

Supporting Information

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SPECTRUM - MS, Her-Rha4.raw, FTMS + p ESI sid=15.00 Full ms [1500.0000-5000.0000], Scan #: 75-137, RT: 2.75-5.02, AV: 63,
NL: 9.44e+005 SIN: 1655

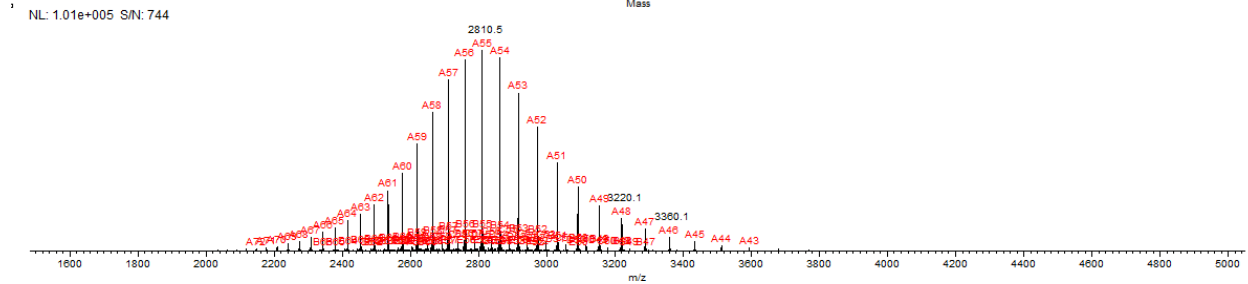
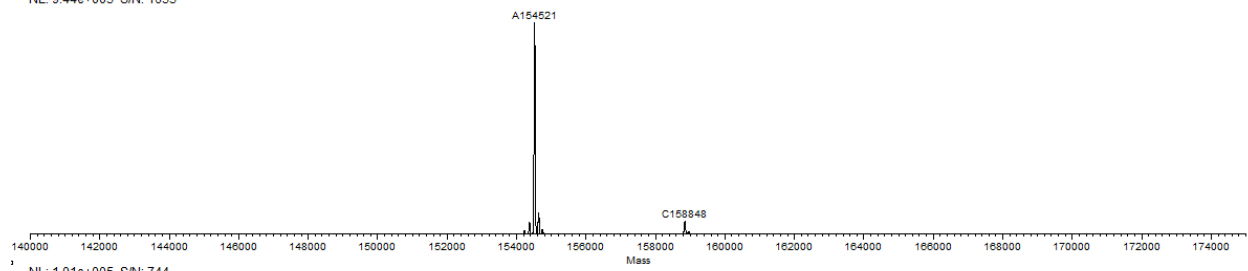


Figure S1. LC-ESI-MS analysis of the intact rhamnose functionalized antibody (**21**)

SPECTRUM - MS, Her-Rha4+IdeS.raw, FTMS + p ESI sid=15.00 Full ms [400.0000-3000.0000], Scan #: 65-92, RT: 2.52-3.02, AV: 28,
NL: 1.06e+006 S/N: 593

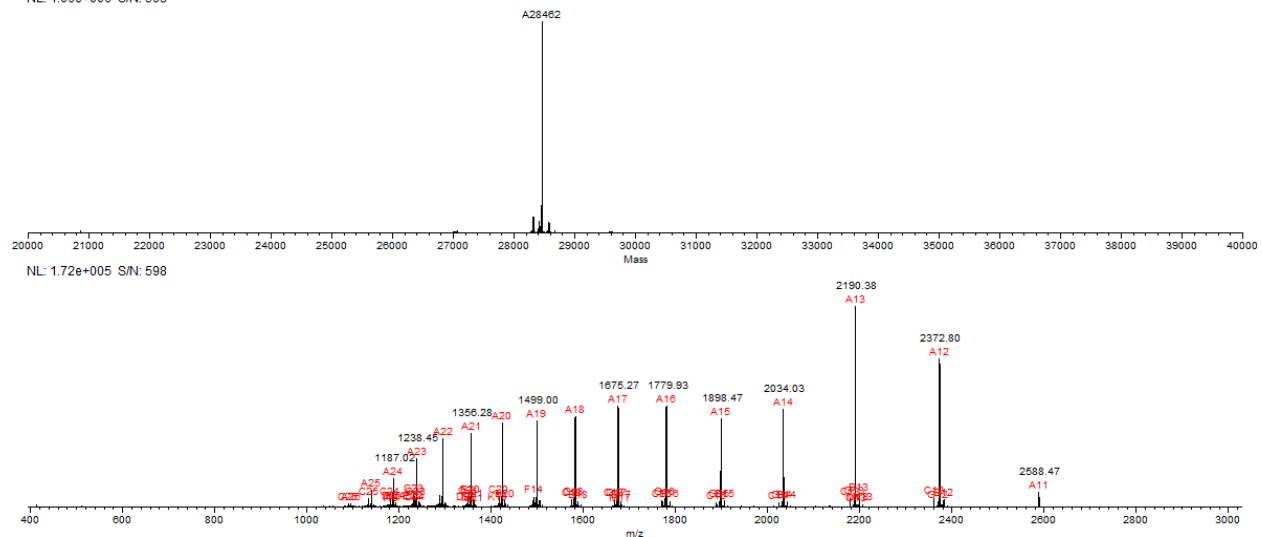


Figure S2. LC-ESI-MS analysis of the Fc domains released by IdeS treatment of the rhamnose functionalized antibody (**21**)

SPECTRUM - MS, Di-N3-SCT-Her+Rha4-DBCO-16h2.raw, FTMS + p ESI sid=40.00 Full ms [1500.0000-5000.0000], Scan #: 110-131, NL: 2.36e+006 S/N: 84

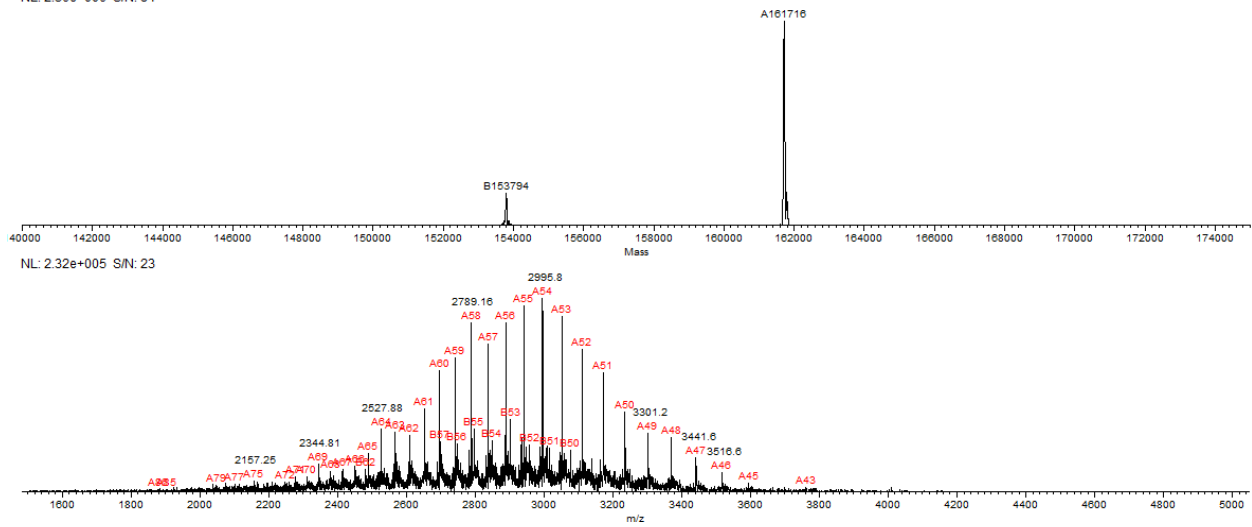


Figure S3. LC-ESI-MS analysis of the intact rhamnose cluster functionalized antibody (**22**)

SPECTRUM - MS, Rha16-Her+Ide-S.raw, FTMS + p ESI sid=40.00 Full ms [400.0000-3000.0000], Scan #: 60-106, RT: 2.28-2.87,
NL: 5.44e+006 S/N: 943

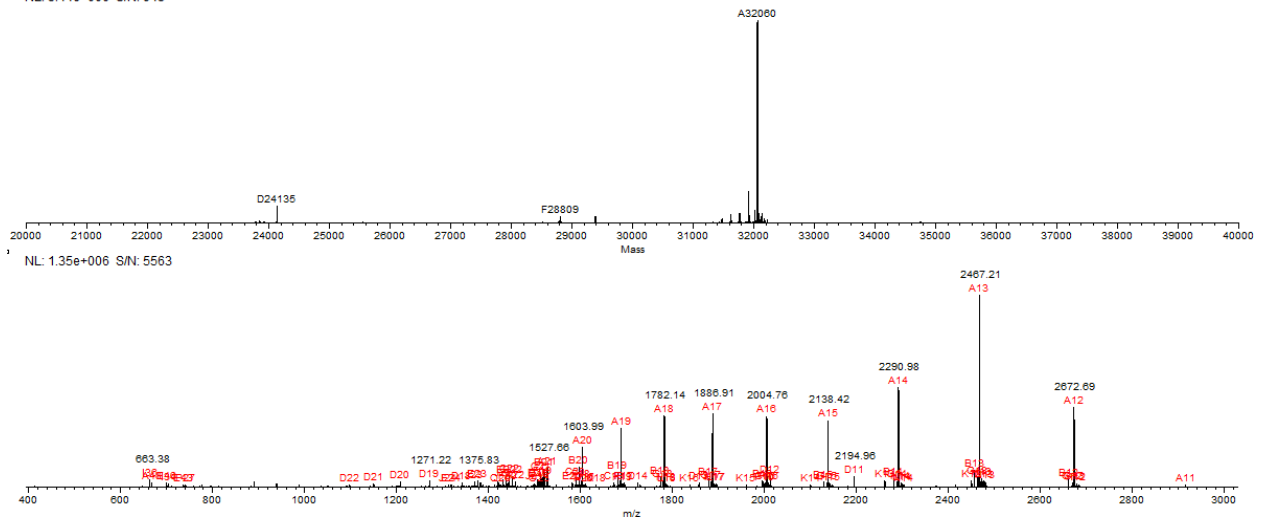


Figure S4. LC-ESI-MS analysis of the Fc domains released by IdeS treatment of the rhamnose cluster functionalized antibody (**22**)

SPECTRUM - MS, Her-a-Gal4.raw, FTMS + p ESI sid=15.00 Full ms [1500.0000-5000.0000], Scan #: 79-123, RT: 2.90-4.51, AV: 45,
NL: 8.45e+005 S/N: 1291

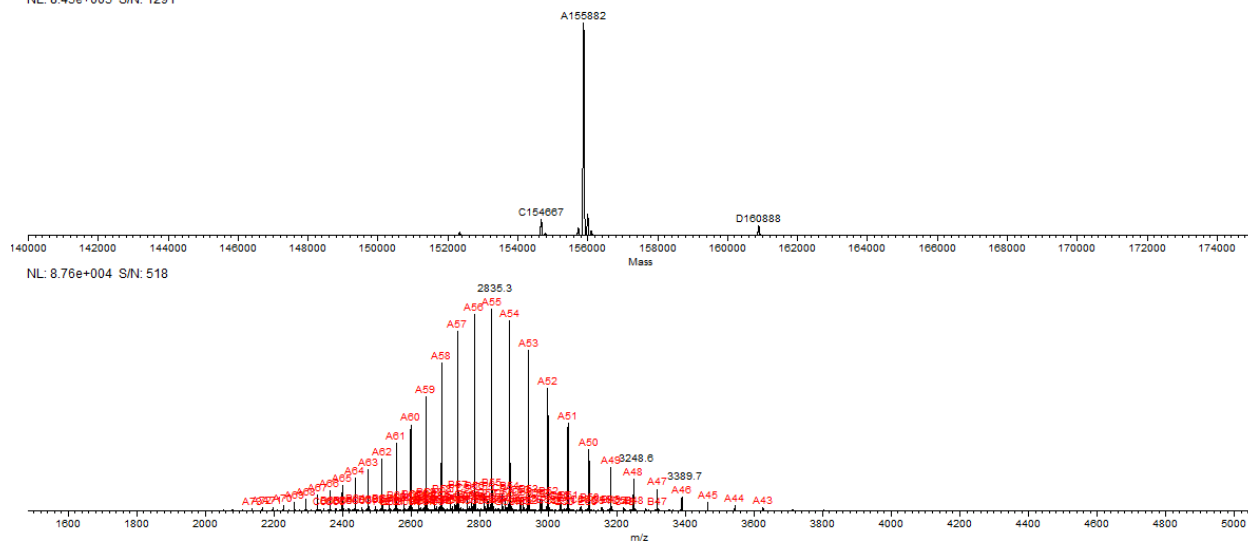


Figure S5. LC-ESI-MS analysis of the intact α -Gal functionalized antibody (**23**)

SPECTRUM - MS, Her-a-Gal4+IdeS.raw, FTMS + p ESI sid=15.00 Full ms [400.0000-3000.0000], Scan #: 60-89, RT: 2.33-2.87, NL: 1.15e+006 S/N: 801

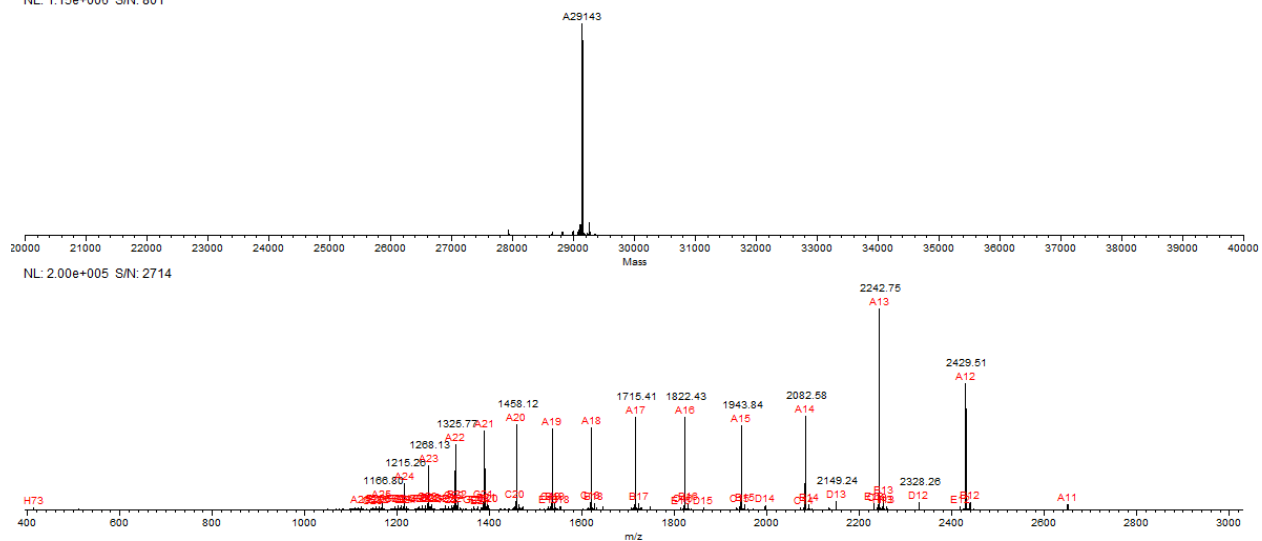


Figure S6. LC-ESI-MS analysis of the Fc domains released by IdeS treatment of the α -Gal functionalized antibody (**23**)

SPECTRUM - MS, DI-N3-SCT-Her+a-Gal4-Lys3-DBCO-44h.raw, FTMS + p ESI sid=40.00 Full ms [1500.0000-5000.0000], Scan #: 81-123,
NL: 6.24e+005 S/N: 594

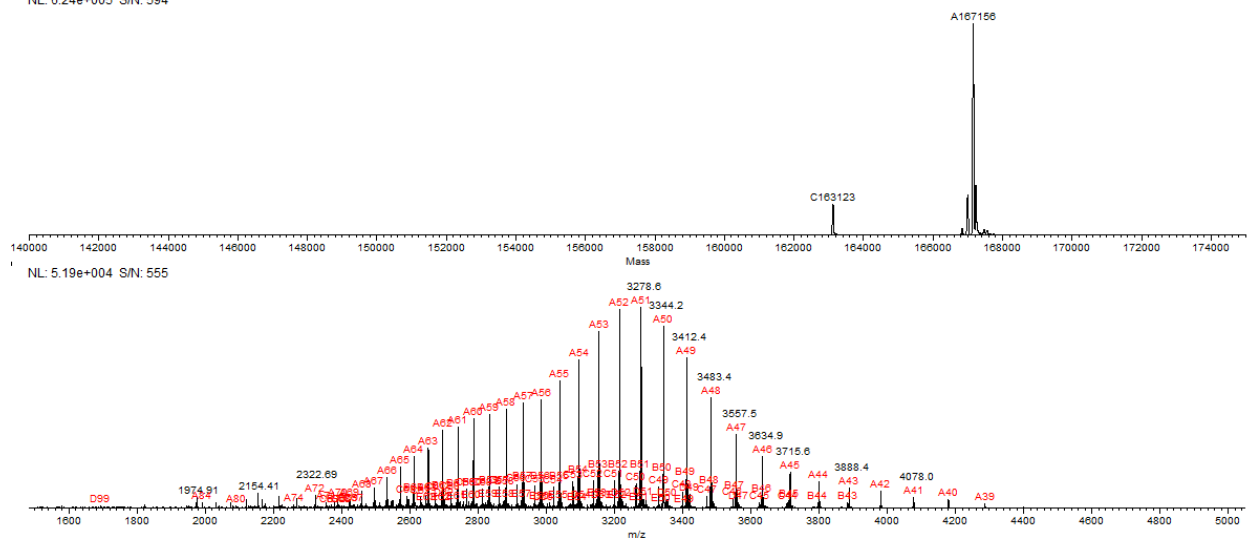


Figure S7. LC-ESI-MS analysis of the intact α -Gal cluster functionalized antibody (**24**)

SPECTRUM - MS, oc-a-Gal16-Her+Ide-S.raw, FTMS + p ESI sid=40.00 Full ms [400.0000-3000.0000], Scan #: 57-93, RT: 2.19-2.66,
NL: 5.04e+006 SIN: 507

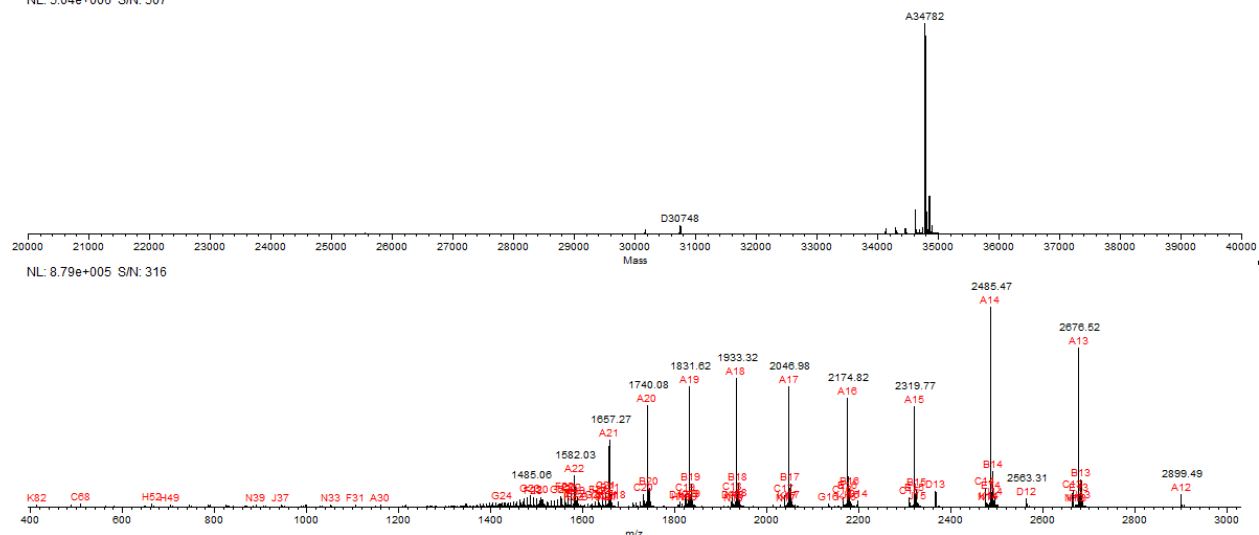


Figure S8. LC-ESI-MS analysis of the Fc domains released by IdeS treatment of the α -Gal functionalized antibody (**24**)

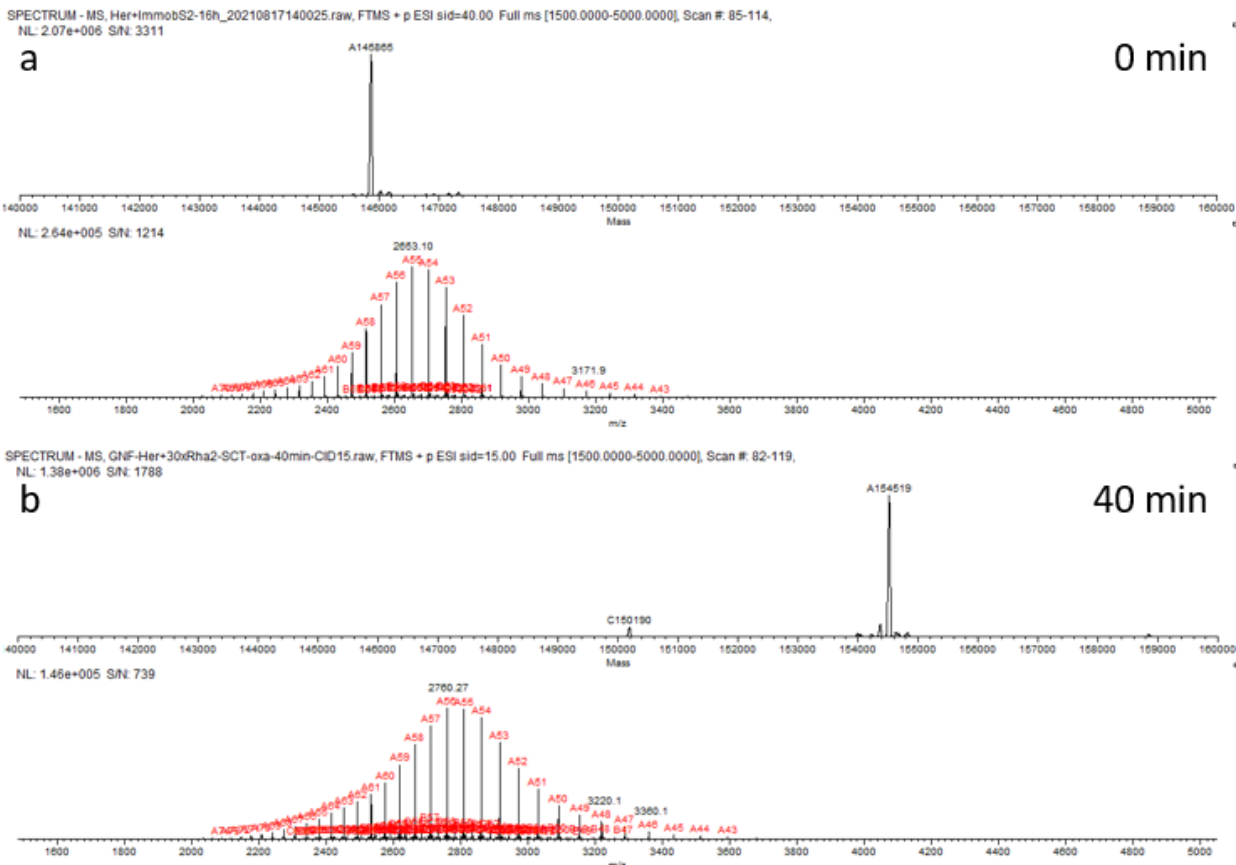


Figure S9. The LC-ESI-MS monitoring of the glycosylation reactions between azide-glycan oxazoline **27a** and antibody **28** catalyzed by Endo-S2 D184M. A mixture of the deglycosylated antibody **28** (0.5 mg, 3.3 nmol, 25 mg/mL), glycan oxazoline **27a** (0.44 mg, 100 nmol, 30 *mol. equiv.* of the antibody), and the mutant enzyme (0.1 mg/mL) in a Tris buffer (100 mM, pH 7.2) was incubated at 30 °C and the reaction was monitored by LC-ESI-MS analysis of the intact antibodies at 20 min intervals. a) the deconvoluted mass of **28**; b) the deconvoluted mass of the reaction mixture catalyzed by Endo-S2 D184M at 40 min.

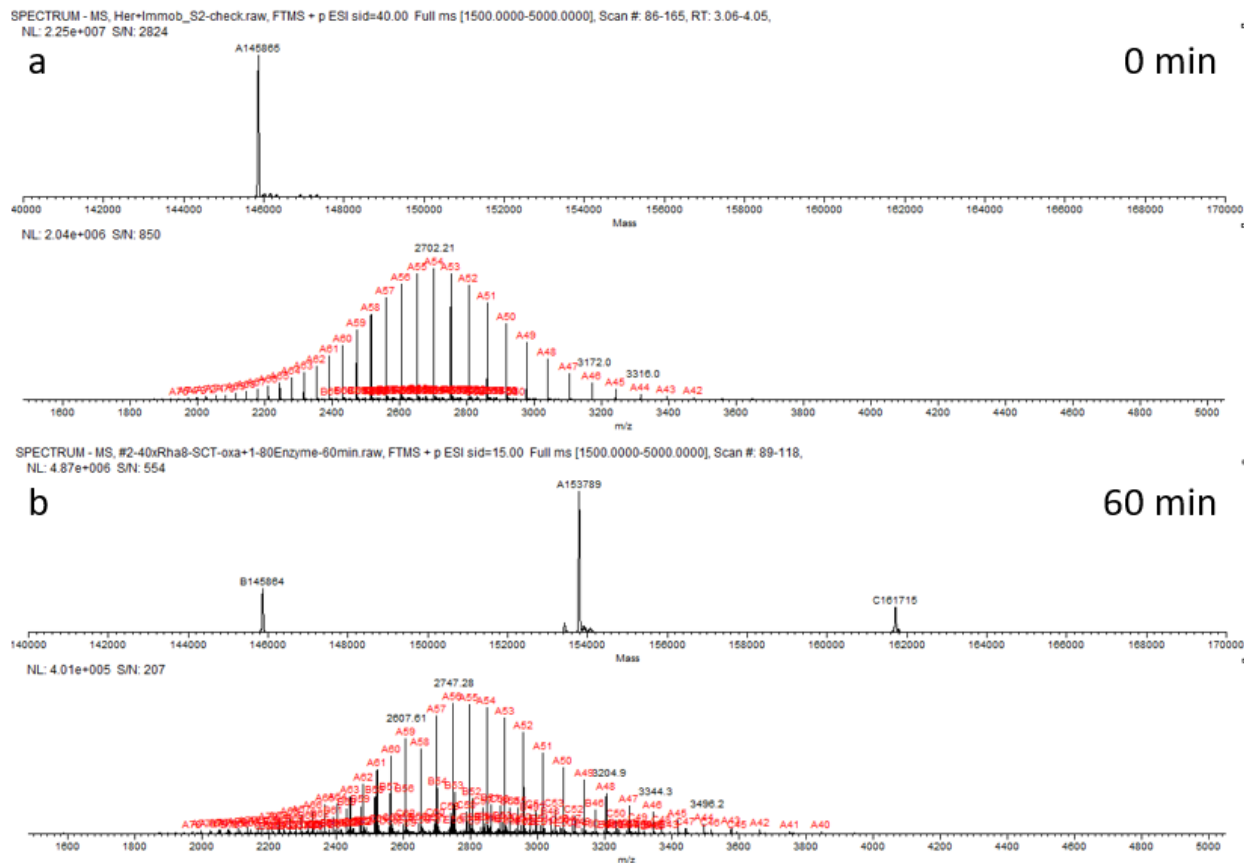


Figure S10. The LC-ESI-MS monitoring of the glycosylation reactions between azide-glycan oxazoline **27b** and antibody **28** catalyzed by Endo-S2 D184M. A mixture of the deglycosylated antibody **28** (0.1 mg, 3.3 nmol, 25 mg/mL), glycan oxazoline **27b** (0.44 mg, 100 nmol, 40 *mol. equiv.* of the antibody), and the mutant enzyme (0.4 mg/mL) in a Tris buffer (100 mM, pH 7.2) was incubated at 30 °C and the reaction was monitored by LC-ESI-MS analysis of the intact antibodies at 20 min intervals. a) the deconvoluted mass of **28**; b) the deconvoluted mass of the reaction mixture catalyzed by Endo-S2 D184M at 60 min.

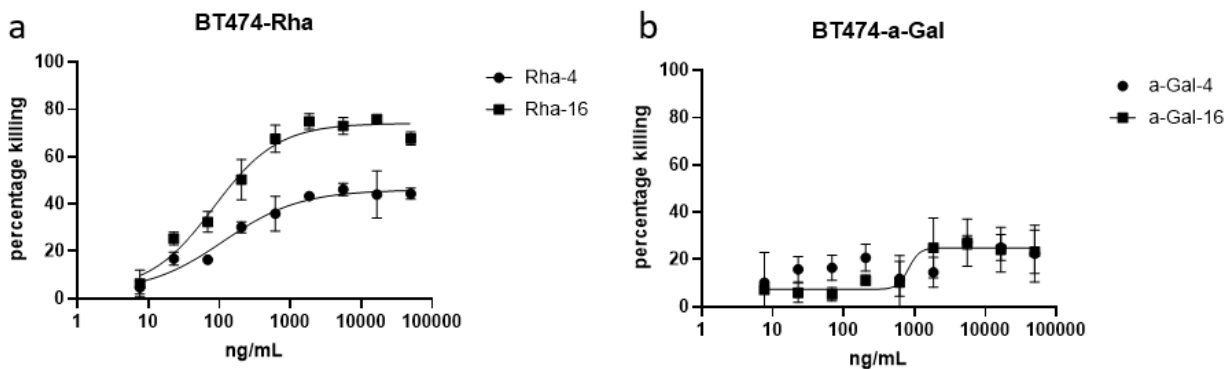


Figure S11. Cell killing assays for breast cancer cell lines a) Rhamnose conjugates with BT-474 (HER2 overexpression); b) α -Gal conjugates with BT-474 (HER2 overexpression). The human serum was purchased from Cosmo Bio USA. All assays were performed in triplicate.

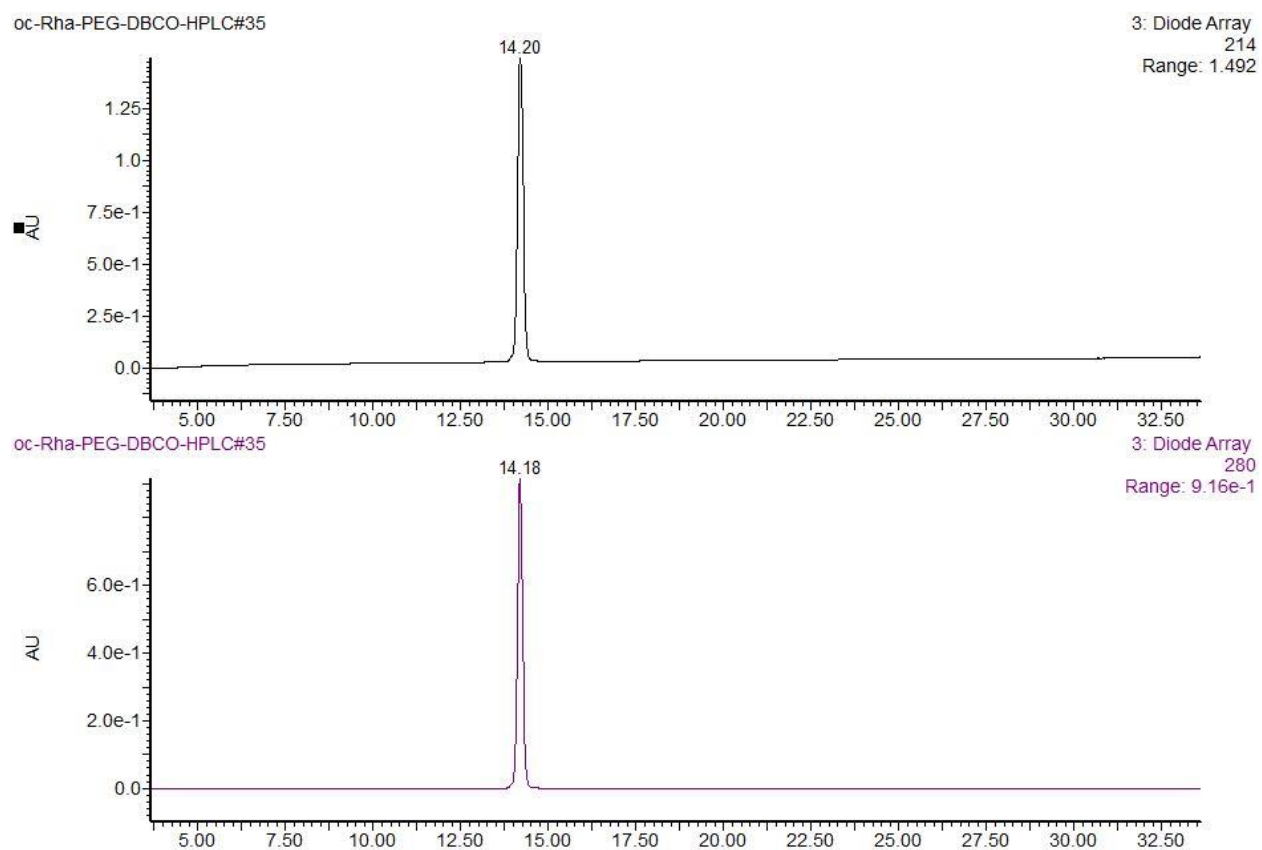
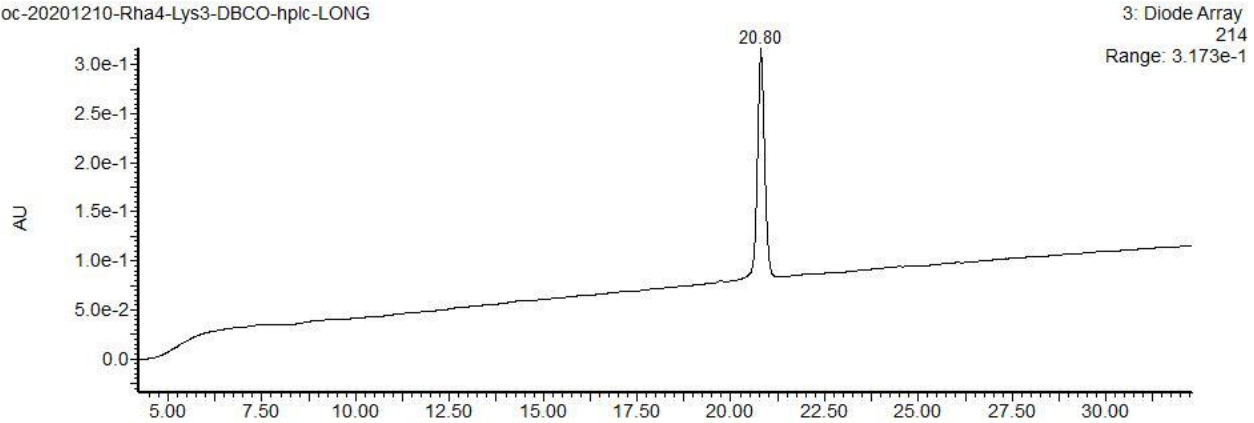


Figure S12. HPLC profile for **3**, 0.4 mL/min, 25-60%B, 30 min

oc-20201210-Rha4-Lys3-DBCO-hplc-LONG



oc-20201210-Rha4-Lys3-DBCO-hplc-LONG

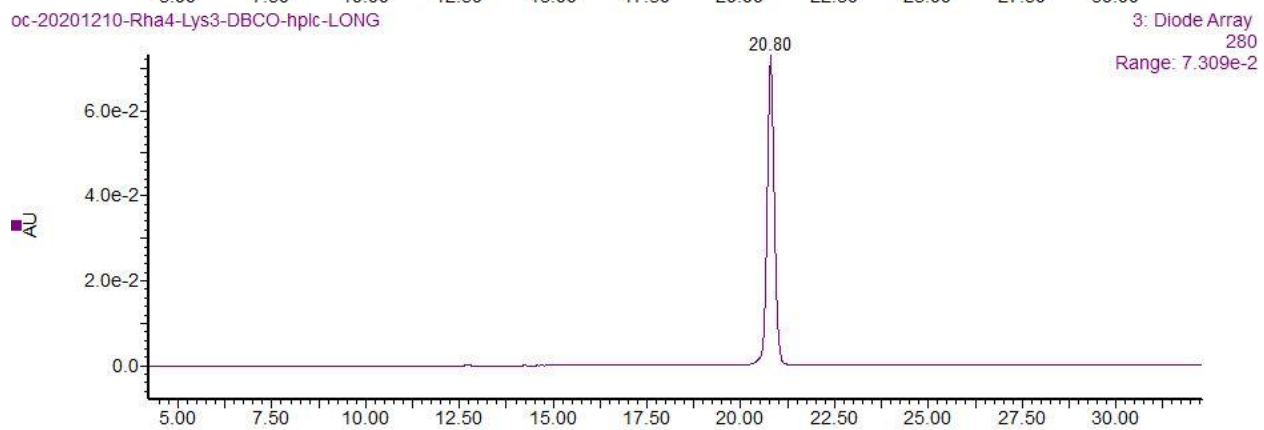


Figure S13. HPLC profile for **13**, 0.4 mL/min, 10-50%B, 30 min

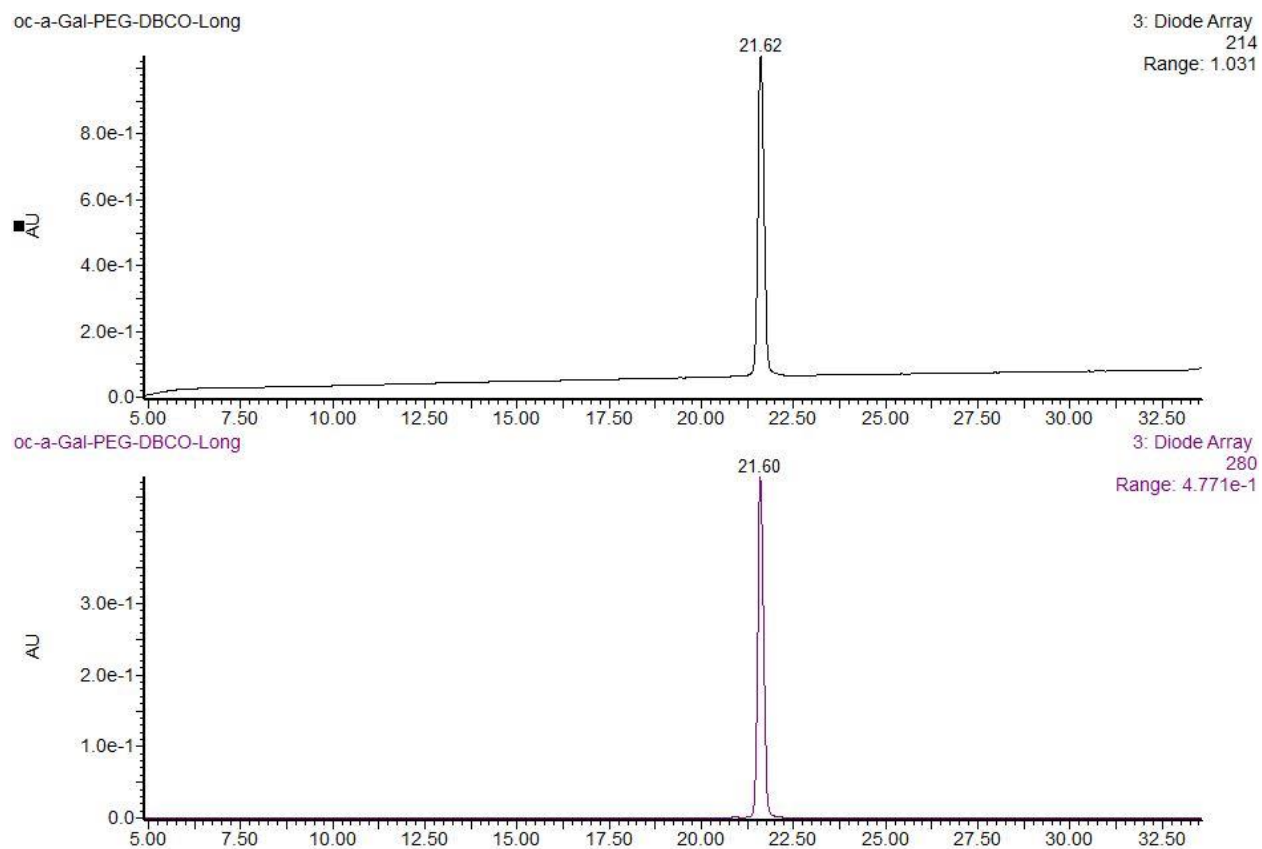


Figure S14. HPLC profile for **15**, 0.4 mL/min, 10-50%B, 30 min

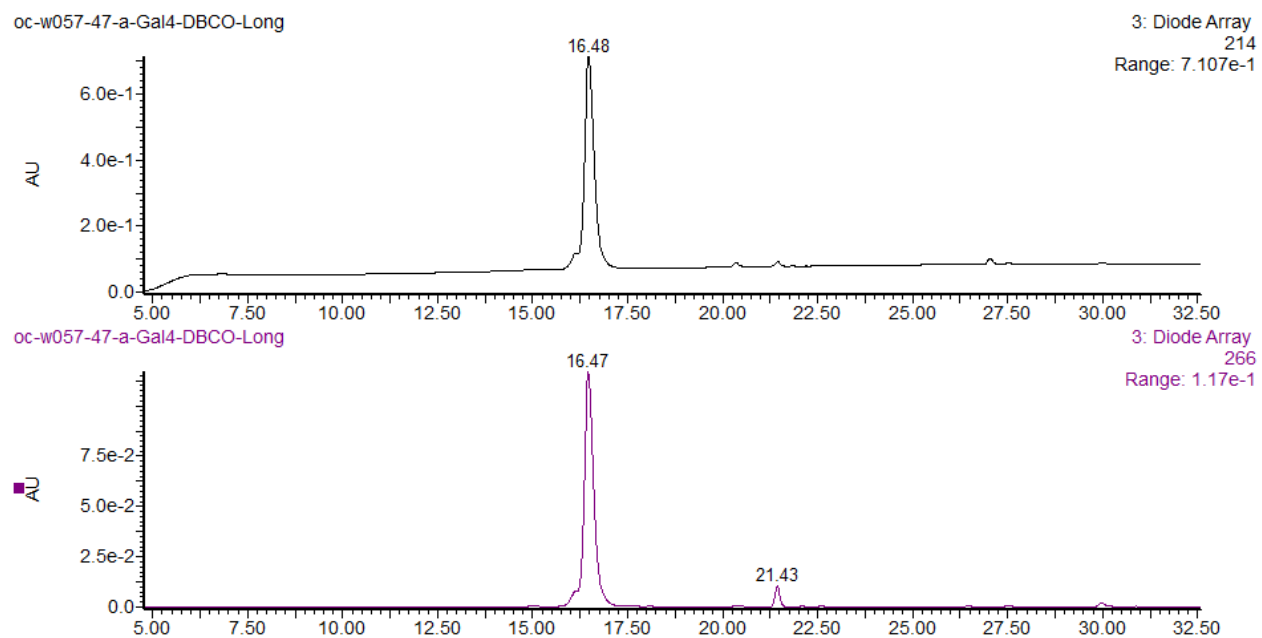
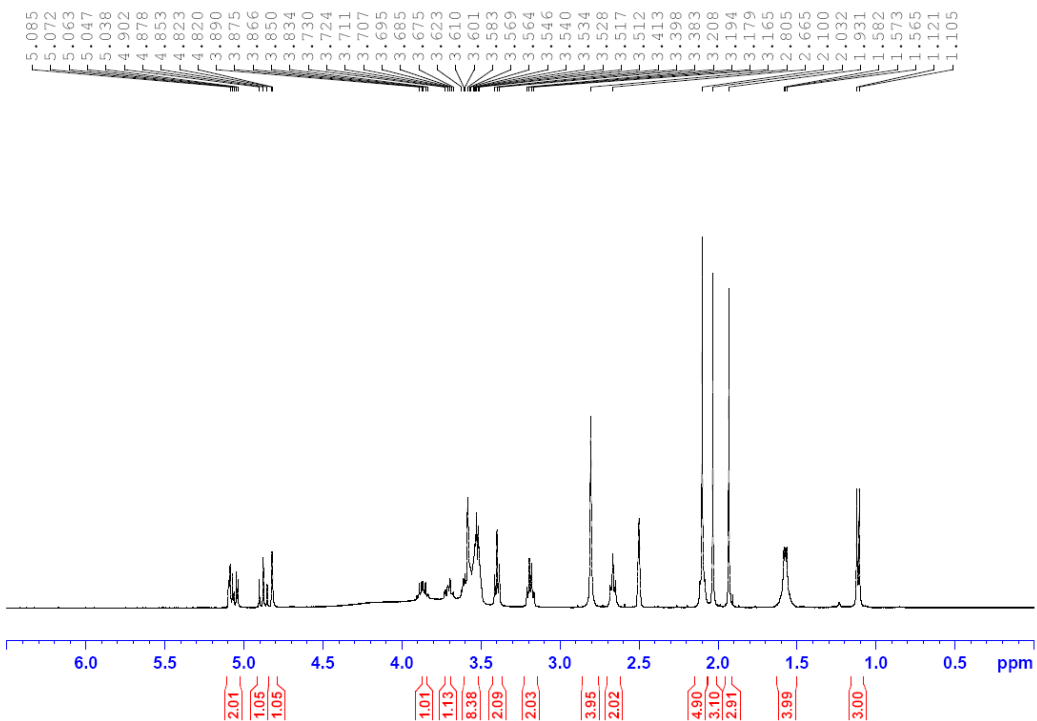


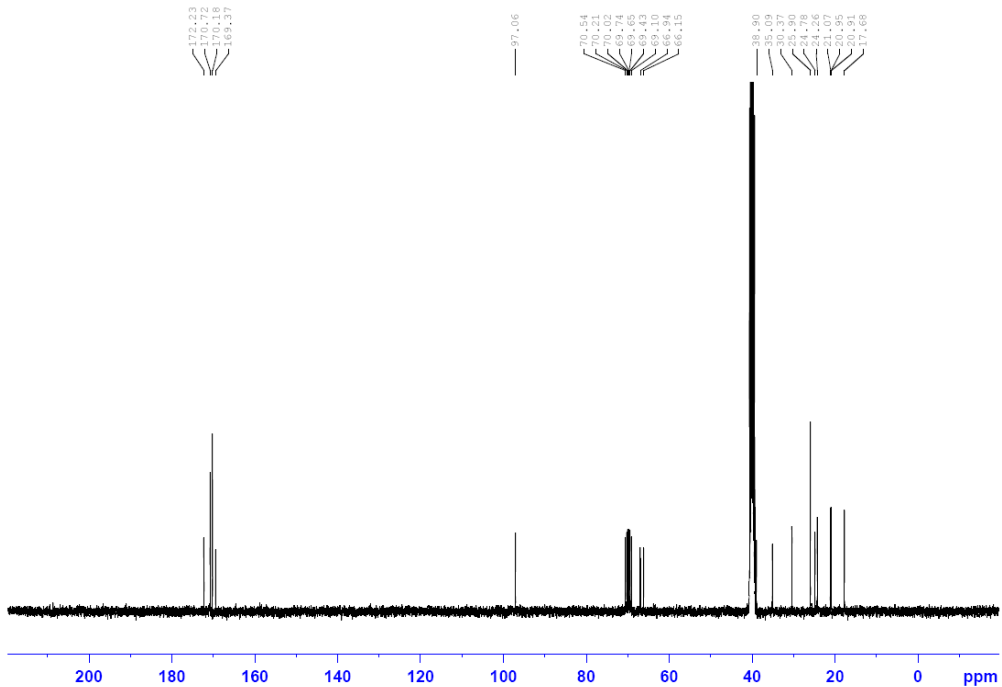
Figure S15. HPLC profile for **18**, 0.4 mL/min, 10-50%B, 30 min

Pre-OAc-Rha-C6-NHS
1H 400MHz DMSO-d6



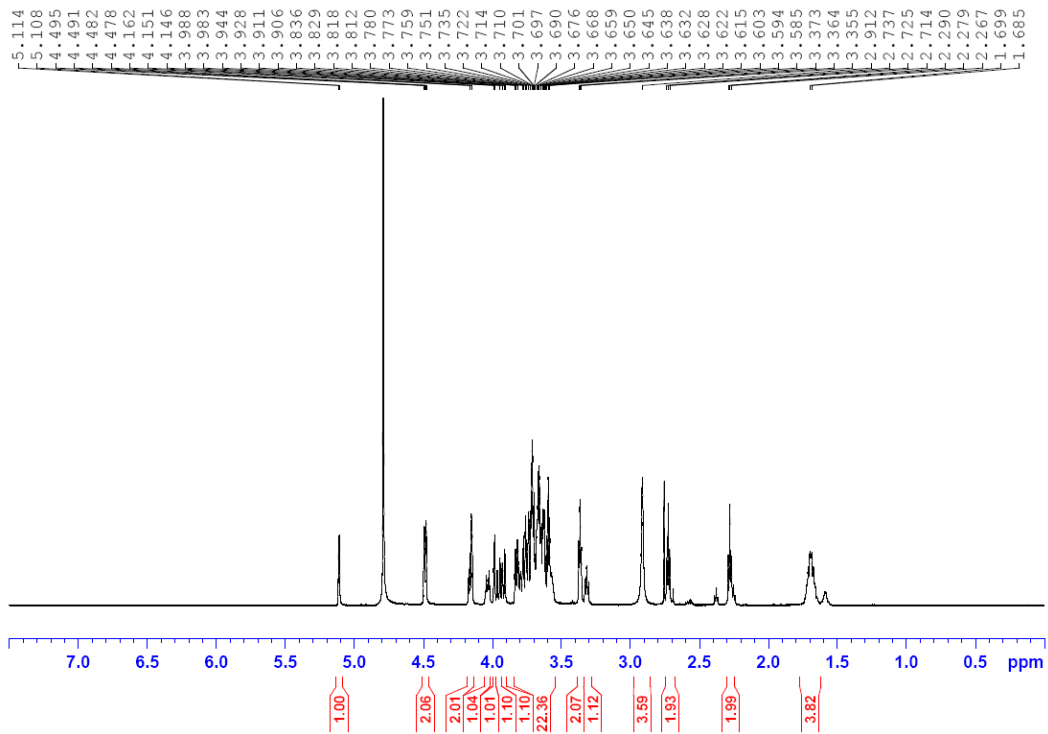
¹H NMR spectrum (400 MHz, DMSO-*d*₆): compound **10**

Per-OAc-Rha-C6-NHS
13C 100MHz DMSO-d6



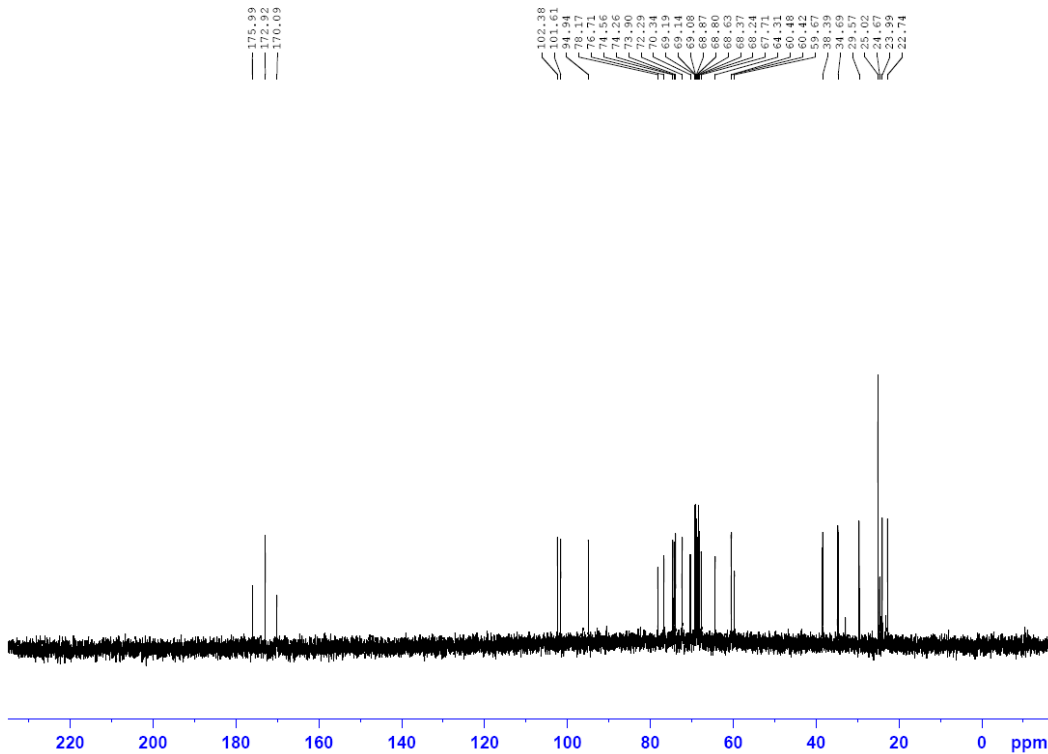
¹³C NMR spectrum (100 MHz, DMSO-*d*₆): compound **10**

oc-a-Gal-PEG-C6-NHS
1H 600MHz D2O

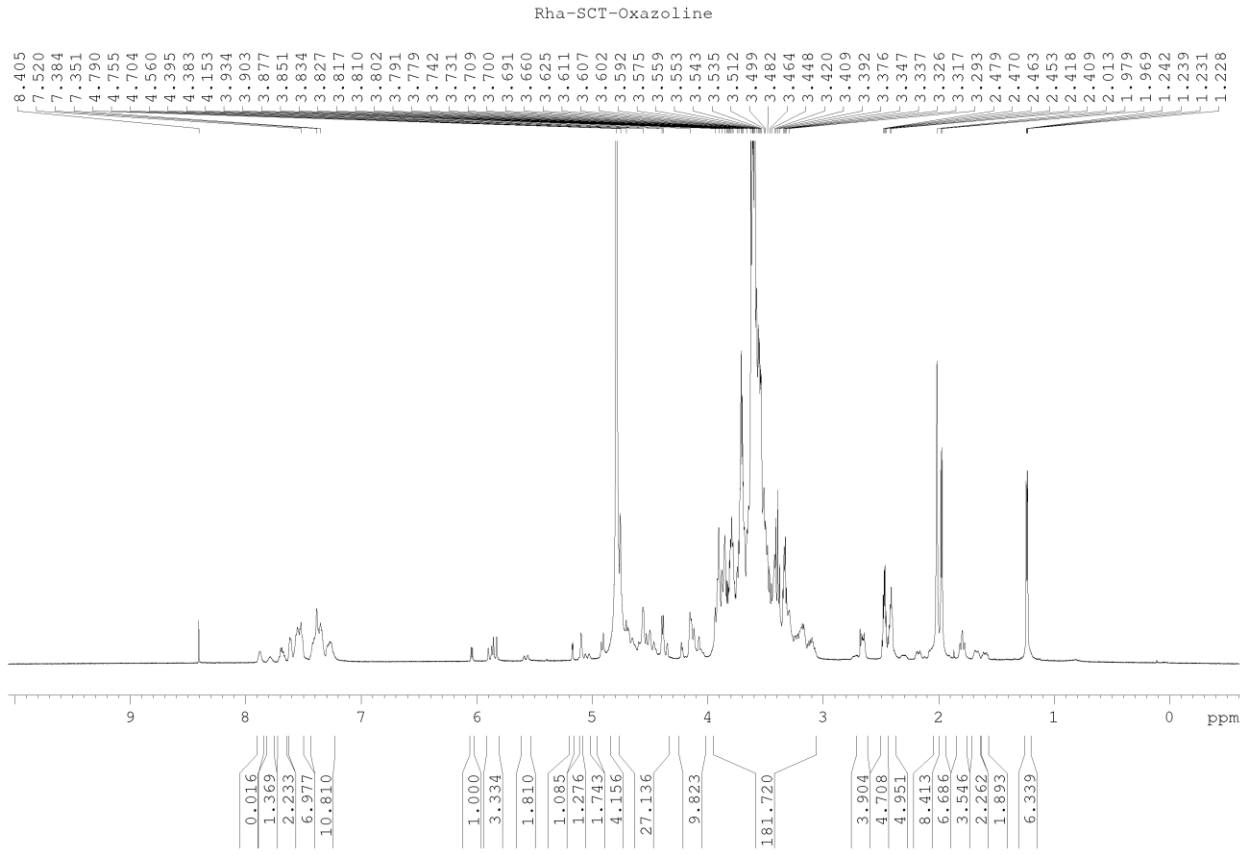


¹H NMR spectrum (600 MHz, D₂O): compound **16**

a-Gal-PEG-C6-NHS
13C 150MHz D2O

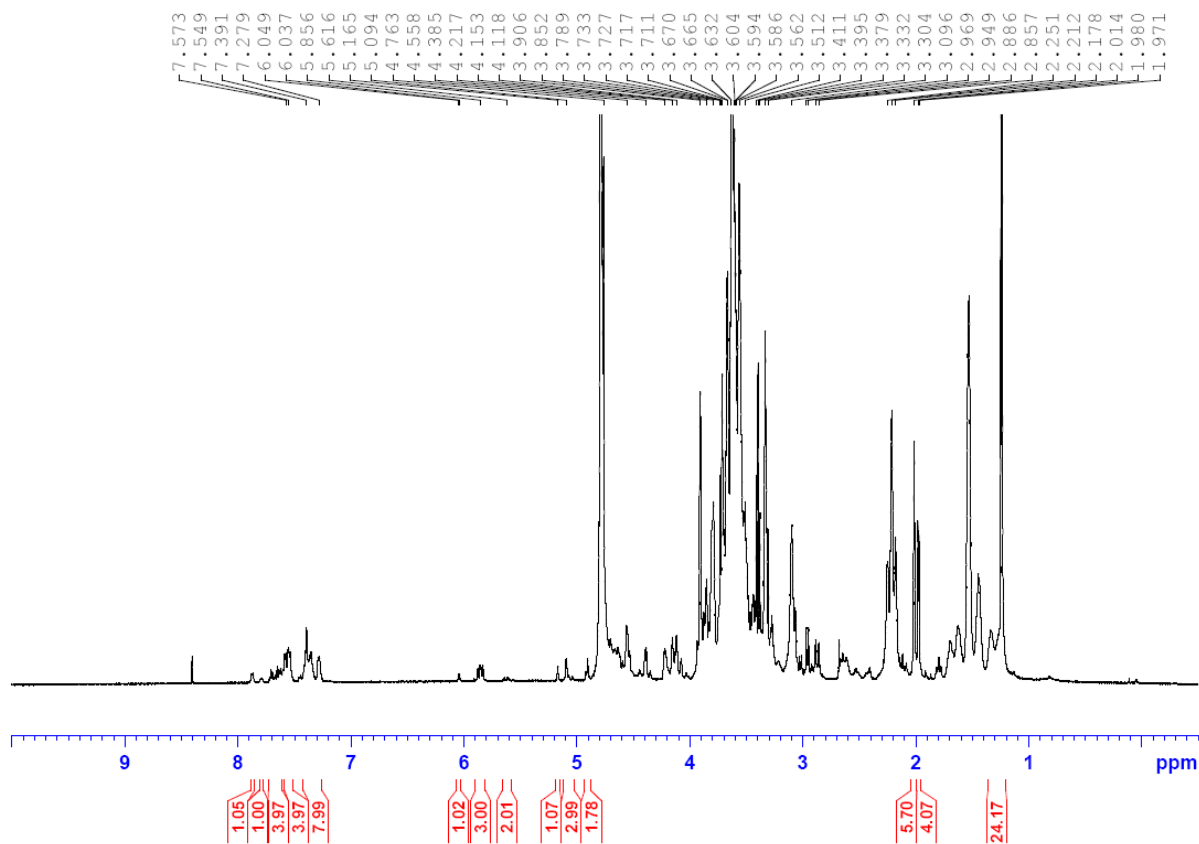


¹³C NMR spectrum (150 MHz, D₂O): compound **16**



^1H NMR spectrum (600 MHz, D_2O): compound **26a**

Rha8-SCT-oxa
1H 600MHz D2O



¹H NMR spectrum (600 MHz, D₂O): compound **26b**