Supplementary Information for

Concise Chemical Synthesis of O-Linked Glycans

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1. Materials and general methods

1.1. Materials:

Unless specifically stated, all commercial reagents and solvents were used without any additional purification. Chromatographic grade acetonitrile (MeCN) was purchased from J&K Scientific Co., Ltd. (Beijing, China). Chromatographic grade water was purchased from Hangzhou Wahaha Group Co., Ltd. (China). Analytical grade hexane, ethyl acetate (EtOAc), acetone, dichloromethane (DCM), tetrahydrofuran (THF), and methanol (MeOH) were purchased from Tongguang Fine Chemical Co., Ltd. (Beijing, China). Dimethylformamide (DMF), anhydrous DCM, anhydrous MeCN, acetic anhydride (Ac_2O), phenylboronic acid ($PhB(OH)_2$), trifluoromethanesulfonic acid (TfOH), trimethylsilyl trifluoromethanesulfonate (TMSOTf), *tert*-butyldimethylsilyl trifluoromethanesulfonate (TBSOTf), Sodium perborate tetrahydrate ($NaBO_3 \cdot 4H_2O$), trifluoroacetic acid (TFA), acetic acid (AcOH), formic acid, pyridine, N,N'-diisopropylcarbodiimide (DIC), Oxyma, 4-methylpiperidine, N,N-diisopropylethylamine (DIEA), triethylamine (Et_3N), hydrazine (NH_2NH_2), sodium hydroxide (NaOH), *tert*-butyl methyl ether, triisopropylsilane (TIS), zinc (Zn), copper sulfate ($CuSO_4$), sodium bicarbonate ($NaHCO_3$), sodium sulfate (Na_2SO_4), and sodium chloride (NaCl) were purchased from Innochem Science & Technology Co., Ltd. (Beijing, China). Rink Amide AM resin, hexafluorophosphate azabenzotriazole tetramethyl uronium (HATU), and Fmoc protected amino acids (FPAAs) were purchased from GL Biochem Co., Ltd. (Shanghai, China). Compounds **Thr6**, **Ser6**, **7a**, **7b**, **7c**, and **8** were purchased from Tianjin Proxino Biology Co., Ltd. (China). Dipeptidyl peptidase-IV (DPP-IV) was purchased from Sigma-Aldrich (Saint Louis, USA).

1.2. Instrumentation:

Unless otherwise specified, all reactions and purifications were performed under an ambient atmosphere at room temperature. All glycosylation reactions were performed in oven-dried round-bottom flasks. Flash column chromatography was carried out using silica gel (60 Å, 230-400 mesh) with analytical grade solvents. Analytical thin-layer chromatography (TLC) was carried out on glass-backed silica gel 60 F_{254} plates (EMD Millipore) and visualized under a UV_{254} lamp or by staining with ceric ammonium molybdate (CAM) solution.

^{1}H, ^{13}C, and 2D NMR spectra were recorded using a Bruker Avance III spectrometer in deuterated solvents. ^{1}H NMR chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (TMS) and referenced to the residual protium of the solvent. Spectral data are reported in the following order: chemical shift (δ, ppm); multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = complex multiplet, br = broad); coupling constants (*J*, Hz); number of protons. High-resolution mass spectra (HRMS) were obtained on a Thermo Scientific Exactive Plus Orbitrap mass spectrometer.

Solid-phase peptide synthesis (SPPS) was performed using a Liberty Blue™ microwave peptide synthesizer (CEM Corporation, Matthews, USA). Centrifugation was carried out using a Sigma 3K15 centrifuge (Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany). Lyophilization was performed using a VirTis BenchTop Pro lyophilizer (SP Scientific, New York, USA) operating at −100 °C and 5 Pa.

Glycopeptide characterization was carried out by ultra-performance liquid chromatography (UPLC), coupled with HRMS. UPLC analysis was performed on a Waters ACQUITY UPLC™ system, equipped with a Waters ACQUITY UPLC® BEH300 C4 column (2.1 × 100 mm, 1.7 μm, 300 Å).

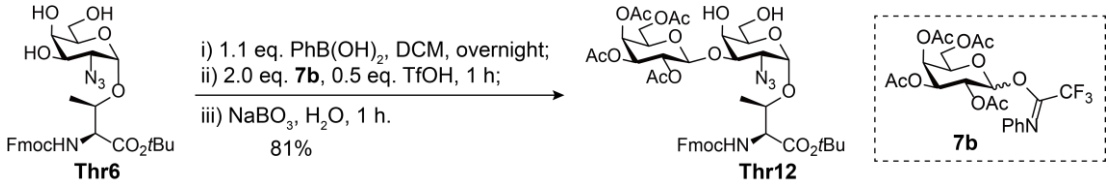
Peptide purification was performed on a Hanbon HPLC system (Huaian, China) equipped with a Newstyle NP7000 serials pump, a Newstyle NU3000 serials UV-Vis detector, a Welch Ultimate XB-C18 column (21.2 × 150 mm, 5 μm, 180 Å) (Yuexu Technology, Shanghai, China), and an EasyChrom™ Liquid Chromatography workstation.

1.3. General procedure of protecting group manipulations:

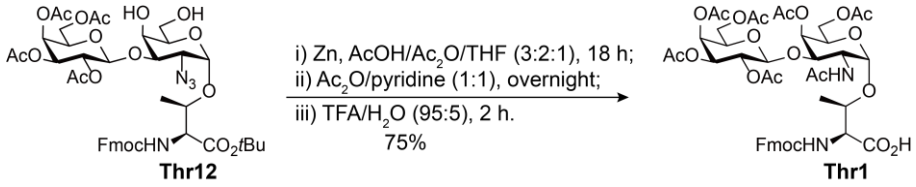
Zinc powder was stirred in 2% aqueous $CuSO_4$ solution for 30 min. After the aqueous phase was decanted, the glycoamino acid intermediate was added together with AcOH/Ac_2O/THF (3:2:1), and the resulting mixture was stirred for 18 h. The mixture was then filtered through a pad of Celite and concentrated under reduced pressure. The residue was dissolved in Ac_2O/pyridine (1:1) and stirred overnight. The reaction mixture was diluted with EtOAc and washed sequentially with saturated aqueous $NaHCO_3$ (×8), 1 M aqueous HCl (×5), distilled water (×2), and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was dissolved in TFA/H_2O (95:5) and stirred for 2 h. The reaction mixture was concentrated, and the crude product was purified by flash column chromatography on silica gel (DCM/MeOH).

2. Synthesis of **Thr**/**Ser1-5**

2.1. Synthesis of **Thr1**

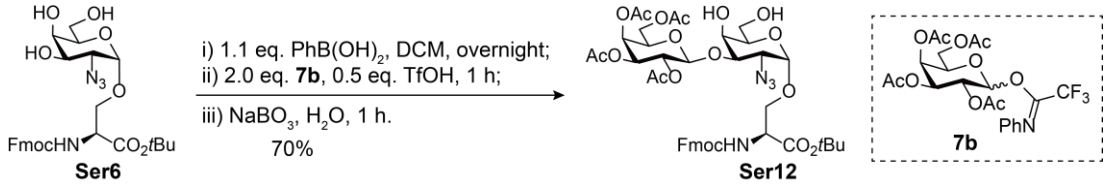


A mixture of **Thr6** (1.85 g, 3.16 mmol), $PhB(OH)_2$ (417 mg, 3.42 mmol), and activated 4 Å molecular sieves (5 g) in anhydrous DCM (100 mL) was stirred under an argon atmosphere overnight. To this reaction mixture, a solution of **7b** (3.29 g, 6.33 mmol) in anhydrous DCM (7 mL) was added, followed by the addition of TfOH (140 µL, 1.58 mmol) in anhydrous DCM (1 mL). The reaction mixture was stirred for an additional 1 h. The reaction mixture was diluted with DCM (40 mL) and subsequently filtered through a pad of Celite. $NaBO_3 \cdot 4H_2O$ (32 eq.) and H_2O (20 mL) were added, and the reaction mixture was stirred for 1 h. The organic phase was separated and washed successively with saturated aqueous $NaHCO_3$ (3 × 60 mL), distilled water (2 × 60 mL), and brine (60 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The crude residue was purified by flash column chromatography on silica gel (hexane/acetone, 3:1) to afford **Thr12** (2.34 g, 2.56 mmol, 81%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 – 7.72 (m, 2H), 7.67 – 7.52 (m, 2H), 7.45 – 7.36 (m, 2H), 7.34 – 7.27 (m, 2H), 5.71 (d, *J* = 9.5, 1H), 5.40 (dd, *J* = 3.5, 1.5 Hz, 1H), 5.29 (dd, *J* = 10.4, 8.0 Hz, 1H), 5.12 – 4.97 (m, 2H), 4.74 (d, *J* = 8.0 Hz, 1H), 4.50 – 4.40 (m, 2H), 4.33 – 4.00 (m, 7H), 3.99 – 3.88 (m, 3H), 3.84 – 3.75 (m, 1H), 3.65 – 3.52 (m, 1H), 2.92 (s, 1H), 2.61 (s, 1H), 2.16 (s, 3H), 2.10 (s, 3H), 2.05 (s, 3H), 1.99 (s, 3H), 1.49 (s, 9H), 1.33 (d, *J* = 6.6 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.58, 170.25, 170.20, 169.75, 169.31, 156.89, 143.98, 143.82, 141.37, 127.85, 127.19, 127.12, 125.33, 125.29, 120.12, 120.09, 101.98, 99.37, 83.08, 77.90, 75.96, 71.39, 70.74, 69.74, 69.27, 68.41, 67.40, 67.04, 62.67, 61.66, 59.13, 58.64, 47.23, 28.08, 20.77, 20.74, 20.71, 20.65, 19.17. HRMS (ESI) m/z calcd for $C_{43}H_{54}N_4O_{18}$ $[M+H]^+$: 915.3506; found: 915.3489.

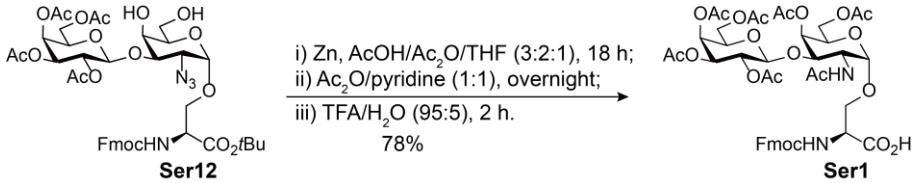


Thr1 was prepared following the general procedure described in Section 1.3 (75% yield). ^{1}H NMR (400 MHz, CD_3OD) δ 7.82 (d, *J* = 7.5 Hz, 2H), 7.69 (d, *J* = 7.4 Hz, 2H), 7.41 (t, *J* = 7.4 Hz, 2H), 7.37 – 7.30 (m, 2H), 5.39 – 5.32 (m, 2H), 5.03 – 4.96 (m, 2H), 4.86 (1H, H-1 overlapped with water signal), 4.65 – 4.47 (m, 3H), 4.39 – 4.31 (m, 2H), 4.29 (t, *J* = 6.2 Hz, 1H), 4.23 – 4.08 (m, 5H), 4.01 – 3.92 (m, 2H), 3.88 (dd, *J* = 11.1, 3.3 Hz, 1H), 2.13 (s, 3H), 2.10 (s, 3H), 2.04 (s, 3H), 2.03 (s, 3H), 2.00 (s, 3H), 1.97 (s, 3H), 1.93 (s, 3H), 1.24 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ 172.31, 172.05, 172.00, 171.48, 171.05, 159.07, 145.32, 145.20, 142.71, 142.69, 128.87, 128.87, 128.23, 126.15, 126.01, 121.06, 121.02, 102.50, 100.97, 77.55, 75.05, 72.19, 71.72, 71.23, 70.14, 68.96, 68.51, 67.66, 64.26, 62.27, 49.99, 48.61, 23.26, 20.84, 20.72, 20.68, 20.47, 20.46, 19.14. HRMS (ESI) m/z calcd for $C_{45}H_{54}N_2O_{21}$ $[M+H]^+$: 959.3292; found: 959.3287.

2.2. Synthesis of **Ser1**

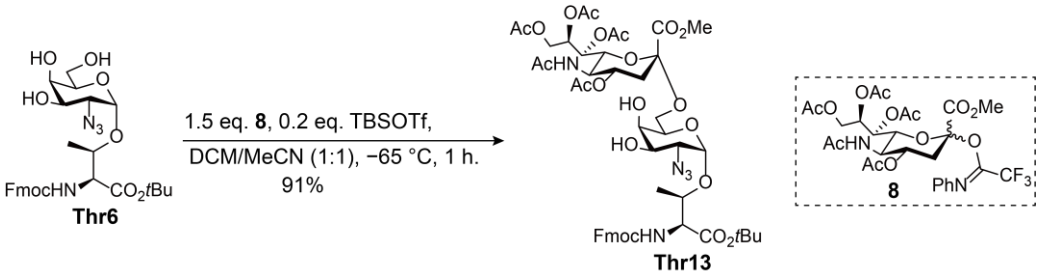


A mixture of **Ser6** (285 mg, 0.50 mmol), $PhB(OH)_2$ (64.0 mg, 0.53 mmol), and activated 4 Å molecular sieves (2 g) in anhydrous DCM (17.5 mL) was stirred under an argon atmosphere overnight. To this reaction mixture, a solution of **7b** (519 mg, 1.0 mmol) in anhydrous DCM (2 mL) was added, followed by the addition of TfOH (22 µL, 0.25 mmol) in anhydrous DCM (0.5 mL). The reaction mixture was stirred for an additional 1 h. The reaction mixture was diluted with DCM (30 mL) and subsequently filtered through a pad of Celite. $NaBO_3 \cdot 4H_2O$ (32 eq.) and H_2O (15 mL) were added, and the reaction mixture was stirred for 1 h. The organic phase was separated and washed successively with saturated aqueous $NaHCO_3$ (3 × 50 mL), distilled water (2 × 50 mL), and brine (50 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The crude residue was purified by flash column chromatography on silica gel (hexane/acetone, 3:1) to afford **Ser12** (313 mg, 0.347 mmol, 70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (d, *J* = 7.6 Hz, 2H), 7.63 (d, *J* = 7.5 Hz, 2H), 7.41 (t, *J* = 7.5 Hz, 2H), 7.36 – 7.28 (m, 2H), 5.96 (d, *J* = 7.9 Hz, 1H), 5.41 – 5.34 (m, 1H), 5.25 (dd, *J* = 10.0, 7.7 Hz, 1H), 5.00 (dd, *J* = 10.5, 3.4 Hz, 1H), 4.90 (d, *J* = 3.6 Hz, 1H), 4.59 (d, *J* = 7.8 Hz, 1H), 4.48 – 4.35 (m, 4H), 4.22 (t, *J* = 7.0 Hz, 1H), 4.15 – 4.02 (m, 4H), 3.98 (dd, *J* = 10.5, 3.0 Hz, 1H), 3.94 – 3.79 (m, 3H), 3.75 (t, *J* = 7.4 Hz, 1H), 3.62 (dd, *J* = 10.5, 3.4 Hz, 1H), 2.16 (s, 3H), 2.07 (s, 3H), 2.01 (s, 3H), 1.99 (s, 3H), 1.50 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.53, 170.24, 169.68, 169.01, 156.02, 143.98, 143.84, 141.48, 127.95, 127.29, 125.31, 120.22, 101.94, 99.82, 83.20, 78.24, 71.46, 70.78, 70.15, 70.05, 69.17, 68.47, 67.15, 67.10, 62.79, 61.71, 58.60, 55.21, 47.33, 28.09, 20.76, 20.67. HRMS (ESI) m/z calcd for $C_{42}H_{52}N_4O_{18}$ $[M+H]^+$: 901.3349; found: 901.3335.

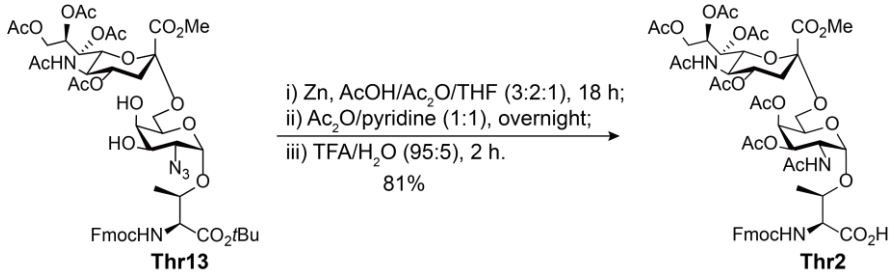


Ser1 was prepared following the general procedure described in Section 1.3 (78% yield). ^{1}H NMR (400 MHz, CD_3OD) δ 7.85 – 7.78 (m, 2H), 7.72 – 7.64 (m, 2H), 7.43 – 7.38 (m, 2H), 7.37 – 7.31 (m, 2H), 5.39 (d, *J* = 3.2 Hz, 1H), 5.34 (dd, *J* = 3.1, 1.2 Hz, 1H), 5.06 – 4.97 (m, 2H), 4.81 – 4.76 (m, 1H), 4.71 (d, *J* = 7.3 Hz, 1H), 4.43 (d, *J* = 6.5 Hz, 2H), 4.38 (dd, *J* = 11.0, 3.5 Hz, 1H), 4.35 – 4.30 (m, 1H), 4.25 (t, *J* = 6.5 Hz, 1H), 4.19 – 3.83 (m, 9H), 2.13 (s, 3H), 2.10 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H), 1.97 (s, 6H), 1.93 (s, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ 172.35, 172.08, 172.06, 171.98, 171.48, 171.14, 145.30, 142.66, 142.64, 128.89, 128.27, 128.24, 126.12, 121.05, 102.48, 99.85, 74.73, 72.19, 71.82, 71.22, 70.20, 69.93, 68.84, 68.62, 67.93, 63.89, 62.41, 50.30, 48.44, 23.05, 20.83, 20.73, 20.68, 20.48, 20.46. HRMS (ESI) m/z calcd for $C_{44}H_{52}N_2O_{21}$ $[M+H]^+$: 945.3135; found: 945.3136.

2.3. Synthesis of **Thr2**

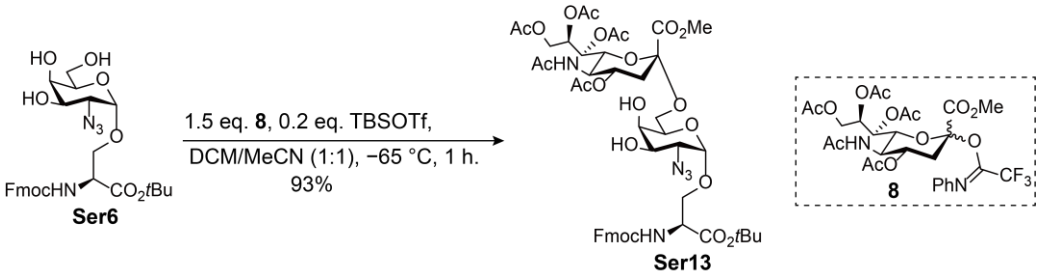


A mixture of **Thr6** (123 mg, 0.21 mmol), **8** (209 mg, 0.32 mmol), and activated 3 Å molecular sieves (600 mg) in anhydrous DCM/MeCN (1:1, 6 mL) was stirred under an argon atmosphere for 30 min and subsequently cooled down to −65 °C. To this reaction mixture, TBSOTf (9.7 µL, 0.042 mmol) in anhydrous DCM/MeCN (1:1, 1 mL) was added. The reaction mixture was stirred for an additional 1 h at −65 °C. Subsequently, the reaction was quenched with Et_3N (0.4 eq.), and the reaction mixture was warmed to room temperature. The resulting reaction mixture was filtered through a pad of Celite and concentrated under reduced pressure. The crude residue was purified by flash column chromatography on silica gel (hexane/acetone, 2:1 → 1:1) to afford **Thr13** (243 mg, 0.23 mmol, 91%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (dq, *J* = 7.6, 1.0 Hz, 2H), 7.63 (d, *J* = 7.5 Hz, 2H), 7.40 (tt, *J* = 7.5, 1.4 Hz, 2H), 7.31 (tt, *J* = 7.4, 1.5 Hz, 2H), 5.75 (d, *J* = 9.5 Hz, 1H), 5.40 (td, *J* = 7.4, 2.7 Hz, 1H), 5.30 (dd, *J* = 7.9, 1.5 Hz, 1H), 5.26 – 5.22 (m, 1H), 5.03 (d, *J* = 3.8 Hz, 1H), 4.89 (ddd, *J* = 12.3, 9.8, 4.7 Hz, 1H), 4.48 – 4.38 (m, 3H), 4.35 – 4.21 (m, 3H), 4.13 – 3.92 (m, 6H), 3.89 (dd, *J* = 10.2, 5.9 Hz, 1H), 3.80 (s, 3H), 3.73 (dd, *J* = 10.1, 5.5 Hz, 1H), 3.50 - 3.41 (m, 2H, H-2, OH), 2.93 (s, 1H, OH), 2.57 (dd, *J* = 12.9, 4.6 Hz, 1H), 2.16 (s, 3H), 2.13 (s, 3H), 2.04 (s, 3H), 2.03 (s, 3H), 2.02 – 1.95 (m, 1H), 1.88 (s, 3H), 1.50 (s, 9H), 1.33 (d, *J* = 6.5 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.43, 171.13, 170.73, 170.45, 170.34, 169.39, 168.23, 157.02, 144.09, 143.93, 141.39, 127.83, 127.25, 127.21, 125.46, 125.41, 120.08, 99.97, 98.87, 82.92, 76.43, 72.95, 69.26, 69.00, 68.88, 67.96, 67.57, 67.46, 63.91, 63.03, 60.88, 59.56, 53.26, 49.44, 47.26, 37.10, 28.10, 23.31, 21.29, 20.99, 20.97, 19.31. HRMS (ESI) m/z calcd for $C_{49}H_{63}N_5O_{21}$ $[M+H]^+$: 1058.4088; found: 1058.4088.

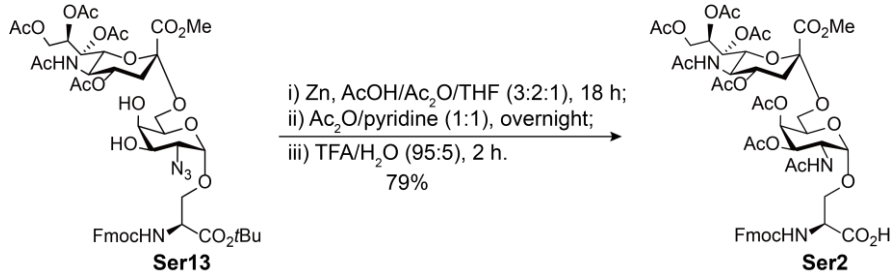


Thr2 was prepared following the general procedure described in Section 1.3 (81% yield). ^{1}H NMR (400 MHz, $CDCl_3$ + 0.5% D_2O) δ 7.80 – 7.71 (m, 2H), 7.66 – 7.58 (m, 2H), 7.43 – 7.35 (m, 2H), 7.34 – 7.28 (m, 2H), 5.43 – 5.24 (m, 3H), 5.17 – 4.99 (m, 2H), 4.90 – 4.81 (m, 1H), 4.55 – 3.99 (m, 11H), 3.86 – 3.80 (m, 1H), 3.77 (s, 3H), 3.29 (dd, *J* = 10.0, 4.9 Hz, 1H), 2.52 (dd, *J* = 12.9, 4.7 Hz, 1H), 2.15 (s, 3H), 2.12 (s, 3H), 2.10 (s, 3H), 2.01 (s, 6H), 2.00 (s, 3H), 1.97 (s, 3H), 1.95 – 1.89 (m, 1H), 1.86 (s, 3H), 1.28 (d, *J* = 6.3 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$ + 0.5% D_2O) δ 171.22, 171.15, 171.06, 170.82, 170.60, 170.57, 170.41, 170.20, 167.97, 157.16, 144.08, 143.92, 141.45, 127.89, 127.28, 125.29, 125.21, 120.14, 120.12, 99.34, 98.73, 77.36, 72.66, 68.97, 68.47, 68.40, 68.30, 67.82, 67.34, 67.03, 63.65, 62.55, 59.21, 53.07, 49.36, 48.09, 47.41, 37.78, 23.22, 21.19, 20.97, 20.93, 20.91, 20.86, 20.83, 18.57. HRMS (ESI) m/z: Calcd for $C_{51}H_{63}N_3O_{24}$ $[M+H]^+$: 1102.3874; found: 1102.3870.

2.4. Synthesis of **Ser2**

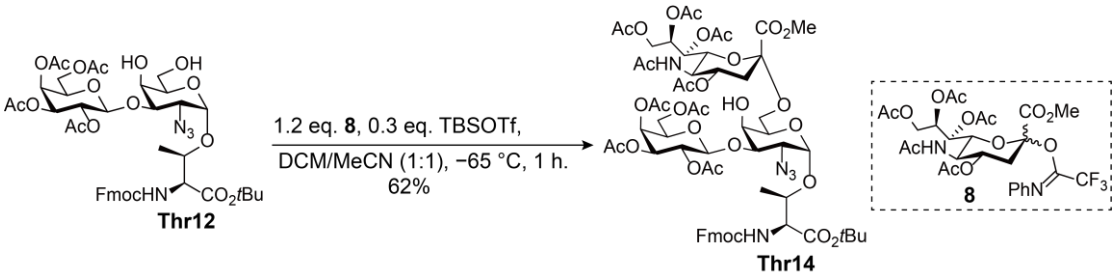


A mixture of **Ser6** (571 mg, 1.00 mmol), **8** (994 mg, 1.50 mmol), and activated 3 Å molecular sieves (3 g) in anhydrous DCM/MeCN (1:1, 30 mL) was stirred under an argon atmosphere for 30 min and subsequently cooled down to −65 °C. To this reaction mixture, TBSOTf (52.9 µL, 0.20 mmol) in anhydrous DCM/MeCN (1:1, 1 mL) was added. The reaction mixture was stirred for an additional 1 h at −65 °C. Subsequently, the reaction was quenched with Et_3N (0.4 eq.), and the reaction mixture was warmed to room temperature. The resulting reaction mixture was filtered through a pad of Celite and concentrated under reduced pressure. The crude residue was purified by flash column chromatography on silica gel (hexane/acetone, 2:1 → 1:1) to afford **Ser13** (971 mg, 0.93 mmol, 93%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, *J* = 7.6 Hz, 2H), 7.62 (dd, *J* = 7.6, 3.8 Hz, 2H), 7.40 (t, *J* = 7.5 Hz, 2H), 7.31 (td, *J* = 7.4, 6.8, 2.5 Hz, 2H), 5.80 (d, *J* = 8.1 Hz, 1H), 5.39 (td, *J* = 7.4, 2.7 Hz, 1H), 5.30 – 5.26 (m, 1H), 5.22 (d, *J* = 8.7 Hz, 1H), 4.94 – 4.81 (m, 2H), 4.47 – 4.20 (m, 5H), 4.10 – 3.83 (m, 9H), 3.77 – 3.65 (m, 4H), 3.50 (dd, *J* = 10.1, 3.6 Hz, 1H), 2.53 (dd, *J* = 12.9, 4.6 Hz, 1H), 2.13 (s, 3H), 2.11 (s, 3H), 2.06 – 1.94 (m, 7H), 1.99 (s, 3H), 1.87 (s, 3H), 1.51 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.35, 171.06, 170.61, 170.39, 170.30, 168.89, 168.25, 156.03, 144.01, 141.40, 127.87, 127.23, 125.35, 120.10, 99.46, 98.87, 83.03, 72.94, 69.18, 69.12, 69.00, 68.92, 68.09, 67.60, 67.37, 63.57, 63.04, 60.67, 55.01, 53.14, 49.49, 47.25, 37.01, 28.08, 23.28, 21.23, 20.93. HRMS (ESI) m/z calcd for $C_{48}H_{61}N_5O_{21}$ $[M+H]^+$: 1044.3932; found: 1044.3938.

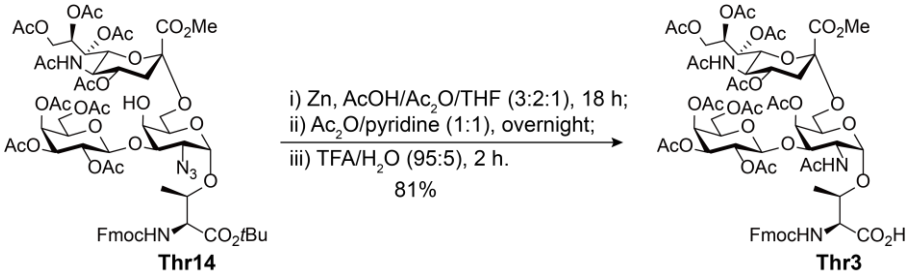


Ser2 was prepared following the general procedure described in Section 1.3 (79% yield). ^{1}H NMR (400 MHz, $CDCl_3$ + 1% D_2O + 2.5% CD_3OD) δ 7.75 (d, *J* = 7.5 Hz, 2H), 7.61 (d, *J* = 7.5 Hz, 2H), 7.38 (t, *J* = 7.5 Hz, 2H), 7.34 – 7.27 (m, 2H), 5.37 – 5.25 (m, 3H), 5.13 – 5.06 (m, 1H), 4.94 – 4.88 (m, 1H), 4.80 (dq, *J* = 10.1, 5.3 Hz, 1H), 4.47 – 4.33 (m, 4H), 4.30 (dd, *J* = 12.4, 2.7 Hz, 1H), 4.21 (t, *J* = 6.8 Hz, 1H), 4.11 – 3.90 (m, 6H), 3.83 – 3.77 (m, 1H), 3.74 (s, 3H), 3.28 (dd, *J* = 10.0, 6.1 Hz, 1H), 2.51 (dd, *J* = 13.0, 4.9 Hz, 1H), 2.13 (s, 3H), 2.11 (s, 3H), 2.08 (s, 3H), 2.00 (s, 3H), 1.98 (s, 3H), 1.96 (s, 3H), 1.93 (s, 3H), 1.91 – 1.87 (m, 1H), 1.84 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$ + 1% D_2O + 2.5% CD_3OD) δ 171.24, 171.20, 171.14, 171.03, 170.62, 170.55, 170.39, 167.96, 143.93, 141.41, 127.86, 127.23, 125.11, 120.09, 98.70, 98.50, 72.60, 69.18, 68.87, 68.51, 68.42, 67.91, 67.60, 67.45, 66.97, 63.167, 62.54, 52.96, 49.00, 47.86, 47.31, 37.78, 22.96, 22.77, 21.10, 20.91, 20.85, 20.76. HRMS (ESI) m/z calcd for $C_{50}H_{61}N_3O_{24}$ $[M+H]^+$: 1088.3718; found: 1088.3727.

2.5. Synthesis of **Thr3**



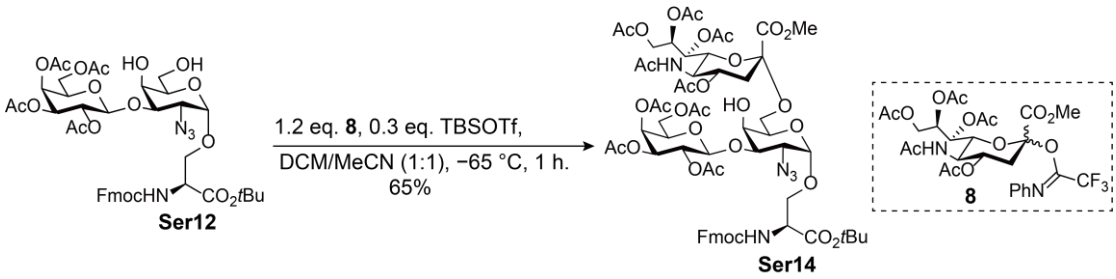
A mixture of **Thr12** (384 mg, 0.42 mmol), **8** (334 mg, 0.50 mmol), and activated 3 Å molecular sieves (800 mg) in anhydrous DCM/MeCN (1:1, 14 mL) was stirred under an argon atmosphere for 30 min and subsequently cooled down to −65 °C. To this reaction mixture, TBSOTf (29 µL, 0.13 mmol) in anhydrous DCM/MeCN (1:1, 0.5 mL) was added. The reaction mixture was stirred for an additional 1 h at −65 °C. Subsequently, the reaction was quenched with Et_3N (0.4 eq.), and the reaction mixture was warmed to room temperature. The resulting reaction mixture was filtered through a pad of Celite and concentrated under reduced pressure. The crude residue was purified by flash column chromatography on silica gel (hexane/acetone, 2:1 → 1:1) to afford **Thr14** (360 mg, 0.26 mmol, 62%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 – 7.74 (m, 2H), 7.66 – 7.60 (m, 2H), 7.45 – 7.37 (m, 2H), 7.34 – 7.28 (m, 2H), 5.66 (d, *J* = 9.6 Hz, 1H), 5.43 – 5.35 (m, 2H), 5.34 – 5.26 (m, 2H), 5.15 – 5.00 (m, 3H), 4.93 – 4.84 (m, 1H), 4.79 (d, *J* = 8.0 Hz, 1H), 4.51 – 4.41 (m, 2H), 4.37 – 4.24 (m, 4H), 4.21 – 3.92 (m, 10H), 3.80 (s, 3H), 3.65 – 3.57 (m, 1H), 3.53 (dd, *J* = 10.6, 3.8 Hz, 1H), 2.61 (s, 1H, OH), 2.57 (dd, *J* = 12.9, 4.7 Hz, 1H), 2.17 (s, 3H), 2.13 (s, 3H), 2.12 (s, 3H), 2.10 (s, 3H), 2.04 (s, 3H), 2.03 (s, 3H), 2.02 (s, 3H), 2.00 (s, 3H), 1.95 (t, *J* = 12.5 Hz, 1H), 1.88 (s, 3H), 1.50 (s, 9H), 1.37 (d, *J* = 6.4 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.08, 170.84, 170.41, 170.37, 170.28, 170.23, 170.19, 169.71, 169.33, 168.18, 156.91, 144.10, 143.93, 141.45, 127.90, 127.25, 127.17, 125.43, 125.35, 120.16, 120.14, 102.13, 100.01, 98.83, 83.03, 77.73, 76.53, 72.99, 71.12, 70.91, 69.15, 69.10, 68.96, 68.62, 68.42, 67.72, 67.49, 66.88, 63.76, 62.55, 61.10, 59.53, 58.92, 53.05, 49.59, 47.34, 37.61, 28.15, 23.36, 21.21, 20.99, 20.96, 20.93, 20.80, 20.77, 20.70, 19.31. HRMS (ESI) m/z calcd for $C_{63}H_{81}N_5O_{30}$ $[M+H]^+$: 1388.5039; found: 1388.5054.



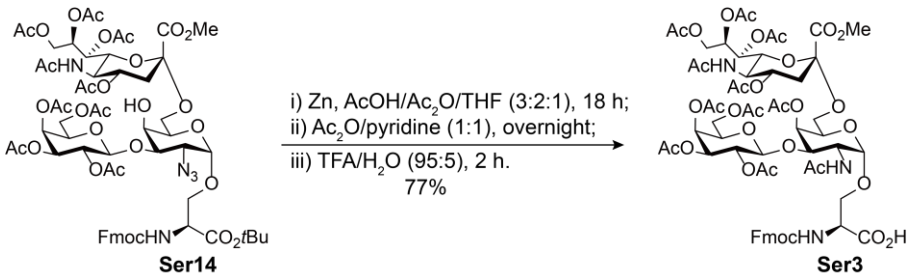
Thr3 was prepared following the general procedure described in Section 1.3 (81% yield). ^{1}H NMR (700 MHz, $CDCl_3$ + 8% CD_3OD) δ 7.72 (d, *J* = 7.5 Hz, 2H), 7.58 (dd, *J* = 7.3, 3.0 Hz, 2H), 7.35 (t, *J* = 7.4 Hz, 2H), 7.29 – 7.25 (m, 2H), 5.31 – 5.21 (m, 4H), 4.97 (dd, *J* = 10.4, 7.9 Hz, 1H), 4.87 (dd, *J* = 10.5, 3.4 Hz, 1H), 4.82 (d, *J* = 3.7 Hz, 1H), 4.75 – 4.70 (m, 1H), 4.53 – 4.47 (m, 3H), 4.32 (qd, *J* = 6.5, 2.0 Hz, 1H), 4.28 – 4.22 (m, 3H), 4.20 (t, *J* = 6.3 Hz, 1H), 4.10 (dd, *J* = 11.2, 5.7 Hz, 1H), 4.06 – 3.93 (m, 5H), 3.80 – 3.71 (m, 5H), 3.69 (dd, *J* = 10.1, 7.4 Hz, 1H), 3.26 (dd, *J* = 10.2, 3.8 Hz, 1H), 2.48 (dd, *J* = 12.8, 4.6 Hz, 1H), 2.09 (s, 3H), 2.06 (s, 3H), 2.05 (s, 3H), 2.03 (s, 3H), 1.97 (s, 3H), 1.96 (s, 3H), 1.95 (s, 3H), 1.95 (s, 3H), 1.91 (s, 3H), 1.88 (s, 3H), 1.82 (t, *J* = 12.4 Hz, 1H), 1.78 (s, 3H), 1.20 (d, *J* = 6.4 Hz, 3H). ^{13}C NMR (176 MHz, $CDCl_3$ + 8% CD_3OD) δ 171.34, 171.17, 171.12, 170.75, 170.61, 170.51,

170.46, 170.19, 169.76, 167.79, 157.23, 143.87, 143.73, 141.34, 141.33, 127.78, 127.15, 127.13, 124.84, 120.02, 120.00, 101.02, 99.51, 98.61, 76.65, 72.64, 72.40, 70.80, 70.29, 69.47, 69.34, 68.80, 68.71, 68.69, 67.42, 66.72, 66.58, 63.94, 62.41, 60.81, 58.88, 52.77, 49.03, 48.66, 47.27, 37.26, 22.62, 22.61, 20.93, 20.79, 20.66, 20.64, 20.60, 20.58, 20.53, 20.47, 18.50. HRMS (ESI) m/z calcd for $C_{63}H_{79}N_3O_{32}$ $[M+H]^+$: 1390.4720; found: 1390.4726.

2.6. Synthesis of **Ser3**



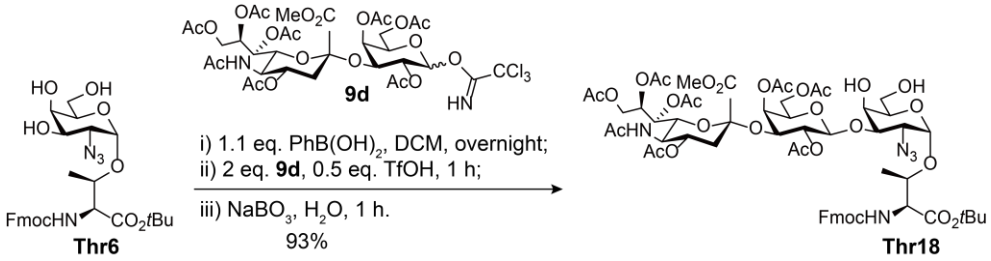
A mixture of **Ser12** (40.5 mg, 0.045 mmol), **8** (35.8 mg, 0.054 mmol), and activated 3 Å molecular sieves (150 mg) in anhydrous DCM/MeCN (1:1, 1.3 mL) was stirred under an argon atmosphere for 30 min and subsequently cooled down to −65 °C. To this reaction mixture, TBSOTf (3.1 µL, 0.0135 mmol) in anhydrous DCM/MeCN (1:1, 0.2 mL) was added. The reaction mixture was stirred for an additional 1 h at −65 °C. Subsequently, the reaction was quenched with Et_3N (0.4 eq.), and the reaction mixture was warmed to room temperature. The resulting reaction mixture was filtered through a pad of Celite and concentrated under reduced pressure. The crude residue was purified by flash column chromatography on silica gel (hexane/acetone, 2:1 → 1:1) to afford **Ser14** (40.1 mg, 0.0292 mmol, 65%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (dt, *J* = 7.5, 0.9 Hz, 2H), 7.63 (t, *J* = 6.4 Hz, 2H), 7.42 (t, *J* = 7.4 Hz, 2H), 7.32 (tdd, *J* = 7.5, 3.1, 1.2 Hz, 2H), 5.76 (d, *J* = 8.2 Hz, 1H), 5.44 – 5.36 (m, 2H), 5.33 – 5.30 (m, 1H), 5.25 (dd, *J* = 10.5, 7.9 Hz, 1H), 5.14 – 5.09 (m, 1H), 5.00 (dd, *J* = 10.5, 3.4 Hz, 1H), 4.93 (d, *J* = 3.6 Hz, 1H), 4.86 (dd, *J* = 10.9, 6.3 Hz, 1H), 4.66 (d, *J* = 7.9 Hz, 1H), 4.50 – 4.45 (m, 1H), 4.44 – 4.29 (m, 3H), 4.25 (t, *J* = 7.4 Hz, 1H), 4.14 – 4.01 (m, 7H), 3.99 – 3.82 (m, 5H), 3.75 (s, 3H), 3.62 – 3.54 (m, 2H), 2.62 – 2.53 (m, 2H, OH & H-3″eq), 2.17 (s, 3H), 2.16 (s, 3H), 2.11 (s, 3H), 2.11 (s, 3H), 2.07 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H), 1.99 (s, 3H), 1.94 (t, *J* = 12.5 Hz, 1H), 1.87 (s, 3H), 1.51 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.06, 170.88, 170.34, 170.28, 170.27, 170.21, 170.18, 169.73, 168.94, 168.18, 156.03, 144.00, 141.45, 127.96, 127.27, 125.35, 120.21, 102.10, 99.27, 98.89, 83.06, 77.88, 72.96, 71.05, 70.88, 69.12, 69.05, 68.83, 68.59, 68.17, 67.65, 67.38, 66.88, 63.56, 62.58, 61.06, 58.81, 54.98, 53.02, 49.54, 47.29, 37.78, 28.12, 23.35, 21.21, 20.96, 20.93, 20.79, 20.74, 20.70. HRMS (ESI) m/z calcd for $C_{62}H_{79}N_5O_{30}$ $[M+H]^+$: 1374.4883; found: 1374.4884.



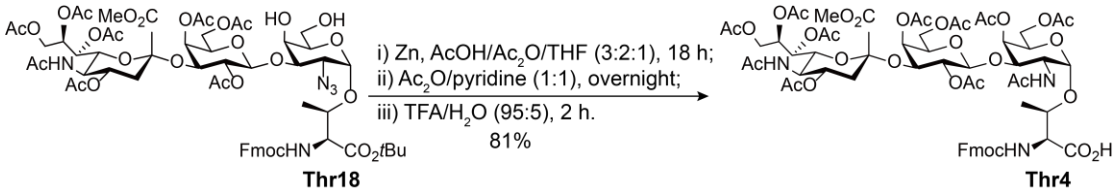
Ser3 was prepared following the general procedure described in Section 1.3 (77% yield). ^{1}H NMR (700 MHz, $CDCl_3$ + 8% CD_3OD) δ 77.73 (d, *J* = 7.5 Hz, 2H), 7.58 (d, *J* = 6.7 Hz, 2H), 7.36 (t, *J* = 7.2 Hz, 2H), 7.29 – 7.26 (m, 2H), 7.13 (d, *J* = 8.5 Hz, 1H), 6.67 (d, *J* = 9.9 Hz, 1H), 6.31 (d, *J* = 7.0 Hz, 1H), 5.32 – 5.21 (m, 4H), 4.99 (dd, *J* = 10.4, 7.7 Hz, 1H), 4.90 (dd, *J*

= 10.4, 3.1 Hz, 1H), 4.84 – 4.81 (m, 1H), 4.74 (td, *J* = 10.8, 5.1 Hz, 1H), 4.58 (d, *J* = 7.7 Hz, 1H), 4.43 (dd, *J* = 10.6, 6.6 Hz, 1H), 4.38 (dd, *J* = 10.7, 6.3 Hz, 1H), 4.29 – 4.21 (m, 3H), 4.18 (t, *J* = 6.4 Hz, 1H), 4.11 – 3.80 (m, 10H), 4.75 – 4.67 (m, 4H), 3.24 (dd, *J* = 10.1, 4.6 Hz, 1H), 2.50 (dd, *J* = 13.2, 5.5 Hz, 1H), 2.10 (s, 3H), 2.06 (s, 3H), 2.05 (s, 6H), 1.98 (s, 3H), 1.97 (s, 3H), 1.96 (s, 3H), 1.95 (s, 3H), 1.92 (s, 3H), 1.91 (s, 3H), 1.86 (t, *J* = 11.8 Hz, 1H), 1.80 (s, 3H). ^{13}C NMR (176 MHz, $CDCl_3$ + 8% CD_3OD) δ 171.44, 171.23, 171.20, 170.81, 170.73, 170.53, 170.40, 169.92, 167.80, 156.73, 143.88, 143.81, 141.35, 141.32, 127.84, 127.19, 127.17, 124.95, 120.06, 100.98, 98.55, 98.32, 72.83, 72.45, 70.84, 70.45, 69.40, 69.31, 68.86, 68.73, 68.52, 67.52, 66.89, 66.83, 63.51, 62.47, 60.98, 55.55, 52.83, 49.26, 48.67, 47.19, 37.55, 22.68, 22.63, 20.94, 20.79, 20.65, 20.64, 20.60, 20.55, 20.52. HRMS (ESI) m/z calcd for $C_{62}H_{77}N_3O_{32}$ $[M+H]^+$: 1376.4563; found: 1376.4564.

2.7. Synthesis of **Thr4**



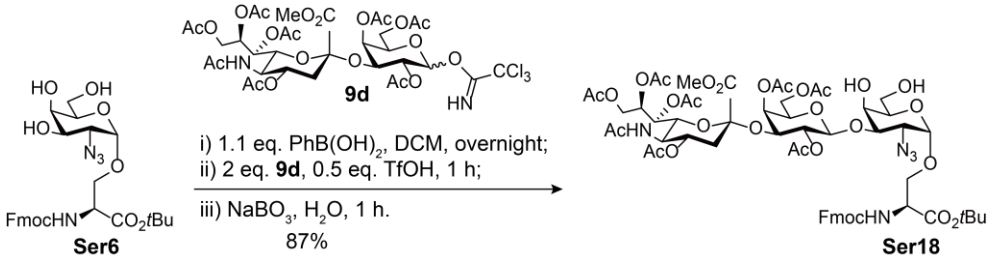
A mixture of **Thr6** (253 mg, 0.43 mmol), $PhB(OH)_2$ (55.4 mg, 0.46 mmol), and activated 4 Å molecular sieves (2 g) in anhydrous DCM (21 mL) was stirred under an argon atmosphere overnight. To this reaction mixture, a solution of **9d** (800 mg, 0.87 mmol) in anhydrous DCM (0.8 mL) was added, followed by the addition of TfOH (19 µL, 0.22 mmol) in anhydrous DCM (0.2 mL). The reaction mixture was stirred for an additional 1 h. The reaction mixture was diluted with DCM (40 mL) and subsequently filtered through a pad of Celite. $NaBO_3 \cdot 4H_2O$ (32 eq.) and H_2O (20 mL) were added, and the reaction mixture was stirred for 1 h. The organic phase was separated and washed successively with saturated aqueous $NaHCO_3$ (3 × 60 mL), distilled water (2 × 60 mL), and brine (60 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The crude residue was purified by flash column chromatography on silica gel (DCM/MeOH, 60:1 → 40:1) to afford **Thr18** (542 mg, 0.40 mmol, 93%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 – 7.71 (m, 2H), 7.64 – 7.58 (m, 2H), 7.42 – 7.34 (m, 2H), 7.33 – 7.24 (m, 2H), 5.76 (d, *J* = 9.6 Hz, 1H), 5.59 – 5.51 (m, 1H), 5.36 (dd, *J* = 9.3, 2.6 Hz, 1H), 5.16 – 5.02 (m, 3H), 4.95 – 4.85 (m, 3H), 4.64 (dd, *J* = 10.2, 3.3 Hz, 1H), 4.48 – 4.19 (m, 7H), 4.15 – 3.91 (m, 8H), 3.87 – 3.76 (m, 4H), 3.67 – 3.59 (m, 2H), 2.92 (s, 1H, OH), 2.59 (dd, *J* = 12.7, 4.6 Hz, 1H), 2.50 (d, *J* = 8.3 Hz, 1H, OH), 2.25 (s, 3H), 2.15, 2.11, 2.06, 2.00, 1.99, 1.96 (s, 3H x 6), 1.84 (s, 3H), 1.75 – 1.68 (m, 1H), 1.50 (s, 9H), 1.34 (d, *J* = 6.3 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.00, 170.59, 170.47, 170.39, 169.91, 169.71, 169.36, 168.16, 156.97, 144.02, 141.41, 127.83, 127.21, 127.15, 125.36, 120.11, 101.41, 99.27, 96.88, 83.09, 78.04, 75.78, 72.21, 71.41, 71.23, 69.72, 69.37, 69.29, 69.14, 67.96, 67.78, 67.63, 67.07, 63.01, 62.45, 62.08, 59.16, 58.60, 53.41, 49.31, 47.23, 37.66, 28.16, 23.32, 21.69, 21.12, 20.91, 20.85, 20.82, 20.76, 19.19. HRMS (ESI) m/z calcd for $C_{61}H_{79}N_5O_{29}$ $[M+H]^+$: 1346.4934; found: 1346.4952.



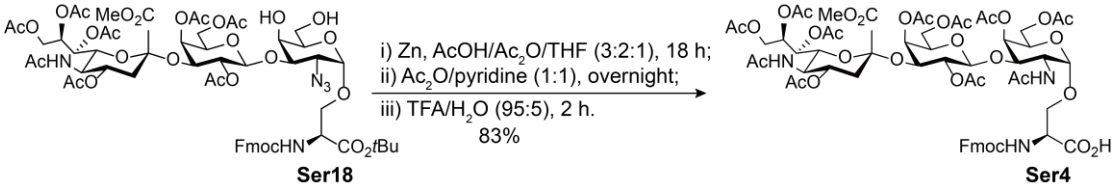
Thr4 was prepared following the general procedure described in Section 1.3 (81% yield). ^{1}H

NMR (700 MHz, $CDCl_3$ + 6% CD_3OD) δ 7.72 (d, *J* = 7.6 Hz, 2H), 7.57 (d, *J* = 7.4 Hz, 2H), 7.35 (t, *J* = 7.4 Hz, 2H), 7.27 – 7.22 (m, 2H), 6.46 (d, *J* = 10.0 Hz, 1H), 6.19 (d, *J* = 9.6 Hz, 1H), 5.56 – 5.48 (m, 1H), 5.33 (s, 1H), 5.29 – 5.22 (m, 1H), 4.97 (d, *J* = 3.1 Hz, 1H), 4.90 – 4.83 (m, 2H), 4.80 (td, *J* = 11.4, 4.4 Hz, 1H), 4.60 (d, *J* = 7.9 Hz, 1H), 4.48 – 4.36 (m, 4H), 4.35 – 4.24 (m, 3H), 4.21 – 4.07 (m, 3H), 4.02 – 3.87 (m, 4H), 3.87 – 3.81 (m, 2H), 3.80 (s, 3H), 3.77 (t, *J* = 6.1 Hz, 1H), 3.63 – 3.57 (m, 1H), 2.52 (dd, *J* = 12.6, 4.3 Hz, 1H), 2.19 (s, 3H), 2.11 (s, 3H), 2.07 (s, 3H), 2.05 (s, 3H), 2.03 (s, 6H), 2.01 (s, 3H), 1.99 (s, 6H), 1.96 (s, 3H), 1.78 (s, 3H), 1.59 (t, *J* = 12.4 Hz, 1H), 1.24 – 1.20 (m, 3H). ^{13}C NMR (176 MHz, $CDCl_3$ + 6% CD_3OD) δ 171.83, 171.67, 171.23, 171.19, 170.94, 170.77, 170.67, 170.52, 170.08, 170.00, 168.08, 157.21, 143.86, 143.79, 141.35, 141.32, 127.80, 127.09, 127.06, 125.05, 124.98, 120.06, 120.04, 101.31, 99.31, 96.78, 76.61, 73.78, 71.77, 71.29, 70.58, 69.51, 69.44, 69.39, 67.81, 67.76, 67.42, 67.34, 67.06, 63.38, 62.97, 61.74, 58.78, 53.13, 49.08, 48.52, 47.24, 37.40, 22.72, 21.47, 20.88, 20.79, 20.71, 20.68, 20.63, 18.62. HRMS (ESI) m/z calcd for $C_{63}H_{79}N_3O_{32}$ $[M+H]^+$: 1390.4720; found: 1390.4714.

2.8. Synthesis of **Ser4**

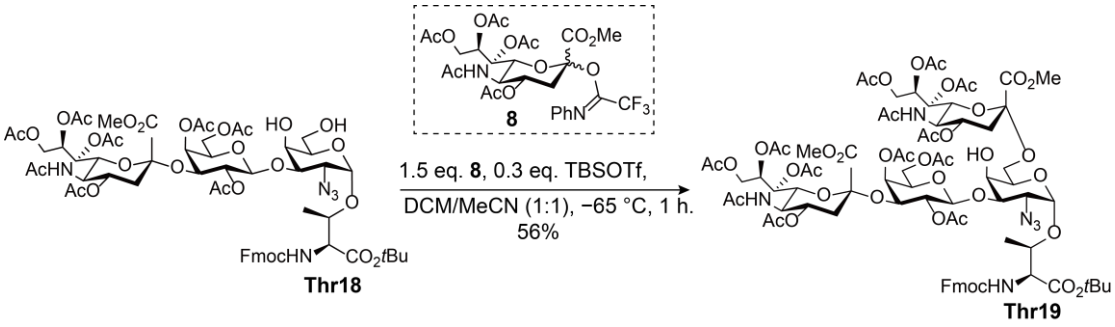


A mixture of **Ser6** (123.5 mg, 0.216 mmol), $PhB(OH)_2$ (27.7 mg, 0.227 mmol), and activated 4 Å molecular sieves (2 g) in anhydrous DCM (20 mL) was stirred under an argon atmosphere overnight. To this reaction mixture, a solution of **9d** (400 mg, 0.433 mmol) in anhydrous DCM (0.8 mL) was added, followed by the addition of TfOH (9.6 µL, 0.108 mmol) in anhydrous DCM (0.2 mL). The reaction mixture was stirred for an additional 1 h. The reaction mixture was diluted with DCM (40 mL) and subsequently filtered through a pad of Celite. $NaBO_3 \cdot 4H_2O$ (32 eq.) and H_2O (20 mL) were added, and the reaction mixture was stirred for 1 h. The organic phase was separated and washed successively with saturated aqueous $NaHCO_3$ (3 × 60 mL), distilled water (2 × 60 mL), and brine (60 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The crude residue was purified by flash column chromatography on silica gel (DCM/MeOH, 60:1 → 40:1) to afford **Ser18** (250 mg, 0.188 mmol, 87%). ^1H NMR (400 MHz, $CDCl_3$) δ 7.79 – 7.72 (m, 2H), 7.65 – 7.58 (m, 2H), 7.44 – 7.35 (m, 2H), 7.31 (td, *J* = 7.5, 1.2 Hz, 2H), 6.06 (d, *J* = 8.3 Hz, 1H), 5.55 (ddd, *J* = 9.0, 4.9, 3.0 Hz, 1H), 5.40 (dd, *J* = 9.1, 2.7 Hz, 1H), 5.16 – 5.06 (m, 2H), 4.96 – 4.81 (m, 4H), 4.64 (dd, *J* = 10.2, 3.4 Hz, 1H), 4.47 – 4.30 (m, 3H), 4.26 – 4.19 (m, 2H), 4.18 – 3.91 (m, 9H), 3.90 – 3.81 (m, 5H), 3.79 – 3.68 (m, 1H), 3.70 – 3.61 (m, 2H), 2.59 (dd, *J* = 12.6, 4.7 Hz, 1H), 2.23 (s, 3H), 2.16 (s, 3H), 2.11 (s, 3H), 2.06 (s, 3H), 2.03 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H), 1.85 (s, 3H), 1.70 (t, *J* = 12.4 Hz, 1H), 1.50 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.01, 170.85, 170.71, 170.58, 170.51, 170.38, 169.91, 169.73, 169.13, 168.16, 156.10, 144.00, 143.93, 141.44, 127.85, 127.23, 125.33, 125.28, 120.13, 101.34, 100.00, 96.89, 83.09, 78.25, 72.25, 71.38, 71.24, 70.61, 70.07, 69.39, 69.31, 68.95, 68.30, 67.96, 67.76, 67.25, 67.11, 62.95, 62.37, 58.61, 55.22, 53.40, 49.30, 47.26, 37.65, 28.09, 23.31, 21.67, 21.11, 20.95, 20.90, 20.81, 20.72. HRMS (ESI) m/z calcd for $C_{60}H_{77}N_5O_{29}$ $[M+H]^+$: 1332.4777; found: 1332.4792.



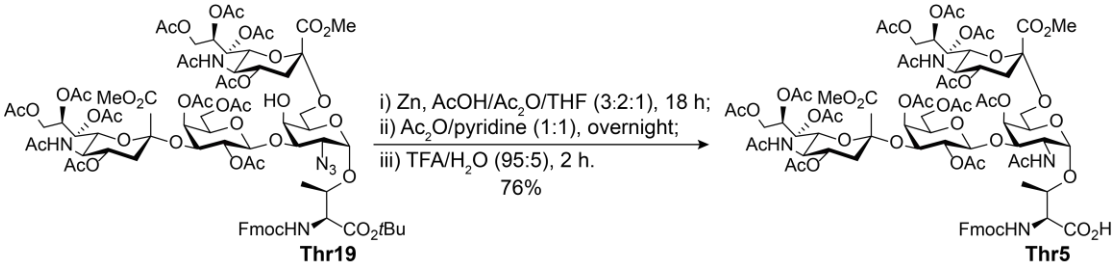
Ser4 was prepared following the general procedure described in Section 1.3 (83% yield). ^{1}H NMR (700 MHz, $CDCl_3$ + 4% CD_3OD) δ 7.73 (d, *J* = 7.4 Hz, 2H), 7.57 (d, *J* = 7.1 Hz, 2H), 7.36 (t, *J* = 7.4 Hz, 2H), 7.29 – 7.25 (m, 2H), 7.13 (d, *J* = 8.0 Hz, 1H), 6.18 (d, *J* = 8.0 Hz, 1H), 5.59 – 5.54 (m, 1H), 5.38 – 5.34 (m, 1H), 5.26 (dd, *J* = 9.1, 2.7 Hz, 1H), 5.02 – 4.99 (m, 1H), 4.90 (dd, *J* = 10.2, 8.1 Hz, 1H), 4.85 (d, *J* = 3.4 Hz, 1H), 4.81 (td, *J* = 11.2, 4.5 Hz, 1H), 4.64 (d, *J* = 7.9 Hz, 1H), 4.49 – 4.24 (m, 6H), 4.18 (t, *J* = 7.0 Hz, 1H), 4.15 (dd, *J* = 11.6, 4.2 Hz, 1H), 4.08 – 3.85 (m, 8H), 3.85 – 3.77 (m, 5H), 3.64 – 3.59 (m, 1H), 2.53 (dd, *J* = 12.6, 4.4 Hz, 1H), 2.21 (s, 3H), 2.14 (s, 3H), 2.08 (s, 3H), 2.06 (s, 3H), 2.05 (s, 3H), 2.01 (s, 6H), 2.00 (s, 3H), 1.99 (s, 3H), 1.97 (s, 3H), 1.80 (s, 3H), 1.62 (t, *J* = 12.3 Hz, 1H). ^{13}C NMR (176 MHz, $CDCl_3$ + 4% CD_3OD) δ 171.90, 171.57, 171.15, 171.07, 171.03, 170.96, 170.79, 170.58, 170.36, 170.19, 170.02, 168.05, 156.26, 143.84, 141.33, 127.84, 127.13, 125.06, 120.10, 101.27, 98.11, 96.80, 73.93, 71.81, 71.29, 70.76, 69.45, 69.35, 69.21, 68.65, 67.63, 67.53, 67.37, 67.11, 63.08, 61.90, 55.07, 53.19, 49.39, 48.66, 47.17, 37.43, 22.95, 22.67, 21.51, 20.98, 20.88, 20.84, 20.77, 20.70, 20.69. HRMS (ESI) m/z calcd for $C_{62}H_{77}N_3O_{32}$ $[M+H]^+$: 1376.4563; found: 1376.4566.

2.9. Synthesis of **Thr5**



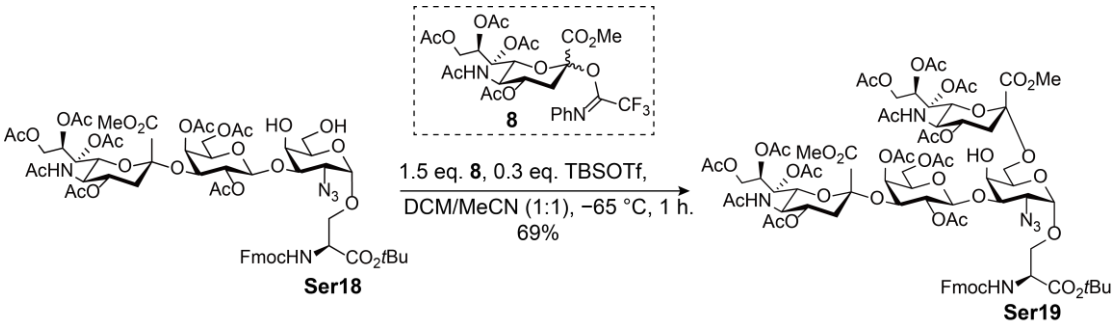
A mixture of **Thr18** (230 mg, 0.17 mmol), **8** (170 mg, 0.26 mmol), and activated 3 Å molecular sieves (500 mg) in anhydrous DCM/MeCN (1:1, 5 mL) was stirred under an argon atmosphere for 30 min and subsequently cooled down to −65 °C. To this reaction mixture, TBSOTf (11.8 µL, 0.051 mmol) in anhydrous DCM/MeCN (1:1, 0.5 mL) was added. The reaction mixture was stirred for an additional 1 h at −65 °C. Subsequently, the reaction was quenched with Et_3N (0.4 eq.), and the reaction mixture was warmed to room temperature. The resulting reaction mixture was filtered through a pad of Celite and concentrated under reduced pressure. The crude residue was purified by flash column chromatography on silica gel (hexane/acetone, 2:1 → 1:1) to afford **Thr19** (175 mg, 0.096 mmol, 56%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (d, *J* = 7.6 Hz, 2H), 7.65 – 7.57 (m, 2H), 7.38 (t, *J* = 7.5 Hz, 2H), 7.31 – 7.26 (m, 2H), 5.74 (d, *J* = 9.6 Hz, 1H), 5.58 – 5.50 (m, 1H), 5.41 – 5.27 (m, 3H), 5.18 – 5.00 (m, 4H), 4.99 – 4.84 (m, 4H), 4.65 (dd, *J* = 10.3, 3.3 Hz, 1H), 4.49 – 4.20 (m, 8H), 4.15 – 3.90 (m, 11H), 3.85 (s, 3H), 3.80 (s, 3H), 3.67 – 3.50 (m, 3H), 2.62 – 2.54 (m, 2H), 2.25 (s, 3H), 2.14 (s, 3H), 2.13 (s, 3H), 2.12 (s, 3H), 2.11 (s, 3H), 2.04 (s, 3H), 2.03 (s, 3H), 2.02 (s, 3H), 2.00 (s, 6H), 1.98 – 1.92 (m, 4H), 1.87 (s, 3H), 1.84 (s, 3H), 1.70 (t, *J* = 12.5 Hz, 1H), 1.51 (s, 9H), 1.38 (d, *J* = 6.3 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.09, 171.01, 170.80, 170.48, 170.42, 170.39, 170.26, 170.15, 169.95, 169.73, 169.36, 168.21, 168.16, 157.05, 144.07, 141.40, 127.83, 127.23, 125.41, 120.10, 101.57, 99.99, 98.82, 96.92, 82.98, 77.83,

76.34, 72.96, 72.26, 71.30, 71.03, 69.48, 69.40, 69.20, 69.12, 68.90, 68.19, 68.16, 67.72, 67.65, 67.54, 63.98, 62.49, 61.88, 59.58, 58.81, 53.36, 53.01, 49.62, 49.33, 47.24, 37.67, 28.15, 23.36, 23.33, 21.66, 21.19, 20.99, 20.96, 20.91, 20.85, 20.78, 19.28. HRMS (ESI) m/z calcd for $C_{81}H_{106}N_6O_{41}$ $[M+H]^+$: 1819.6467; found: 1819.6472.



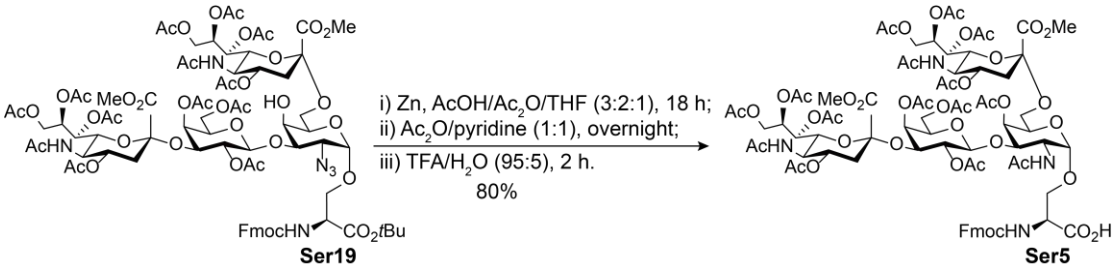
Thr5 was prepared following the general procedure described in Section 1.3 (76% yield). ^{1}H NMR (700 MHz, $CDCl_3$ + 8% CD_3OD) δ 7.71 (d, *J* = 7.5 Hz, 2H), 7.56 (d, *J* = 7.5 Hz, 2H), 7.34 (t, *J* = 7.4 Hz, 2H), 7.24 (t, *J* = 6.5 Hz, 2H), 6.89 (d, *J* = 9.2 Hz, 1H), 6.56 (d, *J* = 9.6 Hz, 2H), 6.26 (d, *J* = 9.6 Hz, 1H), 5.47 (td, *J* = 8.0, 6.9, 2.7 Hz, 1H), 5.30 – 5.22 (m, 4H), 4.91 (d, *J* = 3.4 Hz, 1H, H-1), 4.87 – 4.82 (m, 2H), 4.81 – 4.72 (m, 2H), 4.58 (d, *J* = 8.0 Hz, 1H), 4.42 – 4.34 (m, 4H), 4.32 – 4.24 (m, 4H), 4.19 (t, *J* = 6.8 Hz, 1H), 4.06 – 3.95 (m, 6H), 3.92 (t, *J* = 10.5 Hz, 1H), 3.86 – 3.80 (m, 2H), 3.80 – 3.73 (m, 7H), 3.71 (dd, *J* = 10.2, 7.3 Hz, 1H), 3.58 (dd, *J* = 10.4, 2.6 Hz, 1H), 3.30 (dd, *J* = 10.3, 3.8 Hz, 1H), 2.52 – 2.47 (m, 2H), 2.17 (s, 3H), 2.09 (s, 3H), 2.08 (s, 3H), 2.06 (s, 3H), 2.04 (s, 3H), 2.03 (s, 3H), 2.01 (s, 3H), 1.98 (s, 3H), 1.98 (s, 3H), 1.97 (s, 3H), 1.96 (s, 3H), 1.96 (s, 3H), 1.94 (s, 3H), 1.83 (t, *J* = 12.5 Hz, 1H), 1.79 (s, 3H), 1.76 (s, 3H), 1.57 (t, *J* = 12.4 Hz, 1H), 1.26 (d, *J* = 6.3 Hz, 3H). ^{13}C NMR (176 MHz, $CDCl_3$ + 8% CD_3OD) δ 171.67, 171.63, 171.31, 171.15, 171.09, 170.69, 170.67, 170.54, 170.51, 170.19, 170.09, 169.96, 168.07, 167.89, 157.16, 143.86, 143.77, 141.32, 141.29, 127.77, 127.75, 127.08, 127.04, 125.02, 124.98, 120.02, 120.00, 101.15, 99.47, 98.62, 96.73, 76.65, 73.54, 72.45, 71.71, 71.28, 70.41, 69.58, 69.53, 69.48, 69.35, 68.93, 68.85, 67.83, 67.51, 67.33, 67.24, 67.02, 64.19, 62.80, 62.40, 61.57, 58.68, 53.06, 52.78, 49.04, 48.77, 48.49, 47.22, 37.38, 22.64, 21.44, 20.90, 20.81, 20.78, 20.75, 20.71, 20.68, 20.65, 20.63, 20.59, 20.57, 18.53. HRMS (ESI) m/z calcd for $C_{81}H_{104}N_4O_{43}$ $[M+H]^+$: 1821.7047; found: 1821.6134.

2.10. Synthesis of **Ser5**



A mixture of **Ser18** (700 mg, 0.53 mmol), **8** (522 mg, 0.79 mmol), and activated 3 Å molecular sieves (2 g) in anhydrous DCM/MeCN (1:1, 18 mL) was stirred under an argon atmosphere for 30 min and subsequently cooled down to −65 °C. To this reaction mixture, TBSOTf (30 μL, 0.13 mmol) in anhydrous DCM/MeCN (1:1, 0.5 mL) was added. The reaction mixture was stirred for an additional 1 h at −65 °C. Subsequently, the reaction was quenched with Et_3N (0.4 eq.), and the reaction mixture was warmed to room temperature. The resulting reaction mixture was filtered through a pad of Celite and concentrated under reduced

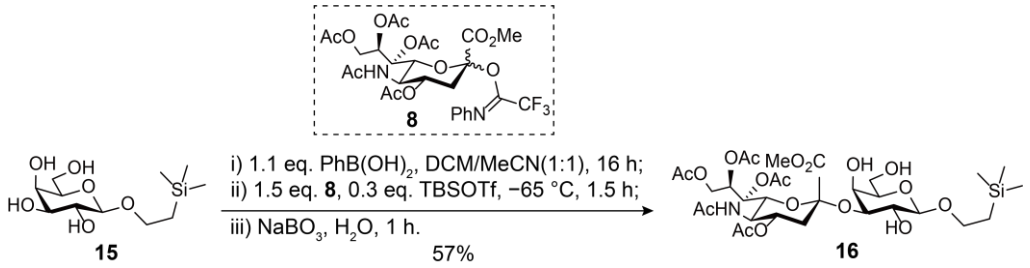
pressure. The crude residue was purified by flash column chromatography on silica gel (hexane/acetone, 2:1 → 1:2) to afford **Ser19** (658 mg, 0.36 mmol, 69%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 – 7.72 (m, 2H), 7.61 (d, *J* = 7.3 Hz, 2H), 7.43 – 7.35 (m, 2H), 7.34 – 7.27 (m, 2H), 5.88 (d, *J* = 8.4 Hz, 1H), 5.60 – 5.51 (m, 1H), 5.44 – 5.35 (m, 2H), 5.31 (dd, *J* = 7.6, 1.7 Hz, 1H), 5.16 – 5.03 (m, 3H), 4.99 – 4.76 (m, 5H), 4.65 (ddd, *J* = 10.2, 5.2, 3.5 Hz, 1H), 4.49 – 4.29 (m, 4H), 4.28 – 4.15 (m, 2H), 4.14 – 3.87 (m, 12H), 3.87 – 3.71 (m, 8H), 3.69 – 3.49 (m, 3H), 2.63 – 2.54 (m, 2H), 2.24 (s, 3H), 2.17 (s, 3H), 2.15 (s, 3H), 2.11 (s, 3H), 2.10 (s, 3H), 2.06 (s, 3H), 2.02 (s, 3H), 2.01 (s, 9H), 1.99 (s, 3H), 1.96 (t, *J* = 12.4 Hz, 1H), 1.87 (s, 3H), 1.85 (s, 3H), 1.70 (t, *J* = 12.4 Hz, 1H), 1.51 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.06, 171.02, 170.83, 170.47, 170.39, 170.33, 170.29, 170.14, 169.99, 169.79, 168.18, 168.15, 156.11, 144.09, 143.99, 141.39, 127.86, 127.23, 125.37, 120.11, 101.51, 99.33, 98.88, 96.92, 82.87, 77.99, 72.98, 72.30, 71.29, 71.06, 69.52, 69.41, 69.15, 68.75, 68.09, 67.93, 67.69, 67.57, 67.44, 67.20, 63.77, 62.89, 62.53, 62.25, 61.82, 58.85, 55.00, 53.35, 52.95, 49.58, 49.33, 47.22, 37.67, 28.12, 23.35, 23.33, 21.64, 21.19, 20.95, 20.92, 20.89, 20.85, 20.75. HRMS (ESI) m/z calcd for $C_{80}H_{104}N_6O_{41}$ $[M+H]^+$: 1805.6310; found: 1805.6312.



Ser5 was prepared following the general procedure described in Section 1.3 (80% yield). ^{1}H NMR (700 MHz, $CDCl_3$ + 8% CD_3OD) δ 7.68 (d, *J* = 7.5 Hz, 2H), 7.53 (d, *J* = 7.2 Hz, 2H), 7.32 (t, *J* = 7.4 Hz, 2H), 7.24 – 7.21 (m, 2H), 5.48 (t, *J* = 8.1 Hz, 1H), 5.31 – 5.18 (m, 4H), 4.91 – 4.87 (m, 1H), 4.83 – 4.79 (m, 2H), 4.75 (dt, *J* = 10.6, 5.4 Hz, 1H), 4.70 (ddd, *J* = 10.3, 9.9, 4.8 Hz, 1H), 4.59 (d, *J* = 7.9 Hz, 1H), 4.38 (dd, *J* = 10.2, 3.4 Hz, 1H), 4.35 (dd, *J* = 10.8, 7.0 Hz, 1H), 4.30 – 4.26 (m, 2H), 4.25 – 4.20 (m, 3H), 4.14 (t, *J* = 6.8 Hz, 1H), 4.01 – 3.84 (m, 10H), 3.79 (dd, *J* = 12.4, 6.6 Hz, 1H), 3.75 – 3.72 (m, 4H), 3.72 – 3.67 (m, 4H), 3.57 – 3.54 (m, 1H), 3.21 (dd, *J* = 9.7, 4.2 Hz, 1H), 2.51 – 2.45 (m, 2H), 2.14 (s, 3H), 2.08 (s, 3H), 2.03 (s, 6H), 2.01 (s, 3H), 2.00 (s, 3H), 1.99 (s, 3H), 1.95 (s, 3H), 1.94 (s, 3H), 1.93 (s, 3H), 1.91 (s, 9H), 1.82 (t, *J* = 12.2 Hz, 1H), 1.76 (s, 3H), 1.73 (s, 3H), 1.53 (t, *J* = 12.3 Hz, 1H). ^{13}C NMR (176 MHz, $CDCl_3$ + 8% CD_3OD) δ 171.57, 171.44, 171.39, 171.29, 171.08, 171.05, 171.01, 170.65, 170.62, 170.40, 170.21, 170.16, 170.14, 169.87, 167.89, 167.63, 156.39, 143.69, 143.66, 141.17, 127.67, 126.98, 124.89, 119.91, 100.93, 98.44, 98.11, 96.58, 73.54, 72.22, 71.45, 71.06, 70.39, 69.52, 69.48, 69.36, 69.17, 68.71, 68.43, 68.30, 67.40, 67.32, 67.11, 66.88, 63.60, 62.63, 62.28, 61.58, 54.97, 52.90, 52.63, 49.18, 48.48, 48.25, 47.02, 37.44, 37.22, 22.43, 22.35, 21.29, 20.77, 20.73, 20.64, 20.61, 20.58, 20.55, 20.51, 20.48, 20.45. HRMS (ESI) m/z calcd for $C_{80}H_{102}N_4O_{43}$ $[M+H]^+$: 1807.5991; found: 1807.5981.

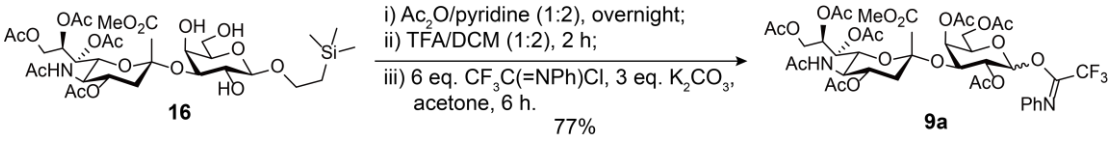
3. Synthesis of sialyl galactosyl donors

3.1. Synthesis of **16**



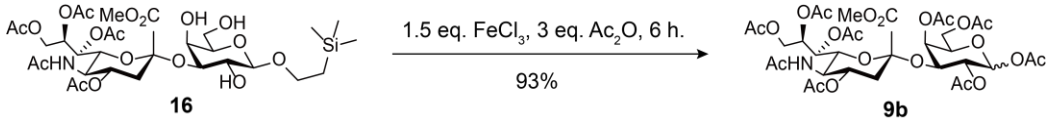
A mixture of **15** (413 mg, 1.47 mmol), $PhB(OH)_2$ (235 mg, 1.55 mmol), and activated 4 Å molecular sieves (4 g) in anhydrous DCM/MeCN (1:1, 34 mL) was stirred under an argon atmosphere overnight and subsequently cooled down to −65 °C. To this reaction mixture, TBSOTf (102 µL, 0.444 mmol) in anhydrous DCM/MeCN (1:1, 1 mL) was added, followed by the addition of **8** (1.47 g, 2.21 mmol) in anhydrous DCM/MeCN (1:1, 5 mL). The reaction mixture was stirred for an additional 1.5 h at −65 °C and quenched by the addition of Et_3N (0.4 eq.) in DCM (1 mL), followed by warming to room temperature. The reaction mixture was then diluted with DCM (80 mL) and subsequently filtered through a pad of Celite. The organic phase was separated and washed successively with saturated aqueous $NaHCO_3$ (3 × 120 mL), distilled water (2 × 120 mL), and brine (120 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The crude residue was purified by flash column chromatography on silica gel (DCM/MeOH, 50:1 → 45:1 → 40:1) to afford **16** (630 mg, 0.835 mmol, 57%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.43 (ddd, *J* = 8.6, 5.8, 2.7 Hz, 1H), 5.30 (dd, *J* = 8.8, 1.9 Hz, 1H), 5.24 (d, *J* = 9.6 Hz, 1H), 4.95 (ddd, *J* = 12.1, 9.9, 4.6 Hz, 1H), 4.42 (d, *J* = 7.7 Hz, 1H), 4.30 (dd, *J* = 12.5, 2.7 Hz, 1H), 4.10 – 3.96 (m, 5H), 3.93 – 3.84 (m, 2H), 3.82 (s, 3H), 3.74 (dd, *J* = 3.4, 1.2 Hz, 1H), 3.71 – 3.61 (m, 2H, H-2), 3.54 (t, *J* = 5.5, 1.1 Hz, 1H), 2.69 (dd, *J* = 13.0, 4.7 Hz, 1H), 2.13 (s, 3H), 2.12 (s, 3H), 2.08 (dd, *J* = 3.8, 2.8 Hz, 1H), 2.03 (s, 3H), 2.03 (s, 3H), 1.89 (s, 3H), 1.15 – 0.93 (m, 2H), 0.01 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.87, 170.70, 170.27, 170.12, 170.03, 168.23, 102.53, 97.70, 76.81, 73.62, 72.66, 69.29, 68.58, 68.55, 68.17, 67.16, 67.03, 62.51, 62.24, 53.21, 49.94, 37.41, 23.15, 21.19 20.80, 20.76, 20.73, 18.18, -1.43. HRMS (ESI) m/z calcd for $C_{31}H_{51}NO_{18}Si$ $[M+Na]^+$: 776.2768; found: 776.2747.

3.2. Synthesis of **9a**, **9b**, **9c** and **9d**

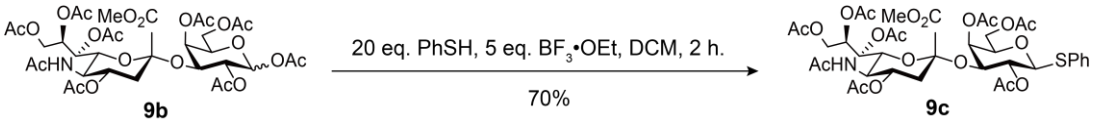


16 (400 mg, 0.531 mmol) was dissolved in a solution composed of Ac_2O (8.8 mL) and pyridine (17.4 mL). The resulting mixture was stirred overnight. Following this, the reaction mixture was diluted with EtOAc (250 mL) and washed successively with 1 M aqueous HCl (10 × 30 mL), saturated aqueous $NaHCO_3$ (5 × 60 mL), distilled water (3 × 120 mL), and brine (250 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was dissolved in a solution composed of TFA (3.6 mL) and DCM (7.2 mL) and allowed to stir for 2 h. The reaction mixture was concentrated, re-dissolved in DCM (50 mL) and washed successively with saturated aqueous $NaHCO_3$ (3 × 50 mL), distilled water (2 × 50 mL), and brine (50 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced

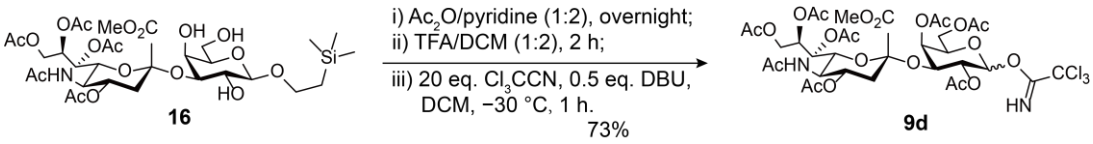
pressure. Azeotropic distillation with toluene followed by vacuum drying was employed to ensure complete removal of residual water. The resulting residue was dissolved in acetone (17.4 mL). K_2CO_3 (220 mg, 1.59 mmol) and $CF_3C(=NPh)Cl$ (0.51 mL, 3.18 mmol) were added. The reaction mixture was stirred for 6 h. Subsequently, it was filtered through a pad of Celite and concentrated under reduced pressure. The crude residue was purified by flash column chromatography on silica gel (hexane/EtOAc, 1:2 → 1:4 → 0:1) to afford **9a** (390 mg, 0.410 mmol, 77%). The spectroscopic data (^1H and ^{13}C NMR) were in good agreement with those reported in the literature.[1]



16 (540 mg, 0.716 mmol) was dissolved in Ac_2O (11 mL), and $FeCl_3$ (180 mg, 1.11 mmol) was added. The resulting mixture was stirred for 1 h. The reaction mixture was then diluted with EtOAc (100 mL) and washed successively with saturated aqueous $NaHCO_3$ (5 × 20 mL), distilled water (5 × 20 mL), and brine (60 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The crude residue was purified by flash column chromatography on silica gel (EtOAc) to afford **9b** (550 mg, 0.669 mmol, 93%). The spectroscopic data (^1H and ^{13}C NMR) were in good agreement with those reported in the literature.[2]



To a solution of **9b** (50 mg, 0.061 mmol) and PhSH (124 µL, 1.22 mmol) in DCM (1.5 mL) was added $BF_3{\cdot}OEt$ (32 µL, 0.304 mmol). The resulting mixture was stirred for 2 h. The reaction mixture was then diluted with DCM (10 mL) and washed successively with saturated aqueous $NaHCO_3$ (3 × 10 mL), distilled water (2 × 10 mL), and brine (10 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The crude residue was purified by flash column chromatography on silica gel (hexane/acetone, 4:1 → 1:1) to afford **9c** (37.4 mg, 0.043 mmol, 70%). The spectroscopic data (^1H and ^{13}C NMR) were in good agreement with those reported in the literature.[3]



16 (390 mg, 0.517 mmol) was dissolved in a solution composed of Ac_2O (8 mL) and pyridine (16 mL). The resulting mixture was stirred overnight. Following this, the reaction mixture was diluted with EtOAc (250 mL) and washed successively with 1 M aqueous HCl (30 mL, ten times), saturated aqueous $NaHCO_3$ (5 × 60 mL), distilled water (3 × 120 mL), and brine (250 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was dissolved in a solution composed of TFA (4 mL) and DCM (8 mL) and allowed to stir for 2 h. The reaction mixture was concentrated, re-dissolved in DCM (50 mL) and washed successively with saturated aqueous $NaHCO_3$ (3 × 50 mL), distilled water (2 × 50 mL), and brine (50 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. Azeotropic distillation with toluene followed by vacuum drying was employed to ensure complete removal of residual water. The resulting residue was dissolved in DCM (10 mL). CCl_3CCN (1.04 mL,

10.4 mmol) was added. The mixture was cooled down to −30 °C and DBU (39 µL, 0.259 mmol) was added. The reaction mixture was stirred at −30 °C for 1 h and then warmed to room temperature. Subsequently, it was filtered through a pad of Celite and concentrated under reduced pressure. The crude residue was purified by flash column chromatography on silica gel (hexane/acetone, 3:1 → 2:1) to afford **9d** (350 mg, 0.379 mmol, 73%). The spectroscopic data (^{1}H and ^{13}C NMR) were in good agreement with those reported in the literature.[4]

4. Synthesis of GHRH glycoforms

4.1. Synthesis of GHRH

Rink Amide AM resin (0.050 mmol, 0.277 mmol/g) was added to the reaction vessel of the peptide synthesizer and washed three times with DMF (3 mL). The resin was allowed to swell in DMF (10 mL) for 5 min. Fmoc deprotection was performed by treating the resin with 20% 4-methylpiperidine in DMF (3 mL) at 50 °C for 5 min, with nitrogen bubbling from the bottom of the vessel to provide agitation. Following deprotection, the resin was washed four times with DMF (4 mL).

For the coupling step, FPAA (0.2 M in DMF, 1.25 mL, 5.0 eq.), DIEA (0.5 M in DMF, 1.0 mL, 10 eq.), and HATU (0.25 M in DMF, 0.9 mL, 4.5 eq.) were added sequentially to the reaction vessel. After incubation at room temperature for 2 min, the reaction mixture was heated to 50 °C and maintained at this temperature for 8 min under continuous nitrogen bubbling. To suppress aspartimide formation, Fmoc-Asp(OMpe)-OH was employed for Asp residues. Double couplings were performed for Arg and for residues following β-branched FPAAs (Ile, Thr, and Val), as well as Pro and Arg, to ensure complete acylation. The deprotection and coupling cycles were repeated until the full peptide sequence was assembled.

Upon completion of SPPS, the resin was washed alternately three times with DMF and DCM (5 mL each). The resin was then treated with a freshly prepared cleavage cocktail (TFA/TIS/H_2O, 95:2.5:2.5, 4 mL) and gently stirred at room temperature for 2 h. The cleavage solution, together with three subsequent washes (0.5 mL each), was collected into a 50 mL centrifuge tube. The crude peptide was precipitated by the addition of ice-cold *tert*-butyl methyl ether (30 mL), followed by vortexing and centrifugation at 8,400 × *g* for 10 min at 4 °C. The supernatant was carefully decanted, and the pellet was dissolved in MeCN/H_2O (1:1, 15 mL) and lyophilized to afford the crude peptide.

The crude peptide was dissolved in MeCN/H_2O (1:4) and purified by preparative HPLC using a linear gradient of 20–40% MeCN (0.05% TFA) in H_2O (0.05% TFA) over 40 min at a flow rate of 4 mL/min, with UV detection at 214 and 254 nm. The desired product was identified by UPLC and HRMS analyses, and the corresponding fractions were collected and lyophilized to furnish GHRH in 33% yield based on resin loading (Fig. S1).

4.2. Synthesis of **G1**

SPPS was performed following the procedure described in Section 4.1. Site-selective glycan introduction at Thr^7 was accomplished using building block **Thr1**. A solution of **Thr1** (0.05 M in DMF, 2 mL, 2.0 eq.) was combined with DIC (0.2 M in DMF, 1 mL, 4.0 eq.) and Oxyma (0.1 M in DMF, 1 mL, 2.0 eq.), incubated at room temperature for 2 min, then heated to 50 °C and maintained for 18 min under continuous nitrogen bubbling. Double coupling was employed for this step. Following cleavage and global deprotection, the crude glycopeptide was purified by preparative HPLC using a linear gradient of 25–40% MeCN (0.05% TFA) in H_2O (0.05% TFA) over 40 min to afford **G1a** in 25% yield (Fig. S2).

G1a (11.2 mg) was dissolved in 40 mM NaOH in MeOH/H_2O (1:3, 6.3 mL) and stirred for 30 min. The reaction was quenched with 20% aq. AcOH, and the product was purified by HPLC using a linear gradient of 20–40% MeCN (0.05% TFA) in H_2O (0.05% TFA) over 40 min to furnish **G1** in 62% yield.

4.3. Synthesis of **G2**

G2a was synthesized in 30% yield following the procedure described for **G1a** in Section 4.2,

with the exception that site-selective glycan introduction at Thr^7 was achieved using **Thr3** in place of **Thr1** (Fig. S3).

G2a (16 mg) was dissolved in 40 mM NaOH in MeOH/H_2O (1:3, 8 mL) and stirred for 1 h. The reaction was quenched with 20% aq. AcOH, and the product was purified by HPLC using a linear gradient of 20–40% MeCN (0.05% TFA) in H_2O (0.05% TFA) over 40 min to afford **G2** in 52% yield.

4.3. Synthesis of **G3**

G3a was synthesized in 28% yield following the procedure described for **G1a** in Section 4.2, with the exception that site-selective glycan introduction at Thr^7 was achieved using **Thr5** in place of **Thr1** (Fig. S4).

G3a (10 mg) was dissolved in 40 mM NaOH in MeOH/H_2O (1:3, 5 mL) and stirred for 2 h. The reaction was quenched with 20% aq. AcOH, and intermediates bearing varying numbers of acetyl groups were partially purified by HPLC using a linear gradient of 20–40% MeCN (0.05% TFA) in H_2O (0.05% TFA,) over 40 min. After lyophilization, the intermediates were subjected to further deprotection with 10% aq. NH_2NH_2 (7 mL). The reaction mixture was stirred for 4 h and then quenched with 0.5% aq. TFA. The desired product **G3** was obtained in 34% yield after HPLC purification using a linear gradient of 20–40% MeCN (0.05% TFA) in H_2O (0.05% TFA) over 40 min.

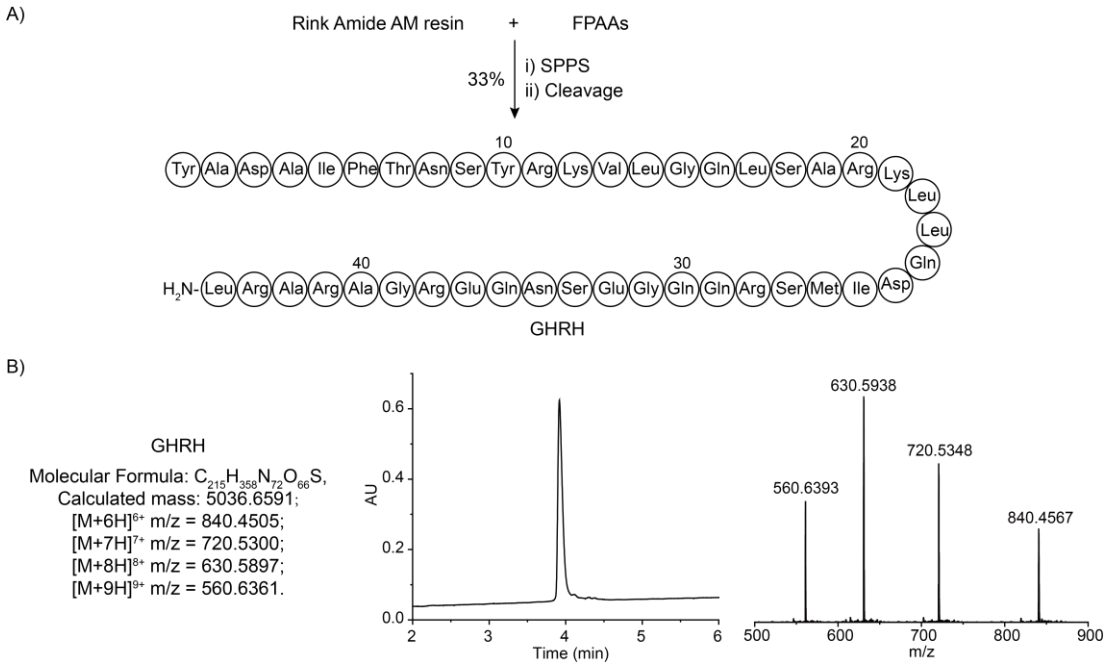


Fig. S1. Synthesis of GHRH. A) Synthesis procedures for GHRH. Reagents and conditions: i) Coupling: HATU, DIEA, DMF, 50 °C, 8 min; Deprotection: 20% 4-methylpiperidine in DMF, 50 °C, 5 min; ii) TFA/TIS/H_2O (95:2.5:2.5), 2 h. B) UPLC and HRMS analysis of purified GHRH using a gradient from 15% to 35% MeCN in H_2O (with 0.1% formic acid) over 6 min and a UV wavelength of 214 nm.

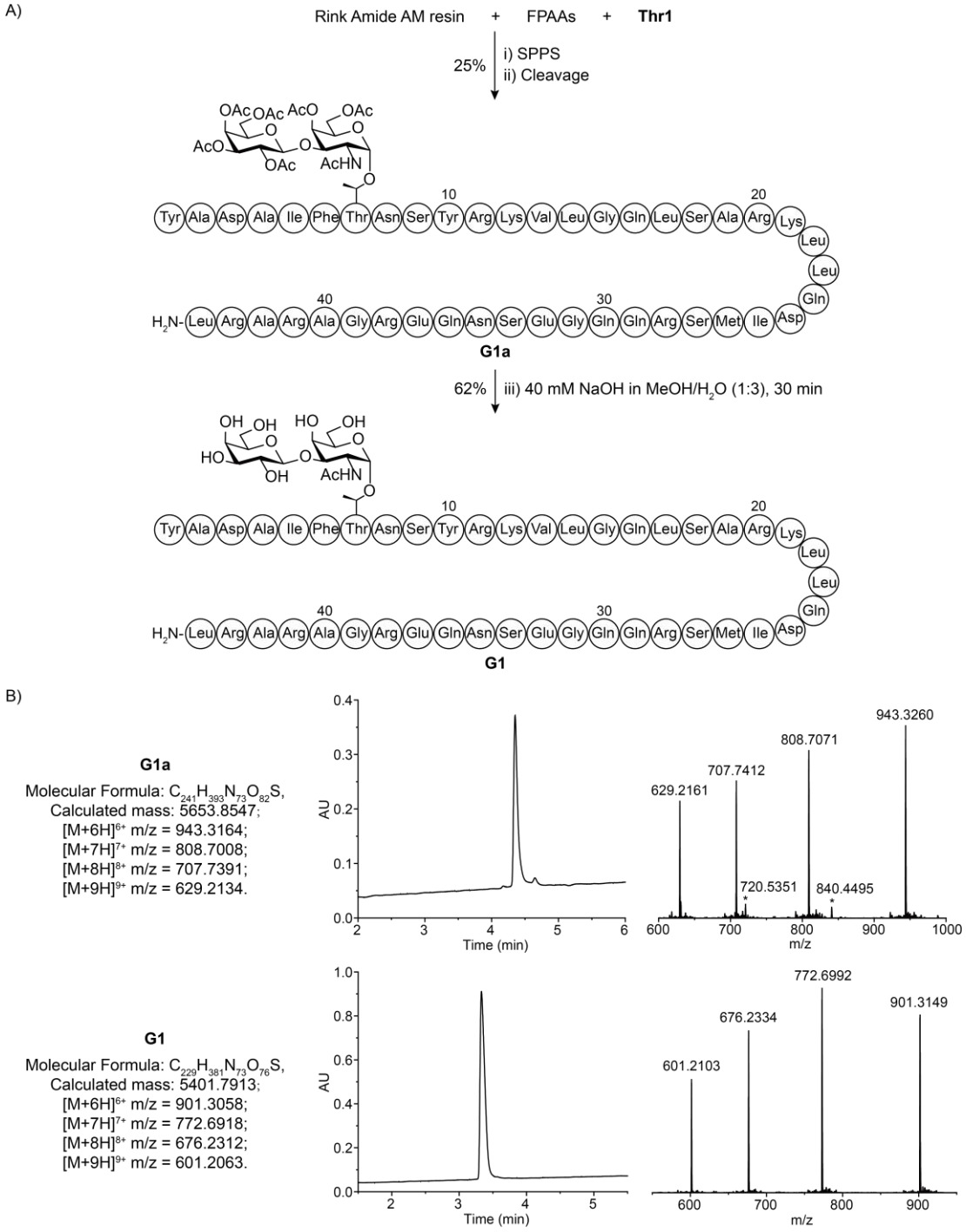


Fig. S2. Synthesis of G1. A) Synthesis procedures for **G1**. Reagents and conditions: i) FPAA coupling: HATU, DIEA, DMF, 50 °C, 8 min; **Thr1** coupling: DIC, Oxyma, DMF, 50 °C, 18 min; Deprotection: 20% 4-methylpiperidine in DMF, 50 °C, 5 min; ii) TFA/TIS/H_2O (95:2.5:2.5), 2 h. B) UPLC and HRMS analysis of purified **G1a** and **G1** using a gradient from 15% to 35% MeCN in H_2O (with 0.1% formic acid) over 6 min and a UV wavelength of 214 nm. The asterisk (*) denotes the glycan-fragmentation ion peak.

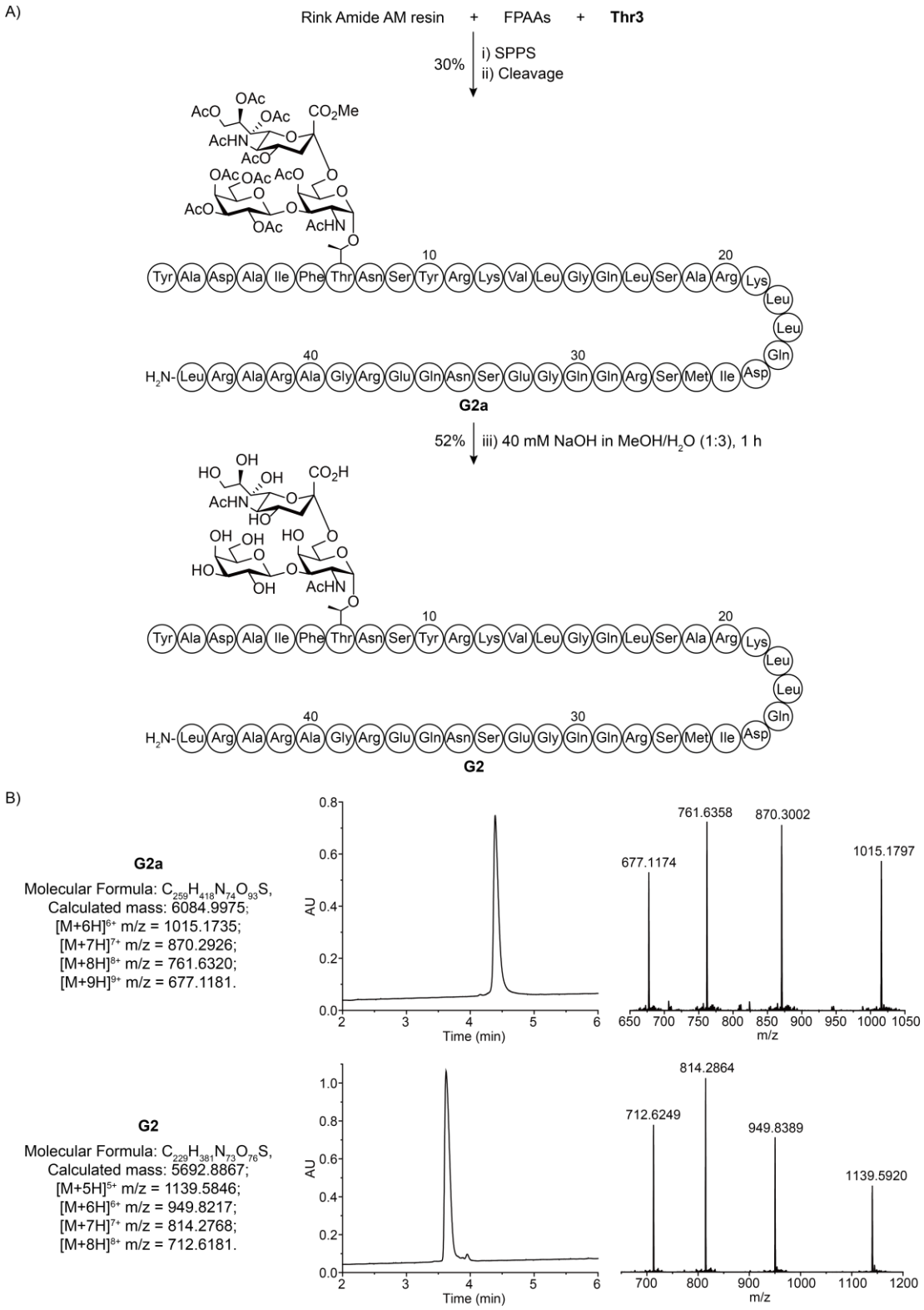


Fig. S3. Synthesis of G2. A) Synthesis procedures for **G2**. Reagents and conditions: i) FPAA coupling: HATU, DIEA, DMF, 50 °C, 8 min; **Thr3** coupling: DIC, Oxyma, DMF, 50 °C, 18 min; Deprotection: 20% 4-methylpiperidine in DMF, 50 °C, 5 min; ii) TFA/TIS/H_2O (95:2.5:2.5), 2 h. B) UPLC and HRMS analysis of purified **G2a** and **G2** using a gradient from 15% to 35% MeCN in H_2O (with 0.1% formic acid) over 6 min and a UV wavelength of 214 nm.

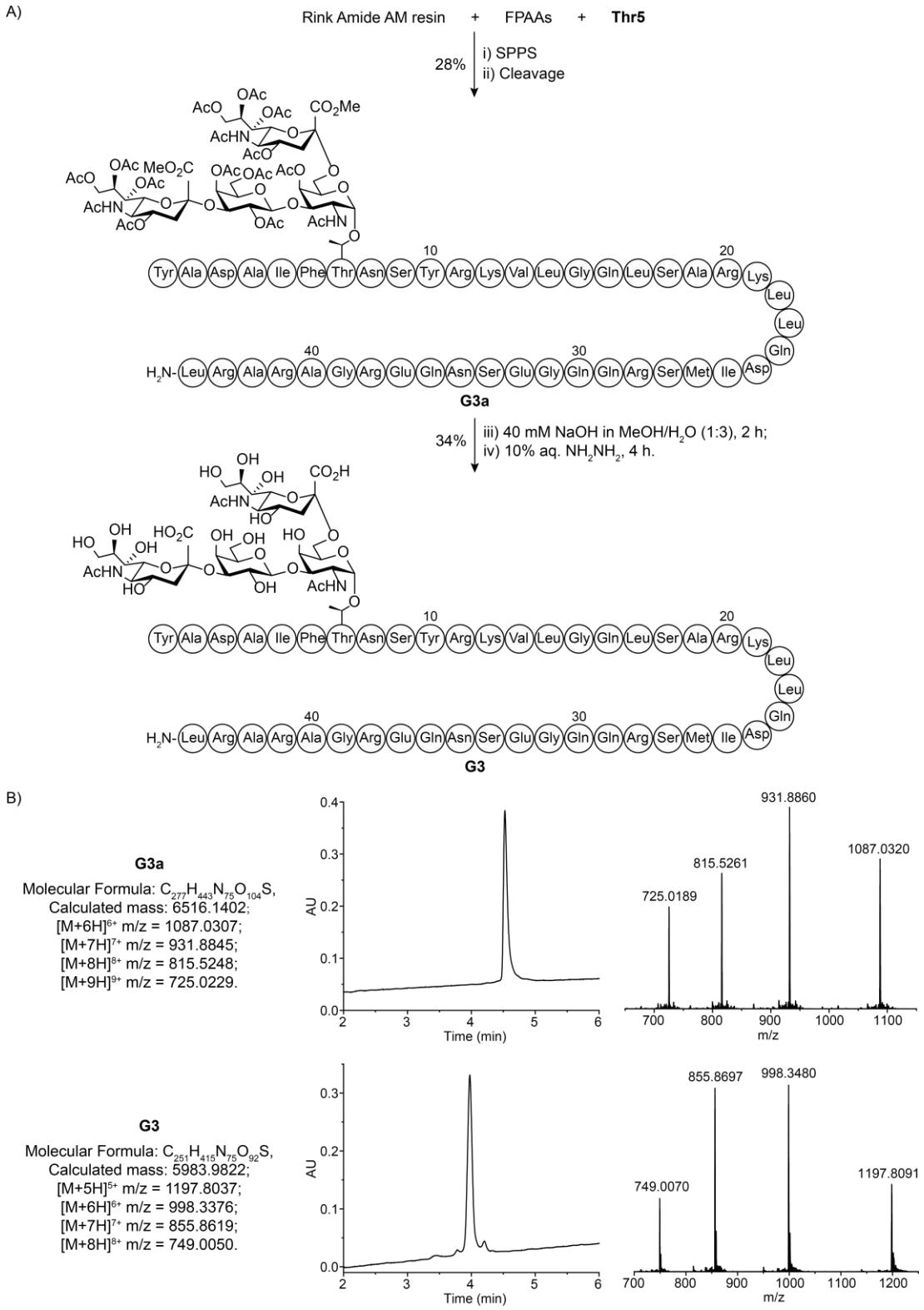


Fig. S4. Synthesis of G3. A) Synthesis procedures for **G3**. Reagents and conditions: i) FPAA coupling: HATU, DIEA, DMF, 50 °C, 8 min; **Thr5** coupling: DIC, Oxyma, DMF, 50 °C, 18 min; Deprotection: 20% 4-methylpiperidine in DMF, 50 °C, 5 min; ii) TFA/TIS/H_2O (95:2.5:2.5), 2 h. B) UPLC and HRMS analysis of purified **G3a** and **G3** using a gradient from 15% to 35% MeCN in H_2O (with 0.1% formic acid) over 6 min and a UV wavelength of 214 nm.

5. Determination of stability of GHRH glycoforms by DPP-IV digestion

A 100 µL solution of each GHRH variant at a concentration of 1 g/L in PBS was incubated at 37 °C for 15 min. Subsequently, 2 µL of DPP-IV stock solution (0.1 µg) was added. The digestion mixture was further incubated at 37 °C for 210 min, during which samples were withdrawn at different time intervals for UPLC and MS analysis. The quantity of the remaining GHRH variants in the digestion mixture was determined based on the UV peak area corresponding to the intact structure. Nonlinear fitting was performed using the equation:

$$y = A1 * e^{-\frac{x}{t1}}$$

where y represents the UV peak area and x is the digestion time. The coefficient t1 derived from the fitting was used to calculate the half-life of each GHRH variant in the presence of DPP-IV using the equation (Table S1):

$$Half\text{-}life = t1 * ln2.$$

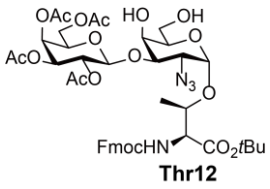
Table S1. Calculated half-lives of the GHRH variants in the presence of DPP-IV.

Variant	**Half-life (min)**			**Mean**	**SD**
	Rep1	Rep2	Rep3		
GHRH	13.5	14.3	17.2	15.0	1.6
G1	22.8	22.7	22.4	22.6	0.2
G2	41.9	44.0	43.0	43.0	0.9
G3	65.7	75.2	85.6	75.5	8.1

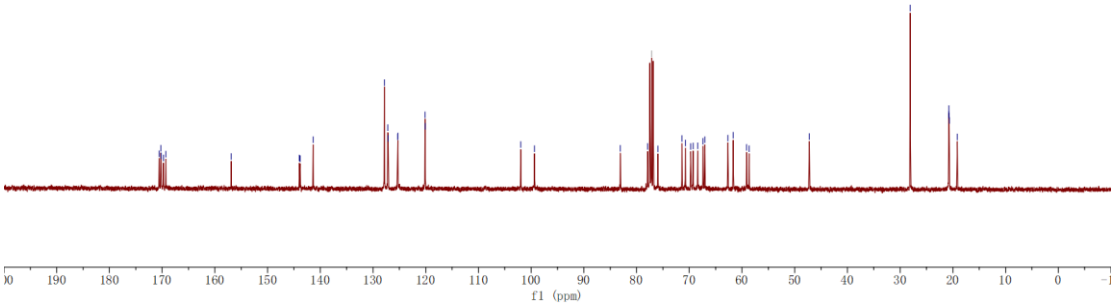
References

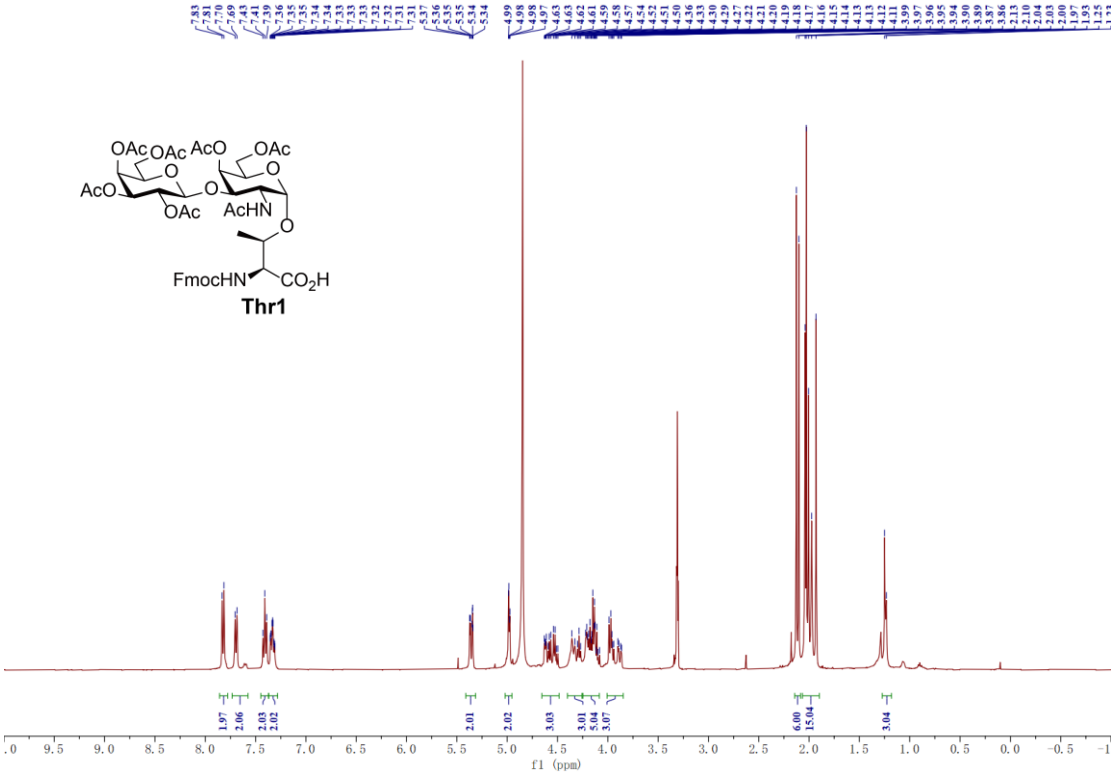
1. Y. Liu, X. Ruan, X. Li and Y. Li, J. Org. Chem., 2008, 73, 4287-4290.
2. A. A. Sherman, O. N. Yudina, B. S. Komarova, Y. E. Tsvetkov, S. Iacobelli and N. E. Nifantiev, Synthesis-Stuttgart, 2005, 11, 1783-1788.
3. S. Mehta, M. Gilbert, W. W. Wakarchuk and D. M. Whitfield, Org. Lett., 2000, 2, 751-753.
4. H. Qu, J.-M. Liu, J. Wdzieczak-Bakala, D. Lu, X. He, W. Sun, M. Sollogoub and Y. Zhang, Eur. J. Med. Chem., 2014, 75, 247-257.

NMR spectra



Thr12

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Thr1
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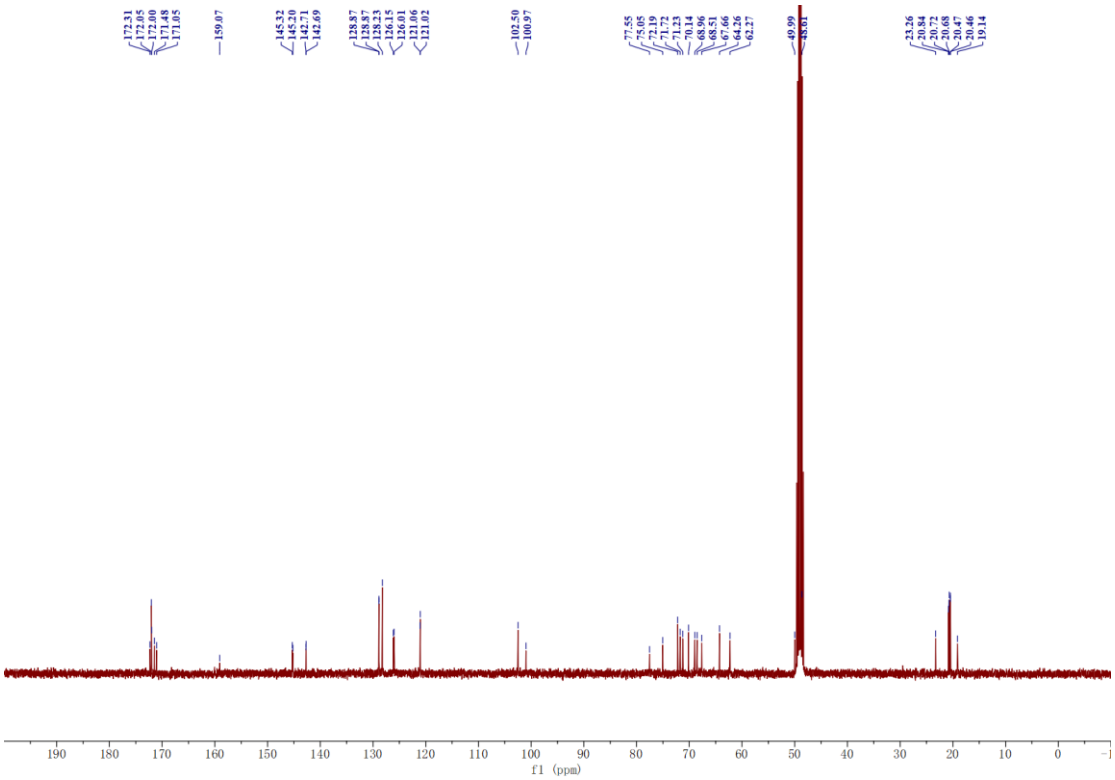
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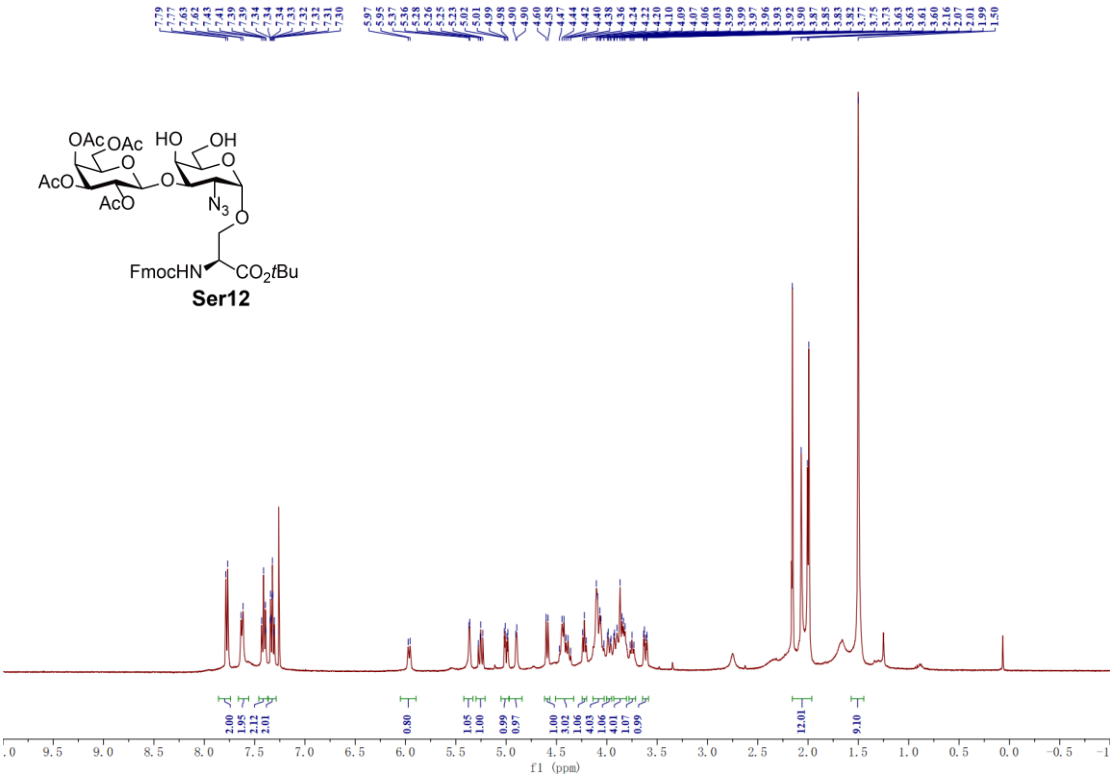
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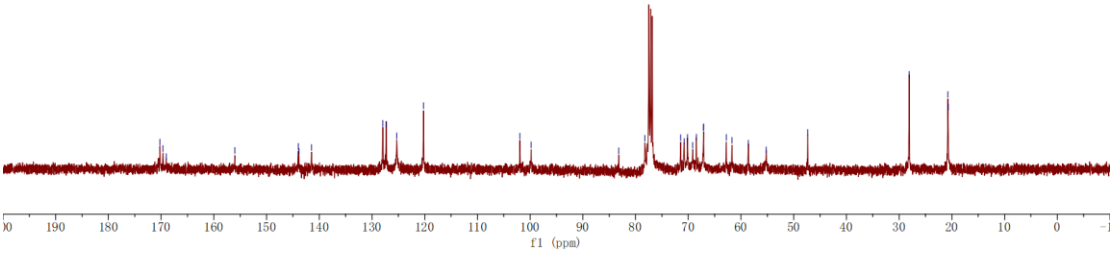
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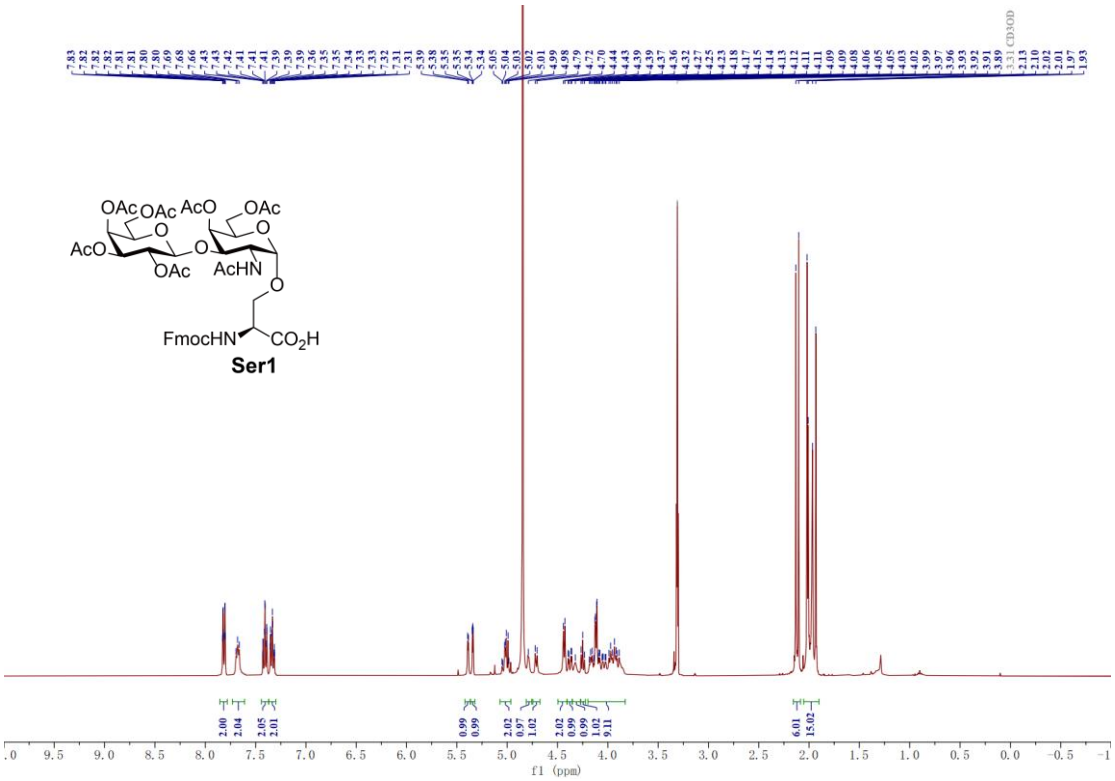
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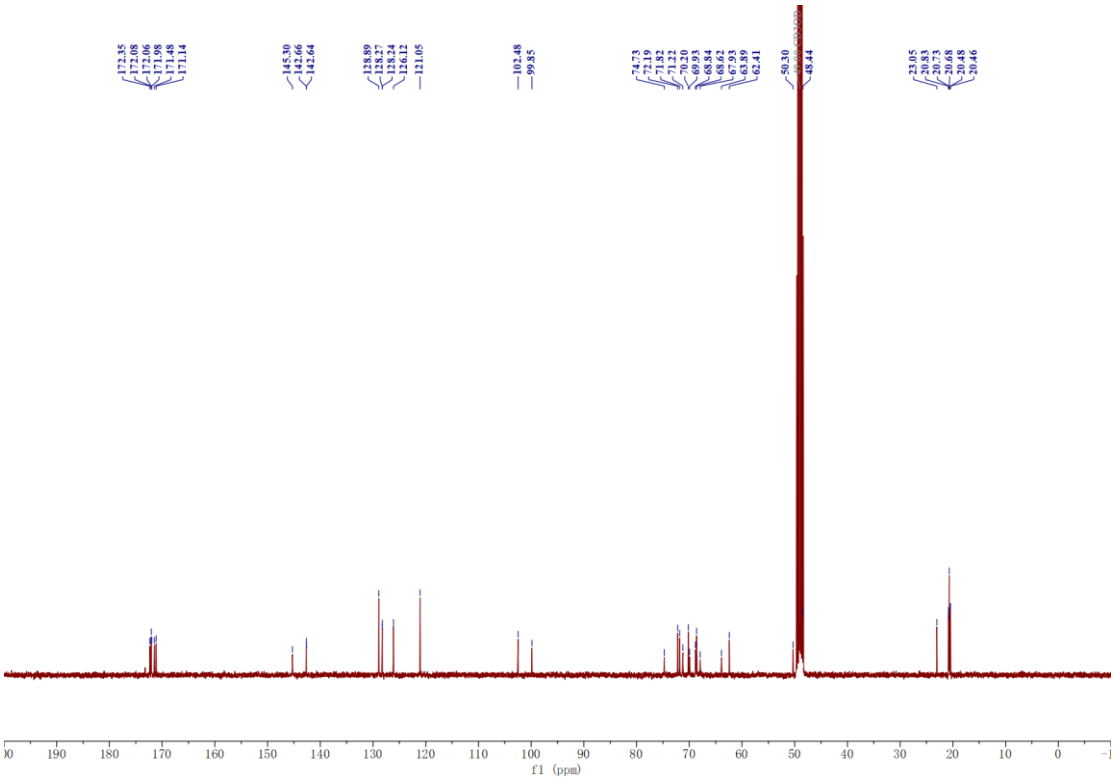
f1 (ppm)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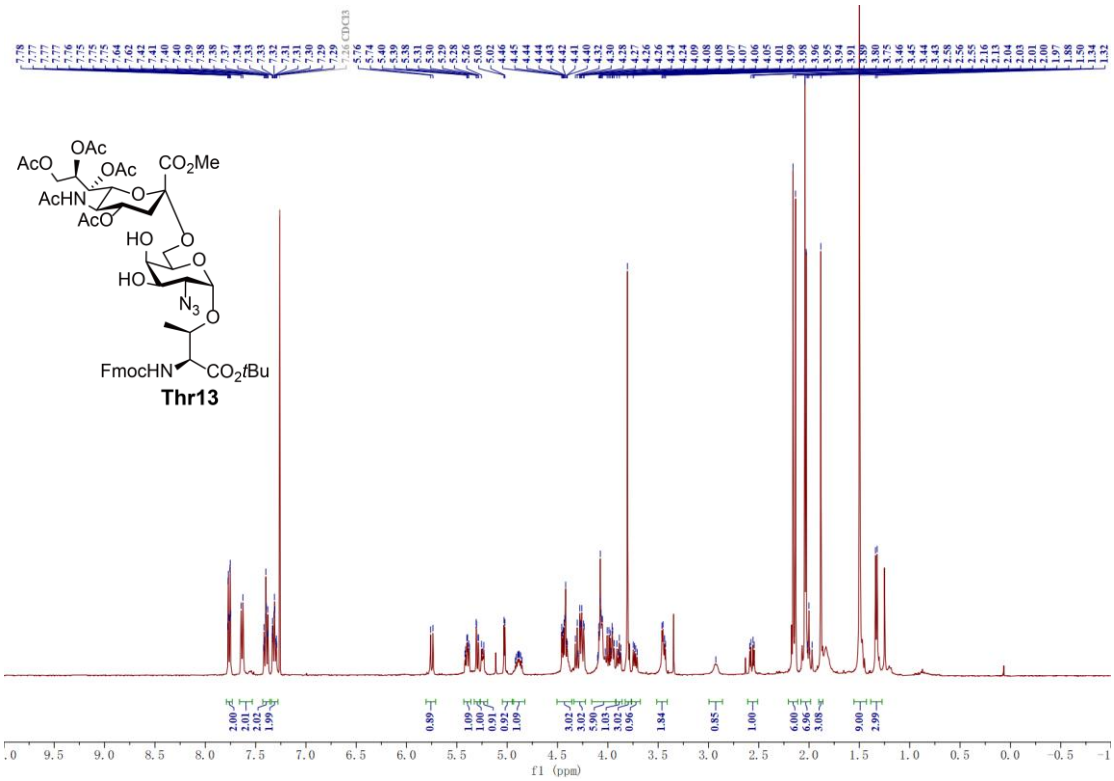
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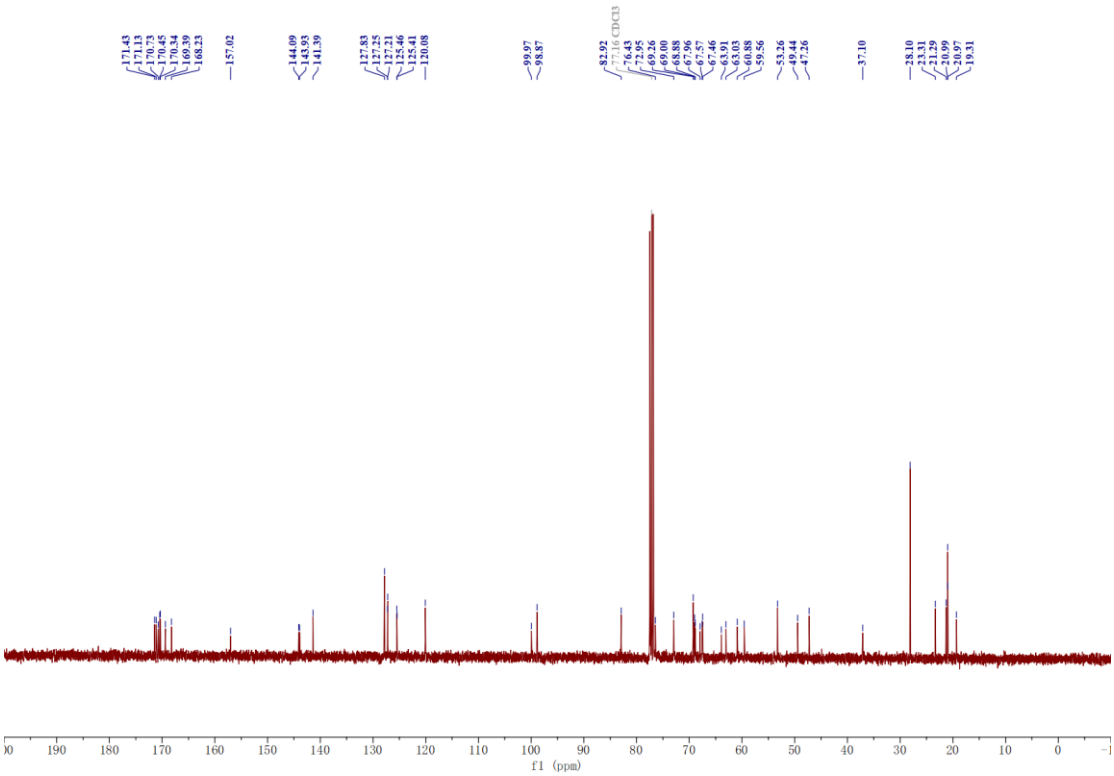
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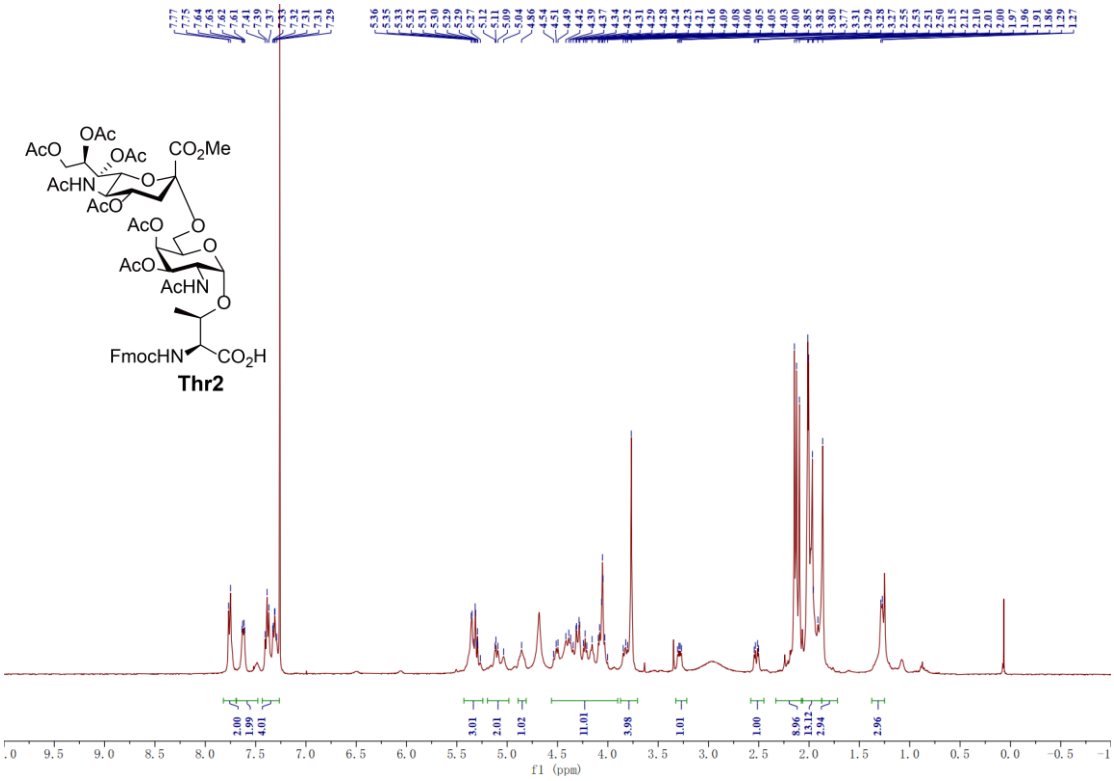
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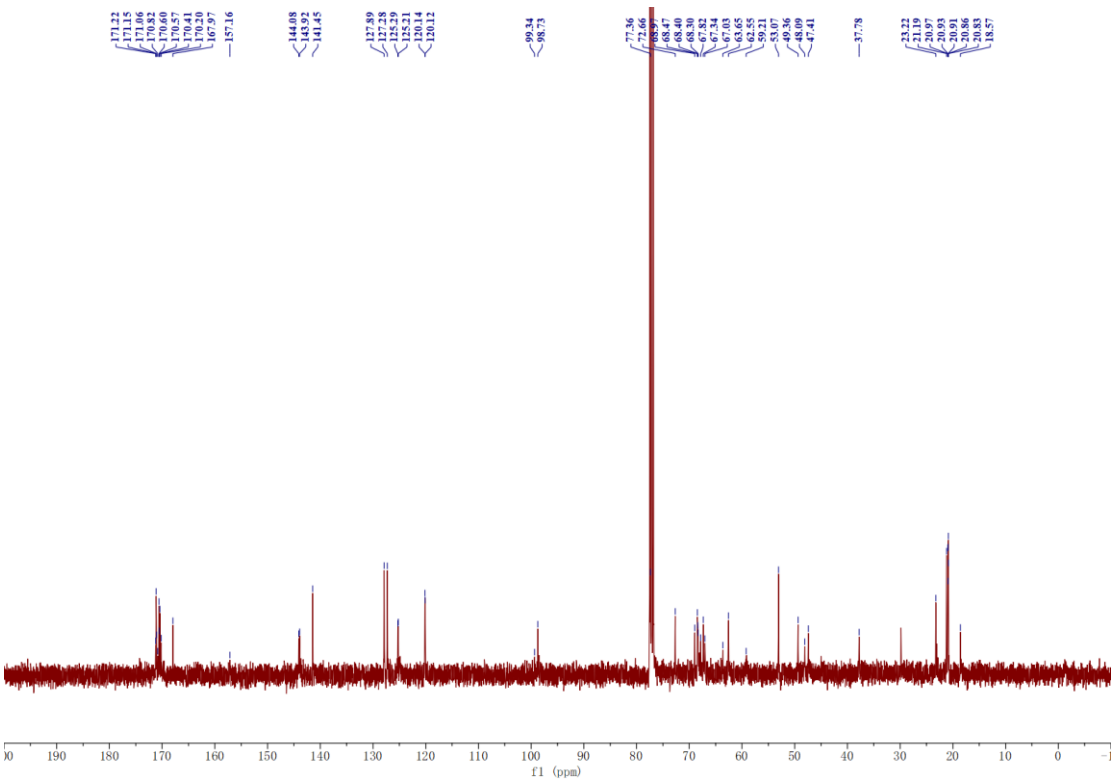
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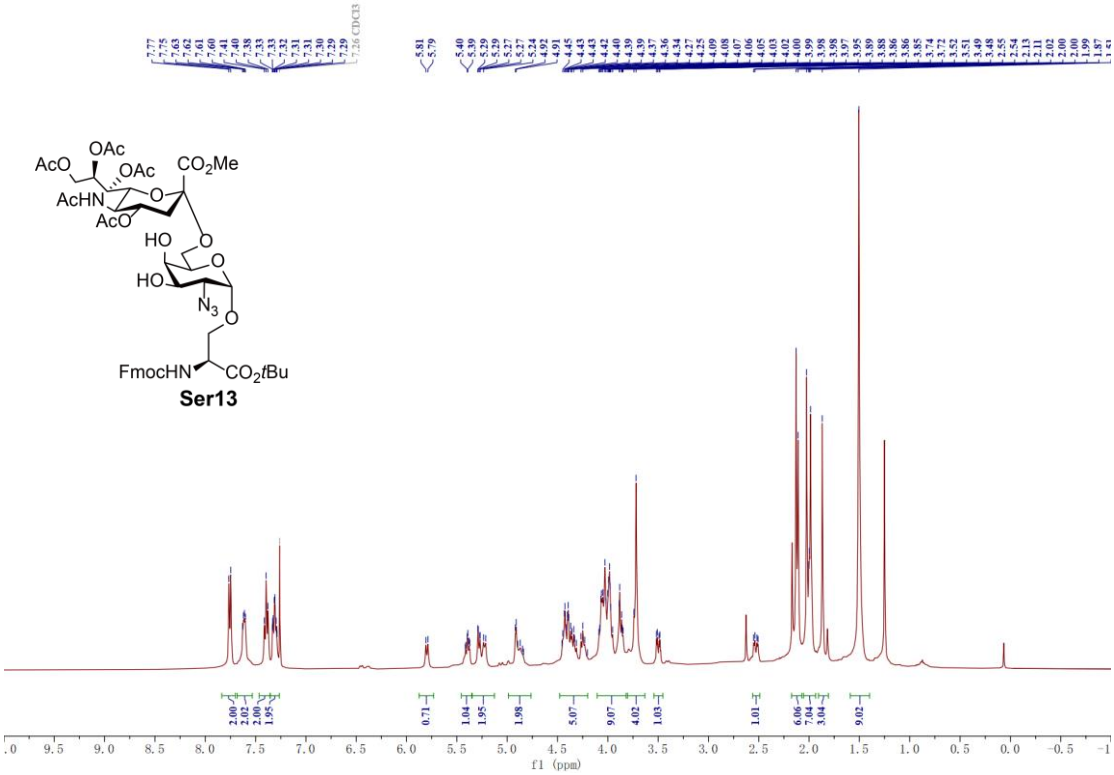
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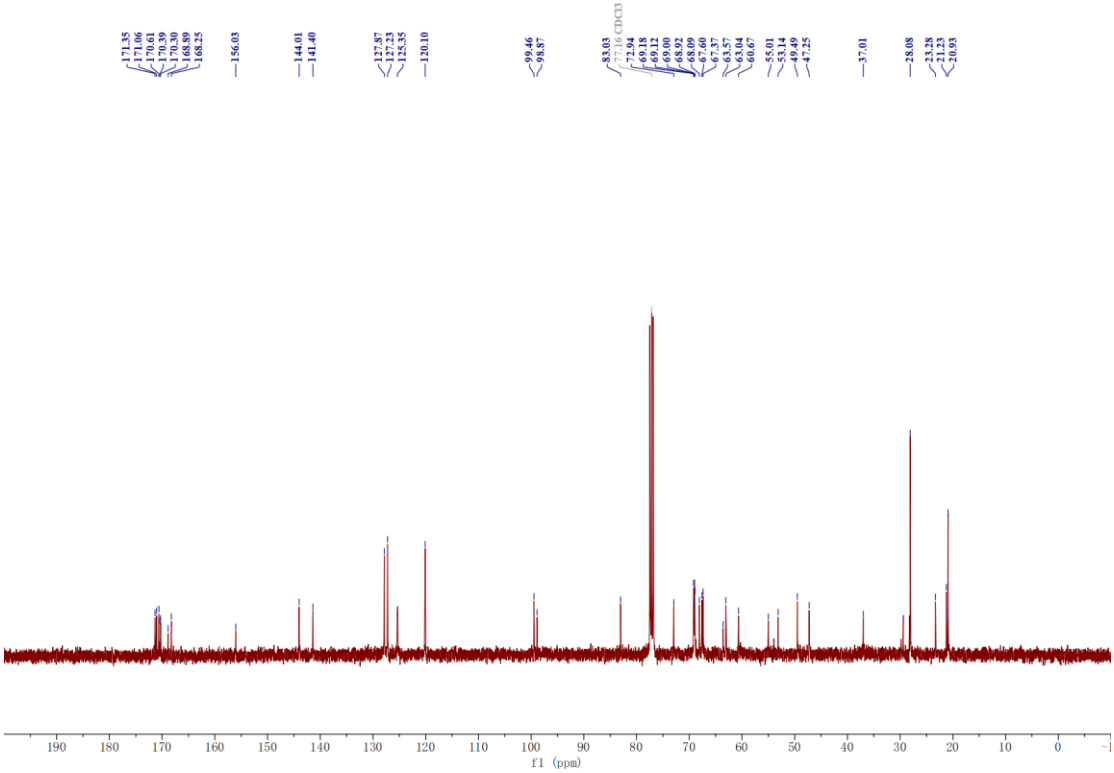
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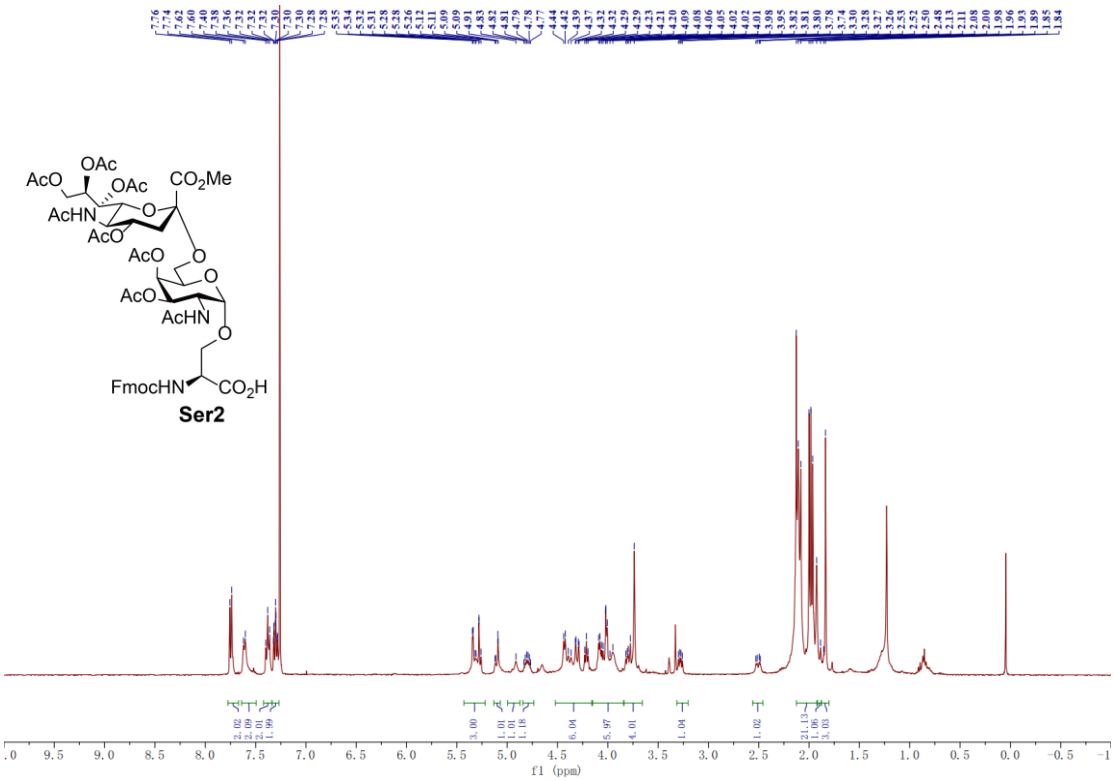
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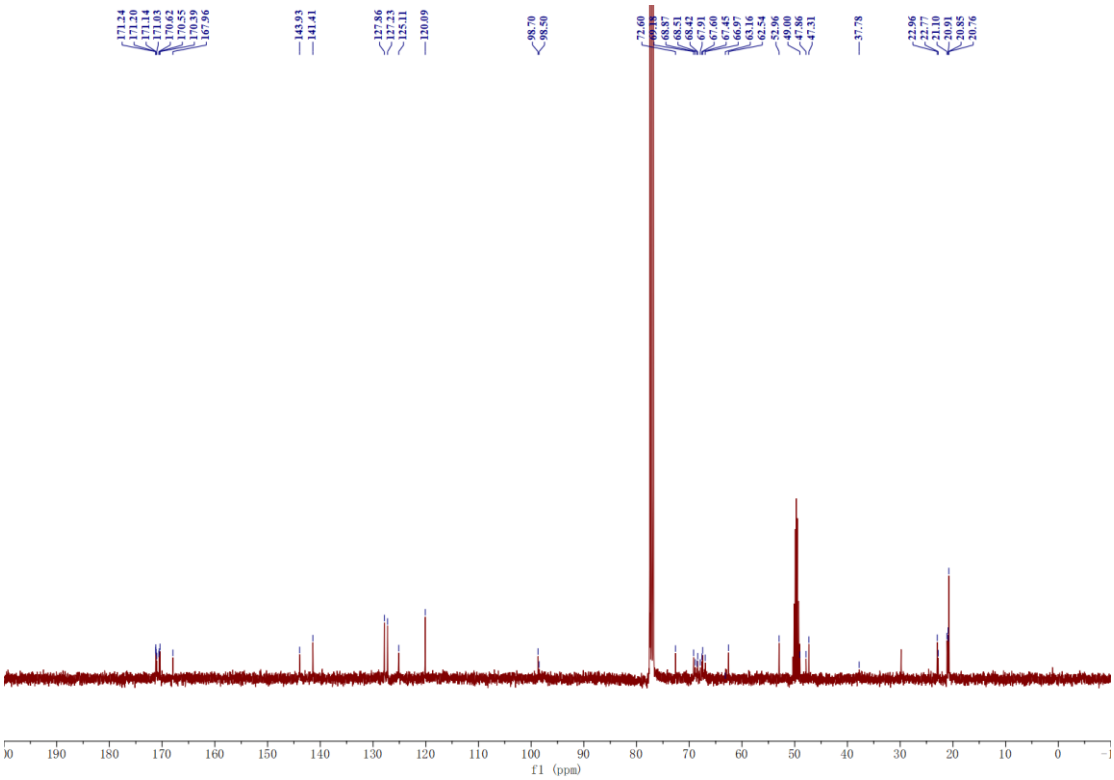
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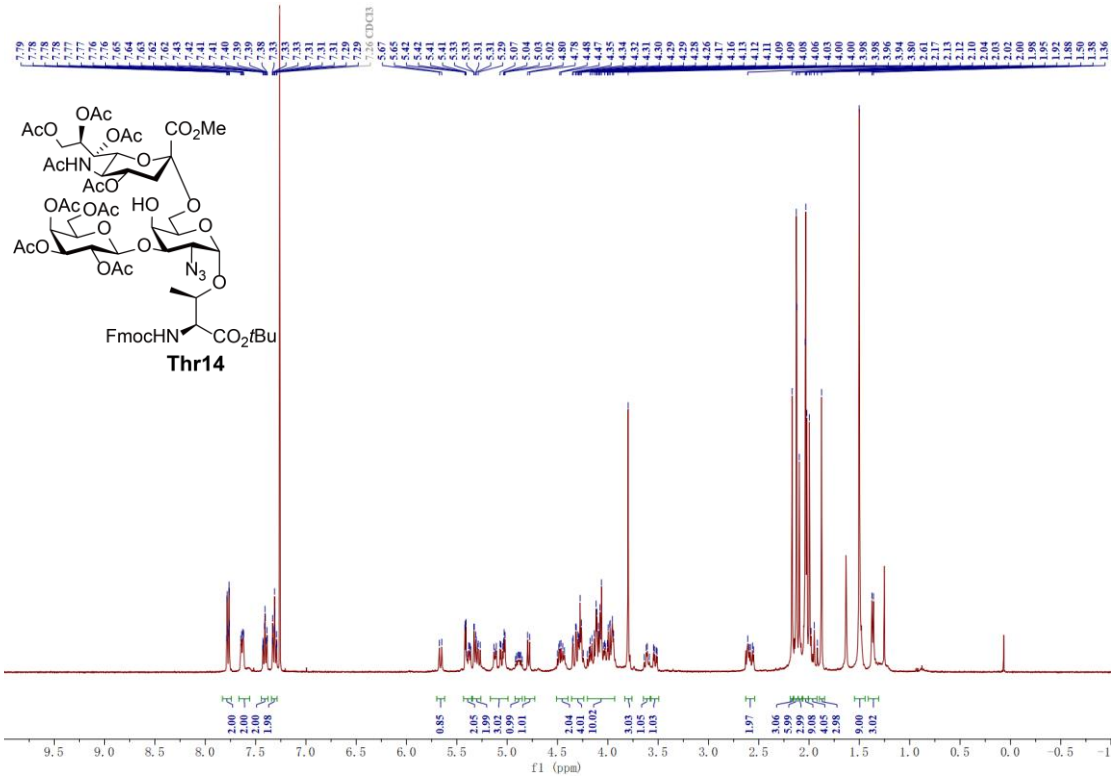
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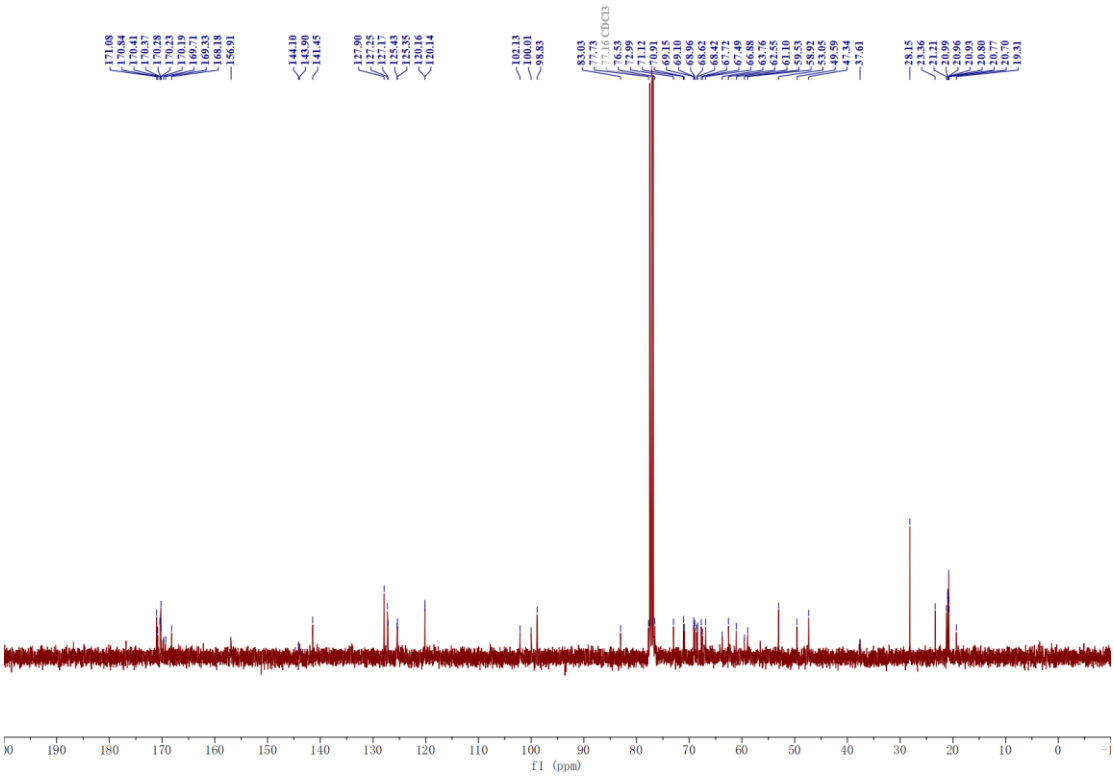
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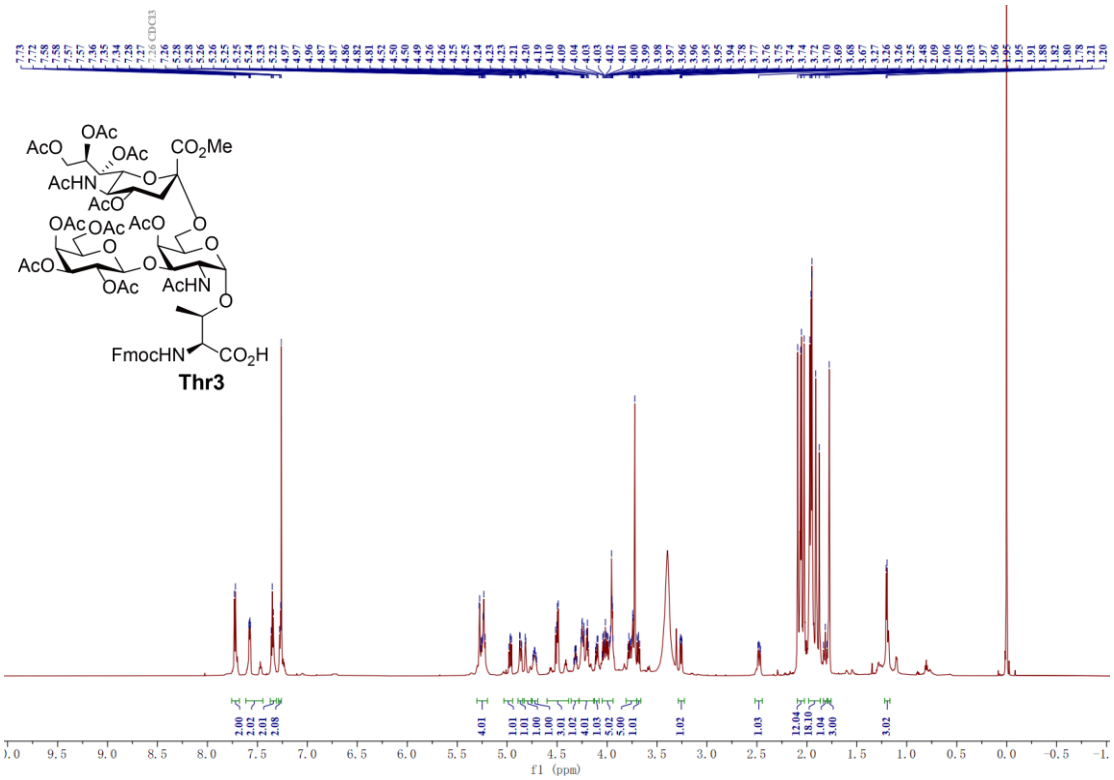
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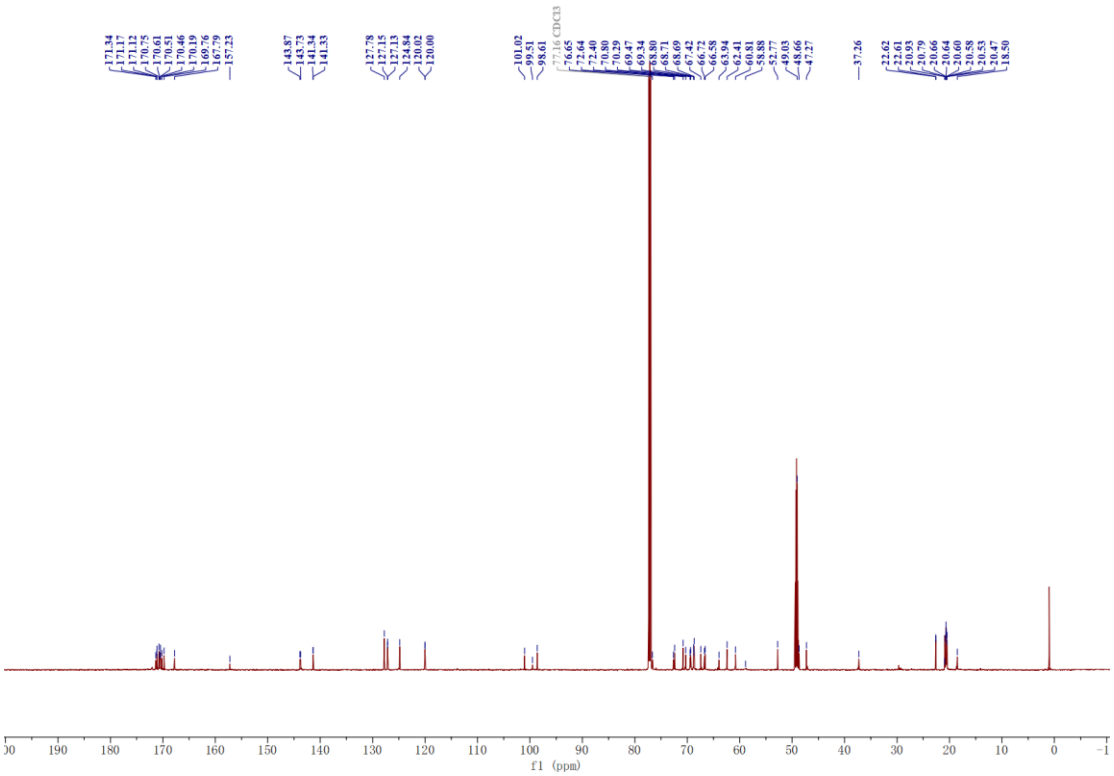
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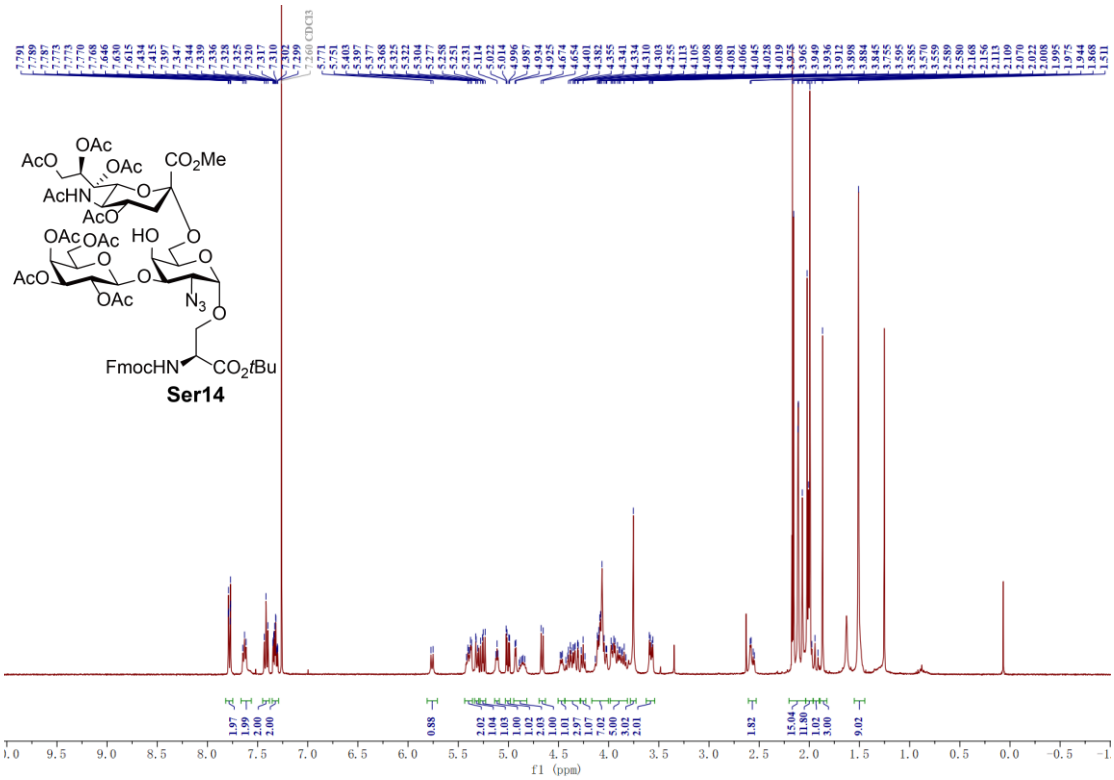
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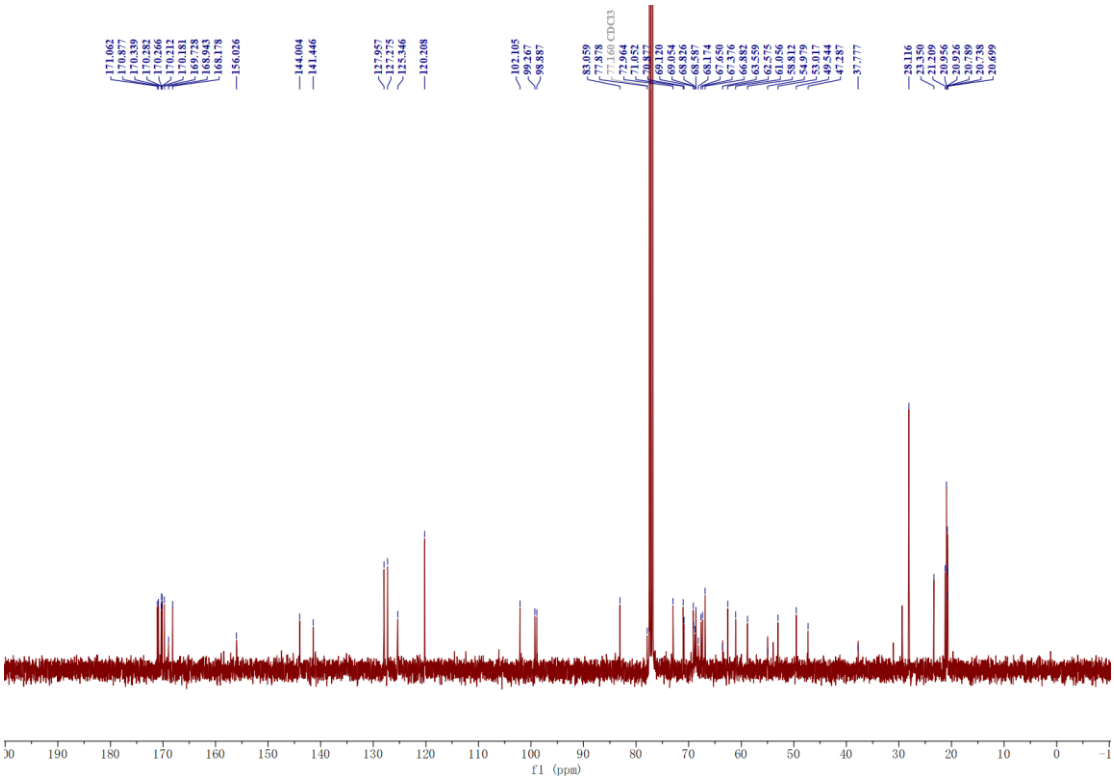
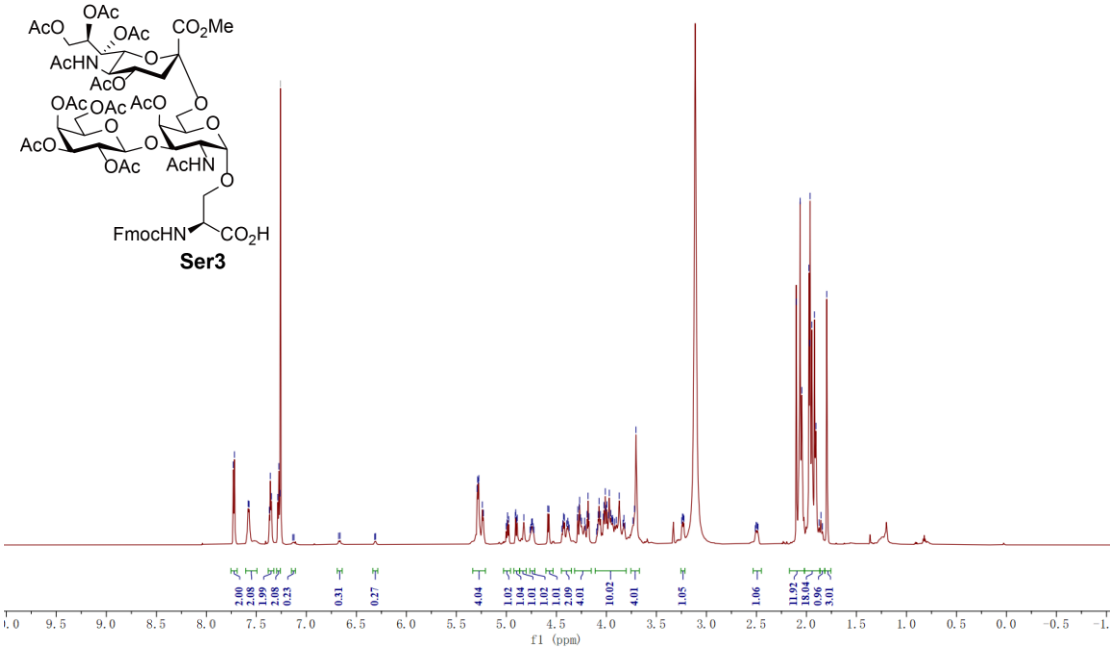
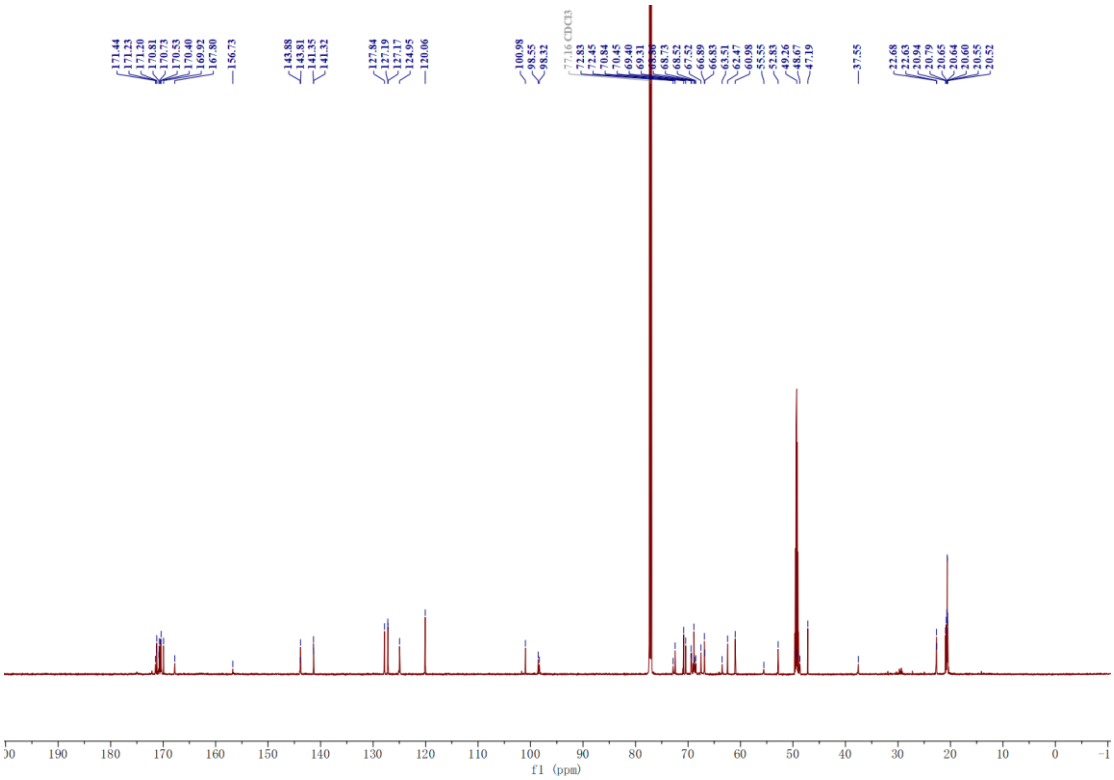
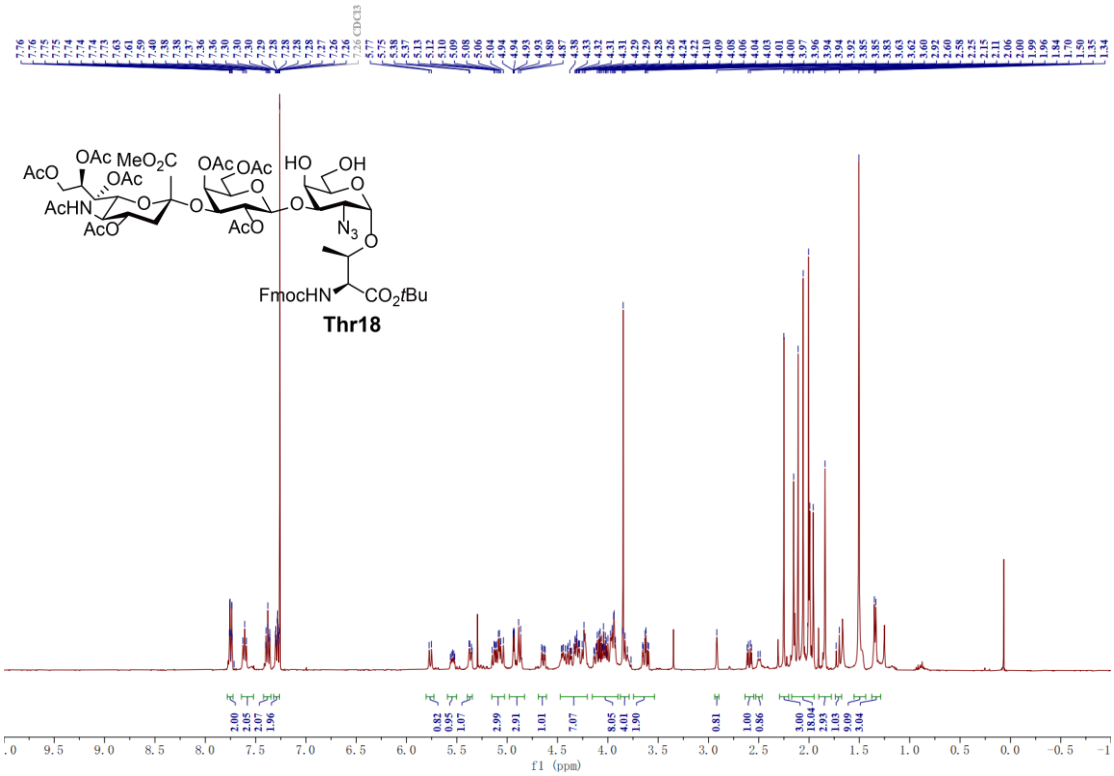
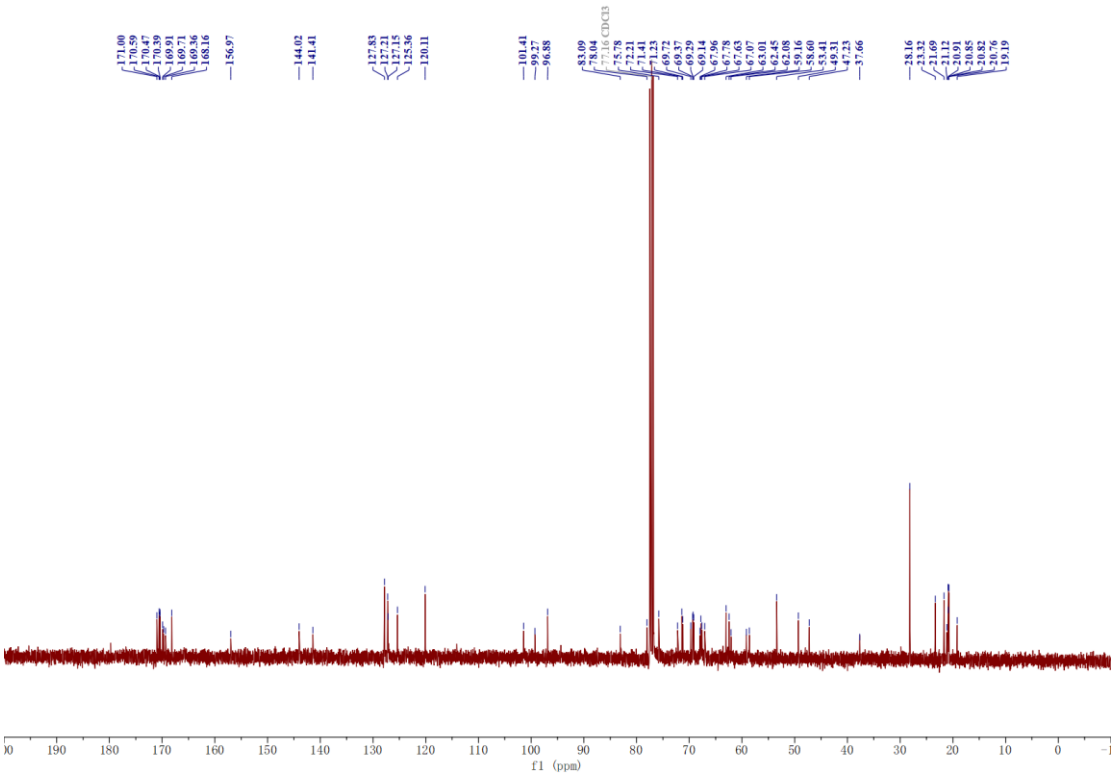
O
OAc
N3
O
FmocHN
CO2tBu
Ser14
f1 (ppm)

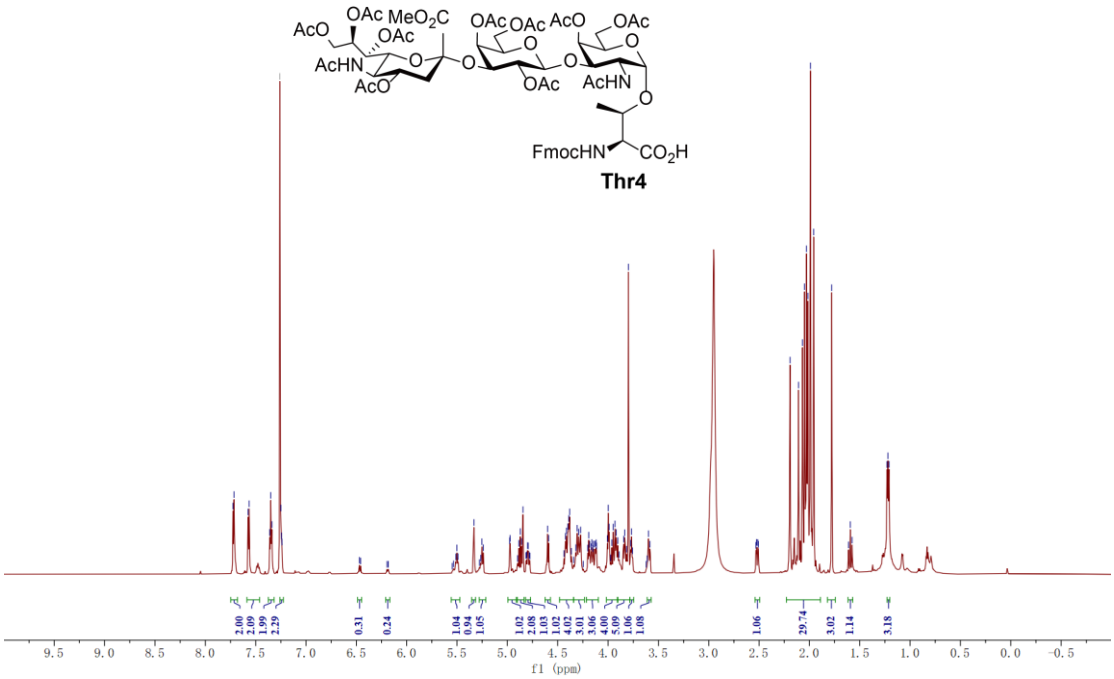

f1 (ppm)

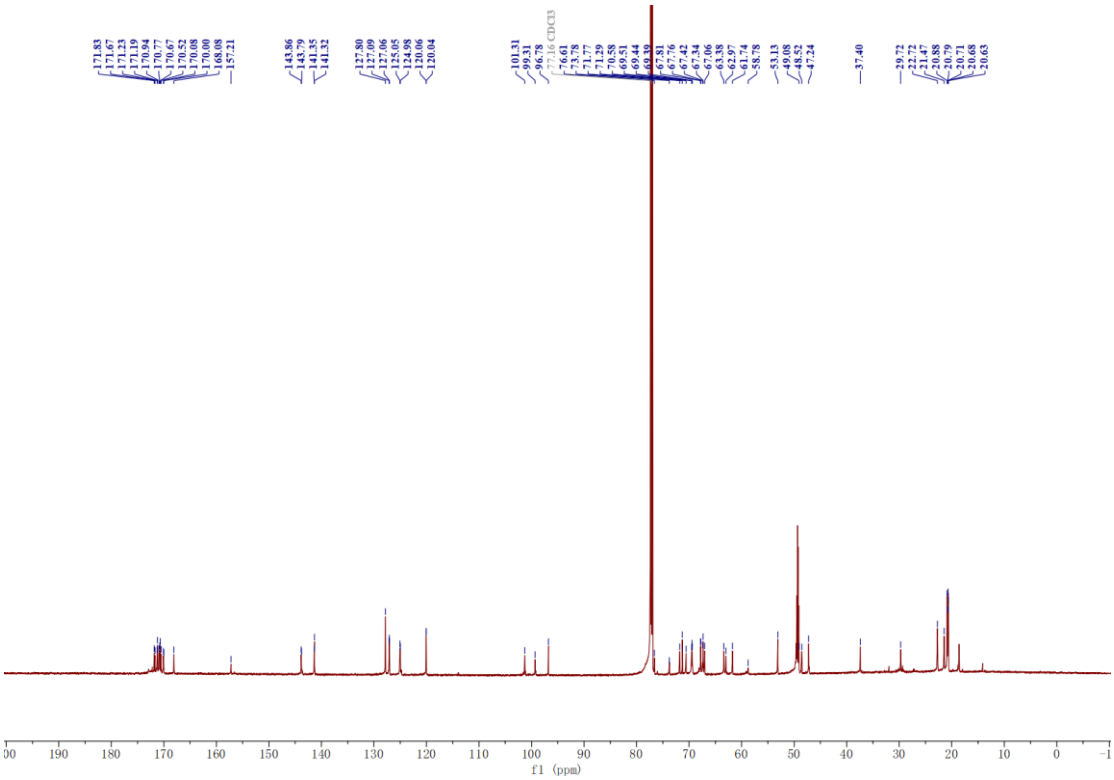

OAc
AcO
OAc
CO2Me
AcHN
O
AcO
OAc
OAc
AcO
O
O
AcO
O
O
OAc
AcHN
O
FmocHN
CO2H
Ser3
f1 (ppm)

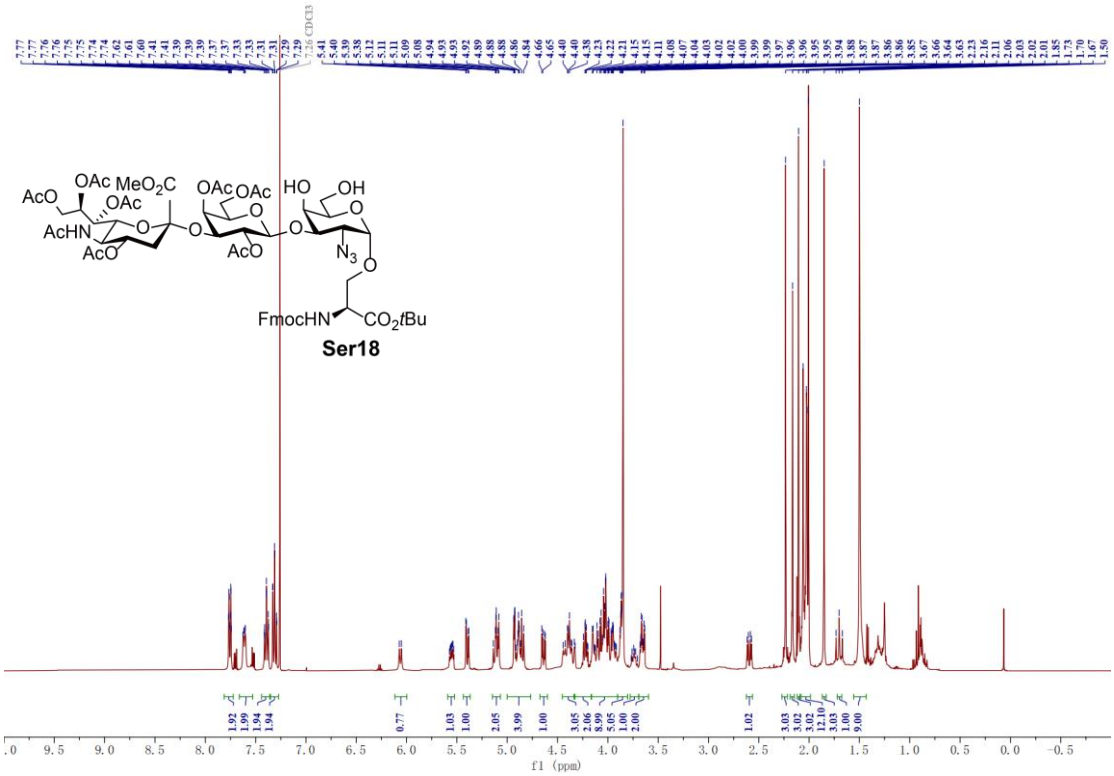

f1 (ppm)

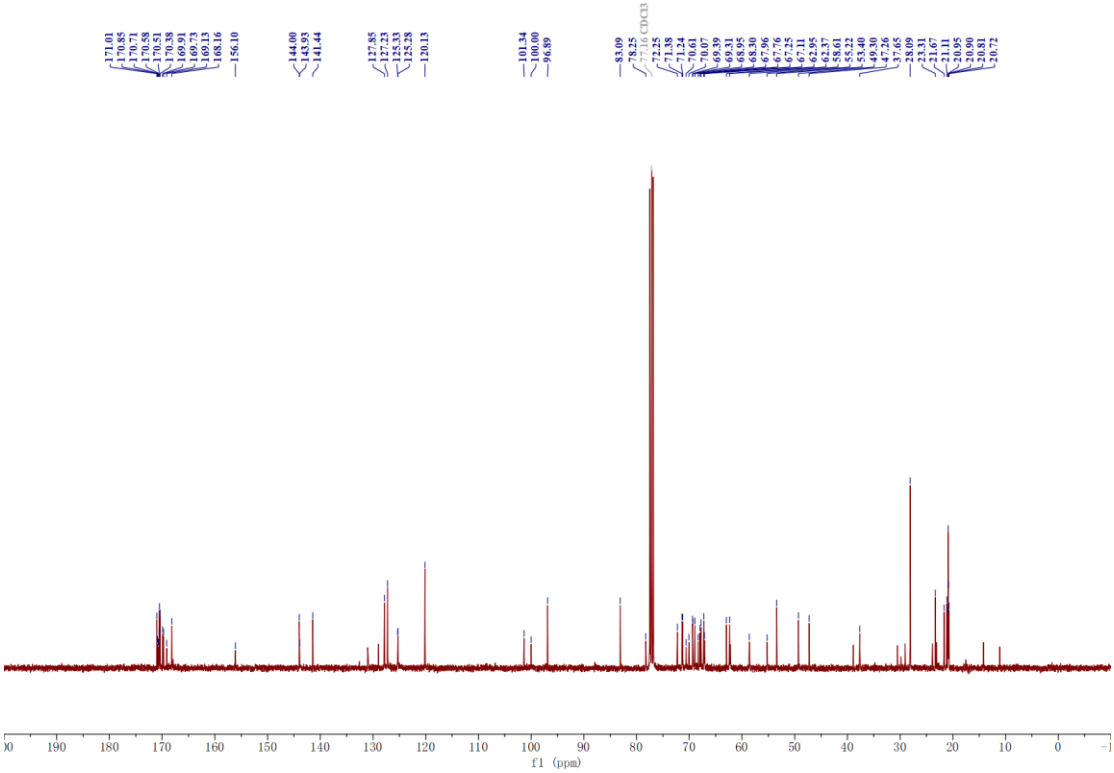

AcO
OAc
AcHN
AcO
OAc MeO2C
OAc
O
O
OAc OAc
O
AcO
HO
OH
O
O
N3
O
FmocHN
CO2tBu
Thr18
f1 (ppm)

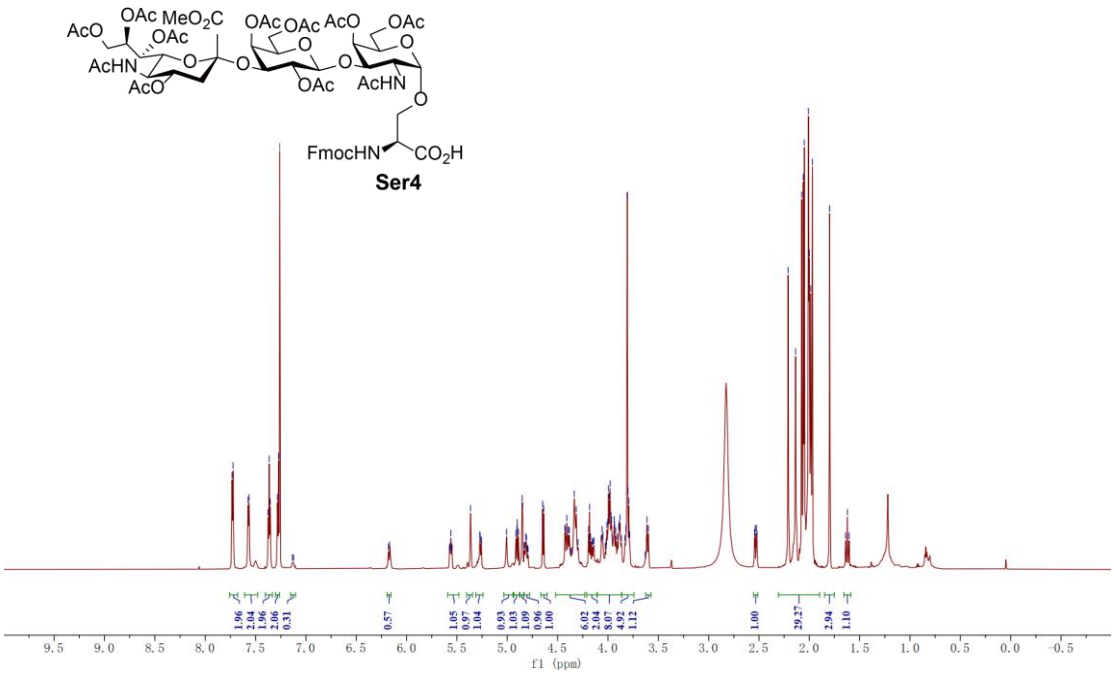

f1 (ppm)

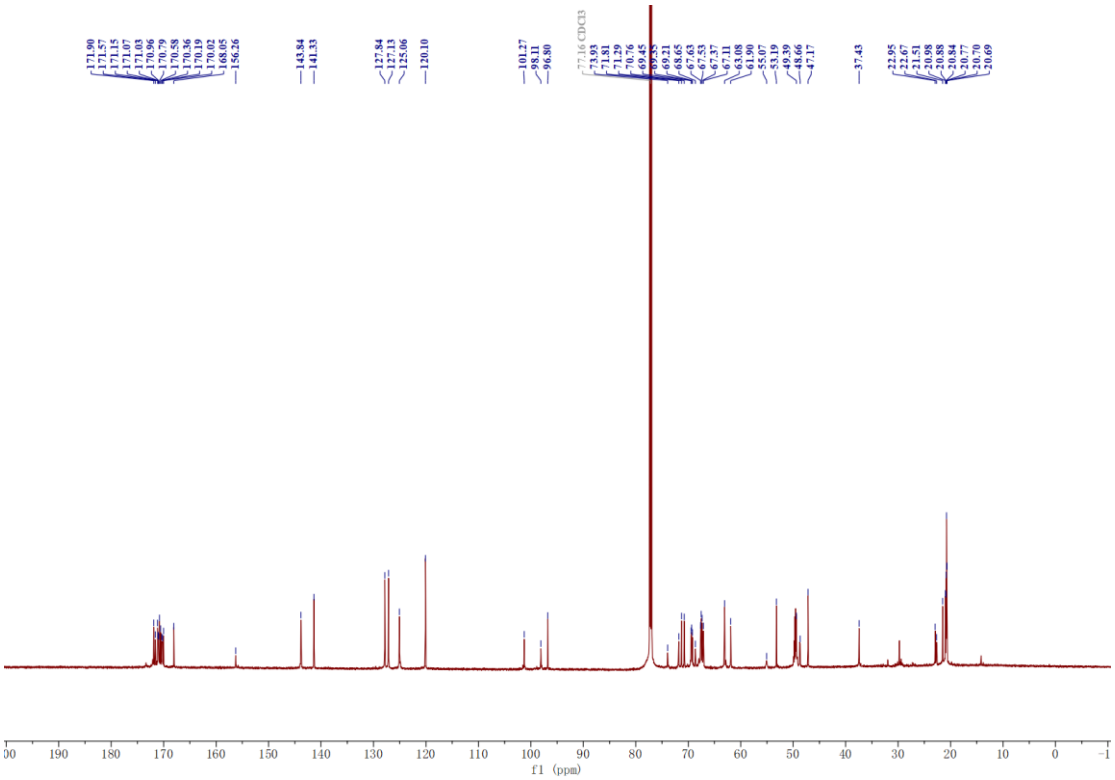

Thr4
f1 (ppm)

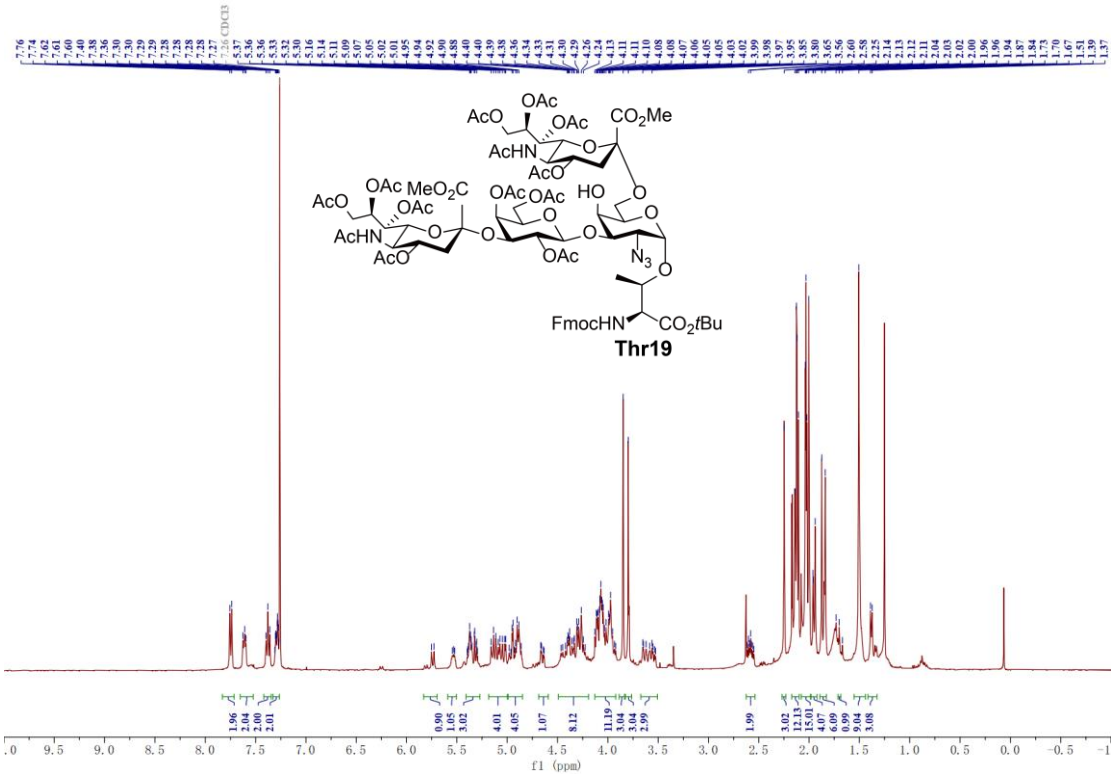

f1 (ppm)

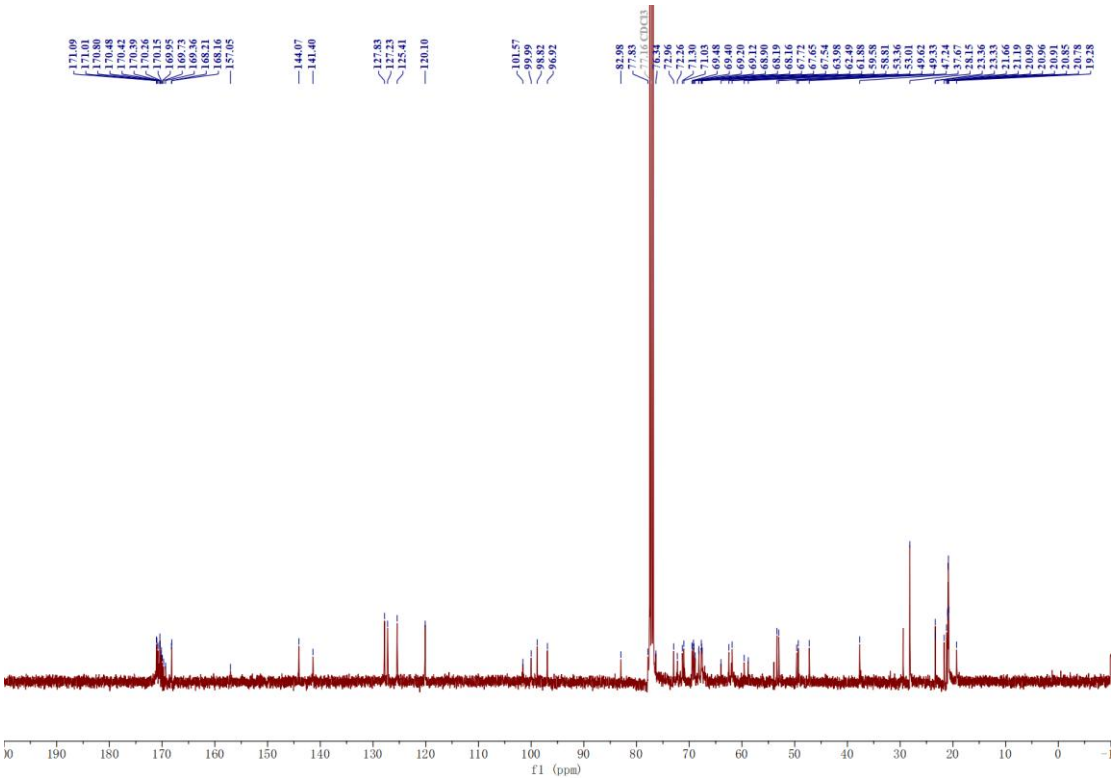

AcO
OAc
OAc MeO2C
AcHN
AcO
OAc OAc
AcO
HO
OH
N3
FmocHN
CO2tBu
Ser18
f1 (ppm)

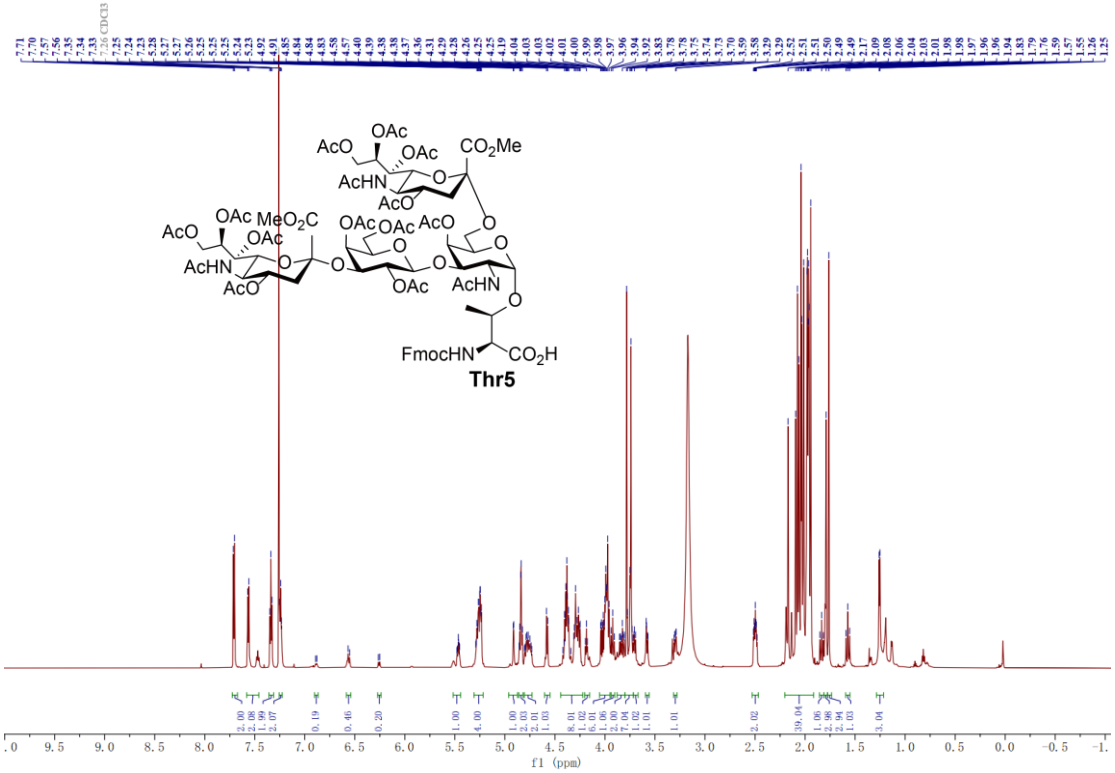

f1 (ppm)

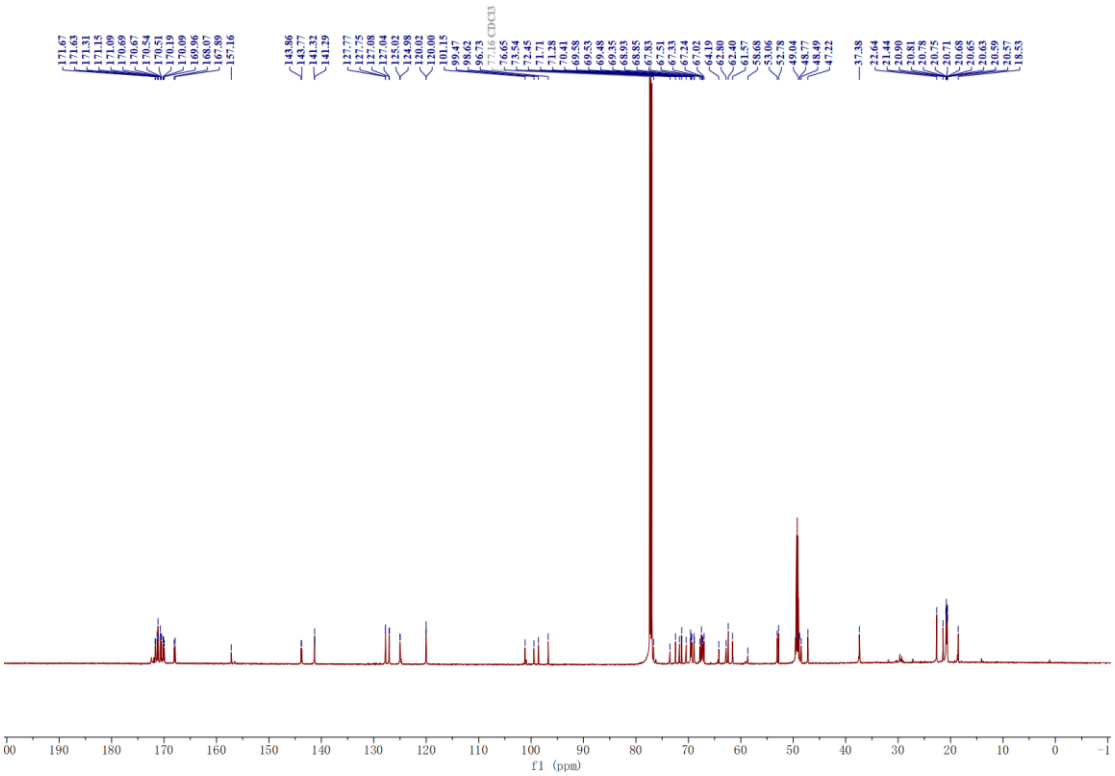

Ser4
f1 (ppm)

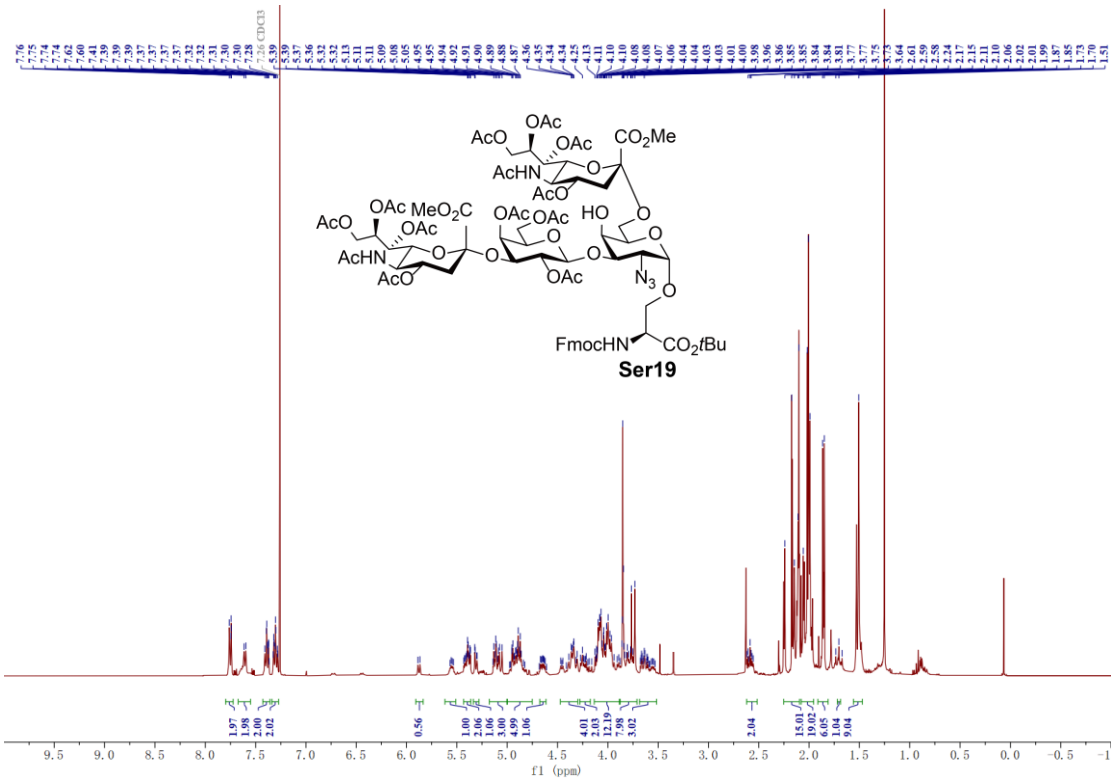

f1 (ppm)

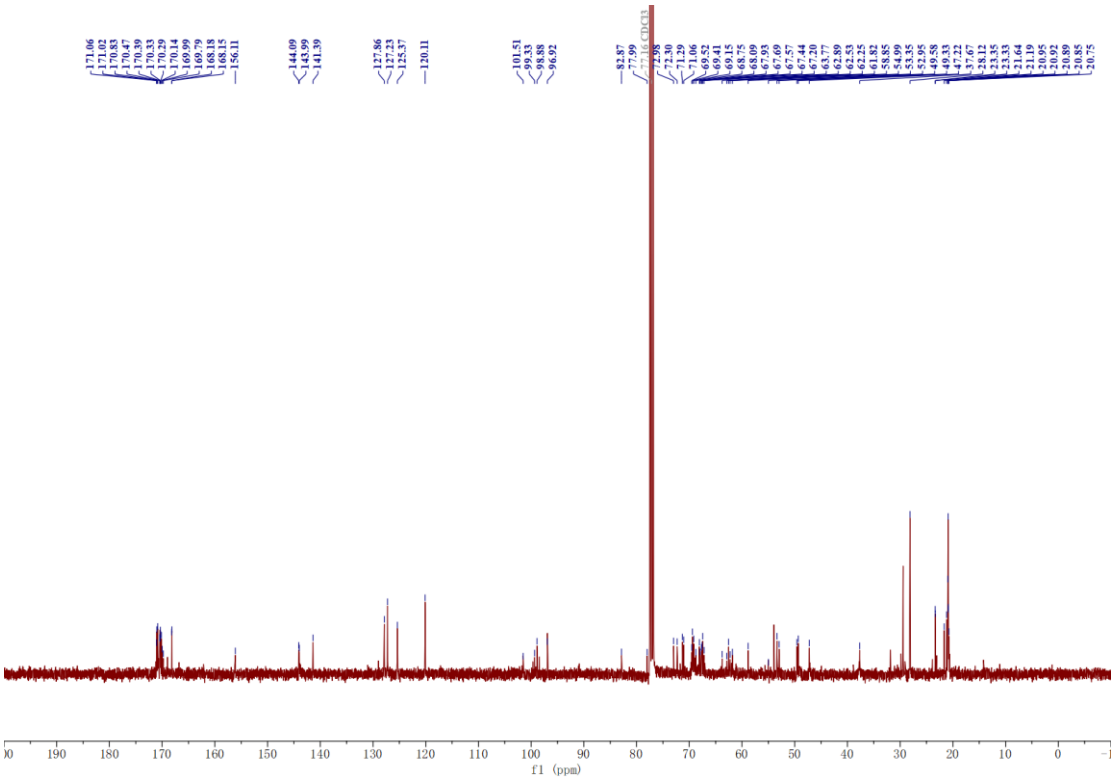

Thr19
f1 (ppm)

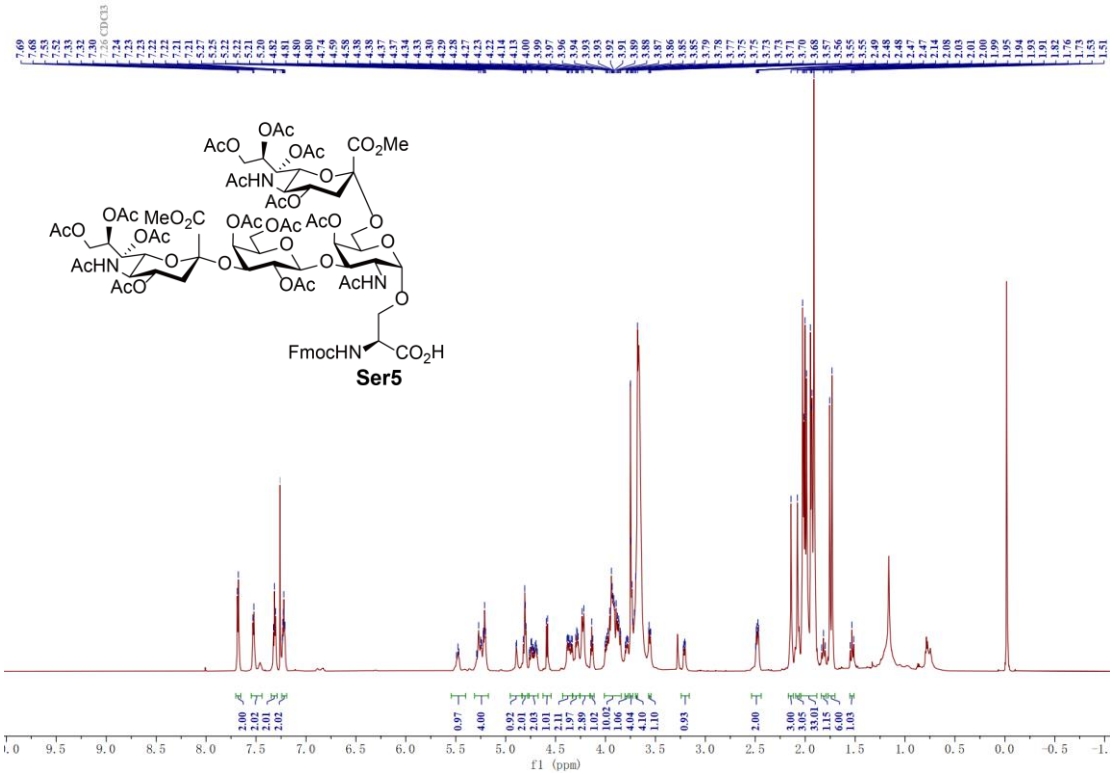

f1 (ppm)

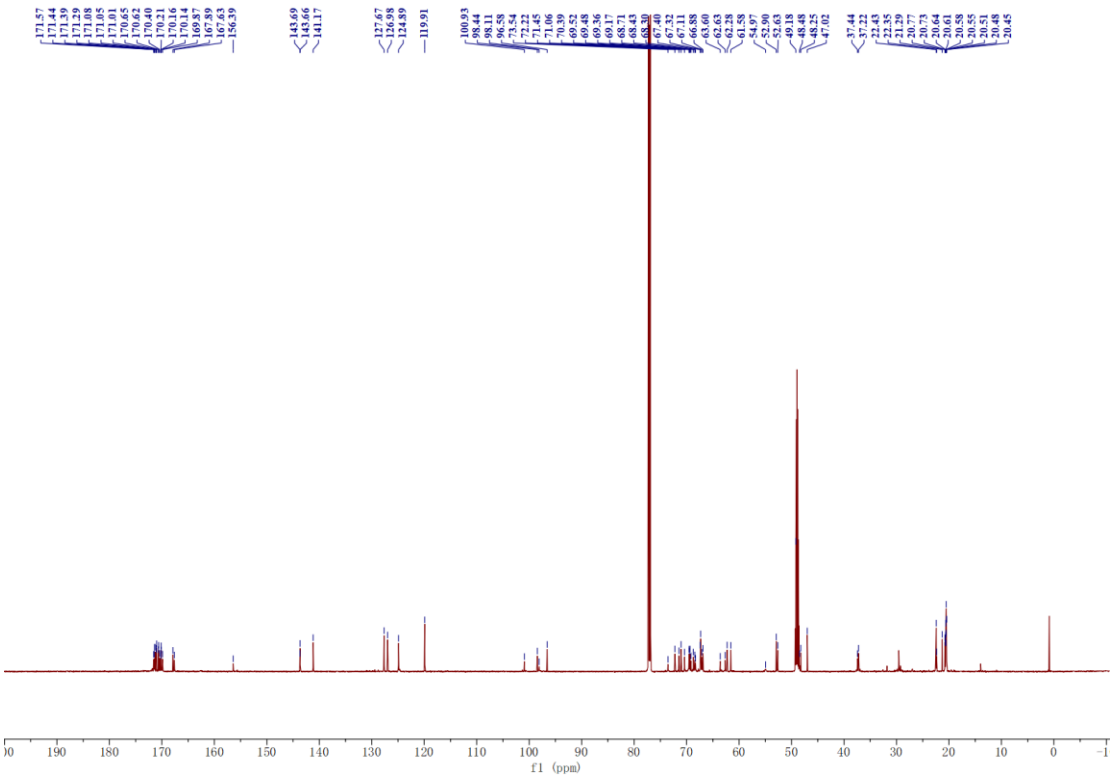

Thr5
f1 (ppm)

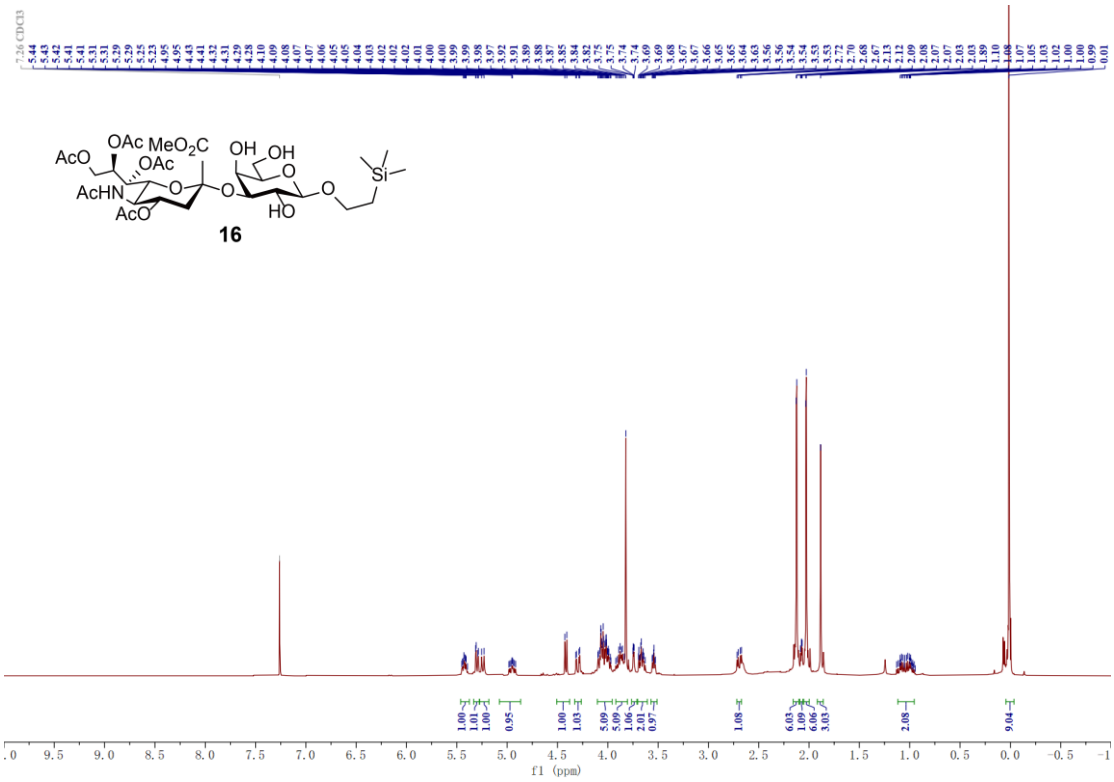

f1 (ppm)

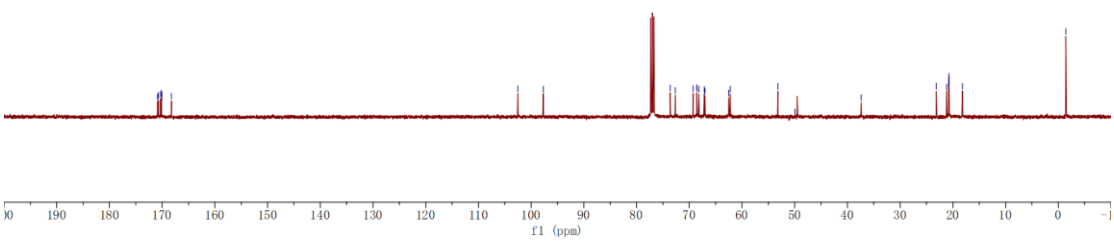

Ser19




Ser5
f1 (ppm)


f1 (ppm)


AcO
OAc
MeO2C
OAc
AcHN
AcO
OH
OH
O
HO
Si
16
f1 (ppm)


f1 (ppm)