Supporting Information

Site-selective Acylations of α and β-Hydroxyamides in Complex Molecules: Application of Template-driven Acylation to Disaccharides and a Glycopeptide

Yasuhiro Nishikawa*, Shione Toda, Takami Matsui, Hanae Takada, Kohei Takemoto and Osamu Hara*

Faculty of Pharmacy, Meijo University, 150 Yagotoyama, Tempaku-ku, Nagoya 468-8503, Japan

E-mail: yasuhiro@meijo-u.ac.jp

oshara@meijo-u.ac.jp

Table of Contents

1. General Information

All reactions were carried out in flame-dried glassware under nitrogen atmosphere and stirred via magnetic stir-plates. All reactions were monitored by analytical thin-layer chromatography using Wako pre-coated silica gel plates with F254 indicator. Visualization was accomplished by UV light (254 nm), phosphomolybdic acid, ninhydrin, anisaldehyde or basic potassium permanganate. Flash column chromatography was performed using Kanto Chemical silica gel 60N (spherical, neutral, 40-50 μm). All reactions were carried out with anhydrous solvents unless otherwise noted. All other reagents and starting materials, unless otherwise noted, were purchased from commercial vendors.

HPLC analysis was performed using the following systems; HPLC system A: Pump: PU-2089 (JASCO), Detector: UV-2075 (JASCO). HPLC system B: Pump: PU-4180 (JASCO) and PU-4185 (JASCO), Detector: MD-4010 (JASCO). HPLC system C: Pump: PU-4086 Binary (JASCO), Detector: UV-2075 (JASCO).

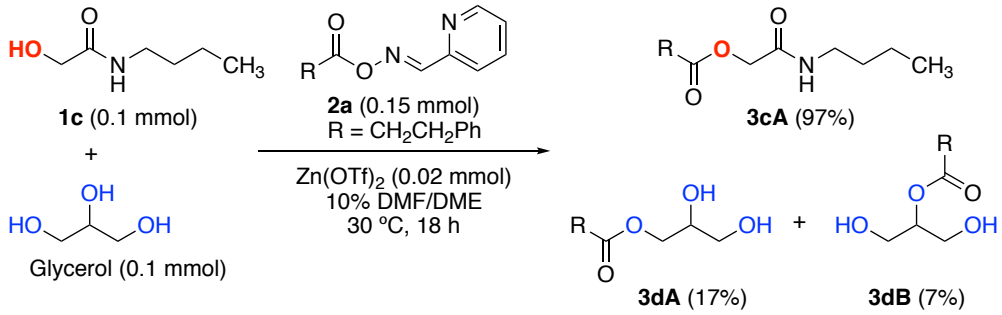
LC-MS analysis was performed using Exactive Plus spectrometer (Thermo Fisher Scientific Inc) connected to Ultimate 3000 HPLC (ThermoFisher Scientific).

^{1}H NMR and ^{13}C NMR spectra were recorded on ECZ-400 spectrometer (JEOL, 400 MHz ^{1}H, 100 MHz ^{13}C) or AVANCE III HD (Bruker, 600 MHz ^{1}H, 151 MHz ^{13}C) with Cryoprobe Prodigy. Chemical shift values (δ) are reported using tetramethylsilane (δH 0.00) and $CDCl_3$ (δC 77.00) in $CDCl_3$ or residual CD_2HOD (δH 3.31) and CD_3OD (δC 49.15) in CD_3OD or residual CHD_2CN (δH 1.94) and CD_3CN (δC 1.39) in CD_3CN or residual DMSO-d_5 (δH 2.49) and DMSO-d_6 (δC 39.51) or residual Acetone-d_5 (δH 2.05) and Acetone-d_6 (δC 29.92). The ^{1}H NMR spectra are reported as follows δ (number of protons, multiplicity, coupling constant J Hz). Multiplicities are indicated by s (singlet), d (doublet), t (triplet), q (quartet), quin (quintet), sext (sextet), sep (septet), dd (doublet of doublet), ABq (AB quartet), m (multiplet) and br (broad).

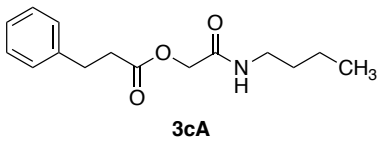
High-resolution mass spectra were obtained on Exactive Plus spectrometer (Thermo Fisher Scientific Inc, ESI-orbitrap) or Triple TOF 6600 system (ABSciex, ESI-TOF).

2. Experimental procedures for control experiments using β-hydroxyamide 1c.

2.1. Intermolecular competition experiments between β-hydroxyamide 1c and glycerol.



To a stirred solution of **1c** (13.5 mg, 0.103 mmol) and glycerol (7.3 μL, 0.10 mmol) in 10% DMF/DME (0.5 mL, 0.2 M) were added pyridine 2-aldoxime ester **2a** (38.2 mg, 0.150 mmol), Zinc(II) Trifluoromethanesulfonate (7.4 mg, 0.02 mmol, 20 mol%) under nitrogen atmosphere. After being stirred at 30 ºC for 18 h, the reaction mixture was evaporated to dryness. The residue was analyzed by ^{1}H-NMR ($CDCl_3$, 600 MHz) to obtain selectivity and yield using dimethyl fumarate as an internal standard (**3cA**: 97%, **3dA**: 17%, **3dB**: 3%). Preparative RP-HPLC of the crude product using HPLC system C afforded an inseparable mixture of **3dA** and **3dB** which were assigned by 1D selective gradient TOCSY and 2D NMR experiments.

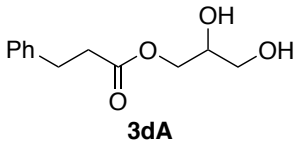


3cA[1]: Colorless oil;

^{1}H-NMR (600 MHz, $CDCl_3$): δ 7.30-7.33 (2H, m), 7.21-7.25 (3H, m), 5.72 (1H, br-s), 4.53 (2H, s), 3.20 (2H, td, *J*=7.2, 6.1), 3.00 (2H, t, *J*=7.5), 2.77 (2H, t, *J*=7.5), 1.39-1.45 (2H, m), 1.27-1.34 (2H, m), 0.92 (3H, t, *J*=7.3);

^{13}C-NMR (151 MHz, $CDCl_3$): δ 171.3, 166.7, 139.9, 128.7, 128.2, 126.6, 63.0, 38.8, 35.5, 31.5, 30.8, 20.0, 13.7;

HRMS (ESI-TOF) *m/z*: calcd for $C_{15}H_{22}NO_3$ $[M+H]^+$: 264.1594, found: 264.1590.

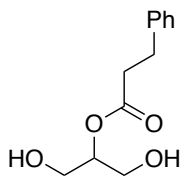


3dA: Colorless oil;

^{1}H-NMR (600 MHz, $CDCl_3$): δ 7.27-7.33 (2H, m), 7.14-7.25 (3H, m), 4.18 (1H, dd, *J*=11.7, 4.6 Hz), 4.12 (1H, dd, *J*=11.7, 6.2 Hz), 3.83-3.89 (1H, m), 3.61 (1H, dd, *J*=11.6, 4.0 Hz), 3.50 (1H, dd, *J*=11.5, 5.8 Hz), 2.97 (2H, t, *J*=7.7 Hz), 2.70 (2H, t, *J*=7.7 Hz);

^{13}C-NMR (151 MHz, $CDCl_3$): δ 173.2, 140.2, 128.6, 128.3, 126.4, 70.1, 65.3, 63.2, 35.7, 30.9;

HRMS (ESI-orbitrap) *m/z*: calcd for $C_{12}H_{16}O_4Na$ $[M+Na]^+$: 247.0941, found: 247.0939.



3dB: Colorless oil;

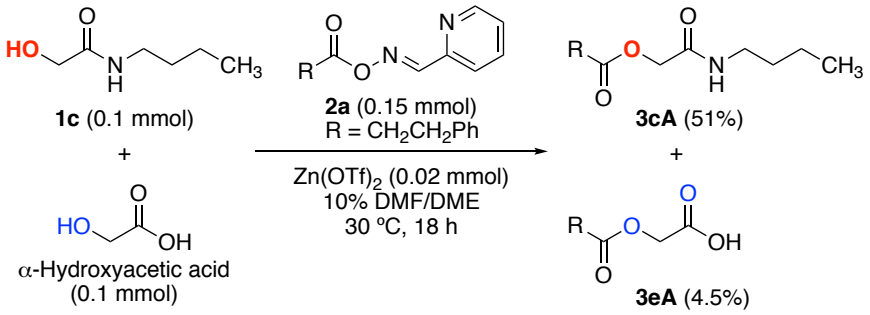
^{1}H-NMR (600 MHz, $CDCl_3$): δ 7.27-7.33 (2H, m), 7.14-7.25 (3H, m), 4.89 (1H, quin, *J*=4.6 Hz), 3.75 (4H, d, *J*=4.6 Hz), 2.89 (2H, t, *J*=7.6 Hz), 2.73 (2H, t, *J*=7.6 Hz);

^{13}C-NMR (151 MHz, $CDCl_3$): δ 173.0, 140.2, 128.6, 128.3, 126.5, 75.2, 62.4, 35.8, 31.0;

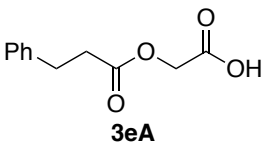
HRMS (ESI-orbitrap) *m/z*: calcd for $C_{12}H_{16}O_4Na$ $[M+Na]^+$: 247.0941, found: 247.0939.

[1] Nishikawa, Y.; Takemoto, K.; Matsuda, K.; Tanaka, R.; Arashima, A.; Ito, K.; Kamezawa, Y.; Hori, Y.; Hara, O. *Org. Lett.* **2018**, *20* , 3367.

2.2. Intermolecular competition experiments between β-hydroxyamide 1c and α-hydroxyacetic acid.



To a stirred solution of **1c** (26.5 mg, 0.202 mmol) and α-hydroxyacetic acid (15.2 mg, 0.200 mmol) in 10% DMF/DME (1.0 mL, 0.30 M) were added pyridine 2-aldoxime ester **2a** (76.3 mg, 0.300 mmol), Zinc(II) Trifluoromethanesulfonate (14.6 mg, 0.04 mmol, 20 mol%) under nitrogen atmosphere. After being stirred at 30 ºC for 18 h, the reaction mixture was evaporated to dryness. The residue was analyzed by ^{1}H-NMR ($CDCl_3$, 600 MHz) to obtain selectivity and yield using dimethyl fumarate as an internal standard (**3cA**: 51%, **3eA**: 4.5%).



The analytical standard of **3eA** was prepared by conventional conditions using 3-phenylpropionyl chloride.

3eA: Yellowish powder;

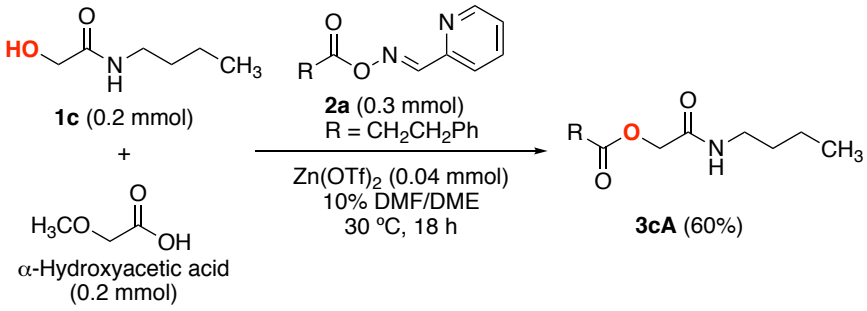
m.p.: 86-89 ºC;

^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.27-7.32 (2H, m), 7.19-7.23 (3H, m), 4.68 (2H, s), 3.00 (2H, t, *J*=7.9 Hz), 2.76 (2H, t, *J*=7.9 Hz);

^{13}C-NMR (150 MHz, acetic acid-d_4): δ 173.7, 173.4, 141.5, 129.4, 129.2, 127.2, 61.2, 36.0, 31.5;

HRMS (ESI-orbitrap) *m/z*: calcd for $C_{11}H_{11}O_4$ $[M–H]^-$: 207.0663, found: 207.0665.

2.3. Intermolecular competition experiments between β-hydroxyamide 1c and α-methoxyacetic acid.

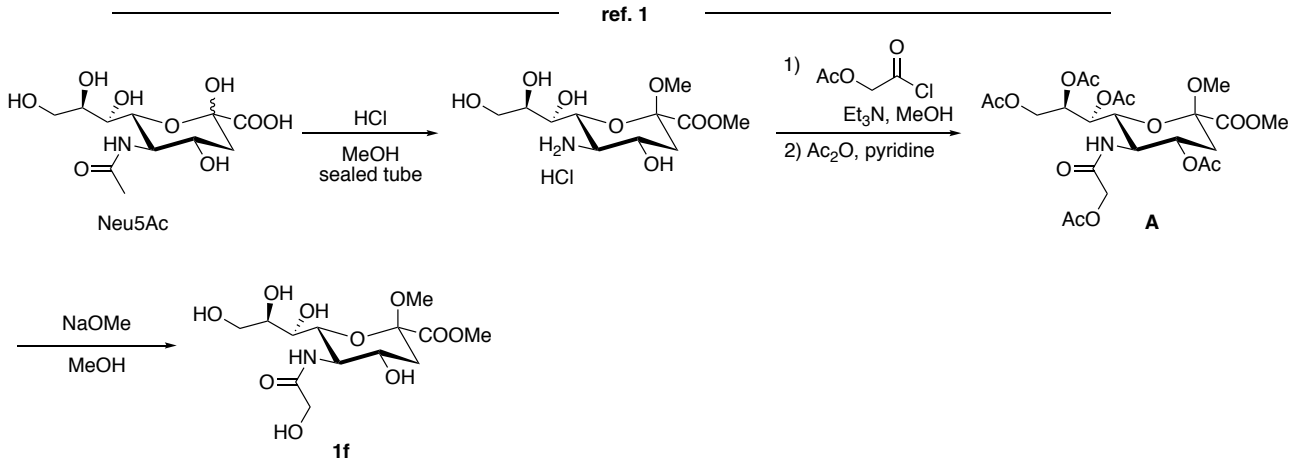


The control experiment between **1c** and α-methoxyacetic acid was done by the same procedure as in the experiment using α-hydroxyacetic acid.

3. Experimental procedures for site-selective acylation of Neu5Gc derivative 1f.

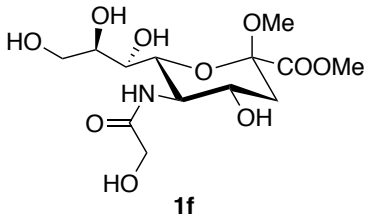
3.1. Summary for the preparation of 1f.

1f was prepared by methanolysis of compound **A** which was prepared by a known procedure from commercially available Neu5Ac[1].



3.2. Synthesis of 1f and its characterization data.

To a stirred solution of a compound **A** (676 mg, 1.20 mmol) in MeOH (6 mL) at 0 ºC was added 28% NaOMe in MeOH (232 µL, 1.20 mmol). After being stirred at 0 ºC for 18 h under nitrogen atmosphere, the reaction was quenched adding acetic acid (82 µL) and stirred for 10 min. The reaction mixture was evaporated to dryness to afford a crude product, which was purified by HPLC system C with InertSustain AQ-C18 (20x250 mm, 5 µm particle size, GL sciences) using a gradient (14.3 mL/min, 0% A over 2.5 min followed by 0-100% A 10 min; A = MeOH, B = water) to give **1f** (372 mg, 1.05 mmol, 88% yield) as a white solid.



m.p.: 198-202 ºC;

^{1}H-NMR (600 MHz, CD_3OD): δ 4.11 (1H, ddd, *J*=11.2, 9.5, 5.0 Hz), 4.03 (2H, s), 3.89-3.96 (2H, m), 3.78-3.83 (5H, m), 3.65 (1H, dd, *J*=12.1, 6.1 Hz), 3.51 (1H, dd, *J*=9.1, 1.0 Hz), 3.28 (3H, s), 2.36 (1H, dd, *J*=13.0, 5.0 Hz), 1.66 (1H, dd, *J*=12.9, 11.3 Hz);

^{13}C-NMR (150 MHz, CD_3OD): δ 176.7, 171.1, 100.6, 72.2, 71.6, 70.2, 67.7, 65.4, 62.8, 53.6, 53.3, 51.8, 41.8;

$[\alpha]_D^{26}$ -47 (MeOH, *c* 0.50);

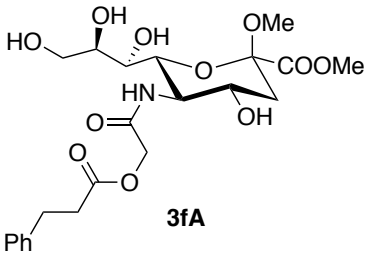
HRMS (ESI-orbitrap) *m/z*: calcd for $C_{13}H_{23}NNaO_{10}^+$ $[M+Na]^+$: 376.1214, found: 376.1215.

HPLC: HPLC system A with InertSustain AQ-C18 (4.6x250 mm, 5 µm), Flow rate 0.75 mL/min, 25 ºC, Gradient: 0-100% A over 10 min; A = MeOH, B = water), t_R = 8.7 min.

3.3. Site-selective acylation of 1f and characterization data of 3fA.

To a mixture of **1f** (60.6 mg, 0.172 mmol), $Zn(OTf)_2$ (12.6 mg, 0.035 mmol) and pyridine aldoxime ester **2a** (52.0 mg, 0.204 mmol) was added DMF/DME (1:1, 0.85 mL) under nitrogen atmosphere. After being stirred at 40 ºC for 41 hour, the reaction mixture was evaporated to afford a crude product of **3fA**, which was purified by HPLC system C with InertSustain AQ-C18 (20x250 mm, 5 µm particle size) using a gradient (14.3 mL/min, 50% A over 2.5min followed by 50-100% A over 18 min; A = MeOH, B = 20 mM

NH_4OAc) to give **3fA** (50.1 mg, 60% yield) as a colorless oil.



3fA

^{1}H-NMR (600 MHz, CD_3OD): δ 7.21-7.29 (4H, m), 7.15-7.20 (1H, m), 4.59, 4.57 (2H, ABq, *J*=15.0 Hz), 4.02-4.08 (1H, m), 3.89-3.95 (2H, m), 3.77-3.86 (5H, m), 3.61-3.66 (1H, m), 3.49 (1H, d, *J*=9.4 Hz), 3.28 (3H, s), 2.96 (2H, t, *J*=7.5 Hz), 2.77 (2H, t, *J*=7.7 Hz), 2.35 (1H, dd, *J*=12.8, 5.0 Hz), 1.64 (1H, dd, *J*=12.9, 11.3 Hz)

^{13}C-NMR (150 MHz, CD_3OD): δ 174.0, 171.5, 171.1, 142.1, 129.7, 129.5, 127.4, 100.6, 72.1, 71.7, 70.3, 67.7, 65.6, 63.7, 53.7, 53.3, 51.8, 41.7, 36.5, 31.8;

$[\alpha]_D^{28}$ -29 (MeOH, *c* 0.16);

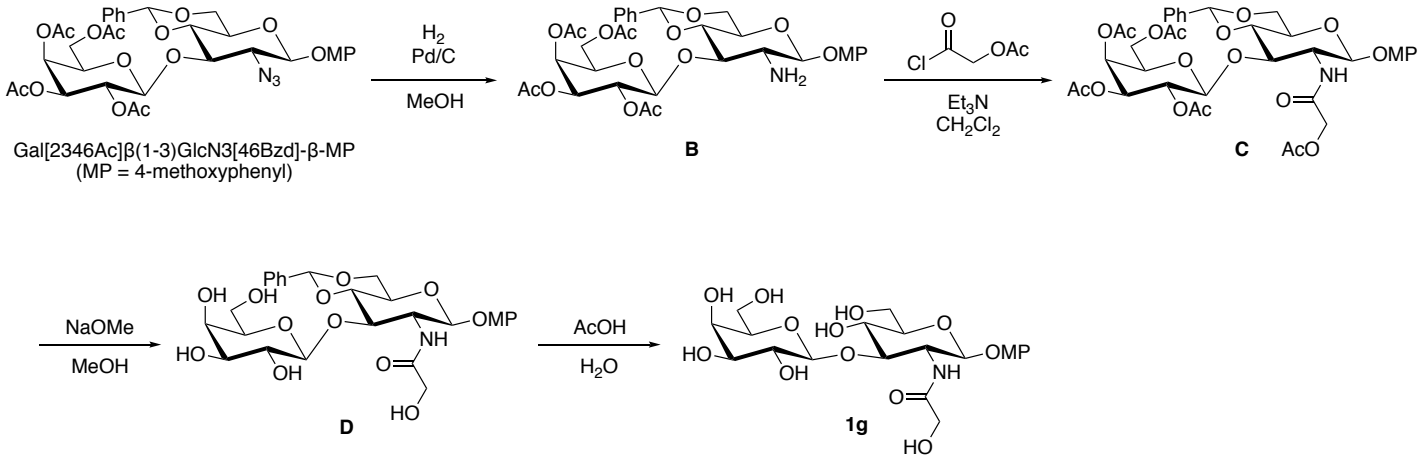
HRMS (ESI-orbitrap) *m/z*: calcd for $C_{22}H_{31}NNaO_{11}^+$ $[M+Na]^+$: 508.1789, found: 508.1796.

HPLC: HPLC system A with InertSustain AQ-C18 (4.6x250 mm, 5 μm), Flow rate 0.75 mL/min, 25 ºC, Gradient: 50-100% A over 18 min, A = MeOH, B = 20 mM NH_4OAc, t_R = 9.9 min.

4. Experimental procedures for site-selective acylation of disaccharide 1g.

4.1. Summary for the preparation of disaccharide 1g.

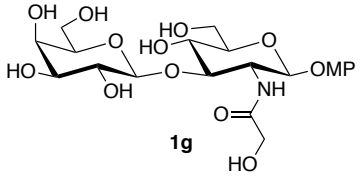
1g was prepared from commercially available Gal[2346Ac]β(1-3)GlcN_3[46Bzd]-β-MP (MP = 4-methoxyphenyl) in 4 steps as shown in the scheme below.



4.2. Synthesis of 1g and its characterization data.

To a mixture of Gal[2346Ac]β(1-3)GlcN_3[46Bzd]-β-MP (500 mg, 0.685 mmol, purchased from TCI chemicals) and 5% Pd/C (500 mg) was added MeOH (13.8 mL) under nitrogen atmosphere. The nitrogen was replaced with hydrogen with stirring. After being stirred at room temperature for 17 h, the reaction mixture was filtered through a pad of celite. The filtrate was evaporated to give a crude product of compound **B** (491 mg), which was dissolved in CH_2Cl_2 (6.9 mL) for the next reaction. To the solution was added Et_3N (114 μL, 0.822 mmol) and acetoxyacetyl chloride (74 μL, 0.685 mmol) under nitrogen atmosphere. After being stirred at room temperature for 17.5 h, the reaction mixture was concentrated to dryness to afford a crude product of compopund **C** (664 mg), which was used without further purification. To a stirred solution of the crude product in MeOH (13.7 mL) was added dropwise 28% NaOMe in MeOH (132 μL, 0.685 mmol) under nitrogen atmosphere. After being stirred at room temperature for 2 h, an additional 28% NaOMe in MeOH (264 μL, 1.37 mmol) was added. After being stirred for 17 h, the reaction was quenched adding acetic acid (120 μL, 2.06 mmol). The mixture was evaporated to give a residue of compound **D**. The residue was suspended in MeOH/$CHCl_3$ (1/1) and filtered.

The filtrate was evaporated to give a crude product of compound **D** (988 mg), which was used further purification. The crude product of compound **D** was dissolved in 80% acetic acid in water (8.1 mL) and stirred at 70 ºC for 2 h. The reaction mixture was evaporated to dryness to afford a crude product, which was purified by HPLC system C with InertSustain AQ-C18 (20x250 mm, 5 µm particle size) using 30% MeOH/water (isocratic) to give **1g** (52.4 mg, 0.104 mmol, 15% yield over 4 steps) as a white solid.



1g

m.p.: 235-238 ºC;

^{1}H-NMR (600 MHz, D_2O): δ 7.00-7.05 (2H, m), 6.91-6.96 (2H, m), 5.12 (1H, d, *J*=8.6 Hz), 4.44 (1H, d, *J*=7.9 Hz), 4.13 (1H, dd, *J*=10.4, 8.5 Hz), 4.08 (2H, s), 3.97 (1H, dd, *J*=10.3, 8.3 Hz), 3.92 (1H, dd, *J*=12.5, 2.0 Hz), 3.88 (1H, d, *J*=3.3 Hz), 3.77-3.80 (1H, m), 3.77 (2H, s), 3.71-3.76 (1H, m), 3.69 (1H, td, *J*=8.1, 3.9 Hz), 3.57- 3.66 (3H, m), 3.50 (1H, dd, *J*=9.9, 7.9 Hz);

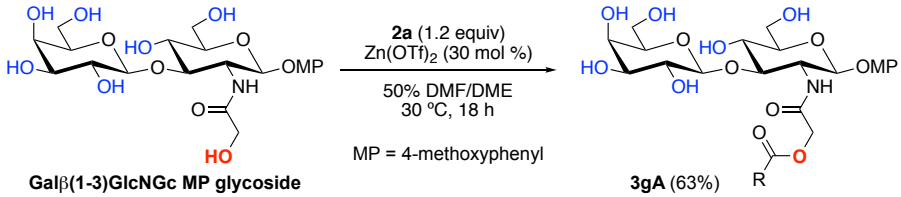
^{13}C-NMR (150 MHz, D_2O+acetone for reference): δ 176.6, 155.6, 151.6, 119.1, 115.7, 104.1, 100.9, 82.3, 76.3, 76.0, 73.2, 71.4, 69.2 (2C), 61.7, 61.6, 61.2, 56.4, 54.9

$[\alpha]_D^{27}$ +0.3 (H_2O, *c* 0.45);

HRMS (ESI-TOF) *m/z*: calcd for $C_{21}H_{31}NNaO_{13}^+$ $[M+Na]^+$: 528.1688, found: 528.1675;

HPLC: InertSustain AQ-C18 (4.6x250 mm, 5 µm), Flow rate 0.75 mL/min, 20% MeOH/H_2O (0-2 min) to 50% MeOH/H_2O (10 min, linear gradient), 50%MeOH/H_2O (10 min-25 min), t_R = 12.7 min.

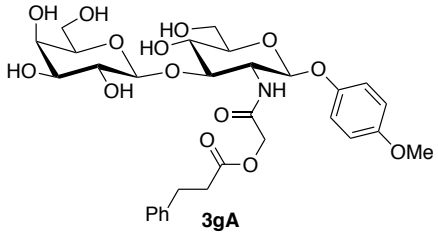
4.3. Site-selective acylation of 1g and characterization data of 3gA.



Galβ(1-3)GlcNGc MP glycoside (1g)

3gA (63%)

To a mixture of **1g** (19.1 mg, 0.0378 mmol), $Zn(OTf)_2$ (4.3 mg, 0.012 mmol) and pyridine aldoxime ester **2a** (11.8 mg, 0.0464 mmol) was added DMF/DME (1:1, 0.6 mL) under nitrogen atmosphere. After being stirred at 30 ºC for 17 hour, the reaction mixture was evaporated to afford a crude product of **3gA**, which was purified by HPLC system C with InertSustain AQ-C18 (20x250 mm, 5 µm particle size) using 70% MeOH/H_2O (isocratic) to give **3gA** (15.2 mg, 63% yield) as a white solid.



3gA

m.p.: 221-224 ºC;

^{1}H-NMR (600 MHz, DMSO-d_6): δ 7.24-7.29 (2H, m), 7.20-7.24 (2H, m), 7.16-7.20 (1H, m), 6.93-6.97 (2H, m), 6.82-6.86 (2H, m), 5.01 (1H, d, *J*=8.1 Hz), 4.79 (1H, d, *J*=5.5 Hz), 4.75 (1H, s), 4.70 (1H, t, *J*=5.9 Hz), 4.65 (1H, t, *J*=5.2 Hz), 4.54 (1H, d, *J*=4.0 Hz), 4.51 (1H, d, *J*=4.6 Hz), 4.46 (2H, s), 4.14 (1H, d, *J*=7.3 Hz), 3.67-3.77 (6H, m), 3.58-3.62 (1H, m), 3.48-3.55 (3H, m), 3.42-3.46 (1H, m), 3.27-3.33 (4H, m), 2.86 (2H, t, *J*=7.9 Hz), 2.67-2.73 (2H, m);

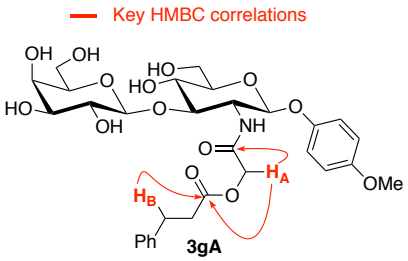
^{13}C-NMR (150MHz, DMSO-d_6): δ 171.6, 167.2, 154.7, 151.4, 140.4, 128.3, 128.2, 126.1, 118.2, 114.4, 104.1, 99.9, 83.8, 76.7, 75.7, 73.1, 70.5, 68.6, 68.2, 62.4, 60.6, 60.5, 55.4, 54.2, 34.8, 30.0;

$[\alpha]_D^{27}$ +3.1 (DMF, *c* 0.50);

HRMS (ESI-TOF) *m/z*: calcd for $C_{30}H_{39}NNaO_{14}^+$ $[M+Na]^+$: 660.2263, found: 660. 2254.

HPLC: HPLC system A with InertSustain AQ-C18 (4.6x250 mm, 5 µm), Flow rate 0.75 mL/min, 25 ºC, Eluent: 70% MeOH/H_2O (isocratic), t_R = 7.7 min.

2D NMR analysis:



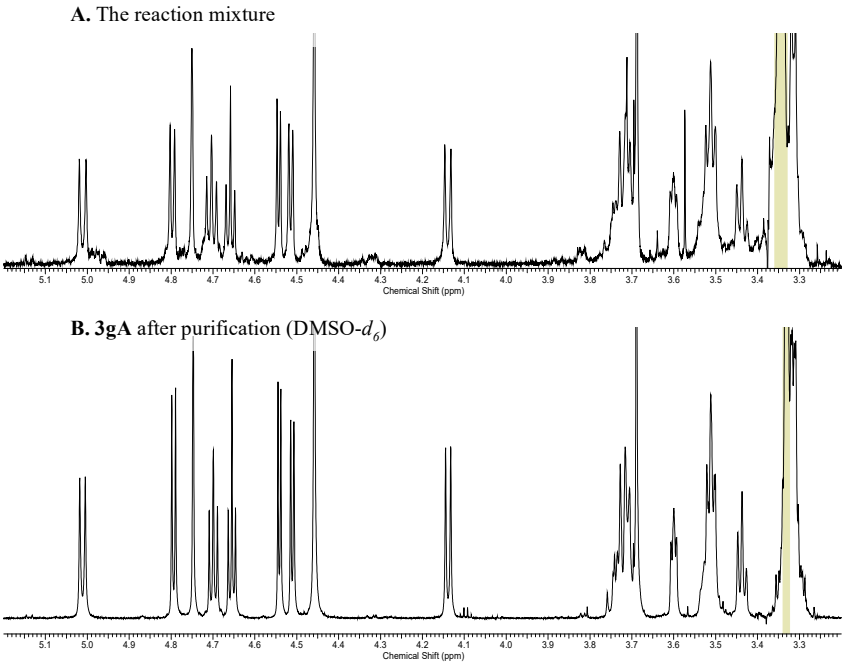
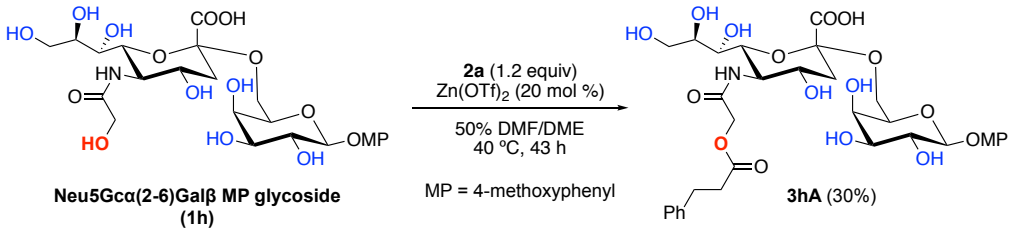


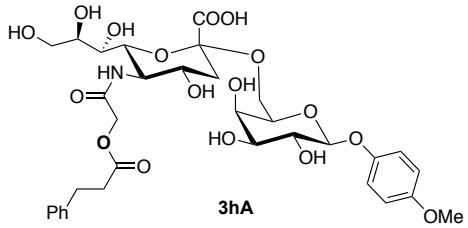
Figure SI-1. ^{1}H NMR analysis for evaluation of the site-selectivity in the reaction of **1g**. (A) ^{1}H NMR spectrum of the reaction mixture in DMSO-d_6, (B) **3gA** after purification

5. Experimental procedures for site-selective acylation of Neu5Gc-containing disaccharide 1h.

5.1. Site-selective acylation of 1h and characterization data of 3hA.



To a mixture of Neu5Gcα(2-6)Galβ MP glycoside (**1h**) (4.9 mg, 8.3 μmol, purchased from TCI chemicals), $Zn(OTf)_2$ (0.6 mg, 1.7 μmol) and pyridine aldoxime ester **2a** (2.5 mg, 9.8 μmol) was added DMF/DME (1:1, 0.2 mL) under nitrogen atmosphere. After being stirred at 40 ºC for 43 hour, the reaction mixture was evaporated to afford a crude product of **3hA**. The residue was dissolved in MeOH (0.5 mL) and 25 μL of the MeOH solution was taken out for HPLC and LCMS analysis. The other MeOH solution was purified by HPLC system C with InertSustain AQ-C18 (20x250 mm, 5 μm particle size) using a gradient (19 mL/min, 20% A over 2.5 min followed by 20-80% A over 16 min; A = MeOH, B = 20 mM aqueous NH_4OAc) to give **3hA** (1.8 mg, 30% yield) as a colorless oil concomitant with NH_4OAc.



^{1}H-NMR (600 MHz, CD_3OD): δ 7.21-7.29 (4H, m), 7.16-7.20 (1H, m), 7.02-7.06 (2H, m), 6.81-6.86 (2H, m), 4.70 (1H, d, *J*=7.7 Hz), 4.58 (2H, s), 3.96, 3.94 (2H, ABq, *J*=5.6 Hz), 3.87 (1H, ddd, *J*=8.9, 6.2, 2.5 Hz), 3.82 (1H, dd, *J*=11.4, 2.6 Hz), 3.69-3.79 (8H, m), 3.60 (1H, dd, *J*=11.6, 6.1 Hz), 3.56 (1H, dd, *J*=9.9, 3.5 Hz), 3.50 (1H, dd, *J*=9.2, 1.8 Hz), 2.92-2.98 (2H, m), 2.85 (1H, dd, *J*=12.4, 4.3 Hz), 2.74-2.78 (2H, m), 1.61 (1H, dd, *J*=12.1, 11.4 Hz);

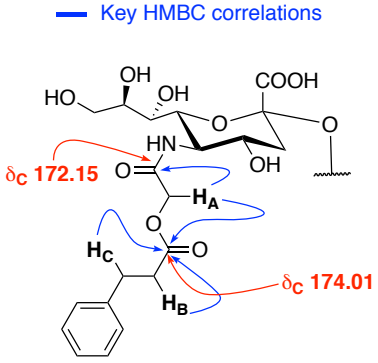
^{13}C-NMR (150 MHz, CD_3OD): δ 174.0, 172.1, 156.7, 153.5, 142.1, 129.7, 129.6, 129.5, 127.5, 119.3, 115.6, 104.1, 102.1, 75.3, 74.9, 74.2, 73.4, 72.6, 70.5, 69.9, 69.6, 65.0, 63.8, 63.6, 56.2, 54.1, 42.9, 36.5, 31.8;

$[\alpha]_D^{26}$ –12 (MeOH, *c* 0.09);

HRMS (ESI-orbitrap) *m/z*: calcd for $C_{33}H_{42}NO_{17}^-$ $[M–H]^-$: 724.2458, found: 724.2469.

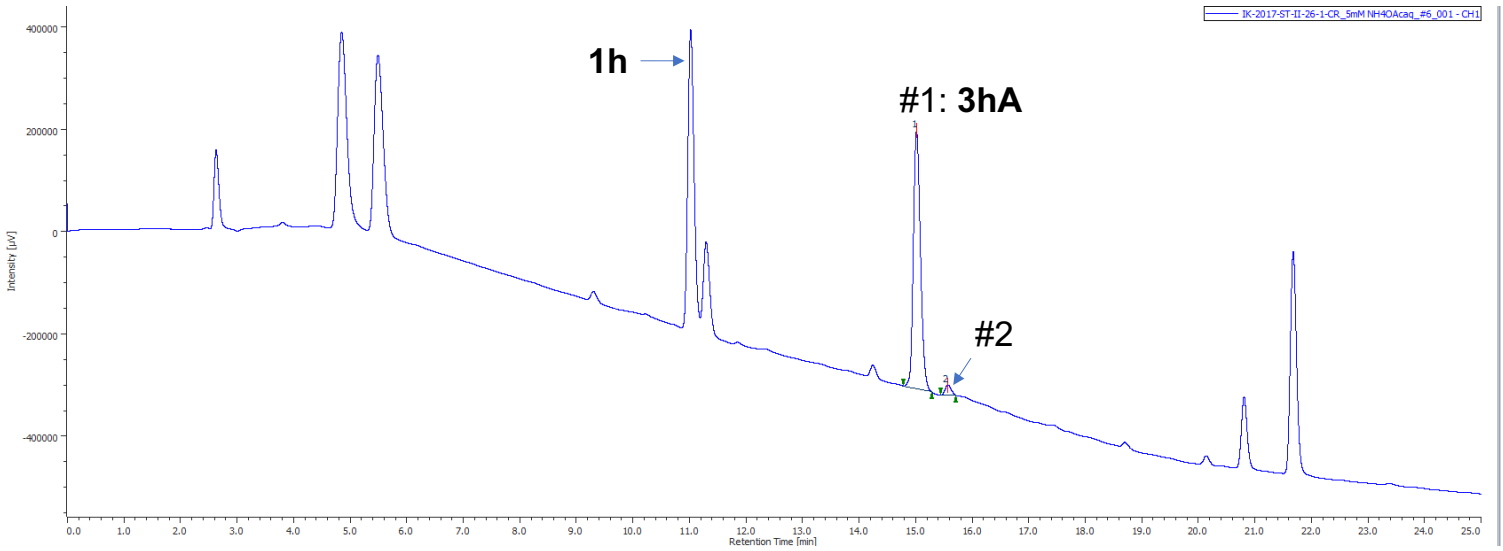
HPLC: HPLC system A with InertSustain AQ-C18 (4.6x150 mm, 3 μm), Flow rate 0.6 mL/min, 25 ºC. Gradient: 20% A over 2.5 min followed by 20-80% A over 16 min; A = MeOH, B = 5 mM aqueous NH_4OAc, t_R = 15.0 min.

2D NMR



5.2. HPLC and LCMS analysis of the mixture in the acylation of 1h.

The analytical sample for HPLC was prepared by diluting 25 μL of the reaction solution in 180 μL of 50% MeOH/H_2O followed by filtration through a nylon syringe filter (ϕ: 13 mm, pore size: 0.22 μm). 10 μL of the diluted solution was injected in HPLC system A and analyzed in the condition described above. The HPLC solution was used after diluting 400 times with 50% MeOH/5 mM aqueous NH_4OAc for the LCMS analysis.



Area%

#	ピーク名	CH	tR	面積	高さ	面積%	高さ%	定量値	NTP	分離度	シンメトリー係数	警告
1	Unknown	1	15.008	4386593	503437	96.717	96.188	N/A	68908	2.567	1.196	
2	Unknown	1	15.564	148923	19954	3.283	3.812	N/A	91896	N/A	1.093	

Figure SI-2. HPLC trace for the mixture in the acylation of 1h

LCMS trace of the mixture in the acylation of 1h.

MS was operated with ESI voltage at -2.0 kV for negative mode; sheath gas flow rate of 50, and capillary temperature of 380 ºC. HPLC was operated under the same conditions as HPLC-UV analysis above.

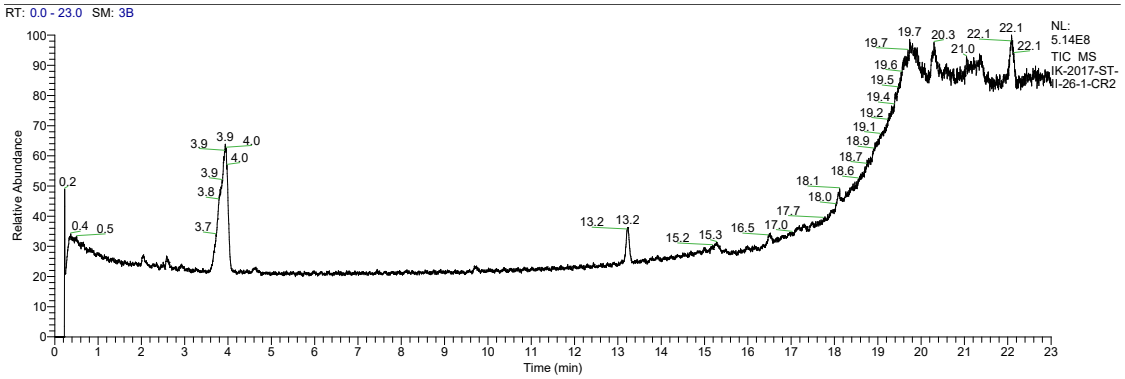


Figure SI-3. Total ion chromatogram in LCMS analysis of the mixture in the acylation of 1h

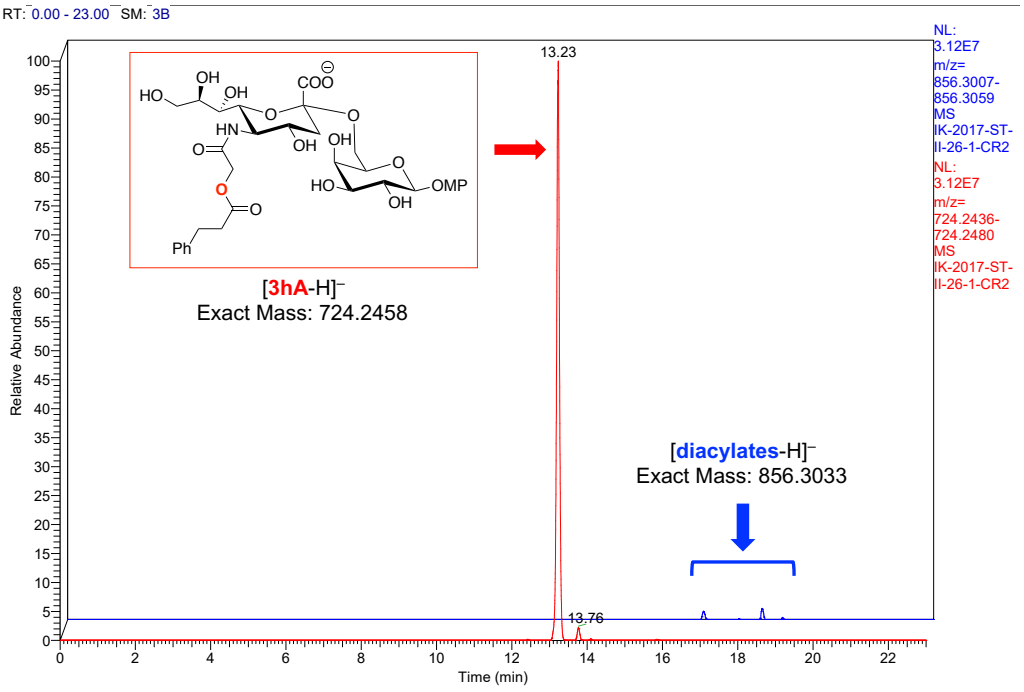


Figure SI-4. Extracted ion chromatogram (XIC) in LCMS analysis of the mixture in the acylation of 1h. The negative ion of *m/z* 724.2458 within 3 ppm error was extracted from total ions detected in all range of analysis times (red trace). The negative ion of *m/z* 856.3033 was also extracted (blue trace).

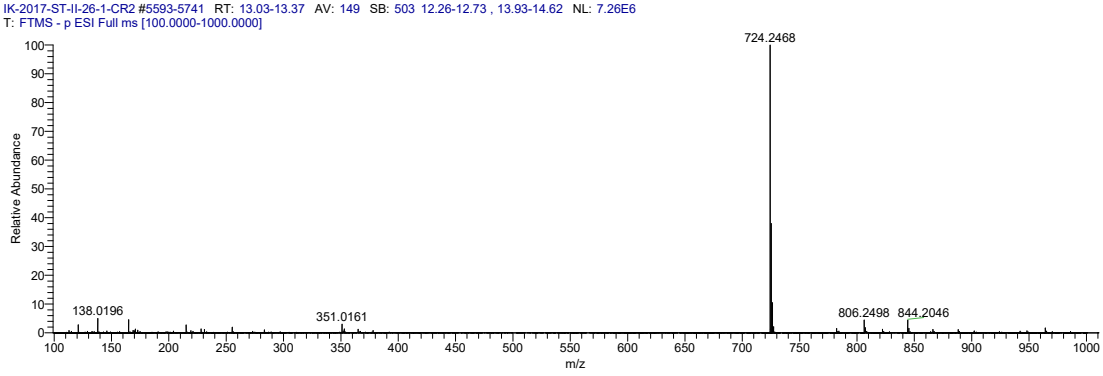
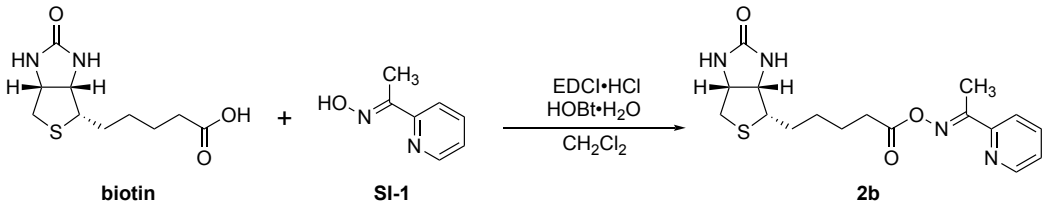


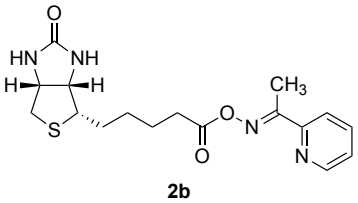
Figure SI-5. Mass spectrum between 13.03 and 13.37 min in LCMS analysis of the acylation of 1h.

6. Experimental procedures for site-selective biotinylation of glycopeptide 1i.

6.1. Synthesis of 2b and its characterization data.



To a stirred solution of biotin (244 mg, 1.00 mmol), ketoxime **SI-1**[2] (163 mg, 1.20 mmol), HOBt•H_2O (30.7 mg, 0.200 mmol) in CH_2Cl_2 (10 mL) at 0 ºC was added 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDCI•HCl, 288 mg, 1.50 mmol). After being stirred at room temperature for 18 h under nitrogen atmosphere, the reaction mixture was evaporated to dryness to afford a crude product. The obtained crude product was suspended in EtOAc then filtered to afford **2b** (263 mg, 0.726 mmol, 73% yield) as a white solid.



m.p.: 168-170ºC;

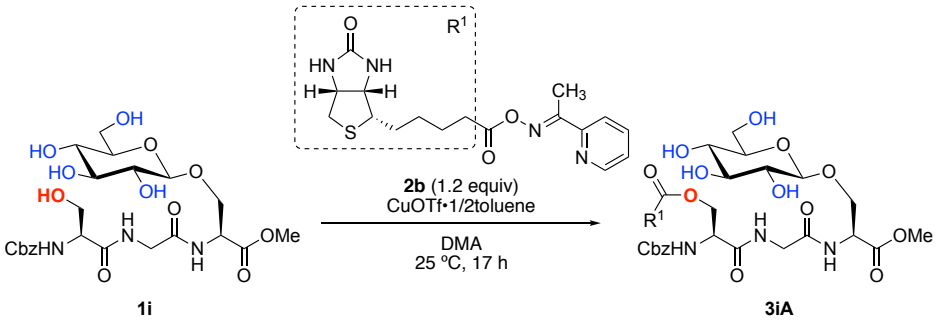
^{1}H-NMR (600 MHz, $CDCl_3$): δ 8.63-8.65 (1H, m), 8.09 (1H, dt, *J*=8.0, 1.1 Hz), 7.74 (1H, td, *J*=7.8, 1.7 Hz), 7.35 (1H, ddd, *J*=7.5, 4.8, 1.3 Hz), 5.10 (1H, br-s), 4.79 (1H, br-s), 4.51-4.55 (1H, m), 4.32-4.36 (1H, m), 3.19 (1H, ddd, *J*=8.6, 6.2, 4.7 Hz), 2.94 (1H, dd, *J*=13.0, 5.1 Hz), 2.75 (1H, d, *J*=13.0 Hz), 2.57 (2H, t, *J*=7.4 Hz), 2.50 (3H, s), 1.49-1.87 (8H, m, overlapped);

^{13}C-NMR (150 MHz, $CDCl_3$): δ 170.9, 163.5, 163.0, 152.7, 149.1, 136.5, 124.9, 121.9, 61.9, 60.1, 55.2, 40.5, 32.6, 28.4, 28.3, 24.7, 12.9;

$[\alpha]_D^{21}$ +49 ($CHCl_3$, *c* 0.53);

HRMS (ESI-orbitrap) *m/z*: calcd for $C_{17}H_{23}N_4O_3S^+$ $[M+H]^+$: 363.1485, found: 363.1488.

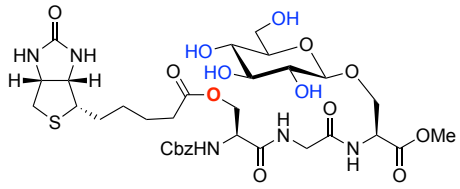
6.2. Site-selective acylation of 1i and characterization data of 3iA.



To a mixture of glycopeptide **1i**[2] (19.4 mg, 34.7 μmol), CuOTf•1/2toluene (9.0 mg, 18.0 μmol) and pyridine aldoxime ester **2a** (15.1 mg, 41.7 μmol) was added DMA (7.0 mL) under nitrogen atmosphere. After being stirred at 25 ºC for 17 hours, the reaction was quenched by adding methanol (1.0 mL) and 20 mM phosphate buffer (pH7, 1.0 mL) followed by being stirred for 30 min. The reaction mixture was evaporated to afford a crude product of **3iA**. A solution of benzamide in MeOH (86.7 μL, 0.2 mmol/mL) was added to the residue as the internal standard for HPLC quantification and then the mixture was used for HPLC and LCMS analysis. After HPLC

[2] Takemoto, K.; Nishikawa, Y.; Moriguchi, S.; Hori, Y.; Kamezawa, Y.; Matsui, T.; Hara, O. *Org. Lett.* **2019**, *21*, 7534.

and LCMS analysis, all of the crude product was purified by HPLC system C with Kinetex EVO C18 (21.2x150 mm, 5 µm particle size) using a gradient (20 mL/min, 10% A over 1 min followed by 10-50% A over 8 min; A = CH_3CN, B = 10 mM aqueous NH_4OAc) to give **3iA** (8.4 mg, 31% yield) as a colorless oil concomitant with NH_4OAc.



3iA

^{1}H-NMR (600 MHz, CD_3OD): δ 7.33-7.41 (4H, m), 7.29-7.33 (1H, m), 5.17, 5.10 (2H, ABq, *J*=12.4), 4.72 (1H, t, *J*=3.9 Hz), 4.51 (1H, dd, *J*=7.0, 4.6 Hz), 4.47 (1H, dd, *J*=7.6, 4.9 Hz), 4.43 (1H, dd, *J*=11.4, 4.6 Hz), 4.26-4.35 (4H, m), 3.99, 3.94 (2H, ABq, *J*=16.7 Hz), 3.84-3.88 (1H, m), 3.81 (1H, dd, *J*=10.3, 3.7 Hz), 3.74 (3H, s), 3.64-3.69 (1H, m), 3.32-3.38 (2H, m), 3.25-3.29 (2H, m), 3.15-3.21 (1H, m), 2.91 (1H, dd, *J*=12.7, 5.0 Hz), 2.70 (1H, d, *J*=12.8 Hz), 2.33 (2H, t, *J*=7.2 Hz), 1.51-1.76 (4H, m), 1.42 (2H, dd, *J*=8.1, 4.4 Hz);

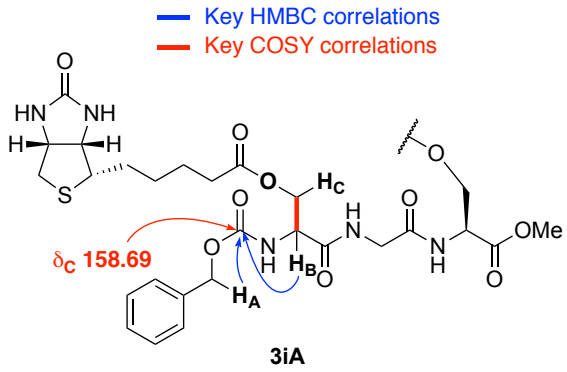
^{13}C-NMR (150 MHz, CD_3OD): δ 175.1, 172.2, 172.0, 171.4, 166.3, 158.7, 138.2, 129.7, 129.3, 129.1, 104.7, 78.2, 78.0, 75.2, 71.6, 70.2, 68.2, 64.8, 63.5, 62.8, 61.8, 57.1, 55.6, 54.2, 53.2, 43.5, 41.2, 34.7, 29.7, 29.5, 25.9;

$[\alpha]_D^{25}$ +16 (MeOH, *c* 0.25);

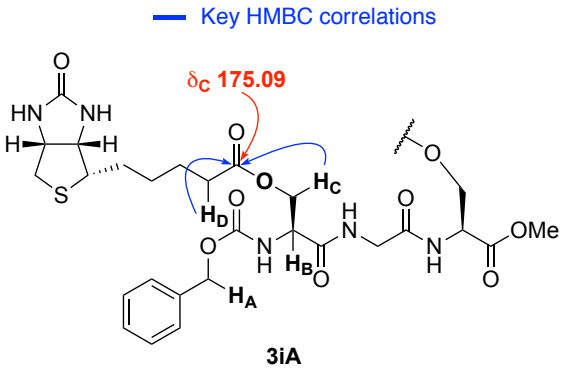
HRMS (ESI$^+$) *m/z*: calcd for $C_{33}H_{48}N_5O_{15}S^+$ $[M+H]^+$: 786.2862, found: 786.2862.

HPLC: HPLC system B with Kinetex EVO C18 (2.1x100 mm, 2.6 µm), Flow rate 0.4 mL/min, 40 ºC, Gradient: 10-50% A over 8 min; A = CH_3CN, B = 10 mM aqueous NH_4OAc, t_R = 6.2 min.

2D NMR



3iA



3iA

6.3. Acylation of 1i in conventional conditions

To a mixture of glycopeptide **1i** (18.2 mg, 32.5 µmol) and *N*-hydroxysuccinimidyl D-biotinate (13.3 mg, 39.0 µmol) was added pyridine (0.33 mL) under nitrogen atmosphere. After being stirred at 40 ºC for 19 hours, the reaction was quenched by adding methanol (1.0 mL) and 20 mM phosphate buffer (pH7, 1.0 mL) followed by being stirred for 30 min. The reaction mixture was evaporated to afford a crude product of **3iA**. HPLC and LCMS analysis was done in the same procedure as in the site-selective acylation and showed that the unselective acylation occurred.

6.4. HPLC and LCMS analysis of the mixture in the acylation of 1i.

The sample for HPLC analysis was prepared as follows. To the reaction solution was added a solution of benzamide in MeOH (86.7 µL, 0.2 mmol/mL) as an internal standard. The solution was diluted with 1648 µL of 50% MeOH/H_2O. and then 100 µL of the resulting solution was taken out and diluted 10 times with 10% CH_3CN/10 mM aqueous NH_4OAc followed by filtration through a nylon syringe filter (ϕ: 13 mm, pore size: 0.22 µm) to afford the solution for HPLC analysis. 3 µL of the diluted solution was injected in HPLC system B and analyzed in the condition described above. The HPLC solution was used after diluting 400 times with 10% CH_3CN/10 mM aqueous NH_4OAc for the LCMS analysis.

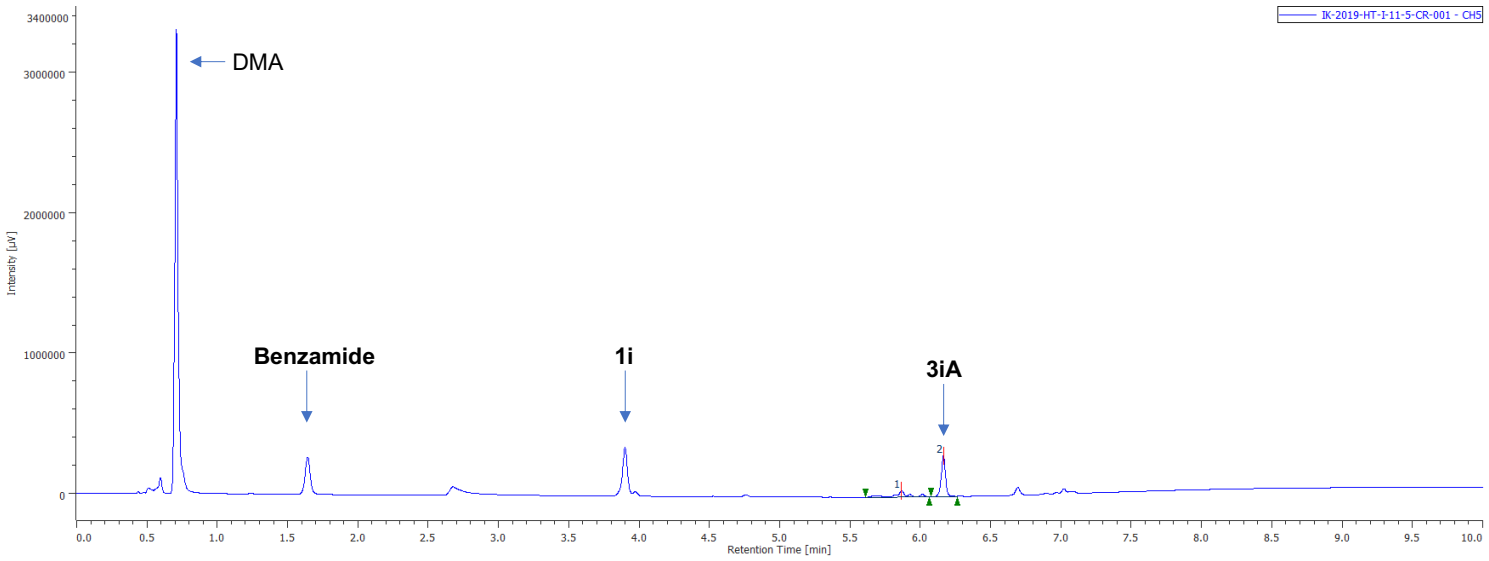
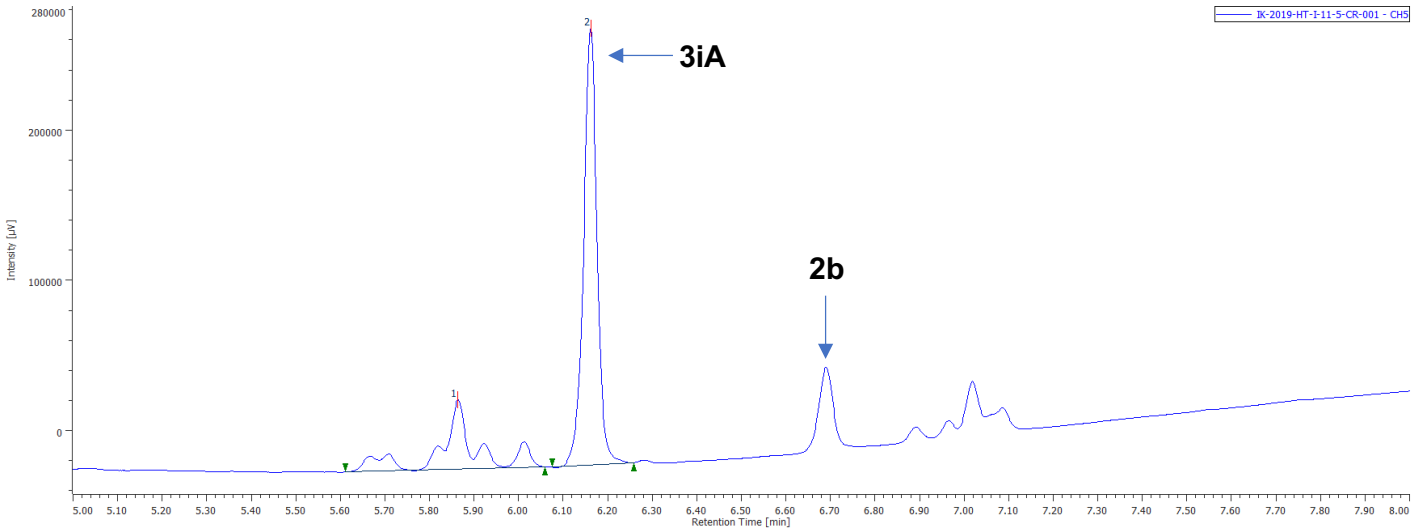
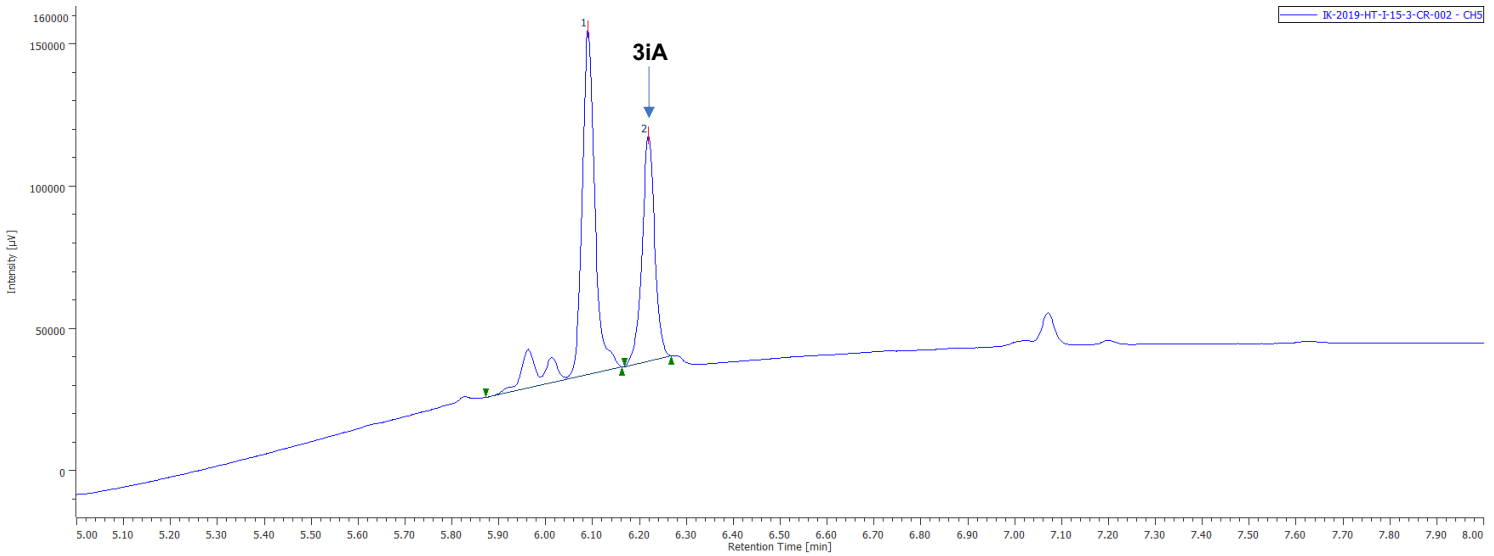


Figure SI-6. HPLC trace for the mixture in the site-selective acylation of 1i



#	ピーク名	CH	tR	Area	Area%	NTP
1	Unknown	5	5.863	239288	28.204	183509
2	Unknown	5	6.162	609131	71.796	223196

Figure SI-7. The enlarged Figure SI-5 in range from 5 min to 8 min.



#	ピーク名	CH	tR	Area	Area%	NTP
1	Unknown	5	6.090	269897	64.535	282770
2	Unknown	5	6.218	148320	35.465	280088

Figure SI-8. HPLC trace between 5 min and 8 min for the mixture in the acylation of 1i in conventional conditions.

LCMS trace of the mixture in the acylation of 1i.

MS was operated with ESI voltage at +3.5 kV for positive mode; sheath gas flow rate of 40, and capillary temperature of 350 ºC. HPLC was operated under the same conditions as HPLC-UV analysis above.

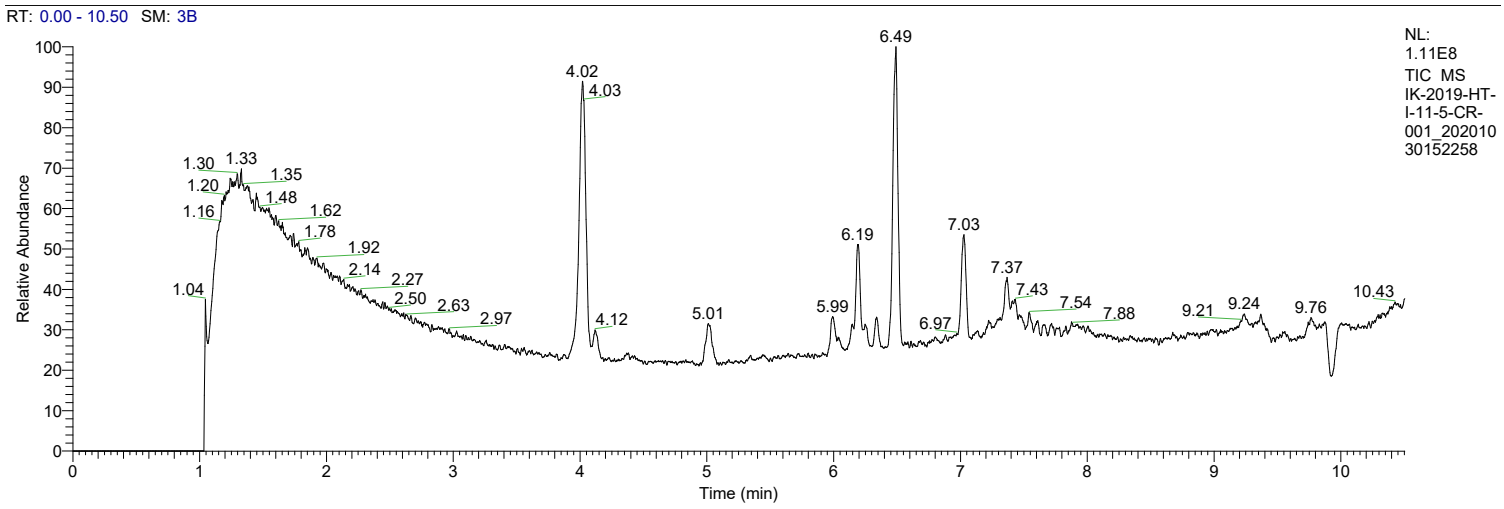


Figure SI-9. Total ion chromatogram in LCMS analysis of the mixture in the acylation of 1i

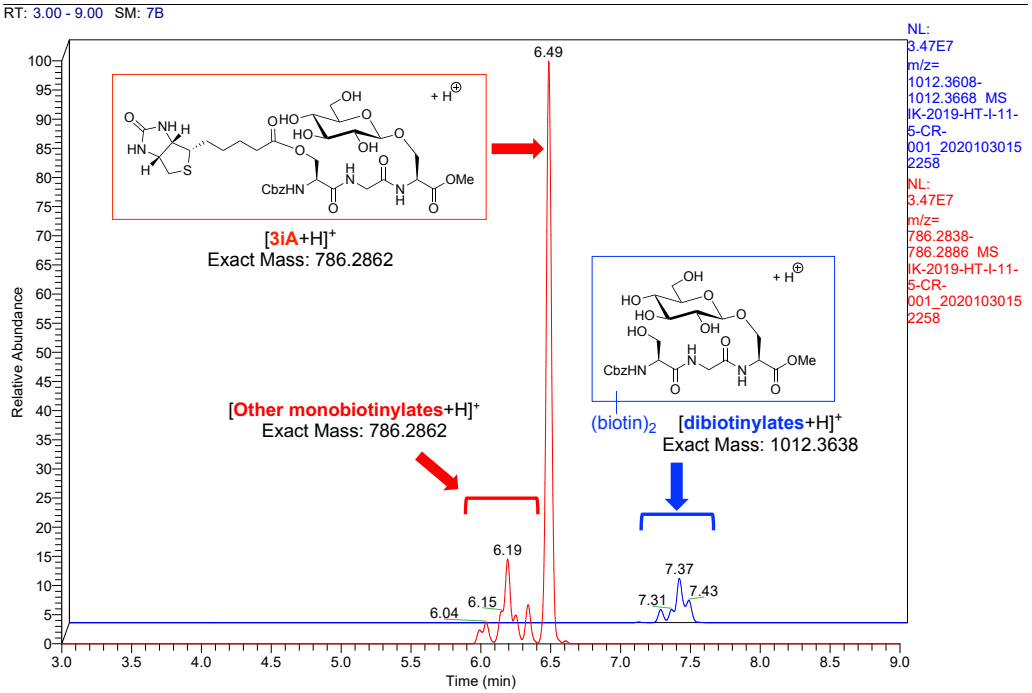


Figure SI-10. Extracted ion chromatogram (XIC) in LCMS analysis of the mixture in the site-selective acylation of 1i. The positive ion of *m/z* 786.2862 within 3 ppm error was extracted from total ions detected in all range of analysis times (red trace). The positive ion of *m/z* 1012.3638 was also extracted (blue trace).

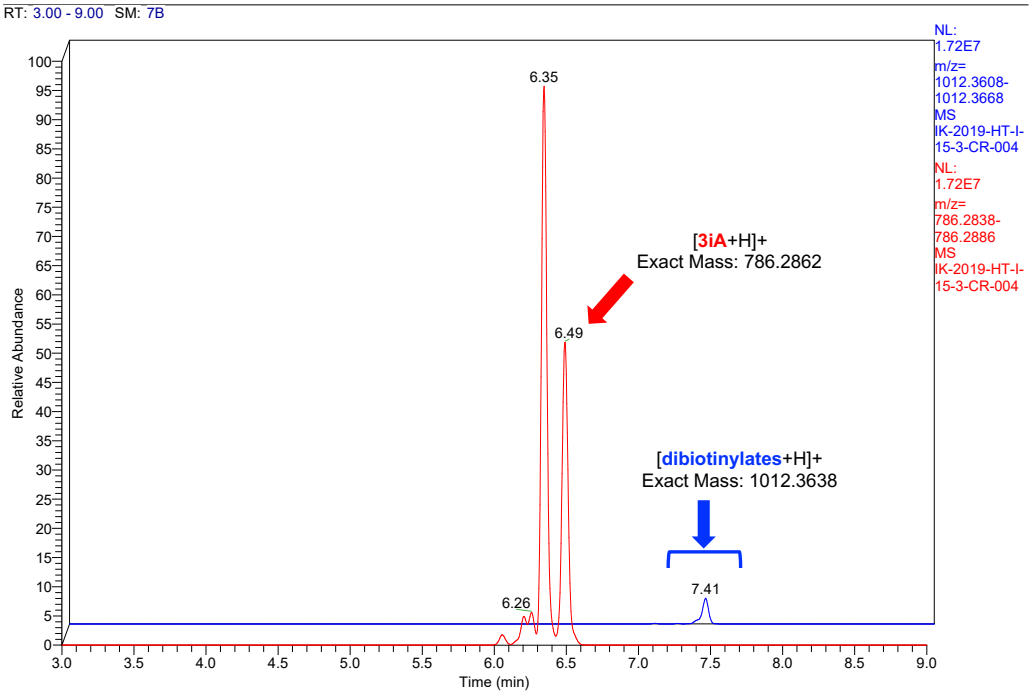


Figure SI-11. Extracted ion chromatogram (XIC) in LCMS analysis of the mixture in the acylation of 1i in conventional conditions. The positive ion of *m/z* 786.2862 within 3 ppm error was extracted from total ions detected in all range of analysis times (red trace). The positive ion of *m/z* 1012.3638 was also extracted (blue trace).

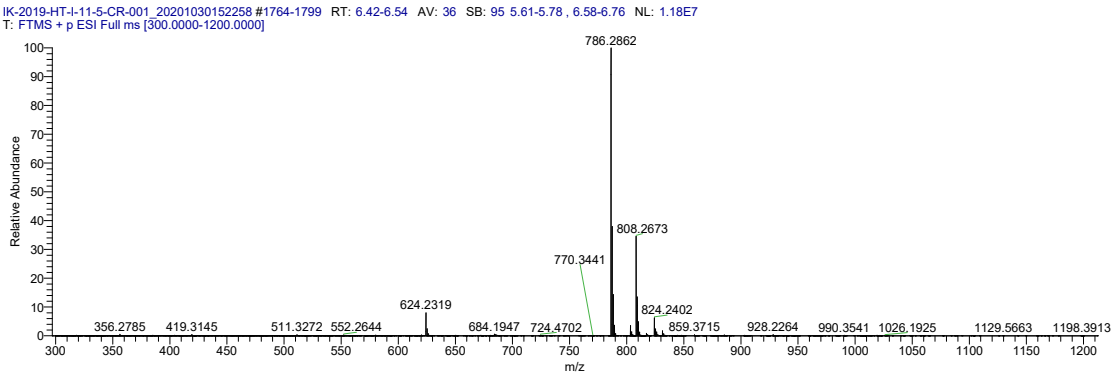
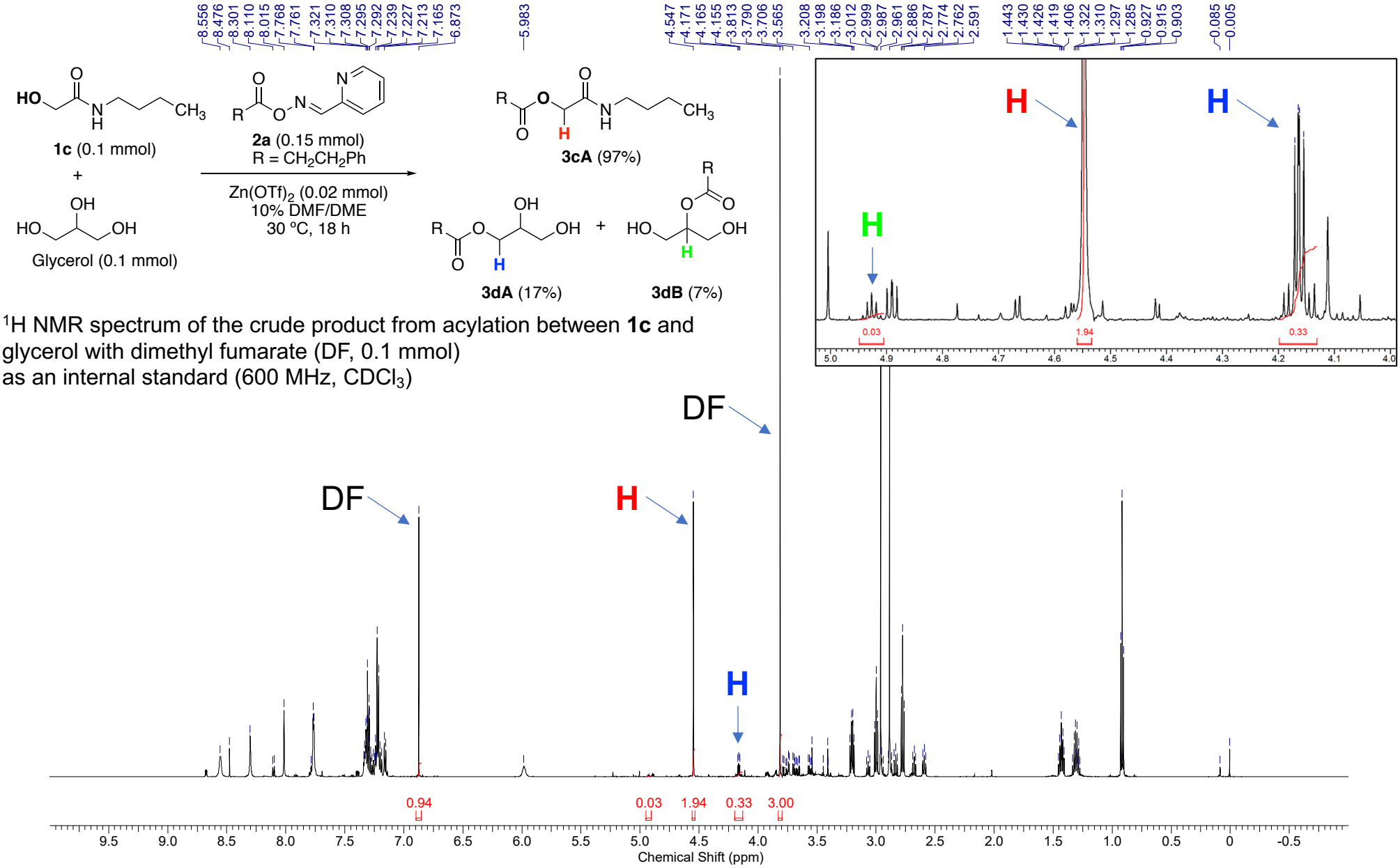


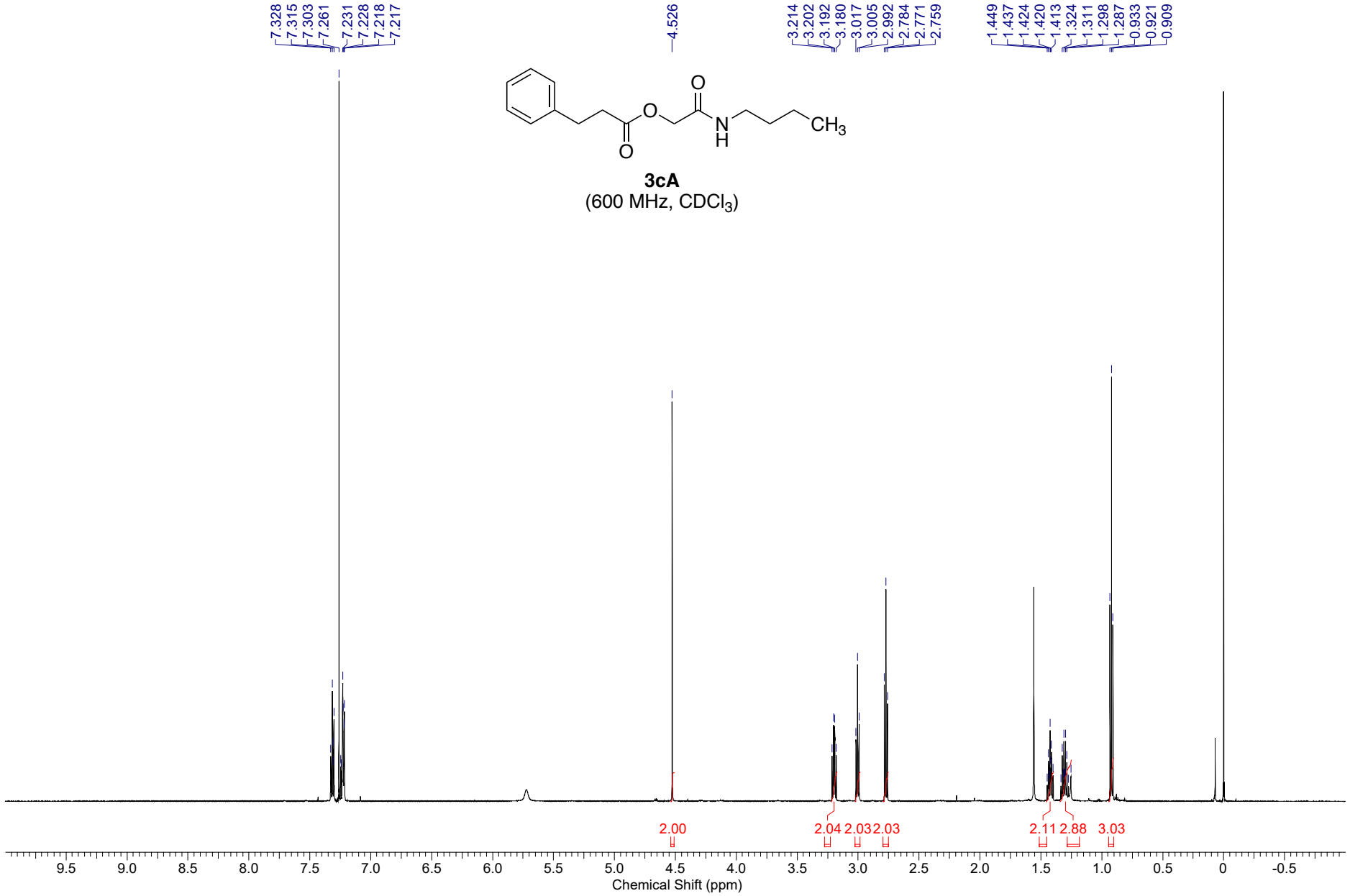
Figure SI12. Mass spectrum between 6.42 and 6.54 min in LCMS analysis of the site-selective acylation of 1i.

^{1}H NMR spectrum of control experiment 1 (Scheme 1a in manuscript)

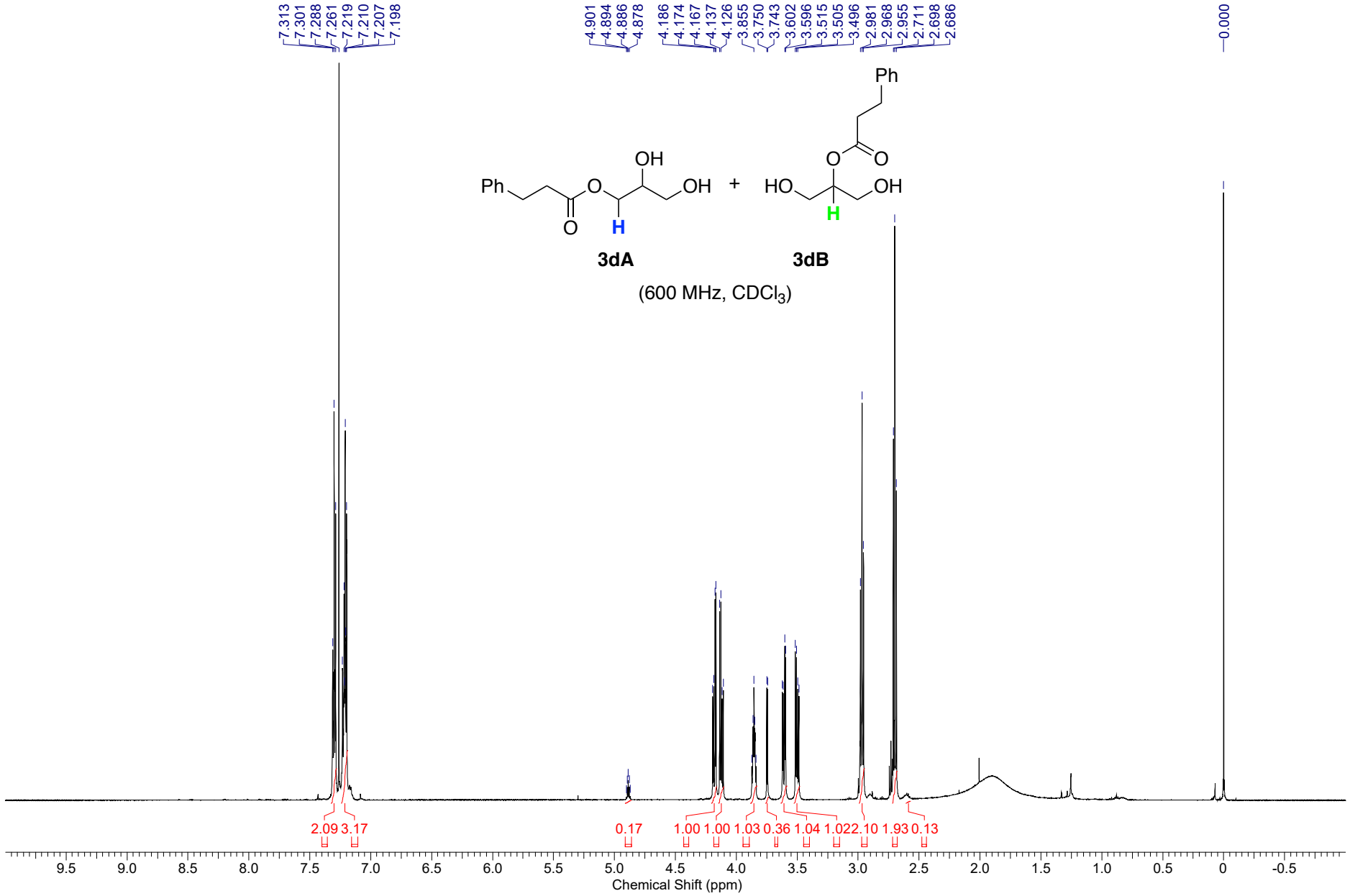


^{1}H NMR spectrum of the crude product from acylation between **1c** and glycerol with dimethyl fumarate (DF, 0.1 mmol) as an internal standard (600 MHz, $CDCl_3$)

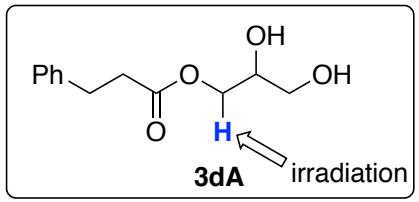
^{1}H NMR spectrum of **3cA**



^{1}H NMR spectrum of **3dA+3dB**

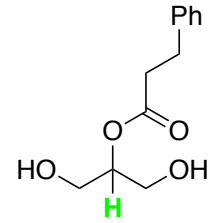


1D selective gradient TOCSY spectrum of **3dA+3dB**



3dA

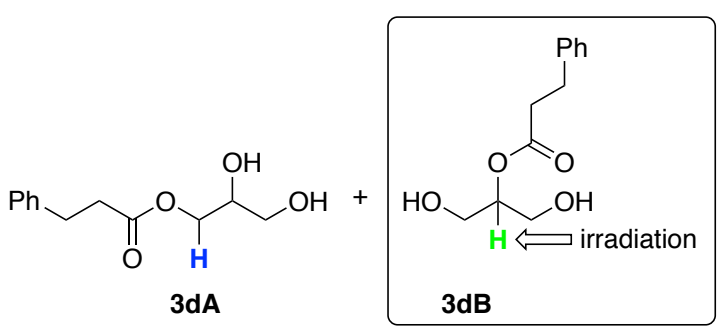
+



3dB

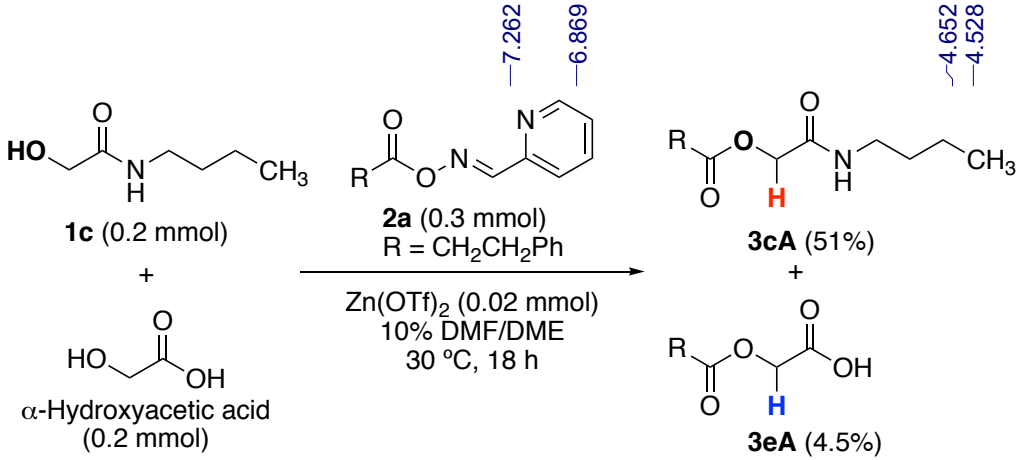
(600 MHz, $CDCl_3$)

1D selective gradient TOCSY spectrum of **3dA+3dB**

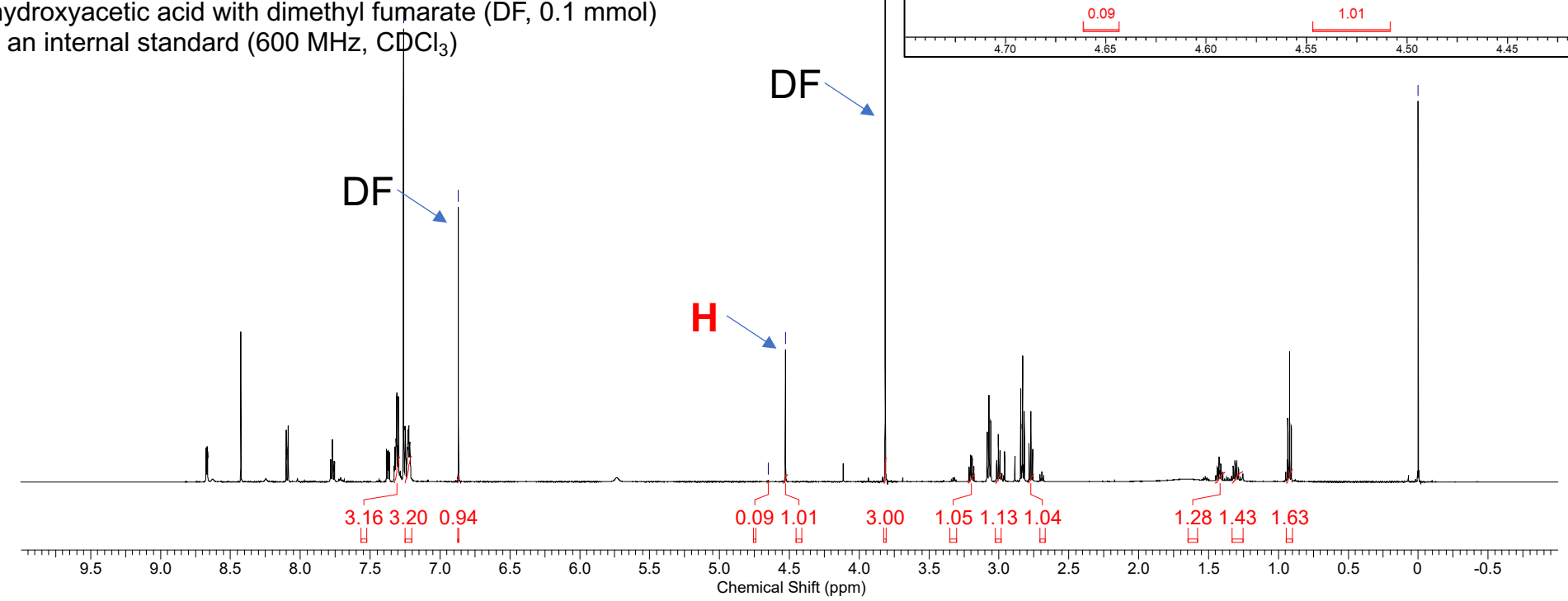


(600 MHz, $CDCl_3$)

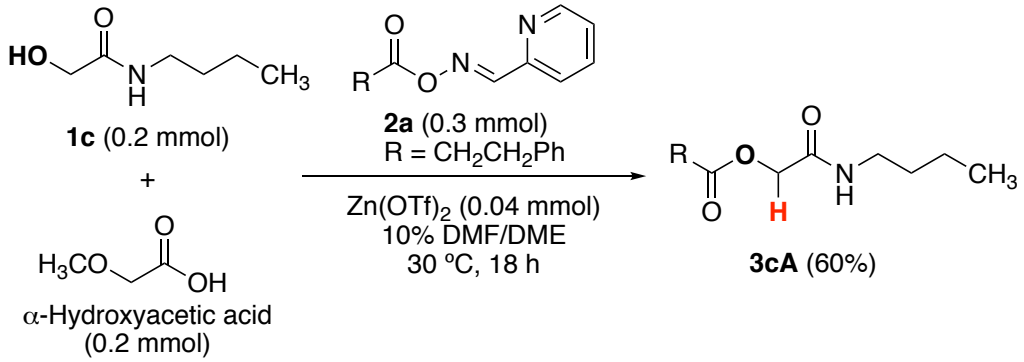
^{1}H NMR spectrum of control experiment 2 (Scheme 1b in manuscript)



^{1}H NMR spectrum of the crude product from acylation between **1c** and α-hydroxyacetic acid with dimethyl fumarate (DF, 0.1 mmol) as an internal standard (600 MHz, $CDCl_3$)

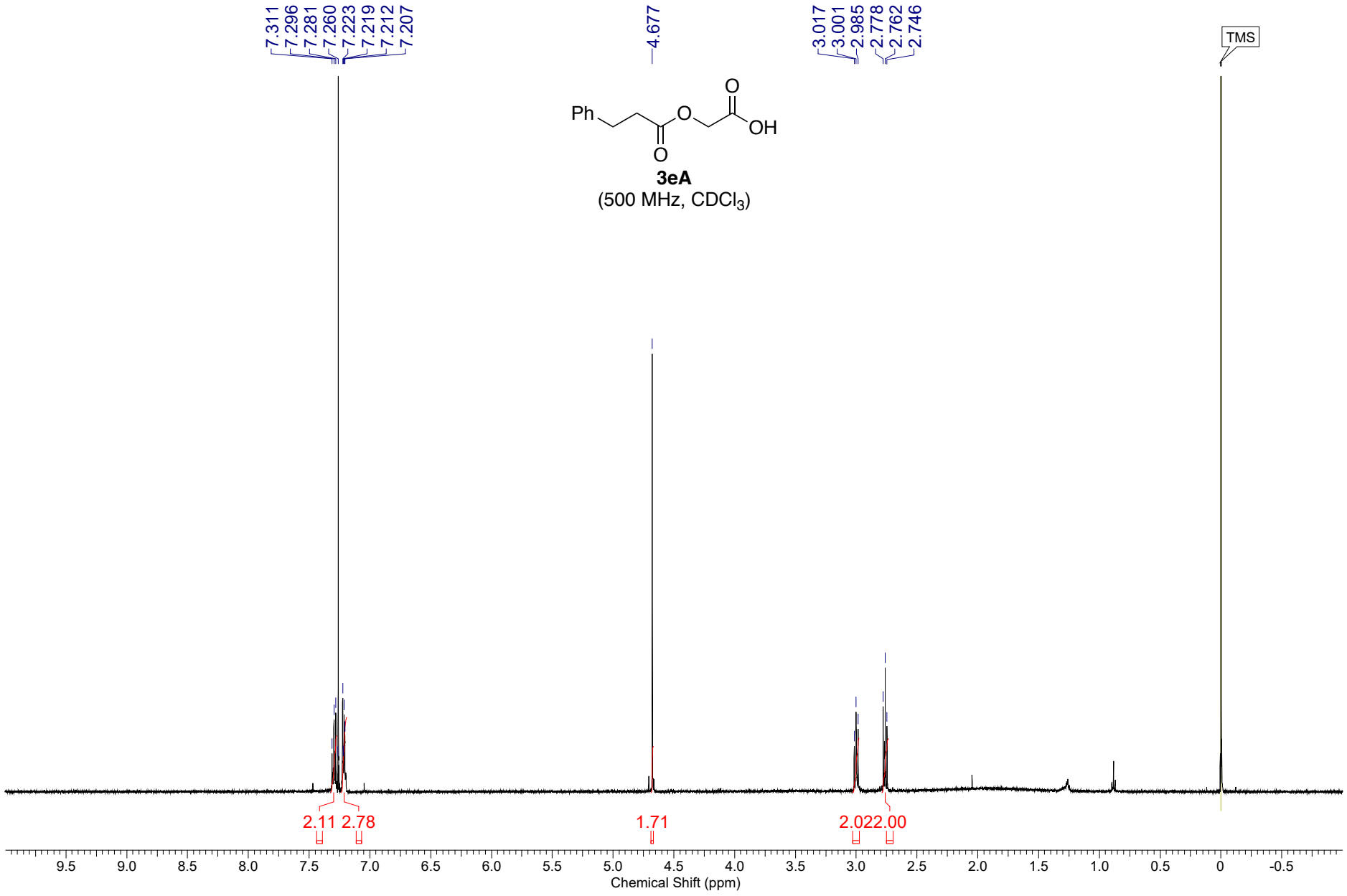


^{1}H NMR spectrum of control experiment 3 (Supporting Infromation 2.3.)

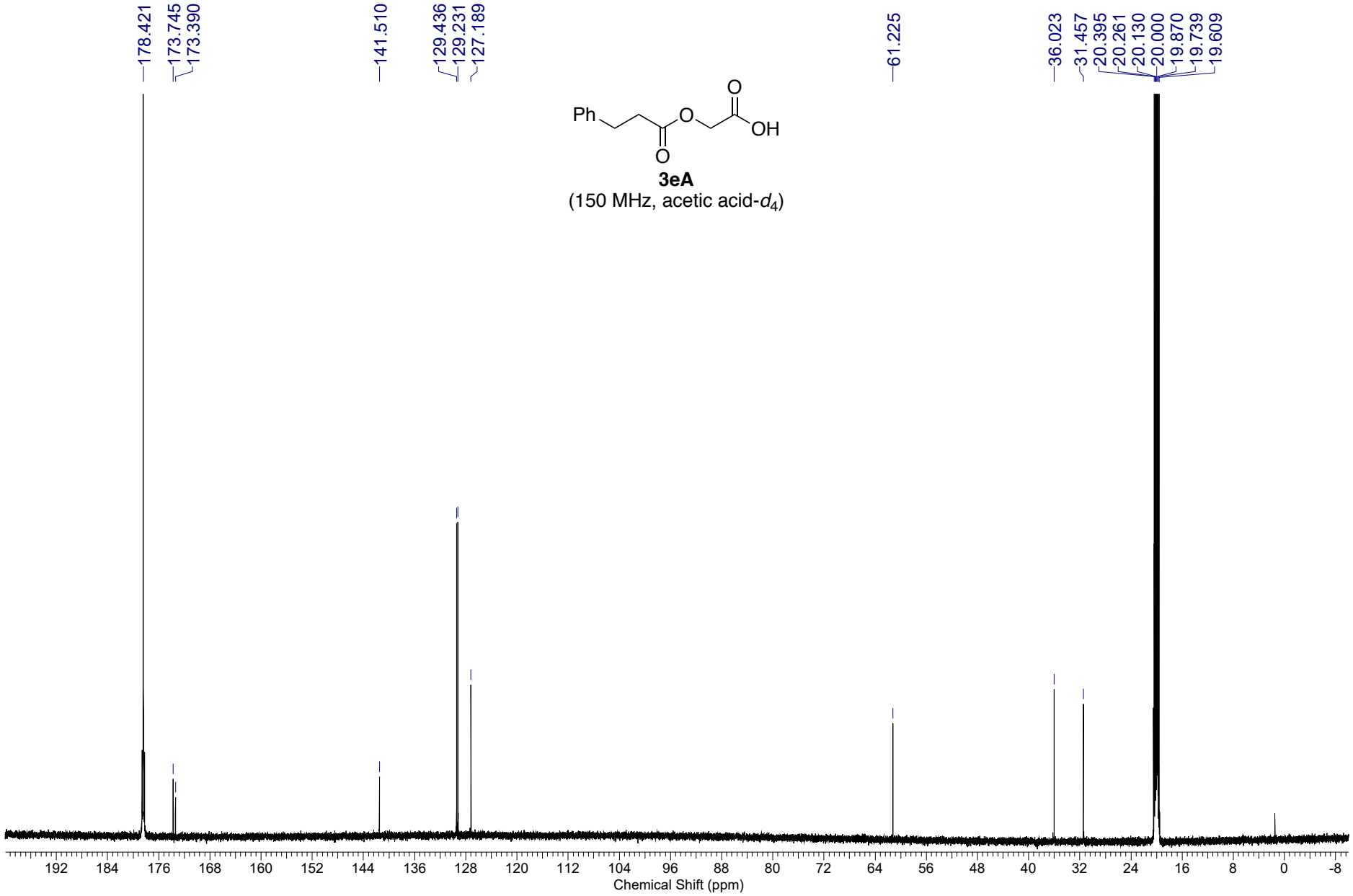


^{1}H NMR spectrum of the crude product from acylation between **1c** and α-methoxyacetic acid with dimethyl fumarate (DF, 0.1 mmol) as an internal standard (600 MHz, $CDCl_3$)

^{1}H NMR spectrum of **3eA**



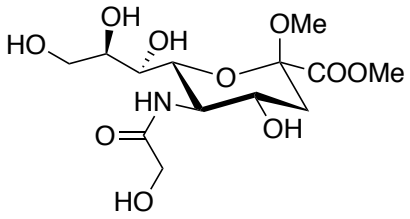
^{13}C NMR spectrum of **3eA**



3eA

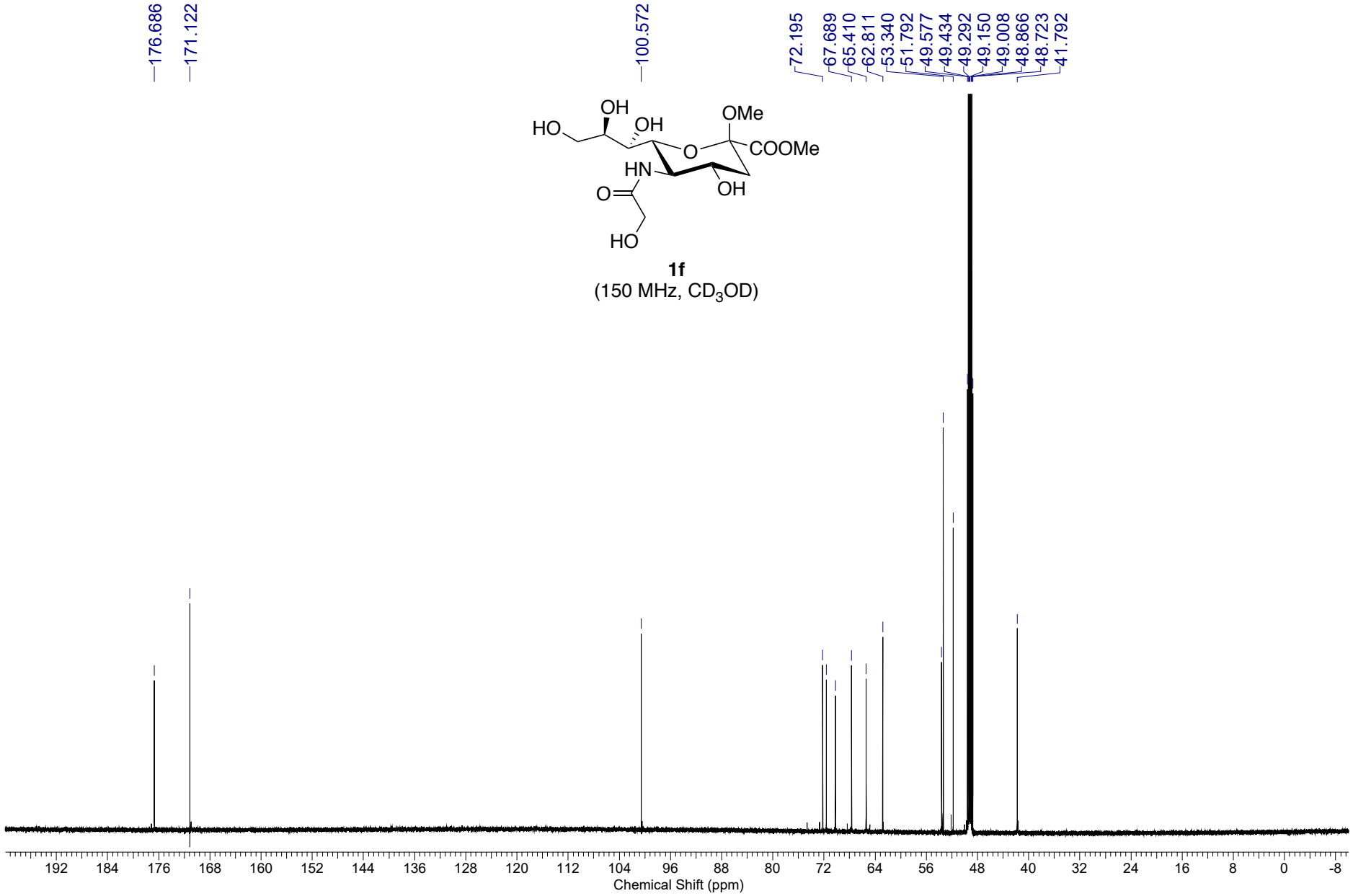
(150 MHz, acetic acid-d_4)

^{1}H NMR spectrum of **1f**



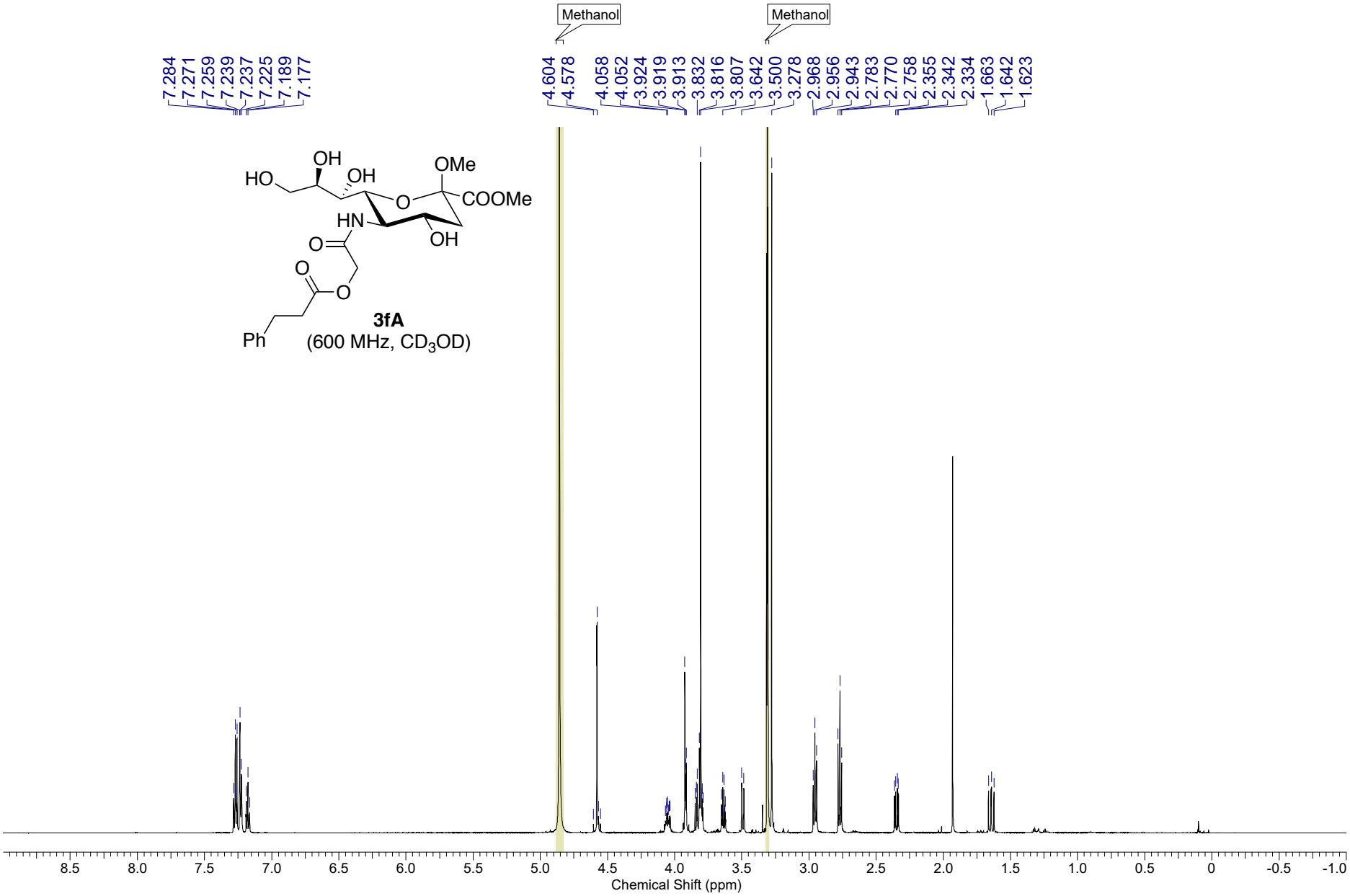
1f
(600 MHz, CD_3OD)

^{13}C NMR spectrum of **1f**

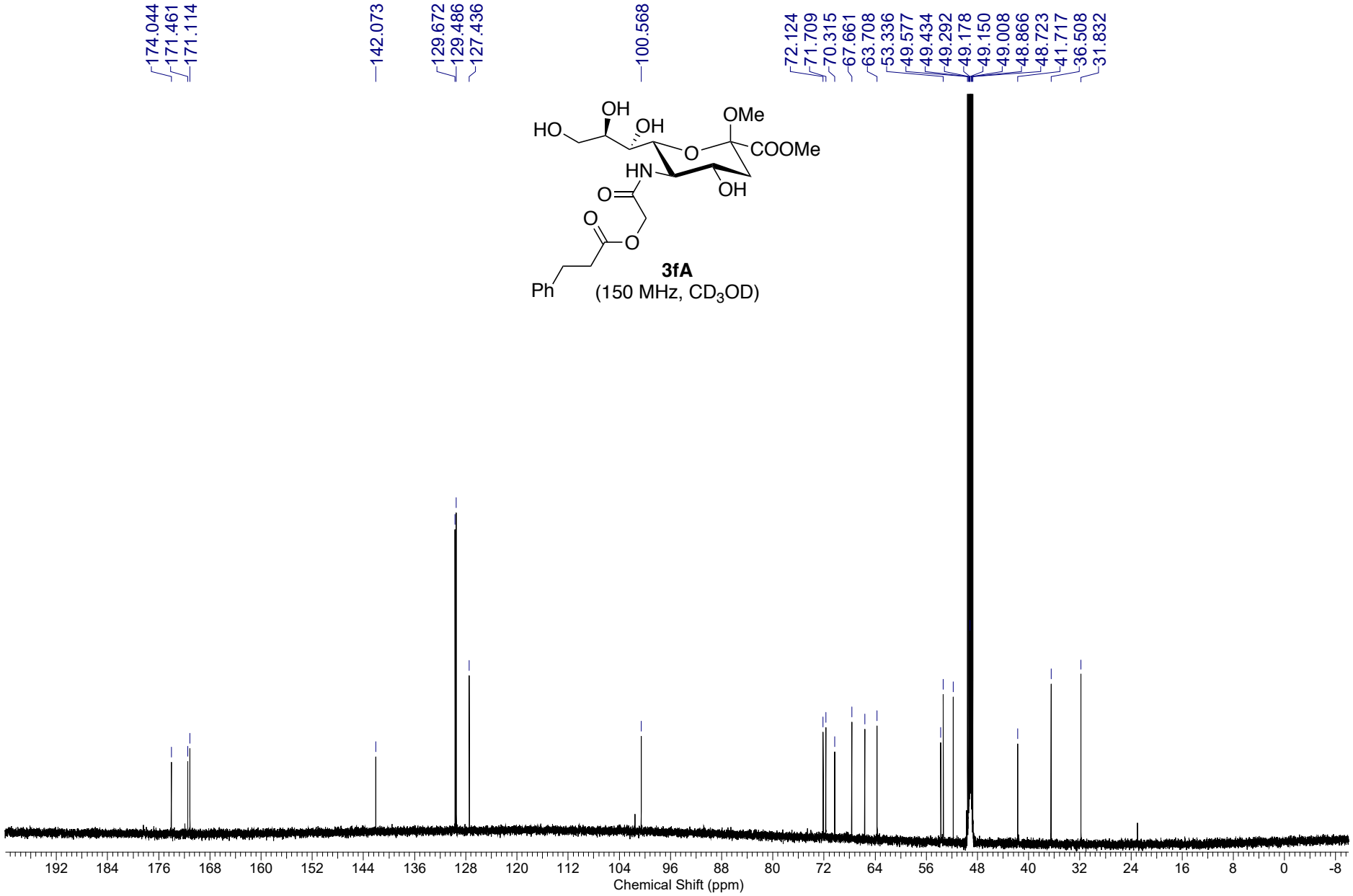


1f
(150 MHz, CD_3OD)

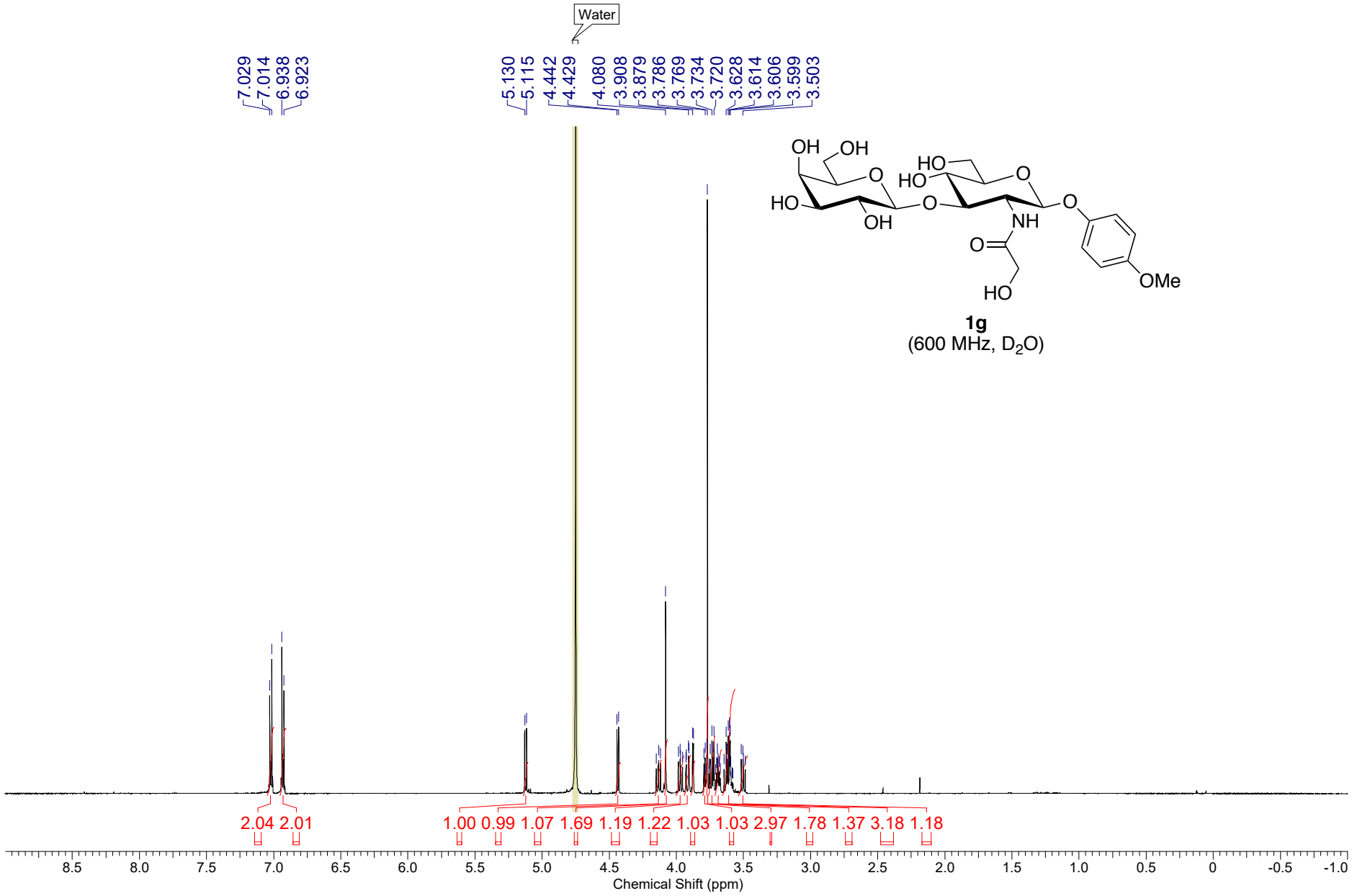
^{1}H NMR spectrum of **3fA**



^{13}C NMR spectrum of **3fA**

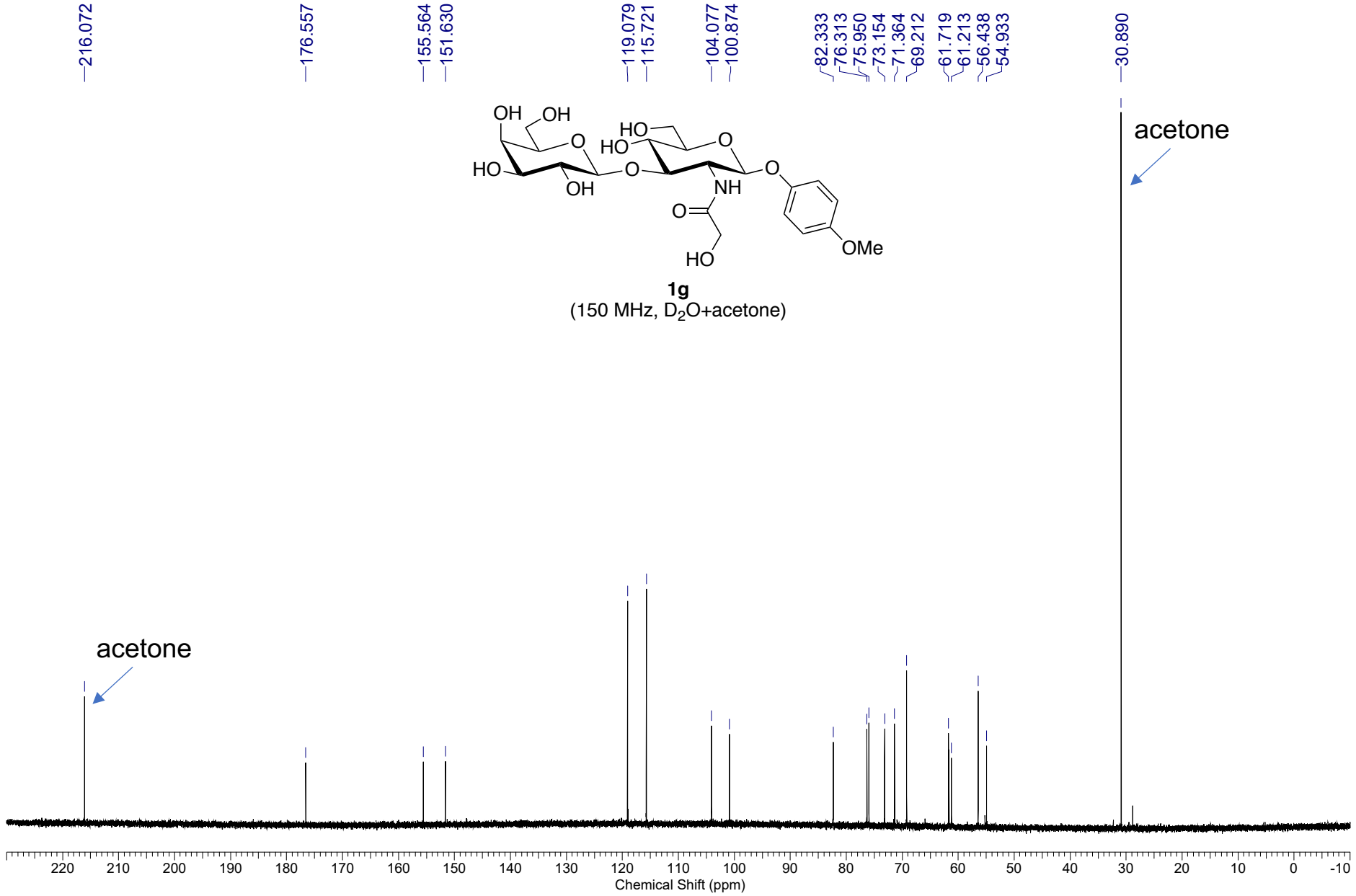


^{1}H NMR spectrum of **1g**



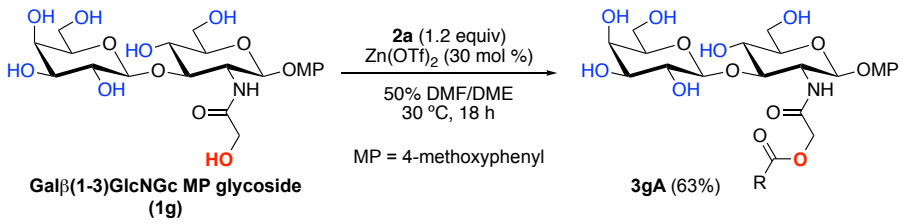
1g
(600 MHz, D_2O)

^{13}C NMR spectrum of **1g**



1g
(150 MHz, D_2O+acetone)

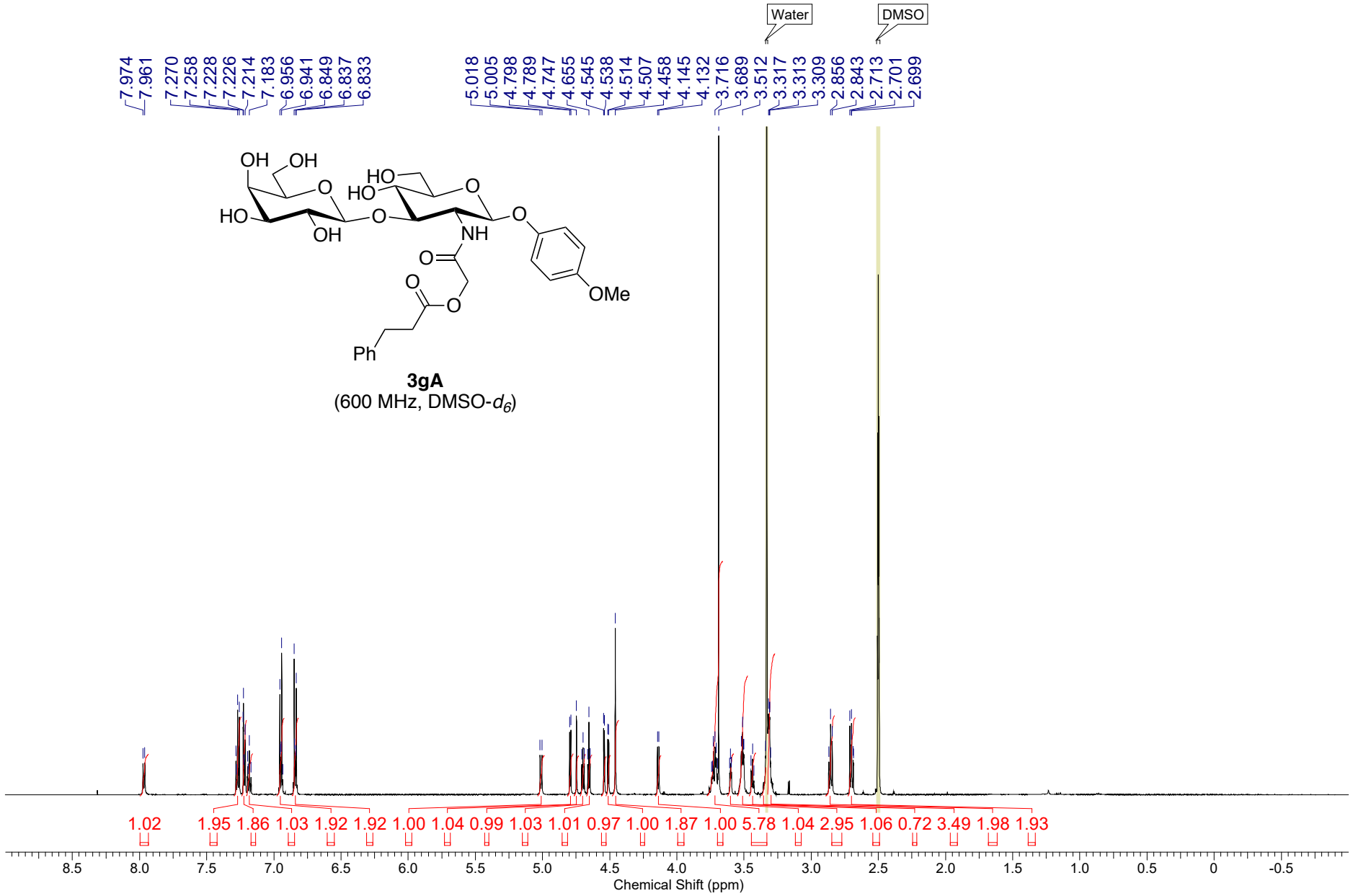
^{1}H NMR spectrum of the reaction mixture producing **3gA**



Galβ(1-3)GlcNGc MP glycoside (1g)

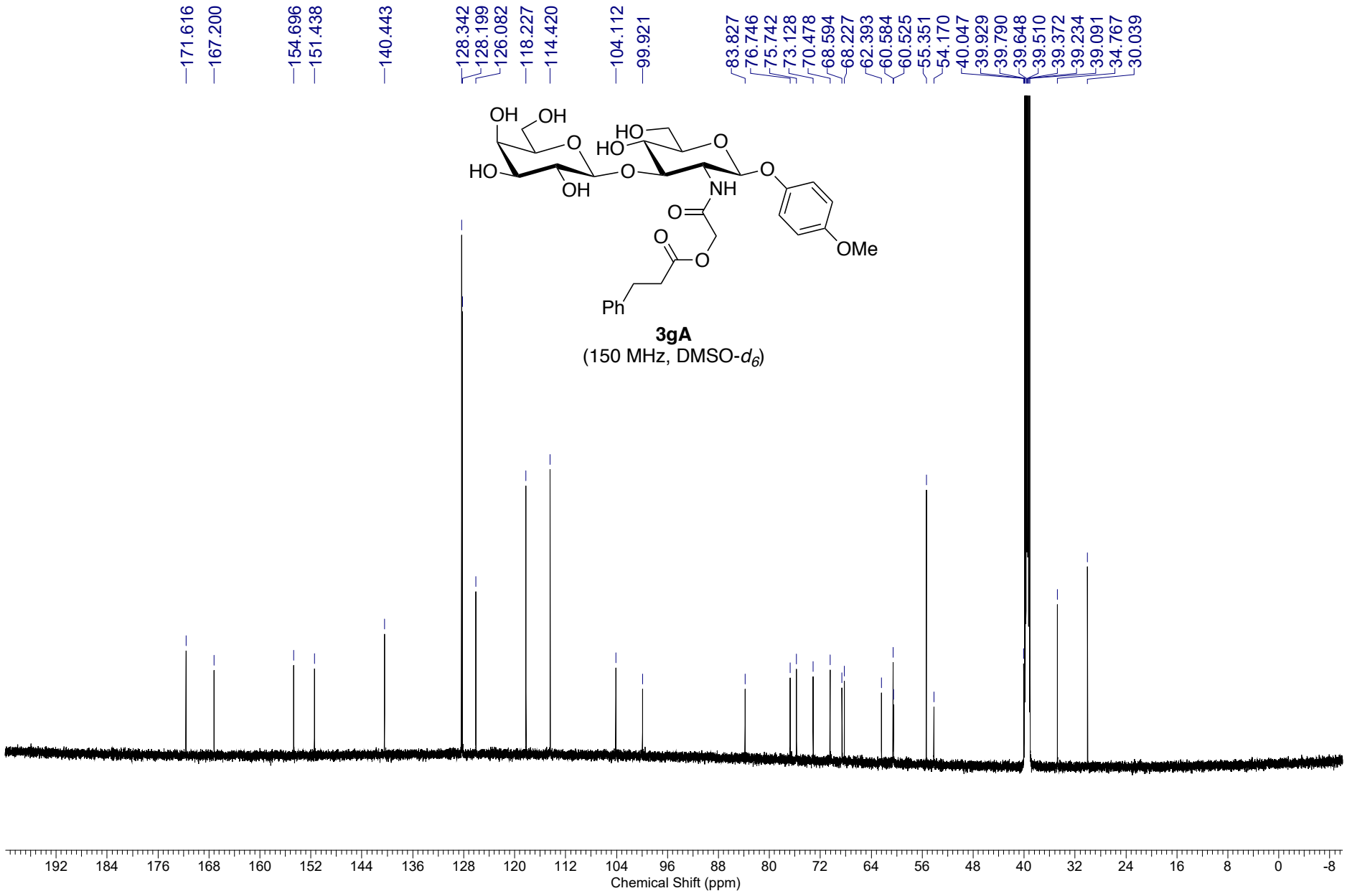
3gA (63%)

^{1}H NMR spectrum of **3gA**



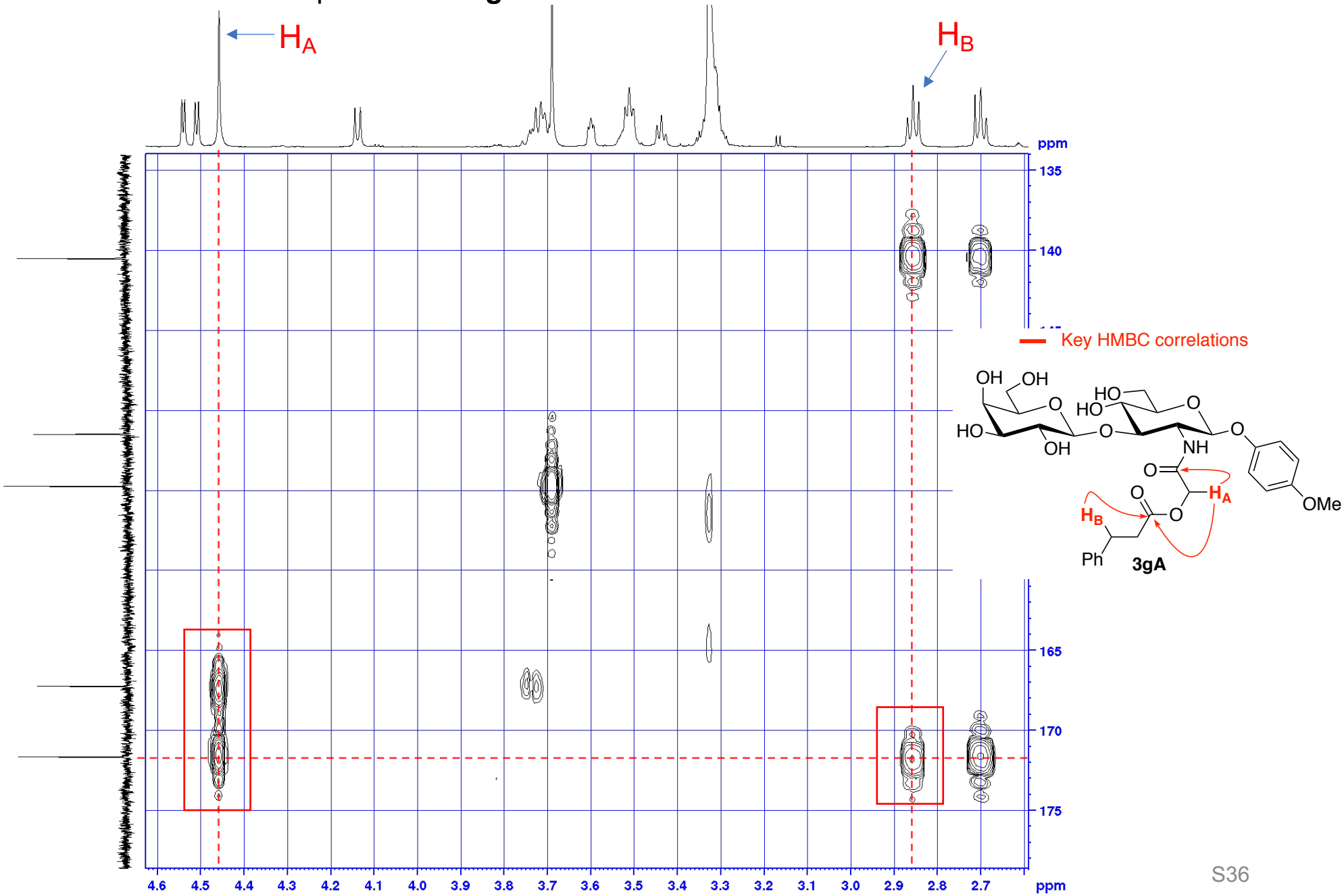
3gA
(600 MHz, DMSO-d_6)

^{13}C NMR spectrum of **3gA**

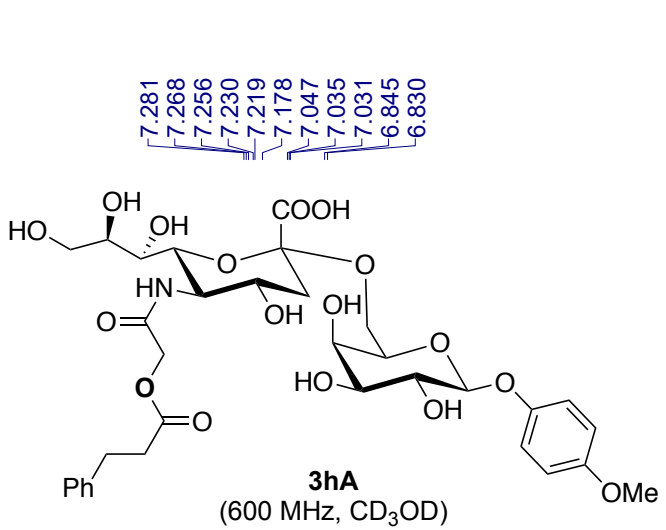


3gA
(150 MHz, DMSO-d_6)

HMBC spectrum of **3gA**

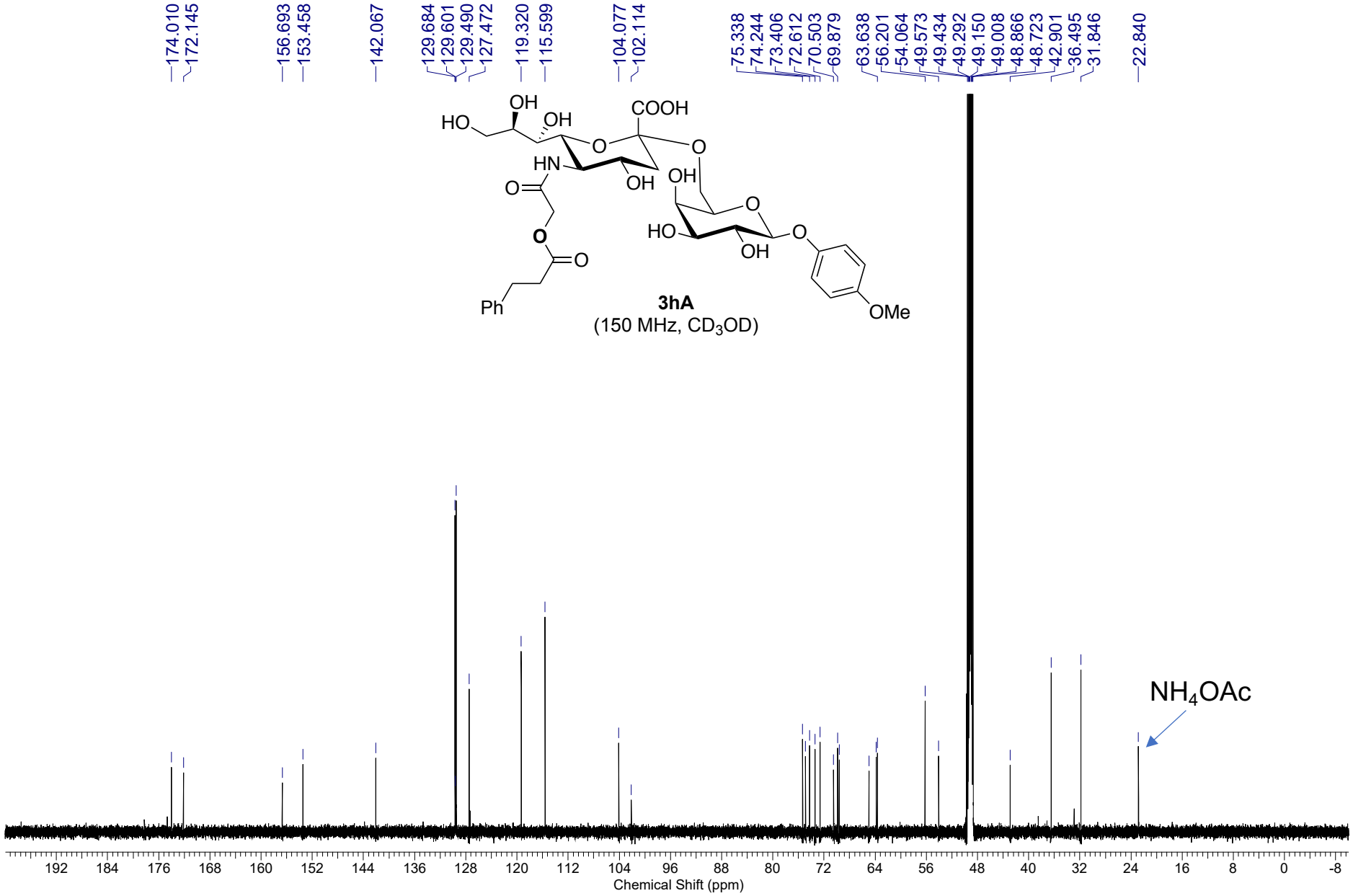


^{1}H NMR spectrum of **3hA**



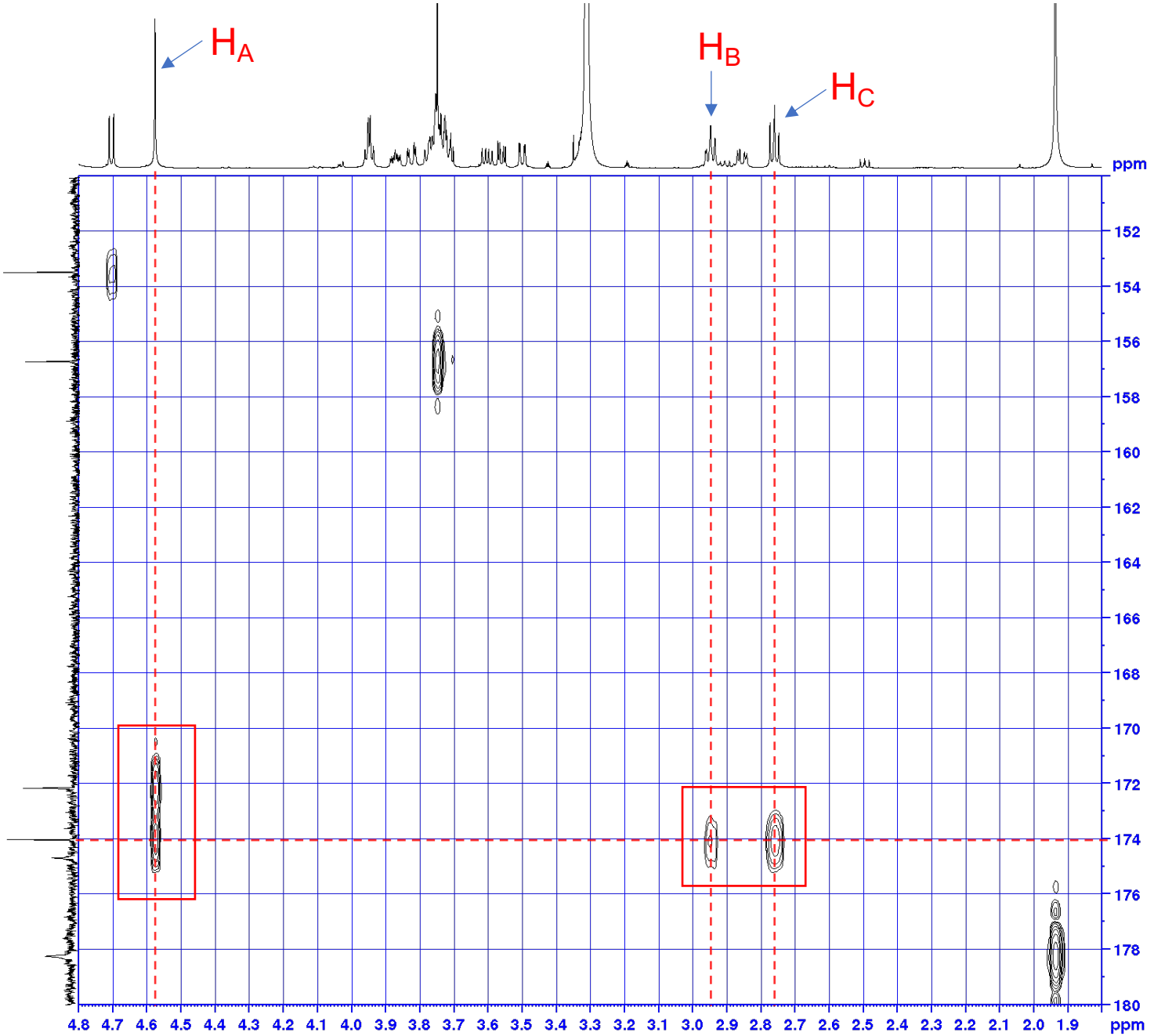
3hA
(600 MHz, CD_3OD)

^{13}C NMR spectrum of **3hA**

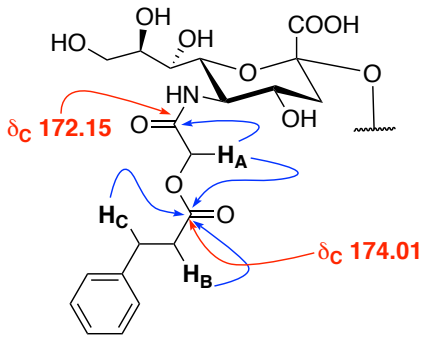


3hA
(150 MHz, CD_3OD)

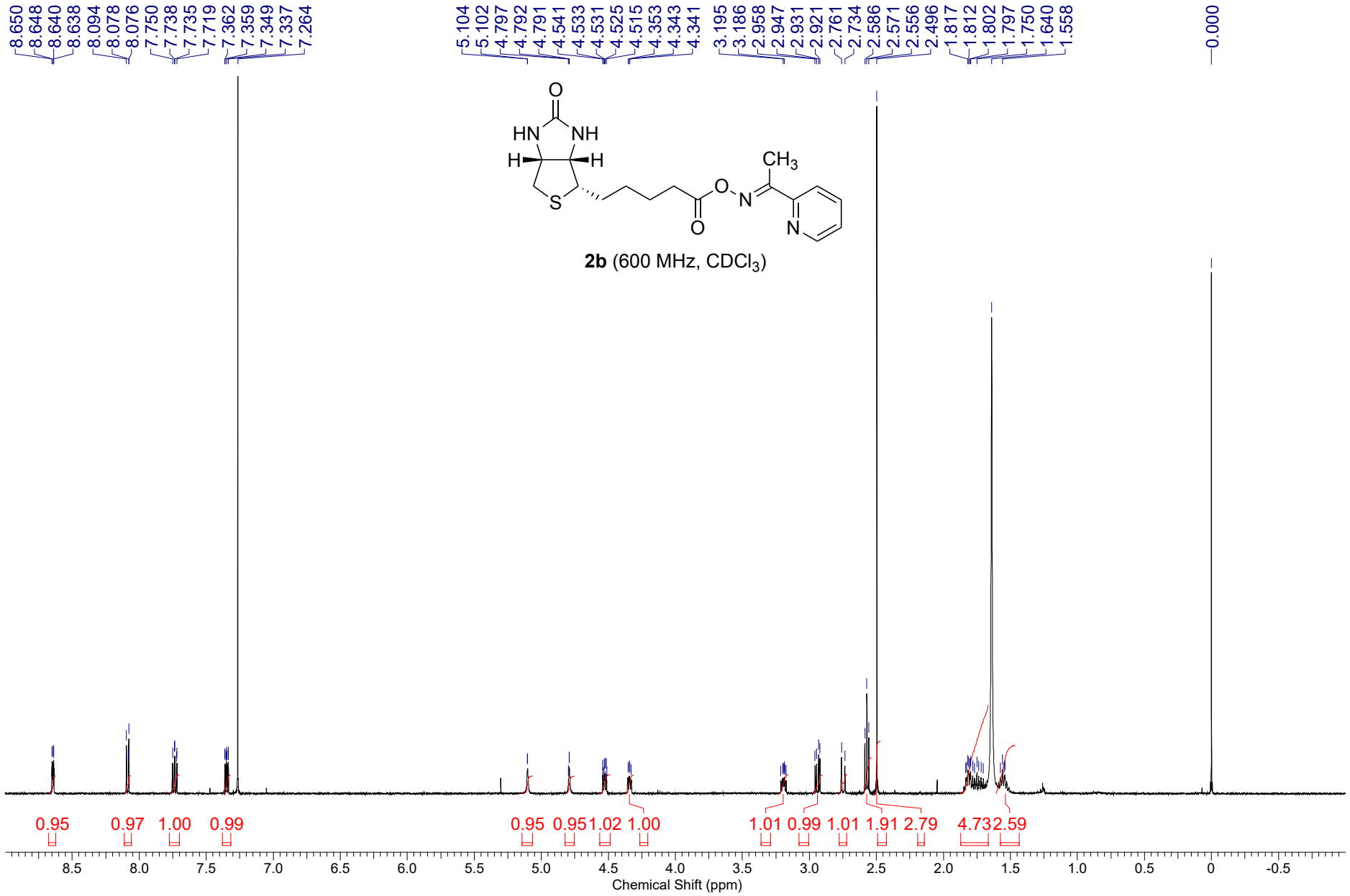
HMBC spectrum of **3hA**



Key HMBC correlations

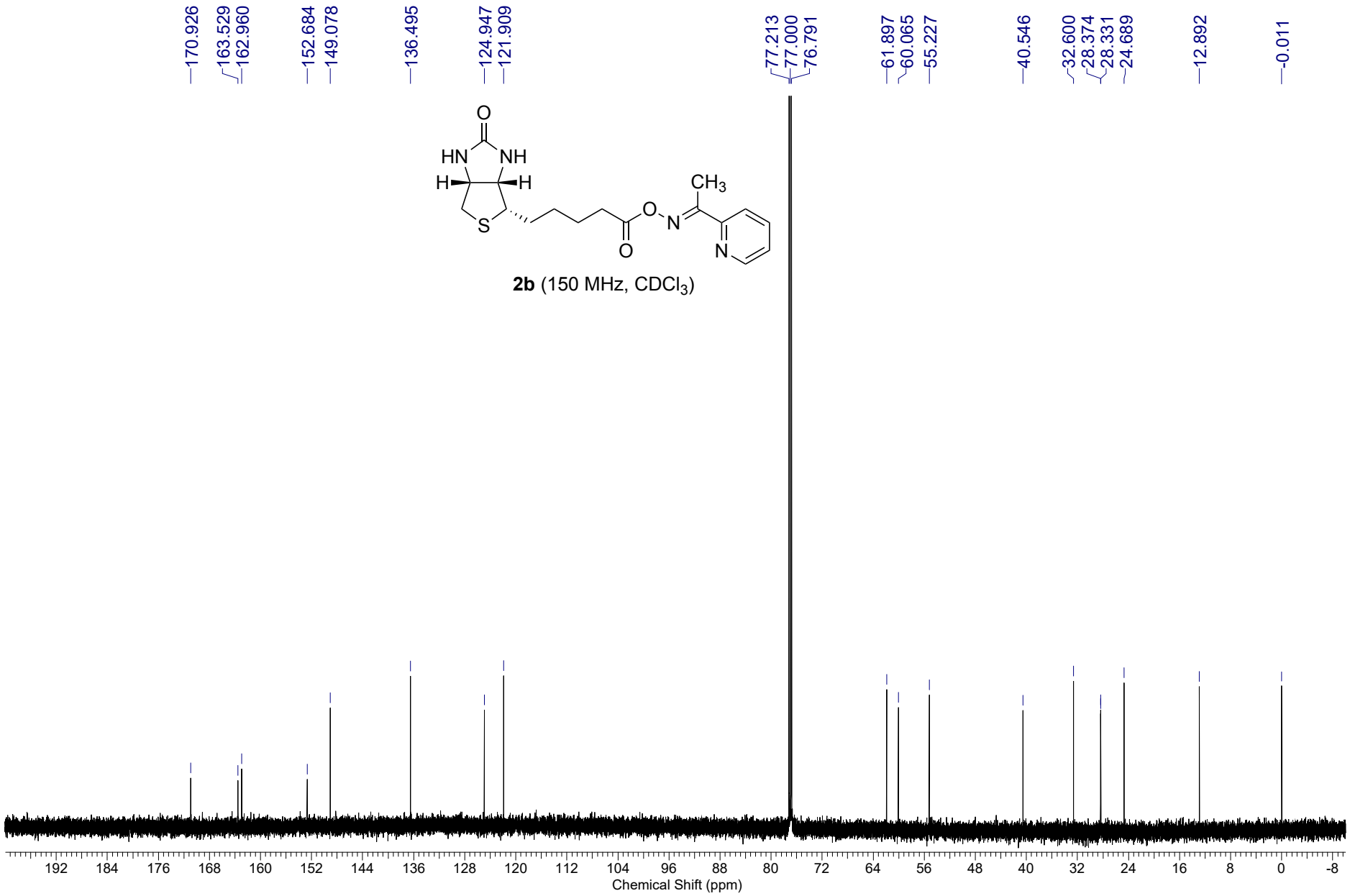


^{1}H NMR spectrum of **2b**

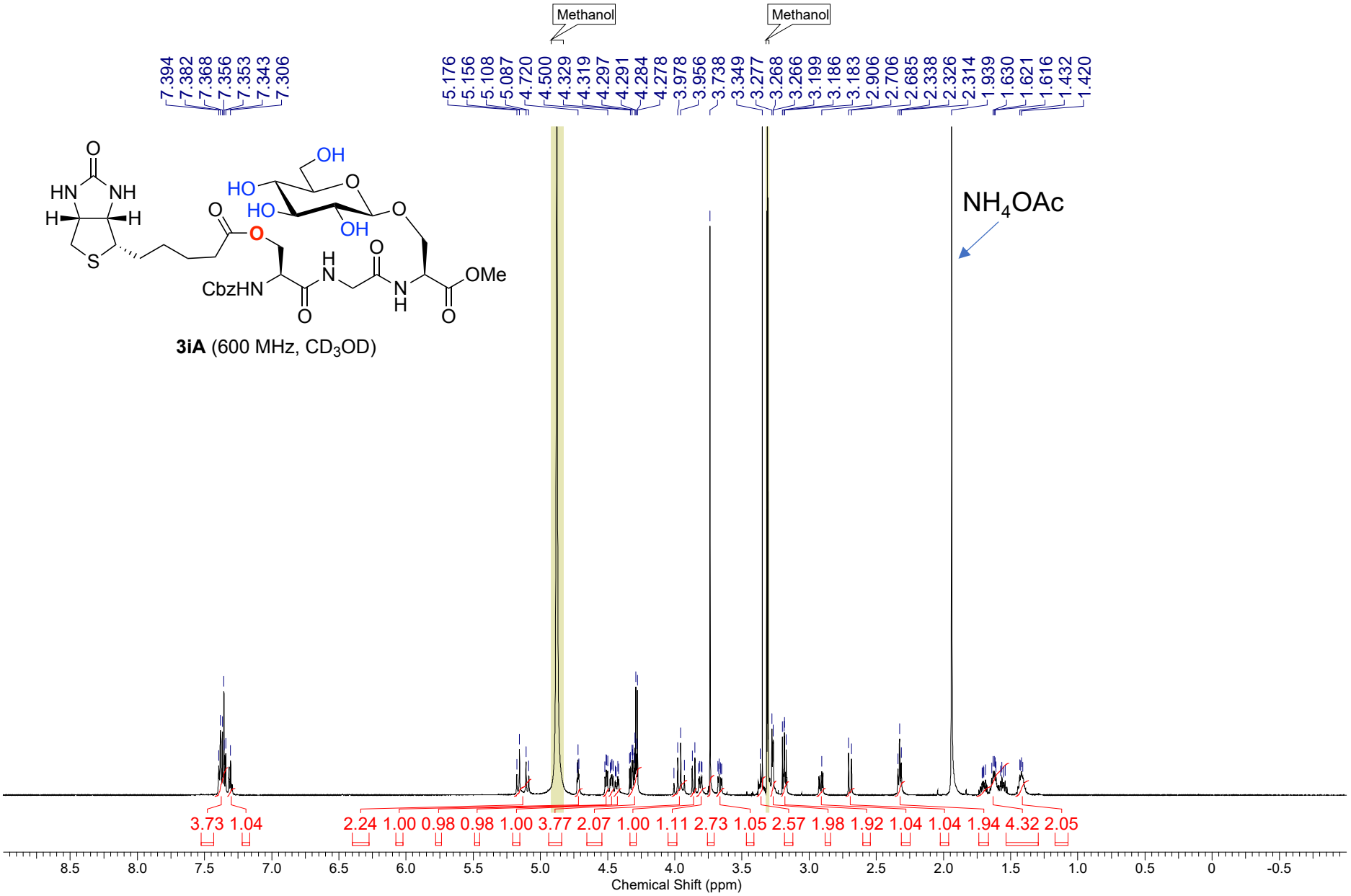


2b (600 MHz, $CDCl_3$)

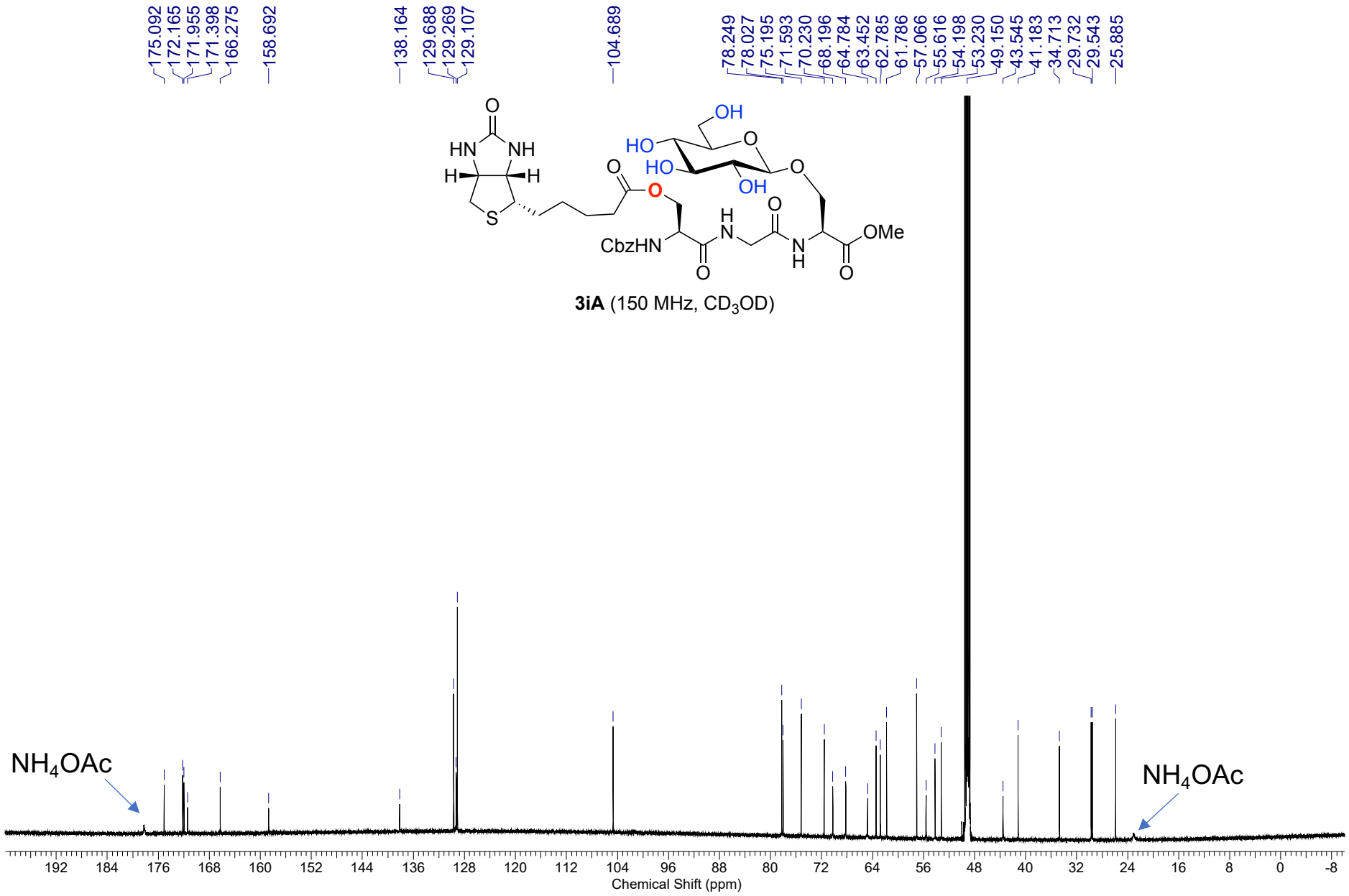
^{13}C NMR spectrum of **2b**



2b (150 MHz, $CDCl_3$)

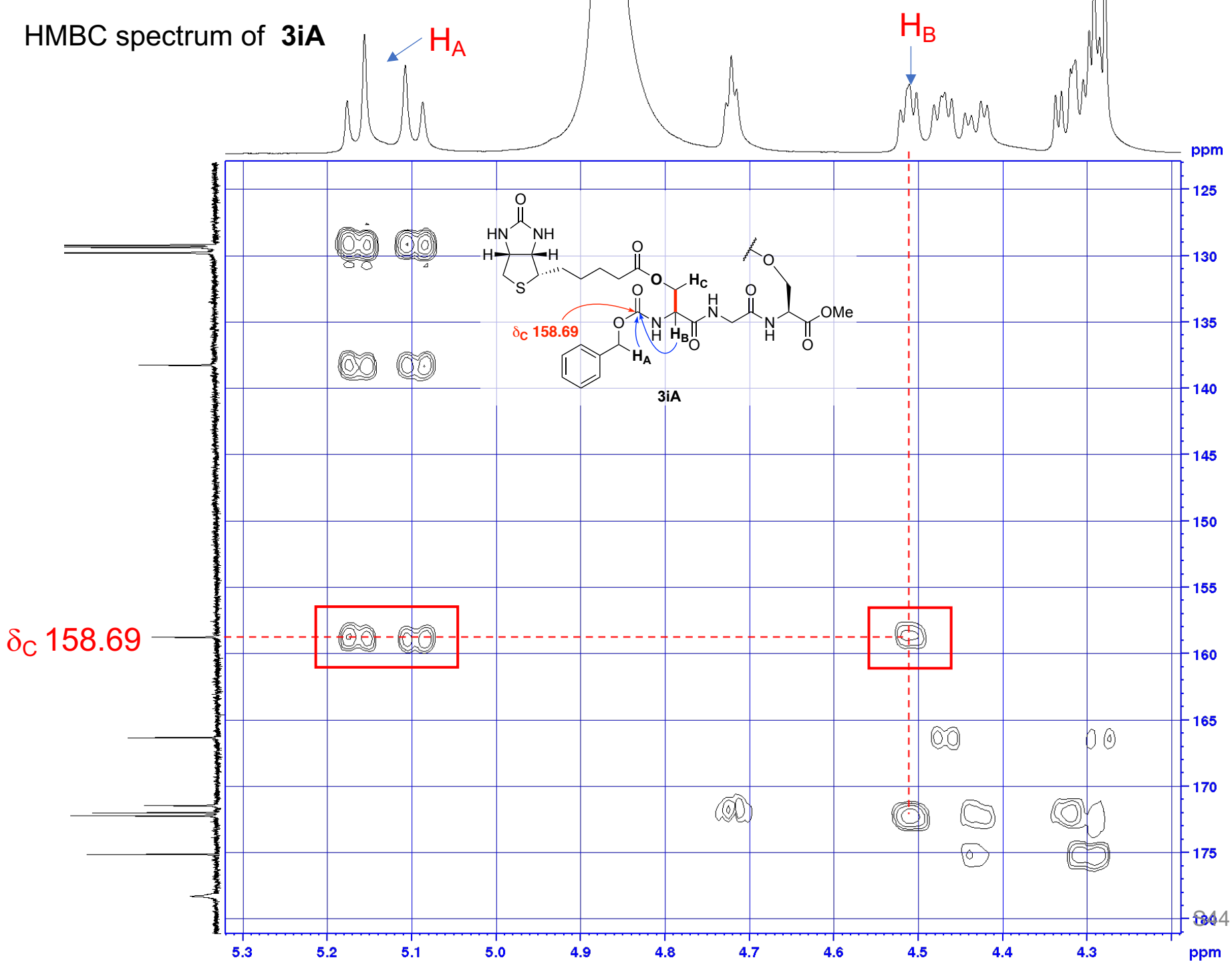


3iA (600 MHz, CD_3OD)

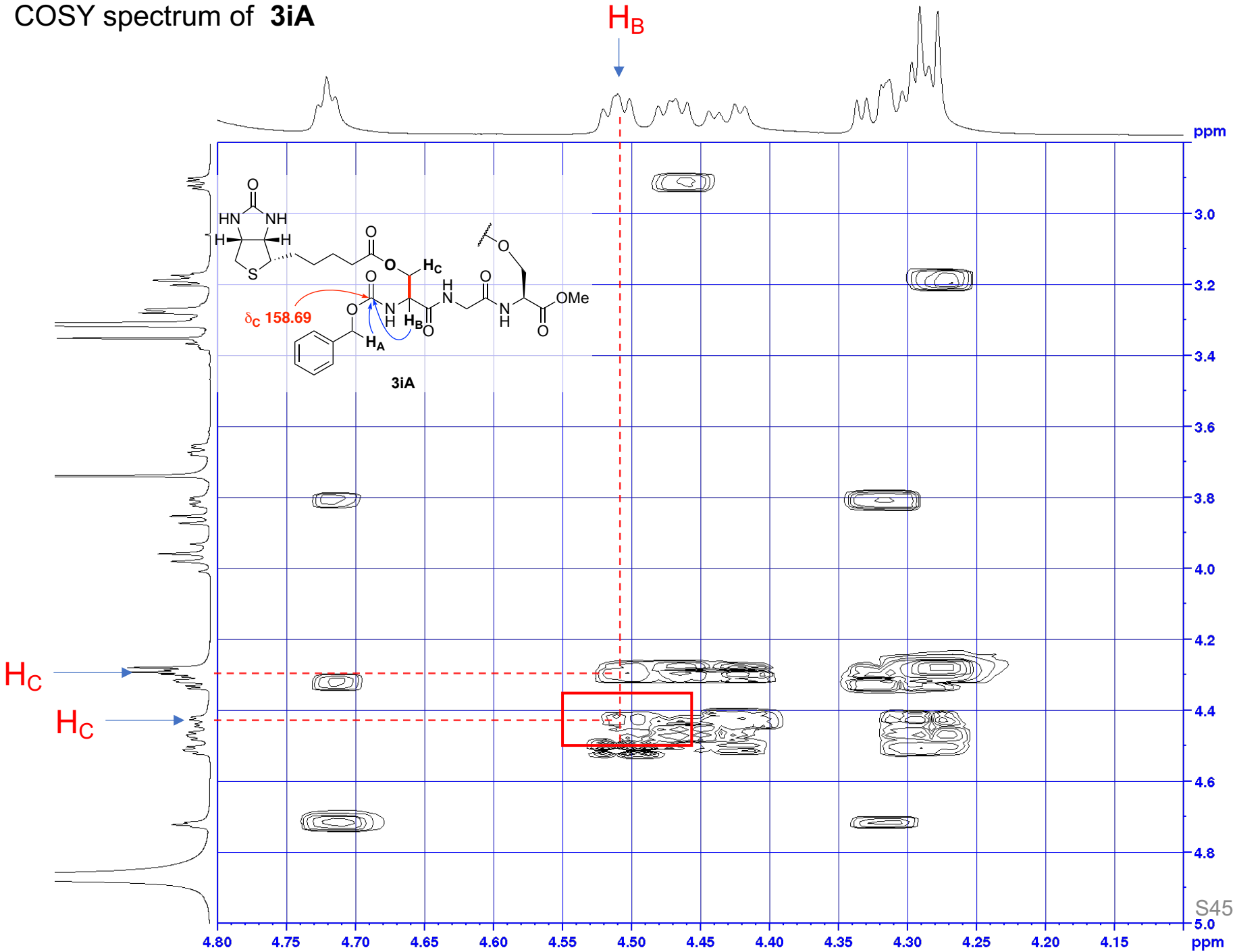


3iA (150 MHz, CD_3OD)

HMBC spectrum of **3iA**



COSY spectrum of **3iA**



HMBC spectrum of **3iA**