Supporting Information

of

Three-dimensional protein assembly directed by orthogonal non-covalent interactions

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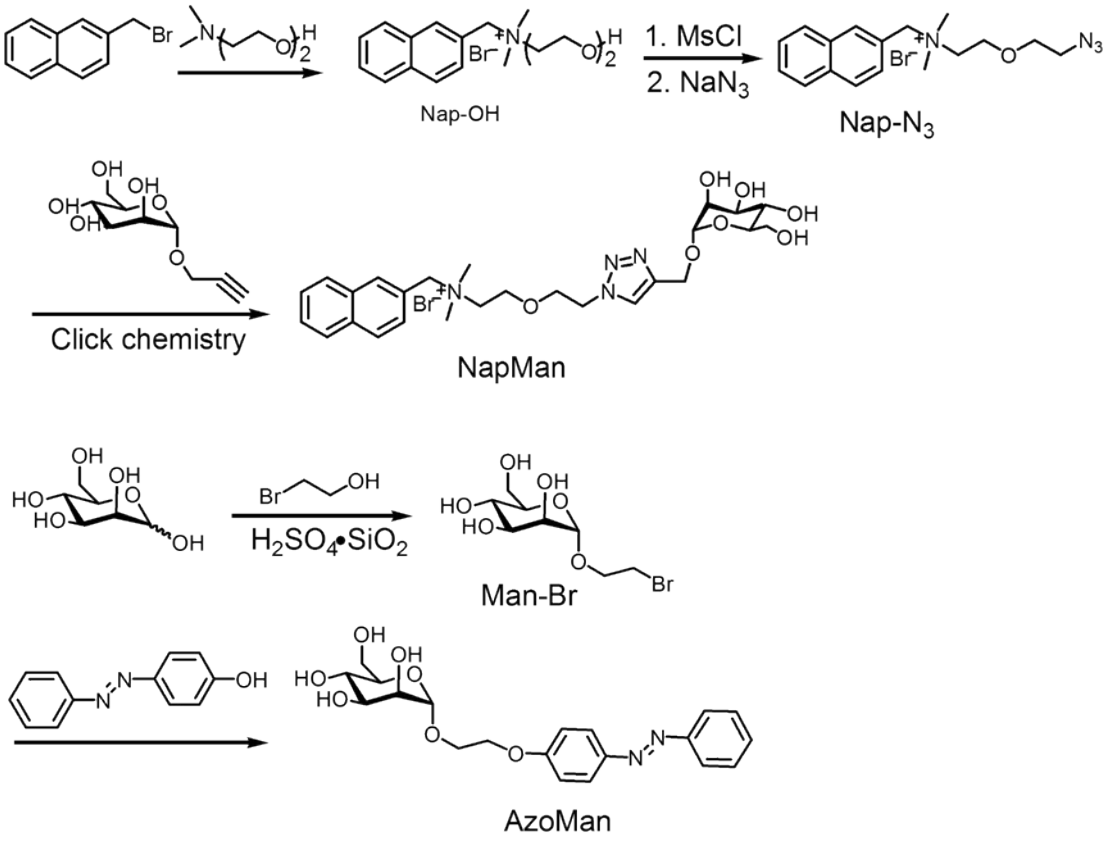
Sample preparation. The small molecules were synthesized and characterized as described in supporting information (Scheme S1 and Figure S10-16). ConA protein was purchased from Sigma-Adrich. All chemicals and proteins are used as received. The buffer solution was prepared with HEPES {4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid} containing 20 mM HEPES, 5 mM $CaCl_2$, 5 mM $MnCl_2$ and 40 mM NaCl.

Characterization. Nuclear magnetic resonance (NMR) was taken by AVANCE III HD 400 MHz of Bruker BioSpin International. UV-vis absorption spectra were recored by Shimadzu UV-2550 spectrophotometer. Isothermal titration calorimetry (ITC) experiments were conducted on a MicroCal VP-ITC system at 20.00±0.01°C. Transmission electron microscopy (TEM) was performed on a JEOL JEM-2100 at 200 kv and by Tecnai G2 20 TWIN at 200 kv. The samples were prepared by dropping sample onto a copper grid and then blotting the excess solvent. Then the samples were subsequently stained with a 1 wt% uranyl acetate. Dynamic light scattering (DLS) was taken by Malvern Nano Sinstrument (Malvern, UK).



Scheme S1. Synthetic procedures of **NapMan** and **AzoMan** used in this paper.

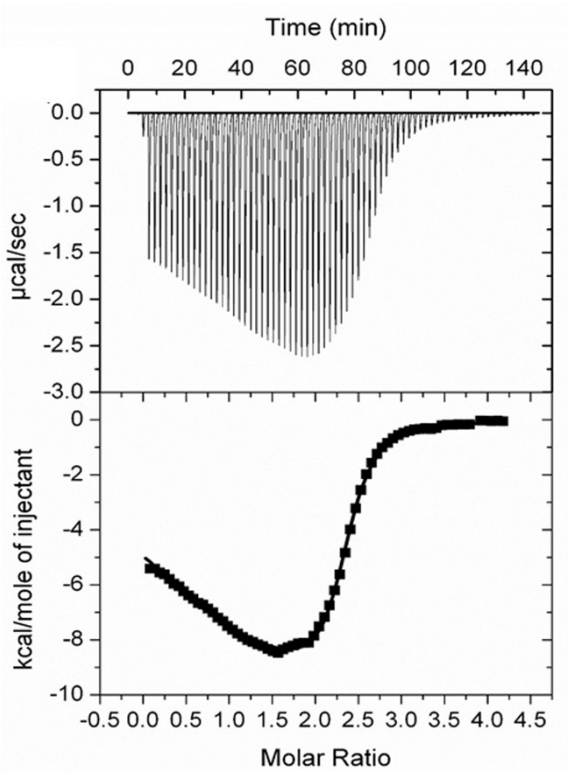


Figure S1. ITC results of raw and integrated data for titration of CB[8] (0.1 mM) to **NapMan** (2.0 mM) in aqueous solution at 20°C.

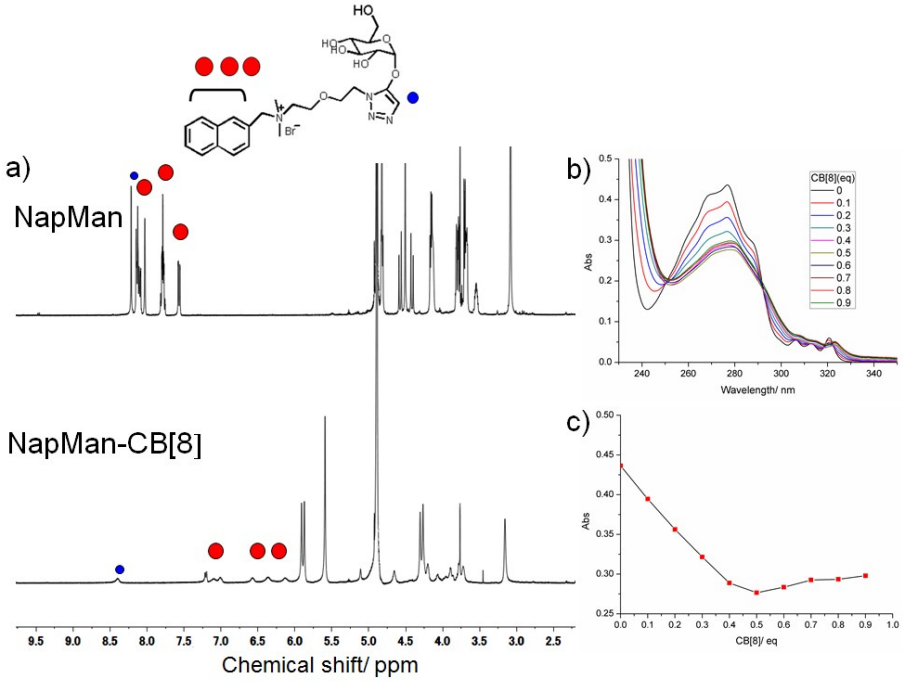


Figure S2. (a) ^{1}H NMR spectra of **NapMan** and the mixture of **NapMan**/CB[8] in D_2O. (b) UV-vis spectra of titration of CB[8] into an aqueous solution of **NapMan** (0.1 mM). (c) UV-vis absorbance at 278 nm was ploted against the equivalent of CB[8].

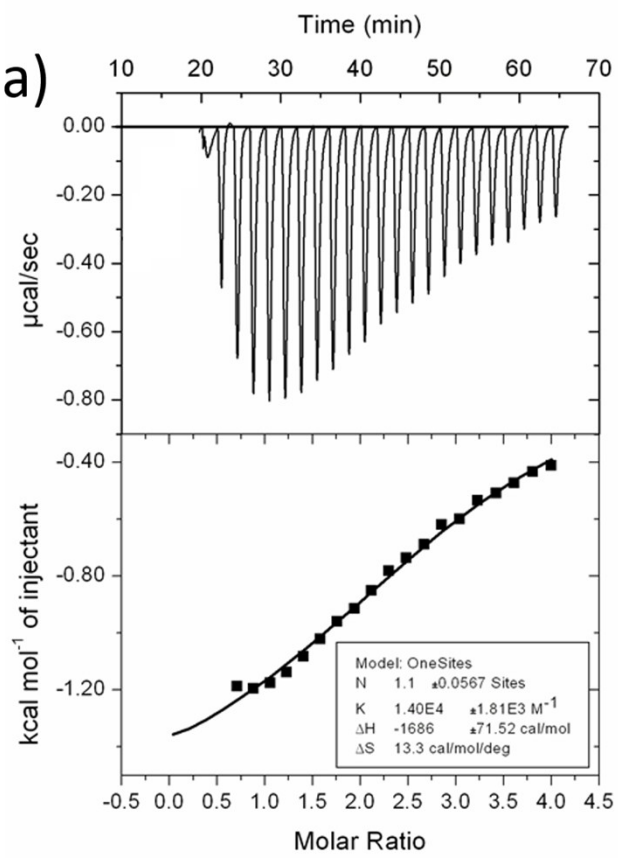


Figure S3. ITC data for the titration of the mixture of CB[8] (0.5 mM) with **NapMan** (1 mM) to ConA (0.05 mM) in aqueous solution at 20°C.

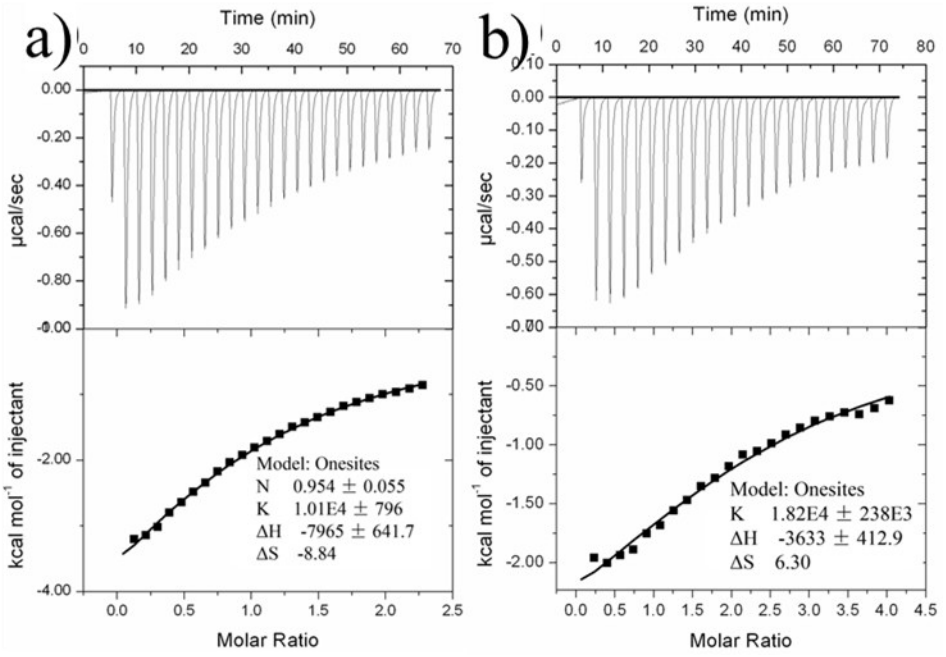


Figure S4. ITC results of raw and integrated data for titration of (a) **AzoMan** (1.0 mM) to ConA (0.08 mM) and (b) mixture of 1.0 mM CB[8] and 0.5 mM **DDPS** to the mixture of 0.05 mM ConA and 0.05 mM **AzoMan** in aqueous solution at 20 °C.

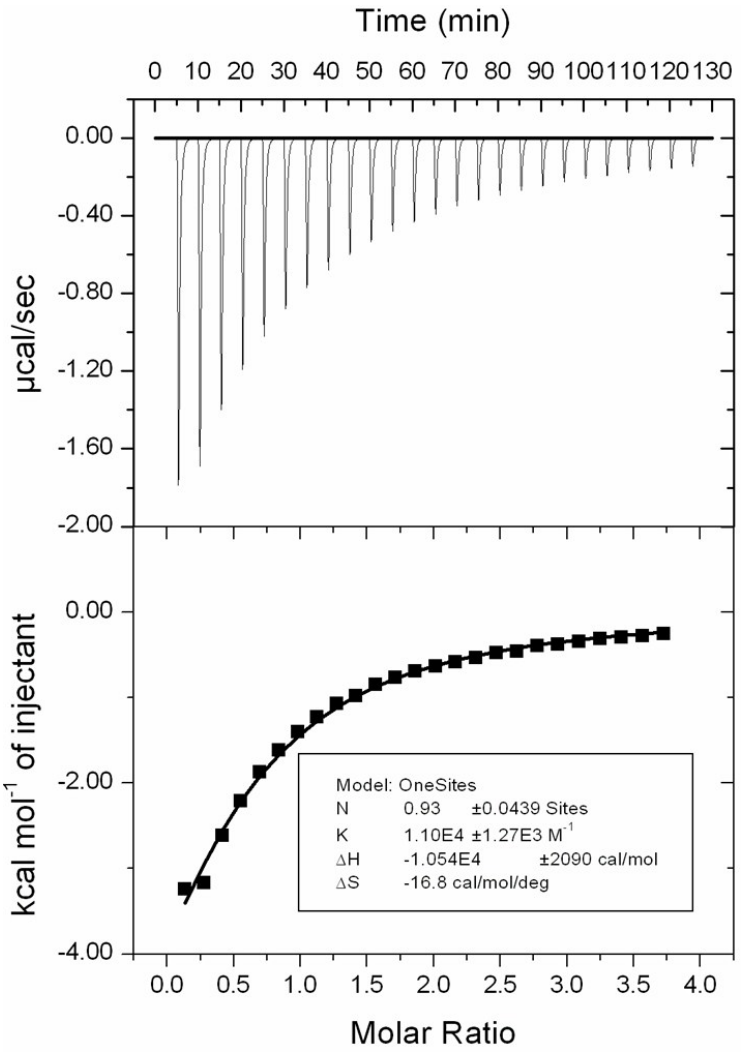


Figure S5. ITC data for the titration of the mixture of CB[8] (1.0 mM) and 0.5 mM **DDPS** to **AzoMan** (0.067 mM) in aqueous solution at 20°C.

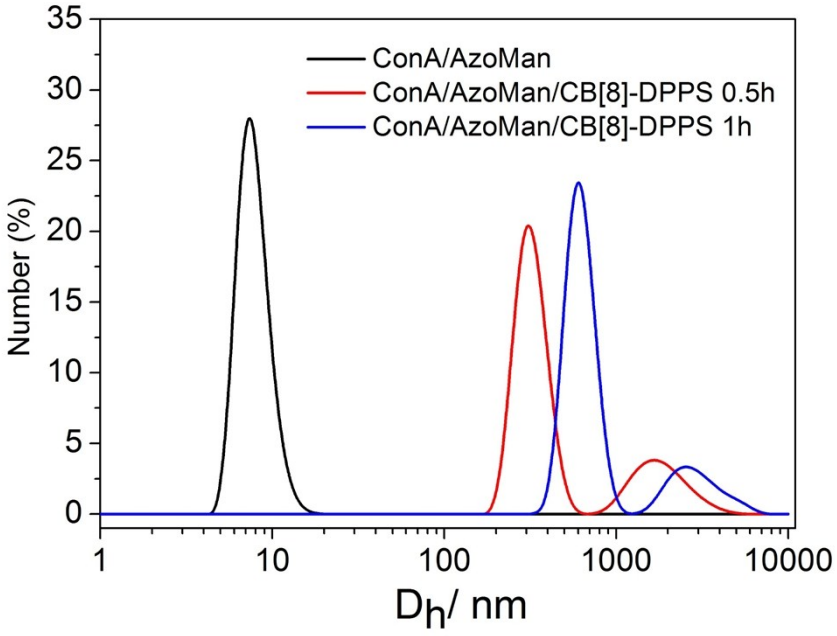


Figure S6. DLS results of ConA(0.2 mM)/**AzoMan**(0.2 mM) solution after addition of same amount of CB[8]/DDPS at different time interval.

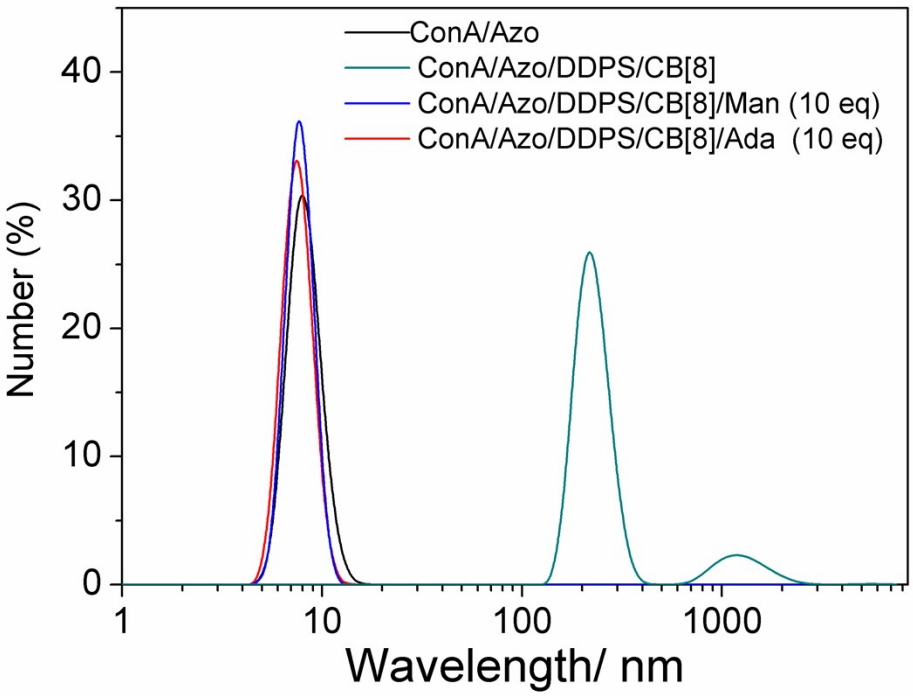


Figure S7. DLS results of ConA (0.2 mM)/**AzoMan** (0.2 mM)/CB[8] (0.2 mM)/DDPS (0.1 mM) assembly solution after the addition of 10 eq free manose or Ada.

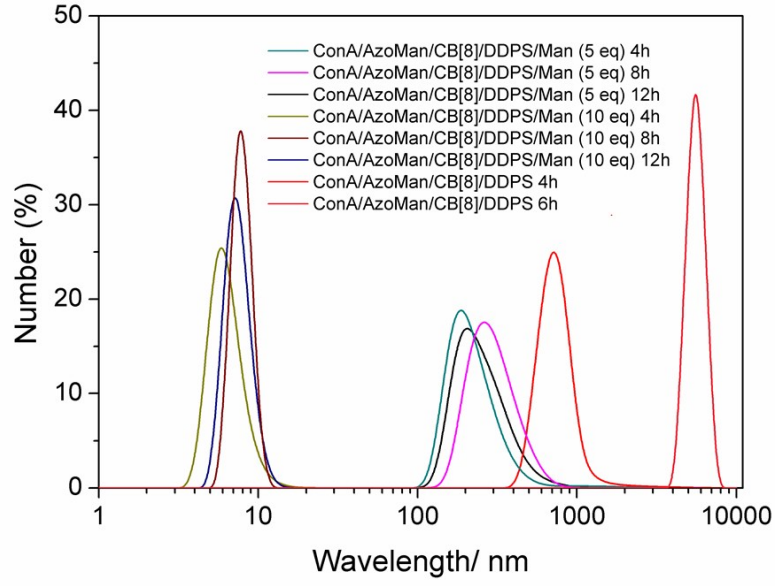


Figure S8. DLS results of ConA(0.2 mM)/**AzoMan**(0.2 mM)/ CB[8] (0.2 mM)/DDPS (0.1 mM) with different amount of free mannose (Man) amount at different time interval.

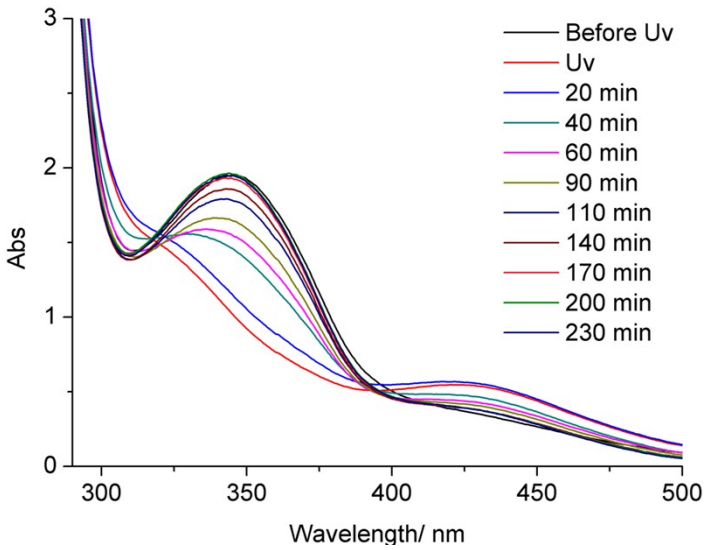


Figure S9. UV-Vis spectra of ConA (0.1 mM)/**AzoMan**/CB[8]/**DDPS** (1:1:1:0.5) before and after 30 min irradiation at 465 nm as well as the time dependent inter-conversion of cis- to trans-**AzoMan** under ambient light.

Synthetic procedures and characterizations:

Synthesis of Nap-OH. 1 g (4.5 mmol) 2-(Bromomethyl)naphthalene and 0.8 g (6 mmol) 2-[2-(Dimethylamino)ethoxy]ethanol were mixed together in 20 mL acetonitrile and then refluxed at 80 °C under Ar for 24 h. After reaction was finished, the solution was evaporated, then the raw product was purified by column chromatography with MeOH/DCM= 1:20 (*v*/*v*) to give compound **1** (1.4 g , 87%) as colourless oil. ^{1}H NMR (400 MHz, CD_3OD) δ 8.17 (s, 1H), 7.96-7.94 (t, 2H), 7.90-7.86 (m, 1H), 7.65-7.63 (m, 1H), 7.57-7.51 (m, 2H), 4.82 (s, 1H), 4.00-3.98 (m, 2H), 3.70-3.60 (m, 6H), 3.15 (s, 6H).

Synthesis of Nap-N_3. 1.3 g (3.6 mmol) Nap-OH and 0.73 g (7.2 mmol) triethylamine were dissoved in 30 mL dry DMF and then 0.9 g methylsufonyl chloride (MsCl) dissoved

in 5 mL dry DMF was added into the above solution in iced water under Ar. The mixture was kept stirring at room temperature for 2 h, then the solvent was removed under rotary evaporator. The oily raw product without further purification was then directly dissolved in 50 mL ethanol/ water (*v*:*v* = 5:1), and then 1.0 g (15 mmol) NaN_3 was added. After refluxing about 24 h, the solution was removed by filtration and the solvent was removed by rotary evaporator. The raw product was purified by column chromatography with MeOH/DCM = 25:1 (*v*/*v*) to give colourless oil (0.9 g, 73%). ^{1}H NMR (400 MHz, CD_3OD) δ 8.13 (s,1H), 7.92-7.85 (m, 3H), 7.6-7.59 (m, 1H), 7.54-7.48 (m, 2H), 4.81 (s, 1H), 3.99-3.98 (t, 2H), 3.68-3.63 (m, 4H), 3.12 (s, 6H).

Synthesis of NapMan. 0.25g (0.67 mmol) Nap-N_3, 0.14 g (0.67 mmol) Alkynyl mannose and 26 mg (0.15 mmol) *N*,*N*,*N*',*N*'',*N*''-Pentamethyldiethylenetriamine (PMDTA) were added into 5 mL DMF and then after bubbling Ar for 15 min, 11 mg (0.075 mmol) CuBr was added under Ar within 10 min. The mixture was heated to 35°C overnight. Then the solvent was removed by evaporation and the raw product was purified by column chromatography with CH_3CN/H_2O from 10:1 (*v*/*v*) to 6:1 (*v*/*v*) to give product (210 mg, 39%) as white solid. ^{1}H NMR (400 MHz, D_2O) δ 8.11 (s,1H), 8.05-7.99 (m, 3H), 7.93 (s, 1H), 7.70-7.65 (m, 2H), 7.48-7.45 (m, 1H), 4.82-4.81 (d, 1H), 4.72 (s, 2H), 4.49-4.30 (m, 4H), 4.07-4.05 (t, 4H), 3.73-3.66 (m, 3H), 3.61-3.57 (m, 4H), 2.98 (s, 6H). ^{13}C NMR (400 MHz, D_2O) δ 165.0, 133.6, 132.5, 129.1, 128.8, 128.0, 127.8, 124.5, 99.4, 72.9, 70.1, 69.9, 68.5, 66.6, 64.4, 63.1, 60.8, 50.6, 50.4, 37.0, 31.4.

Synthesis of Man-Br. Man-Br was synthesied according to the literature.[1] D-mannose was dried under vaccum at 50 °C before use. The dried D-mannose 3.6 g (20 mmol) and 0.4 g $SiO_2 \cdot H_2SO_4$ were mixed together and then heated to 60 °C overnight under Ar.

Finally the raw product was purified by column chromatography with DCM/CH_3OH = 6:1 (*v*/*v*) to give light yellow oil (2.7g, 47%). ^1H NMR (400 MHz, CD_3OD) δ 4.96-4.94 (d,1H), 4.07-4.03 (m, 1H), 4.01-3.98 (m, 1H), 3.96-3.88 (m, 2H), 3.87-3.83 (m, 2H), 3.69-3.64 (m, 3H). ^{13}C NMR (400 MHz, D_2O) 99.7, 73.0, 70.5, 69.9, 67.6, 66.7, 61.1, 31.3.

Synthesis of AzoMan. 0.5g (2.5 mmol) 4-(Phenylazo)phenol, 0.5g (1.7 mmol) Man-Br and 1.0 g K_2CO_3 (7 mmol) were added into 10 mL DMF and then the mixture was heated at 70°C for 24 h under Ar. After the reation, the product was purified by column chromatography with DCM/CH_3OH = 10:1 (*v*/*v*) to give yellow solid (0.4 g, 40%). ^1H NMR (400 MHz, CD_3OD) δ 7.93-7.86 (m, 4H), 7.56-7.48 (m, 3H), 7.13-7.11 (d, 2H), 4.92 (d, 1H), 4.30-4.28 (t, 2H), 4.12-4.09 (m, 1H), 3.92-3.87 (m, 2H), 3.84-3.80 (m, 1H), 3.78-3.73 (m, 2H), 3.69-3.67 (m, 2H).

DDPS, CB[8] and Alkynyl mannose were synthsized in our previous literature[2,3].

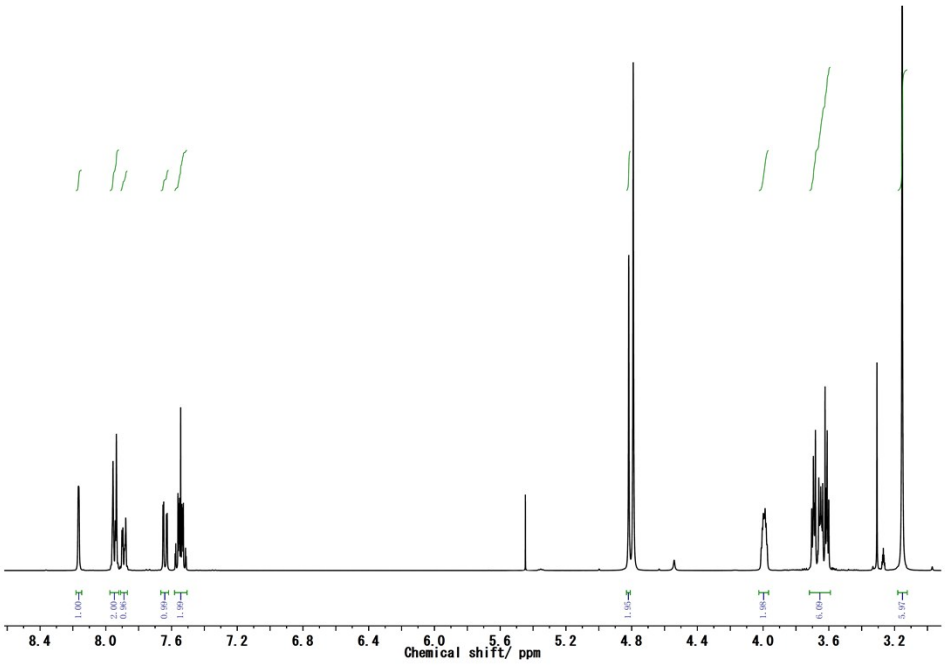


Figure S10. ^{1}H NMR of **NapMan** in CD_3OD.

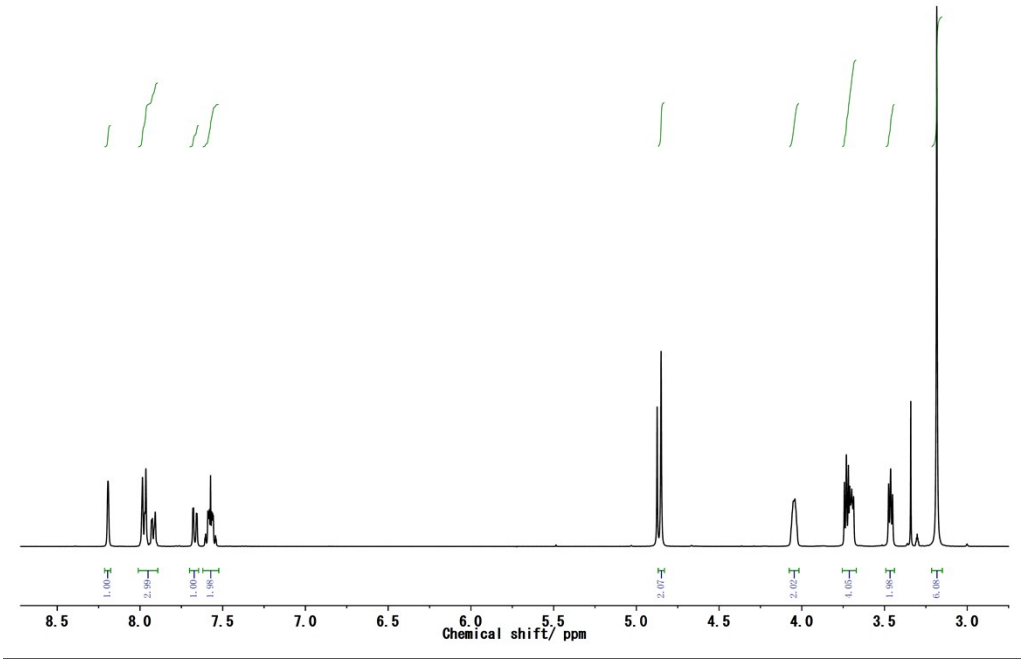


Figure S11. ^{1}H NMR of **NapMan** in CD_3OD.

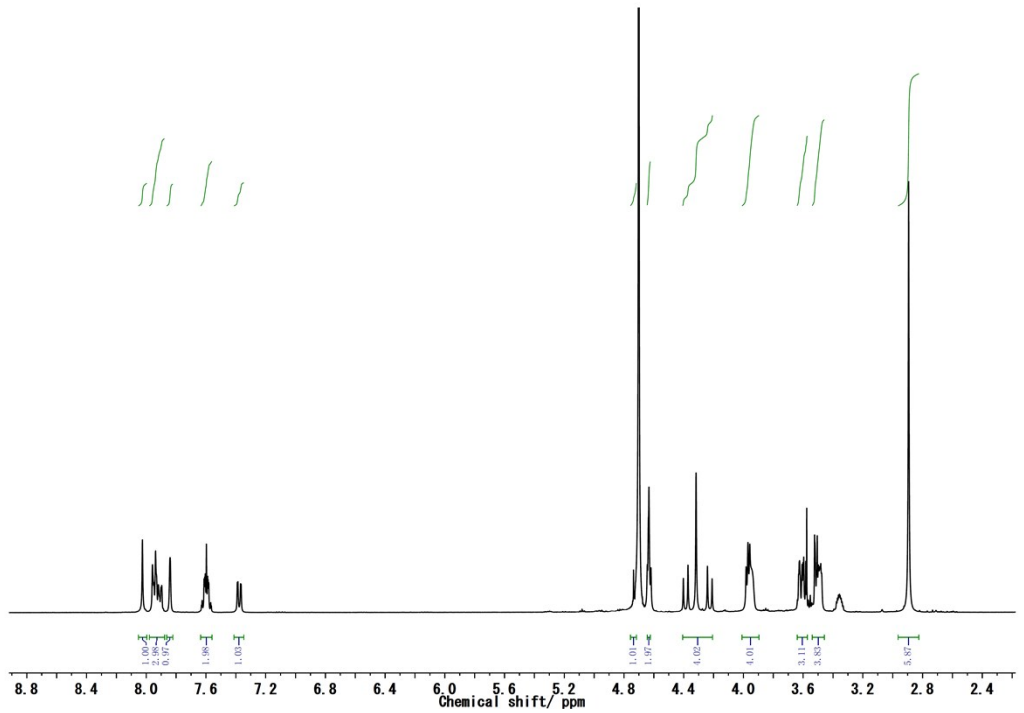


Figure S12. ^{1}H NMR of NapMan in D_2O.

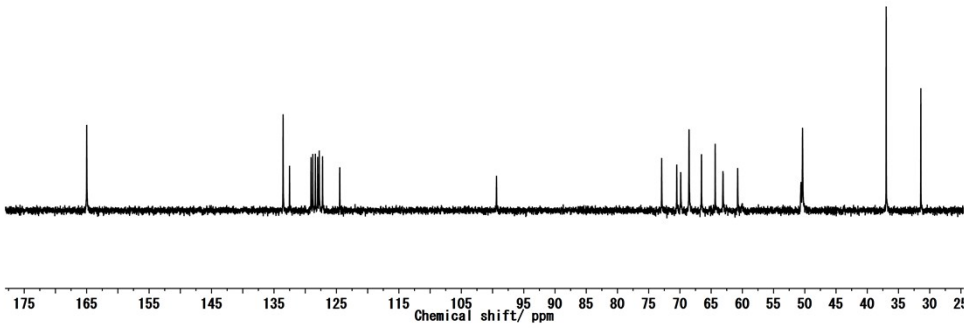


Figure S13. ^{13}C NMR of **NapMan** in D_2O.

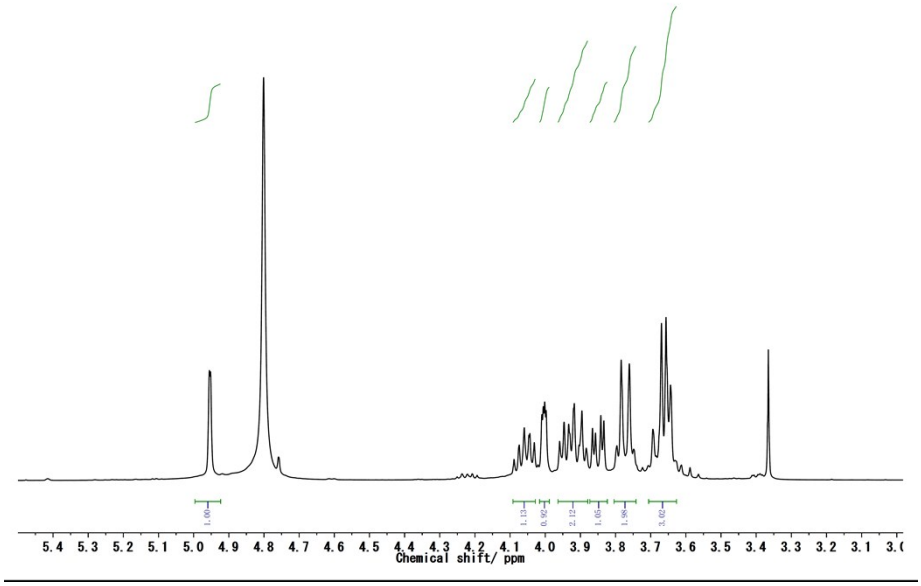


Figure S14. ^{1}H NMR of Man-Br in CD_3OD.

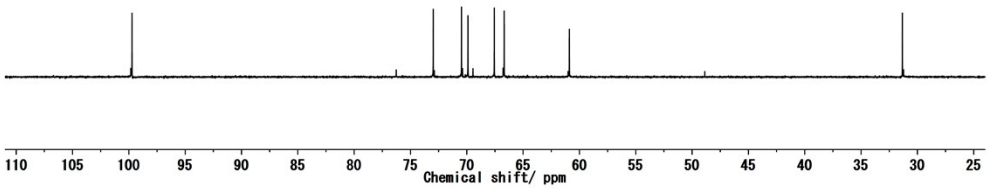


Figure S15. ^{13}C NMR of Man-Br in D_2O.

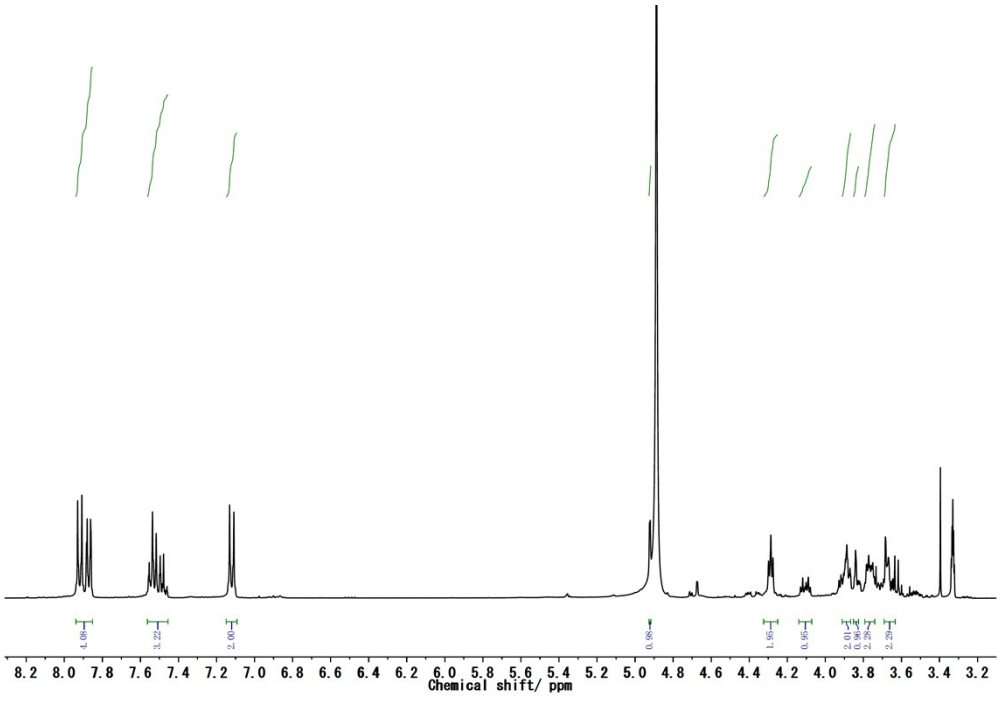


Figure S16. ^{1}H NMR of **AzoMan** in CD_3OD.

Reference

1 J. Geng, F. Biedermann, J. M. Zayed, F. Tian and O. A. Scherman, *Macromolecules*, 2011, **44**, 4276.
2 Z. Ji, J. Liu, G. Chen and M. Jiang, *Polym. Chem.*, 2014, **5**, 2709.
3 F. Sakai, G. Yang, M. S. Weiss, Y. Liu, G. Chen and M. Jiang, *Nat. Commun.*, 2014, **5**. 4634