Synthesis, Self-aggregate and Cryopreservation Effects of Perylene Bisimide-Glycopeptide Conjugates

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S1. Experimental section

Measurements. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker 600 spectrometer. HRMS analysis was performed on a FT-MALDI MS (Bruker Company). UV−Vis spectra were recorded in a quartz cell (light path 10 mm) on a Cary 5000 spectrophotometer equipped with a temperature controller. Transmission Electron Microscope (TEM) images were recorded on a FEI Tecnai G^2 F20 instrument. Circular dichroism (CD) results were measured on a JASCO J-1500 spectrometer. The fluorescent imaging was acquired by a confocal laser scanning microscope (Zeiss Company). IRI activity analysis was performed on a Nikon polarized optical microscope (LV100ND, Japan) equipped with a Linkman (LTS420) cooling stage. The Dynamic Light Scattering (DLS) result was performed on a light scattering spectrometer (Brookhaven BI-APDV, USA) equipped with a He−Ne laser working at 4 mW (λ = 633 nm).

Solvent-dependent and concentration-dependent absorption and CD spectra:

Perylene bisimide-glycopeptide conjugates were dissolved in DMSO solvent with the concentration of 1×10^{-3} M. A series of 50 μL of the above standard solution (1×10^{-3} M) were added to a 5 mL volumetric flask, and which were diluted to the final concentration of 1×10^{-5} M with various ratios of DMSO and H_2O. Solvent-dependent absorption and CD spectra were studied.

Perylene bisimide-glycopeptide conjugates were dissolved in H_2O solvent with the concentration of 1×10^{-3} mol/L, then which was diluted to the final concentration of 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 μmol/L. Concentration-dependent UV-Vis and CD spectra were studied.

Ice Recrystallization Inhibition (IRI) Activity: IRI activity analysis was performed via the splat cooling method. The experimental apparatus used to investigate the IRI activity was equipped with a Nikon polarized optical microscope (LV100ND, Japan) and a Linkman (LTS420) cooling stage. In a typical IRI activity measurement, a 10 μL droplet of solution at room temperature (25 °C) was dropped onto the surface of a silicon substrate precooled to -60.0 °C with liquid nitrogen from a height of 1.5 m to form a thin solid ice film. Then the temperature was increased to -6.0 °C at a rate of 15 °C min^{-1}, and the samples annealed at this temperature for 30 min. The ice wafer was imaged randomly with a digital camera (Nikon Y-TV55, Japan) to obtain the grain size of the ice crystals. The number of ice crystals in the field of view was determined, and this determination was repeated for three independent wafers. The average (mean) of these four measurements was then used to find the mean grain size (MGS) relative to that of the PBS buffer solution. The average value and error were compared to those of PBS buffer solution as a negative control.

Cytotoxicity of PBI-AFF-Man, PBI-AFF-Gal and PBI-AFF-Glu in HeLa cells: HeLa cells were seeded in a 96-well plate at a density of 7×10^3 cells per well (with 180 μL of DMEM). After 24 h of incubation, 20 μL of medium containing different concentrations of **PBI-AFF-Man, PBI-AFF-Gal** and **PBI-AFF-Glu** was added to the wells with cells. Then, all the cells were cultured for an additional 48 h. Cell viability was assessed by the MTT method. First, cells were incubated with MTT (0.5 mg mL^{-1}) for 4 h at 37 °C. During this incubation period, water-insoluble crystals formed, which were then dissolved by the addition of 100 μL of a hydrochloric acid-isopropanol mixed solution to each well. The optical density at 650 nm in each well was measured using an enzyme-linked immunosorbent assay plate reader. Wells containing culture medium and MTT but no cells acted as blanks. The percent of cell viability was calculated as follows: $(A_{compound} - A_{blank})/(A_{control} - A_{blank}) \times 100\%$.

Cryopreservation of HeLa Cells: HeLa cells were cultured under standard conditions (5% CO_2, 37 °C). HeLa cells were grown in DMEM supplemented with 10% FBS. After 48 h of incubation under standard conditions (5% CO_2, 37 °C), the cells were washed from the plate using trypsin and centrifuged for 3 min at 1200 rpm. For cryopreservation, the cells were diluted into the cryoprotectants (80% DMEM, 10% FBS and 10% DMSO) containing different concentrations of compounds **PBI-AFF-Man, PBI-AFF-Gal** and **PBI-AFF-Glu** (0.5, 1 and 2 mg/mL) at a density of 5×10^5 cells·mL^{-1}. The mixtures were first cooled from room temperature to 4 °C and equilibrated for 5 min. Then, they were further frozen in a -20 °C freezer and equilibrated for 0.5 h. Finally, the mixtures were frozen in a -80 °C freezer and equilibrated for 24 h to completely freeze the cells. To thaw the cells, the mixtures were placed in a water bath at 37 °C for 2 min. After removing of the cryoprotectants, the cells were cultured in DMEM with 10% FBS under standard conditions. After 24 h of incubation, adherent cells (alive) were counted, and the proportion of surviving cells compared to the total number of cells was calculated and compared with the group without compound. Five parallels in each group were repeated three times.

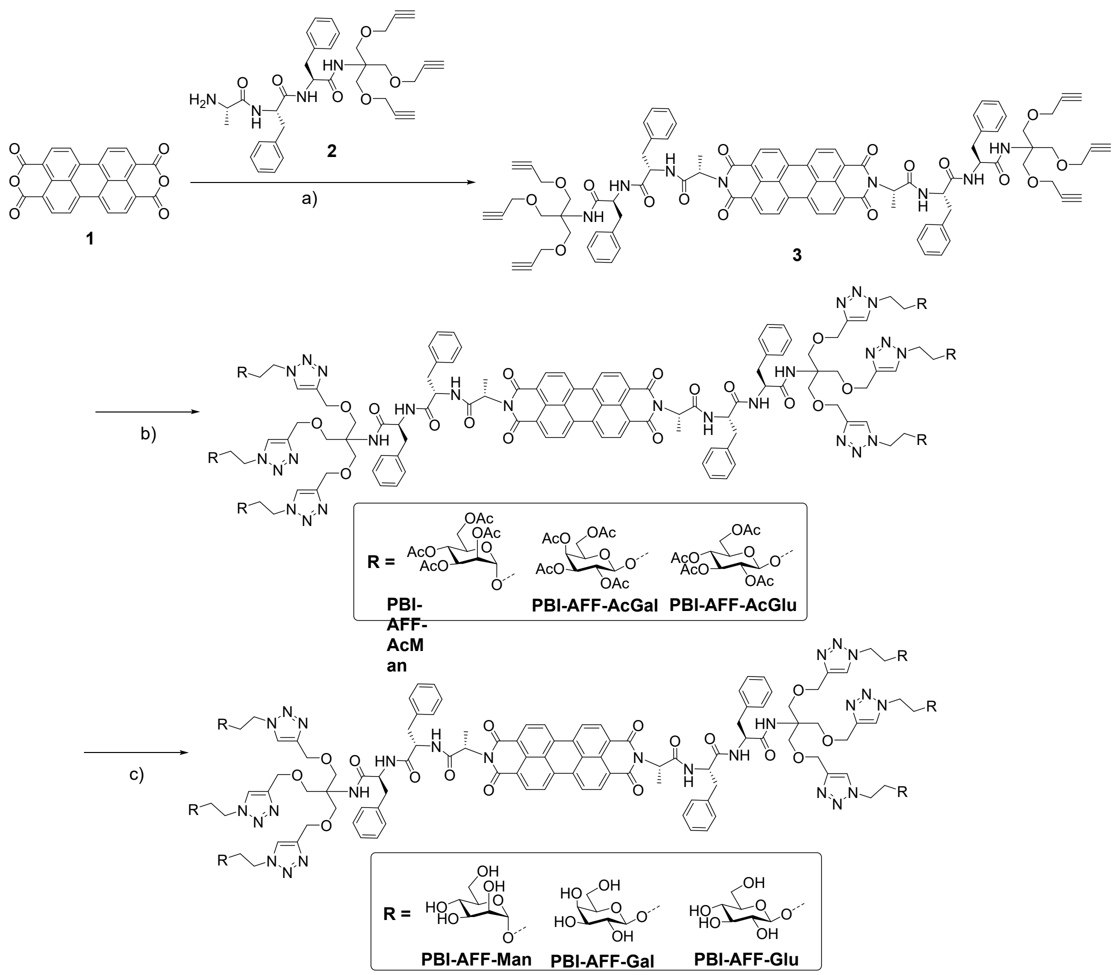
Confocal microscopy assay: HeLa cells were seeded in a culture dish at a density of 1×10^5 per culture dish (with 1.5 mL DMEM). After 24 h, the medium was removed, and 1 mL of medium containing 10 μM **PBI-AFF-Man, PBI-AFF-Gal** and **PBI-AFF-Glu** was added to the culture dish. Then, all the cells were cultured for an additional 6 h, washed three times with PBS and new medium was added. Confocal microscopy was performed to observe the cells.

Calcein AM cell staining assay: Frozen HeLa cells at a density of 5×10^5 cells·mL^{-1} were placed in a water bath at 37 °C for 2 min. After removing the cryoprotectants, the cells were cultured in confocal dishes at 37 °C in DMEM with 10% FBS under standard conditions. After 24 h of incubation, nonadherent cells were removed. Calcein

AM dye was used to stain live cells. Then, the cells were washed with PBS four times. Confocal images were acquired with a Carl Zeiss (Germany) microscope.

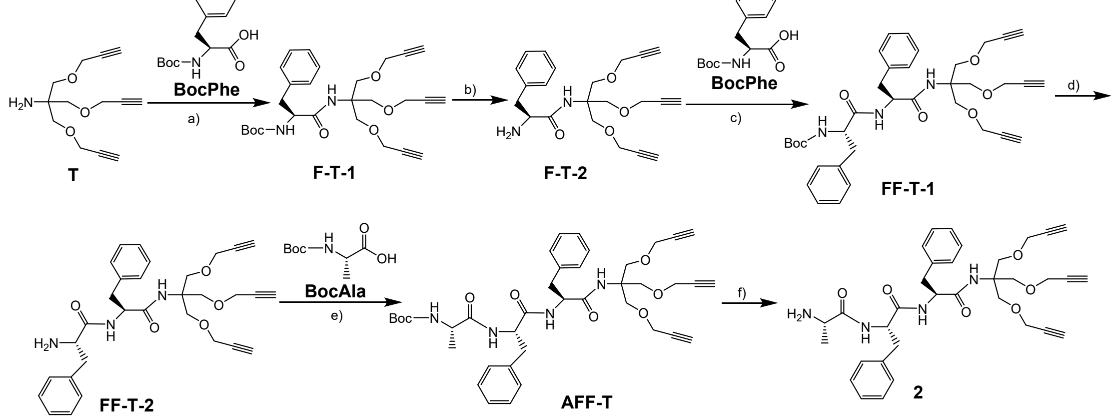
S2. Synthesis.

A series of perylene bisimide-glycopeptide conjugates (**PBI-AFF-Man, PBI-AFF-Gal** and **PBI-AFF-Glu**) were designed and synthesized (Scheme S1). 3,4;9,10-Perylenetetracarboxy dianhydride (**1**) was reacted with the peptide sequence Ala-Phe-Phe (**2**) to give perylene bisimide derivative **3**. Then, the acetyl carbohydrate (mannose, galactose or glucose)-modified perylene bisimide-triglycopeptide derivatives (**PBI-AFF-AcMan**, **PBI-AFF-AcGal** and **PBI-AFF-AcGlu**) were obtained by reacting compound **3** with 2-azido-ethyl-2,3,4,6-tetra-*O*-acetyl-*α*-D-mannopyranoside (**Man-N_3**), 2-azido-ethyl-2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranoside (**Gal-N_3**) or 2-azido-ethyl-2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranoside (**Glu-N_3**), respectively. Finally, three triglycopeptide-modified perylene bisimide derivatives (**PBI-AFF-Man**, **PBI-AFF-Gal** and **PBI-AFF-Glu**) were produced by deprotection of the acetyl group. ^{1}H NMR, ^{13}C NMR and HRMS analyses were used to characterize the intermediates and target molecules (Fig. S1~S24).



Scheme S1 (a) $Zn(OAc)_2$, pyridine, 115 °C; (b) $CuSO_4 \cdot 5H_2O$, L-ascorbic acid sodium salt, THF-H_2O (v /v = 1/1), 55 °C; (c) CH_3OH, CH_3ONa, r. t.

The Phe-Phe peptides are good self-assembling backbones that are widely used to construct the ice growth inhibitors[1]. And the Ala residue existed in AFGPs for ice binding[2]. As shown in Scheme 2, compound **2** was synthesized in six steps according to a similar method as reference[3].



Scheme S2. (a) Boc-L-phenylalanine, HATU, DIPEA, DCM, r.t.; (b) DCM, TFA, r.t.; (c) Boc-L-phenylalanine, HATU, DIPEA, DCM, r.t.; (d) DCM, TFA, r.t.; (e) Boc-L-alanine, HATU, DIPEA, DCM, r.t.; (f) DCM, TFA, r.t.

Compound **AFF-T**: m. p. 126.3-127.2. ^{1}H NMR ($CDCl_3$，600 MHz): δ (ppm) 7.32-7.14 (m, 11H, Ar-H and -CONH-), 6.63 (d, 1H, J = 9.6 Hz, -CONH-), 6.60 (d, 1H, J = 11.4 Hz, -CONH-), 5.77 (s, 1H, -CONH-), 4.86 (s, 1H), 4.61 (m, 1H), 4.52 (m, 1H), 4.10-4.08 (d, 6H, J = 3.6 Hz), 3.76-3.68 (m, 6H), 3.10-2.89 (m, 4H), 2.43 (t, 3H, -C≡CH, J = 3.6Hz), 1.43 (s, 9H, -Boc), 1.24 (d, 3H, $-CH_3$, J = 10.8 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 172.53, 170.06, 136.65, 136.24, 129.54, 129.33, 128.74, 128.60, 127.14, 126.94, 79.58, 74.71, 68.18, 59.52, 58.61, 54.78, 54.19, 50.30, 38.61, 38.57, 37.86, 28.33, 18.14. HRMS: Calcd. for $C_{39}H_{49}N_4O_8$ ($[M+H]^+$): 701.3550, found: 701.3545.

Compound **3**: First, 218 mg (0.56 mmol) of perylenetetracarboxy dianhydride (**1**), 308 mg (1.68 mmol) of $Zn(AcO)_2$, and 1.0 g (1.68 mmol) of **2** in 200 mL of pyridine solution, were added into a single-neck round-bottom flask. The reaction mixture was stirred at 115 °C for 16 h under a N_2 atmosphere. The reaction was monitored by TLC. The pyridine was then evaporated under vacuum. The residue was redissolved CH_2Cl_2 and purified by silica gel column chromatography (CH_2Cl_2-CH_3OH (v/v = 70/1)). Compound **3** (191 mg) was obtained in a yield of 21.9%. m.p. 162.4-162.7 ºC. ^{1}H NMR ($CDCl_3$, 600 MHz): δ (ppm) 1.57 (d, 6H, J = 6.6 Hz, $-CH_3$), 2.32 (s, 6H, -C≡CH), 3.08-3.18 (m, 4H), 3.23-3.28 (m, 4H), 3.71 (q, 12H, J = 9.6 Hz, $-CH_2$), 4.03 (q, 12H, J = 16.2 Hz, $-CH_2$), 4.63 (q, 2H, J = 7.2 Hz), 4.76 (q, 2H, J = 6.0 Hz), 5.60 (q, 2H, J = 6.6 Hz), 6.20 (s, 2H), 6.24 (d, 2H, J = 0.6 Hz), 7.10 (d, 4H, J = 6.6 Hz), 7.19-7.24 (m, 8H), 7.27-7.31 (m, 10H), 8.51 (s, 4H, perylene-H), 8.63 (d, 4H, J = 7.2 Hz, perylene-H); ^{13}C NMR ($CDCl_3$, 150 MHz): δ (ppm) 14.23, 29.72, 36.58, 37.71, 50.54, 53.93, 55.63, 58.63, 59.42, 68.28, 74.54, 79.71, 122.80, 123.55, 126.12, 126.84, 127.08, 128.90, 129.11, 129.26, 129.41, 132.17, 134.74, 136.31, 137.52, 163.00, 169.37, 170.62, 170.64; HRMS: calcd. for $C_{92}H_{85}N_8O_{16}$, 1557.6078, found 1567.6047.

General synthesis of compounds **PBI-AFF-AcMan**, **PBI-AFF-AcGal** and **PBI-**

AFF-AcGlu. A mixture of **3** (100 mg, 0.064 mmol) and compounds **Man-N_3**, **Gal-N_3** or **Glu-N_3** (192 mg, 0.46 mmol) was dissolved in THF (10 mL). Then, water solutions of $CuSO_4 \cdot 5H_2O$ (58 mg, 0.23 mmol) and sodium ascorbate (46 mg, 0.23 mmol) were added. The reaction mixture was stirred for 12 h at 55 °C under a N_2 atmosphere. The reaction solvents were then evaporated under vacuum. The residue was redissolved in CH_2Cl_2 and purified by silica gel column chromatography using CH_2Cl_2/CH_3OH (v/v = 25/1) as the eluent. Compounds **PBI-AFF-AcMan** (160 mg), **PBI-AFF-AcGal** (162 mg) and **PBI-AFF-AcGlu** (173 mg) were obtained in yields of 61.5%, 62.3% and 66.5%, respectively.

Compound **PBI-AFF-AcMan**: m.p. 143.4-144.1 ºC. ^{1}H NMR ($CDCl_3$, 600 MHz): δ (ppm) 1.60 (d, 6H, J = 10.2 Hz, -CH_3), 2.01 (s, 18H, -$COCH_3$), 2.06 (s, 18H, -$COCH_3$), 2.11 (s, 18H, -$COCH_3$), 2.16 (s, 18H, -$COCH_3$), 3.00-3.11 (m, 4H), 3.18 (dd, 2H, J = 13.2 Hz, 7.8 Hz), 3.33 (dd, 2H, J = 9.0 Hz, 12.6 Hz), 3.65-3.72 (12H), 3.89-3.93 (m, 6H), 4.05-4.13 (12H), 4.25 (dd, 6H, J = 7.8 Hz, 10.8 Hz), 4.50 (s, 12H), 4.55-4.69 (14H), 4.83 (s, 6H), 5.23-5.31 (18H), 5.64 (dd, 2H, J = 9.6 Hz, 10.2 Hz), 6.31 (d, 1H, J = 10.2 Hz), 6.59 (s, 1H), 7.06-7.13 (11H), 7.22-7.29 (9H), 7.53 (d, 2H, J = 12.6 Hz), 7.75 (s, 6H, Triaz-H), 6.71 (d, 4H, J = 12.0 Hz, perylene-H), 8.87 (d, 4H, J = 12.0 Hz, perylene-H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 14.09, 20.66, 20.69, 20.72, 20.82, 29.68, 36.39, 37.42, 49.43, 50.32, 54.13, 55.36, 59.87, 62.15, 64.61, 65.66, 66.25, 68.57, 68.84, 68.90, 69.14, 97.51, 122.70, 123.87, 126.41, 126.65, 126.89, 128.39, 128.68, 128.97, 129.36, 132.21, 135.15, 136.40, 137.59, 145.06, 163.08, 169.66, 169.95, 170.60, 170.85, 170.86; HRMS: calcd. for $C_{188}H_{223}N_{26}O_{76}$, 4060.4378, found 4060.4337.

Compound **PBI-AFF-AcGal**: m.p. 144.5-145.6 ºC. ^{1}H NMR ($CDCl_3$, 600 MHz): δ (ppm) 1.57 (d, 6H, J = 6.6 Hz, -CH_3), 1.90 (s, 18H, -$COCH_3$), 1.96 (s, 18H, -$COCH_3$), 2.02 (s, 18H, -$COCH_3$), 2.13 (s, 18H, -$COCH_3$), 2.99-3.07 (m, 4H), 3.16 (dd, 2H, J = 9.6 Hz, 13.8 Hz), 3.29 (dd, 2H, J = 5.4 Hz, 9.0 Hz), 3.69 (d, 6H, J = 9.6 Hz), 3.75 (d, 6H, J = 9.0 Hz), 3.90-3.92 (m, 12H), 4.08-4.15 (m, 12H), 4.20-4.23 (m, 6H), 4.42-4.59 (32H), 4.65 (dd, 2H, J = 8.4 Hz, 6.0 Hz), 5.00 (dd, 6H, J = 3.6 Hz, 7.2 Hz), 5.16 (dd, 6H, J = 8.4 Hz, 1.8 Hz), 5.37 (d, 6H, J = 3.0 Hz), 5.61 (dd, 2H, J = 6.6 Hz, 7.2 Hz), 6.34 (d, 2H, J = 4.8 Hz), 6.60 (d, 2H, J = 12.6 Hz), 7.03-7.10 (m, 10H), 7.18 (t, 2H, J = 7.2 Hz), 7.23 (t, 4H, J = 7.2 Hz), 7.28 (2H), 7.54 (d, 2H, J = 8.4 Hz), 7.61 (s, 6H, Triaz-H), 8.68 (d, 4H, J = 7.8 Hz), 8.84 (d, 4H, J = 7.8 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 14.17, 20.56, 20.64, 20.66, 22.68, 29.34, 29.68, 31.91, 36.36, 37.51, 49.82, 50.40, 53.48, 54.30, 55.56, 59.86, 61.17, 64.67, 66.95, 67.59, 68.45, 68.76, 70.59, 70.81, 100.93, 122.73, 123.93, 124.02, 126.32, 126.69, 128.43, 128.65, 129.05, 129.26, 129.43, 132.18, 135.07, 136.59, 137.58, 144.66, 163.09, 169.51, 169.56, 170.05, 170.88, 170.94; HRMS: calcd. for $C_{188}H_{222}N_{26}NaO_{76}$, 4083.4237, found 4083.5924.

Compound **PBI-AFF-AcGlu**: m.p. 146.2-147.2 ºC. ^{1}H NMR ($CDCl_3$, 600 MHz): δ (ppm) 1.57 (d, 6H, J = 6.6 Hz, -CH_3), 1.89 (s, 18H, -$COCH_3$), 1.98 (s, 18H, -$COCH_3$), 2.01 (s, 18H, -$COCH_3$), 2.06 (s, 18H, -$COCH_3$), 300-3.07 (m, 4H), 3.16 (dd, 2H, J = 9.0 Hz, 4.8 Hz), 3.30 (dd, 2H, J = 5.4 Hz, 8.4 Hz), 3.67-3.70 (12H), 3.75 (d, 6H, J = 9.6 Hz), 4.12 (dd, 6H, J = 1.8 Hz, 10.2 Hz), 4.41-4.56 (32H), 4.64 (dd, 2H, J = 8.4 Hz, 6.0 Hz), 4.95 (t, 6H, J = 7.8 Hz), 5.05 (t, 6H, J = 10.2 Hz), 5.17 (t, 6H, J = 9.0 Hz), 5.60 (dd, 2H, J = 6.6 Hz, 7.2 Hz), 6.36 (d, 2H, J = 4.8 Hz), 6.60 (s, 2H), 7.03-7.11 (10H), 7.18-7.25 (8H), 7.28 (2H), 7.53 (d, 2H, J = 8.4 Hz), 7.60 (s, 6H, Triaz-H), 8.68 (d, 4H, J = 7.8 Hz, perylene-H), 8.84 (d, 4H, J = 8.4 Hz, perylene-H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 14.20, 22.73, 29.73, 31.96, 36.34, 37.48, 49.79, 50.40, 54.27, 55.63, 59.81, 61.77, 64.68, 67.78, 68.24, 68.76, 70.84, 71.89, 72.46, 100.54, 122.78, 123.96, 126.39, 126.73, 126.88, 128.46, 128.68, 129.07, 129.33, 129.44, 132.24, 135.14, 136.60, 137.63, 144.71, 163.13, 169.40, 169.48, 169.56, 170.19, 170.67, 170.91, 171.98; HRMS: calcd. for $C_{188}H_{223}N_{26}O_{76}$, 4060.4378, found 4060.4521.

General synthesis of compounds **PBI-AFF-Man**, **PBI-AFF-Gal** and **PBI-AFF-Glu**. Compounds **PBI-AFF-AcMan** (118 mg, 0.029 mmol), **PBI-AFF-AcGal** (118 mg, 0.029 mmol) or **PBI-AFF-AcGlu** (118 mg, 0.029 mmol), and MeONa (9.2 mg, 0.58 mmol) were dissolved in MeOH (10 mL). The reaction mixture was stirred at room temperature for 12 h. The reaction mixture was then put in a cellulose dialysis tube (cutoff 1000) and dialyzed against water for 2 d. Compounds **PBI-AFF-Man** (85 mg), **PBI-AFF-Gal** (80 mg) and **PBI-AFF-Glu** (83 mg) were obtained through lyophilization in yields of 95.9%, 90.3% and 93.8%, respectively.

Compound **PBI-AFF-Man**: m.p. 148.2-148.5 ºC. ^{1}H NMR (DMSO-d_6 + D_2O, 600 MHz): δ (ppm) 1.60 (s, 6H), 2.61-2.63 (m, 2H), 2.79-2.80 (4H), 2.99 (d, 2H, J = 10.2 Hz), 3.13-3.16 (m, 6H), 3.35-3.43 (18H), 3.53 (6H), 3.59-3.64 (18H), 3.76-3.78 (m, 6H), 3.92-3.94 (m, 6H), 4.40-4.55 (26H), 4.81 (s, 6H), 4.81 (dd, 1H, J = 3.6 Hz, 9.6 Hz), 5.38 (d, 2H, J = 6.6 Hz), 6.97-7.07 (10h), 7.22-7.29 (10H), 7.99 (s, 6H, Triaz-H), 8.36 (s, 4H, perylene-H), 8.74 (s, 4H, perylene-H); ^{13}C NMR (DMSO-d_6 + D_2O, 100 MHz): δ (ppm) 14.40, 22.58, 29.19, 36.35, 49.70, 54.55, 54.75, 60.27, 61.60, 64.59, 65.32, 67.20, 68.32, 70.53, 71.27, 74.60, 100.27, 123.21, 124.22, 124.60, 125.53, 126.35, 128.26, 128.48, 128.76, 129.36, 129.81, 131.14, 134.08, 138.19, 138.58, 144.35, 162.64, 169.68, 171.59, 171.78; HRMS: calcd. for $C_{140}H_{174}N_{26}NaO_{52}$, 3074.1662, found 3074.1640.

Compound **PBI-AFF-Gal**: m.p. 149.1-149.9 ºC. ^{1}H NMR (DMSO-d_6 + D_2O, 600 MHz): δ (ppm) 1.60 (s, 6H, -CH_3), 2.59-2.61 (m, 2H), 2.78 (m, 4H), 2.98 (d, 2H, J = 10.2 Hz), 3.27-3.28 (m, 12H), 3.33-3.35 (m, 6H), 3.47-3.50 (12H), 3.83-3.85 (m, 6H), 4.02-4.04 (m, 6H), 4.14 (d, 6H, J = 6.6 Hz), 4.38-4.51 (32H), 4.69-4.78 (m, 2H), 4.99 (d, 1H, J = 4.2 Hz), 5.36 (d, 2H, J = 7.2 Hz), 6.95-6.96 (m, 8H), 7.03-7.04 (m, 2H), 7.21-7.22 (m, 2H), 7.26-7.29 (m, 8H), 8.06 (s, 6H, Triaz-H), 8.31 (s, 4H, perylene-H), 8.67 (s, 4H, perylene-H); ^{13}C NMR (DMSO-d_6 + D_2O, 100 MHz): δ (ppm) 14.40, 22.59, 29.51, 36.36, 38.24, 50.06, 54.54, 60.30, 60.91, 64.62, 67.63, 68.36, 68.58, 70.87, 73.78, 75.83, 103.93, 123.19, 124.23, 125.08, 126.33, 126.71, 128.25, 128.48, 129.38, 129.83, 130.13, 131.08, 134.06, 138.22, 138.58, 144.23, 144.53, 162.62, 169.62, 171.64, 171.75; HRMS: calcd. for $C_{140}H_{175}N_{26}O_{52}$, 3052.1843, found 3052.1724.

Compound **PBI-AFF-Glu**: m.p. 147.4-148.0 ºC. ^{1}H NMR (DMSO-d_6 + D_2O, 600 MHz) : δ (ppm) 1.59 (s, 6H, -CH_3), 2.58-2.61 (m, 2H), 2.76-2.78 (m, 4H), 2.94 (t, 6H, J = 8.4 Hz), 3.01 (t, 6H, J = 9.0 Hz), 3.10-3.15 (12H), 3.40 (dd, 6H, J = 6.0 Hz, 5.4 Hz), 3.60-3.64 (10H), 3.85-3.88 (m, 6H), 4.03-4.05 (m, 6H), 4.20 (d, 6H, J = 7.8 Hz), 4.37-

4.50 (30 H), 4.67-4.69 (m, 1H), 5.04 (t, 1H, *J* = 7.8 Hz), 5.17 (d, 1H, *J* = 4.8 Hz), 5.35 (d, 2H, *J* = 6.6 Hz), 6.94-7.04 (10H), 7.21-7.28 (10H), 8.05 (s, 6H, Triaz-H), 8.31 (s, 4H, perylene-H), 8.66 (s, 4H, perylene-H); ^{13}C NMR (DMSO-d_6 + D_2O, 100 MHz): δ (ppm) 14.41, 22.59, 29.51, 36.36, 38.24, 50.05, 54.53, 60.31, 61.52, 63.53, 64.61, 67.76, 68.36, 70.44, 72.97, 7375, 77.04, 77.42, 103.32, 123.15, 124.20, 125.13, 126.34, 126.72, 128.25, 128.48, 129.37, 129.83, 131.06, 134.00, 138.20, 138.58, 144.21, 144.50, 162.60, 169.64, 171.63, 171.76; HRMS: calcd. for $C_{140}H_{175}N_{26}O_{52}$, 3052.1843, found 3052.1836.

S3. NMR and HRMS results

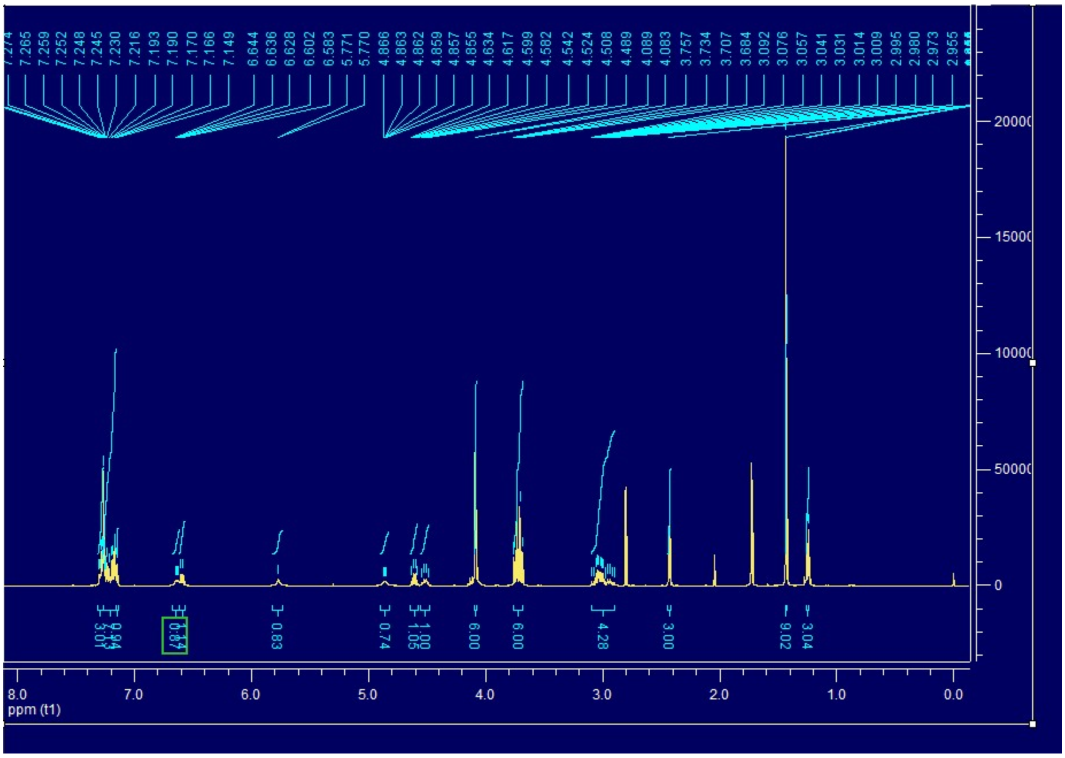


Fig. S1 ^{1}H NMR ($CDCl_3$, 600 MHz) of compound **AFF-T**.

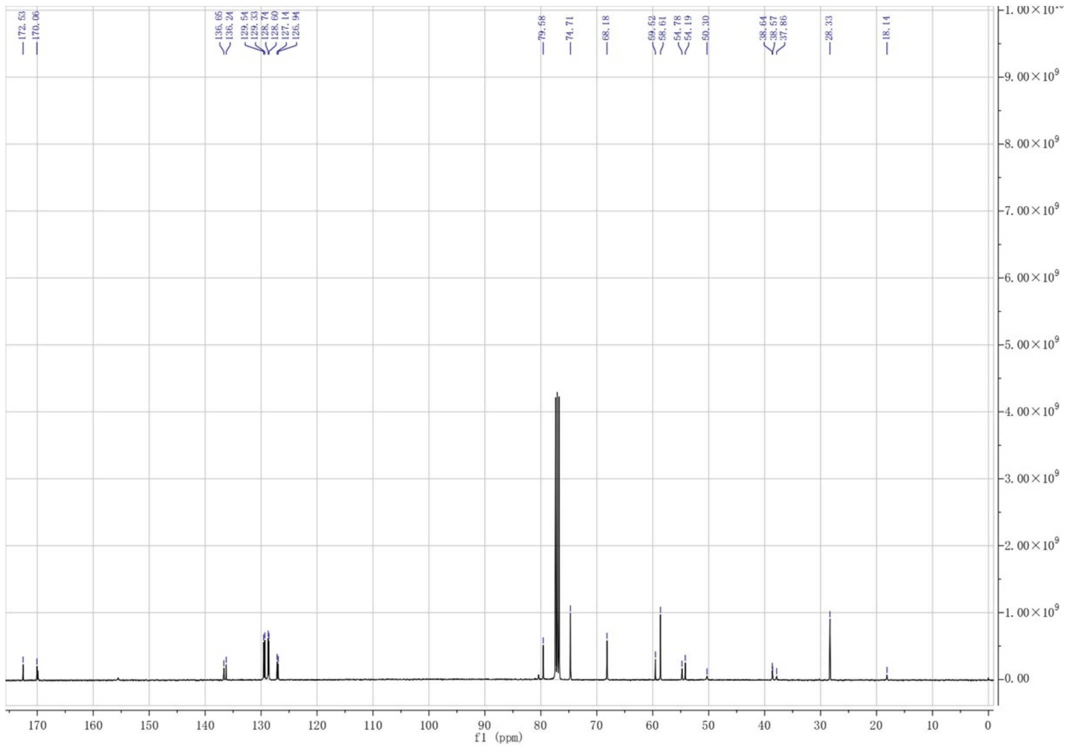


Fig. S2 ^{13}C NMR ($CDCl_3$, 100 MHz) of compound **AFF-T**.

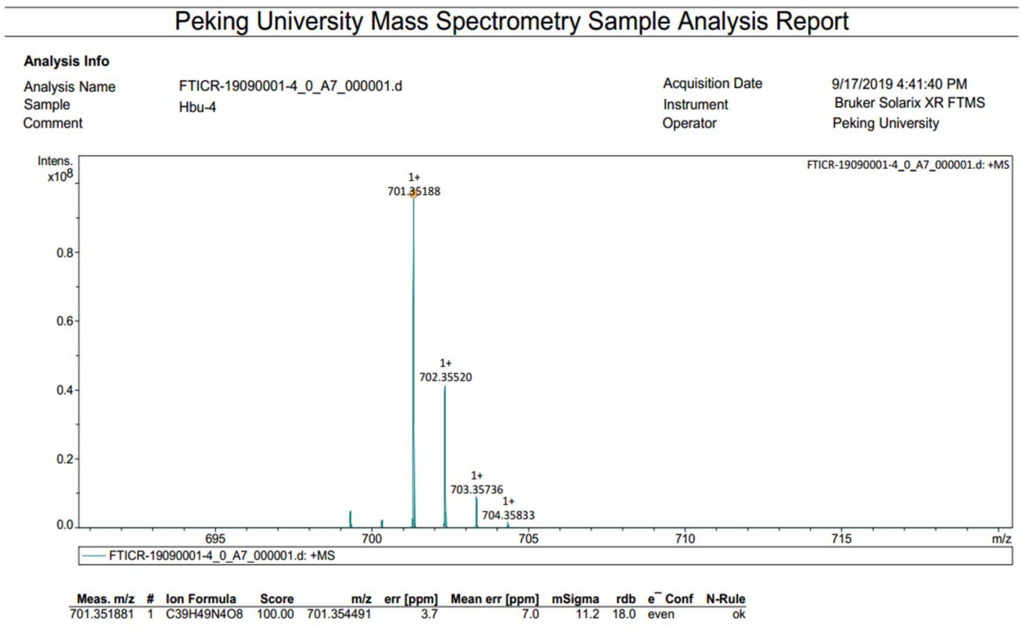


Fig. S3 HRMS (MALDI-TOF) of compound **AFF-T**.

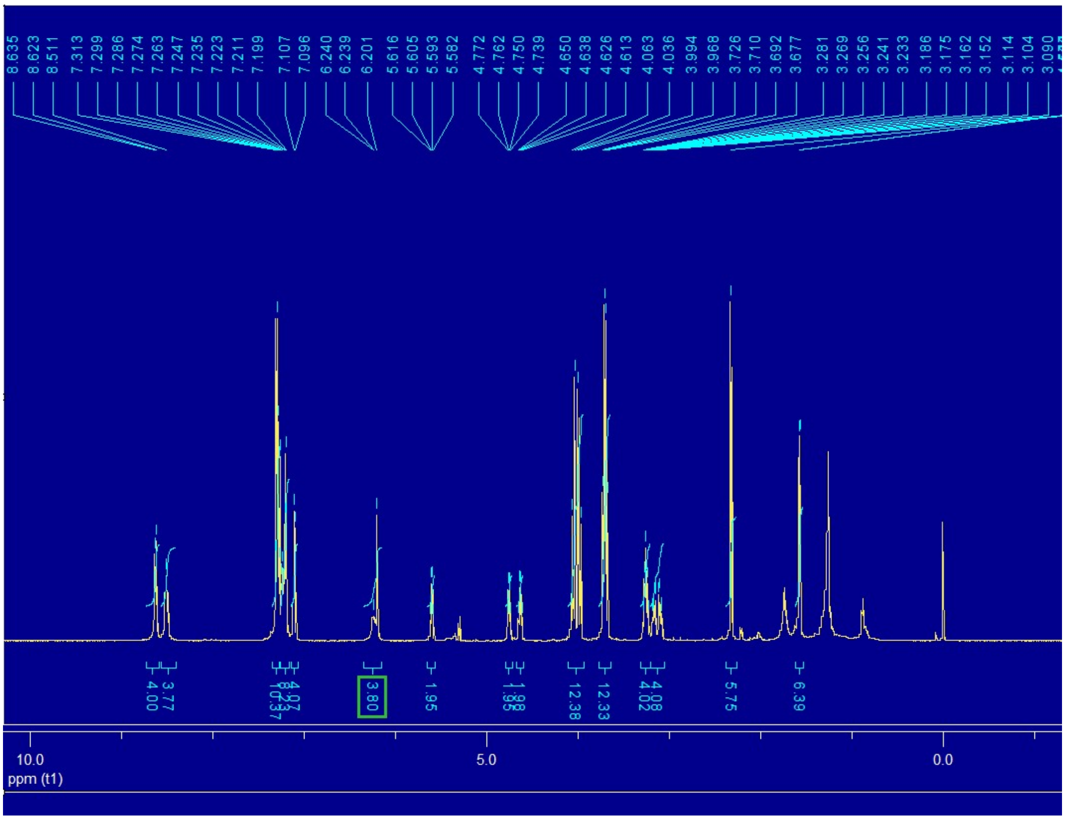


Fig. S4 ^{1}H NMR ($CDCl_3$, 600 MHz) of compound **3**.

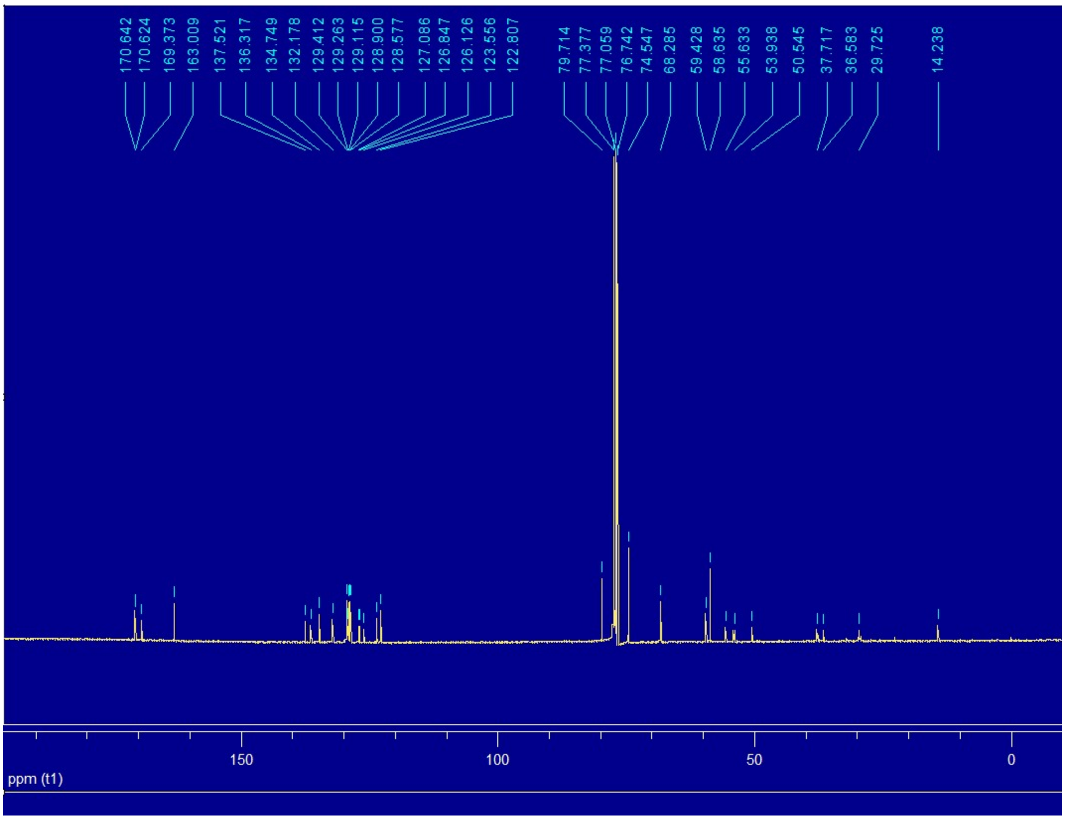


Fig. S5 ^{13}C NMR ($CDCl_3$, 150 MHz) of compound **3**.

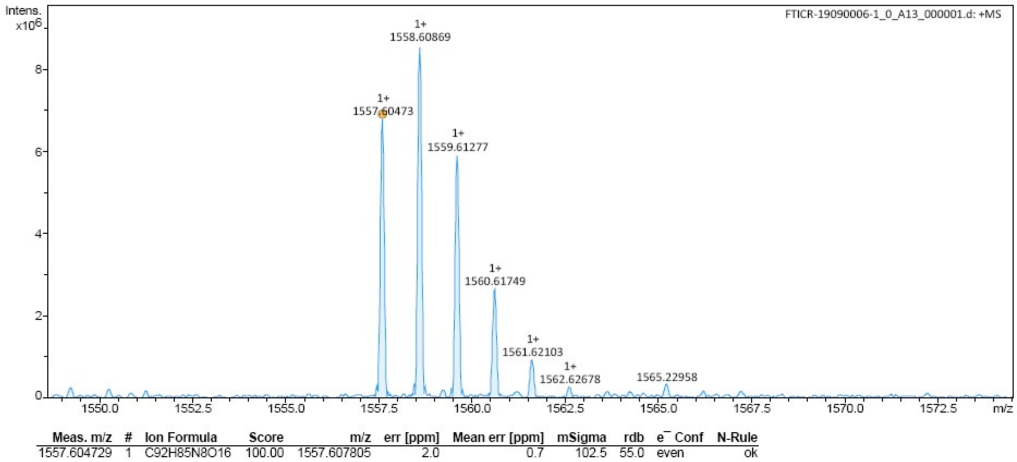


Fig. S6 HRMS (MALDI-TOF) of compound **3**.

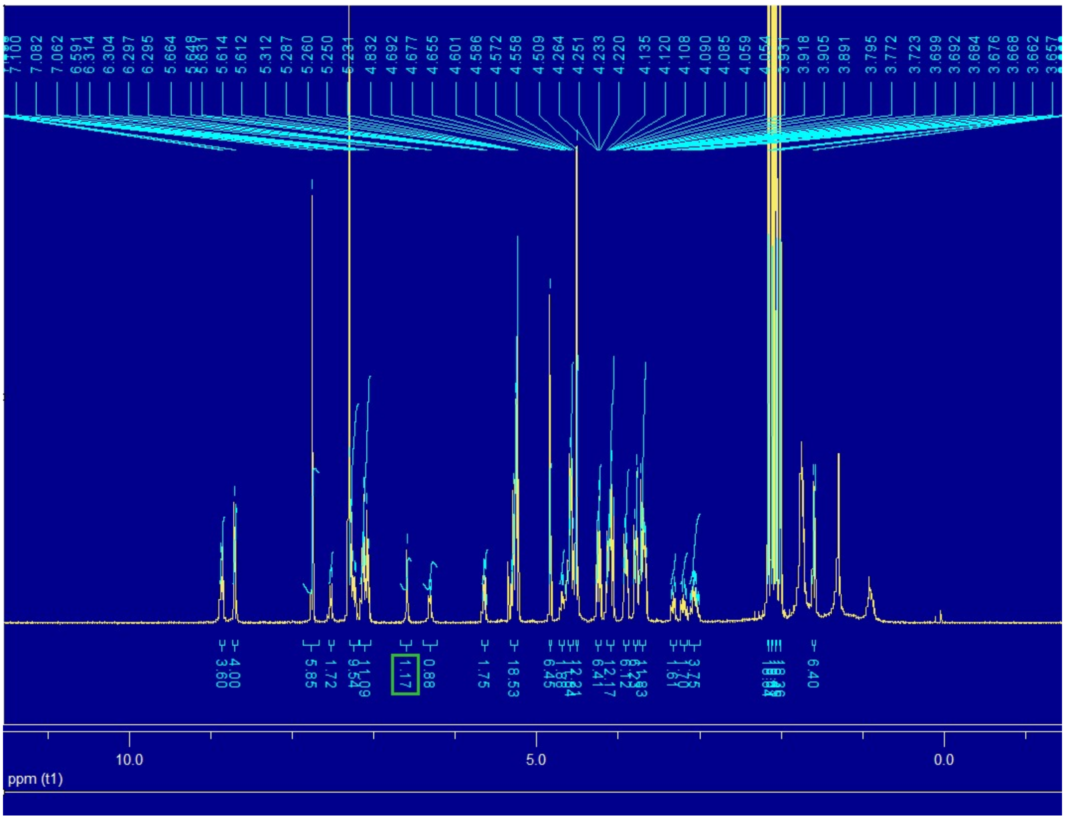


Fig. S7 ^{1}H NMR ($CDCl_3$, 600 MHz) of compound **PBI-AFF-AcMan**.

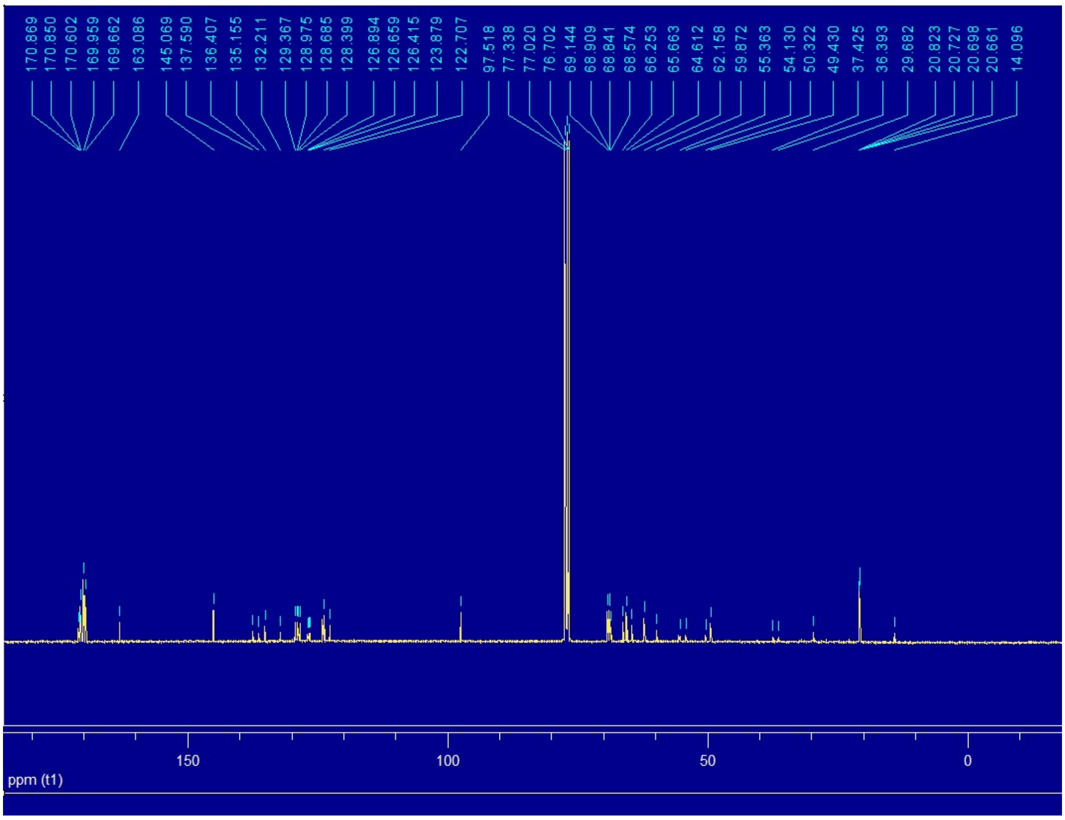
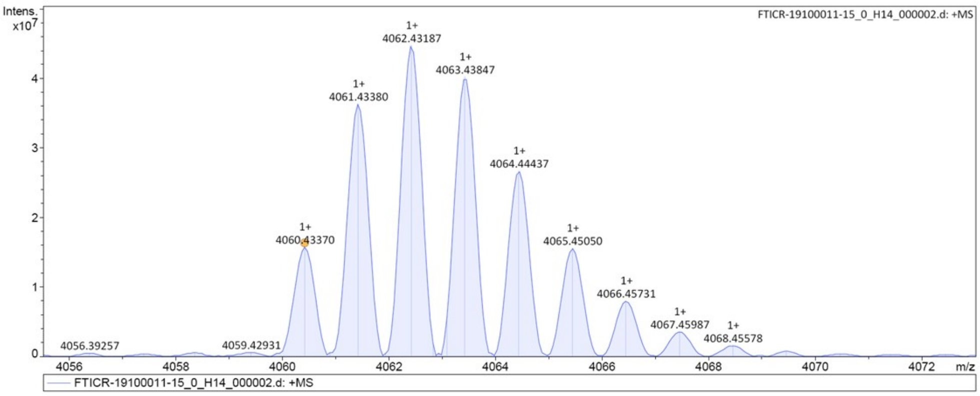


Fig. S8 ^{13}C NMR ($CDCl_3$, 100 MHz) of compound **PBI-AFF-AcMan**.



Meas. m/z	#	Ion Formula	Score	m/z	err [ppm]	Mean err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
4060.433702	1	C188H223N26O76	100.00	4060.437869	1.0	1.4	56.8	91.0	even	ok

Fig. S9 HRMS (MALDI-TOF) of compound **PBI-AFF-AcMan**.

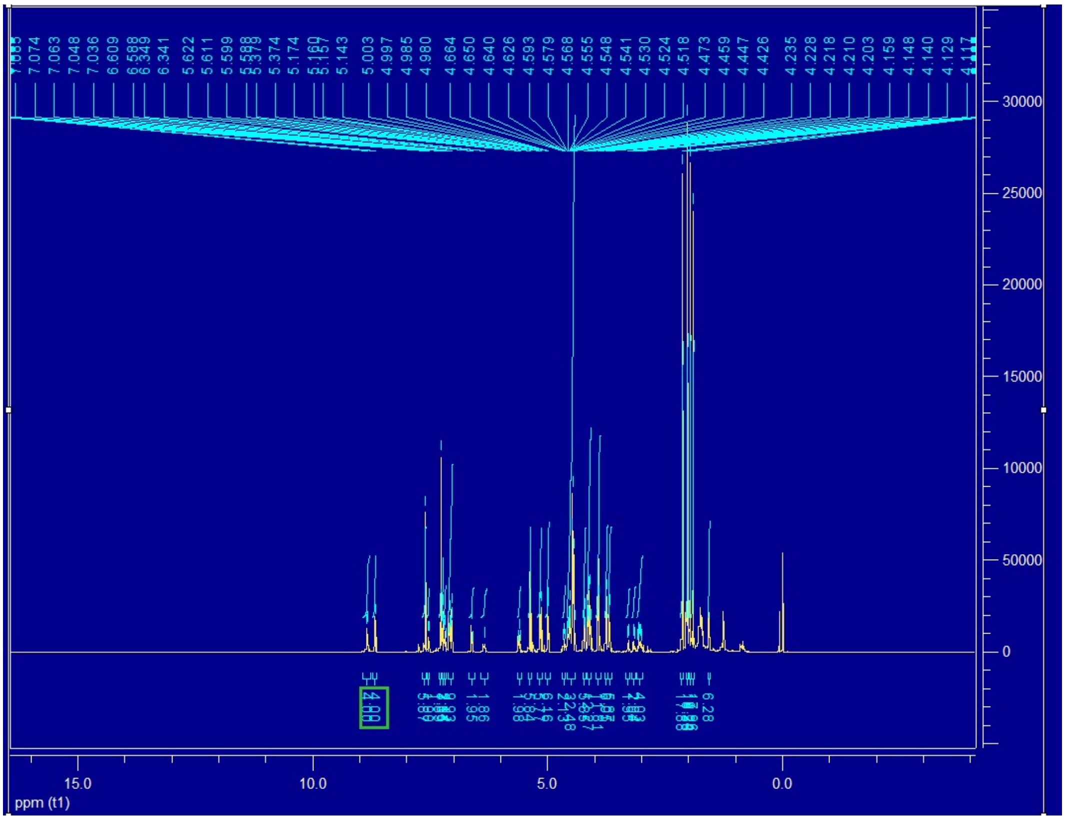


Fig. S10 ^{1}H NMR ($CDCl_3$, 600 MHz) of compound **PBI-AFF-AcGal**.

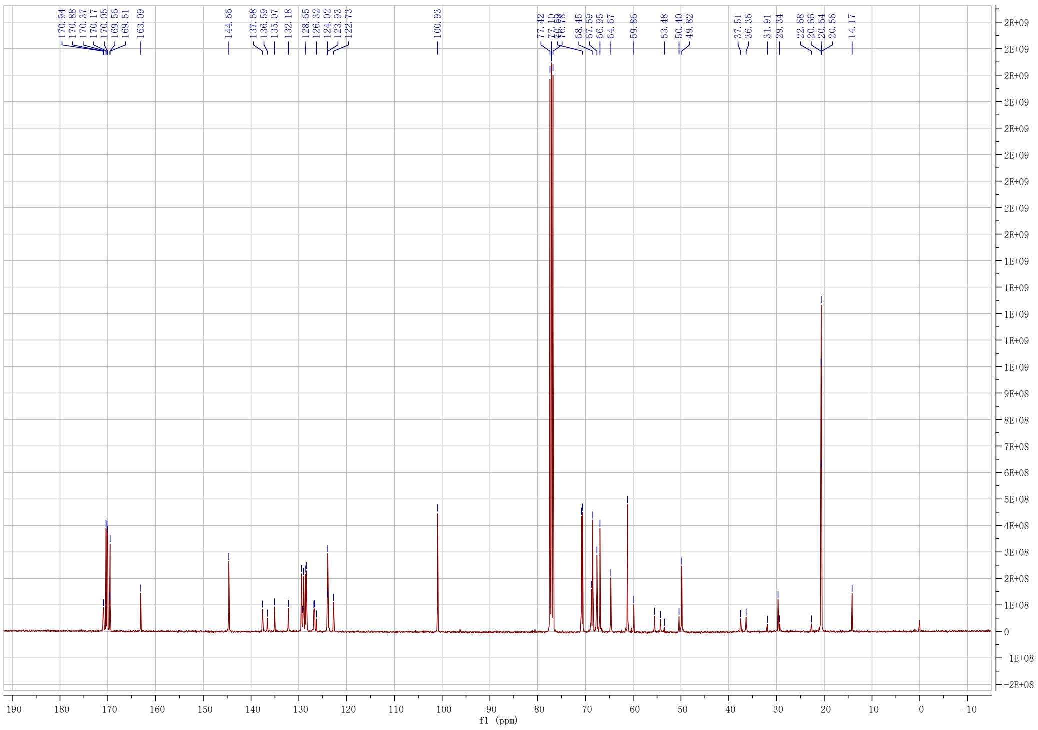


Fig. S11 ^{13}C NMR ($CDCl_3$, 100 MHz) of compound **PBI-AFF-AcGal**.

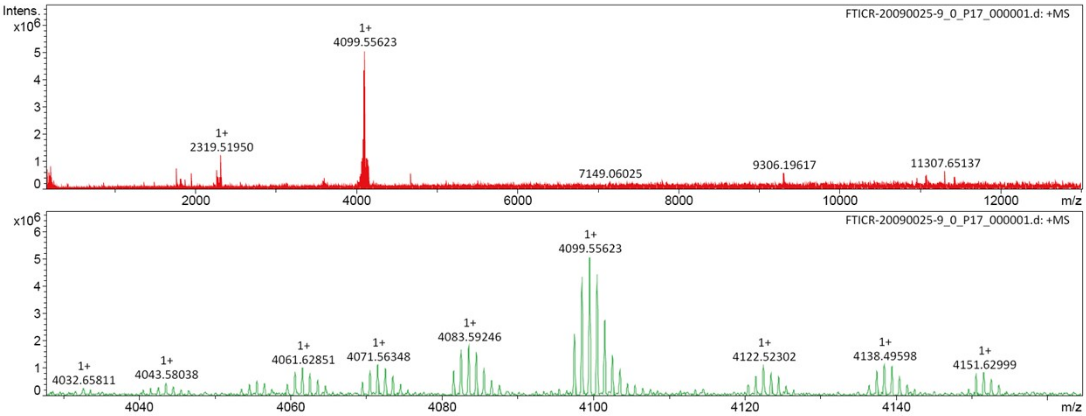


Fig. S12 HRMS (MALDI-TOF) of compound **PBI-AFF-AcGal**.

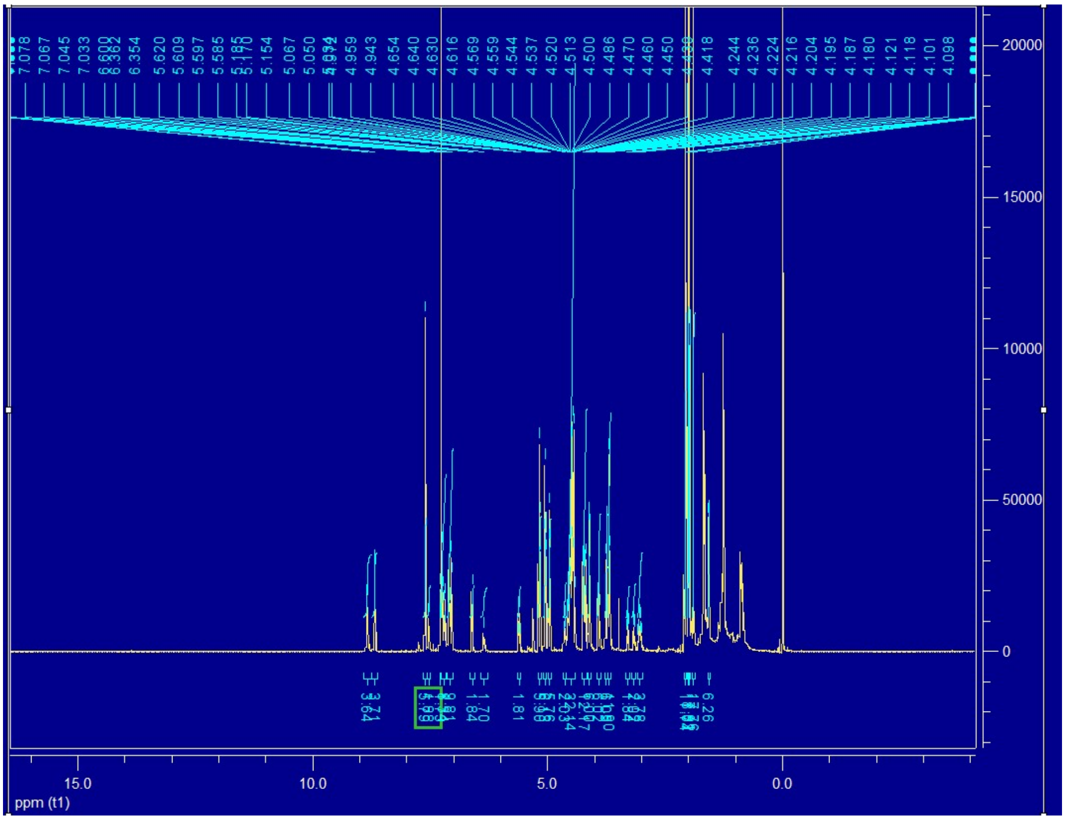


Fig. S13 ^{1}H NMR ($CDCl_3$, 600 MHz) of compound **PBI-AFF-AcGlu**.

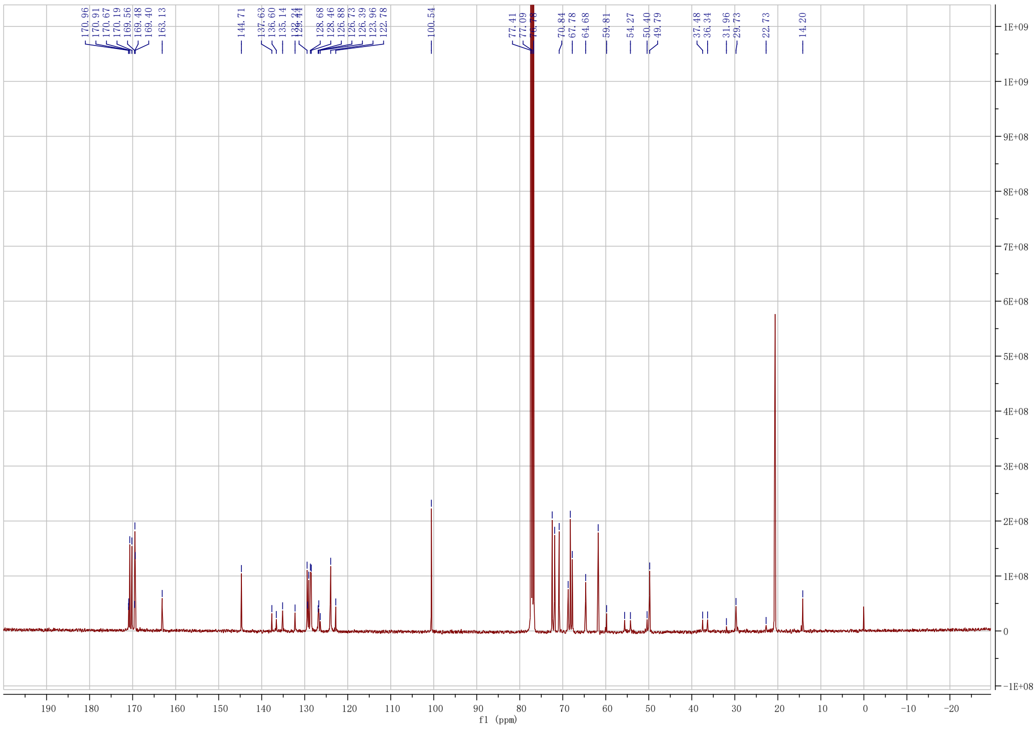
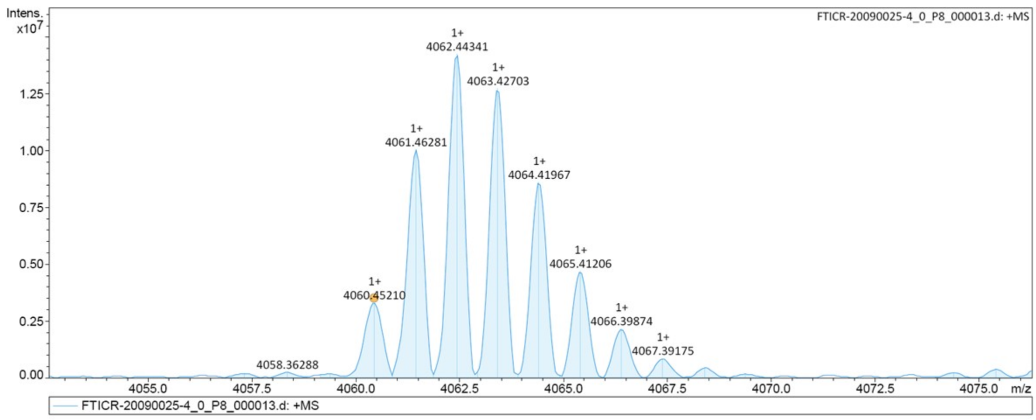


Fig. S14 ^{13}C NMR ($CDCl_3$, 100 MHz) of compound **PBI-AFF-AcGlu**.



Meas. m/z	#	Ion Formula	Score	m/z	err [ppm]	Mean err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
4060.452100	1	C188H223N26O76	100.00	4060.437869	-3.5	4.2	88.7	91.0	even	ok

Fig. S15 HRMS (MALDI-TOF) of compound **PBI-AFF-AcGlu**.

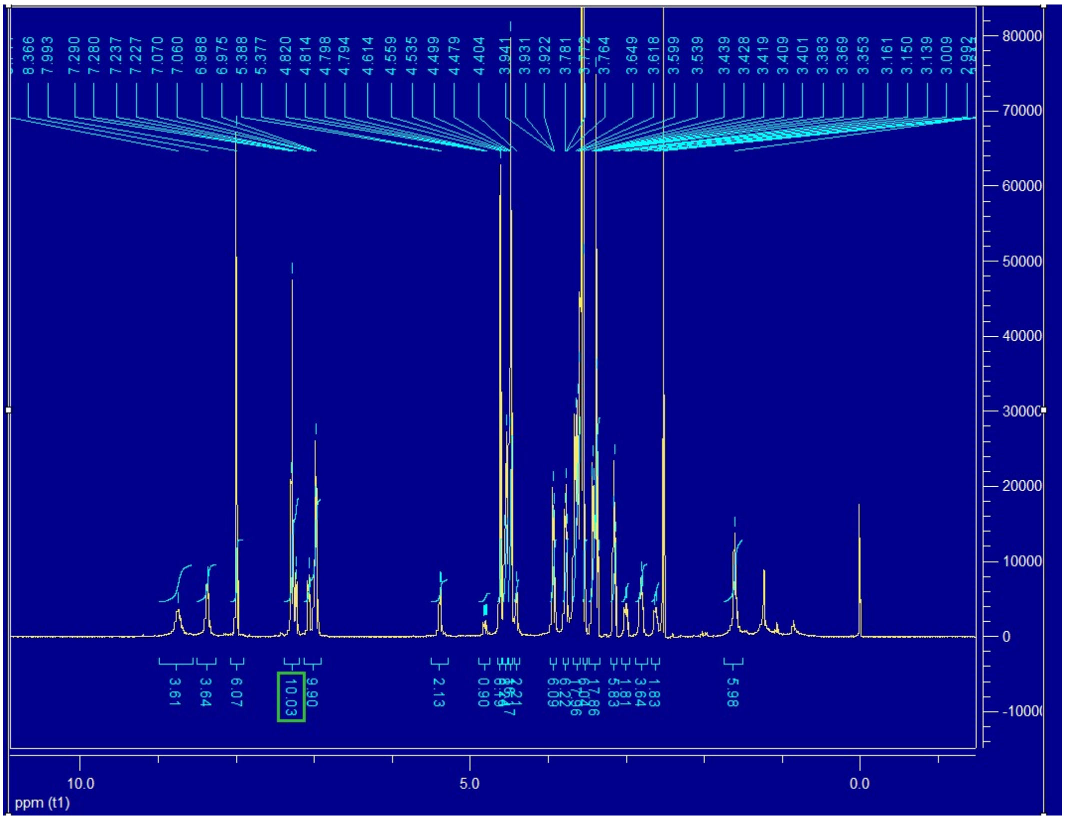


Fig. S16 ^{1}H NMR (DMSO-d$_6$+D$_2$O, 600 MHz) of compound **PBI-AFF-Man**.

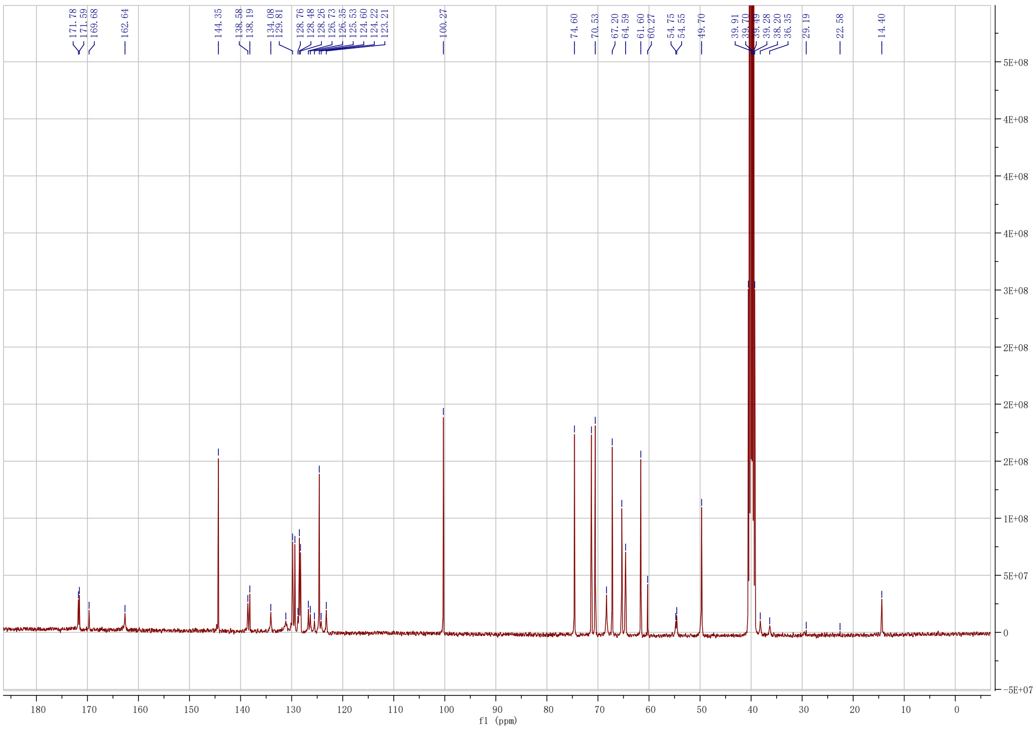
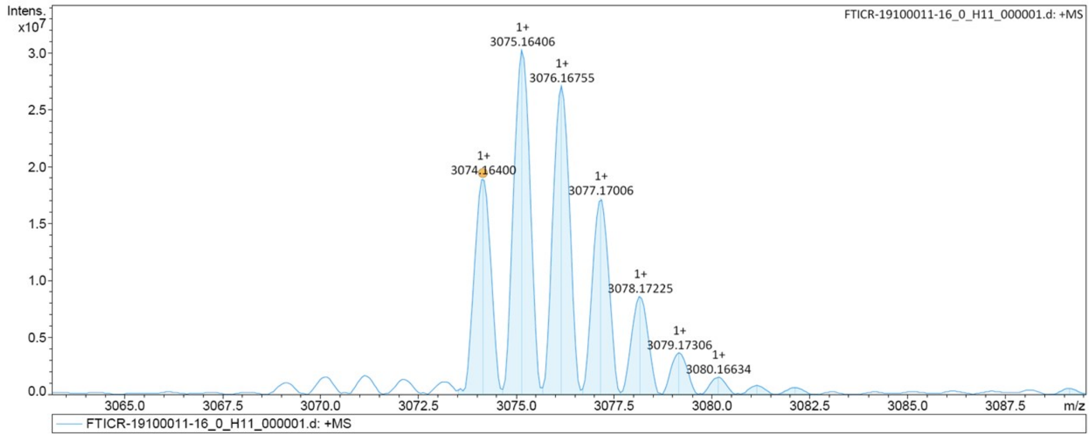


Fig. S17 ^{13}C NMR (DMSO-d$_6$+D$_2$O, 100 MHz) of compound **PBI-AFF-Man**.



Meas. m/z	#	Ion Formula	Score	m/z	err [ppm]	Mean err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
3074.164000	1	C140H174N26NaO52	-1.#J	3074.166261	0.7	26.8	14.1	67.0	even	ok

Fig. S18 HRMS (MALDI-TOF) of compound **PBI-AFF-Man**.

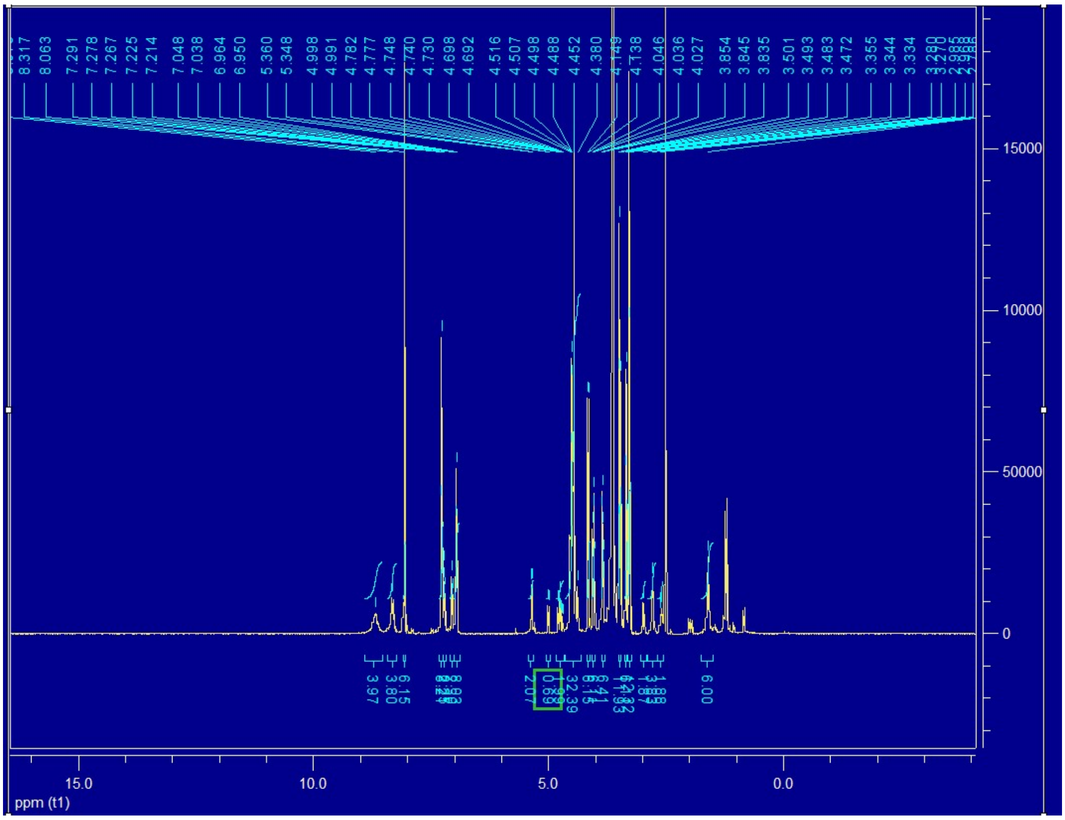


Fig. S19 ^{1}H NMR (DMSO-d_6+D_2O, 600 MHz) of compound **PBI-AFF-Gal**.

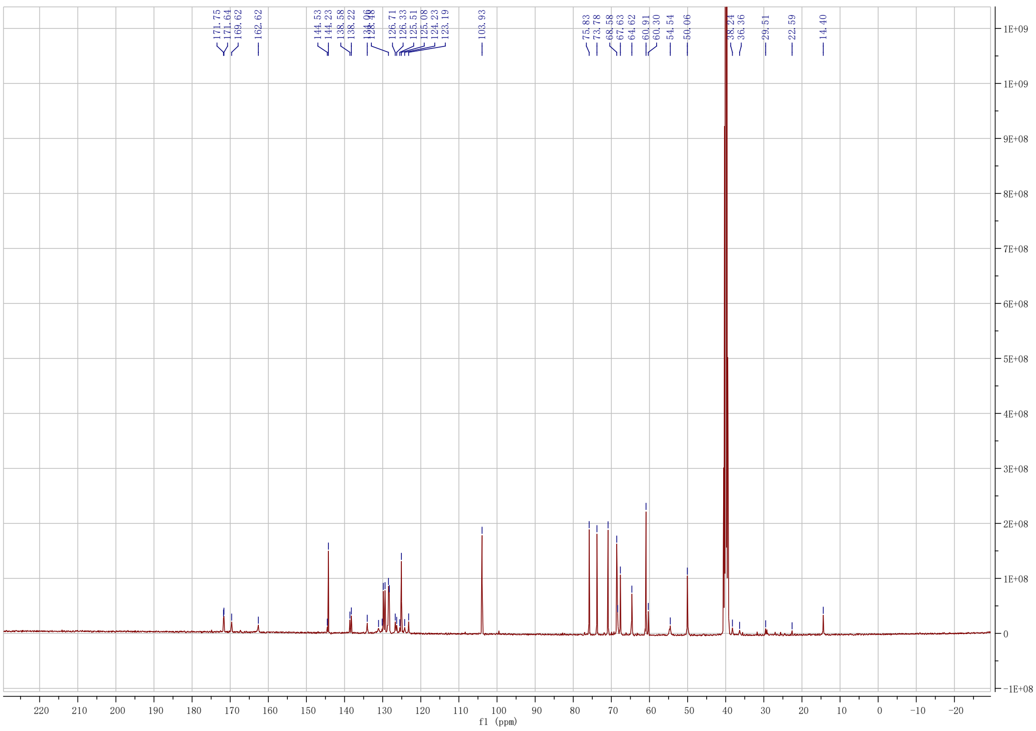
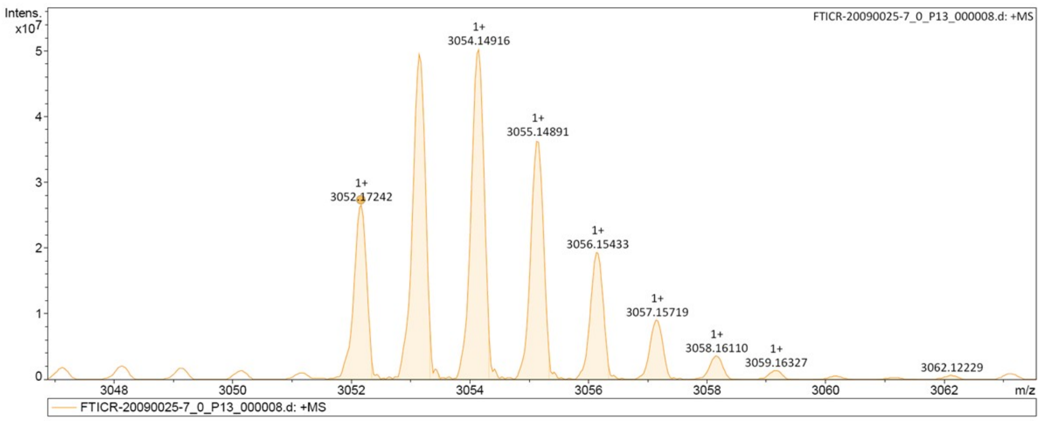


Fig. S20 ^{13}C NMR (DMSO-d$_6$+D$_2$O, 100 MHz) of compound **PBI-AFF-Gal**.



Meas. m/z	#	Ion Formula	Score	m/z	err [ppm]	Mean err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
3052.172415	1	C140H175N26O52	100.00	3052.184316	3.9	11.1	98.7	67.0	even	ok

Fig. S21 HRMS (MALDI-TOF) of **PBI-AFF-Gal**.

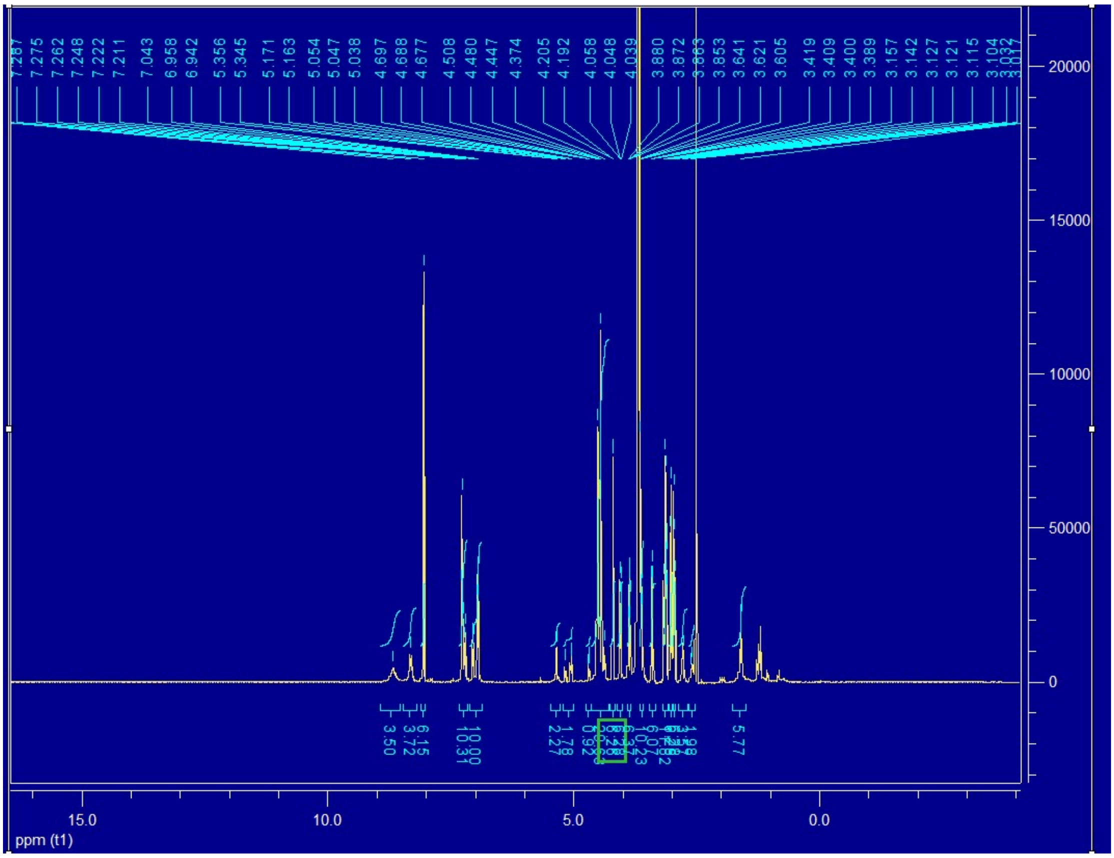


Fig. S22 [1]H NMR (DMSO-d_6+D_2O, 600 MHz) of compound **PBI-AFF-Glu**.

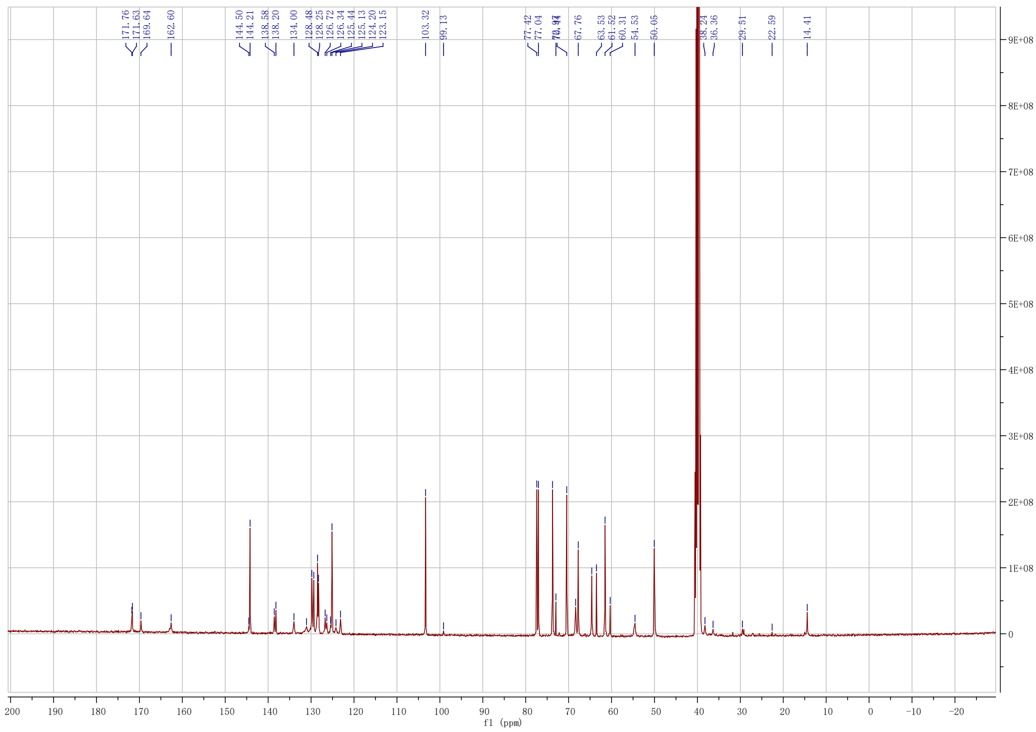
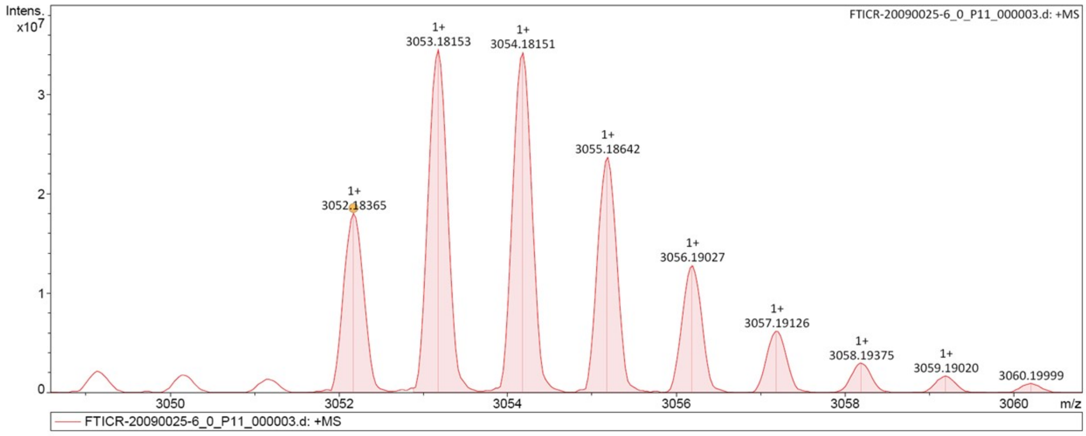


Fig. S23 [13]C NMR (DMSO-d_6+D_2O, 100 MHz) of compound **PBI-AFF-Glu**.



Meas. m/z	#	Ion Formula	Score	m/z	err [ppm]	Mean err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
3052.183651	1	C140H175N26O52	100.00	3052.184316	0.2	2.0	87.7	67.0	even	ok

Fig. S24 HRMS (MALDI-TOF) of compound **PBI-AFF-Glu**.

S4. Additional Figures

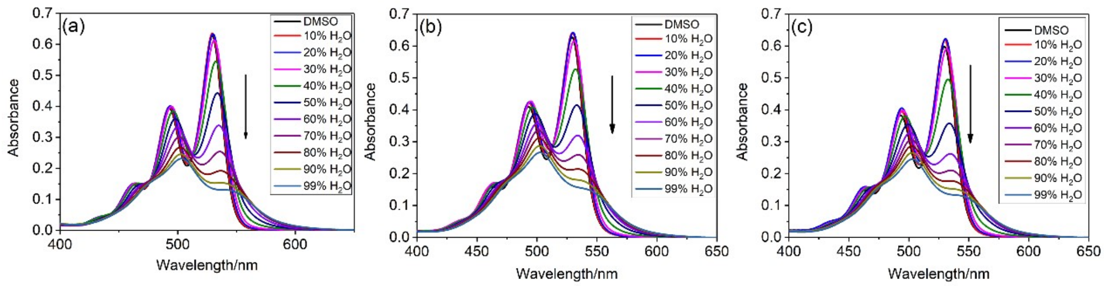


Fig. S25 Solvent-dependent UV-Vis spectra of **PBI-AFF-Man**, **PBI-AFF-Gal** and **PBI-AFF-Glu** in various ratio of DMSO-H_2O under the concentration of 1 × 10^{-5} M.

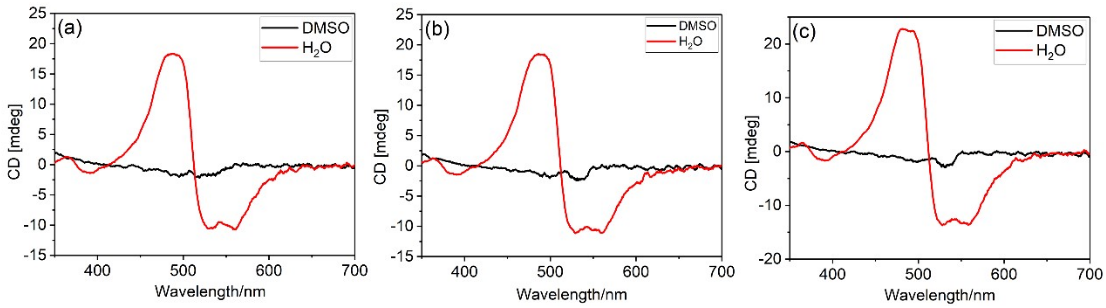


Fig. S26 Solvent-dependent CD spectra of **PBI-AFF-Man**, **PBI-AFF-Gal** and **PBI-AFF-Glu** in DMSO and H_2O solution under the concentration of 2 × 10^{-5} M.

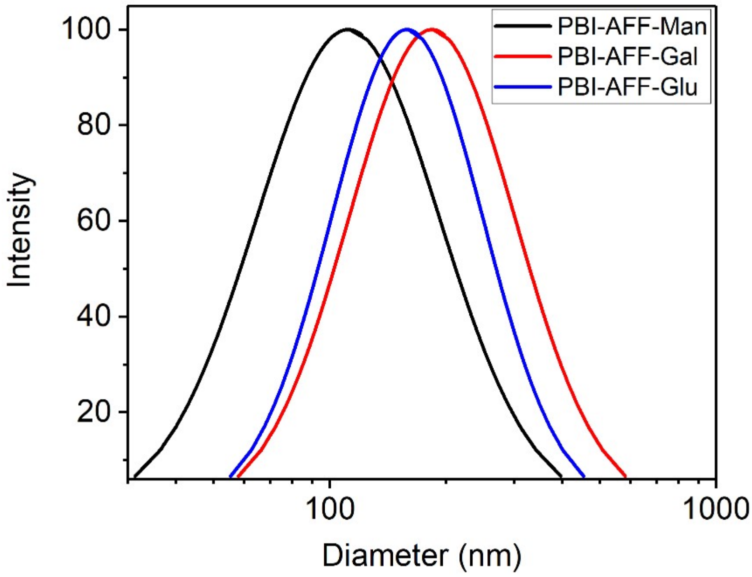


Fig. S27 DLS data (a) of **PBI-AFF-Man**, **PBI-AFF-Gal** and **PBI-AFF-Glu** at the concentration of 1 × 10^{-4} M.

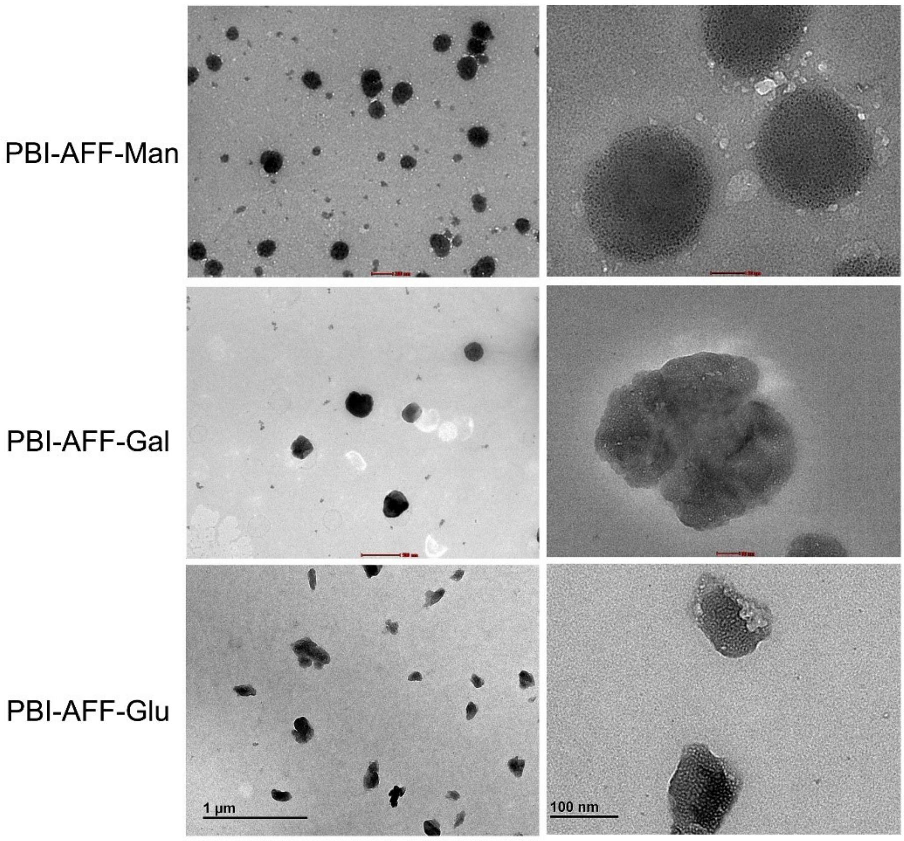


Fig. S28 TEM images of **PBI-AFF-Man**, **PBI-AFF-Gal** and **PBI-AFF-Glu**.

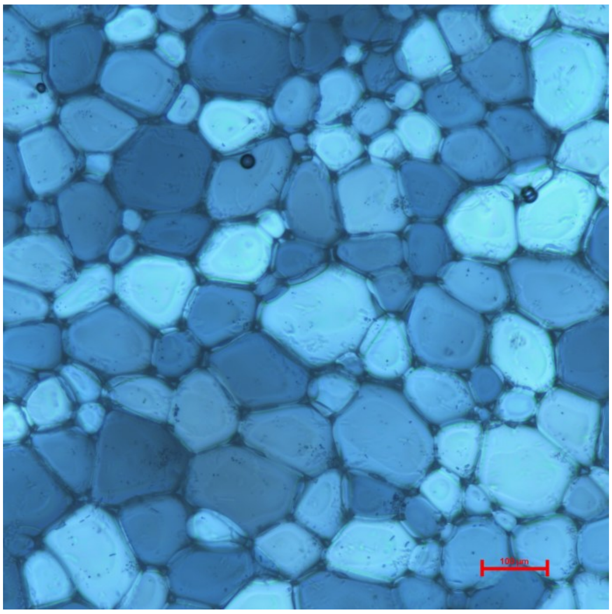


Fig. S29 Representative polarized light microscopy images of ice wafers for PBS buffer (pH = 7.3) after 30 min annealing at -6 °C.

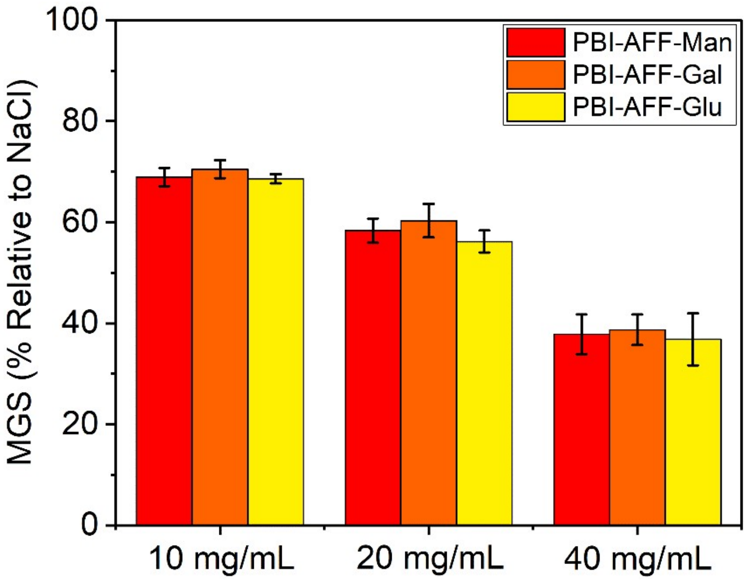


Fig. S30 IRI activities of **PBI-AFF-Man**, **PBI-AFF-Gal** and **PBI-AFF-Glu** under the concentrations of 10 mg/mL, 20 mg/mL and 40 mg/mL calculated by the % MGS values relative to PBS buffer (pH = 7.3). Each value represents the mean ± SEM (n = 5).

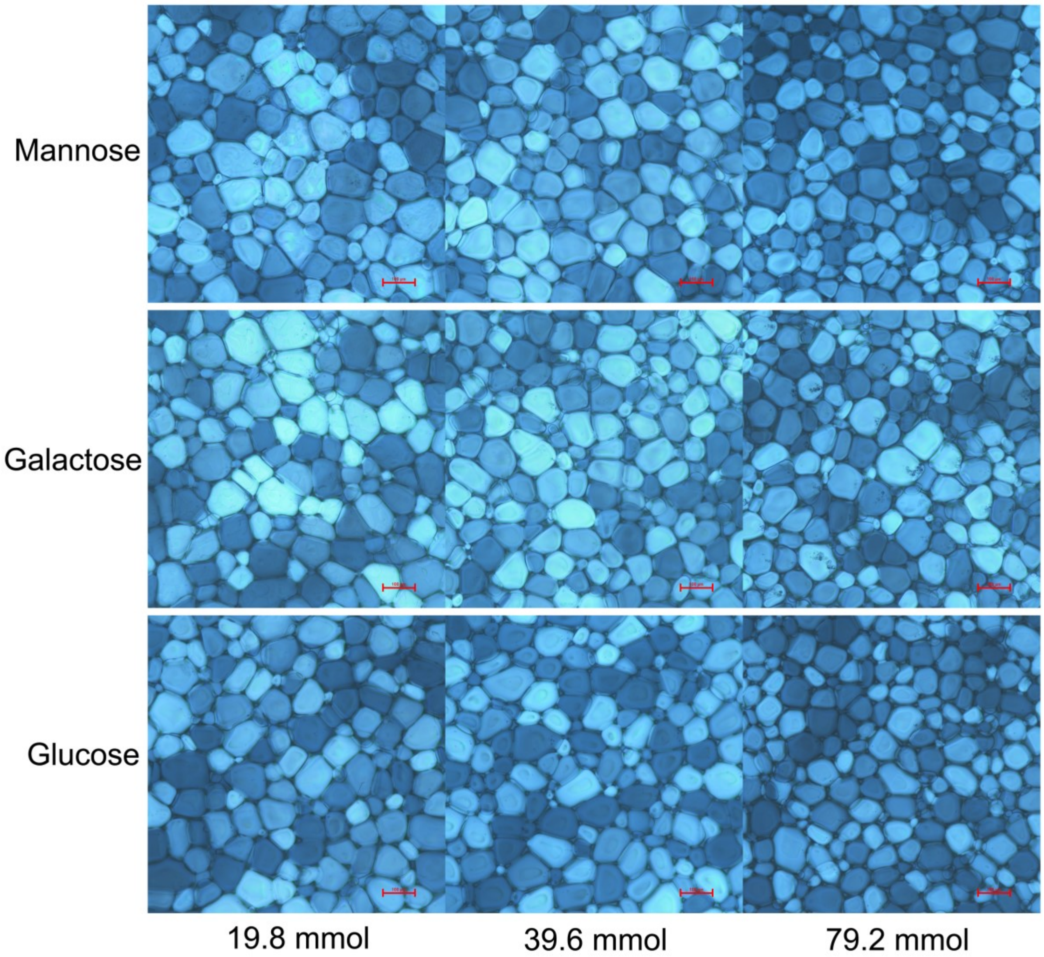


Fig. S31 Representative polarized light microscopy images of ice wafers for mannose, galactose and glucose after 30 min annealing at -6 °C under the concentrations of 19.8 mmol/mL, 39.6 mmol/mL and 79.2 mmol/mL.

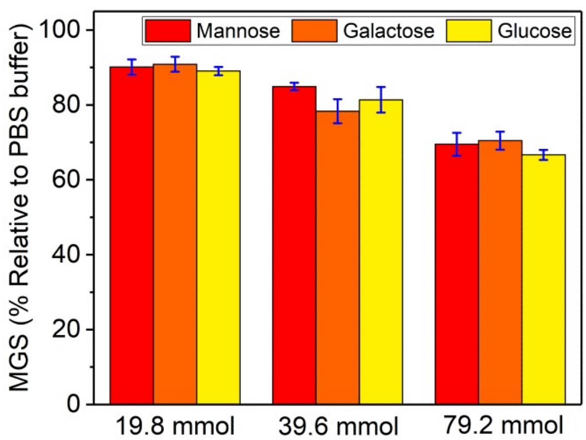


Fig. S32 IRI activities of mannose, galactose and glucose under the concentrations of 10 mg/mL, 20 mg/mL and 40 mg/mL calculated by the % MGS values relative to PBS buffer (pH = 7.3). Each value represents the mean ± SEM (n = 5).

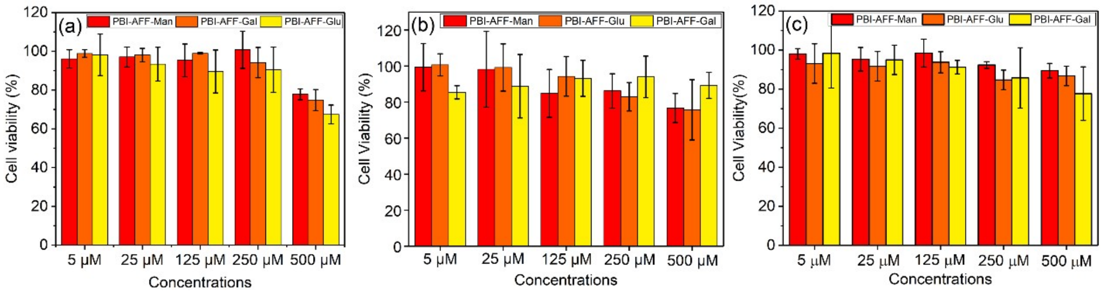


Fig. S33 Cell viability of **PBI-AFF-Man**, **PBI-AFF-Gal** and **PBI-AFF-Glu** against HeLa cells (a), A549 cells (b) and GES-1 cells (c) at different concentrations (5.0, 25.0, 125.0, 250.0 and 500 μM). Each value represents the mean ± SEM (n = 5).

Table S1 Viability and enhanced cell viability of **PBI-AFF-Man**, **PBI-AFF-Gal** and **PBI-AFF-Glu** for HeLa cells, A549 cellsand GES-1 cells under the concentrations of 0.5 mg/mL, 1.0 mg/mL and 2.0 mg/mL after cryopreservation for 24 h in the solution of 10% DMSO (or 5% DMSO), 80% DMEM (or 85% DMEM) and 10% FBS (control).

Compounds	**Dose**	**HeLa cells**		**A549 cells**		**GES-1 cells**	
		Cell viability (%)	Enhanced cell viability (%)	Cell viability (%)	Enhanced cell viability (%)	Cell viability (%)	Enhanced cell viability (%)
PBI-AFF-Man	Control	30.14±8.78	-	71.90±2.87	-	65.63±0.47	-
	0.5 mg/mL	39.79±8.05	9.65±1.05	79.10±1.51	7.10±1.47	74.80±0.40	9.20±0.40
	1.0 mg/mL	46.91±1.05	16.29±8.43	86.96±1.81	15.06±1.81	83.63±0.51	18.03±0.51
	2.0 mg/mL	26.58±3.91	-	75.16±0.45	-	71.06±1.15	-
PBI-AFF-Glu	Control	31.24±3.84	-	72.33±2.22	-	63.10±5.20	-
	0.5	43.30±6.36	12.06±4.03	85.20±0.36	12.90±0.36	69.86±3.46	9.40±0.81

	mg/mL						
	1.0 mg/mL	54.02±3.58	22.77±3.33	91.73±1.90	19.43±1.90	75.83±1.22	16.63±1.76
	2.0 mg/mL	38.43±0.64	-	78.26±1.53	-	68.73±1.38	-
PBI-AFF-Gal	Control	33.10±1.16	-	71.03±2.57	-	66.76±5.20	-
	0.5 mg/mL	38.09±3.97	5.54±2.63	78.73±4.35	7.73±4.35	69.86±3.46	3.96±3.46
	1.0 mg/mL	44.52±7.84	11.42±6.98	86.03±1.40	15.03±1.40	75.83±1.22	9.93±1.22
	2.0 mg/mL	35.89±4.51	-	85.40±7.27	-	68.73±1.38	-

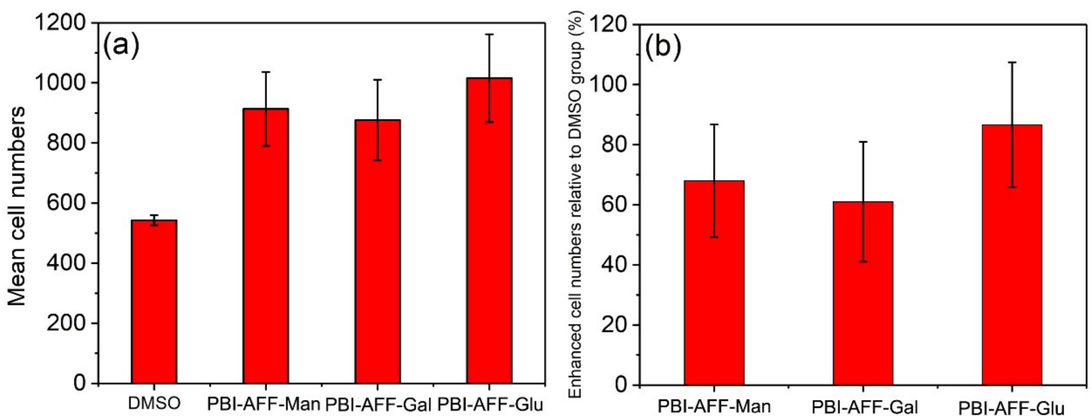


Fig. S34 (a) The mean cell numbers of DMSO solution with containing 1.0 mg/mL of **PBI-AFF-Man**, **PBI-AFF-Gal** and **PBI-AFF-Glu** calculated by three random confocal images; (b) the enhanced percentages of the cell numbers relative to DMSO group for **PBI-AFF-Man**, **PBI-AFF-Gal** and **PBI-AFF-Glu**. Each value represents the mean ± SEM (n = 5).

References

1. B. Xue, L. Zhao, X. Qin, M. Qin, J. Lai, W. Huang, H. Lei, J. Wang, W. Wang, Y. Li, Y. Cao. *ACS Macro Lett.*, 2019, **8**, 1383.

2. K. Meister, A. L. DeVries, H. J. Bakker, R. Drori. *J. Am. Chem. Soc.*, 2018, **140**, 9365.

3. R. F. Li, J. X. Yang, H. Y. Zhang, K. R. Wang, X. L. Li. *Sci. Sin. Chim.*, 2021, **51**, DOI: 10.1360/ssc-2021-0128.