Electronic Supplementary Information

Ratiometric fluorescence sensing of sugars via a reversible disassembly and assembly of the peptide aggregates mediated by sugars

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1. Experimental Section

1.1. Reagents:

Fmoc-Orn(alloc)-OH was purchased from Bachem. Fmoc-Trp(Boc)-OH, Rink Amide MBHA resin, 1-hydroxybenzotriazole (HOBt) and *N*,*N*''-diisopropylcarbodiimide (DIC) were purchased from Bead Tech. 1-pyreneacetic acid, 4-Carboxyphenylboronic acid pinacol ester, trifluoroacetic acid (TFA), *N*,*N'*-dimethylformamide (DMF), Dichloromethane (DCM), Tetrakis(triphenylphosphine) palladium(0) ($Pd(PPh_3)_4$), Phenylsilane, (1S,2S,3R,5S)-(+)-pinanediol, piperidine were purchased from Sigma Aldrich.

1.2. Solid phase synthesis of 1 and 2

Compound **1** was synthesized in solid phase synthesis with Fmoc chemistry.[1] Fmoc protect ed L-Trp(Boc)-OH (0.03 mmol, 3 equiv) was assembled on Rink Amide MBHA resin (0.1 m mol) for the synthesis of compound **1**. After deprotection of Fmoc group of the Trp(Boc) on t he resin, Fmoc-Orn(alloc)-OH (0.03 mmol, 3 equiv) was coupled with the amino acid on the r esin (Scheme S1). After deprotection of Fmoc group, coupling of 4-carboxyphenylboronic ac id pinacol ester acid was performed by the following procedure. 4-carboxyphenylboronic acid pinacol ester (75 mg, 0.3 mmol), HOBt (40 mg, 0.3 mmol) and DIC (47 μL, 0.3 mmol) in D MF (3mL) were added into the resin and the resulting solution containing the resin was for 4 h at room temperature. The deportection of alloc group of the side chain of ornithine was carr ied out by the following literature procedure.[2] 1-Pyreneacetic acid (78 mg, 0.3 mmol), HOBt (40 mg, 0.3 mmol) and DIPC (47 μL, 0.3 mmol) in DMF (3mL) were added into the resin an d the resulting solution was mixed for 4 h at room temperature. Deprotection and cleavage of the compound **1** from the resin was achieved by treatment with a mixture of TFA/H_2O (95:5, v/v) at room temperature for 5 h. After cleavage of the product from resin, compound **1** was t riturated with diethyl ether chilled at -20 °C and then centrifuged at 3,000 rpm for 10 min at -10 °C. The crude product of **1** was purified by HPLC with a Vydac C_{18} column using a water (0.1% TFA)-Acetonitrile (0.1% TFA) gradient to give the 79% of final product. The successf ul synthesis of **1** was confirmed by ESI mass spectrometry (platform II, micromass, Manchest er, UK) and the high purity (>95%) was confirmed by analytical HPLC with C_{18} column. Co mpound **1** was characterized by melting point, ^{1}HNMR, ^{13}C NMR, and elemental analysis. C ompound **2** was synthesized in solid phase synthesis with Fmoc chemistry.[1] Fmoc protected L

-Gly-OH (0.03 mmol, 3 equiv) was assembled on Rink Amide MBHA resin (0.1 mmol) for th e synthesis of compound **2**. The successful synthesis of **2** was confirmed by ESI mass spectro metry (platform II, micromass, Manchester, UK) and the high purity (>95%) was confirmed b y analytical HPLC with C_{18} column. Compound **2** was characterized by melting point, ^{1}HNM R, ^{13}C NMR, and elemental analysis.

Compound 1. White solid, Yield : 79 %, M.P. 260 ºC –262 ºC, ^{1}H NMR (400 MHz, DMSO-d6) δ: 10.8 (1H, s), 8.43 (1H, d, *J* = 8.4 Hz), 8.36 (1H, d, *J* = 8.4 Hz), 8.25 (1H, d, J = 8.4 Hz), 8.23 (3H, m), 8.17 (1H, d, *J* = 8.4 Hz), 8.15–8.12 (2H, m), 8.17 (1H, d, *J* = 8.4 Hz), 7.91 (1H, d, *J* = 8.4 Hz), 7.87–7.85(2H, m), 7.82–7.80 (2H, m), 7.56 (1H, d, *J* = 8.5 Hz), 7.12 (1H, brs), 7.05–6.99 (2H, m), 6.92 (1H, t, *J* = 8.5 Hz), 4.5–4.4 (2H, m), 4.2 (2H, s), 3.19–3.0 (4H, m), 1.8–1.7 (2H, m). ^{13}C NMR (50 MHz, DMSO-d6), δ: 173.94, 172.19, 170.71, 167.46, 138.23, 136.67, 136.09, 134.56, 131.74, 131.49, 131.02, 130.37, 129.66, 129.32, 128.11, 127.89, 127.47, 127.11, 126.83, 125.73, 125.59, 125.44, 124.78, 124.71, 124.61, 124.16, 121.47, 119.14, 118.87, 111.87, 110.69, 54.13, 53.82, 39.58, 39.15, 29.71, 28.34, 26.70. ESI-Mass (m/z) calculated for $C_{41}H_{38}BN_5O_6$ $[M + Na^+]^+$, 730.28; found, 730.16.

Compound 2. White solid, Yield : 81%, M.P. = 264 ºC–265 ºC, ^{1}H NMR (400 MHz, DMSO-d6) δ: 8.57 (1H, d, *J* = 8.5 Hz), 8.36 (1H, d, *J* = 8.4 Hz), 8.26 (2H, d, *J* = 8.4 Hz), 8.24 – 8.18 (4H, m), 8.12 – 8.11 (2H, m), 8.04 (1H, t, *J* = 8.5 Hz), 7.98 (1H, d, *J* = 8.4 Hz), 7.88 – 7.86 (4H, m), 7.22 (1H, s), 7.11 (1H, s), 4.42 – 4.41 (1H, m), 4.17 (2H, s), 3.69 (1H, dd, *J* = 8.6 Hz, 2.0), 3.57 (1H, dd, *J* = 8.6 Hz, 2.0), 3.14 – 3.09 (2H, m), 1.83 – 1.72 (2H, m), 1.53 –1.51 (2H, m). ^{13}C NMR (50 MHz, DMSO-d6), δ: 172.01, 170.96, 169.99, 166.97, 135.24, 133.85, 131.06, 130.82, 130.35, 129.69, 128.98, 128.63, 127.38, 127.20, 126.79, 126.47, 126.15, 125.06, 124.90, 124.75, 124.10, 124.02, 123.93, 53.60, 42.03, 38.52, 28.76, 26.06. ESI-Mass (m/z) calculated for $C_{32}H_{31}BN_4O_6$ $[M + H^+]^+$, 578.23; found, 576.64.

1.3. General fluorescence measurements

A stock solution of compound **1** and **2** at the concentration of 1×10^{-3} M was prepared in DMSO, respectively and stored in a cold and dark place. The concentration of stock solution was confirmed by UV absorbance at 342 nm for pyrene. Fluorescence emission spectrum of the sample in a 10 mm path length quartz cuvette was measured in 50 mM phosphate buffer solution containing 1% DMSO (pH 7.4) using a PerkineElmer luminescence spectrophotometer (model LS 55). Emission spectra of the compound in the presence of sugar were measured by excitation with 342 nm for pyrene. The slit size for excitation and emission was 12/8 nm respectively.

1.4. Determination of association constant

The association constant for the 1:1 complex was calculated based on the titration curve of the fluorescent chemosensor with sugar. Association constants (K_a) was determined by a nonlinear least squares fitting of the data with the following equation.[3]

$$I = \frac{I_{min} + I_{max}K_a[S]}{1 + K_a[S]} + k_a^{'}[s]$$

Where I_{min} and I_{max} are the initial (no sugar) and final (plateau) fluorescence intensities of the titration curves and ka' is slope of the linear equation for the fitting of the linearly increased emission intensity as function of the concentration of sugar.

1.5. Determination of pK_a

The pK_a value was calculated based on the Fluorescence intensity of probe with and without D-Fructose in 50 mM phosphate buffer solution containing 1% DMSO at different pH. The pK_a values were obtained by simulating the pH and Fluorescence intensity to the Henderson-Hasselbalch equation.[4]

$$I = \frac{I_{max} \times 10^{(pH - pK_a)} + I_{min}}{1 + 10^{(pH - pK_a)}}$$

Where, I, I_{min} and I_{max}, are observed, minimum and maximum fluorescence intensity respectively.

1.6. Measurement of the size of the aggregates of the compound in solutions

The size distribution of the aggregates of **1** in aqueous solution was characterized by using a particle size analyzer (Brookhaven Instruments Corporation, New York, USA, model 986 3) equipped with He-Ne laser (633 nm). The measurements of the aggregates in the soluti on were carried out by 90º dynamic light scattering at 25ºC. The compound **1** (30 μM) and **2** (30 μM) was dissolved in 50 mM phosphate buffer solution containing 3 % DMSO at pH 7.4 for the size measurement, respectively.

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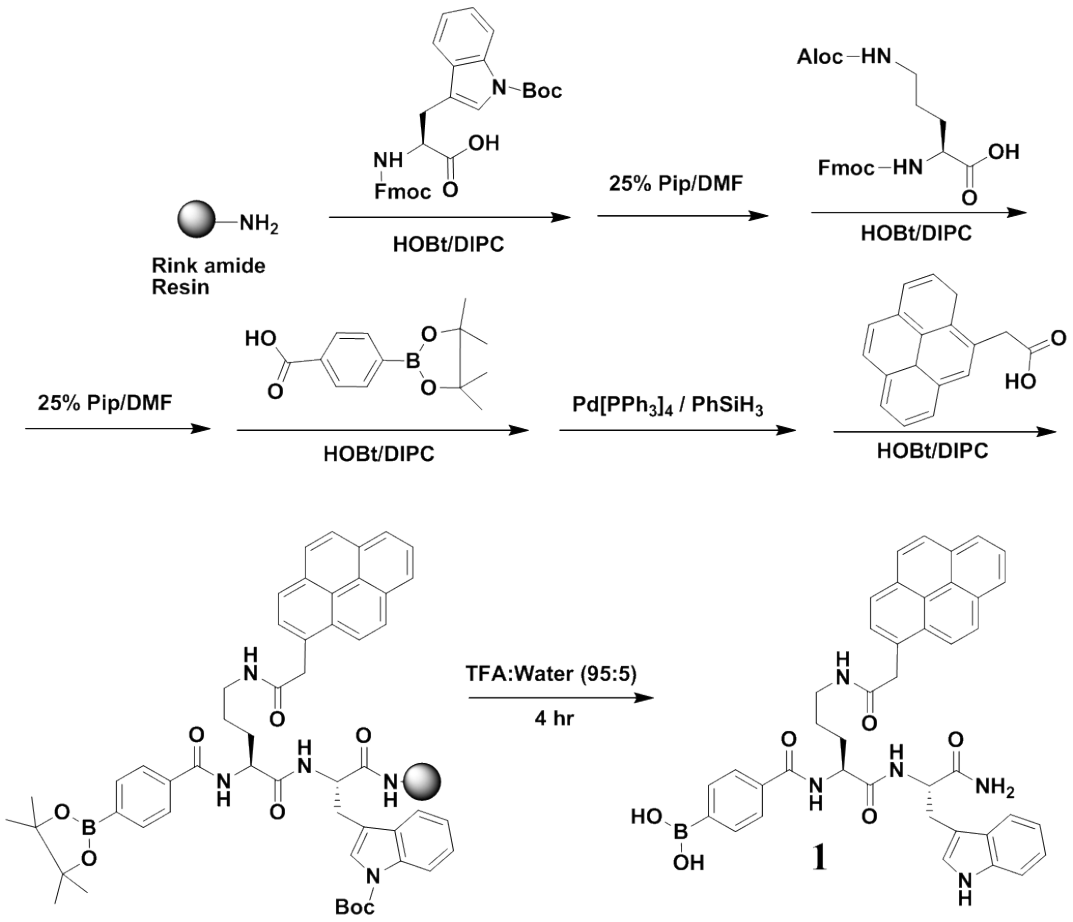

Scheme S1. Synthesis of the compound **1** and compound **2**.

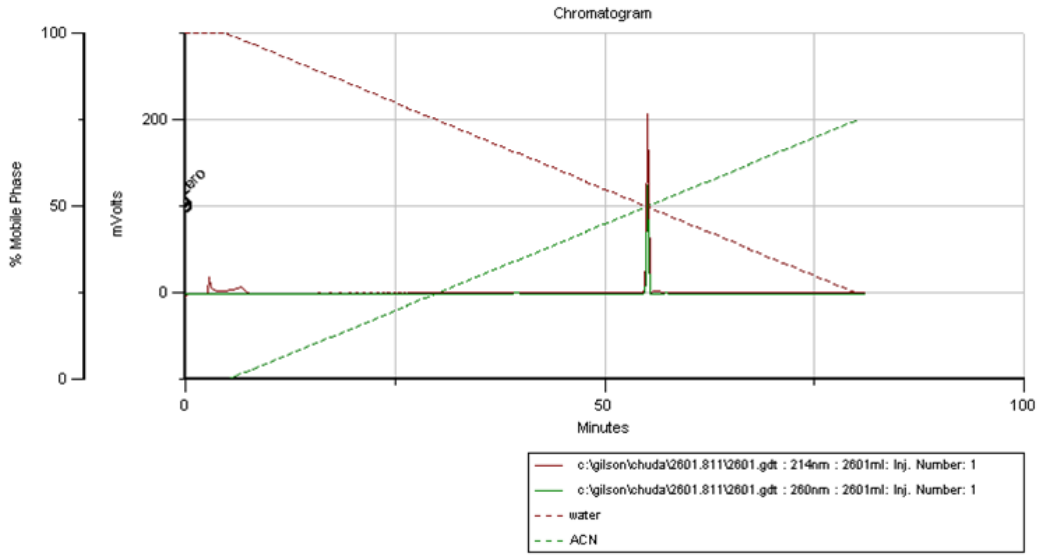

Figure S1. HPLC chromatogram of **1**.

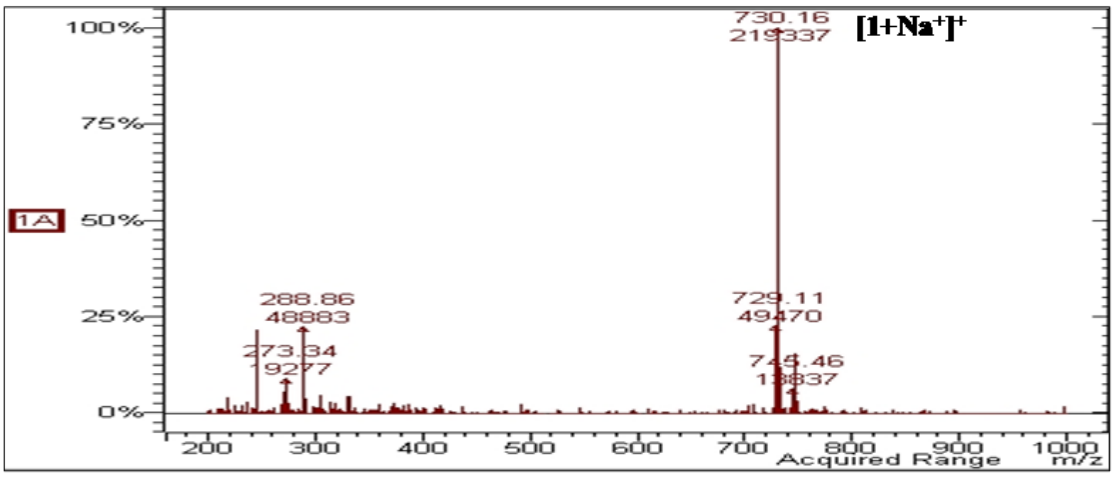

Figure S2. ESI-Mass spectrum of 1.

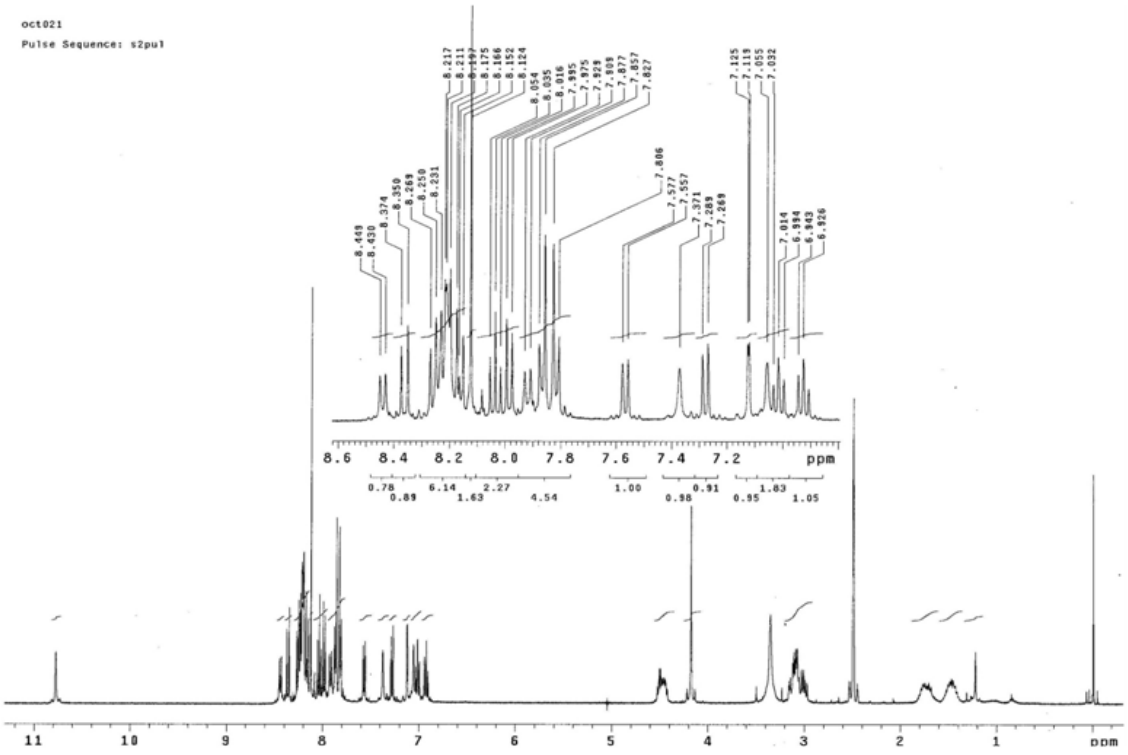

Figure S3. ^{1}H NMR of **1**.

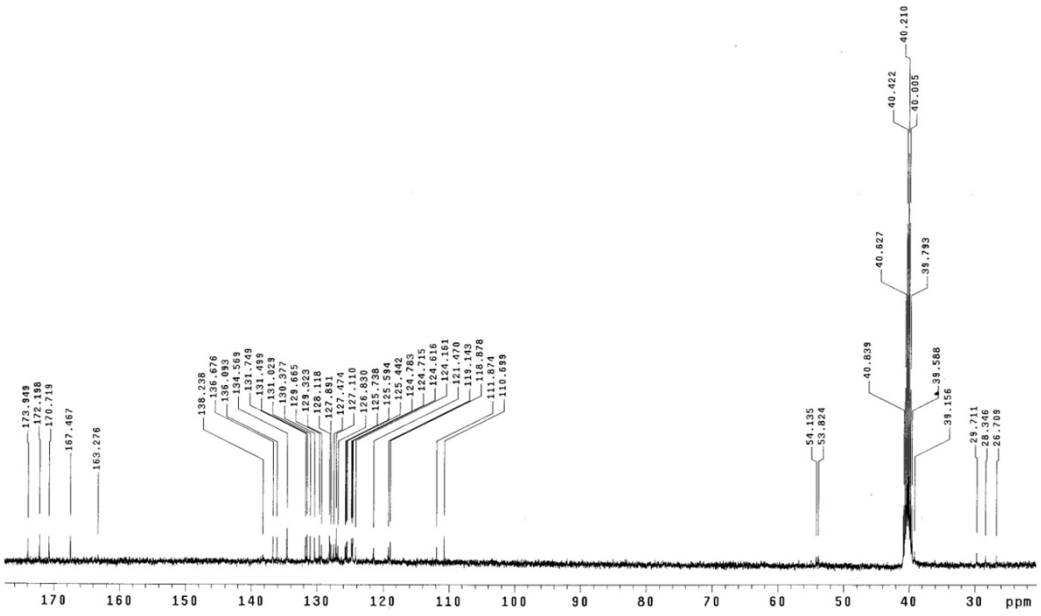

Figure S4. ^{13}C NMR of **1**.

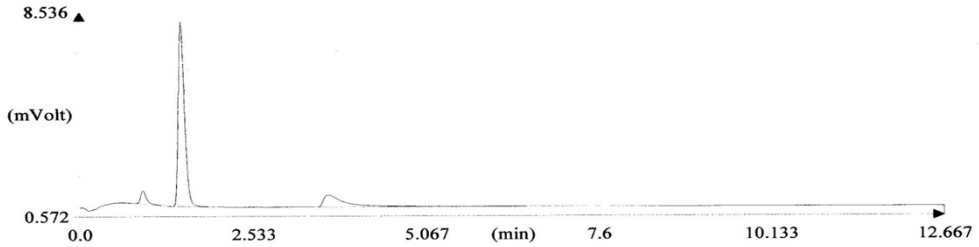

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Figure S5. Elemental analysis of **1**.

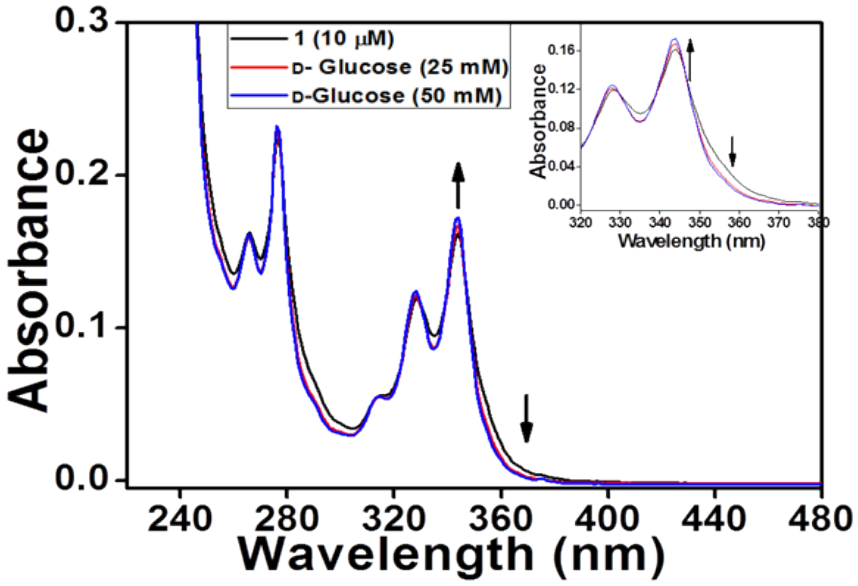


Figure S6. UV-vis absorbance spectra of **1** (10 μM) with the gradual addition of D-glucose (0~0.05 M) in 50 mM phosphate buffer solution containing 1 % DMSO at pH 7.4.

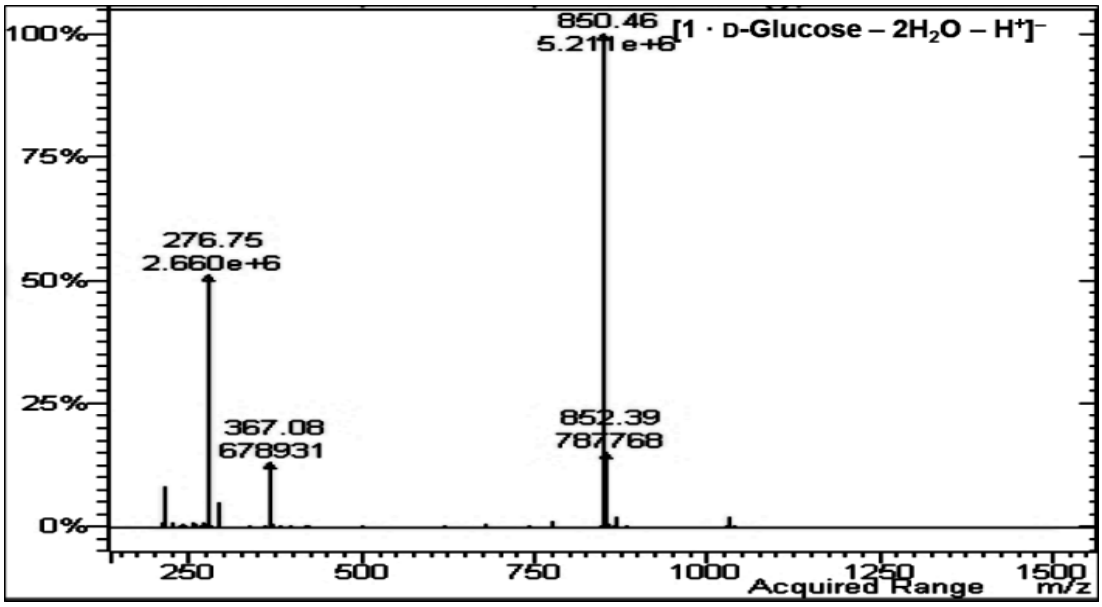


Figure S7. ESI mass spectrum of **1** (500 μM) in the presence of D-glucose in 30% CH_3CN/H_2O.

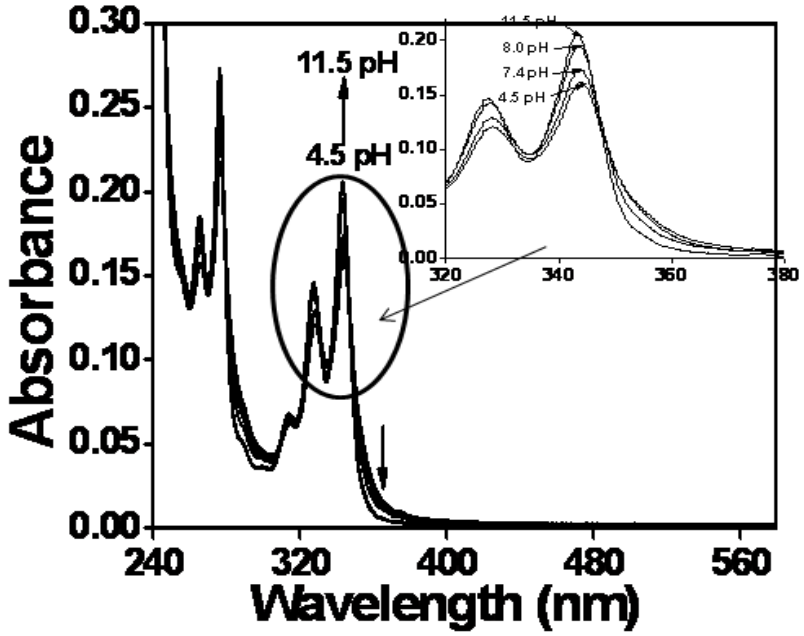


Figure S8. UV-vis abosrbance spectra of compound **1** (10 μM) at different pH (4.5, 5.5, 6.5, 7.0, 7.4, 8.0, 8.5, 9.5, 10.5, 11.5).

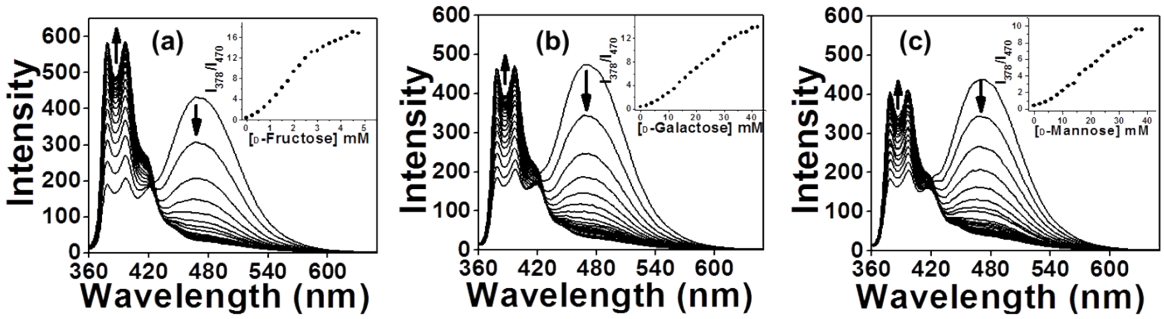


Figure S9. Fluorescence emission spectra of compound **1** (10 μM) with the gradual additio n of (a) D-fructose, (b) D-Galactose, and (c) D-Mannose in 50 mM phosphate buffer solution containing 1% DMSO at 7.4 pH (λ_{ex} = 342, slit = 12/8).

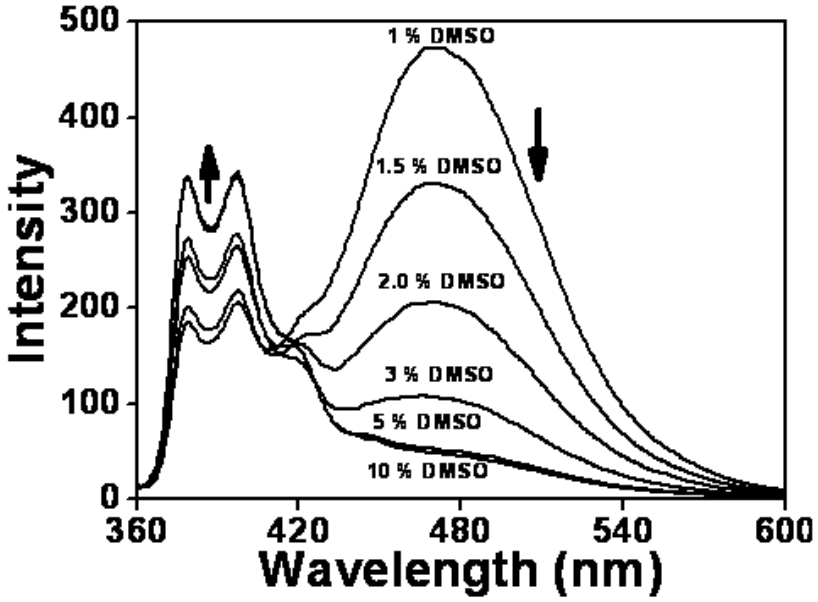


Figure. S10. Fluorescence emission spectra of compound **1** (10 μM) in 50 mM phosphate b uffer solution at 7.4 pH containing different percent of DMSO solvent.

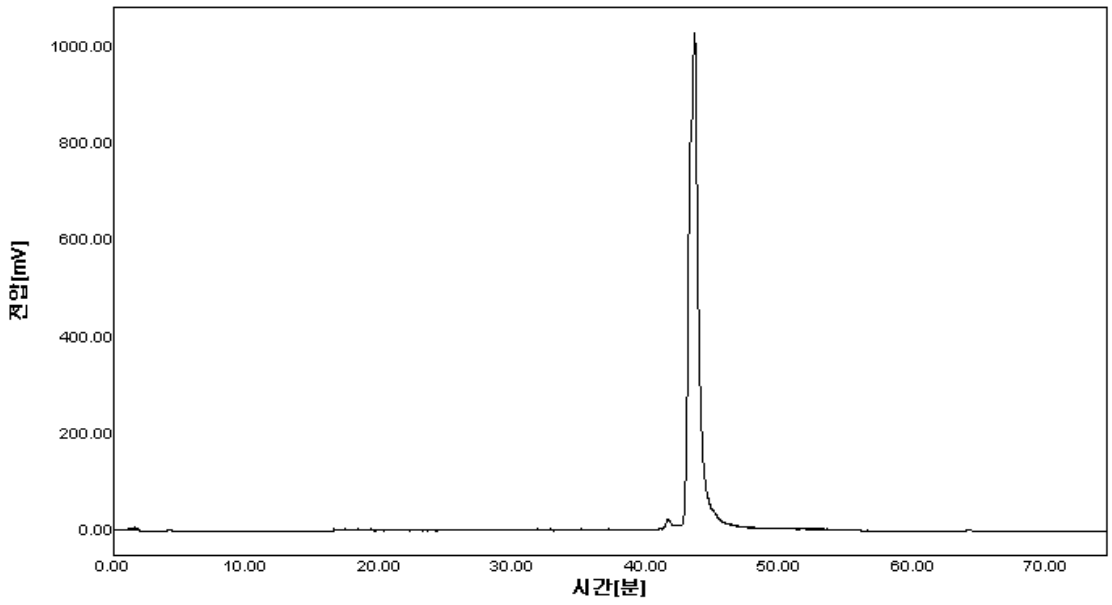


Figure S11. HPLC chromatogram of **2**.

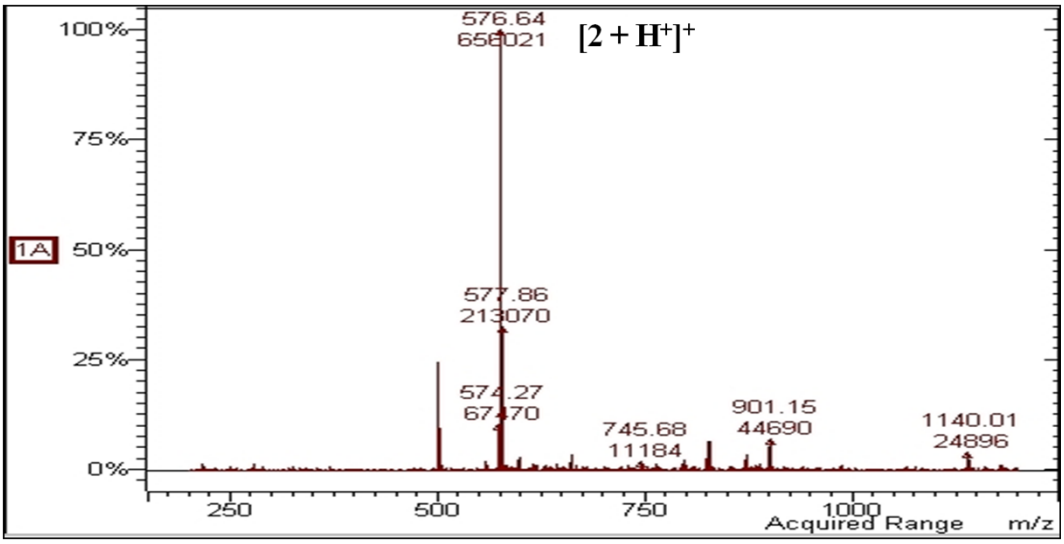


Figure S12. Mass spectrum of **2**.

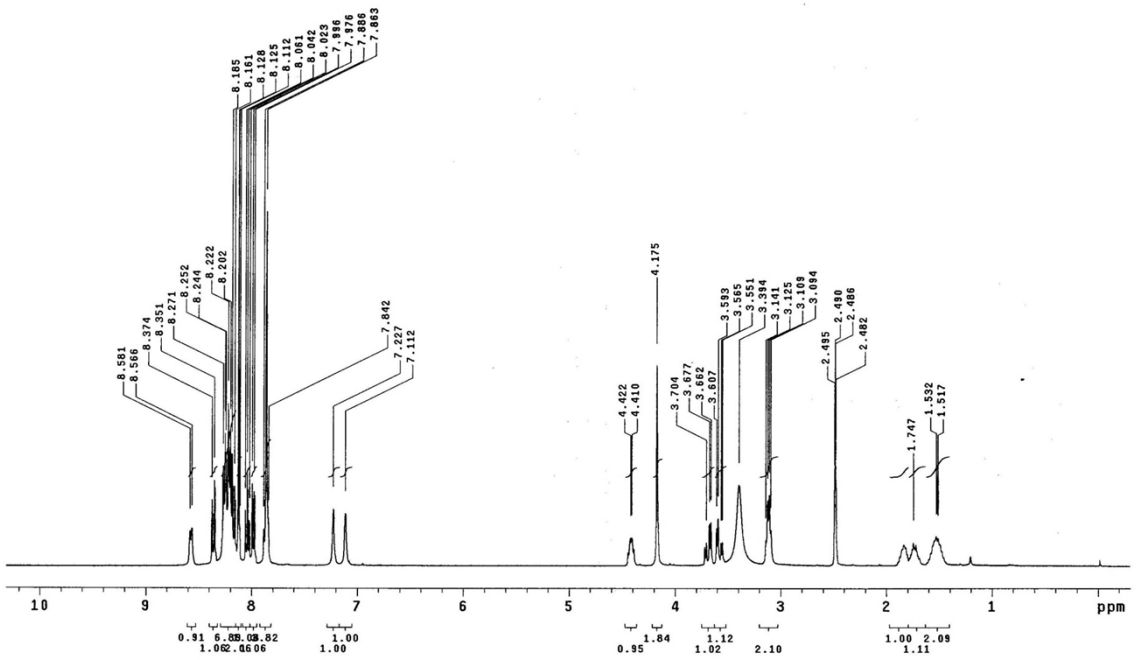


Figure S13. ^{1}H NMR of **2**.

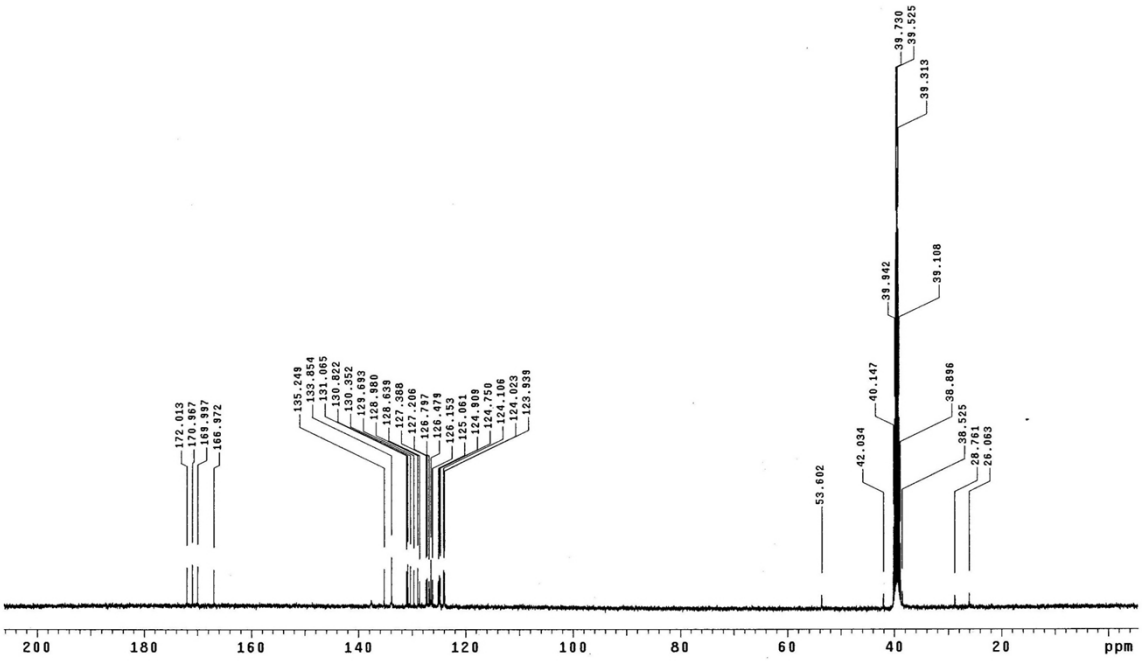
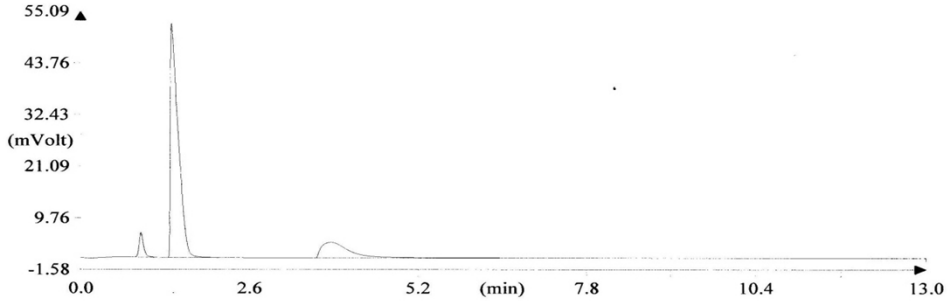


Figure S14. ^{13}C NMR of **2**.



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Carbon	64.7695	84	4338097	RS	1.000000	.
Hydrogen	5.4752	231	1104321	RS	3.928293	.
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Figure S15. Elemental analysis of **2**.

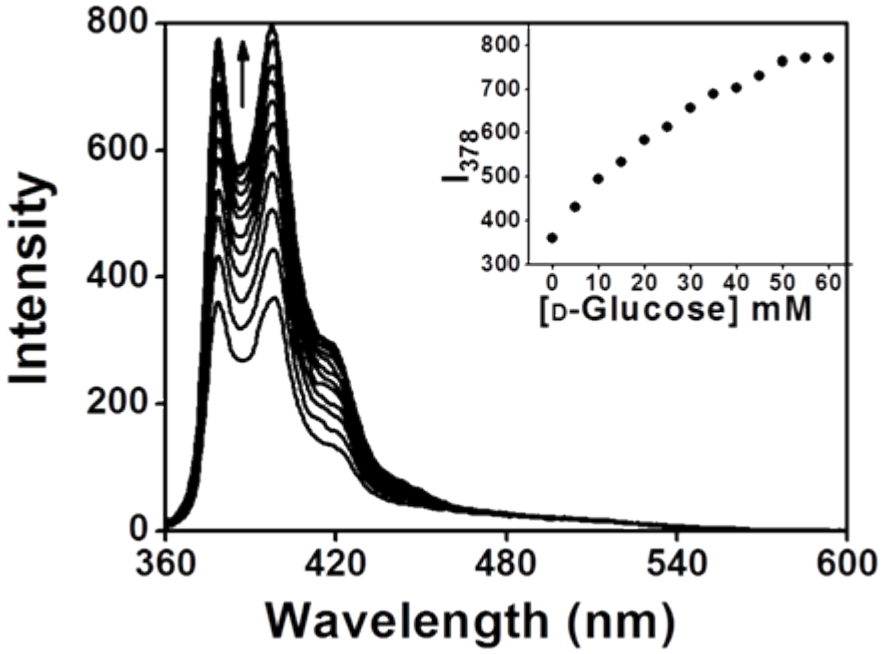

Figure S16. Fluorescence emission spectra of **2** (10 μM) upon addition of D-glucose in 50 mM phosphate buffer solution containing 1% DMSO at pH 7.4, (λ_{ex} = 342 nm, slit = 12/6).

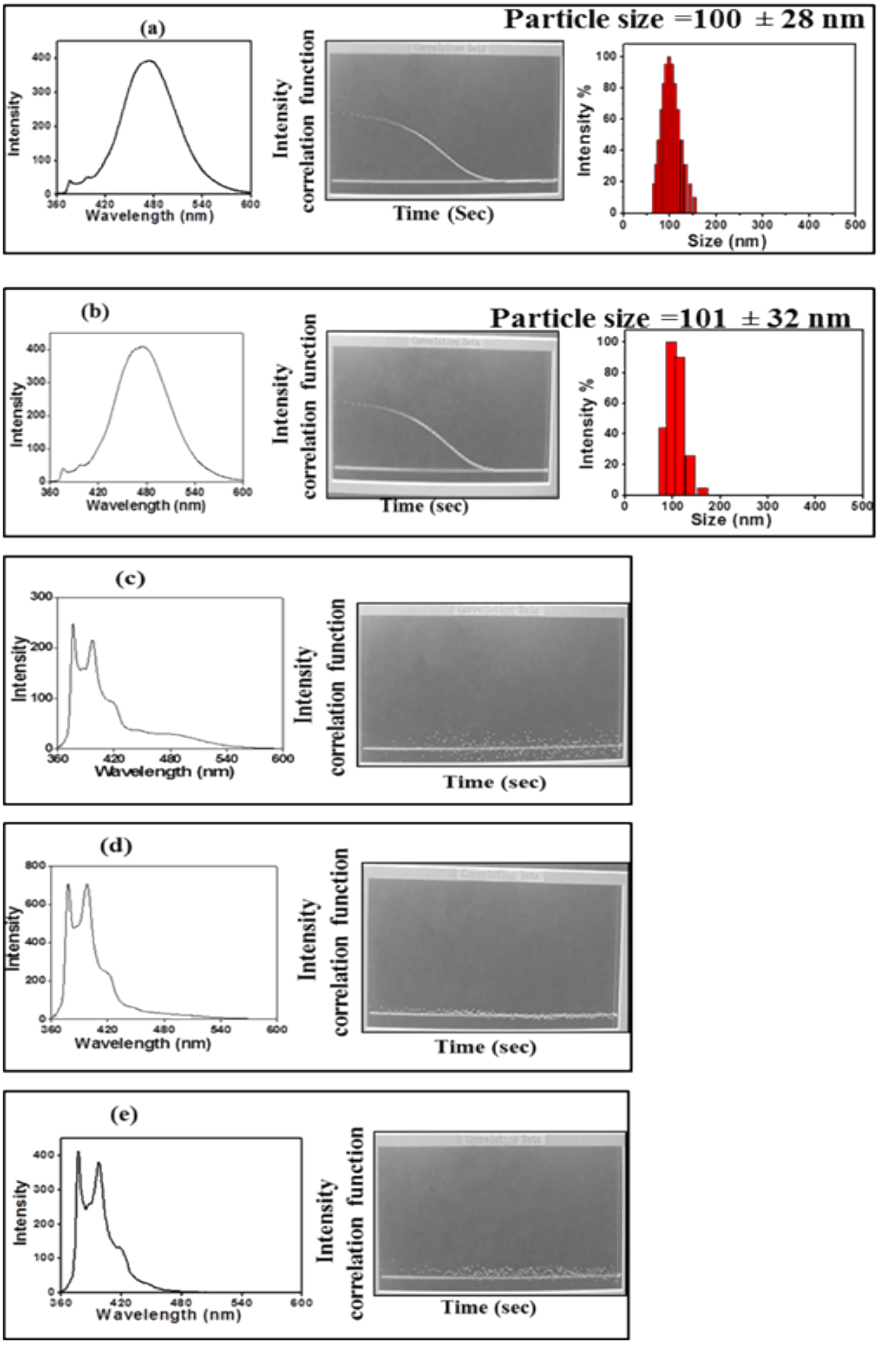

Figure S17. The size analysis of aggregates of **1** and **2** in solution.

Fluorescence emission spectrum and size distribution of the aggregate (a) compound **1** (30 μM) in buffered aqueous solution at pH 7.4 containing 3 % DMSO (b) compound **1** (30 μ M) in buffered aqueous solution at pH 4.5 containing 3 % DMSO (c) compound **1** (30 μM) in the presence of glucose (0.15 M) in buffered aqueous solution (pH 7.4) containing 3 % D MSO (d) compound **1** (30 μM) in buffered aqueous solution at pH 10.5 containing 3 % D MSO (e) compound **2** (30 μM) in buffered aqueous solution at pH 7.4 containing 3 % DM SO.

References

1. G.B. Fields, R.L. Nobel, Int. J. Pept. Protein Res. **1990**, *35*, 161–214

2. Thieriet, N.; Alsina, J.; giralt, E.; Guibe, F. and Albericio, F. *Tetrahedron Letters*, **1997**, *38*, 7275

3. N. D.Cesare , J. R. Lakowicz, *J. Photochem. Photobiol. A* **2001**, *143*, 39.

4. K. Ma, E. L. Clancy, Y. Zhang, D. G. Ray, K. Wollenberg, M. G. Zagorski. *Am. Chem. Soc.* **1999**, *121,* 8698-8706.