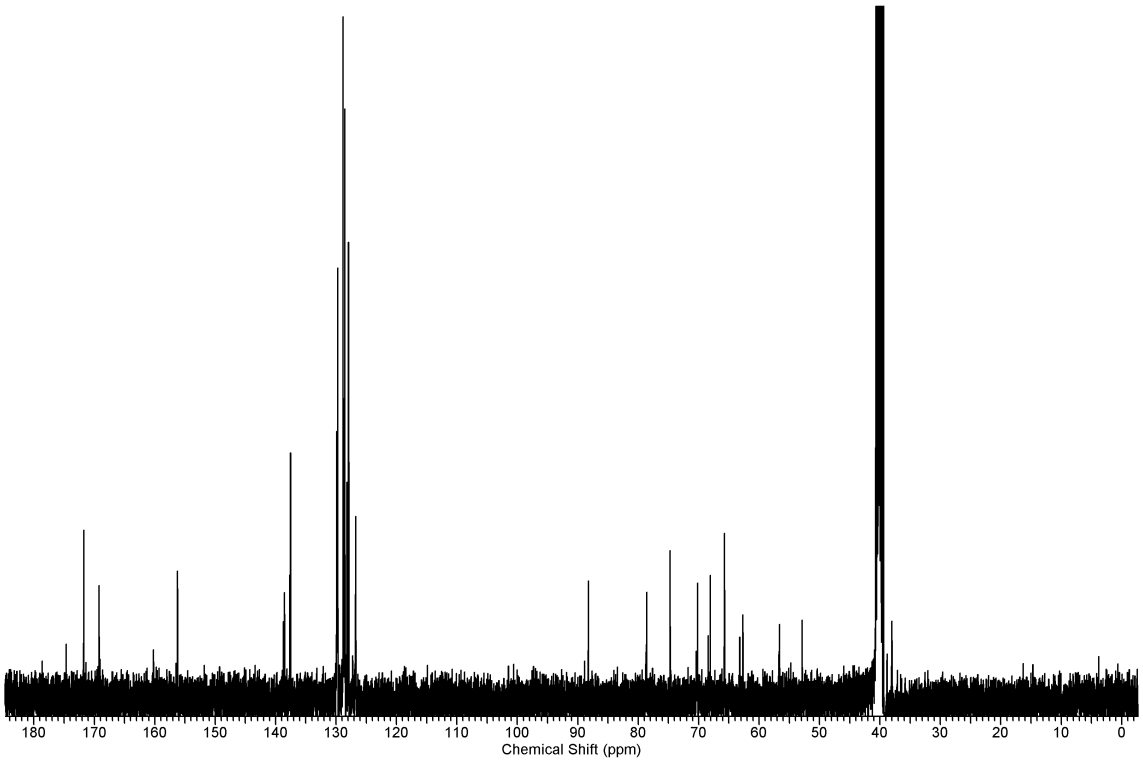


Figure S10. ^{13}C NMR spectrum (100 MHz, DMSO-d_6) of **Z-F$_2$-Man**.

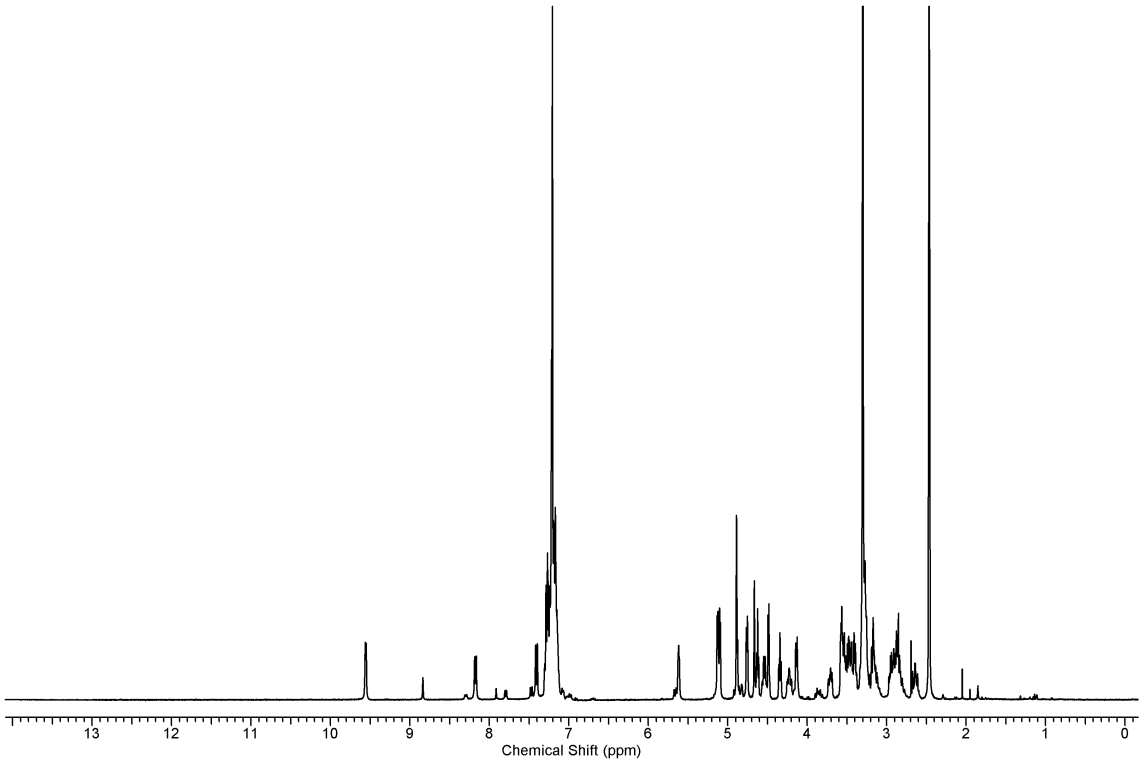


Figure S11. ^{1}H NMR spectrum (400 MHz, DMSO-*d*$_6$) of **Z-F$_2$-Lac**.

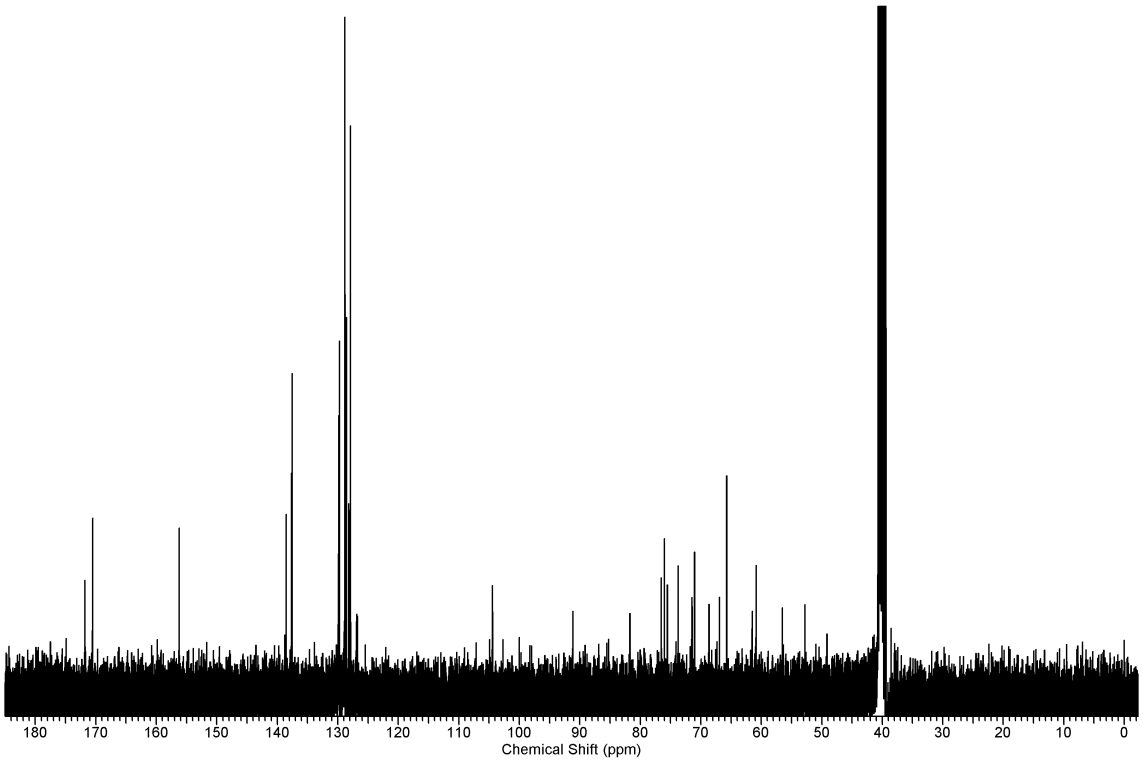


Figure S12. ^{13}C NMR spectrum (100 MHz, DMSO-*d*$_6$) of **Z-F$_2$-Lac**.

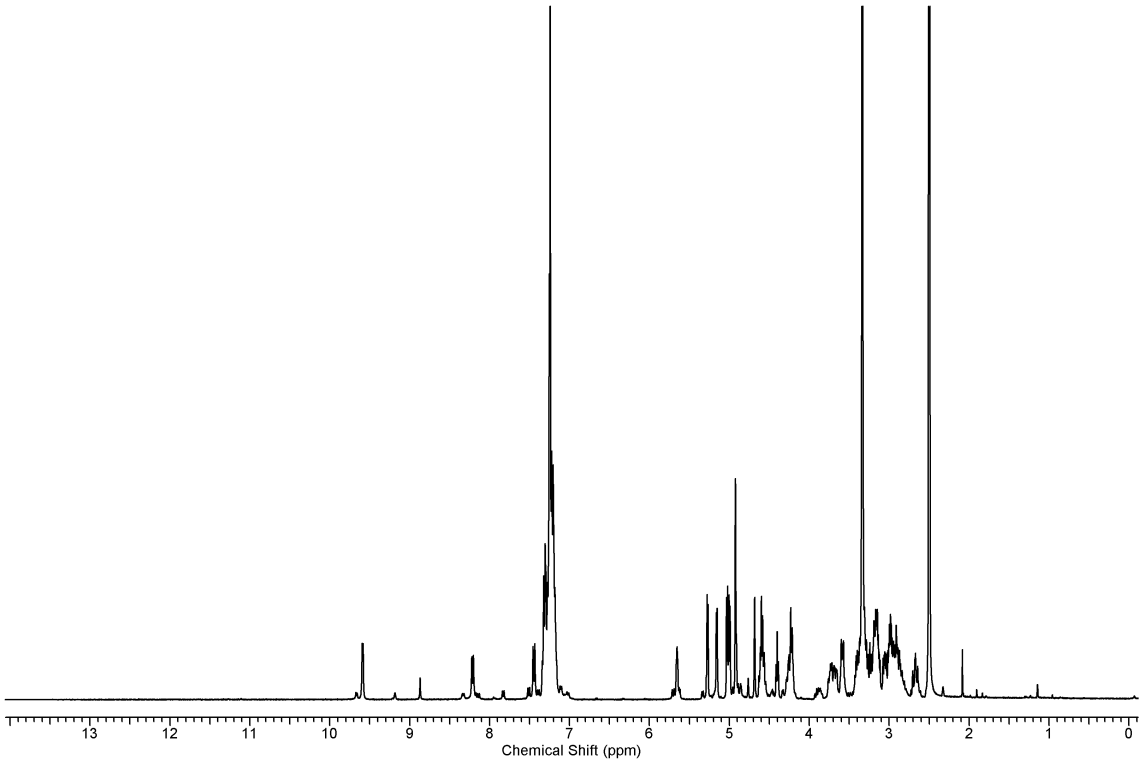


Figure S13. ^{1}H NMR spectrum (400 MHz, DMSO-*d*$_6$) of **Z-F$_2$-Cel**.

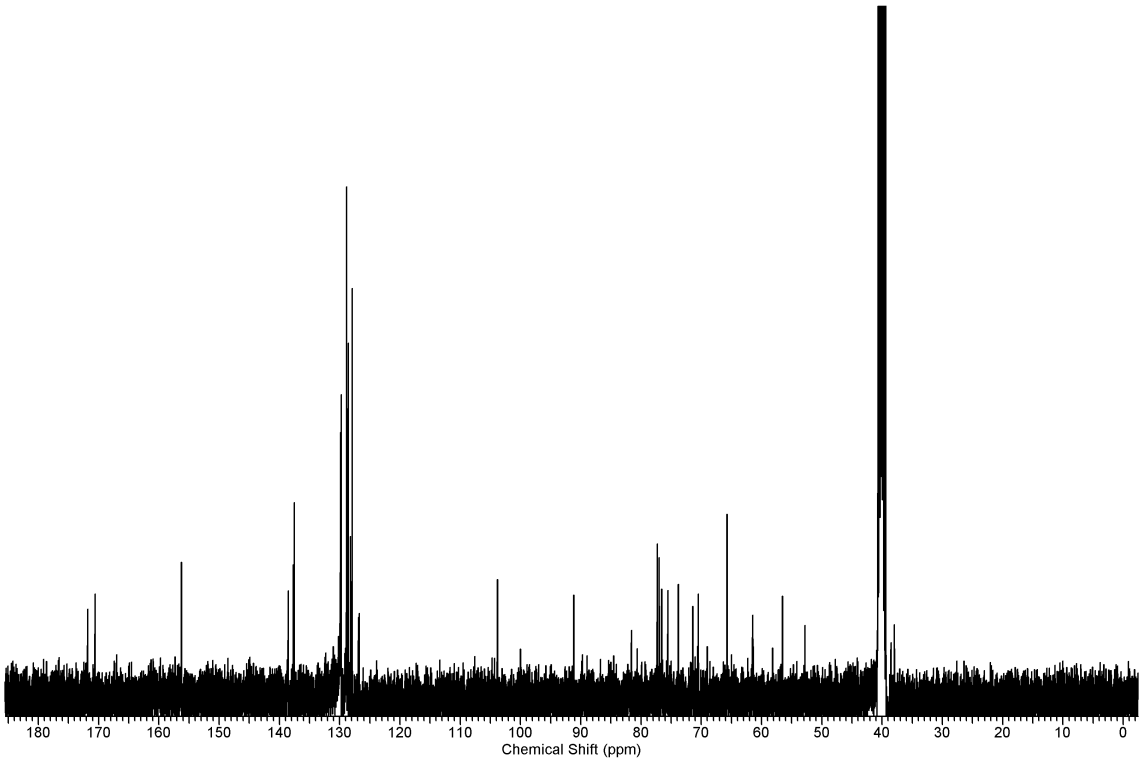


Figure S14. ^{13}C NMR spectrum (100 MHz, DMSO-*d*$_6$) of **Z-F$_2$-Cel**.

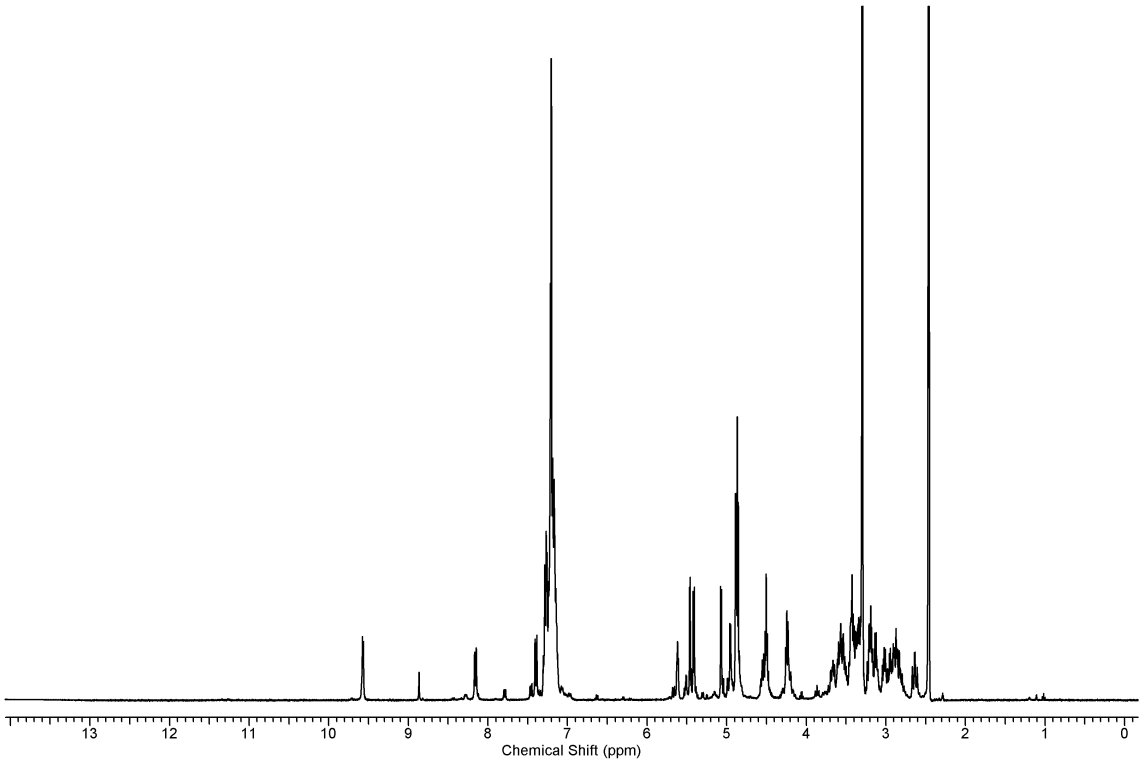


Figure S15. ^{1}H NMR spectrum (400 MHz, DMSO-*d*$_6$) of **Z-F$_2$-Mal**.

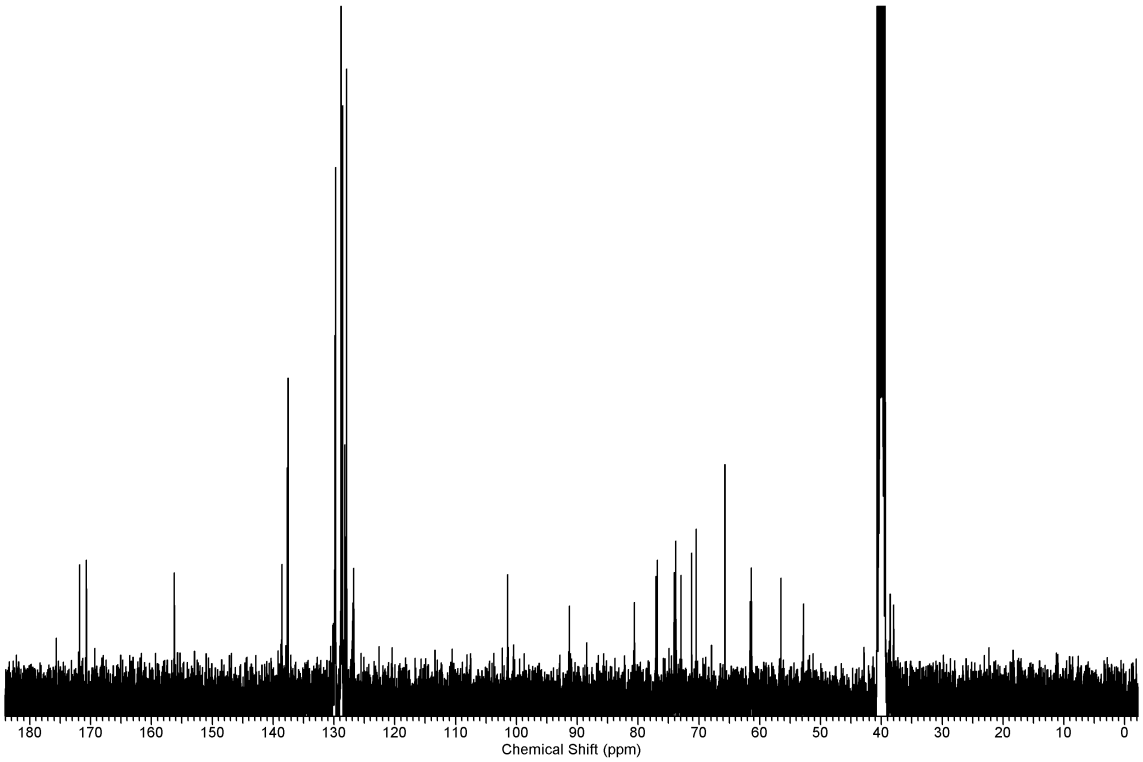


Figure S16. ^{13}C NMR spectrum (100 MHz, DMSO-*d*$_6$) of **Z-F$_2$-Mal**.

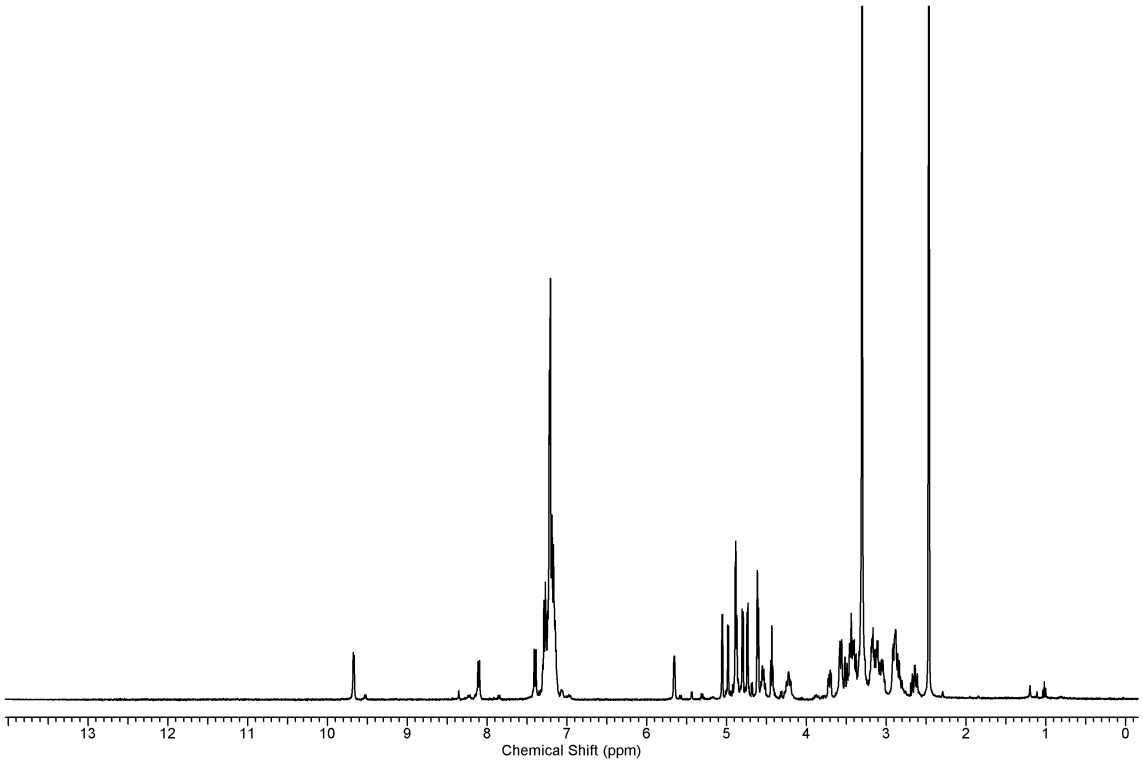


Figure S17. ^{1}H NMR spectrum (400 MHz, DMSO-d_6) of **Z-F$_2$-iMal**.

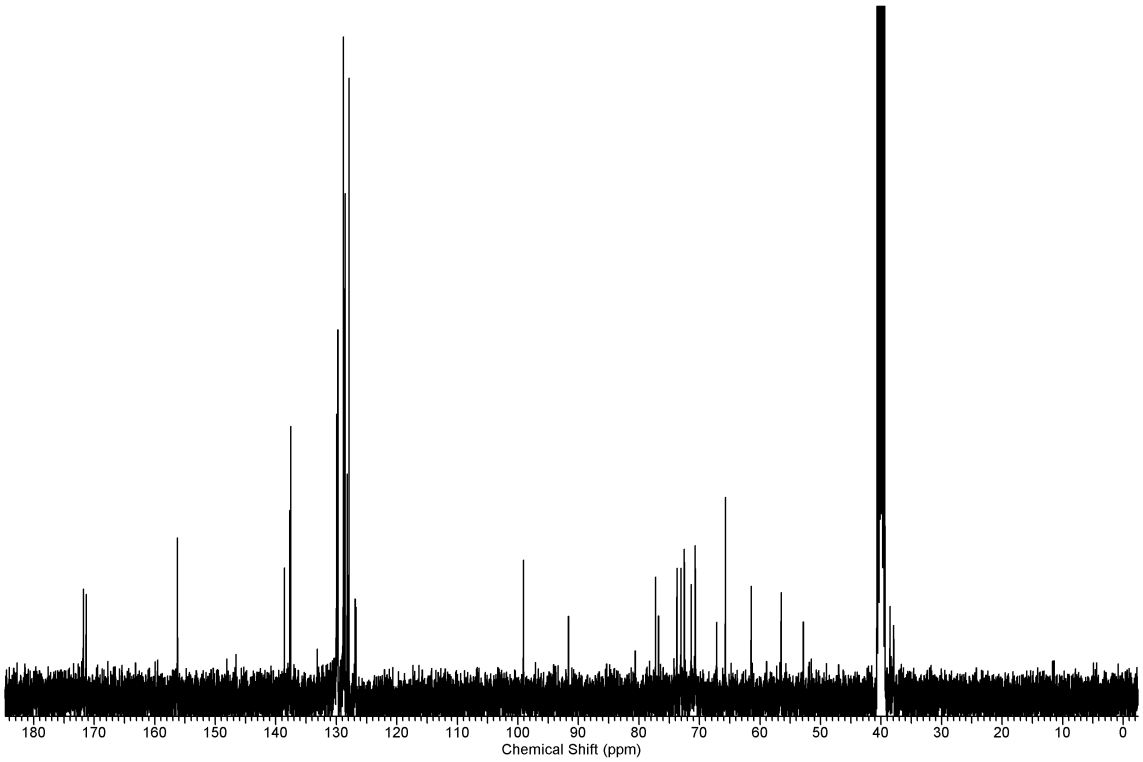


Figure S18. ^{13}C NMR spectrum (100 MHz, DMSO-d_6) of **Z-F$_2$-iMal**.

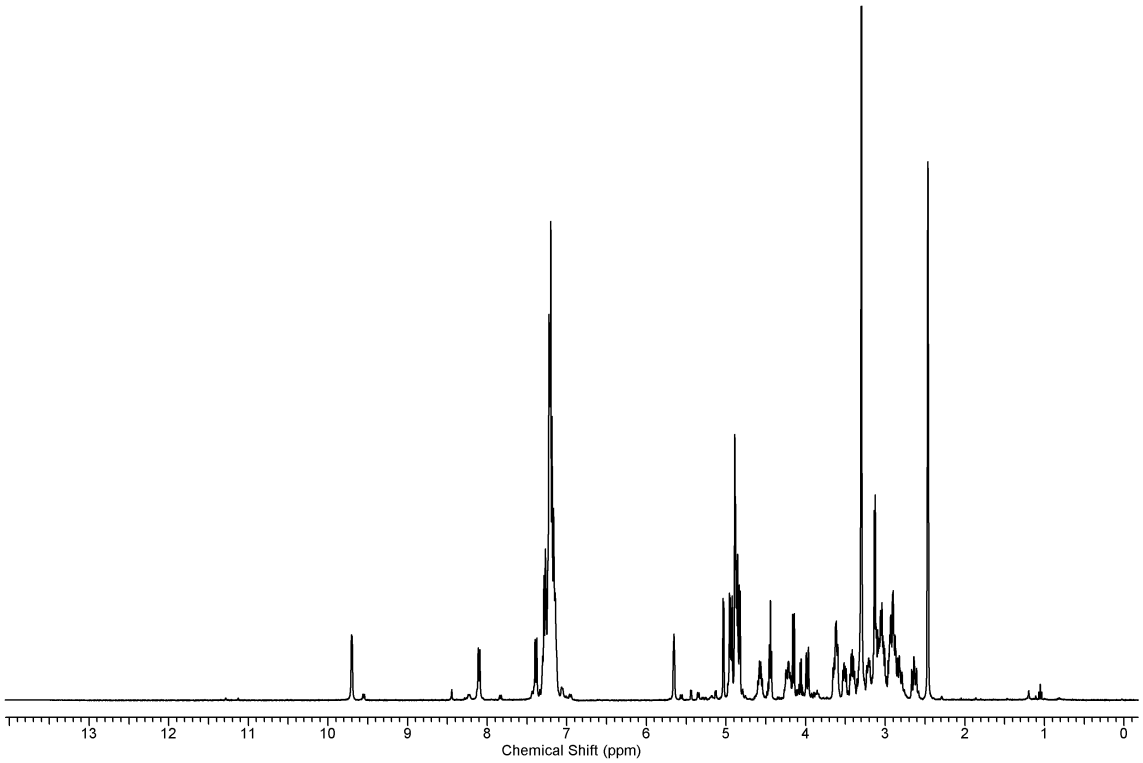


Figure S19. ^{1}H NMR spectrum (400 MHz, DMSO-d_6) of **Z-F$_2$-Gen**.

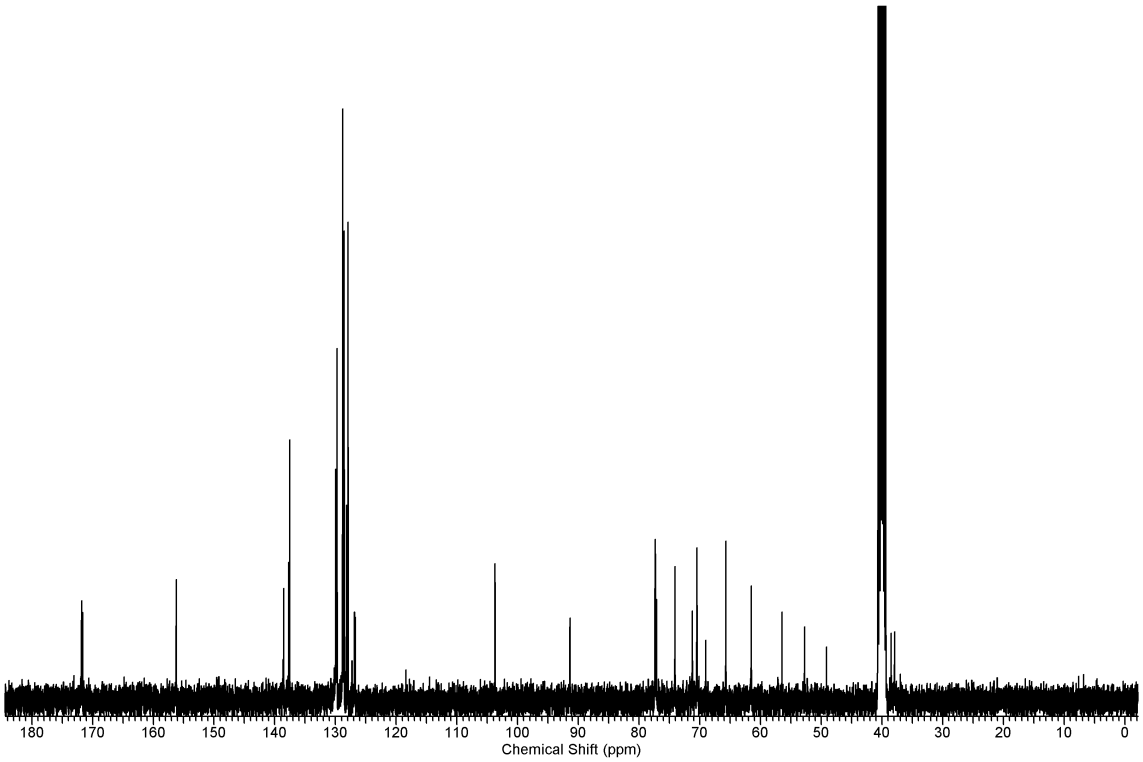


Figure S20. ^{13}C NMR spectrum (100 MHz, DMSO-d_6) of **Z-F$_2$-Gen**.

5. References

[S1] R. R. Hill, D. Birch, G. E. Jeffs and M. North, *Org. Biomol. Chem.,* 2003, **1**, 965–972.