Supplementary Information

A General Strategy for the Synthesis of Homogeneous Hyaluronan Conjugates and Their Biological Applications

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Experimentals and Materials

Materials. Uridine 5'-diphosphate *N*-acetylglucosamine (UDP-GlcNAc), Uridine 5'-diphosphateglucuronic acid (UDP-GlcA), GlcA-*p*NP and avidin-FITC were from Sigma. Oroxylin A 7-O-β-D-glucuronide was supplied by Ruifensi Biological Technology Co. Ltd. (Chengdu, China). Cell culture mediums were purchased from HyClone, USA. All other reagents unless otherwise stated were all of analytical grade and purchased from Sinopharm Chemical Reagent Co. (Shanghai, China). In addition, all solutions were prepared with double-distilled water.

PmHAS expression. The recombinant *E. coli* BL21 (DE3) harboring the pET15b-*PmHAS* vector was constructed and stored in our Lab. Protein expression was performed in E. coli BL21 (DE3), using Luria Bertani broth supplemented with 100 μg/mL ampicillin at 37 ℃ with shaking until OD600 reached 0.6-0.8. After 20 h of induction at 16 ℃ with 0.2 mM isopropyl-1-thio-β-D-galactopyranoside, the cells were harvested by centrifugation at 12,000 rpm for 5 min, and the pellet was stored at -80 ℃ until needed. Protein purification was performed utilizing a Ni-NTA resin (Amersham) according to the manufacturer's instructions at 4 ℃. The purified fraction was dialyzed with a Millipore Amicon ultra 10 K centrifugal filter to remove the imidazole and buffer components. The protein was kept in a pH 7.5 Tris-HCl buffer containing 20% glycerol and showed no loss of activity after storing for 1 month at -20 ℃.

Capillary Electrophoresis Condition. The products were characterized by a P/ACE MDQ Capillary Electrophoresis System. Electrophoresis was run in 75 μm×50 cm (40 cm to detector) bare fused silica capillary, under 25 kV with UV detection at 254 nm.

HA oligosaccharides step-wise synthesis. HA oligosaccharides (**2, 3, 4, 6, 7, 9, 10**) were synthesized using a step-wise synthetic route as mentioned above. The reaction system contains 50 mM Tris-HCl buffer, pH 7.5, 20 mM $MgCl_2$, 10 mM acceptor, 10 mM sugar nucleotide donor and PmHAS, and incubation time was 24 h at 30 °C. The reaction quenched by boiling in water bath for 5 min followed by centrifuged at 12,000 rpm for 5 min, the supernatant was purified by Bio-gel P2 gel size-exclusion column chromatographic, followed by freeze drying.

HA polymers one-pot synthesis. HA polymers including homogeneous HA-*p*NP, HA-biotin, and HA-drug conjugates were synthesized using a one-pot synthetic route as mentioned above, respectively. The reaction system contains 50 mM Tris-HCl buffer, pH 7.5, 20 mM $MgCl_2$, and PmHAS, adding oligosaccharide substrates and sugar nucleotide donors in proportion to the required ratio. The reaction incubated at 30 °C for

20 h, then quenched by boiling in water bath for 5 min followed by centrifuged at 12,000 rpm for 5 min, the supernatant was purified with a dialysis by 3K centrifugal filter purification process to remove buffer components, byproduct UDP, and remained reaction substrates. Subsequently, all the HA polymers were well characterized by gel electrophoresis. The average molecular masses and polydispersity index of HA conjugates were also determined by SEC-MALLS (*Mw* and *Mw/Mn*), respectively (Table S1).

Table S1. Molecular weights and Polydispersity index of formed homogeneous HA polymers.

Acceptor	Acceptor/ Donor molar ratio	*Mw* (Da)	*Mn* (Da)	polydispersity index (*Mw/Mn*)
GlcA-*p*NP	1:100	1.085×10^5	2.022×10^4	5.366
	1:500	1.791×10^5	3.147×10^4	5.561
	1:1000	1.761×10^5	2.908×10^4	6.056
HA2-*p*NP	1:100	3.347×10^5	1.267×10^5	2.642
	1:500	1.626×10^5	5.591×10^4	2.908
	1:1000	2.057×10^5	7.027×10^4	2.027
HA3-*p*NP	1:100	4.64×10^4	4.421×10^4	1.05
	1:500	1.076×10^5	8.108×10^4	1.327
	1:1000	1.552×10^5	1.114×10^5	1.393
HA4-*p*NP	1:100	7.474×10^4	7.24×10^4	1.032
	1:500	1.922×10^5	1.534×10^5	1.253
	1:1000	2.243×10^5	1.583×10^5	1.416

Homogeneous HA-biotin conjugates one-pot synthesis. Homogeneous HA-biotin conjugates were synthesized using a one-pot synthetic route as mentioned above, respectively. The reaction system contains 50 mM Tris-HCl buffer, pH 7.5, 20 mM $MgCl_2$, and PmHAS, adding HA-biotin trisaccharide **7** and sugar nucleotide donors. Homogeneous HA-biotin conjugates (20, 50, 100) were synthesized by controlling the reaction stoichiometry (molar ratio of substrate and acceptor molecules). The reaction incubated at 30 °C for 20 h, then quenched by boiling in water bath for 5 min followed by centrifuged at 12,000 rpm for 5 min, the supernatant was purified with a dialysis by 3K centrifugal filter purification process to remove buffer components, byproduct UDP, and remained reaction substrates.

Homogeneous HA-oroxylin conjugates one-pot synthesis. Homogeneous HA-oroxylin conjugates were synthesized using a one-pot synthetic route as mentioned above, respectively. The reaction system contains 50 mM Tris-HCl buffer, pH 7.5, 20 mM $MgCl_2$, and PmHAS, adding HA-oroxylin trisaccharide **10** and sugar nucleotide donors by controlling reaction stoichiometry. The reaction incubated at 30 °C for 20 h, then quenched by boiling in water bath for 5 min followed by centrifuged at 12,000 rpm for 5 min, the supernatant was purified with a dialysis by 3K centrifugal filter purification process to remove buffer components,

byproduct UDP, and remained reaction substrates. The concentrations of total HA-oroxylin conjugates were calibrated based on oroxylin contained in the conjugate by measurement of UV absorption at 275 nm with oroxylin as standard.

Gel electrophoresis of HA polymers. All the HA polymers were separated by electrophoresis on a 1% agarose gel. Electrophoresis for 6 h under the condition of 4 V/cm, followed by detection of HA polymers using the cationic dye stain-all overnight. LMW-HA polymers were separated by SDS-PAGE and detected by silver-alcian blue double-staining. The molecular weight of homogeneous HA was determined by Multi Angle Laser light Scattering detection and Refractive Index detection (MALLS-RI). The chromatography system consisted of a Waters Alliance HPLC equipped with Wyatt's Multi Angle Laser Light Scattering (Dawn HELEOS, Santa Barbara, CA). The fused silica column was eluted with water at a flow rate of 0.6 mL min^{-1} at 30 °C. Dn/dc and A2 values used were 0.153 and 0, respectively. All data were calculated to measure polydispersity index (Mw/Mn).

Cell culture. Human non-small-cell lung cancer cell line A549 was obtained from the Type Culture Collection of the Chinese Academy of Sciences, Shanghai, China. A549 was cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum at 37 °C in a 5% CO_2 humidified atmosphere.

Exogenous HA fragments cell assay. A convenient HA detection method which combined HA-CD44 interaction and biotin-avidin specific recognition was designed in order to visually monitor the metabolic process of exogenous HA fragments (**Fig. 4a**). ① HA-biotin conjugate could be recognized by HA specific receptor CD44 (the major HA receptor on A549 cell surface) to form CD44-HA-biotin complex; ②biotin motif of HA-biotin conjugates could be recognized by avidin-FITC to form CD44-HA-biotin-avidin-FITC complex. ③ A549 cell take up exogenous HA complex and accumulate intracellularly. ④ Images were captured by a laser scanning confocal microscope.

A549 cells (2×10^4 cells per well) were seeded onto a 15 mm coverslip in a 24-well plate overnight. HA-biotin (32 μg/mL) (HA_{20}-biotin, HA_{50}-biotin, and HA_{100}-biotin) were then added to the culture medium and incubated for 30 min. Cells were washed with PBS buffer, fixed with 4% paraformaldehyde (Sigma-Aldrich) for 15 min, and permeabilized with 0.5% (v/v) Triton X-100 (Sigma) in PBS for 10 min before staining. Cells were blocked in 1% (w/v) bovine serum albumin at room temperature for 30 min. After extensive washing, the cells were stained with 8 μg/mL avidin-FITC for 30 min in a dark place. After three additional washes, the DAPI solution was added to the culture and further incubated for 5 min followed by washing with PBS thrice. The

coverslips were mounted on microscope slides and sealed with a synthetic mount. Images were captured by a laser scanning confocal microscope (LSM700, Zeiss, Germany).

Cell proliferation assay. Cell proliferation was measured using the CCK-8 kit (Dojindo Laboratories, Kumamoto, Japan). A549 cells (5×10^3 cells/well) were seeded into the wells of 96-well plate and cultured overnight. After treatment with HA-oroxylin conjugates and oroxylin at the concentration of 40 μM, 20 μM, 10 μM, 5 μM and 2.5 μM, the cells were cultured 48 h. Then, 10 μL CCK-8 solution was added to each well, and the cells incubated at 37 °C for 2 h. Absorbance was read at 450 nm using a Bio-Rad iMark plate reader. Cell growth rate was calculated as (Abs of treated cells/Abs of untreated cells) × 100.

Statistical Analysis. Statistical analysis was performed using the Graphpad Prism 5 Project. The data were analyzed by two-ways ANOVA. Differences between the variables were considered significant for p values less than 0.05.

Figure Legends:

Figure S1. $^{-}$Q ESI-MS profile of GlcNAcβ1-4GlcAβ-*p*NP **2**

Figure S2. ^{1}H NMR of GlcNAcβ1-4GlcAβ-*p*NP **2**

Figure S3. ^{13}C NMR of GlcNAcβ1-4GlcAβ-*p*NP **2**

Figure S4. $^{-}$Q ESI-MS profile of GlcAβ1-3GlcNAcβ1-4GlcAβ-*p*NP **3**

Figure S5. ^{1}H NMR of GlcAβ1-3GlcNAcβ1-4GlcAβ-*p*NP **3**

Figure S6. ^{13}C NMR of GlcAβ1-3GlcNAcβ1-4GlcAβ-*p*NP **3**

Figure S7. $^{-}$Q ESI-MS profile of GlcNAcβ1-4GlcAβ1-3GlcNAcβ1-4GlcAβ-*p*NP **4**

Figure S8. ^{1}H NMR of GlcNAcβ1-4GlcAβ1-3GlcNAcβ1-4GlcAβ-*p*NP **4**

Figure S9. ^{13}C NMR of GlcNAcβ1-4GlcAβ1-3GlcNAcβ1-4GlcAβ-*p*NP **4**

Figure S10. TLC profiles of formed HA by different HA oligosaccharide acceptor

Figure S11. SDS-PAGE profiles of formed homogeneous LMW-HA polymers

Figure S12. $^{-}$Q ESI-MS profile of GlcNAcβ1-4GlcAβ-biotin **6**

Figure S13. ^{1}H NMR of GlcNAcβ1-4GlcAβ-biotin **6**

Figure S14. ^{13}C NMR of GlcNAcβ1-4GlcAβ-biotin **6**

Figure S15. $^{-}$Q ESI-MS profile of GlcAβ1-3GlcNAcβ1-4GlcAβ-biotin **7**

Figure S16. ^{1}H NMR of GlcAβ1-3GlcNAcβ1-4GlcAβ-biotin **7**

Figure S17. ^{13}C NMR of GlcAβ1-3GlcNAcβ1-4GlcAβ-biotin **7**

Figure S18. Agarose gel profiles of HA-biotin conjugates synthesized by using the general synthetic strategy.

Figure S19. Agarose gel electrophoresis profiles of homogeneous HA-biotin conjugates. The acceptor/precursor molar ratios were marked above. The loading amount was 50 μg per sample.

Figure S20. $^{-}$Q ESI-MS profile of GlcNAcβ1-4GlcAβ-oroxylin **9**

Figure S21. ^{1}H NMR of GlcNAcβ1-4GlcAβ-oroxylin **9**

Figure S22. ^{13}C NMR of GlcNAcβ1-4GlcAβ-oroxylin **9**

Figure S23. $^{-}$Q ESI-MS profile of GlcAβ1-3GlcNAcβ1-4GlcAβ-oroxylin **10**

Figure S24. ^{1}H NMR of GlcAβ1-3GlcNAcβ1-4GlcAβ-oroxylin **10**

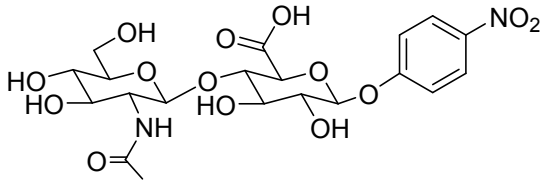
Figure S25. ^{13}C NMR of GlcAβ1-3GlcNAcβ1-4GlcAβ-oroxylin **10**

Figure S26. Agarose gel electrophoresis profiles of HA-oroxylin conjugates

Figure S27. Confocal microscopy fluorescence profiles of HA-biotin conjugates detected on A549 cell.

Figure S28. The growth effects on A549 cells of HA_{50}-oroxylin and HA_{100}-oroxylin conjugates.

GlcNAcβ1-4GlcAβ-*p*NP 2



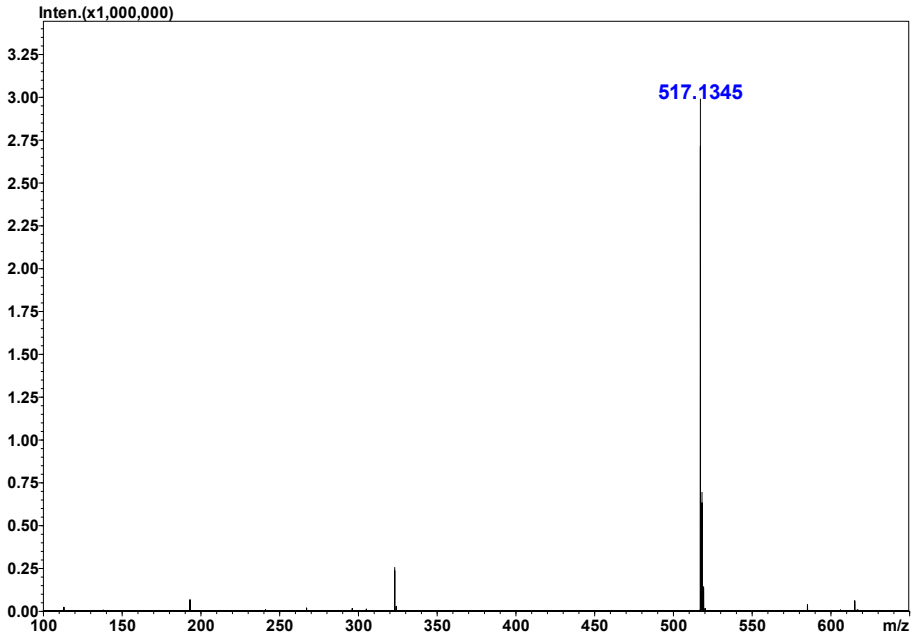


Figure S1. -Q ESI-MS profile of GlcNAcβ1-4GlcAβ-*p*NP **2**

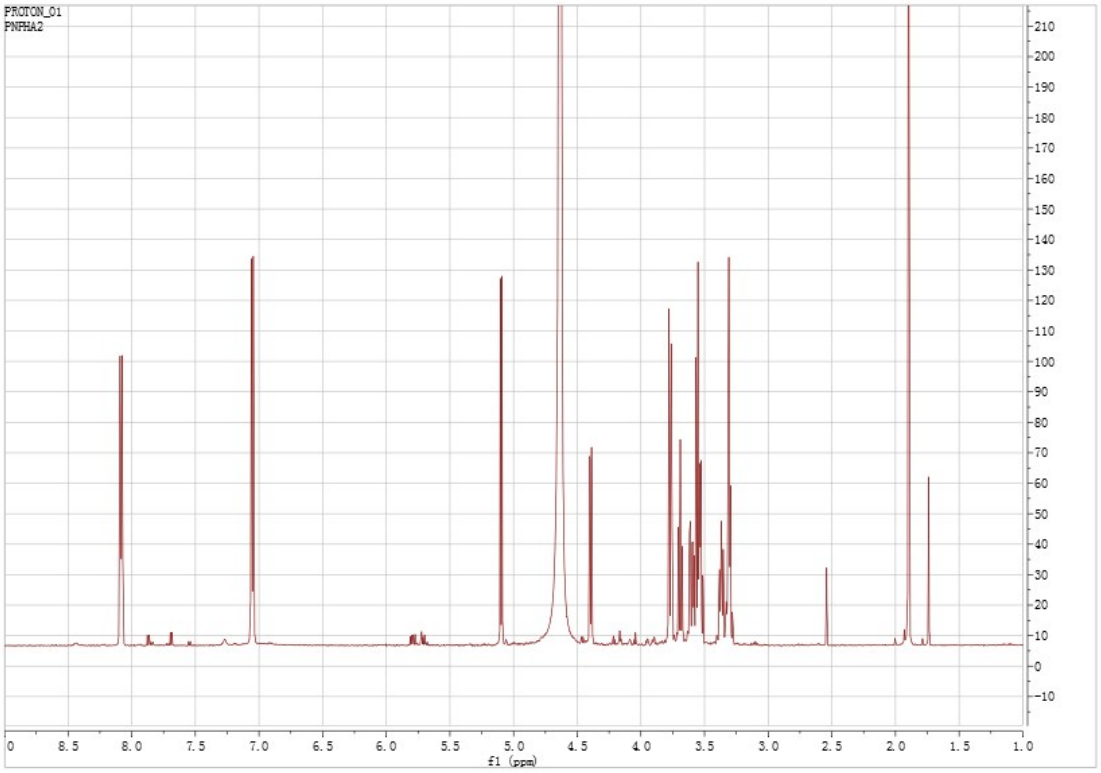


Figure S2. ^{1}H NMR of GlcNAcβ1-4GlcAβ-*p*NP **2**

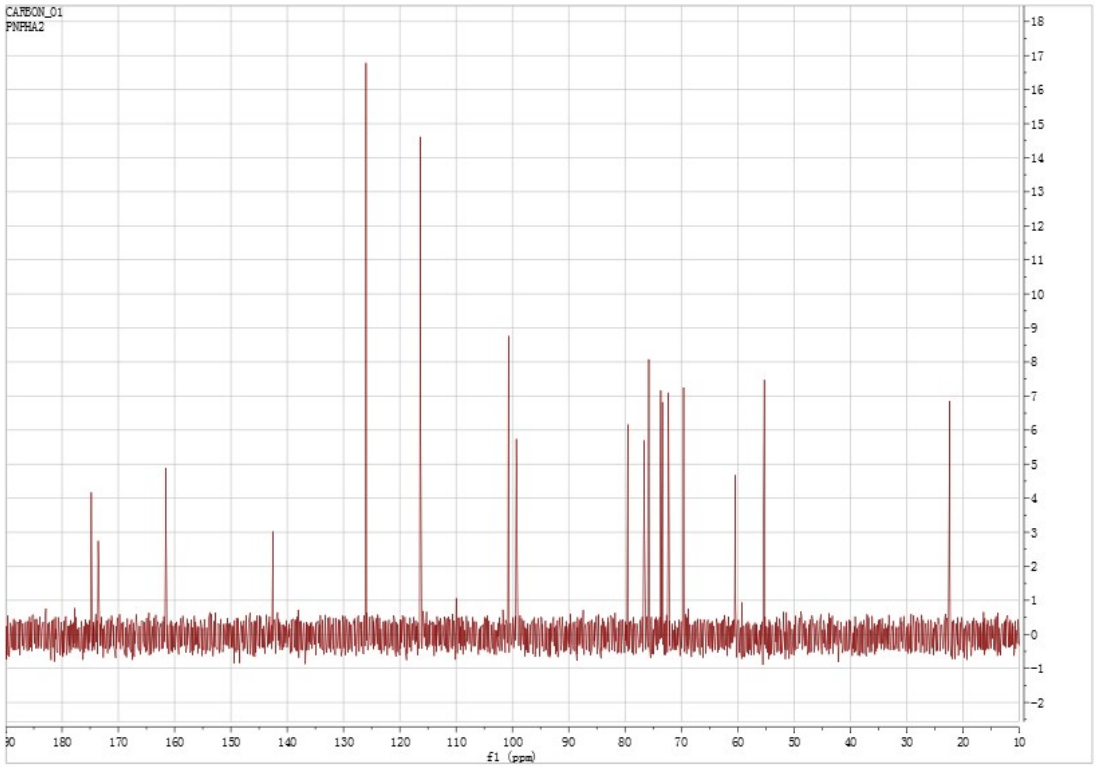
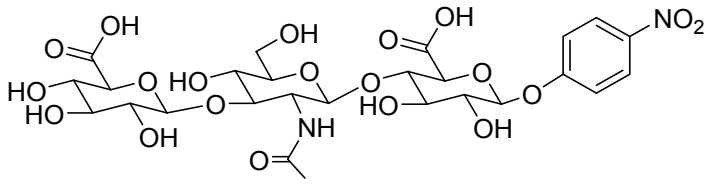


Figure S3. [13] C NMR of GlcNAcβ1-4GlcAβ-*p*NP **2**

Spectroscopic data of **GlcNAcβ1-4GlcAβ-*p*NP 2**:

^{1}H NMR (600 MHz, D_2O) δ 8.23-8.25 (m, 2 H), 7.20-7.22 (m, 2 H), 5.25 (d, J = 7.5 Hz, 1 H), 4.55 (dd, J = 8.4, 1 .7 Hz, 1 H), 3.93-3.49 (m, 1 H), 3.91-3.92 (m, 1 H), 3.85 (t, J = 8.7 Hz, 1 H), 3.75-3.77 (m, 1 H), 3.71-3.72 (m, 2 H), 3.68-3.69 (m, 1 H), 3.50-3.54 (m, 1 H), 3.45-3.48 (m, 2 H), 2.05 (s, 3 H); ^{13}C NMR (150 MHz, D_2O) δ 174.8, 173.6, 161.5, 142.5, 126.0, 116.3, 100.7, 99.3, 79.5, 76.6, 75.8, 73.7, 73.4, 72.3, 69.6, 60.4, 55.3, 22.3. ESI-MS (negative ion): Calcd for $C_{20}H_{26}N_2O_{14}$: 518.14 [M]; Found: 517.1345 $[M-H]^-$.

GlcAβ1-3GlcNAcβ1-4GlcAβ-*p*NP 3



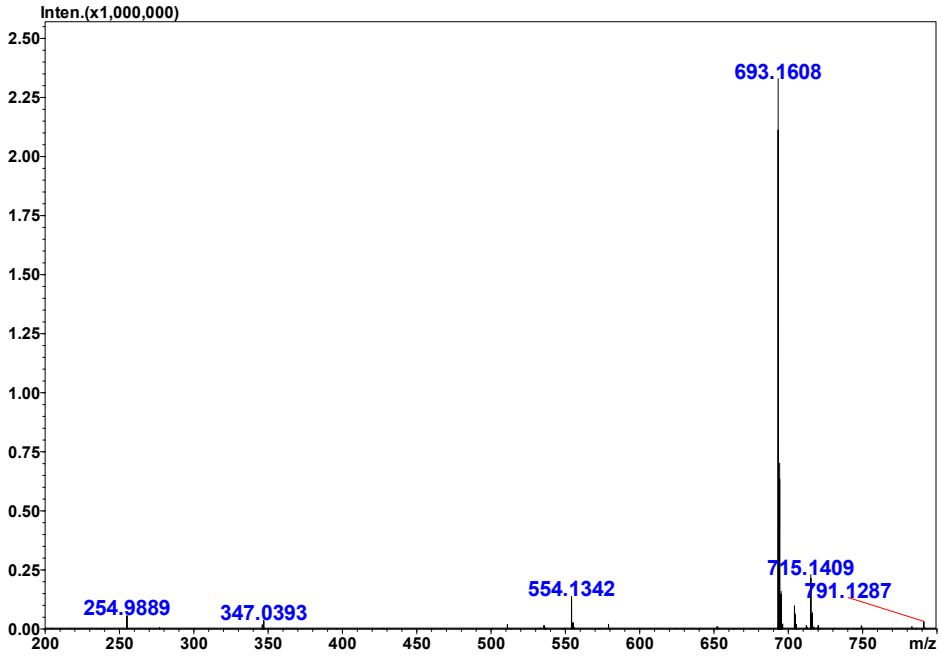


Figure S4. -Q ESI-MS profile of GlcAβ1-3GlcNAcβ1-4GlcAβ-*p*NP **3**

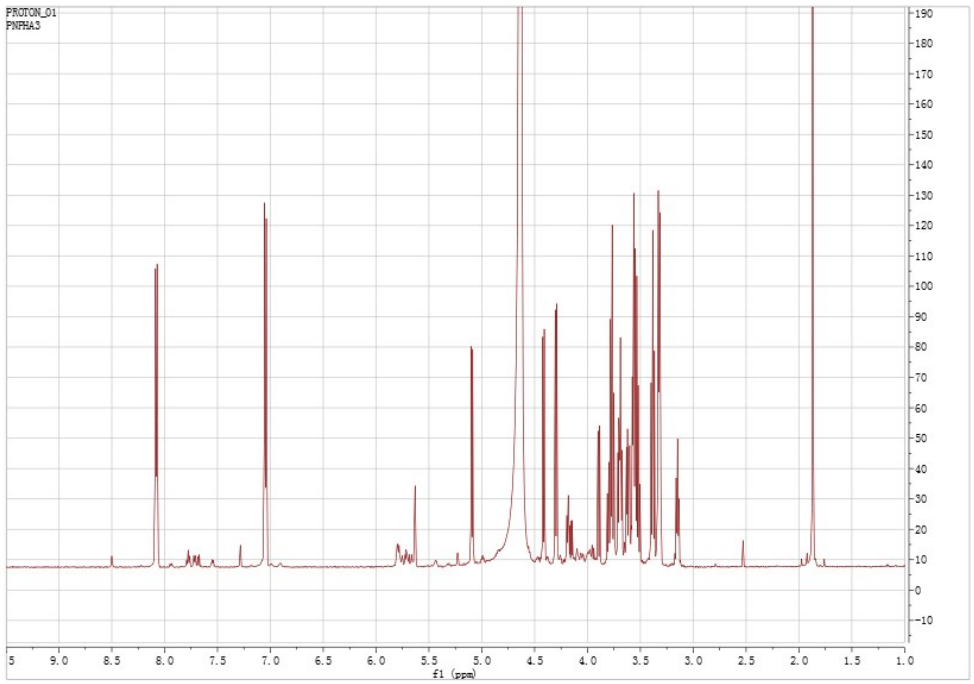


Figure S5. ^{1}H NMR of GlcAβ1-3GlcNAcβ1-4GlcAβ-*p*NP **3**

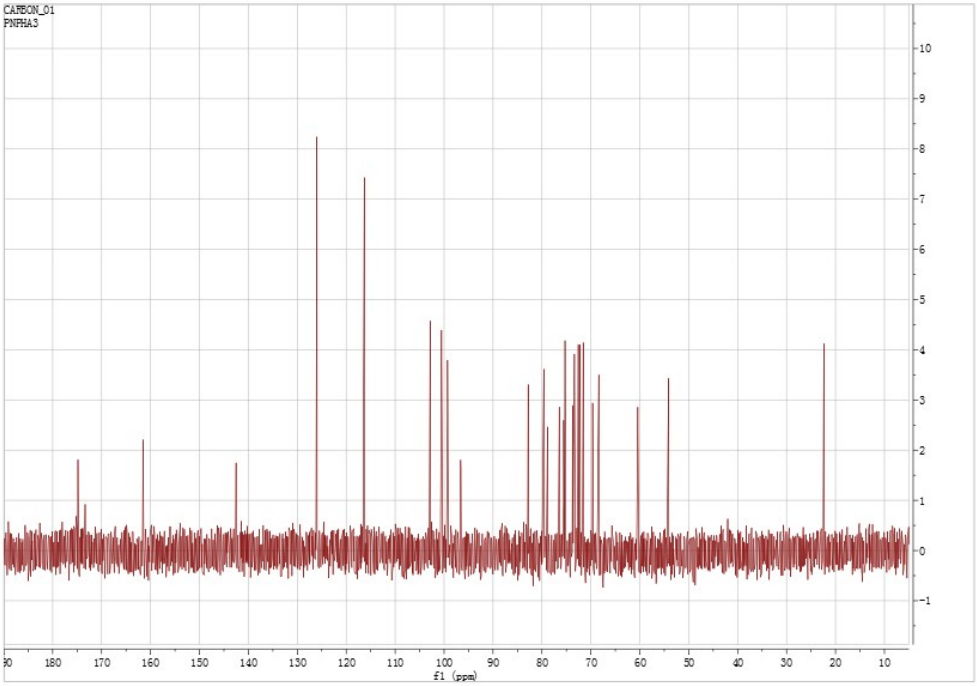
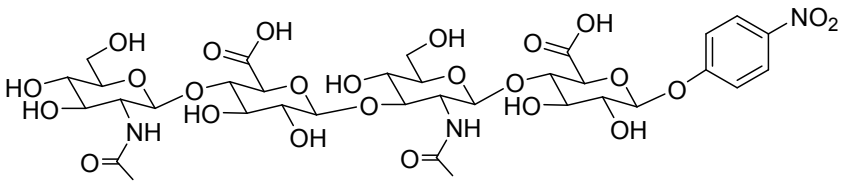


Figure S6. ^{13}C NMR of GlcAβ1-3GlcNAcβ1-4GlcAβ-*p*NP **3**

Spectroscopic data of **GlcAβ1-3GlcNAcβ1-4GlcAβ-*p*NP 3**:

^{1}H NMR (600 MHz, D_2O) δ 8.22-8.25 (m, 2 H), 7.19-7.21 (m, 2 H), 5.25 (d, J = 7.6 Hz, 1 H), 4.57 (d, J = 8.5 Hz, 1 H), 4.45 (d, J = 7.9 Hz, 1 H), 4.05 (d, J = 9.7 Hz, 1 H), 3.91-3.97 (m, 3 H), 3.83-3.87 (m, 2 H), 3.77 (dd, J = 12.5, 5.2 Hz, 1 H), 3.66-3.74 (m, 3 H), 3.54 (t, J = 9.5 Hz, 1 H), 3.47-3.49 (m, 2 H), 3.29-3.32 (m, 1 H), 2.03 (s, 3 H); ^{13}C NMR (150 MHz, D_2O) δ 174.8, 161.5, 142.5, 126.0, 116.3, 102.8, 100.5, 99.3, 82.8, 79.6, 76.5, 75.3, 75.2, 73.4, 72.6, 72.3, 71.6, 69.6, 68.4, 60.4, 54.1, 22.4. ESI-MS (negative ion): Calcd for $C_{26}H_{34}N_2O_{20}$: 694.17 [M]; Found: 693.1608 $[M-H]^-$, 715.1409 $[M-H+Na]^-$.

GlcNAcβ1-4GlcAβ1-3GlcNAcβ1-4GlcAβ-*p*NP 4



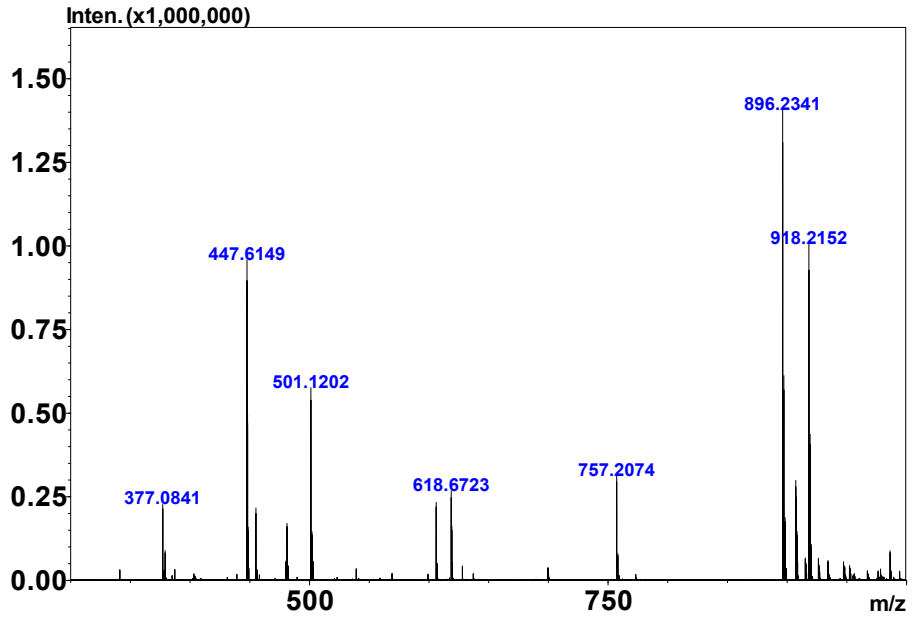


Figure S7. -Q ESI-MS profile of GlcNAcβ1-4GlcAβ1-3GlcNAcβ1-4GlcAβ-*p*NP **4**

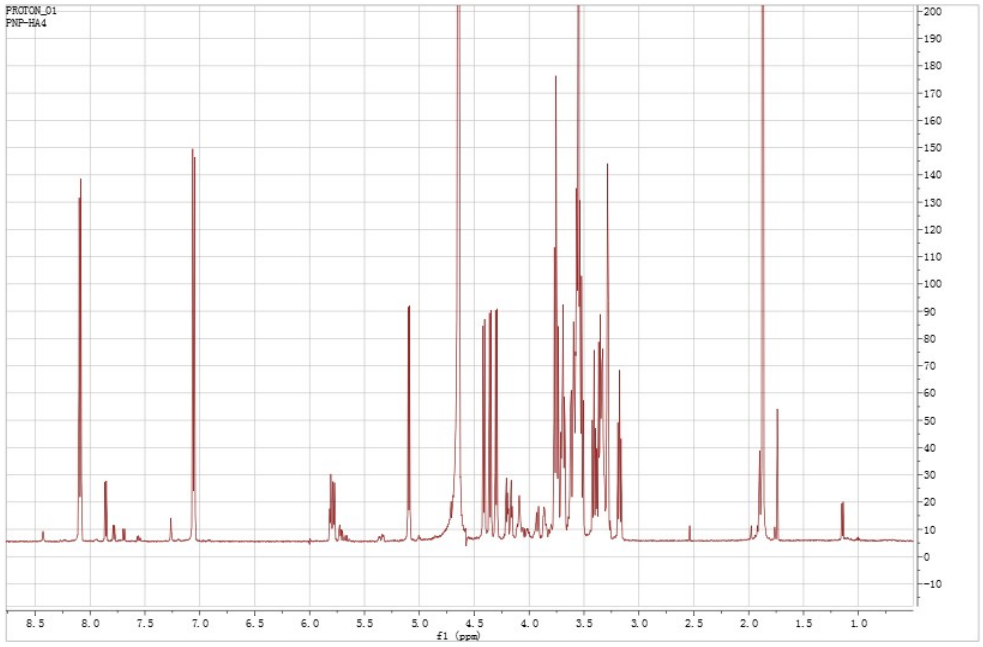


Figure S8. ^{1}H NMR of GlcNAcβ1-4GlcAβ1-3GlcNAcβ1-4GlcAβ-*p*NP **4**

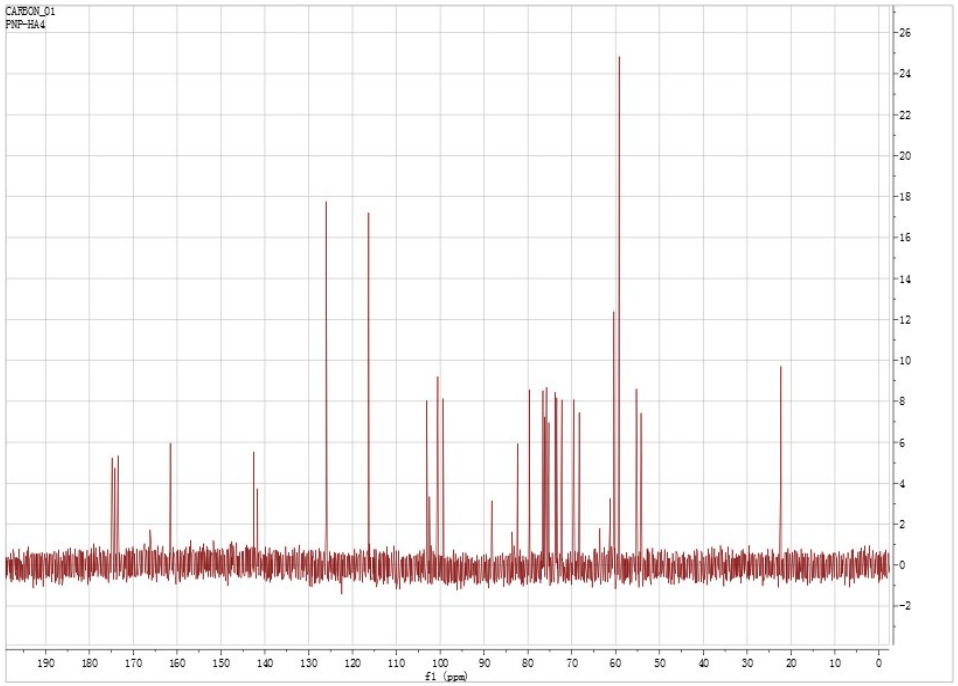


Figure S9.[13] C NMR of GlcNAcβ1-4GlcAβ1-3GlcNAcβ1-4GlcAβ-*p*NP **4**

Spectroscopic data of **GlcNAcβ1-4GlcAβ1-3GlcNAcβ1-4GlcAβ-*p*NP 4**:
^{1}H NMR (600 MHz, D_2O) *d* 8.23 (d, *J* = 9.2 Hz, 2 H), 7.19 (d, *J* = 9.2 Hz, 2 H), 5.23 (d, *J* = 7.4 Hz, 1 H), 4.55 (d, *J* = 8.5 Hz, 1 H), 4.49 (d, *J* = 8.5 Hz, 1 H), 4.44 (d, *J* = 7.9 Hz, 1 H), 3.87-3.91 (m, 3 H), 3.81-3.85 (m, 2 H), 3.76 (d, *J* = 5.2 Hz, 1 H), 3.73 (d, *J* = 5.0 Hz, 1 H), 3.64-3.71 (m, 5 H), 3.55 (t, *J* = 9.1 Hz, 1 H), 3.50 (t, *J* = 9.8 Hz, 1 H), 3.45-3.49 (m, 2 H), 3.41-3.42 (m, 2 H), 3.31 (t, *J* = 8.7 Hz, 1 H), 2.01 (s, 6 H, 2 Ac); ^{13}C NMR (150 MHz, D_2O) *d* 174.9, 174.8, 174.2, 173.5, 161.5, 142.5, 141.7, 126.0, 116.3, 103.1, 100.6, 100.5, 99.3, 88.5, 82.4, 79.7, 79.6, 76.6, 76.2, 75.8, 75.2, 73.7, 73.4, 72.4, 72.3, 69.6, 68.3, 60.4, 59.2, 55.3, 54.2, 22.4, 22.3. ESI-MS (negative ion): Calcd for $C_{34}H_{47}N_3O_{25}$: 897.25 [M]; Found: 896.2341 $[M-H]^-$, 918.2152 $[M-H+Na]^-$, 447.6149 $[M-2H]^{2-}$, 501.1202 $[M-3H+Na]^{2-}$.

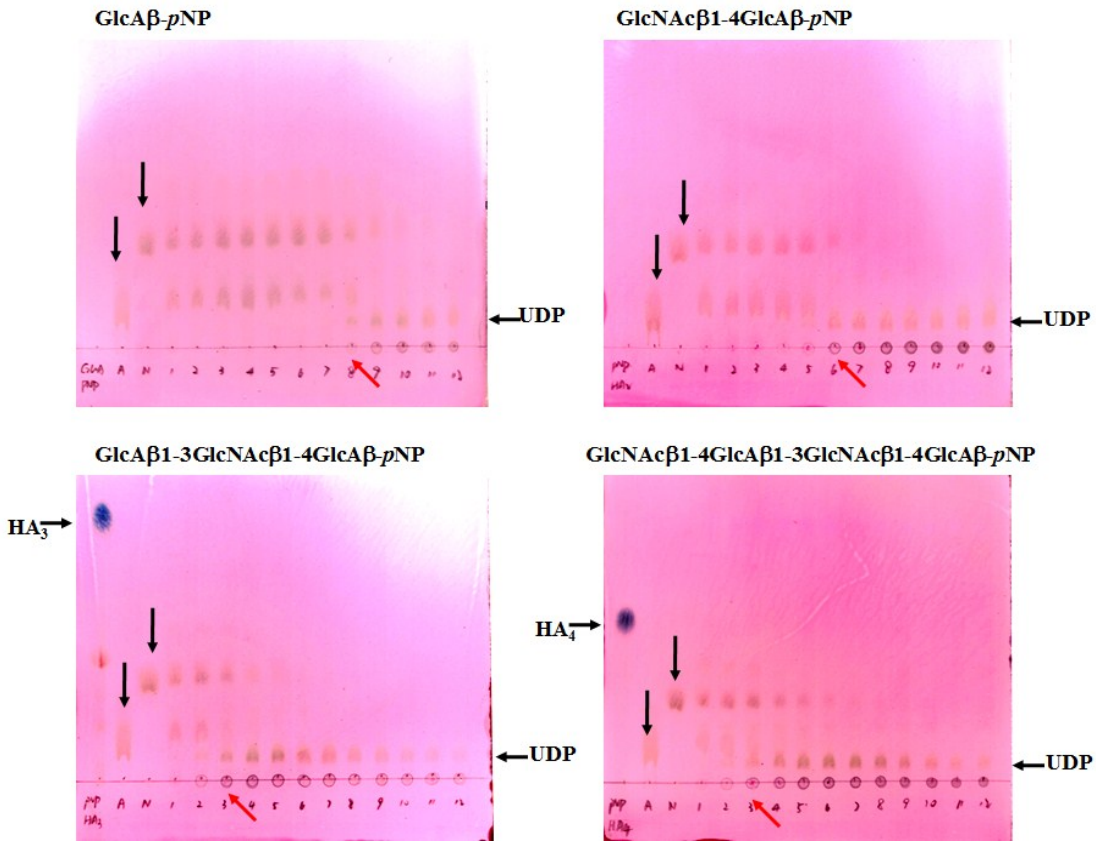


Figure S10. TLC profiles of formed HA by different HA oligosaccharide acceptor. Upper left: **1** as the acceptor, upper right: **2** as the acceptor; low left: **3** as the acceptor; low right: **4** as the acceptor. Black arrow: the UDP-GlcNAc, UDP-GlcA, the starting acceptor, and byproduct UDP; red arrow, the detectable HA product.

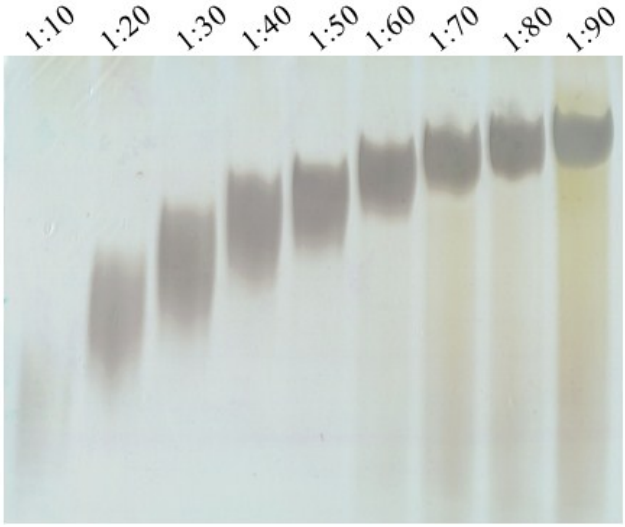
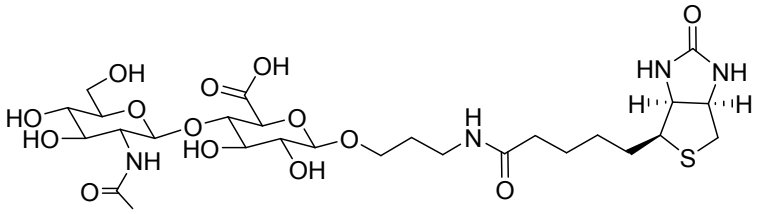


Figure S11. SDS-PAGE profiles of formed homogeneous LMW-HA polymers. Acceptor/ donor molar ratios varied from 1:10 to 1:100 as marked above.

GlcNAcβ1-4GlcAβ-biotin 6



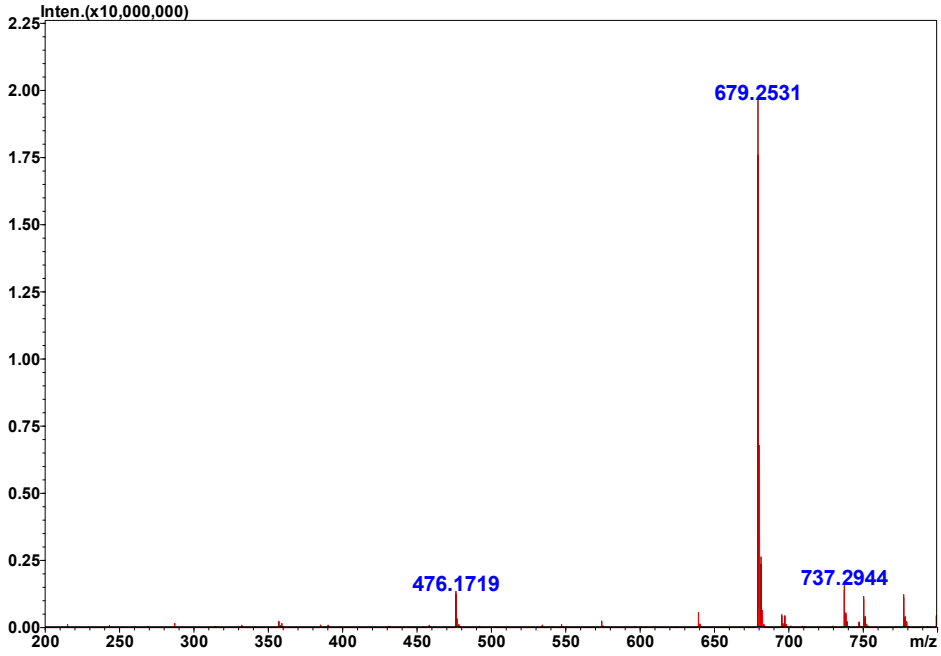


Figure S12. -Q ESI-MS profile of GlcNAcβ1-4GlcAβ-biotin **6**

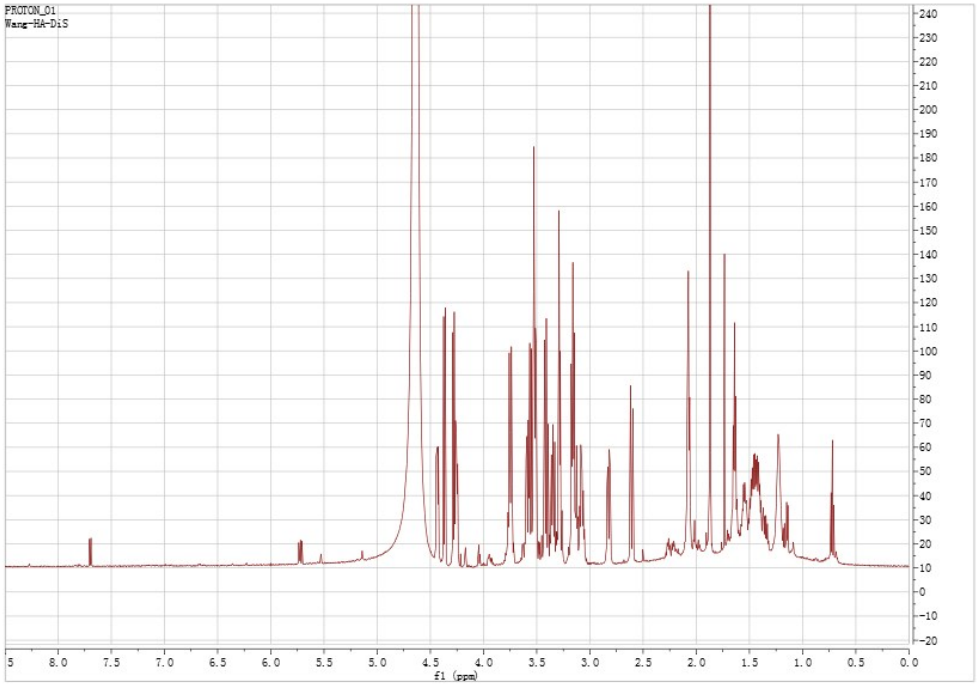


Figure S13. ^{1}H NMR of GlcNAcβ1-4GlcAβ-biotin **6**

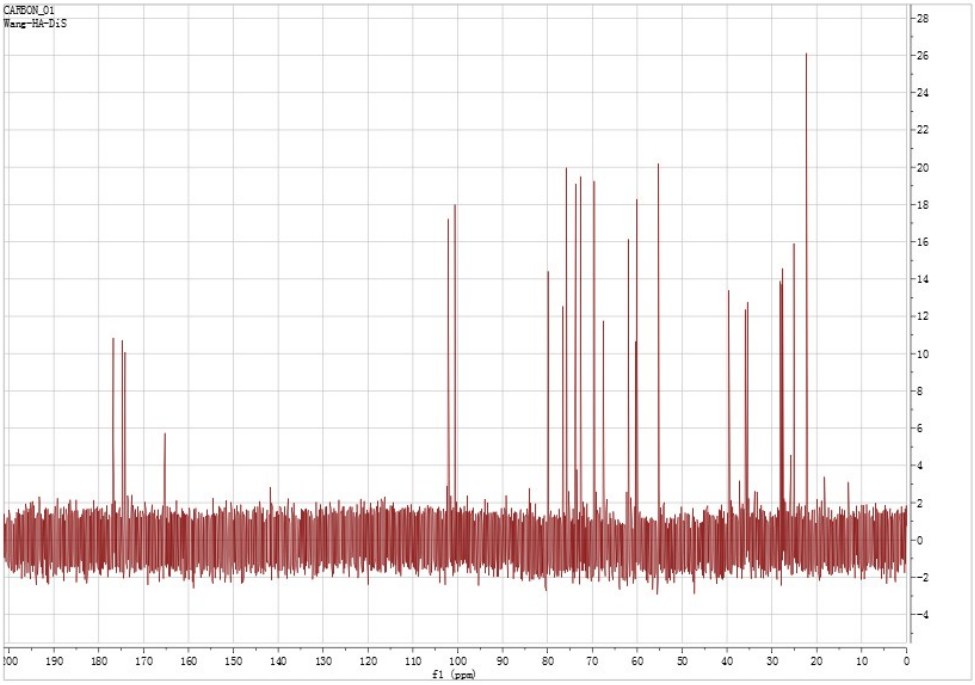


Figure S14. ^{13}C NMR of GlcNAcβ1-4GlcAβ-biotin **6**

Spectroscopic data of **GlcNAcβ1-4GlcAβ-biotin 6**:

^{1}H NMR (600 MHz, D_2O) 4.44 (dd, J = 7.8, 5.4 Hz, 1 H), 4.37 (d, J = 8.4 Hz, 1 H), 4.28 (d, J = 7.8 Hz, 1 H), 4.25 (dd, J = 7.8, 4.2 Hz, 1 H), 3.74-3.77 (m, 1 H), 3.59-3.60 (m, 1 H), 3.57-3.58 (m, 1 H), 3.54-3.55 (m, 1 H), 3.52-3.53 (m, 2 H), 3.50-3.51 (m, 1 H), 3.41 (t, J = 9.3 Hz, 1 H), 3.35 (t, J = 9.3 Hz, 1 H), 3.28-3.29 (m, 2 H), 3.15-3.18 (m, 2 H), 3.13 (t, J = 6.6 Hz, 1 H); 3.06-3.09 (m, 1 H), 2.82 (dd, J = 12.6, 4.8 Hz, 1 H), 2.61 (d, J = 12.6 Hz, 1 H), 2.06-2.09 (m, 2 H), 1.87 (s, 3 H, Ac), 1.63-1.65 (m, 2 H), 1.40-1.56 (m, 4 H), 1.20-1.25 (m, 2 H); ^{13}C NMR (150 MHz, D_2O) 176.8, 174.7, 174.1, 165.2, 102.2, 100.6, 79.9, 76.5, 75.8, 73.7 (2 C), 72.7, 69.6, 67.6, 62.0, 60.4, 60.1, 55.3, 55.2, 39.6, 35.9, 35.4, 28.1, 27.7, 27.6, 25.1, 22.3. ESI-MS (negative ion): Calcd for $C_{27}H_{44}N_4O_{14}S$: 680.26 [M]; Found: 679.2531 $[M-H]^-$.

GlcAβ1-3GlcNAcβ1-4GlcAβ-biotin 7

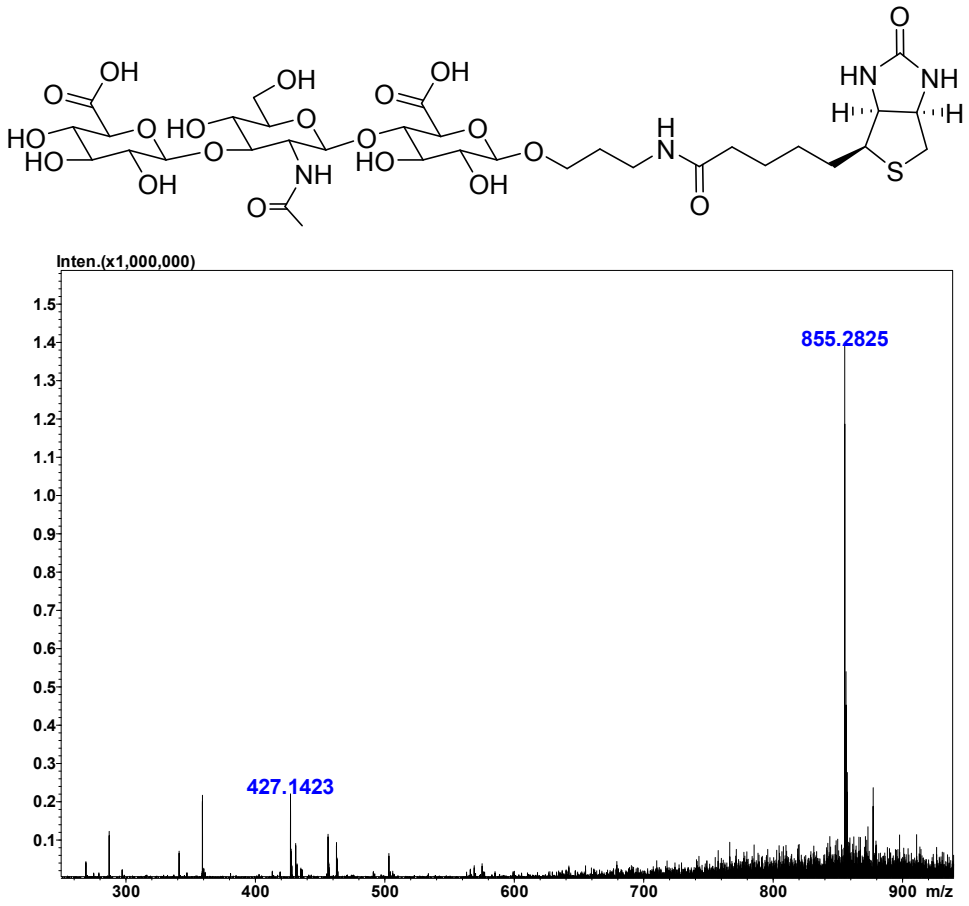


Figure S15. -Q ESI-MS profile of GlcAβ1-3GlcNAcβ1-4GlcAβ-biotin **7**

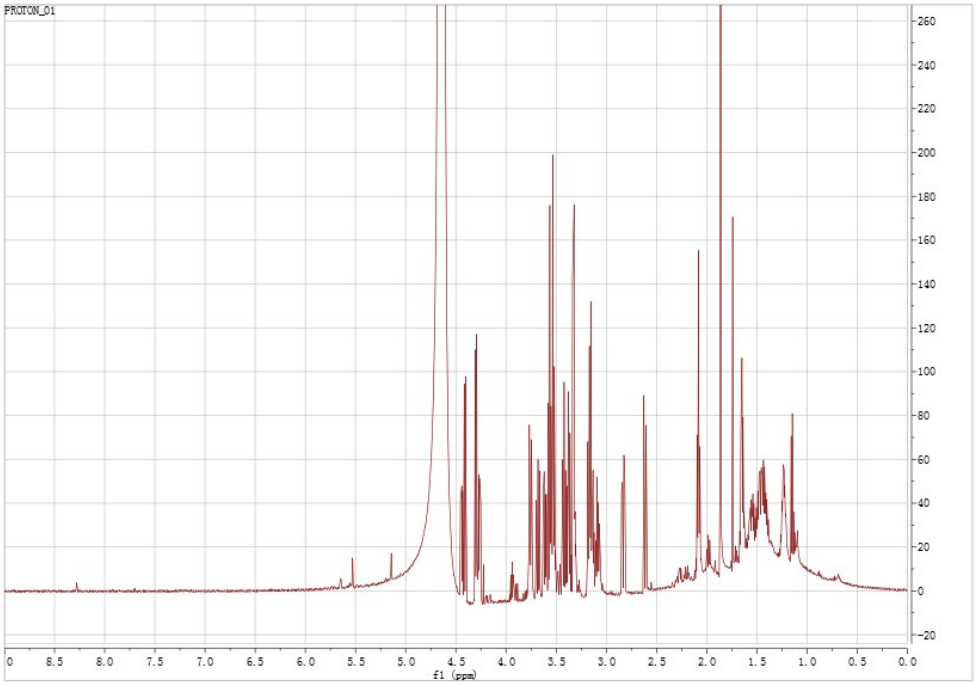


Figure S16. ^{1}H NMR of GlcAβ1-3GlcNAcβ1-4GlcAβ-biotin **7**

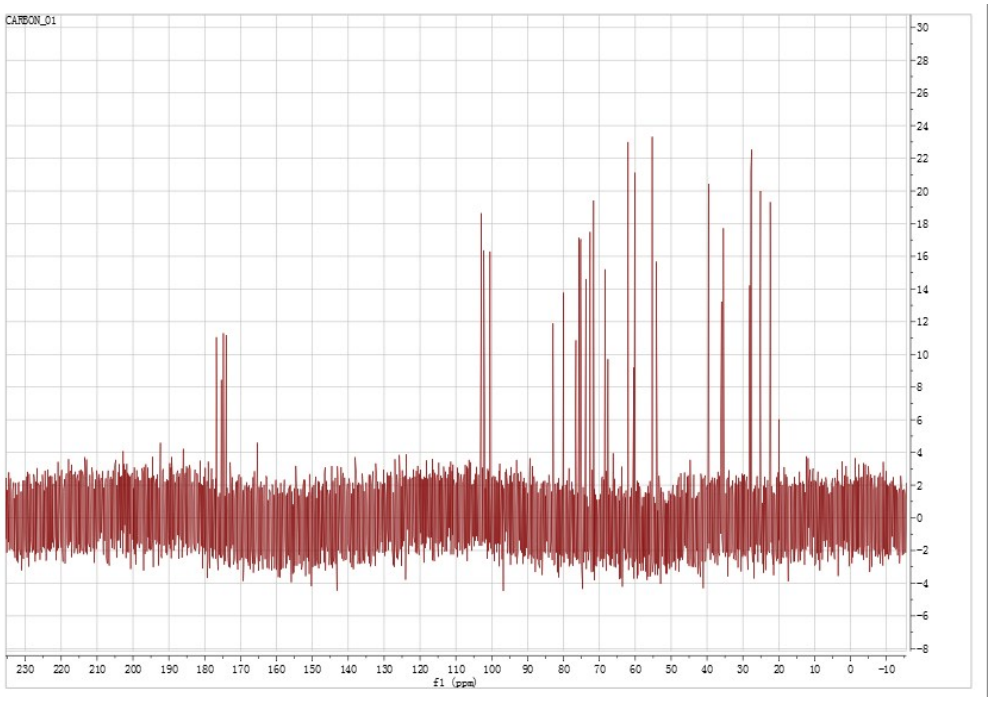


Figure S17.[13] C NMR of GlcAβ1-3GlcNAcβ1-4GlcAβ-biotin **7**

Spectroscopic data of**GlcAβ1-3GlcNAcβ1-4GlcAβ-biotin 7**:

^{1}H NMR (600 MHz, D_2O) 4.44 (dd, J = 7.8, 4.8 Hz, 1 H), 4.41 (d, J = 8.4 Hz, 1 H), 4.30 (d, J = 7.8 Hz, 1 H), 4.29 (d, J = 7.8 Hz, 1 H), 3.76-3.77 (m, 1 H), 3.74-3.75 (m, 1 H), 3.68 (dd, J = 10.2, 8.4 Hz, 1 H), 3.61-3.63 (m, 1 H), 3.59-3.61 (m, 1 H), 3.58 (t, J = 7.8 Hz, 1 H), 3.55-3.56 (m, 1 H), 3.54-3.55 (m, 1 H), 3.51-3.53 (m, 1 H), 3.50-3.52 (m, 1 H), 3.42 (t, J = 9.6 Hz, 1 H), 3.38 (t, J = 9.6 Hz, 1 H), 3.33-3.34 (m, 1 H), 3.32-3.33 (m, 1 H), 3.17-3.18 (m, 1 H), 3.16-3.17 (m, 1 H), 3.14-3.16 (m, 1 H), 3.12-3.13 (m, 1 H), 3.06-3.09 (m, 2 H), 2.83 (dd, J = 13.2, 4.8 Hz, 1 H), 2.61 (t, J = 12.6 Hz, 1 H), 2.08 (t, J = 7.2 Hz, 2 H), 1.86 (s, 3 H, Ac), 1.63-1.66 (m, 2 H), 1.37-1.50 (m, 4 H), 1.20-1.26 (m, 2 H); ^{13}C NMR (150 MHz, D_2O) 176.8, 175.4, 174.8, 174.0, 165.3, 102.9, 102.2, 100.5, 82.9, 80.0, 76.5, 75.6, 75.3, 75.2, 73.8, 72.6 (2 C), 71.6, 68.4, 67.6, 62.0, 60.4, 60.1, 55.3, 54.1, 39.6, 36.0, 35.4, 28.1, 27.8, 27.6, 25.1,22.3. ESI-MS (negative ion): Calcd for $C_{33}H_{52}N_4O_{20}S$: 856.29 [M]; Found: 855.2825 $[M-H]^-$.

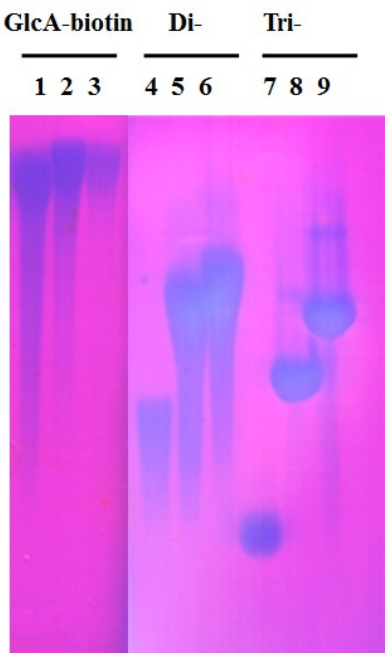


Figure S18. Agarose gel electrophoresis profiles of HA-biotin conjugates synthesized by using the general synthetic strategy. Acceptor used: lane 1, 2, 3: GlcAβ-biotin; 4, 5, 6: HA2- biotin; 7, 8, 9: HA3- biotin; A/P: 1, 4, 7 = 1:100; 2, 5, 8 = 1:500; 3, 6, 9 = 1:1000. The loading amount was 50 μg per sample.

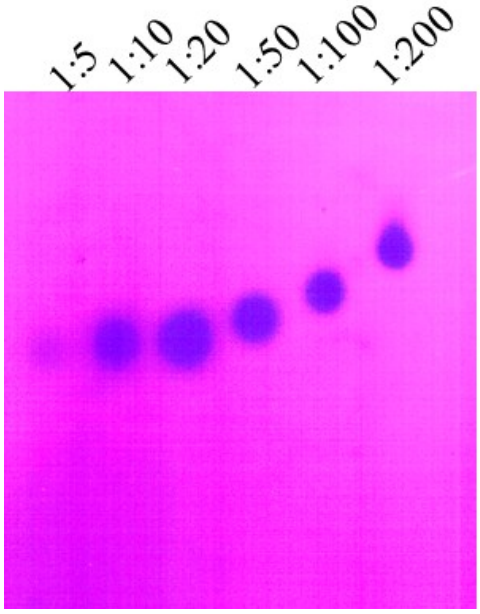
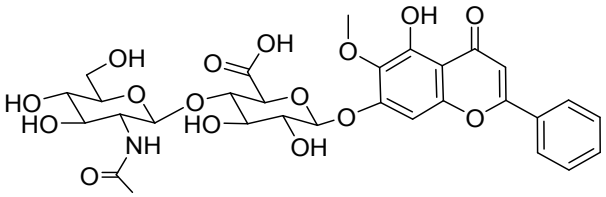


Figure S19. Agarose gel electrophoresis profiles of homogeneous HA-biotin conjugates. The acceptor/precursor molar ratios were marked above. The loading amount was 50 μg per sample.

GlcNAcβ1-4GlcAβ-oroxylin 9



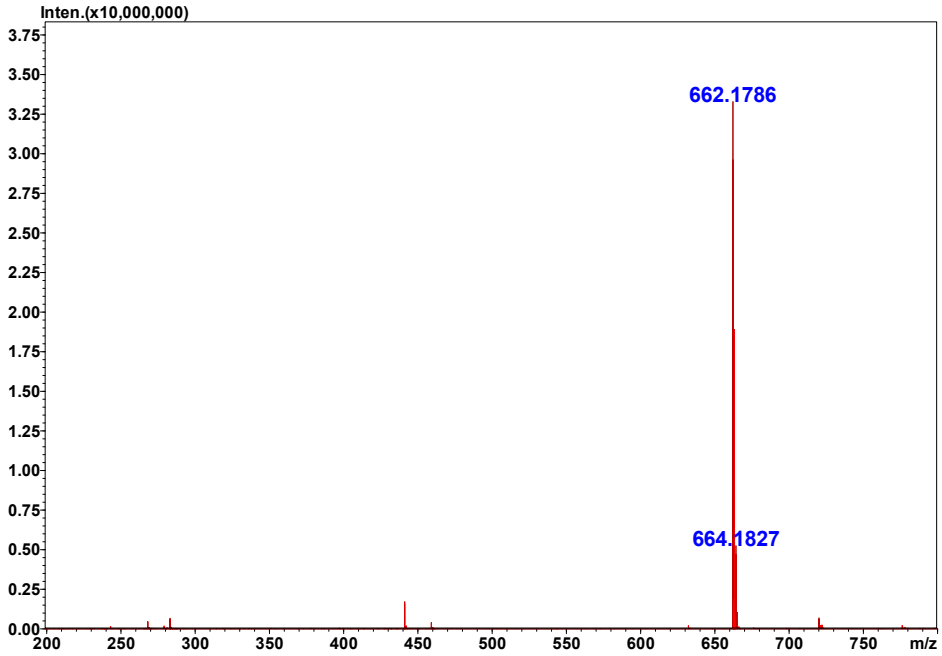


Figure S20. -Q ESI-MS profile of GlcNAcβ1-4GlcAβ-oroxylin **9**

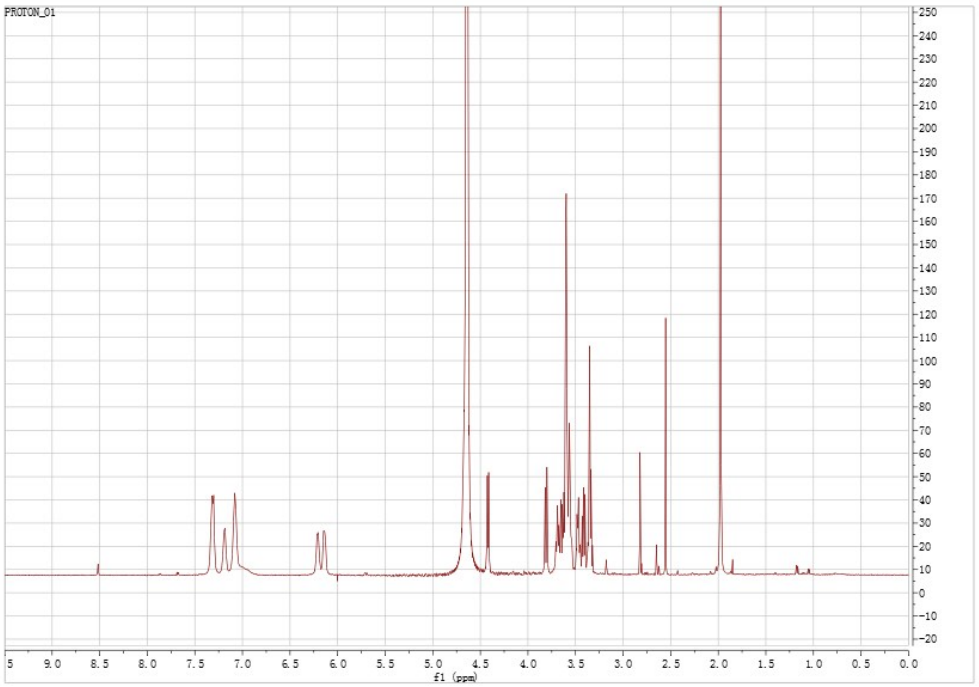


Figure S21. ^{1}H NMR of GlcNAcβ1-4GlcAβ-oroxylin **9**

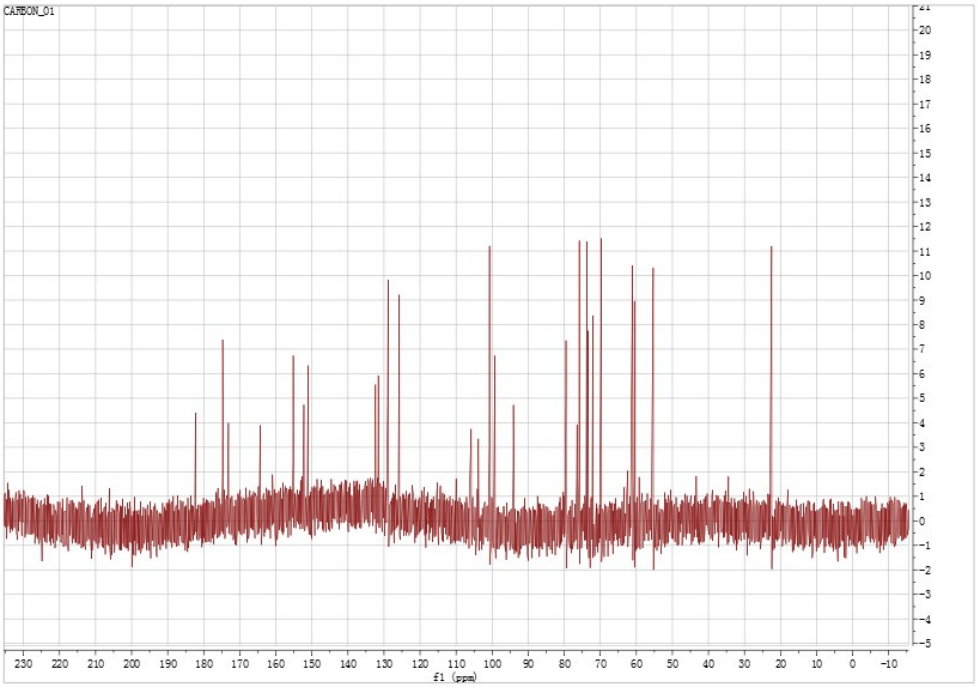
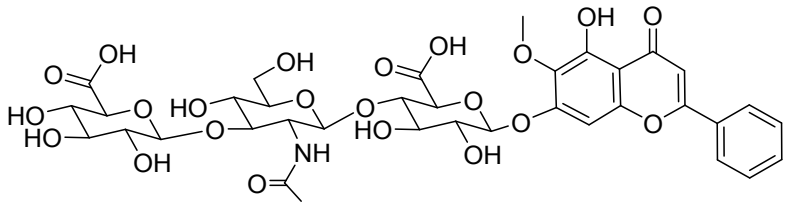


Figure S22. ^{13}C NMR of GlcNAcβ1-4GlcAβ-oroxylin **9**

Spectroscopic data of **GlcNAcβ1-4GlcAβ-oroxylin 9**:

^{1}H NMR (600 MHz, D_2O) 7.32-7.35 (m, 2 H), 7.21 (d, J = 4.2 Hz, 1 H), 7.11 (brs, 2 H), 6.23 (d, J = 4.8 Hz, 1 H), 6.16 (d, J = 6.6 Hz, 1 H), 4.43 (d, J = 8.4 Hz, 1 H), 3.85 (d, J = 11.4 Hz, 1 H), 3.71 (t, J = 9.0 Hz, 1 H), 3.68 (d, J = 4.2 Hz, 1 H), 3.64-3.66 (m, 1 H), 3.62 (s, 3 H, -OCH3), 3.56-3.59 (m, 2 H), 3.47-3.51 (m, 2 H), 3.44 (t, J = 9.3 Hz, 1 H), 3.34-3.38 (m, 2 H), 2.00 (s, 3 H, -Ac);^{13}C NMR(150 MHz, D_2O)182.3, 174.7, 173.1, 164.3, 155.2, 152.2 (2 C), 151.0, 132.4, 131.6, 129.0, 128.9, 125.8 (2 C), 100.7, 99.2, 79.5, 75.8, 73.7, 73.4, 72.1, 69.7, 61.0, 60.5, 55.3, 22.5. ESI-MS (negative ion): Calcd for $C_{30}H_{33}NO_{16}$: 663.18 [M]; Found: 662.1786 $[M-H]^-$.

GlcAβ1-3GlcNAcβ1-4GlcAβ-oroxylin 10



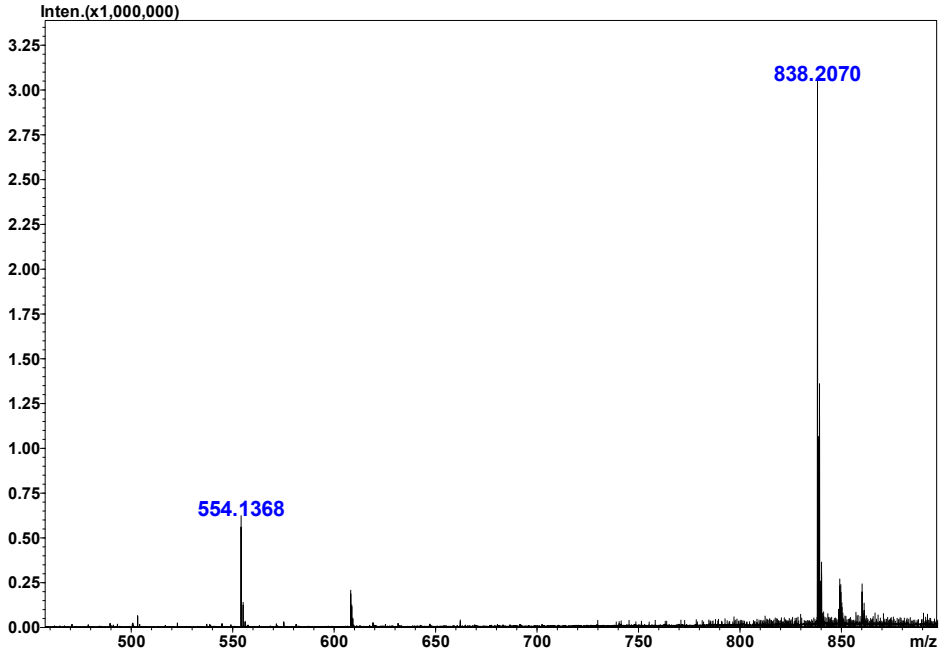


Figure S23. -Q ESI-MS profile of GlcAβ1-3GlcNAcβ1-4GlcAβ-oroxylin **10**

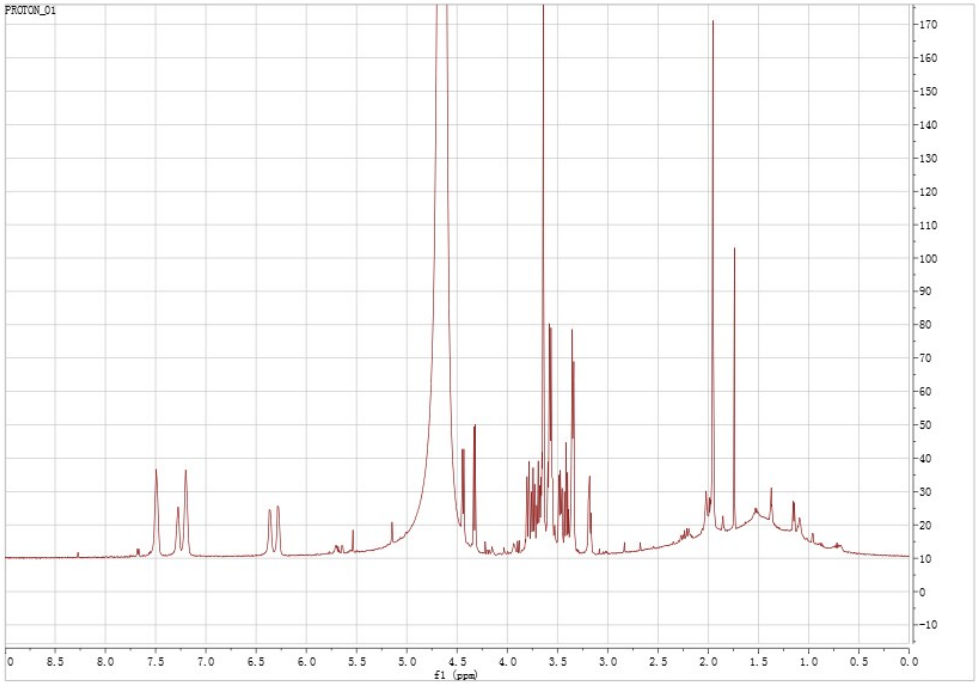


Figure S24. ^{1}H NMR of GlcAβ1-3GlcNAcβ1-4GlcAβ-oroxylin **10**

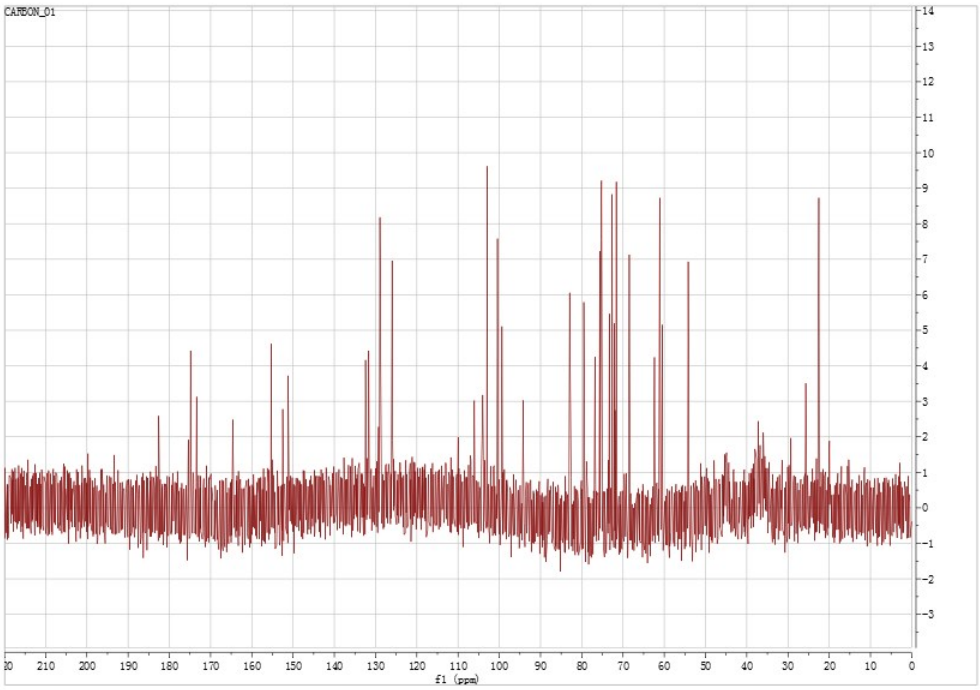


Figure S25.[13] C NMR of GlcAβ1-3GlcNAcβ1-4GlcAβ-oroxylin **10**

Spectroscopic data of **GlcAβ1-3GlcNAcβ1-4GlcAβ-oroxylin 10:**

^{1}H NMR (600 MHz, D_2O) 7.50-7.52 (m, 2 H), 7.29-7.31 (m, 1 H), 7.21-7.23 (m, 2 H), 6.37-6.39 (m, 1 H), 6.29-6.31 (m, 1 H), 4.46 (d, J = 8.4 Hz, 1 H), 4.34 (d, J = 7.8 Hz, 1 H), 3.81 (d, J = 10.8 Hz, 1 H), 3.76 (t, J = 9.6 Hz, 1 H), 3.71 (t, J = 9.6 Hz, 1 H), 3.66-3.68 (m, 1 H), 3.66 (s, 3 H, -OCH3), 3.55-3.61 (m, 3 H), 3.47-3.50 (m, 2 H), 3.43 (t, J = 9.3 Hz, 1 H), 3.39-3.41 (m, 1 H), 3.34-3.39 (m, 3 H), 3.19-3.21 (m, 1 H), 1.97 (s, 3 H, -Ac); ^{13}C NMR (150 MHz, D_2O) 182.6, 175.4, 174.9, 173.4, 164.6, 155.3, 152.5, 151.2, 132.4, 131.7, 129.3, 126.0, 102.9, 100.4, 99.4, 82.9, 79.5, 75.6, 75.3, 75.2, 73.3, 72.6, 72.1, 71.9, 71.6, 68.5, 62.4, 61.1, 60.5, 54.2, 22.5. ESI-MS (negative ion): Calcd for $C_{36}H_{41}NO_{22}$: 839.21 [M]; Found: 838.2070 $[M-H]^-$.

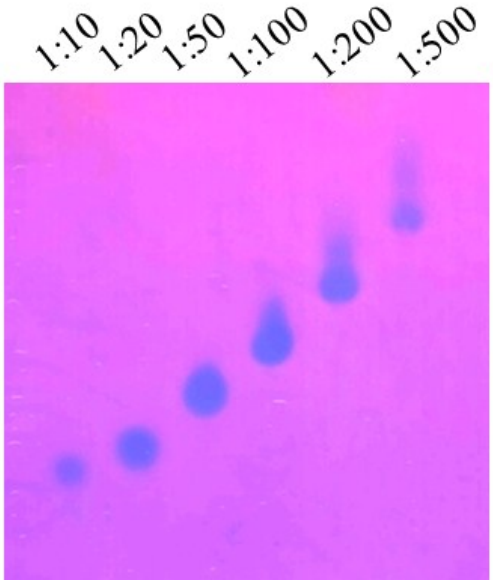


Figure S26. Agarose gel electrophoresis profiles of HA-oroxylin conjugates. The acceptor/precursor molar ratios were marked above. The loading amount was 50 μg per sample.

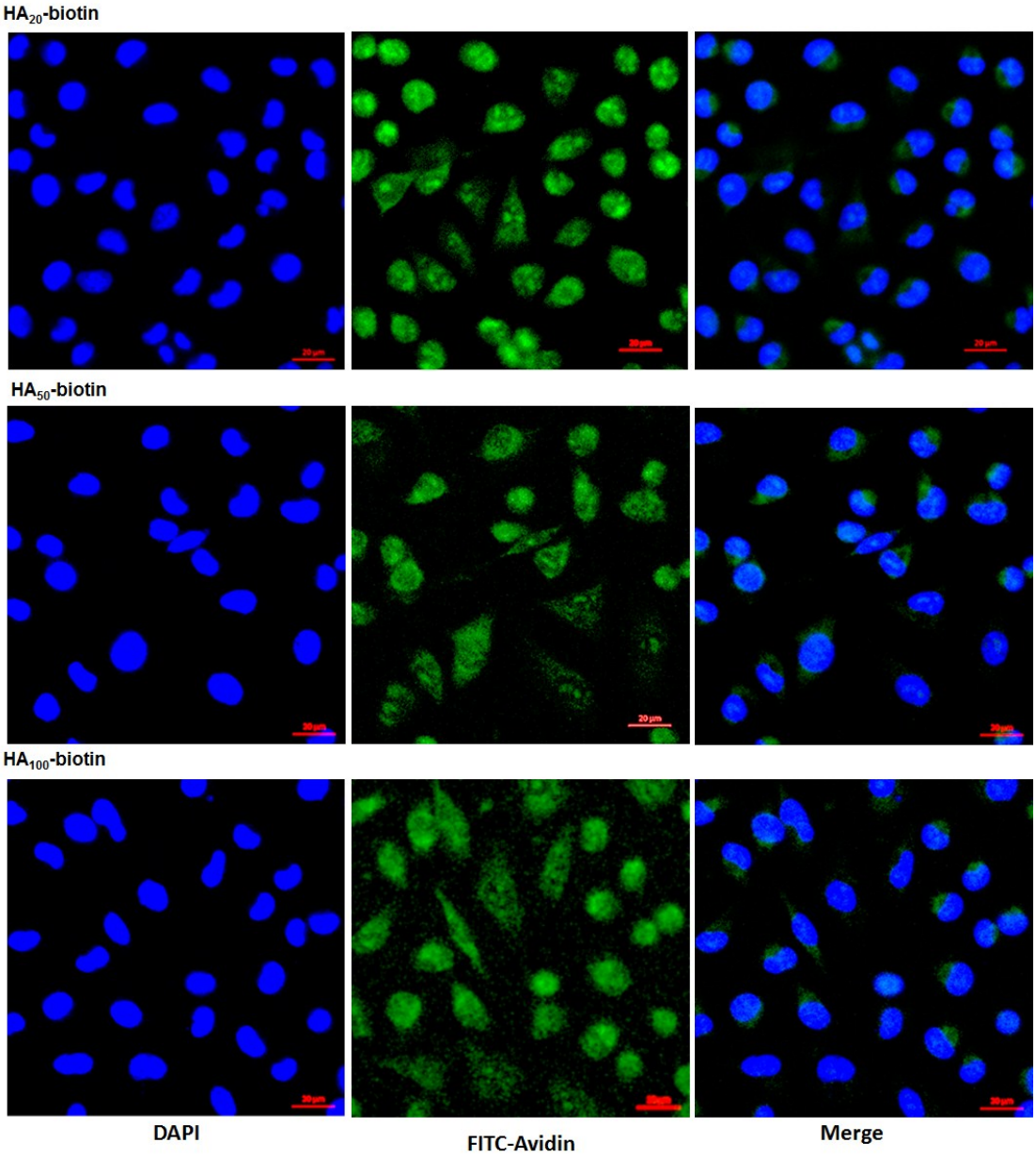


Figure S27. Confocal microscopy fluorescence profiles of exogenous HA-biotin conjugates detected on A549 cell lines.

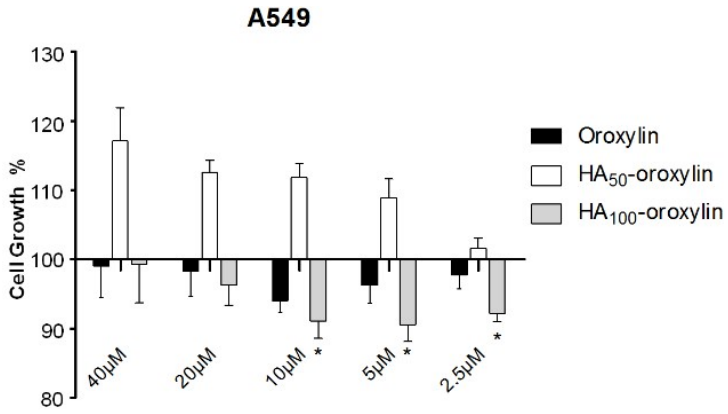


Figure S28. The growth effects on A549 cells of HA_{50}-oroxylin and HA_{100}-oroxylin conjugates. The data shown are means ± SD from three independent experiments (*: $p<0.05$).