Supplementary Information

β-Galactosidase-dependent metabolic glycoengineering of tumor cells for imaging and immunotherapy

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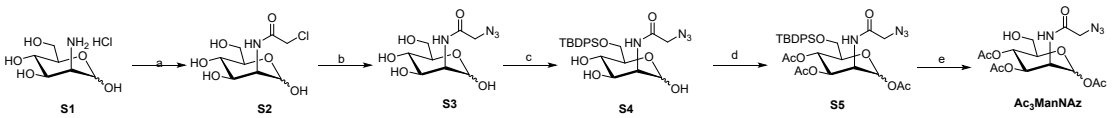
I. General Information

Materials All chemicals were obtained from commercial resource and used without further purification. All solvents were dried and purified by known procedures and freshly distilled under nitrogen from appropriate drying agents prior to use. The products were isolated by column chromatography on silica gel (200-300 mesh or 100-200 mesh) by using petroleum ether (30-60°C), ethyl acetate, dichloromethane, methyl alcohol as eluents. Dibenzylcyclooctyne-Cy5 (DBCO-Cy5) were purchased from Xi'an confluore Biological Technology. Dulbecco's Modified Eagle Medium (DMEM, Cat. No. SH30022.01), Roswell Park Memorial Institute medium (1640 medium, Cat. No. SH30809.01), Phosphate-buffered saline (PBS), Fetal Bovine Serum (FBS, Cat. No. SH30084.03), Penicillin-Streptomycin solution (Cat. No. SV30010) and HiPrep Desalting column were all from GE Healthcare. Anti-Rha antibody was obtained from rabbit serum. Rabbit total complement was from Sigma-Aldrich (Santa Clara, USA). The BCA kit, CCK8 assay kit and LDH cytotoxicity assay kit were purchased from Beyotime. The human peripheral blood mononuclear cells (PBMCs), mainly including lymphocytes and monocytes, is derived from the peripheral blood of healthy people by Ficoll density gradient centrifugation.

Instrumentation Reaction progress and product mixtures were routinely monitored by TLC using TLC SiO_2 sheets, and compounds were visualized under ultraviolet light. ^{1}H NMR, ^{13}C NMR spectra were recorded on a Bruker AVANCE III 400 spectrometer, Mass spectra (ESI) were recorded on a Bruker Amazon SL. Flow cytometry analyses of cells were conducted with an Accuri C6 flow cytometer (BD Biosciences), Confocal laser scanning microscopy (CLSM) images were taken on a Carl Zeiss LSM880 Confocal Microscope (Carl Zeiss, LSM880, Germany).

Cell culture. MDA-MB-231 and MDA-MB-468 (human triple-negative breast cancer line), MCF-7 (human breast cancer line), HELA (human Cervical cancer line), NCM-460 (Human normal colonic epithelial cells). All the above lines were cultured in DMEM medium supplemented with 10% FBS and 1% penicillin-streptomycin OVCAR3 (primary ovarian cancer cell) was maintained in 1640 medium supplemented with 20% FBS, 1% penicillin-streptomycin and 1% insulin. All cells were cultured at 37°C in a 5% CO_2 humidified atmosphere.

II. Synthesis of compound $Ac_3ManNAz$



Scheme S1. Reagents and conditions: （a）(1) MeOH, MeONa, ; (2) TEA, chloroacetic anhydride, r.t, 12 h, 80%;（b）DMF, NaN_3, 12 h, 80%; (c) TBDPSCl, Py, DMAP, 50°C, 24 h; (d) Py, AC_2O, r.t, 12 h, 76%; (e) TBAF, THF, r.t, 12 h, 76%;

Compound S2: To a solution of compound **S1** (10 g, 46 mmol) in MeOH (80 mL) were added NaOMe (80 mL), the reaction was stirred at r.t for 1 h and then TEA (6.4 mL) and 2-chloroacetic anhydride (9.3 g, 55 mmol) was added, the reaction mixture was stirred for a further 12 h at r.t. the solution was concentrated under reduced pressure and was purified by silica gel chromatography (DCM:MeOH=4:1, R_f=0.4) to give the compound **S2**[1] (8 g, 80%.).

Compound S3: To a solution of compound **S2** (6 g, 23.5 mmol) in DMF (30 mL) were added NaN_3 (3 g, 47 mmol), the reaction was stirred at 50°C for 12 h. After the completion of the reaction, the solution was concentrated under reduced pressure and was purified by silica gel chromatography (DCM:MeOH=4:1, R_f=0.45) to give the compound **S3** (4.8 g, 80%.).

Compound S5 To a solution of compound **S3** (4.5 g, 17 mmol) in Py (20 mL) were added TBDPSCl (7 g, 25.7 mmol), DMAP (4.1 g, 34 mmol), the reaction was stirred at 50°C for 24 h, then the solution was cooled to 0°C, and was added AC_2O (9.6 mL), the reaction was stirred at r.t for 12 h. After the completion of the reaction, the reaction was concentrated and extracted with DCM and washed with saturated NaCl solution, the organic layer was dried over anhydrous Na_2SO_4. The concentrated residue was purified by silica gel chromatography (PE:EA=1:1, R_f =0.5) to give the compound **S5** as a mixture of anomers [2](2.8 g, 76%).

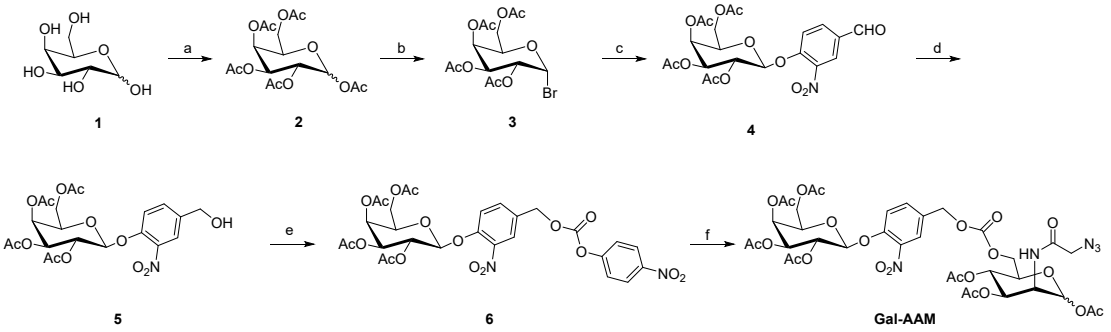
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 – 7.60 (m, 4H), 7.51 – 7.35 (m, 6H), 6.48 (dd, J = 19.8, 9.1 Hz, 1H), 6.30 – 5.75 (m, 1H), 5.36 – 5.18 (m, 2H), 4.50 – 4.21 (m, 1H), 3.94 (m, 2H), 3.79 – 3.66 (m, 2H), 2.17- 1.94 (m, 9H), 1.06 (s, 9H). MS-ESI (m/z): $[M+Na]^+$ calculated for $C_{24}H_{32}N_4NaO_6Si$, 649.32; observed, 649.14.

Compound $Ac_3ManNAz$: To a solution of compound **S5** (2.8 g, 4.4 mmol) in THF (25 mL) were added TBAF (6.7 mL, 6.7 mmol), AcOH (0.5 mL, 8.8 mmol), the reaction was stirred at r.t for 24

h After the completion of the reaction, the reaction was concentrated and extracted with DCM and washed with saturated NaCl solution, the organic layer was dried over anhydrous Na_2SO_4. The solution was concentrated under reduced pressure and was purified by silica gel chromatography (DCM:MeOH=20:1, R_f=0.5) to give the compound **Ac_3ManNAz** (1.3 g,76%.).

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.63 (d, J = 8.7 Hz, 1H), 6.18 (d, J = 3.7 Hz, 1H), 5.18 (dd, J = 11.0, 9.3 Hz, 1H), 4.54 (dd, J = 12.5, 3.6 Hz, 1H), 4.33 (m, 1H), 4.23 (m, 1H), 3.91 (s, 2H), 3.67 (m, 1H), 3.46 (d, J = 5.2 Hz, 1H), 2.19 (s, 3H), 2.14 (s, 3H), 2.13 (s, 3H). MS-ESI (m/z): $[M+Na]^+$ calculated for $C_{14}H_{20}N_4O_9$, 411.11; observed, 411.12

III. Synthesis of compound Gal-AAM



Scheme S2. Reagents and conditions: (a) Py, AC_2O, r.t, 12 h, 93%; (b) DCM, 33% HBr/AcOH, 0°C 4 h; (c) Ag_2O, CH_3CN, 4-hydroxy-3-nitrobenzaldehyde, r.t, 3 h, 69%; (d) $NaBH_4$, iPrOH/$CHCl_3$, r.t, 3 h, 88%; (e) 4-nitrophenyl carbonochloridate, DCM, TEA, r.t, 3 h, 64%; (f) Ac_3ManNAz, DMAP, Py, 50°C, 18 h, 42%.

Compound 4: Galactose **1** (5 g, 27.7 mmol) was dissolved in 34 mL pyridine, and then added 17 mL acetic anhydride dropwise slowly at 0°C. The mixture was stirred overnight, after the completion of the reaction, the solvent was removed in vacuo, and the resulting syrup was taken up in 200 mL of H_2O and extracted with EtOAc (three to four times), the organic phase was washed with saturated $NaHCO_3$ solution and NaCl solution. The organic layer was dried over sodium sulfate, and the solvent was evaporated to give compound **2** (10 g, 93%.) as a yellow oil. It was used for the next step directly without further purification.

Compound **2** (4 g, 10.2 mmol) was dissolved in DCM (25 mL), and then added HBr/AcOH (20 mL) dropwise slowly at 0°C. The mixture was stirred 4 h, after the completion of the reaction, the reaction was diluted with DCM and the organic phase was washed with saturated $NaHCO_3$ solution and NaCl solution. The organic layer was dried over sodium sulfate, and the solvent was evaporated to

give compound **3** as a yellow oil. It was used for the next step directly without further purification. Compound **3** was dissolved in CH_3CN (25 mL), and then was added 4-hydroxy-3-nitrobenzaldehyde (1.8 g, 11.2 mmol), Ag_2O (2.3 g, 10.2 mmol). The mixture was stirred at r.t for 3 h under argon atmosphere. After the completion of the reaction, the reaction was concentrated and then diluted with DCM, the organic phase was washed with saturated NaCl solution. The organic layer was dried over sodium sulfate, the solvent was evaporated and the crude residue was purified by silica gel column chromatography to give compound **4**[3](3.4 g, 69%, over the two steps) as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.99 (s, 1H), 8.31 (d, J = 2.0 Hz, 1H), 8.08 (dd, J = 8.7, 2.0 Hz, 1H), 7.50 (d, J = 8.6 Hz, 1H), 5.59 (dd, J = 10.4, 7.9 Hz, 1H), 5.50 (d, J = 3.1 Hz, 1H), 5.23 (d, J = 7.9 Hz, 1H), 5.14 (dd, J = 10.4, 3.4 Hz, 1H), 4.28 – 4.13 (m, 3H), 2.20 (s, 3H), 2.13 (s, 3H), 2.09 (s, 3H), 2.03 (s, 3H). MS-ESI (m/z): $[M+Na]^+$ calculated for $C_{21}H_{23}NNaO_{13}$, 520.11, observed, 520.10

Compound 5: Compound **4** (3.4 g, 6.8 mmol) was dissolved in iPrOH-$CHCl_3$ (1:5, 30 mL), and then added $NaBH_4$ (555 mg, 15 mmol) slowly at 0^{o}C and then was stirred 4 h, After the completion of the reaction, the reaction was quenched with 1M HCl and was concentrated, then diluted with DCM, the organic phase was washed with saturated $NaHCO_3$ solution and NaCl solution. The organic layer was dried over sodium sulfate, the solvent was evaporated and the crude residue was purified by silica gel column chromatography to give compound **5**[3] (3 g, 88%) as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (d, J = 1.9 Hz, 1H), 7.52 (m, 1H), 7.35 (d, J = 8.6 Hz, 1H), 5.54 (dd, J = 10.5, 8.0 Hz, 1H), 5.47 (d, J = 2.6 Hz, 1H), 5.10 (dd, J = 10.5, 3.4 Hz, 1H), 5.06 (d, J = 7.9 Hz, 1H), 4.73 (d, J = 5.0 Hz, 2H), 4.26 (m, 1H), 4.20 – 4.13 (m, 1H), 4.07 (m, 1H), 2.19 (s, 3H), 2.13 (s, 3H), 2.07 (s, 3H), 2.02 (s, 3H). MS-ESI (m/z): $[M+Na]^+$ calculated for $C_{21}H_{25}NNaO_{13}$, 522.12, observed, 522.05

Compound 6: To a solution of **5** (3 g, 6 mmol) in dry DCM (20 mL) were added 4-nitrophenyl carbonochloridate (1.3 g, 6.6 mmol), TEA (1.6 mL, 12 mmol). The mixture was stirred at r.t for 3 h, after the completion of the reaction, the reaction was concentrated and then diluted with DCM, the organic phase was washed with saturated NaCl solution. The organic layer was dried over sodium sulfate, the solvent was evaporated and the crude residue was purified by silica gel column chromatography to give compound **6** (2.5 g, 64%) as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.33 – 8.24 (m, 2H), 7.91 (d, J = 2.1 Hz, 1H), 7.61 (dd, J = 8.6, 2.2 Hz, 1H), 7.40 (dd, J = 8.8, 6.5 Hz, 3H), 5.56 (dd, J = 10.5, 7.9 Hz, 1H), 5.48 (d, J = 3.3 Hz, 1H),

5.29 (s, 2H), 5.15 – 5.09 (m, 2H), 4.29 – 4.24 (m, 1H), 4.16 (m, 1H), 2.19 (s, 3H), 2.13 (s, 3H), 2.08 (s, 3H), 2.02 (s, 3H).

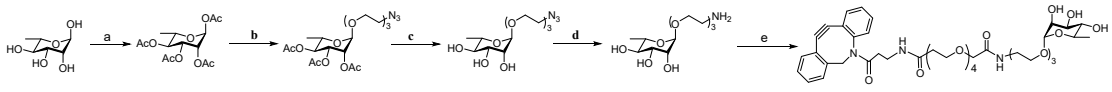
^{13}C NMR (100 MHz, $CDCl_3$) δ 170.28, 170.13, 170.11, 169.33, 155.2, 152.31, 149.67, 145.61, 141.37, 133.77, 130.24, 125.42, 125.39, 121.70, 119.95, 100.68, 71.57, 70.49, 68.86, 67.82, 66.67, 61.31, 20.67, 20.63(2C), 20.56. MS-ESI (m/z): $[M+Na]^+$ calculated for $C_{28}H_{28}N_2NaO_{17}$, 687.13; observed, 687.04.

Compound Gal-AAM: To a solution of **Ac_3ManNAz** (200 mg, 0.5 mmol) in dry Py (8 mL) was added **6** (664 mg, 1 mmol), DMAP (122 mg, 1 mmol). The mixture was stirred at 50°C for 24 h, the reaction was concentrated and then diluted with DCM, the organic phase was washed with saturated $NaHCO_3$ and NaCl solution. The organic layer was dried over sodium sulfate, the solvent was evaporated and the crude residue was purified by silica gel column chromatography to give compound **Gal-AAM**[2] (200 mg, 42%) as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82 (d, J = 2.1 Hz, 1H), 7.53 (m, 1H), 7.38 (d, J = 8.6 Hz, 1H), 6.48 (d, J = 8.8 Hz, 1H), 6.19 (d, J = 3.7 Hz, 1H), 5.54 (m, 1H), 5.47 (m, 1H), 5.32 (m, 1H), 5.15 (s, 2H), 5.13 – 5.08 (m, 2H), 5.02 (t, J = 9.9 Hz, 1H), 4.44 (m, 1H), 4.32 (m, 1H), 4.25 (m, 1H), 4.18 (m, 1H), 4.11 – 4.04 (m, 3H), 3.93 (s, 2H), 2.20 (s, 3H), 2.19 (s, 3H), 2.12 (s, 3H), 2.08 (s, 6H), 2.02 (s, 3H), 1.97 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 171.33, 170.56, 170.28, 170.14, 170.10, 169.33, 168.60, 166.97,153.71, 149.43, 141.30, 133.49, 130.71, 124.99, 119.87, 100.70, 90.11, 71.80, 71.52, 70.49, 70.08, 69.48, 68.39, 67.81, 66.69, 61.29, 52.41, 51.29, 20.85, 20.65, 20.64, 20.61(2C), 20.54, 20.52. HRMS-ESI (m/z): $[M+H]^+$ calculated for $C_{36}H_{44}N_5NaO_{23}$, 914.2427 ; observed, 914.2401.

IV: Synthesis of compound DBCO-Rha



Scheme S3. Reagents and conditions: (a) Ac_2O, Py, r.t, 12 h, 94%; (b) 2-(2-(2-azidoethoxy)ethoxy)ethan-1-ol, $BF_3{\cdot}Et_2O$, DCM, r.t, 68%; (c) MeONa, MeOH, 95%; (d) 10% Pd/C, H_2, MeOH, r.t, 95%; (p) DBCO-PEG_4-NHS, DMF, r.t, 30 min, 75%.

Compound 8: L-rhamnose **7** (5 g, 30 mmol) was dissolved in 20 mL pyridine, and then added 17 mL acetic anhydride dropwise slowly at 0°C. The mixture was stirred overnight, after the completion of the reaction, the solvent was removed in vacuo, and the resulting syrup was taken up

in 200 mL of H_2O and extracted with EtOAc (three times), the organic phase was washed with 1M HCl, saturated $NaHCO_3$ solution and NaCl solution. The organic layer was dried over sodium sulfate, and the solvent was evaporated to give **8** (9.3 g, 94%.) as a yellow oil. It was used for the next step without further purification.

Compound 9 (1 mL, 3 eq) $BF_3 \cdot OEt_2$ was added to a mixture of **8** (1.1 g, 3.3 mmol) and 2-(2-(2-azidoethoxy)ethoxy)ethan-1-ol (0.7 g,1.2 eq) in anhydrous CH_2Cl_2 (15 mL) at 0°C. The mixture was stirred for 2 h at 0°C before being allowed to warm to room temperature followed by continuous stirring overnight. After the completion of the reaction, the reaction mixture was quenched with saturated $NaHCO_3$ and diluted with CH_2Cl_2 (100 mL), washed with saturated $NaHCO_3$ and then with brine. It was then dried over $NaSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel（PE/EtOAc=3:1）to afford compound **9** (1 g, 68%.). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.26 – 5.15 (m, 2H), 4.99 (m, 1H), 4.71 (d, *J* = 1.8 Hz, 1H), 3.86 (dd, *J* = 9.8, 6.3 Hz, 1H), 3.73 (dd, *J* = 7.5, 2.3 Hz, 1H), 3.67 – 3.49 (m, 8H), 3.33 (t, *J* = 5.0 Hz, 2H), 2.08 (s, 3H), 1.98 (d, *J* = 2.2 Hz, 3H), 1.92 (d, *J* = 2.9 Hz, 3H), 1.15 (dd, *J* = 6.3, 2.5 Hz, 3H).

Compound 10: NaOMe (0.1 eq 50 μL) was added to a stirred solution of **9** (250 mg，0.56 mmol) in MeOH (5 mL) for 12 h at room temperature, After the completion of the reaction, the reaction mixture was acidified with Amberlite® IR120 hydrogen form followed by filtration, and solvent was evaporated under reduced pressure. The residue was dried over high vacuum to afford compound **10** (170 mg, 95%.). ^{1}H NMR (400 MHz, D_2O) δ 4.85 (d, *J* = 1.5 Hz, 1H), 3.99 (m, 1H), 3.92 – 3.85 (m, 1H), 3.83 – 3.67 (m, 12H), 3.57 – 3.51 (m, 2H), 3.47 (t, *J* = 9.7 Hz, 1H), 1.32 (d, *J* = 6.3 Hz, 3H).

Compound 11: To a solution of **10** (110 mg, 0.3 mmol) in MeOH (5 mL) and was added Pd/C (10% w/w, wet 10 mg), the solution was stirred at r.t. under H_2 atmosphere for 4 h. After the completion of the reaction, the mixture was filtered over Celite and thoroughly washed with CH_2Cl_2 and MeOH. Solvents were removed in vacuo to afford compound **11** (96 mg, 95%). ^{1}H NMR (400 MHz, D_2O) δ 4.84 (s, 1H), 3.98 (d, J = 3.3 Hz, 1H), 3.88 (dd, J = 11.6, 4.3 Hz, 1H), 3.82 – 3.60 (m, 11H), 3.46 (t, J = 9.6 Hz, 1H), 3.37 (d, J = 1.6 Hz, 1H), 2.86 (m, 2H), 1.31 (d, J = 6.2 Hz, 3H).

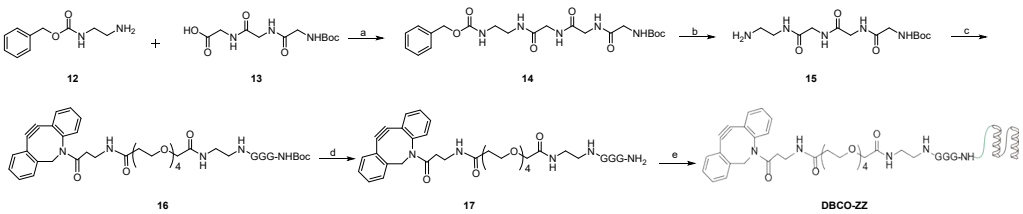
Compound DBCO-Rha: To a solution of compound **11** (11 mg, 0.036 mmol) in DMF (2 mL) were added DBCO-PEG_4-NHS (20 mg, 0.03 mmol), the reaction mixture was stirred at r.t. for 30 min.

After the completion of the reaction, the reaction was concentrated and was purified by silica gel chromatography (DCM:MeOH=10:1, R_f=0.4) to give the compound **DBCO-Rha** (18 mg, 75%).

^{1}H NMR (400 MHz, Methanol-d_4) δ 7.67 (dd, J = 7.4, 1.6 Hz, 1H), 7.65 – 7.59 (m, 1H), 7.51 – 7.43 (m, 3H), 7.40 – 7.31 (m, 2H), 7.27 (m, 1H), 5.15 (d, J = 14.0 Hz, 1H), 4.74 (d, J = 1.7 Hz, 1H), 3.85 – 3.77 (m, 2H), 3.75 – 3.70 (m, 3H), 3.69 – 3.64 (m, 6H), 3.63 – 3.60 (m, 10H), 3.59 – 3.53 (m, 4H), 3.48 – 3.41 (m, 2H), 3.40 – 3.35 (m, 3H), 3.33 (p, J = 1.7 Hz, 2H), 3.26 (dd, J = 6.2, 4.8 Hz, 2H), 2.71 (dd, J = 15.8, 8.2 Hz, 1H), 2.46 (t, J = 6.2 Hz, 2H), 2.43 – 2.29 (m, 1H), 2.23 – 2.11 (m, 1H), 1.99 (m, 1H), 1.28 (d, J = 6.2 Hz, 3H).

^{13}C NMR (100 MHz, Methanol-d_4) δ 173.18, 172.63, 172.61, 151.32, 148.10, 132.09, 129.22, 128.61, 128.24, 127.79, 127.49, 126.72, 125.06, 123.00, 122.32, 114.19, 107.42, 100.42, 72.59, 70.98, 70.82, 70.29, 70.15, 70.13, 70.05, 69.95, 69.87, 69.24, 69.05, 68.41, 66.88, 66.31, 55.30, 39.04, 38.99, 36.20, 30.52, 30.01, 16.69. MS-ESI (m/z): $[M+Na]^+$ calculated for $C_{42}H_{59}N_3NaO_{14}$, 852.39; observed, 852.43

V: Synthesis of compound DBCO-ZZ



Scheme S4. Reagents and conditions: （a）EDCI, HOBt, TEA, DCM, r.t, 4 h, 76%; (b) Pd/C, H_2, MeOH, r.t, 12 h; (c) DBCO-PEG_4-NHS, DMF, r.t, 30 min, 64%; (d) 10%TFA/DCM, 0^{o}C, 1.5 h, 94%; (e) ZZ domain, SrtA, 16^{o}C, 4 h, 57%.

Compound 14: To a solution of the compound **12** (310 mg, 1.6 mmol) in DMF (10 mL) were added (tert-butoxycarbonyl)glycylglycylglycine (compound **13**) (578 mg, 2 mmol), HOBt (411 mg, 3 mmol), TEA (0.6 mL, 4 mmol) and EDC·HCl (573 mg, 3 mmol) at 0°C. The reaction mixture was then warmed to r.t. and stirred for 4 h. After the completion of the reaction, the reaction was concentrated and extracted with DCM and washed with saturated NaCl solution, the organic layer was dried over anhydrous Na_2SO_4. The concentrated residue was purified by silica gel chromatography (DCM:MeOH=50:1, R_f=0.3) to give the compound **14** (670 mg, 76%).

^{1}H NMR (400 MHz, Methanol-d_4) δ 7.41 – 7.25 (m, 5H), 5.05 (s, 2H), 3.88 (s, 2H), 3.83 (s, 2H), 3.73 (s, 2H), 3.32–3.24 (m, 4H), 1.43 (s, 9H). MS-ESI (m/z): $[M+Na]^+$ calculated for $C_{21}H_{31}N_5NaO_7$, 488.21; observed, 488.18.

Compound 15: Compound **14** was dissolved in MeOH (20 mL) and Pd/C（120 mg）, the reaction mixture was stirred at room temperature under H_2 atmosphere for 12 h, after the completion of the reaction, the product mixture was filtered through Celite, washed with MeOH, and then the solvent was concentrated to afford compound **15** and was used for the next step directly without purification.

Compound 16: To a solution of the compound **15** (70 mg, 0.21 mmol) in DMF (10 mL) were added DBCO-PEG_4-NHS (140 mg, 0.21 mmol), the reaction mixture was stirred at r.t. for 30 min. After the completion of the reaction, the reaction was concentrated and was purified by silica gel chromatography (DCM:MeOH=10:1, R_f=0.4) to give the compound **16** (120 mg, 64%).

^{1}H NMR (400 MHz, Methanol-d_4) δ 7.71 – 7.59 (m, 2H), 7.48 (d, *J* = 2.5 Hz, 3H), 7.37 (m, 2H), 7.31 – 7.25 (m, 1H), 5.16 (d, *J* = 14.0 Hz, 1H), 3.94 (s, 2H), 3.86 (s, 2H), 3.79 (s, 2H), 3.77 – 3.69 (m, 3H), 3.67 – 3.54 (m, 12H), 3.46 (m, 2H), 3.34 (m, 3H), 3.27 (m, 2H), 2.72 (m, 1H), 2.47 (t, *J* = 6.1 Hz, 2H), 2.39 (m, 1H), 2.20 (m, 1H), 2.01 (m, 1H), 1.48 (s, 9H).

^{13}C NMR (100 MHz, Methanol-d_4) δ 173.19, 173.08, 172.62, 172.23, 170.87, 170.44, 157.35, 151.31, 148.10, 132.07, 129.20, 128.61, 128.25, 127.79, 127.49, 126.72, 125.07, 123.00, 122.33, 114.19, 107.41, 79.61, 70.11 (2C), 70.08, 70.01, 69.87, 69.85, 69.07, 66.80, 55.31, 43.55, 42.48, 42.19, 39.06, 38.97, 38.35, 36.25, 30.54, 30.02, 27.34 (3C). MS-ESI (m/z): $[M+Na]^+$ calculated for $C_{43}H_{59}N_7NaO_{12}$, 888.41; observed, 888.29.

Compound 17: To a solution of the compound **16** (120 mg, 0.14 mmol) in DCM (6 mL), then 20% TFA/DCM (6 mL) was added dropwise at 0^{o}C, then the reaction mixture was stirred at 0^{o}C for 1.5 h. After the completion of the reaction, the reaction was diluted with toluene (5 mL), and concentrated in vacuo. The residual TFA was removed from the crude residue by azeotroping with toluene (3×5 mL) and was concentrated to give the compound **23** (100 mg, 94%).

^{1}H NMR (400 MHz, Methanol-d_4) δ 7.67 – 7.59 (m, 2H), 7.47 (q, J = 2.9 Hz, 3H), 7.39 – 7.26 (m, 3H), 5.15 (d, J = 14.0 Hz, 1H), 3.98 (s, 2H), 3.86 (s, 2H), 3.81 (s, 2H), 3.74 – 3.70 (m, 3H), 3.66 – 3.60 (m, 12H), 3.45 (m, 2H), 3.35 – 3.33 (m, 3H), 3.26 (t, J = 5.5 Hz, 2H), 2.71 (m, 1H), 2.46 (m, 2H), 2.40 – 2.32 (m, 1H), 2.22 (m, 1H), 2.04 (m, 1H).

^{13}C NMR (100 MHz, Methanol-d_4) δ 173.27, 173.22, 172.67, 170.41, 167.01, 151.28, 148.10, 132.03, 129.18, 128.62, 128.28, 127.79, 127.50, 126.73, 125.08, 123.00，122.33， 114.18,107.40, 70.03, 70.00（4C）, 69.81, 69.79, 69.14, 66.82, 55.33, 42.34, 42.29, 40.25, 38.93, 38.90, 38.26, 36.04, 30.55, 30.01. MS-ESI (m/z): $[M+H]^+$ calculated for $C_{43}H_{57}N_6NaO_{12}$, 766.38; observed, 766.19.

Compound DBCO-ZZ: Compound **17** (500 μM), ZZ domain (20 μM), and SrtA (5 μM) were mixed in a reaction buffer (Tris/HCl 50 mM, NaCl 150 mM, $CaCl_2$ 5 mM, pH = 7.5, 1.0 mL), the mixture was incubated at 16°C for 5 h with constant shaking at 200 rpm. Then, nickel-magnetic beads (500 μL) were added into the mixture to capture and remove SrtA and unreacted ZZ domain, which was vortexed at 4°C for 30 min. The supernatant containing the desired conjugates was collected and subjected to purification with a Sephadex G25 column using PBS as the eluent to provide DBCO-ZZ conjugate

VI: General procedure of immunological assay

General procedures for flow cytometry analysis of surface azido sugar

Cells were seeded in a 6-well plate at a density of $3x10^4$ cells per well and allowed to attach for 12 h, then the cells were washed twice using DPBS and further incubated with fresh medium, Azido sugars were added in stock solution of DMSO (100 mM) into cell culture wells to reach final concentration (100 μM) and the cells were cultured at 37°C in 5% CO_2 humidified air for 24 h, the culture media were removed and cells were washed with DPBS for three times. Cells were lifted by incubating with trypsin solution and were added into 1.5 mL Eppendorf tubes, washed with DPBS for three times. Fresh culture media with 50 μM DBCO-Cy5 were treated to cells at 37°C for 1 h, free dye was washed with flow cytometry buffer (2% BSA in PBS, 2 × 500 μL) and then resuspended in 200 μL of flow cytometry buffer. Finally, they were analyzed using an Accuri C6 flow cytometer (BD Biosciences), and data analyses were performed with the FlowJo software.

General procedures for confocal imaging of azido sugar labeled cells.

Cells were seeded in a 24-well plate at a density of $8×10^3$ cells per well and allowed to attach for 12 h, Azido sugar (100 μM) was added and the cells were incubated at 37°C for 24 h. After washing with PBS，Cells were fixed with 4% paraformaldehyde for 10 min, and blocked using 1% BSA (in

PBS) for 1 h at 37°C.Then, cells were treated with DBCO-Cy5 (50 μM in 1% BSA) at 37°C for 1 h, washed with PBS, after permeabilization with 0.1% TritonX-100 (in 1% BSA), cells were treated with a drop of antifade mounting medium (containing 4',6-diamidino-2-phenylindole (DAPI)) and finally imaged using a confocal microscope.

Inhibition assay of azido sugar cell labeling

Cells were seeded in a 6-well plate at a density of 3×10^4 cells per well and allowed to attach for 12 h，then the cells were washed twice using DPBS, 50 μM of galactose was treated to MDA-MB-231 cells for 12 h before treatment of Gal-AAM and Ac_3ManNAz was added，the cells were further cultured for another 24 h, washed with DPBS twice and incubated with 50 μM of DBCO-Cy5 for 1 h at 37°C. then the fluorescence analysis was performed using flow cytometer and confocal imaging

Labeling kinetics of Gal-AAM in vitro

Concentration-dependent: MDA-MB-231cells were seeded in a 6-well plate at a density of 3×10^4 cells per well and allowed to attach for 12 h, then the cells were washed twice using DPBS and incubated with various concentrations of Gal-AAM (0 μM, 10 μM, 25 μM, 50 μM, 75 μM, 100 μM) for 24 h, cells were lifted by incubating with trypsin solution and were added into 1.5mL Eppendorf tubes, washed with DPBS for three times, and incubated with 50 μM of DBCO-Cy5 for 1 h at 37 °C, Cells were then washed 3 times with FACS buffer (2% BSA in PBS), suspended in 200 μL FACS buffer for flow cytometry analysis.

Time-dependent: Cell samples for flow cytometry measurements was similar to the concentration-dependent procedures. MDA-MB-231 cells were treated with 100 μM Gal-AAM for different time points (0 h, 6 h, 12 h, 24 h, 36 h, 48 h, 72 h).

Cell viability assays of compound Gal-AAM as determined by CCK8 MDA-MB-231 and HEK293 cells were seeded in 96-well plates at a density of 8×10^3 cells per well and allowed to attach for overnight. Then cells were treated with different concentrations supplemented with various concentrations (0 μM, 10 μM, 25 50 μM, 75 μM and 100 μM) of Gal-AAM for 48 h. Subsequently, 10% (v/v) CCK-8 was added to each well and cells were incubated for 4 h. The cell viability was measured on a microplate reader at 450 nm wavelength.

Gal-AAM mediated cancer labeling *in vivo*

All animal procedures were carried out in accordance with the guidelines listed in the Guide for the

Care and Use of Laboratory Animals. This study has been reviewed and approved by the Institutional Animal Care and Use Committee of Jiangnan University (JN.No20210630b0250910[234], JN.No20210630b0251130[239]). To evaluate the in vivo tumor metabolic labeling, the female BALB/c nude mice were implanted with 5×10^6 cells (MDA-MB-231 cells) subcutaneously in the right flank. When the tumors reached ~50 mm^3, Gal-AAM (25 mM, 20 μL) was injected into the tumors once daily for three consecutive days, while the same amount of $Ac_4ManNAc$ and $Ac_3ManNAz$ were injected to the tumors as control. One day after that, DBCO–Cy5 (5 mg/kg) was intravenously injected, and its biodistribution was monitored at 4 h and 48 h post-injection of DBCO–Cy5 using the Maestro In Vivo Fluorescence Imaging System. The excitation filter was 575–605 nm. Meanwhile, to further confirm the selectivity of Gal-AAM *in vivo,* the above experiments were performed in non-tumor bearing mice as well.

Analysis the abilities to recruiting endogenous antibodies by Rha or ZZ domain engineered on the target cell surface

MDA-MB-231 and MCF-7 cells were seeded in a 6-well plate at a density of 4×10^4 cells per well and allowed to attach for 12 h. The old culture media were removed and cells were washed with PBS for three times. Then cells were incubated with PBS, 100 μM Gal-AAM, 100 μM $Ac_4ManNAc$, respectively, for 12 h, and then washed and treated with 50 μM DBCO-Rha/DBCO-ZZ for 2 h. After washing, cells were treated with 100 μL of diluted rabbit serum (1% in PBS) at 37°C for 1 h. Subsequently, cells were treated with 100 μL of diluted Dylight 647-conjugated goat anti- rabbit IgG antibodies (0.05% in PBS) at 37°C for 1 h, washed with flow cytometry buffer (2% BSA in PBS, 2× 500 μL) and then resuspended in 200 μL of flow cytometry buffer. Finally, they were analyzed using an Accuri C6 flow cytometer (BD Biosciences), and data analysis were performed with the FlowJo software.

Complement-dependent cytotoxicity (CDC) assay: MDA-MB-231 and MCF-7 cells were seeded in a 96-well plate at a density of 4×10^3 cells per well and allowed to attach for 12 h. The old culture media were removed and cells were washed with DPBS for three times and were incubated with 100 μM Gal-AAM for 12 h, and then washed and labeled by 50 μM DBCO-Rha/DBCO-ZZ for 2 h. After washing, cells were treated with 100 μL of diluted rabbit serum (1% in PBS) at 37°C for 1 h. Subsequently, cells were treated with 100 μL of diluted rabbit complement (2% in PBS) at 37°C for 4 h. The groups treated with $Ac_4ManNAc$ and $Ac_3ManNAz$ was employed as negative and positive

control. The cell maximum killing was achieved by adding 100 μL of 1% Triton X-100 to the cell culture. The cell viability was measured using a CCK8 assay kit on a microplae reader at 450 nm wavelength. The negative control was the cells without Rha labelling on surface for excluding non-specific killing by rabbit complement. Cell cytotoxicity was calculated by the following equation.

$$CDC(\%) = 1 - \frac{A\,(\text{experimental}) - A(\text{maximum})}{A\,(\text{negative}) - A\,(\text{maximum})} \times 100$$

Where A(negative) is the OD_{450} value of cells treated with Gal-AAM in the absence of rabbit complement; A(maximum) is the OD_{450} value of cells completely lysed with 1% Triton X-100; A(experimental) is the OD_{450} value of cells treated with Gal-AAM conjugates or $Ac_4ManNAc$ or $Ac_3ManNAz$

Antibody-dependent cell-mediated cytotoxicity (ADCC) assay: MDA-MB-231 and MCF-7 cells were seeded in a 96-well plate at a density of 4×10^3 cells per well and allowed to attach for 12 h. Cells were washed with DPBS for three times and incubated with 100 μM Gal-AAM for 12 h. In a parallel study, MDA-MB-231 and MCF-7 cells were incubated with 100 μM $Ac_3ManNAc$ and $Ac_3ManNAz$ for 12 h, respectively, and then washed and labeled by 50 μM DBCO-Rha/DBCO-ZZ for 2 h. After washing, cells were treated with 100 μL of diluted rabbit serum (1% in PBS) at 37°C for 1 h. After washing, cells were then treated with 8×10^4 freshly isolated PBMCs at 37°C for 4 h. The cell maximum killing was achieved by adding LDH release reagent to a final concentration of 1%. Cytotoxicity was then detected using an LDH cytotoxicity assay kit. Spontaneous LDH released by PBMC were also detected. Cell lysis was calculated using the following equation:

$$ADCC(\%) = \frac{A\,(\text{experimental}) - A(\text{spontaneous})}{A\,(\text{maximum})} \times 100$$

Where A(experimental) is the OD490 value of LDH released by cells treated with Gal-AAM, $Ac_3ManNAc$ and $Ac_3ManNAz$ in the presence of rabbit serum and PBMC; A(spontaneous) is the OD490 value of LDH spontaneously released by PBMC; A(maximum) is the OD490 value of cells completely lysed with 10% LDH release reagent.

VII: Supporting figures

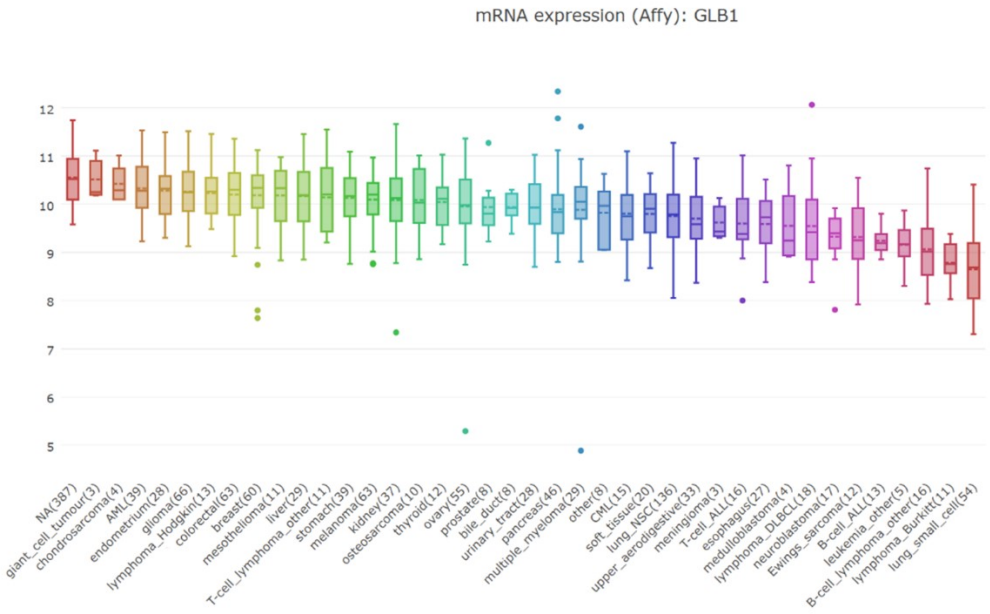
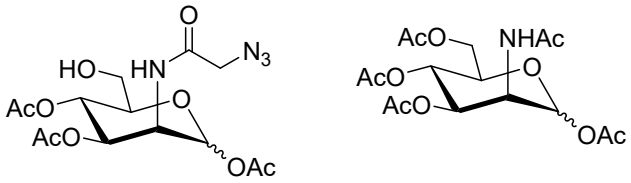


Fig. S1: The expression analysis of β-galactosidase (mRNA expression (Affy): GLB1) in various cells through Gene Expression Profiling Interactive Analyses (GEPIA, http://gepia.cancer-pku.cn/), a web server for cancer and normal gene expression profiling and interactive analyses.



AC_3ManNAz **AC_4ManNAc**

Fig. S2**.** the structure of Ac_3ManNAz and Ac_4ManNAc

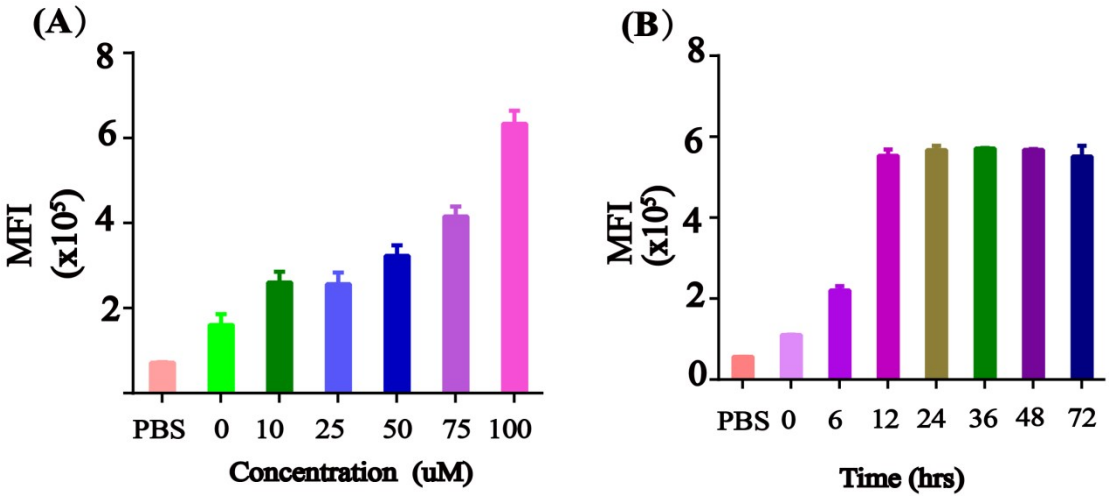


Fig. S3 (A): Concentration-and (B) time-dependent Gal-AAM-mediated labeling in MDA-MB-231 cells

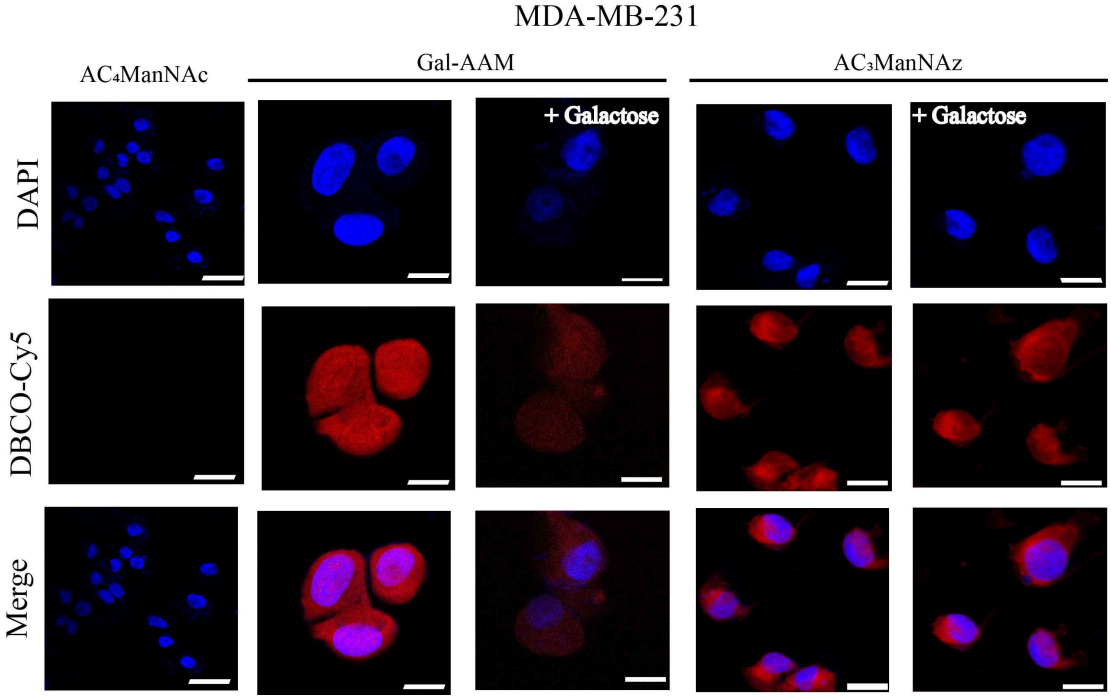


Fig.S4 Confocal microscopy images of the specificity of Gal-AAM in response to the endogenous β-Gal. MDA-MB-231 cells treated with $Ac_4ManNAc$ (100 μM), $Ac_3ManNAz$ (100 μM), $Ac_3ManNAz$ (100 μM) + galactose (50 μM), Gal-AAM (100 μM), Gal-AAM (100 μM) + galactose (50 μM) and labeled by DBCO-Cy5 (50 μM) at 37°C for 1 h. Red=DBCO-Cy5 channel; blue=DAPI channel. Scale bar: 20 μm.

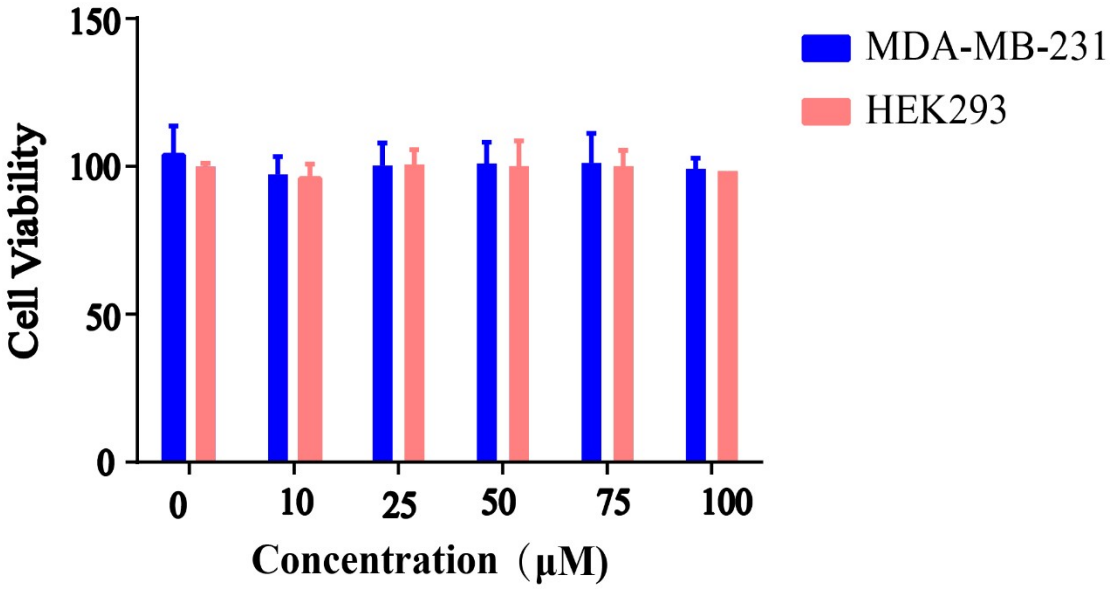


Fig. S5 Cell viability of MDA-MB-231 cells and HEK293 cells after treated with different concentrations of Gal-AAM for 72 h, as determined by CCK8 assay.

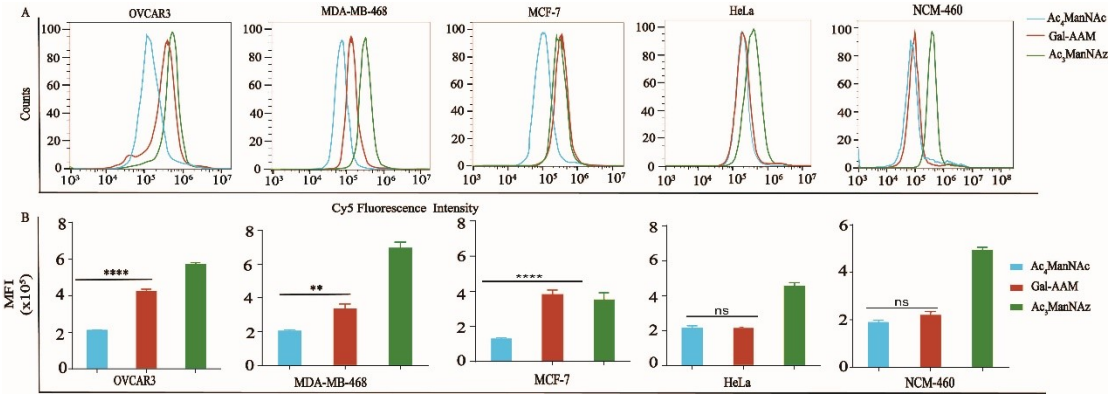


Fig. S6 (A) Flow cytometry assays and (B) the corresponding MFI of tumor cells OVCAR3 MDA-MB-468, MCF-7, HeLa and normal cells NCM-460 treated with $Ac_4ManNAc$ (100 μM), $Ac_3ManNAz$ (100 μM) and Gal-AAM (100 μM), and labeled by DBCO-Cy5 (50 μM) at 37°C for 1 h. Error bars show the SD of three parallel experiments. ****: $P < 0.0001$; **: $P < 0.01$.

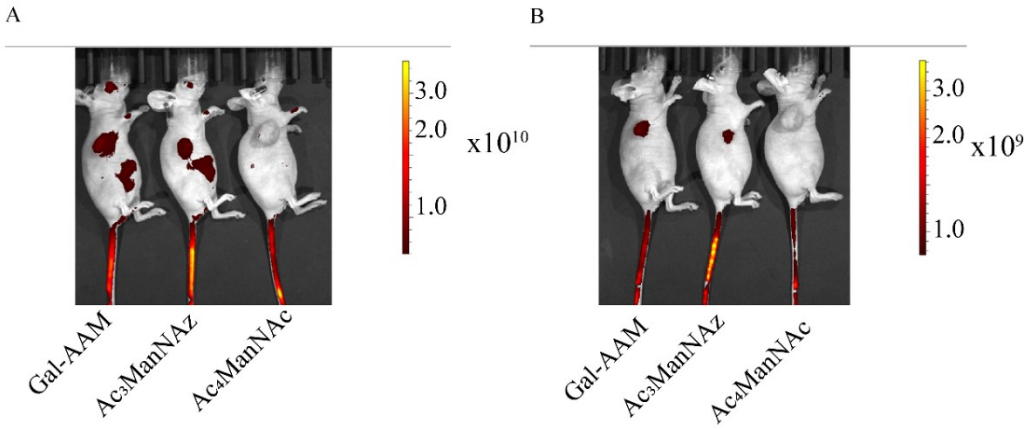


Fig. S7. In vivo fluorescence imaging of tumor bearing mice pre-treated with Gal-AAM, $Ac_3ManNAz$ and $Ac_4ManNAc$, respectively, at (A) 4 h and (B) 48 h post-injection (i.v.) of DBCO–Cy5.

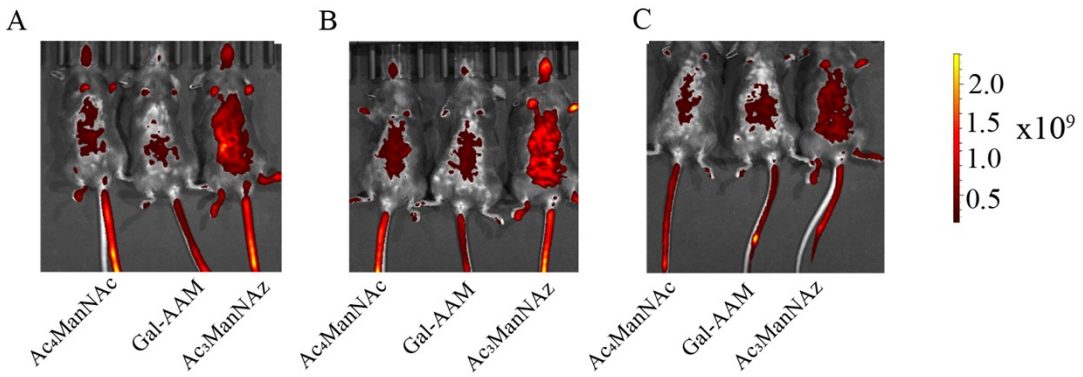


Fig. S8. In vivo fluorescence imaging of non-tumor bearing mice pre-treated with $Ac_4ManNAc$, Gal-AAM and $Ac_3ManNAz$, respectively, at (A) 4 h and (B) 24 h and (C) 48 h post-injection (i.v.) of DBCO–Cy5.

VIII: Reference

[1] J. Boutet, C. Guerreiro and L. A. Mulard, *Tetrahedron* **2008**, *64*, 10558-10572.

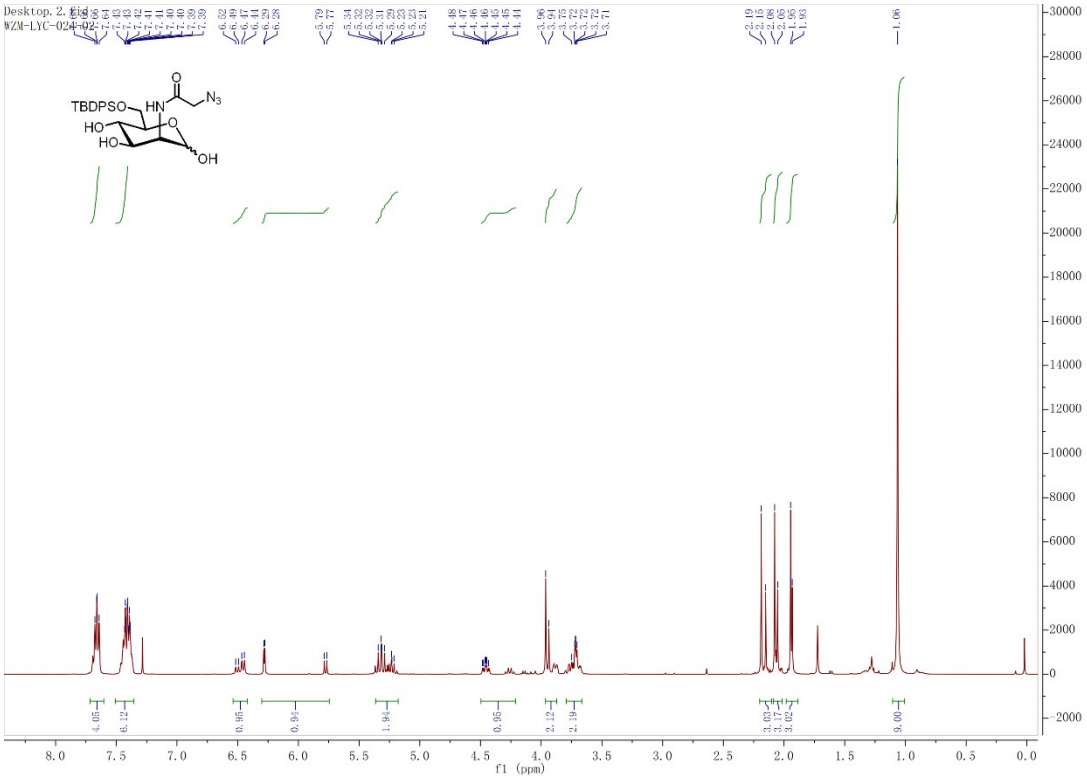
[2] P. V. Chang, D. H. Dube, E. M. Sletten and C. R. Bertozzi, *Journal of the American Chemical Society* **2010**, *132*, 9516-9518.

[3] P. R. Adiyala, V. Tekumalla, I. B. Sayeed, V. L. Nayak, A. Nagarajan, M. A. Shareef, B. Nagaraju and A. Kamal, *Bioorg Chem* **2018**, *76*, 288-293.

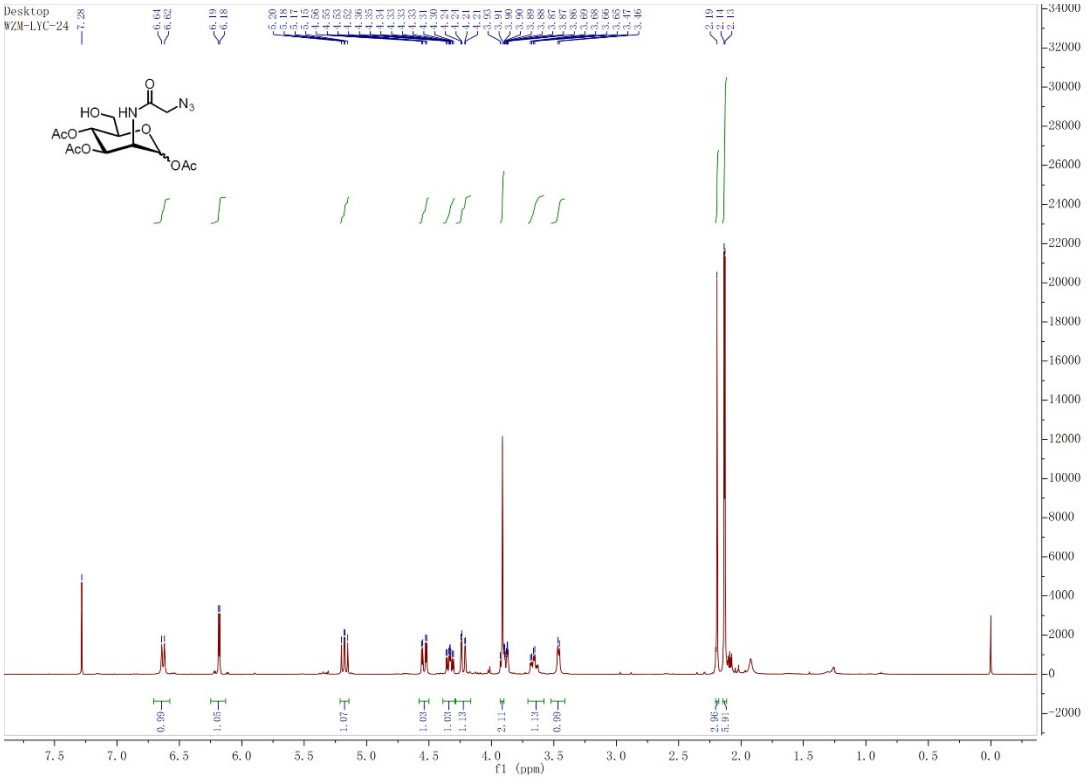
[4] H. Lin, H. Hong, J. Wang, C. Li, Z. Zhou and Z. Wu, *Chem Commun (Camb)* **2020**, *56*, 13959-13962.

[5] Z. F. Zhou, W. Z. Ding, C. Li and Z. M. Wu, *Journal of Carbohydrate Chemistry* **2017**, *36*, 205-219.

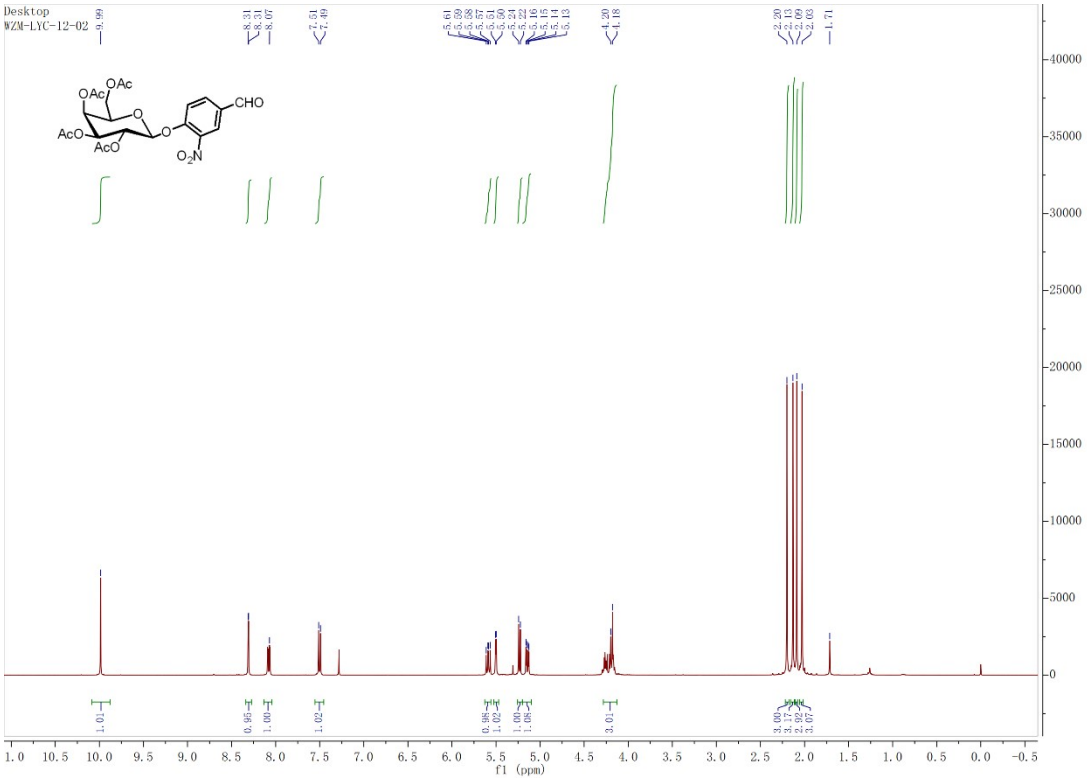
IX: NMR spectra of Synthesized Compounds



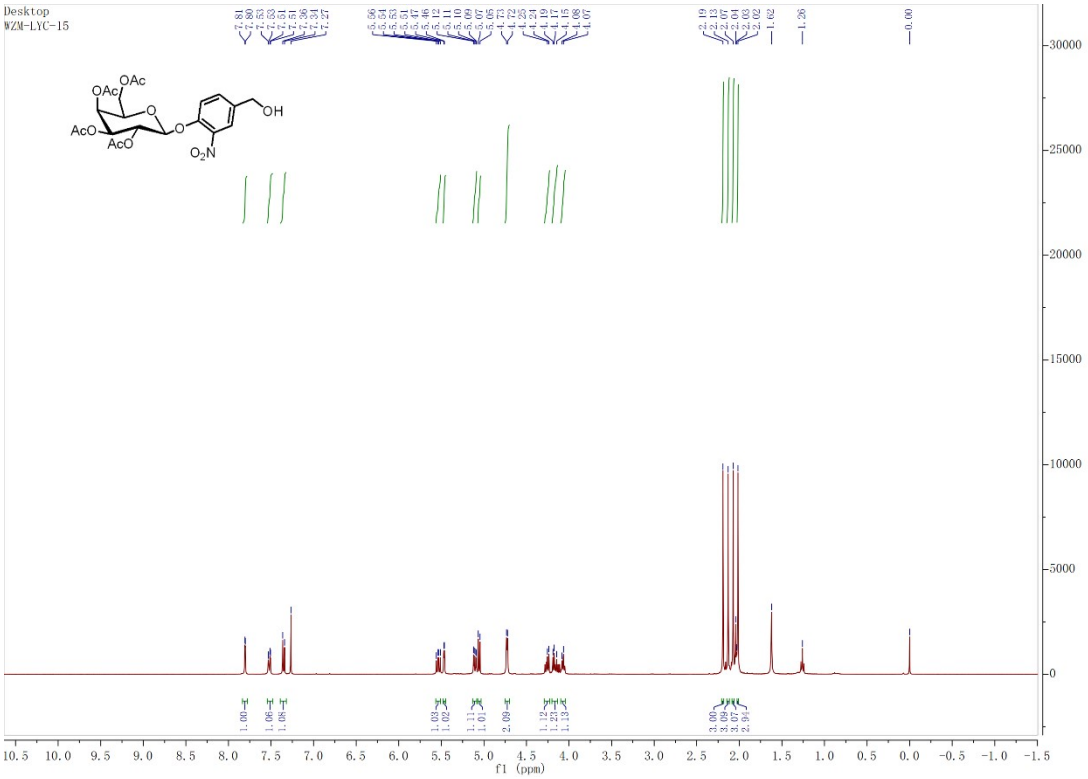
^{1}H NMR spectrum of compound **S5** in $CDCl_3$



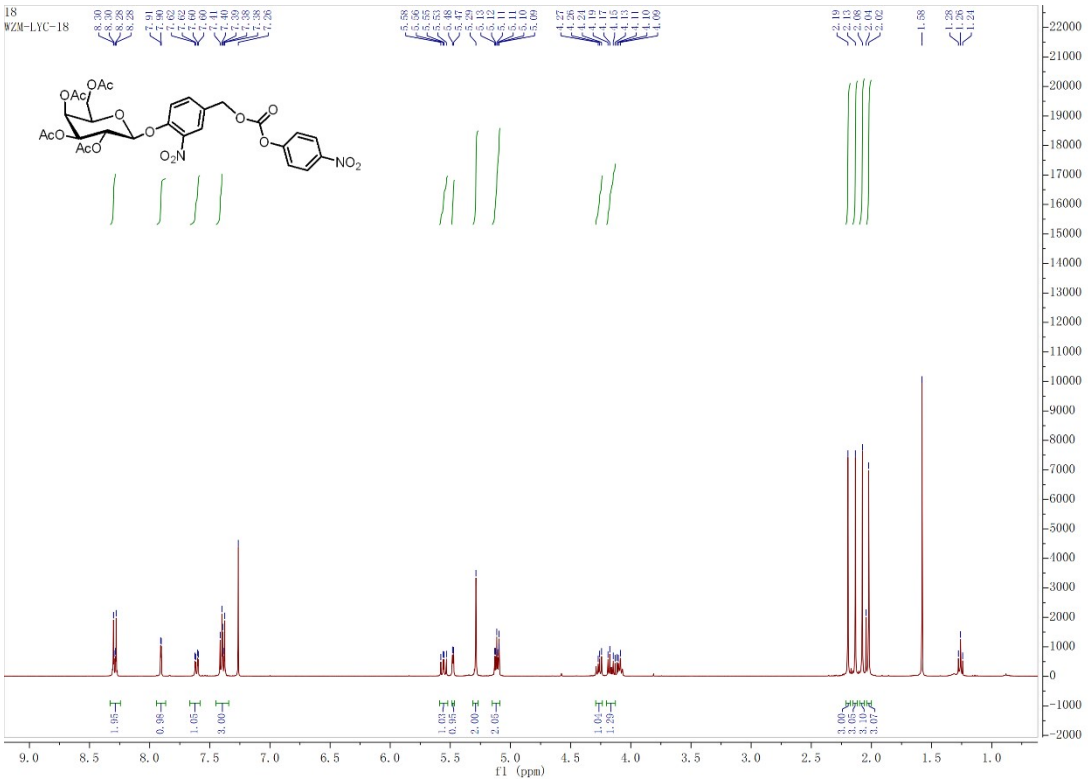
^{1}H NMR spectrum of compound **Ac_3ManNAz** in $CDCl_3$



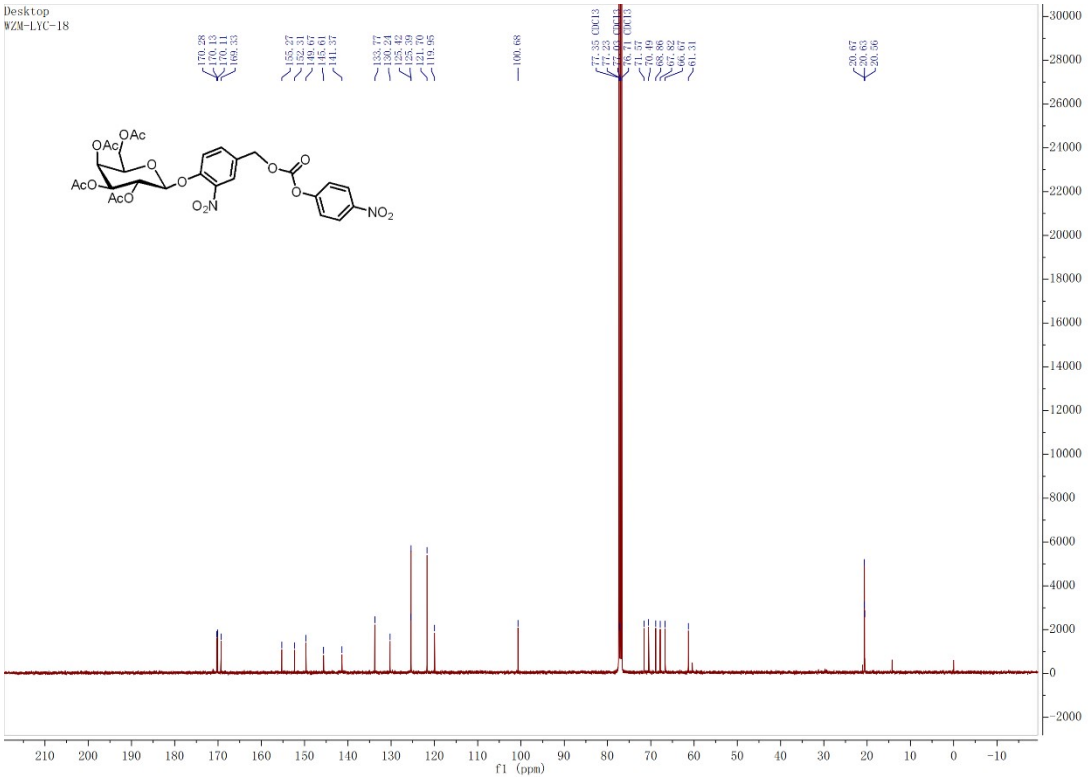
^{1}H NMR spectrum of compound **4** in $CDCl_3$



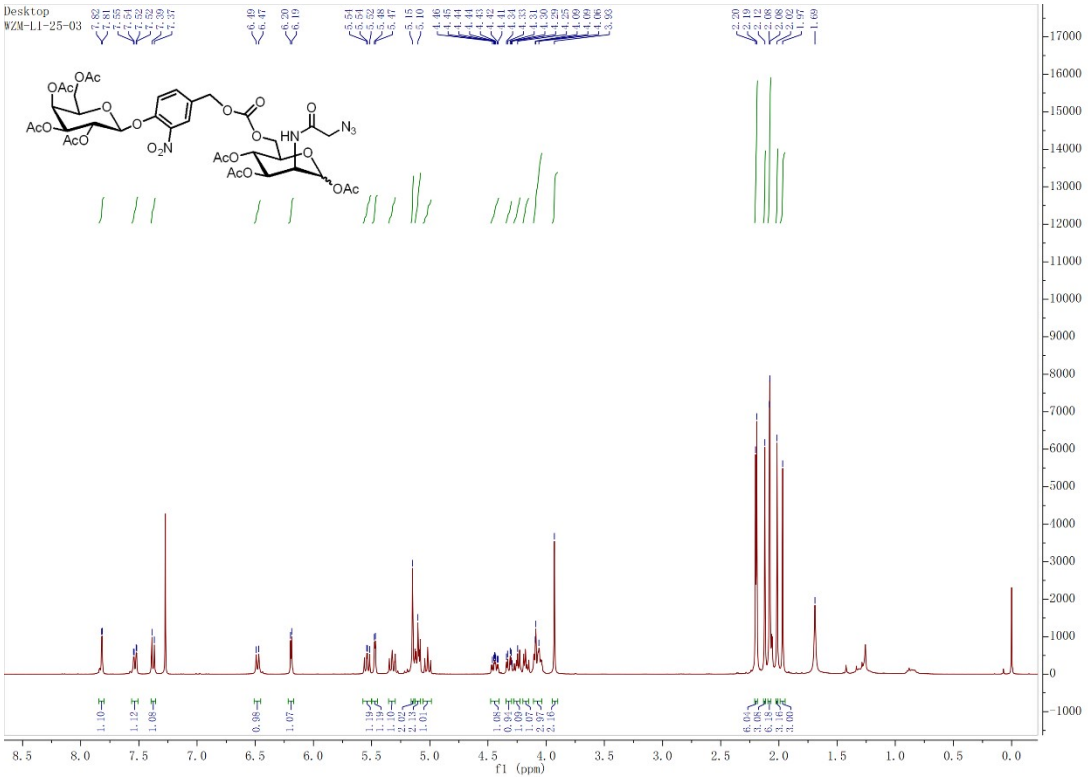
^{1}H NMR spectrum of compound **5** in $CDCl_3$



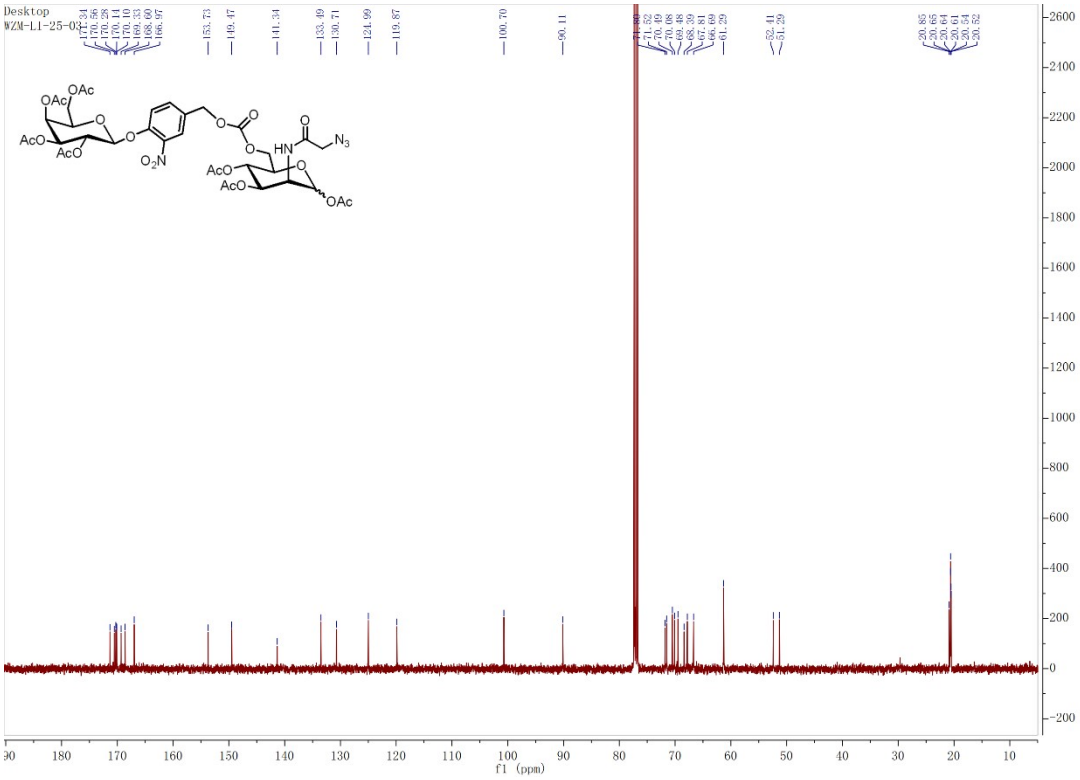
^{1}H NMR spectrum of compound **6** in $CDCl_3$



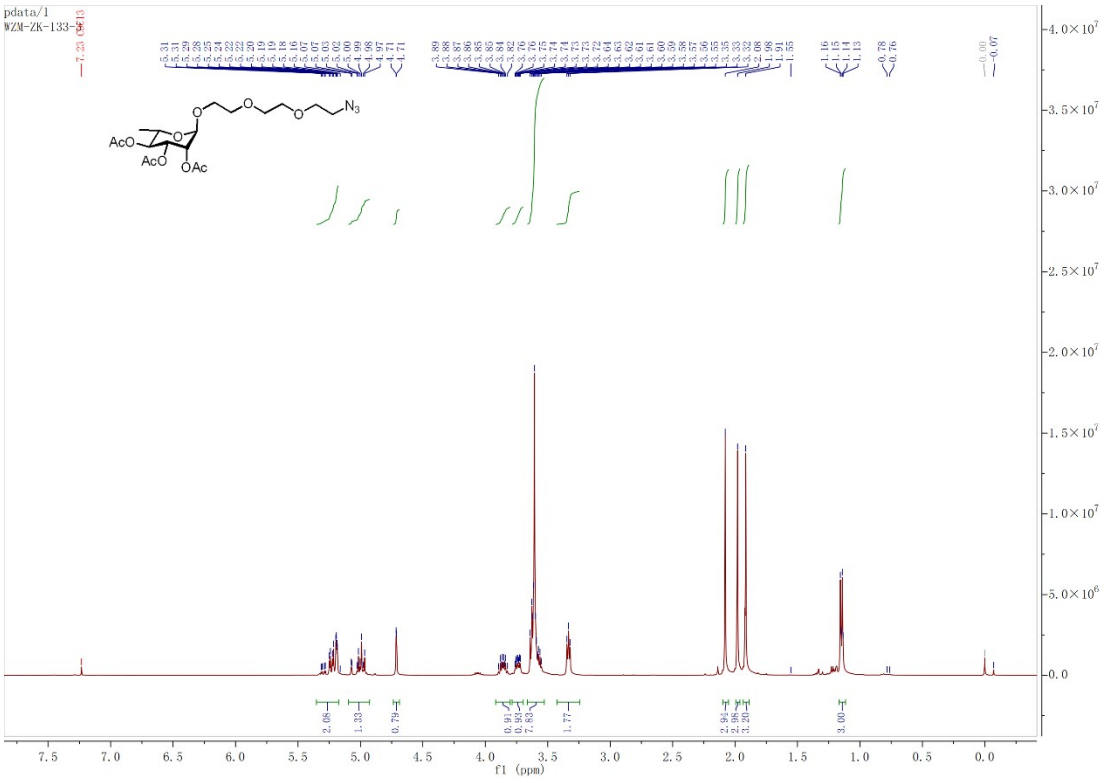
^{13}C NMR spectrum of compound **6** in $CDCl_3$



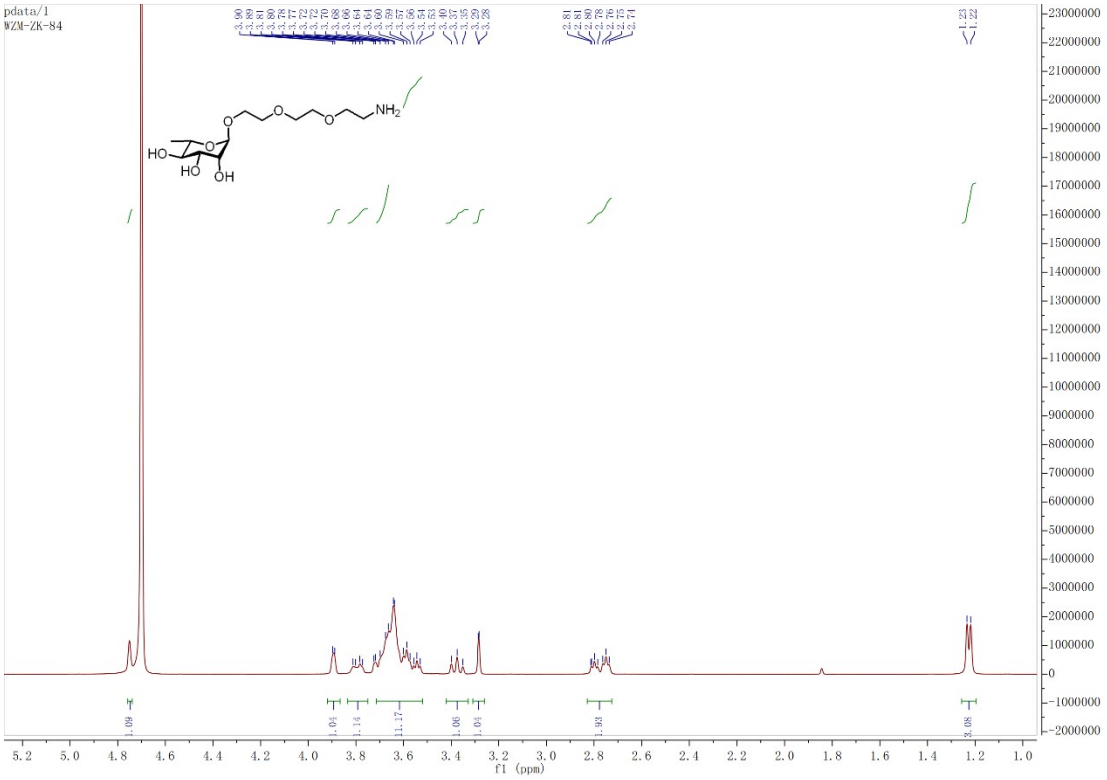
^{1}H NMR spectrum of compound **Gal-AAM** in $CDCl_3$



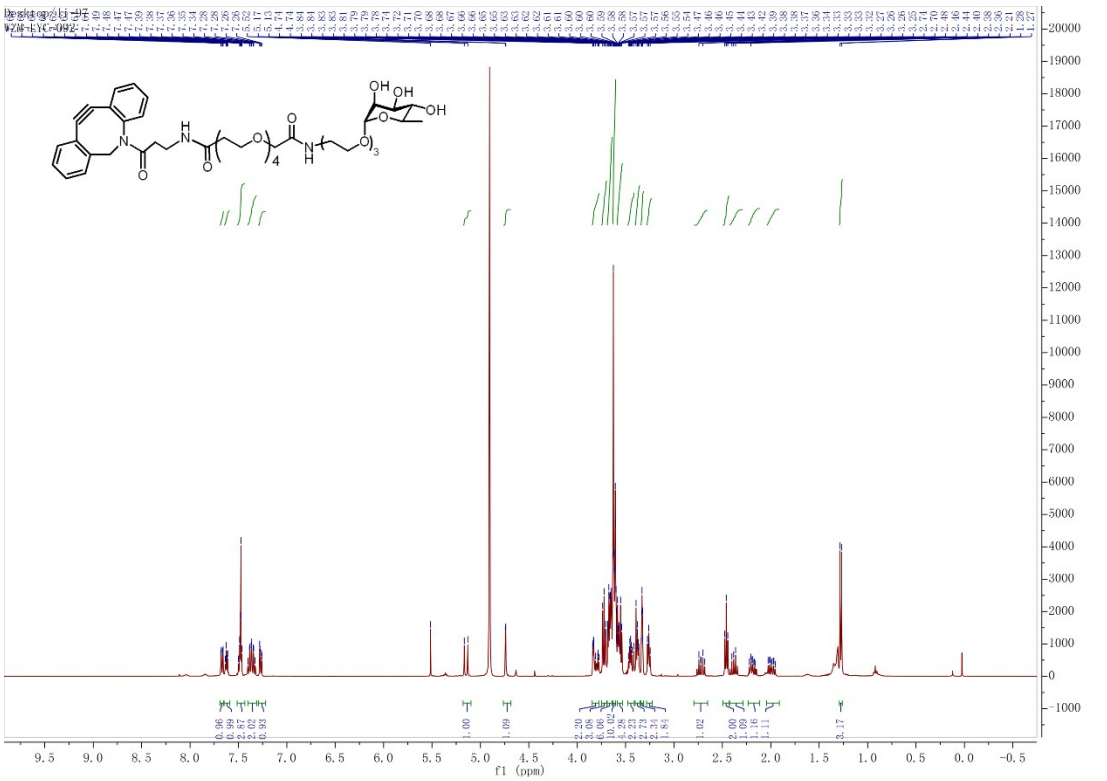
^{13}C NMR spectrum of compound **Gal-AAM** in $CDCl_3$



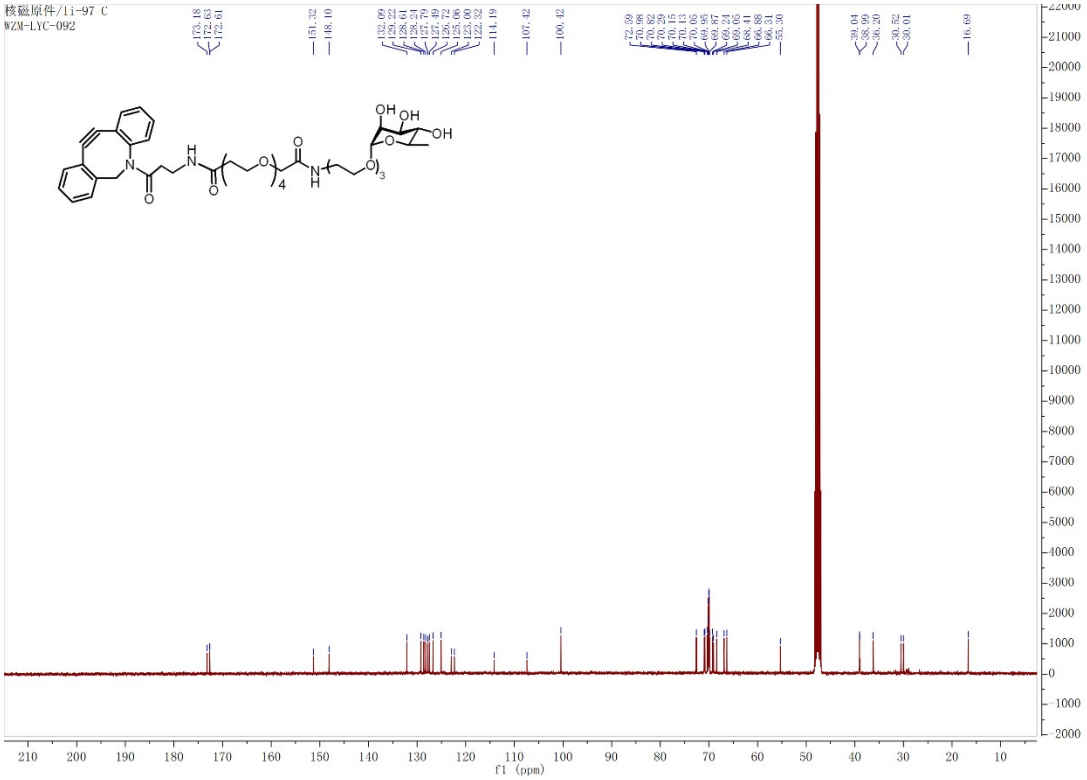
^{1}H NMR spectrum of compound **9** in $CDCl_3$



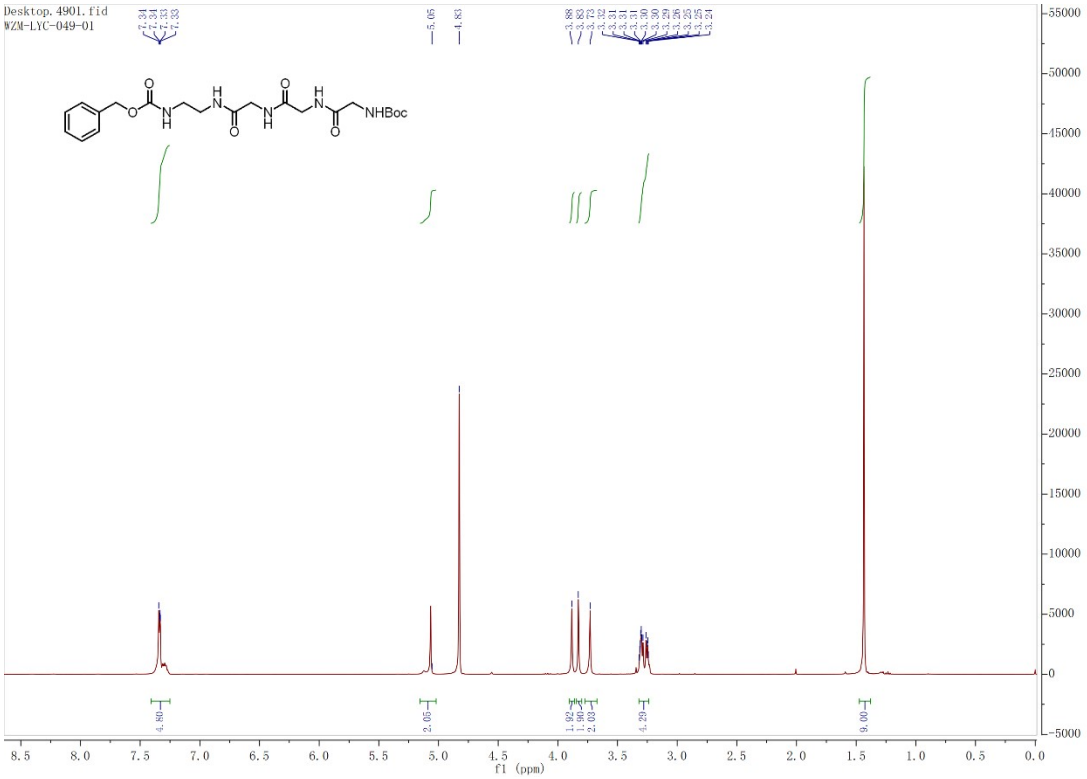
^{1}H NMR spectrum of compound **10** in MeOD



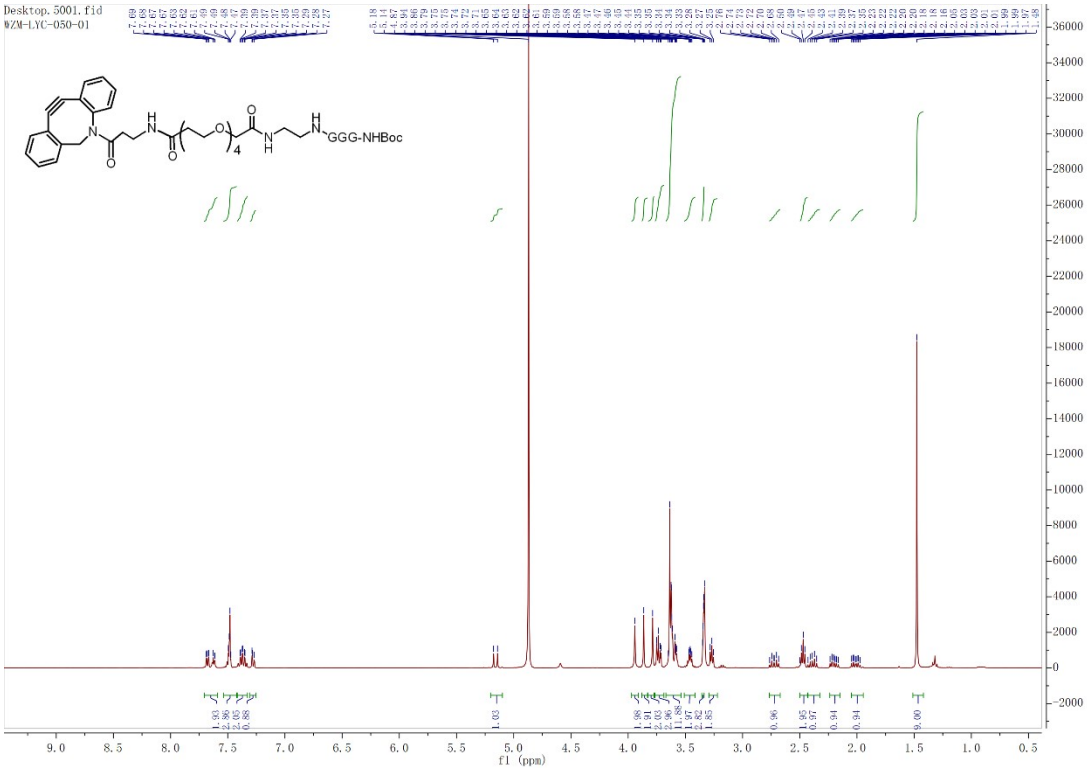
^{1}H NMR spectrum of compound **DBCO-Rha** in MeOD



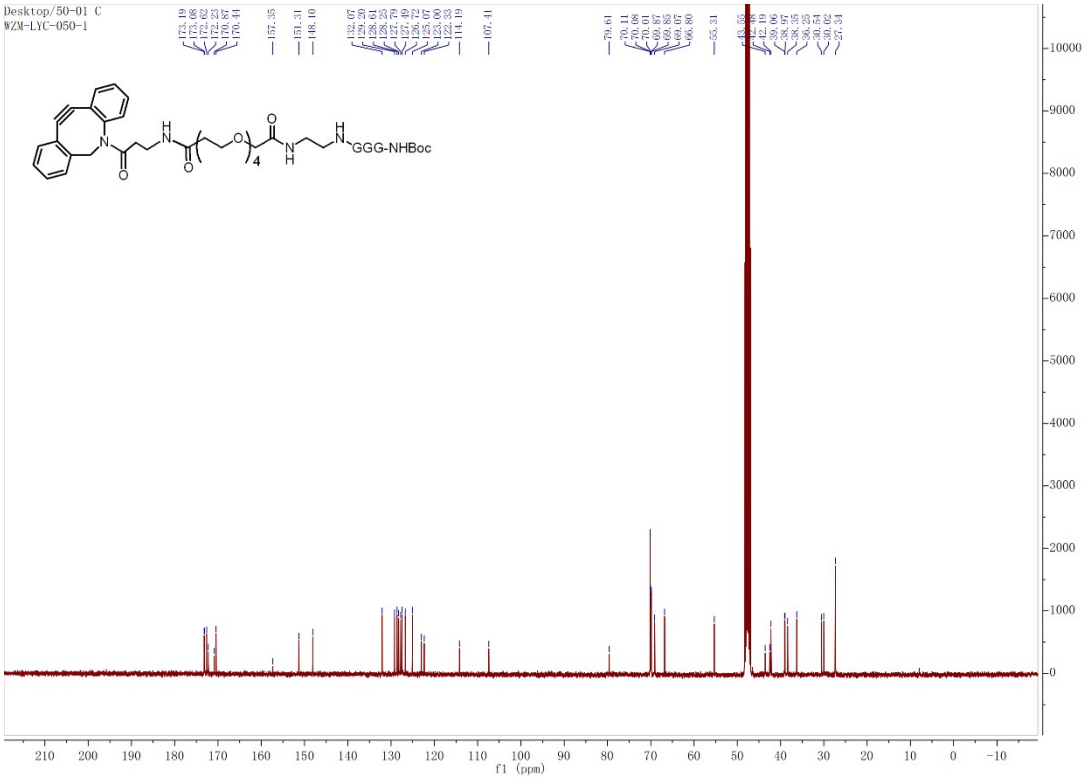
^{13}C NMR spectrum of compound **DBCO-Rha** in MeOD



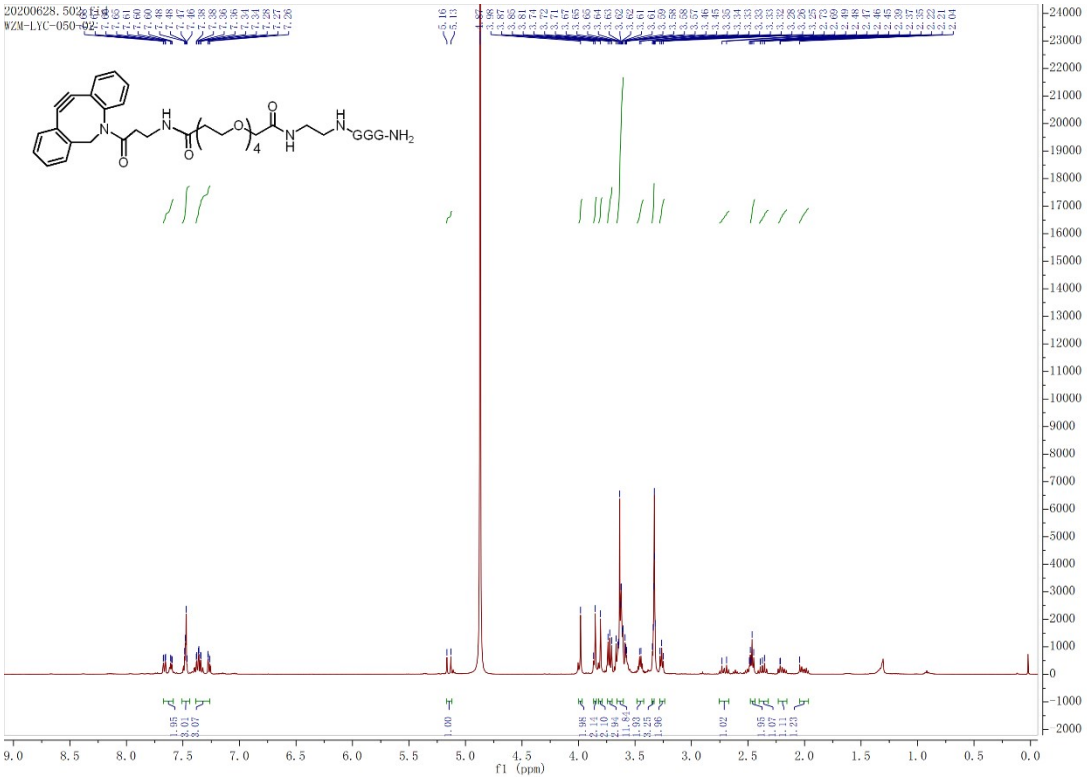
^{1}H NMR spectrum of compound **14** in MeOD



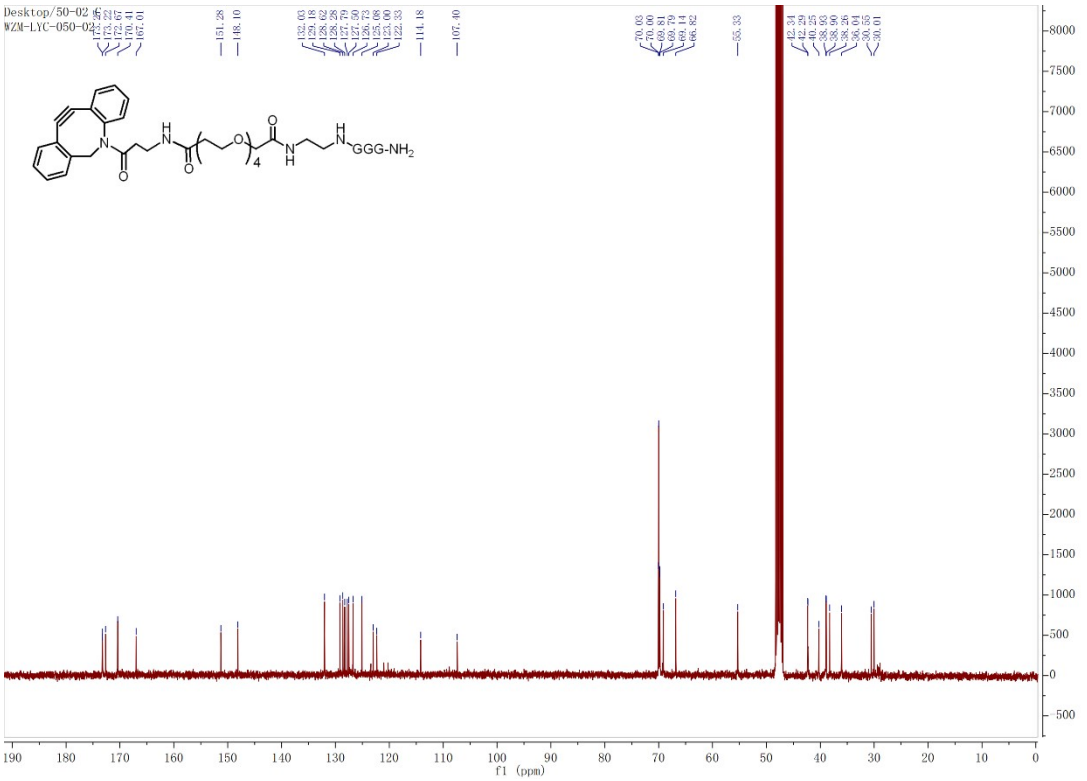
^{1}H NMR spectrum of compound **16** in MeOD



^{13}C NMR spectrum of compound **16** in MeOD

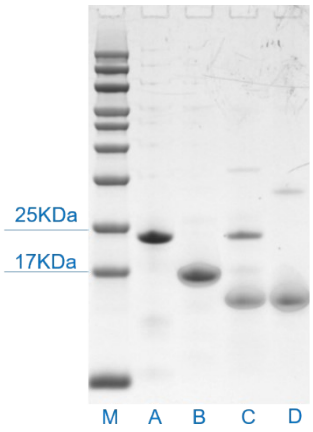


^{1}H NMR spectrum of compound **17** in MeOD



^{13}C NMR spectrum of compound **17** in MeOD

SDS-PAGE analysis of DBCO-ZZ



M: Pre-stained marker
A: SrtA
B: ZZ domain
C: 5h enzyme reaction product
D:purified product

SDS-PAGE analysis of purified proteins: lanes A–D, SrtA, ZZ domain, 5 h enzyme reaction product and purified DBCO-ZZ, respectively; lane M, marker