Supporting Information

A direct functionalization of the C5 position of Neu5Ac2en achieved by an efficient 4,5-oxazoline ring-opening

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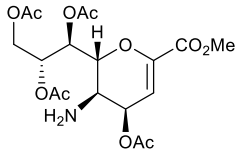
General

All chemicals and solvents used were of analytical grade and purchased from Sigma-Aldrich (St. Louis, MO, USA). The progress of all reactions was monitored by thin-layer chromatography (TLC) carried out on 0.25 mm Sigma-Aldrich silica gel plates (60 F254) using UV light, anisaldehyde/H_2SO_4/EtOH solution or 0.2% ninhydrin in ethanol and heat as the developing agent. Flash chromatography was performed with normal phase silica gel (Sigma-Aldrich 230–400 mesh silica gel). Nuclear magnetic resonance spectra were recorded at 298K on a Bruker AM-500 spectrometer equipped with a 5-mm inverse-geometry broadband probe and operating at 500.13 MHz for ^{1}H and 125.76 MHz for ^{13}C. Chemical shifts are reported in parts per million and are referenced for ^{1}H spectra, to a solvent residue proton signal (δ = 7.26 ppm for $CDCl_3$) and for ^{13}C spectra, to solvent carbon signal (central line at δ = 77.0 ppm, for $CDCl_3$). The chemical shifts for the spectra collected in CD_3CN-D_2O (1:1, v/v) are referenced to the internal CD_3CN residue proton signal (δ = 1.94 ppm for ^{1}H spectra and δ = 1.24 ppm for ^{13}C spectra). Otherwise, during the NMR study on compound **5** the ^{1}H spectra, collected in CD_3CN-D_2O (1:1 v/v), are referenced to the methyl ester signal of the analyzed compound, fixed at δ = 3.80 ppm. The ^{1}H and ^{13}C resonances were assigned by ^{1}H–^{1}H (COSY) and ^{1}H–^{13}C (HSQC and HMBC) correlation 2D experiments. The ^{1}H NMR data are tabulated in the following order: multiplicity (s=singlet, d=doublet, t=triplet, br s=broad singlet, m=multiplet, app=apparent), coupling constant(s) are given in Hz, number of protons, and assignment of proton(s). Optical rotations were taken on a Perkin-Elmer 241 polarimeter equipped with a 1 dm tube and the $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. Mass spectrometry spectra were obtained on an ABSciex 4000Qtrap mass spectrometer equipped with an ESI ion source. The spectra were collected in a continuous flow mode by connecting the infusion pump directly to the ESI source. Solutions of the compounds were infused at a flow rate of 0.01 mL min^{-1}, the spray voltage was set at 4.5 kV in the negative ion mode with a capillary temperature of 550°C. Full-scan mass spectra were recorded by scanning an *m/z* range of 100–2000.

Preparation of methyl 4,7,8,9-tetra-*O*-acetyl-5-amino-2,6-anhydro-3,5-dideoxy-D-*glycero*-D-*talo*-non-2-enoate (5).

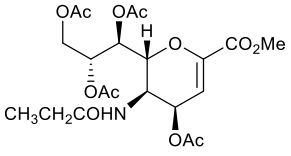


5

Preparation and physicochemical properties are reported as footnote in the paper.

Further data:^{1}H NMR (500 MHz, CD_3CN-D_2O, 1:1 v/v): δ=6.11 (d, $J_{3,4}$ = 5.6 Hz, 1H; H-3), 5.59 (dd, $J_{7,6}$ = 1.6, $J_{7,8}$ = 7.1 Hz, 1H; H-7), 5.43 (ddd, $J_{8,9a}$ = 2.6, $J_{8,9b}$ = 5.6, $J_{8,7}$ = 7.1 Hz, 1H; H-8), 5.25 (dd, $J_{4,5}$ = 4.2, $J_{4,3}$ = 5.6 Hz, 1H; H-4), 4.56 (dd, $J_{9a,8}$ = 2.6, $J_{9a,9b}$ = 12.6 Hz, 1H; H-9a), 4.30-4.23 (overlapping with water signal, H-9b), 4.12 (dd, $J_{6,7}$ = 1.6, $J_{6,5}$ = 10.7 Hz, 1H; H-6), 3.80 (s, 3H; $COOCH_3$), 3.04 (dd, $J_{5,4}$ = 4.2, $J_{5,6}$ = 10.7 Hz, 1H; H-5), 2.17 (s, 3H; $OCOCH_3$), 2.11 (s, 3H; $OCOCH_3$), 2.10 (s, 3H; $OCOCH_3$), 2.08 ppm (s, 3H; $OCOCH_3$).

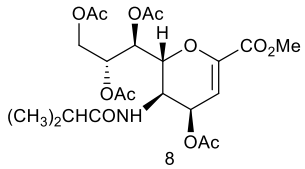
Preparation of methyl 4,7,8,9-tetra-*O*-acetyl-2,6-anhydro-3,5-dideoxy-5-propionamido-D-*glycero*-D-*talo*-non-2-enoate (6).



6

To a solution of amine **5** (345 mg, 0.8 mmol) in CH_2Cl_2 (4 mL) a weak basic resin (IRA-67), in excess compared to acylating agent, was added and the reaction mixture was immediately treated with propionyl chloride (0.175 ml, 2.0 mmol) at 0°C. The reaction mixture was stirred at 23°C until the complete formation of the compound **6** (0.5 h). At this time, MeOH (1.0 mL) was added and the mixture was stirred for 15 minutes, filtered (washing with 8 mL of CH_2Cl_2) and evaporated. After flash chromatographic purification (eluting with AcOEt/hexane, 8:2 v/v) compound **6** was obtained as a white solid (304 mg, 78%): $[\alpha]_D^{23}$=-136.5 (c=1.0 in chloroform); ^{1}H NMR (500 MHz, $CDCl_3$): δ=6.19 (d, $J_{3,4}$ = 5.6 Hz, 1H; H-3), 5.51 (d, $J_{NH,5}$ = 10.2 Hz, 1H; N*H*COCH$_2$CH$_3$), 5.47 (dd, $J_{7,6}$ = 2.2, $J_{7,8}$ = 4.2 Hz, 1H; H-7), 5.30 (ddd, $J_{8,9a}$ = 2.6, $J_{8,7}$ = 4.2, $J_{8,9b}$ = 7.6 Hz, 1H; H-8), 5.14 (dd, $J_{4,5}$ = 4.1, $J_{4,3}$ = 5.6 Hz, 1H; H-4), 4.77 (dd, $J_{9a,8}$ = 2.6, $J_{9a,9b}$ = 12.5 Hz, 1H; H-9a), 4.60 (m, 1H; H-5), 4.28 (dd, $J_{6,7}$ = 2.2, $J_{6,5}$ = 11.0 Hz, 1H; H-6), 4.17 (dd, $J_{9b,8}$ = 7.6, $J_{9b,9a}$ = 12.5 Hz, 1H; H-9b), 3.79 (s, 3H; COOCH$_3$), 2.18-2.11 (overlapping, 2H; NHCOC*H*$_2$CH$_3$), 2.10 (s, 3H; OCOCH$_3$), 2.09 (s, 3H; OCOCH$_3$), 2.07 (s, 3H; OCOCH*3*), 2.05 (s, 3H; OCOCH$_3$), 1.09 ppm (m, 3H; NHCOCH$_2$C*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$): δ=173.2 (NH*C*OCH$_2$CH$_3$), 170.5 (2C; O*C*OCH$_3$ at C-8, O*C*OCH$_3$ at C-9), 170.0 (O*C*OCH$_3$ at C-7), 169.5 (O*C*OCH$_3$ at C-4), 161.7 (C-1), 146.3 (C-2), 105.9 (C-3), 74.0 (C-6), 71.9 (C-8), 67.7 (C-7), 64.9 (C-4), 62.1 (C-9), 52.5 (COO*C*H$_3$), 44.0 (C-5), 29.5 (NHCO*C*H$_2$CH$_3$), 20.9 (2C; 2 X OCO*C*H$_3$), 20.7 (2C; 2 X OCO*C*H$_3$), 9.2 ppm (NHCOCH$_2$*C*H$_3$); MS (ESI positive): *m/z* 488.2 $[M+H]^+$, 510.2 $[M+Na]^+$; elemental analysis calcd (%) for $C_{21}H_{29}NO_{12}$: C 51.74, H 6.00, N 2.87; found: C 51.69, H 6.12, N 3.10.

Preparation of methyl 4,7,8,9-tetra-*O*-acetyl-2,6-anhydro-3,5-dideoxy-5-isobutyramido-D-*glycero*-D-*talo*-non-2-enoate (8).

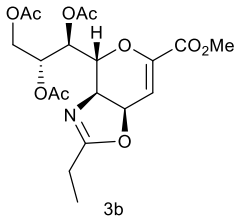


8

To a solution of oxazoline **3a**[1] (413 mg, 1.0 mmol) in CH_3CN-H_2O, 1:1 v/v (15 mL) TFA (0.2 mL) was added and the reaction was stirred at 23°C until the complete disappearance of the starting material (5-15 minutes), by monitoring with TLC (AcOEt). At this time, a weak basic resin (IRA-67), in excess compared to the acylating agent, was added and the reaction mixture was immediately treated with isobutyric anhydride (0.730 mL, 4.5 mmol) at 0°C. The mixture was stirred at 23°C until the complete formation of the desired compound **8** (1 h). MeOH (3 mL) was added and the reaction mixture was stirred for 15 minutes, filtered (washing with 20 mL AcOEt) and evaporated. After purification by flash chromatography (eluting with AcOEt/hexane, 6:4 to 7:3 v/v), the compound **8** (391 mg, 78%) was obtained as a white amorphous solid: $[\alpha]_D^{23}$ =-123.0 (c=1.0 in chloroform); ^{1}H NMR (500 MHz, $CDCl_3$): δ=6.17 (d, $J_{3,4}$ = 5.6 Hz, 1H; H-3), 5.49 (d, $J_{NH,5}$ = 10.1 Hz, 1H; N*H*COCH(CH$_3$)$_2$), 5.44 (dd, $J_{7,6}$ = 2.2, $J_{7,8}$ = 4.2 Hz, 1H; H-7), 5.28 (ddd, $J_{8,9a}$ = 2.6, $J_{8,7}$ = 4.2, $J_{8,9b}$ = 7.5 Hz, 1H; H-8), 5.17 (dd, $J_{4,5}$ = 4.2, $J_{4,3}$ = 5.6 Hz, 1H; H-4), 4.74 (dd, $J_{9a,8}$ = 2.6, $J_{9a,9b}$ = 12.4 Hz, 1H; H-9a), 4.57 (m, 1H; H-5), 4.26 (dd, $J_{6,7}$ = 2.2, $J_{6,5}$ = 10.9 Hz, 1H; H-6), 4.17 (dd, $J_{9b,8}$ = 7.5, $J_{9b,8}$ = 12.4 Hz, 1H; H-9b), 3.78 (s, 3H; COOCH$_3$), 2.33-2.24 (sept, $^3J_{H,H}$ = 6.9 Hz, 1H; NHCOC*H*(CH$_3$)$_2$), 2.09 (s, 3H; OCOCH*3*), 2.08 (s, 3H; OCOCH*3*), 2.07 (s, 3H; OCOCH*3*), 2.04 (s, 3H; OCOCH$_3$), 1.11-1.06 ppm (overlapping, 6H; NHCOCH(C*H*$_3$)$_2$); ^{13}C NMR (125 MHz, $CDCl_3$): δ=176.2 (NH*C*OCH(CH$_3$)$_2$), 170.5 (2C; O*C*OCH$_3$ at C-8, O*C*OCH$_3$ at C-9), 169.8 (O*C*OCH$_3$ at C-7), 169.5 (O*C*OCH$_3$ at C-4), 161.7 (C-1), 146.3 (C-2), 105.9 (C-3), 74.0 (C-6), 71.9 (C-8), 67.7 (C-7), 64.7 (C-4), 62.1 (C-9), 52.5 (COO*C*H$_3$), 43.9 (C-5), 35.6 (NHCO*C*H(CH$_3$)$_2$), 20.9 (OCO*C*H$_3$), 20.8 (OCO*C*H$_3$), 20.7 (OCO*C*H$_3$), 20.6 (OCO*C*H$_3$), 19.5 (NHCOCH(*C*H$_3$)$_2$), 18.9 ppm (NHCOCH(*C*H$_3$)$_2$); MS (ESI positive): *m/z* 502.1 $[M+H]^+$; elemental analysis calcd (%) for $C_{22}H_{31}NO_{12}$: C 52.69, H 6.23, N 2.79; found: C 52.75, H 6.08, N 2.55.

General procedure for the synthesis of the oxazolines 3b,c: To a solution of glycal **6** or **8** (0.7 mmol) in CH_2Cl_2 (3.5 mL), $BF_3 \cdot Et_2O$ (0.35 mL, 2.8 mmol) was added at 23°C and the mixture was stirred at 80 °C for 20 min in a sealed tube. Then, the reaction mixture was diluted with CH_2Cl_2 (15 mL) containing Et_3N (1.94 mL, 14 mmol), washed with saturated $NaHCO_3$ solution and brine, dried over anhydrous Na_2SO_4 and evaporated. Then, the crude was purified by a flash chromatography eluting with AcOEt/hexane, 7:3 v/v containing the 0.3% of Et_3N.

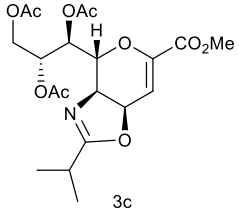
Preparation of methyl oxazolo[5,4]-fused 7,8,9-tri-*O*-acetyl-2,3,4,5-tetradeoxy-2,3-didehydro-4',5'-dihydro-2'-ethyl-D-*glycero*-D-*talo*-non-2-enoate (3b).



3b

Starting from compound **6** (341 mg, 0.7 mmol), according to general oxazoline procedure, compound **3b** (209 mg, 70%) was achieved as a white amorphous solid: $[\alpha]_D^{23}$=-18.0 (c=1.0 in chloroform); ^{1}H NMR (500 MHz, $CDCl_3$): δ=6.37 (d, $J_{3,4}$ = 4.0 Hz, 1H; H-3), 5.61 (dd, $J_{7,6}$ = 3.0, $J_{7,8}$ = 5.6 Hz, 1H; H-7), 5.46 (ddd, $J_{8,9a}$ = 2.6, $J_{8,7}$ = 5.6, $J_{8,9b}$ = 6.6 Hz, 1H; H-8), 4.81 (dd, $J_{4,3}$ = 4.0, $J_{4,5}$ = 8.6 Hz, 1H; H-4), 4.56 (dd, $J_{9a,8}$ = 2.6, $J_{9a,9b}$ = 12.4 Hz, 1H; H-9a), 4.24 (dd, $J_{9b,8}$ = 6.6, $J_{9b,9a}$ = 12.4 Hz, 1H; H-9b), 3.98 (m, 1H; H-5), 3.81 (s, 3H; $COOCH_3$), 3.44 (dd, $J_{6,7}$ = 3.0, $J_{6,5}$ = 9.9 Hz, 1H; H-6), 2.30 (overlapping, 2H; CCH_2CH_3), 2.14 (s, 3H; $OCOCH_3$), 2.05-2.03 (overlapping, 6H; 2 X $OCOCH_3$), 1.15 ppm (s, 3H; CCH_2CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ=171.0, 170.5, 169.7, 169.5 (4C; 3 X $OCOCH_3$ and CCH_2CH_3), 161.8 (C-1), 146.9 (C-2), 107.5 (C-3), 76.7 (C-6), 71.9 (C-4), 70.3 (C-8), 69.2 (C-7), 61.9 (C-9), 61.8 (C-5), 52.3 ($COOCH_3$), 21.5 ($OCOCH_2CH_3$), 20.7 ($OCOCH_3$), 20.6 ($OCOCH_3$), 20.5 ($OCOCH_3$), 10.1 ppm ($OCOCH_2CH_3$); MS (ESI positive): *m/z* 428.1 $[M+H]^+$; elemental analysis calcd (%) for $C_{19}H_{25}NO_{10}$: C 53.39, H 5.90, N 3.28; found: C 53.55, H 6.13, N 2.99.

Preparation of methyl oxazolo[5,4]-fused 7,8,9-tri-*O*-acetyl-2,3,4,5-tetradeoxy-2,3-didehydro-4',5'-dihydro-2'-isopropyl-D-*glycero*-D-*talo*-non-2-enoate (3c).

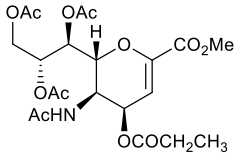


3c

Starting from compound **8** (351 mg, 0.7 mmol), according to general oxazoline procedure, compound **3c** (210 mg, 68%) was achieved as a white amorphous solid: $[\alpha]_D^{23}$=-43.6 (c=1.0 in chloroform); ^{1}H NMR (500 MHz, $CDCl_3$): δ=6.34 (d, $J_{3,4}$ = 4.0 Hz, 1H; H-3), 5.56 (dd, $J_{7,6}$ = 3.5, $J_{7,8}$ = 5.2 Hz, 1H; H-7), 5.44 (ddd, $J_{8,9a}$ = 2.6, $J_{8,7}$ = 5.2, $J_{8,9b}$ = 6.8 Hz, 1H; H-8), 4.76 (dd, $J_{4,3}$ = 4.0, $J_{4,5}$ = 8.6 Hz, 1H; H-4), 4.52 (dd, $J_{9a,8}$ = 2.6, $J_{9a,9b}$ = 12.4 Hz, 1H; H-9a), 4.20 (dd, $J_{9b,8}$ = 6.8, $J_{9b,9a}$ = 12.4 Hz, 1H; H-9b), 3.95 (m, 1H; H-5), 3.77 (s, 3H; $COOCH_3$), 3.39 (dd, $J_{6,7}$ = 3.5, $J_{6,5}$ = 9.7 Hz, 1H; H-6), 2.26 (sept, $^3J_{H,H}$ = 7.0 Hz, 1H; $CCH(CH_3)_2$), 2.11 (s, 3H; $OCOCH_3$ at C-7), 2.04-2.00 (overlapping, 6H; 2 X $OCOCH_3$), 1.15-1.09 ppm (overlapping, 6H; $NHCOCH(CH_3)_2$); ^{13}C NMR (125 MHz, $CDCl_3$): δ=174.0 ($CCH(CH_3)_2$), 170.5 ($OCOCH_3$ at C-9), 169.8 ($OCOCH_3$ at C-8), 169.5 ($OCOCH_3$ at C-7), 161.8 (C-1), 146.9 (C-2), 107.5 (C-3), 76.9 (C-6), 71.9 (C-4), 70.4 (C-8), 69.6 (C-7), 62.0 (C-9), 61.8 (C-5), 52.4 ($COOCH_3$), 28.2 ($CCH(CH_3)_2$), 20.8 ($OCOCH_3$), 20.6 (2C; 2 X $OCOCH_3$), 19.5

(CCH(CH_3)$_2$), 19.4 ppm (CCH(CH_3)$_2$); MS (ESI positive): *m/z* 442.3 $[M+H]^+$; elemental analysis calcd (%) for $C_{20}H_{27}NO_{10}$: C 54.42, H 6.17, N 3.17; found: C 54.58, H 6.08, N 3.35.

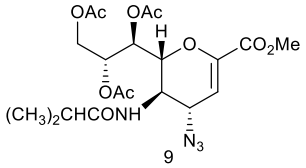
Preparation of methyl 5-acetamido-7,8,9-tri-*O*-acetyl-2,6-anhydro-3,5-dideoxy-4-*O*-propionyl -D-*glycero*-D-*talo*-non-2-enoate (7).



7

To a solution of oxazoline **3b** (171 mg, 0.4 mmol) in CH_3CN-H_2O, 1:1 v/v (6 mL), TFA (0.080 mL) was added and the mixture was stirred at 23°C until the complete disappearance of the starting material (5-15 minutes), by monitoring with TLC (AcOEt). At this time, a weak basic resin (IRA-67), in excess compared to the acylating agent, was added and the reaction mixture was immediately treated with acetyl chloride (0.128 ml, 1.8 mmol) at 0°C. The mixture was stirred at 23°C, until the complete transformation into the desired compound **7** (0.5 h). Then, MeOH (1.5 mL) was added and the reaction mixture was stirred for 15 minutes, filtered (washing with 8 mL AcOEt) and evaporated. After a flash chromatographic purification (eluting with AcOEt/hexane, 8:2 v/v), the compound **7** (156 mg, 80%) was obtained as a white amorphous solid: $[\alpha]_D^{23}$=-131.0 (c=1.0 in chloroform); ^{1}H NMR (500 MHz, $CDCl_3$): δ=6.18 (d, $J_{3,4}$ = 5.6 Hz, 1H; H-3), 5.65 (m, 1H; N*H*COCH$_3$), 5.47 (dd, $J_{7,6}$ = 2.2, $J_{7,8}$ = 4.1 Hz, 1H; H-7), 5.29 (ddd, $J_{8,9a}$ = 2.6, $J_{8,7}$ = 4.1 Hz, $J_{8,9b}$ = 7.6 Hz, 1H; H-8), 5.14 (dd, $J_{4,5}$ = 4.1, $J_{4,3}$ = 5.6 Hz, 1H; H-4), 4.76 (dd, $J_{9a,8}$ = 2.6, $J_{9a,9b}$ = 12.4 Hz, 1H; H-9a), 4.56 (dd, $J_{5,4}$ = 4.1, $J_{5,6}$ = $J_{5,NH}$ = 10.9 Hz, 1H; H-5), 4.25 (dd, $J_{6,7}$ = 2.2, $J_{6,5}$ = 10.9 Hz, 1H; H-6), 4.16 (dd, $J_{9b,8}$ = 7.6, $J_{9b,9a}$ = 12.4 Hz, 1H; H-9b), 3.77 (s, 3H; COOCH$_3$), 2.38-2.30 (overlapping, 2H; OCOC*H*$_2$CH$_3$), 2.08 (s, 3H; OCOCH$_3$), 2.05 (s, 3H; OCOCH$_3$), 2.03 (s, 3H; OCOCH$_3$), 1.91 (s, 3H; NHCOC*H*$_3$), 1.14 ppm (t, $^3J_{H,H}$ = 7.5 Hz, 3H; OCOCH$_2$C*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$): δ=173.0 (O*C*OCH$_2$CH$_3$), 170.5 (O*C*OCH$_3$ at C-8 or C-9), 170.4 (O*C*OCH$_3$ at C-8 or C-9), 170.1 (O*C*OCH$_3$ at C-7), 169.6 (NH*C*OCH$_3$), 161.7 (C-1), 146.2 (C-2), 106.0 (C-3), 73.9 (C-6), 71.8 (C-8), 67.7 (C-7), 64.6 (C-4), 62.1 (C-9), 52.5 (COO*C*H$_3$), 44.3 (C-5), 27.4 (OCO*C*H$_2$CH$_3$), 23.1 (NHCO*C*H$_3$), 20.9 (OCO*C*H$_3$), 20.7 (OCO*C*H$_3$), 20.6 (OCO*C*H$_3$), 8.9 (OCOCH$_2$*C*H$_3$); MS (ESI positive): *m/z* 488.2 $[M+H]^+$, 510.2 $[M+Na]^+$; elemental analysis calcd (%) for $C_{21}H_{29}NO_{12}$: C 51.74, H 6.00, N 2.87; found: C 51.56, H 6.16, N 2.61.

Preparation of methyl 7,8,9-tri-*O*-acetyl-2,6-anhydro-4-azido-3,4,5-trideoxy-5-isobutyramido-D-*glycero*-D-*galacto*-non-2-enoate (9)

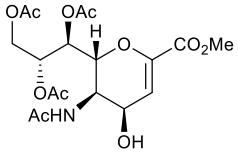


9

Starting from oxazoline **3c** (177 g, 0.4 mol) in *tert*-butyl alcohol (3.0 mL) containing azidotrimethylsilane (0.158 mL, 1.2 mmol), according to the literature procedure[2] (performed on a different oxazoline), compound **9** (145 mg, 75%) was achieved as a white solid: $[\alpha]_D^{23}$=+85.4 (c=1.0 in chloroform); ^{1}H NMR (500 MHz, $CDCl_3$): δ=5.96 (d, $J_{NH,5}$ = 8.3 Hz, 1H; N*H*COCH(CH$_3$)$_2$), 5.94 (d, $J_{3,4}$ = 2.6 Hz, 1H; H-3), 5.38 (dd, $J_{7,6}$ = 1.9, $J_{7,8}$ = 5.6 Hz, 1H; H-7), 5.28 (ddd, $J_{8,9a}$ = 2.7, $J_{8,7}$ = 5.6, $J_{8,9b}$ = 6.3 Hz, 1H; H-8), 4.66-4.55 (overlapping, 3H; H-4, H-6 and H-9a), 4.18 (dd, $J_{9b,8}$ = 6.3, $J_{9b,9a}$ = 12.5 Hz, 1H; H-9b), 3.78 (s, 3H; COOCH$_3$), 3.67 (m, 1H; H-5), 2.36 (sept, $^3J_{H,H}$ = 6.9 Hz, 1H; CC*H*(CH$_3$)$_2$), 2.12 (s, 3H; OCOCH$_3$), 2.04 (s, 3H; OCOCH$_3$), 2.02 (s, 3H; OCOCH$_3$), 1.16 (d, $^3J_{H,H}$

= 6.9 Hz, OCOCH(C*H*$_3$)$_2$), 1.13 (d, $^3J_{H,H}$ = 6.9 Hz, OCOCH(C*H*$_3$)$_2$); MS (ESI positive): *m/z* 485.2 $[M+H]^+$. Other chemical-physical properties were superimposable with those previously reported.[3,4]

Preparation of methyl 5-acetamido-7,8,9-tri-*O*-acetyl-2,6-anhydro-3,5-dideoxy-D-*glycero*-D-*talo*-non-2-enoate (4b).



4b

To a solution of oxazoline **3a**[1] (413 mg, 1.0 mmol) in CH_3CN-H_2O (15 mL, 1:1 v/v), TFA (0.2 mL) was added and the mixture was stirred at 23°C until the complete disappearance of the starting material (5-15 minutes), by monitoring with TLC (AcOEt). At this time, the reaction was treated with Et_3N (0.7 mL, 5.0 mmol) and stirred at 23°C for 40 minutes. Then, the reaction was quenched by the addition of a strong acidic resin (DOWEX 50WX8 H^+) until acid pH, filtered (washing with 7 ml of MeOH), and evaporated to give quantitatively the crude product **4b** and trace amount of triethylammonim salts (NMR evidence, see S21). After flash chromatography on silica gel (eluting from AcOEt to AcOEt/MeOH, 95:5 v/v) compound **4b** (384 mg, 89%) was obtained as a white solid: m.p. 100-103°C (dec.) [lit. 101-103°C[5]], $[\alpha]_D^{23}$=-34.9 (c=1.0 in chloroform) [lit. $[\alpha]_D^{23}$=-34.14 (c=1.4 in chloroform)[5] $[\alpha]_D^{23}$=+64.0 (c=1.41 in chloroform)[6]]; ^{1}H NMR (500 MHz, $CDCl_3$): δ=6.16 (d, $J_{3,4}$ = 5.6 Hz, 1H; H-3), 6.01 (d, $J_{NH,5}$ = 9.8 Hz, 1H; N*H*COCH$_3$), 5.45 (dd, $J_{7,6}$ = 2.1, $J_{7,8}$ = 4.3 Hz, 1H; H-7), 5.31 (ddd, $J_{8,9a}$ = 2.5, $J_{8,7}$ = 4.3, $J_{8,9b}$ = 7.6 Hz, 1H; H-8), 4.79 (dd, $J_{9a,8}$ = 2.5, $J_{9a,9b}$ = 12.4 Hz, 1H; H-9a), 4.36 (m, 1H; H-5), 4.27-4.21 (overlapping, 2H; H-4 and H-6), 4.18 (dd, $J_{9b,8}$ = 7.6, $J_{9b,9a}$ = 12.4 Hz, 1H; H-9b), 3.81 (s, 3H; COOCH$_3$), 2.11 (s, 3H; OCOCH$_3$), 2.08 (s, 3H; OCOCH$_3$), 2.06 (s, 3H; OCOCH$_3$), 1.96 ppm (N*H*COCH$_3$); ^{1}H NMR (500 MHz, CD_3CN:D_2O, 1:1 v/v): δ=6.07 (d, $J_{3,4}$ = 5.2 Hz, 1H; H-8), 4.44 (dd, $J_{9a,8}$ = 2.7, $J_{9a,9b}$ = 12.4 Hz, 1H; H-9a), 4.17 (overlapping with water signal; H-6), 4.10 (dd, $J_{9b,8}$ = 6.1, $J_{9b,9a}$ = 12.4 Hz, 1H; H-9b), 4.09-4.02 (overlapping, 2H; H-5 and H-4), 3.71 (s, 3H; COOCH$_3$), 1.99 (s, 3H; OCOCH$_3$), 1.98 (overlapping, 6H; 2 X OCOCH$_3$), 1.84 ppm (s, 3H; NHCOC*H*$_3$); ^{13}C NMR (125 MHz, CD_3CN:D_2O, 1:1 v/v): δ=173.5 (NH*C*OCH$_3$), 173.3 (O*C*OCH$_3$ at C-9), 172.4 (2C; 2 X O*C*OCH$_3$ at C-7 and C-8), 164.0 (C-1), 145.2 (C-2), 111.4 (C-3), 72.7 (C-6), 70.9 (C-8), 68.8 (C-7), 63.1 (C-9), 61.3 (C-4), 53.4 (COO*C*H$_3$), 48.0 (C-5), 22.8 (NHCO*C*H$_3$), 21.0 (2 X OCO*C*H$_3$), 20.9 ppm (OCO*C*H$_3$). Other chemical-physical properties were superimposable with those previously reported.[6,7]

NMR study on the compound 5.

Oxazoline **3a**[1] (36 mg, 0.087 mmol) was dissolved in a mixture of CD_3CN/D_2O, 1:1 v/v (0.750 mL), and ^{1}H-NMR and ^{13}C-NMR spectra were acquired, showing signals coherent with its structure: ^{1}H-NMR (500 MHz, CD_3CN:D_2O, 1:1 v/v): δ=6.42 (d, $J_{3,4}$ = 4.1 Hz, 1H; H-3), 5.52 (dd, $J_{7,6}$ = 1.6, $J_{7,8}$ = 6.9 Hz, 1H; H-7), 5.34 (ddd, $J_{8,9a}$ = 2.6, $J_{8,9b}$ = 5.7, $J_{8,7}$ = 6.9 Hz, 1H; H-8), 4.96 (dd, $J_{4,3}$ = 4.1, $J_{4,5}$ = 8.5 Hz, 1H; H-4), 4.51 (dd, $J_{9a,8}$ = 2.6, $J_{9a,9b}$ = 12.5 Hz, 1H; H-9a), 4.24 (dd, overlapping with water signal, $J_{9b,8}$ = 5.7, $J_{9b,9a}$ = 12.5 Hz, 1H; H-9b), 3.99 (m, 1H; H-5), 3.80 (s, 3H; COOCH$_3$), 3.56 (dd, $J_{6,7}$ = 1.6, $J_{6,5}$ = 10.1 Hz, 1H; H-6), 2.16 (s, 3H; OCOCH$_3$), 2.06 (s, 3H; OCOCH$_3$), 2.04 (s, 3H; OCOCH$_3$), 1.99 ppm (s, 3H; CC*H*$_3$); ^{13}C NMR (125 MHz, CD_3CN:D_2O, 1:1 v/v): δ=173.0, 172.1, 172.7, 169.7 (4C; 3 X O*C*OCH$_3$ and *C*CH$_3$), 163.3 (C-1), 147.5 (C-2), 108.6 (C-3), 76.7 (C-6), 73.4 (C-4), 70.6 (C-8), 69.1 (C-7), 62.8 (C-9), 61.6 (C-5), 53.4 (COO*C*H$_3$), 21.0 (OCO*C*H$_3$), 20.9 (OCO*C*H$_3$), 20.8 (OCO*C*H$_3$), 14.1 ppm (C*C*H$_3$).

Then, TFA (0.017 mL) was added directly in NMR tube solution, mixed with a vortex (30 s) and ^{1}H-NMR and ^{13}C-NMR were immediately acquired, showing signals attributable to the structure of the compound **5** as ammonium salt: ^{1}H NMR (500 MHz, CD_3CN-D_2O, 1:1 v/v): δ=6.16 (d, $J_{3,4}$ = 4.7 Hz,

1H; H-3), 5.50 (t app, $J_{4,5} = J_{4,3} = 4.7$ Hz, 1H; H-4), 5.41 (ddd, $J_{8,9a} = 2.6$, $J_{8,9b} = 5.1$, $J_{8,7} = 6.9$ Hz, 1H; H-8), 5.34 (dd, $J_{7,6} = 2.8$, $J_{7,8} = 6.9$ Hz, 1H; H-7), 4.59 (dd, $J_{6,7} = 2.8$, $J_{6,5} = 8.9$ Hz, 1H; H-6), 4.56-4.41 (overlapping with water signal, H-9a), 4.28 (dd, $J_{9b,8} = 5.1$, $J_{9b,9a} = 12.6$ Hz, 1H; H-9b), 3.82 (dd, $J_{5,4} = 4.7$, $J_{5,6} = 8.9$ Hz, 1H; H-5), 3.80 (s, 3H; $COOCH_3$), 2.18 (s, 3H; $OCOCH_3$), 2.13 (s, 3H; $OCOCH_3$), 2.09 (s, 3H; $OCOCH_3$), 2.06 ppm (s, 3H; $OCOCH_3$); ^{13}C NMR (125 MHz, CD_3CN-D_2O, 1:1 v/v): δ =173.7, 173.1, 172.2, 172.1 (4C; 4 X O*C*OCH$_3$), 162.8 (C-1), 146.2 (C-2), 106.1 (C-3), 72.7 (C-6), 70.1 (C-8), 68.9 (C-7), 63.1 (C-4), 62.7 (C-9), 53.7 (COO*C*H$_3$), 47.1 (C-5), 21.1 (OCO*C*H$_3$), 21.0 (OCO*C*H$_3$), 20.9 ppm (2C; 2 X OCO*C*H$_3$).
Subsequently, the solution was filtered on a small column containing IRA-67 to remove TFA acid, and ^{1}H-NMR was immediately acquired, showing signals consistent with compound **5** in amine form and traces of a second product superimposable to those of the alcohol **4b**.
The monitoring of the solution, by acquiring ^{1}H NMR spectra at different times (see S22), showed that after 6 days the only product present was the alcohol **4b**.

Oxazoline 3a hydrolysis with acetic acid according to the literature.[7]
To a solution of oxazoline **3a**[1] (413 mg, 1.0 mmol) in ethyl acetate (4.4 mL), acetic acid in water (0.370 mL, 1:1 v/v) was added and the reaction mixture was stirred at 23°C overnight.[7] Noteworthy, after 10 minutes, the partial transformation of the oxazoline **3a** into the intermediate **5** was observed by monitoring in TLC (AcOEt). However, after stirring overnight, these compounds were completely transformed into the final reaction products. Then, the reaction mixture was diluted with ethyl acetate (15 mL) and neutralized with $NaHCO_3$ solution. The organic layer was dried on Na_2SO_4 and evaporated.
The obtained crude was chromatographed on silica gel (eluting with AcOEt), affording a first fraction (125 mg), formed by two compounds (NMR evidence), and a second one constituted by the compound **4b** (220 mg, 51%). Compound **4b** showed all the chemical-physical properties superimposable with those above reported.
The first fraction was chromatographed on silica gel eluting with CH_2Cl_2-acetone, 7:3 v/v, to afford, at first, the compound **10a** (30 mg, 7%), as a white amorphous solid: $[\alpha]_D^{23}$=-116.4 (c=0.5 in chloroform); ^{1}H NMR (500 MHz, $CDCl_3$): δ=6.20 (d, $J_{3,4} = 5.7$ Hz, 1H; H-3), 5.88 (d, $J_{NH,5} = 8.6$ Hz, 1H; N*H*COCH$_3$), 5.34 (ddd, $J_{8,9a} = 2.3$, $J_{8,9b} = 5.7$, $J_{8,7} = 7.3$ Hz, 1H; H-8), 5.21 (dd, $J_{4,5} = 4.3$, $J_{4,3} = 5.7$ Hz, 1H; H-4), 4.77 (dd, $J_{9a,8} = 2.3$, $J_{9a,9b} = 12.3$ Hz, 1H; H-9a), 4.68 (br s, 1H; OH at C-7), 4.41 (ddd, $J_{5,4} = 4.3$, $J_{5,NH} = 8.6$, $J_{5,6} = 11.4$ Hz, 1H; H-5), 4.33 (dd, $J_{9b,8} = 5.7$, $J_{9b,9a} = 12.3$ Hz, 1H; H-9b), 3.94 (d app, $J_{6,5} = 11.4$ Hz, 1H; H-6), 3.83 (d app, $J_{7,8} = 7.3$ Hz, 1H; H-7), 3.78 (s, 3H; $COOCH_3$), 2.14 (s, 3H; $OCOCH_3$), 2.13 (s, 3H; NHCOC*H*$_3$), 2.07 (s, 3H; $OCOCH_3$), 2.05 ppm (s, 3H; $OCOCH_3$); ^{13}C NMR (125 MHz, $CDCl_3$): δ=172.2 (NH*C*OCH$_3$), 170.7 (O*C*OCH$_3$ at C-9), 169.8 (O*C*OCH$_3$ at C-8), 169.5 (O*C*OCH$_3$ at C-4), 161.9 (C-1), 147.3 (C-2), 103.9 (C-3), 73.8 (C-6), 71.2 (C-8), 67.4 (C-7), 65.2 (C-4), 62.9 (C-9), 52.6 (COO*C*H$_3$), 45.7 (C-5), 23.3 (NHCO*C*H$_3$), 21.0 (2 X OCO*C*H$_3$), 20.8 ppm (OCO*C*H$_3$); MS (ESI positive): *m/z* 432.0 $[M+H]^+$; elemental analysis calcd (%) for $C_{18}H_{25}NO_{11}$: C 50.12, H 5.84, N 3.25; found: C 49.89, H 6.05, N 3.42.
Further elution yielded the isomeric compound **10b** (78 mg, 18 %) as a white amorphous solid: $[\alpha]_D^{23}$=-133.2 (c=1.0 in chloroform); ^{1}H NMR (500 MHz, $CDCl_3$): δ=6.20 (d, $J_{3,4} = 5.7$ Hz, 1H; H-3), 5.76 (d, $J_{NH,5} = 10.1$ Hz, 1H; N*H*COCH$_3$), 5.19-5.14 (overlapping, 2H; H-4 and H-7), 4.60 (m, 1H; H-5), 4.46 (dd, $J_{6,7} = 2.0$, $J_{6,5} = 11.3$ Hz, 1H; H-6), 4.30 (ddd, $J_{8,9a} = 3.1$, $J_{8,9b} = 6.2$, $J_{8,7} = 8.1$ Hz, 1H; H-8), 4.19 (dd, $J_{9a,8} = 3.1$, $J_{9a,9b} = 11.9$ Hz, 1H; H-9a), 4.09 (dd, $J_{9b,8} = 6.2$, $J_{9b,9a} = 11.9$ Hz, 1H; H-9b), 3.80 (s, 3H; $COOCH_3$), 2.09 (s, 3H; $OCOCH_3$ at C7), 2.09 (s, 3H; $OCOCH_3$ at C8), 2.08 (s, 3H; $OCOCH_3$ at C4), 1.94 ppm (s, 3H; N*H*COCH$_3$); ^{13}C NMR (125 MHz, $CDCl_3$): δ=171.3 (O*C*OCH$_3$ at C-9), 170.4 (O*C*OCH$_3$ at C-4 or at C-7), 169.8 (NH*C*OCH$_3$), 169.7 (O*C*OCH$_3$ at C-4 or at C-7), 162.1 (C-1), 146.2 (C-2), 106.0 (C-3), 73.2 (C-6), 68.8 (C-7), 68.0 (C-8), 65.5 (C-9), 65.0 (C-4), 52.6 (COO*C*H$_3$), 44.2 (C-5), 23.2 (NHCO*C*H$_3$), 20.9 (2 X OCO*C*H$_3$), 20.8 ppm (OCO*C*H$_3$); MS (ESI positive): *m/z* 432.2 $[M+H]^+$; elemental analysis calcd (%) for $C_{18}H_{25}NO_{11}$: C 50.12, H 5.84, N 3.25; found: C 50.01, H 5.96, N 3.15.

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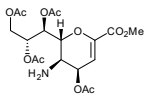

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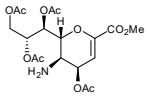

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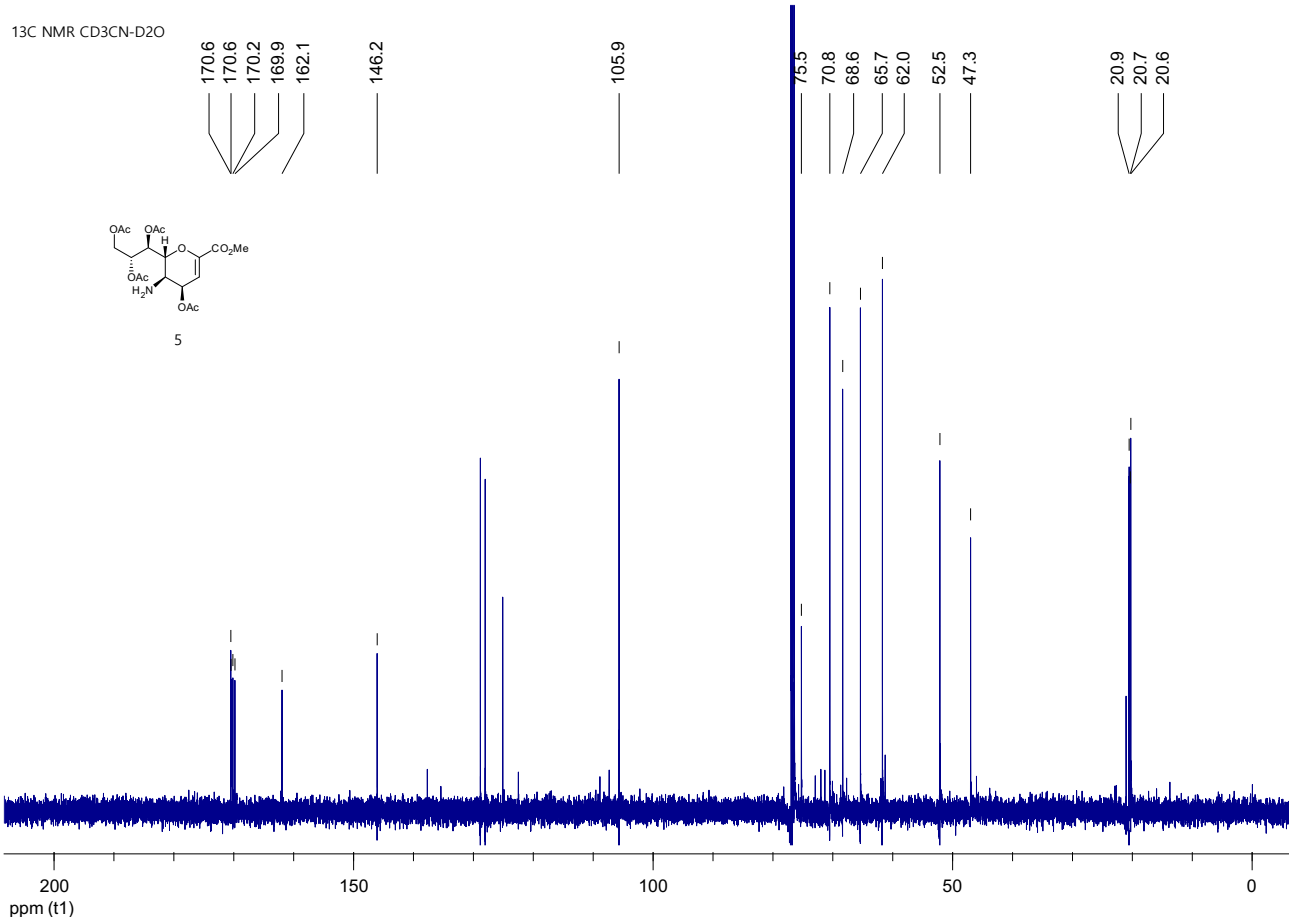
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62.0
52.5
47.3
20.9
20.7
20.6
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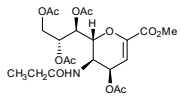
1H NMR CDCl3



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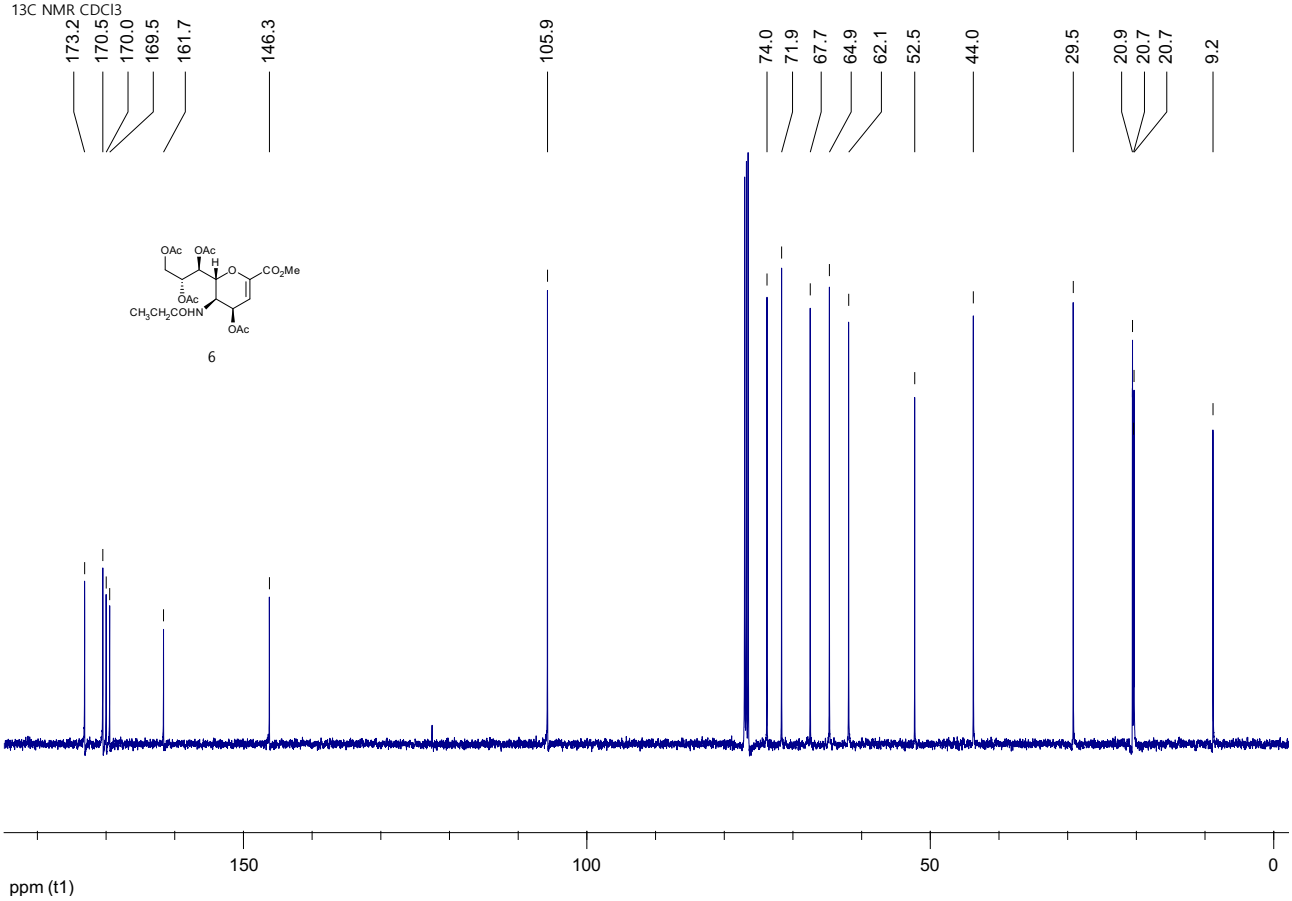

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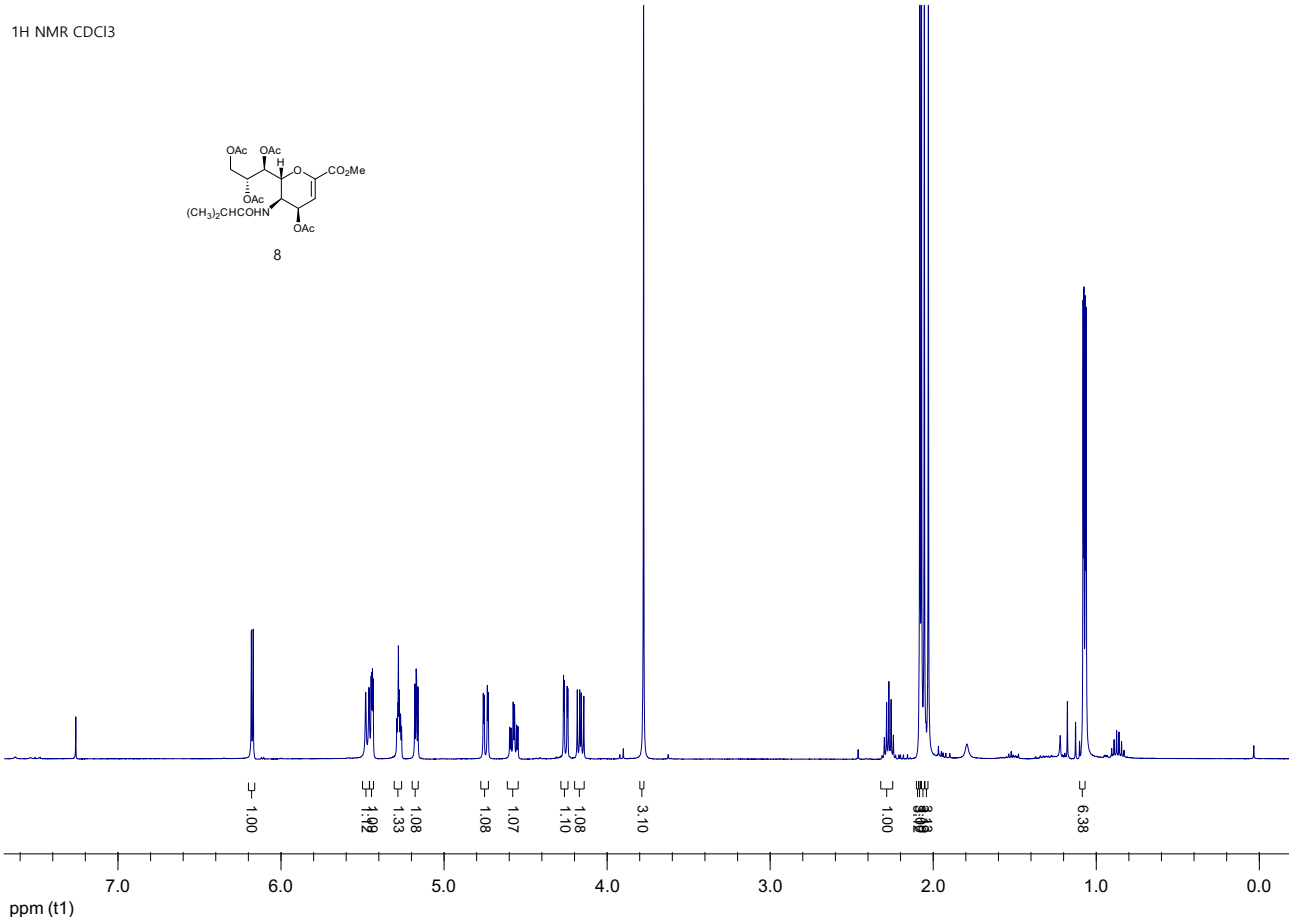
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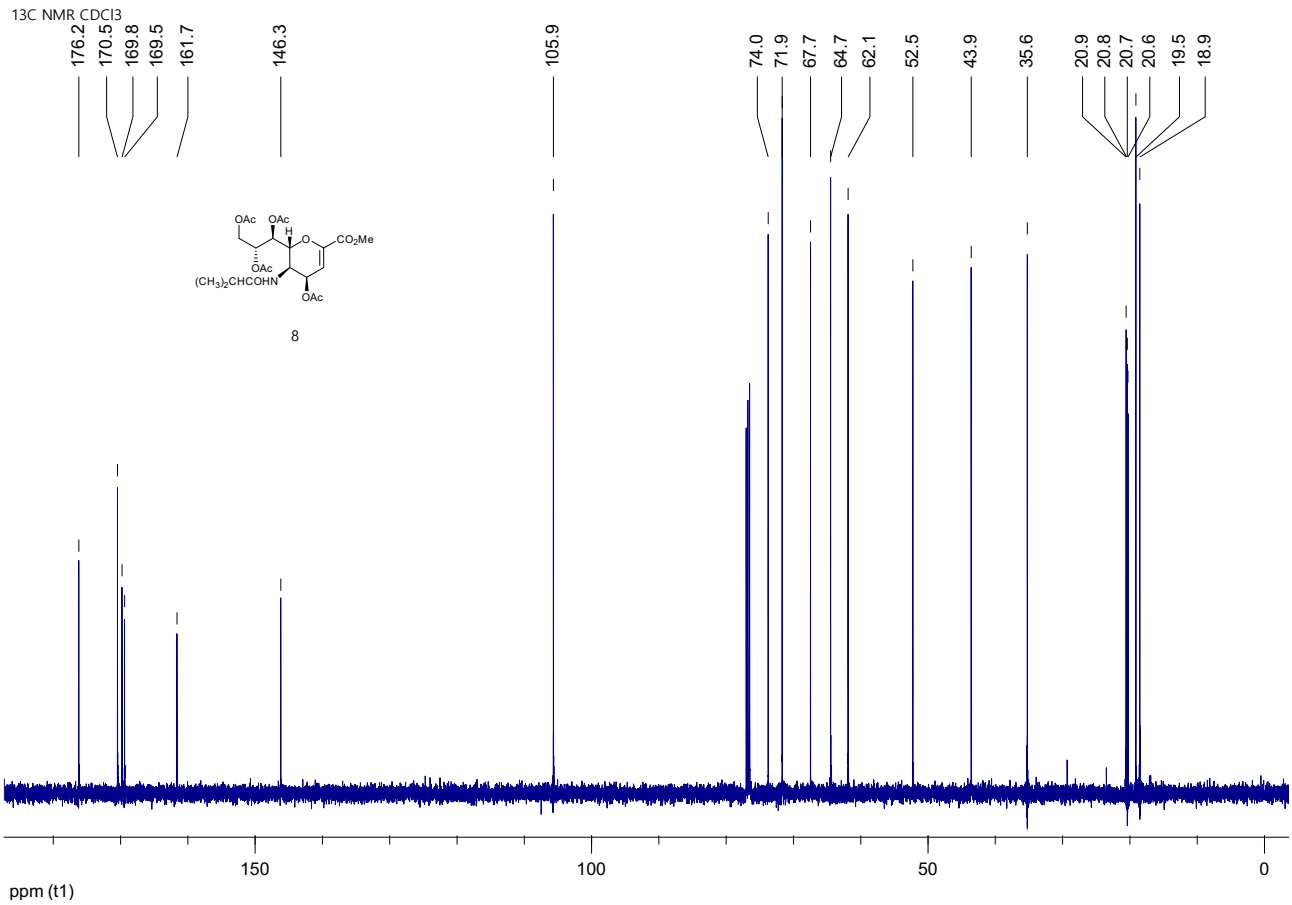
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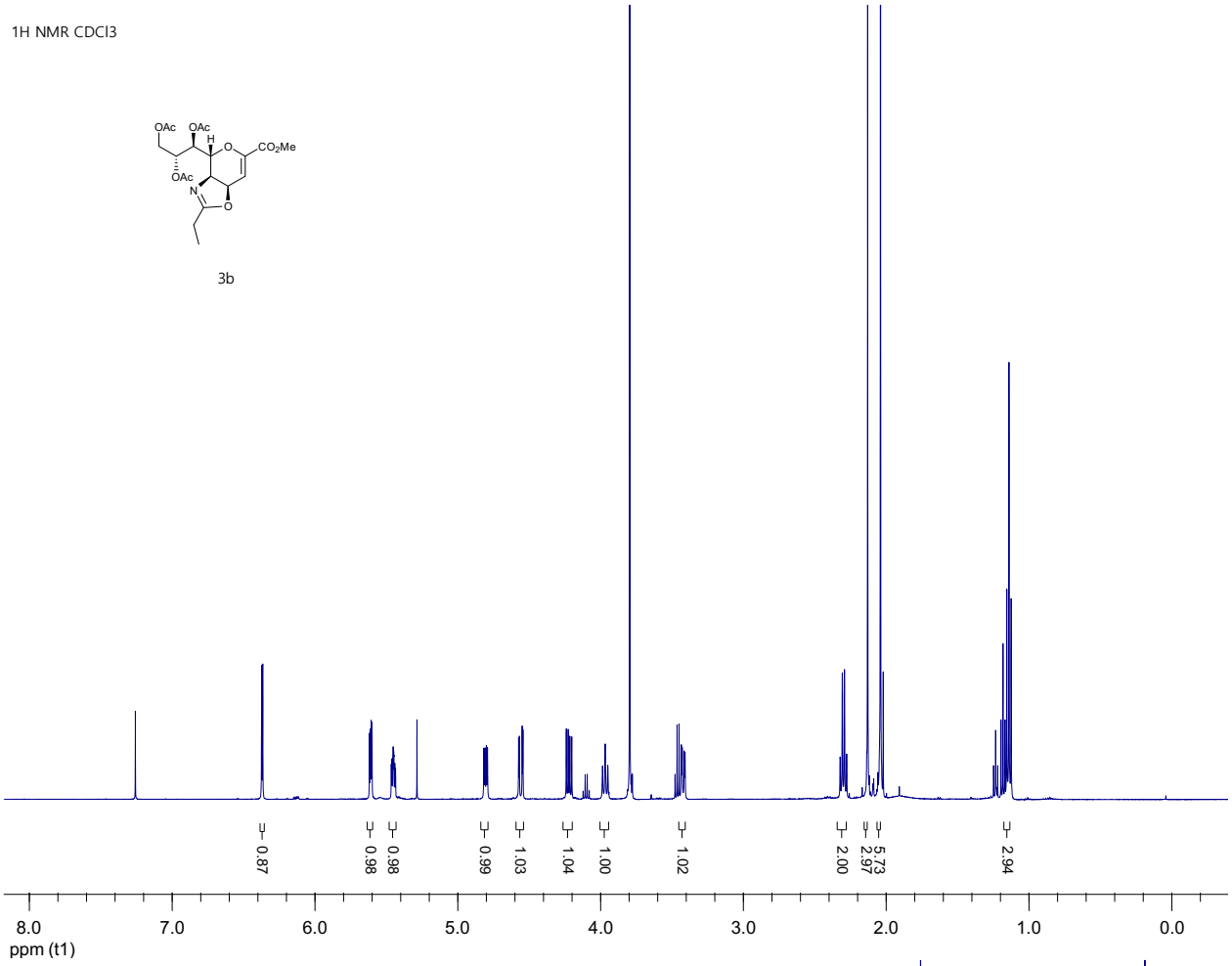
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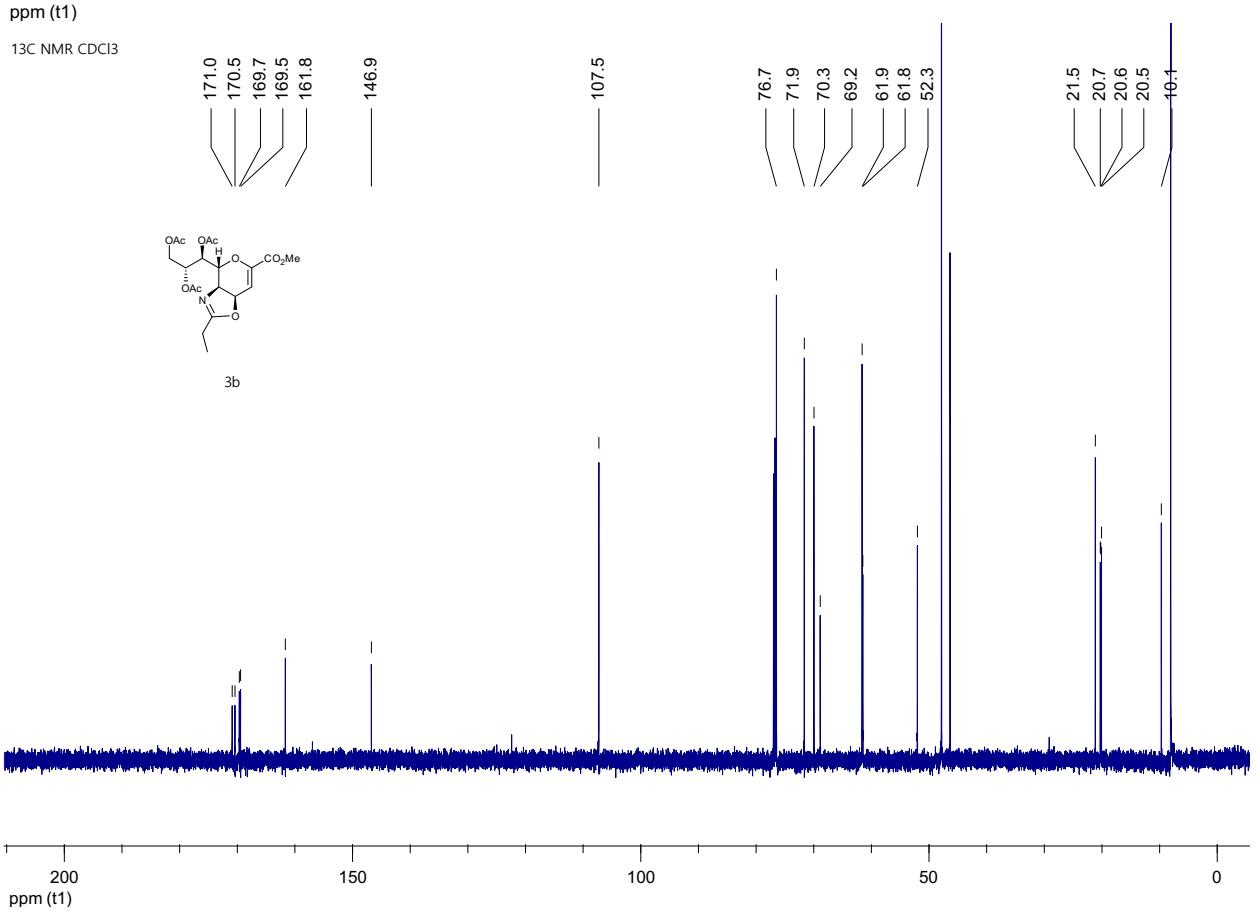
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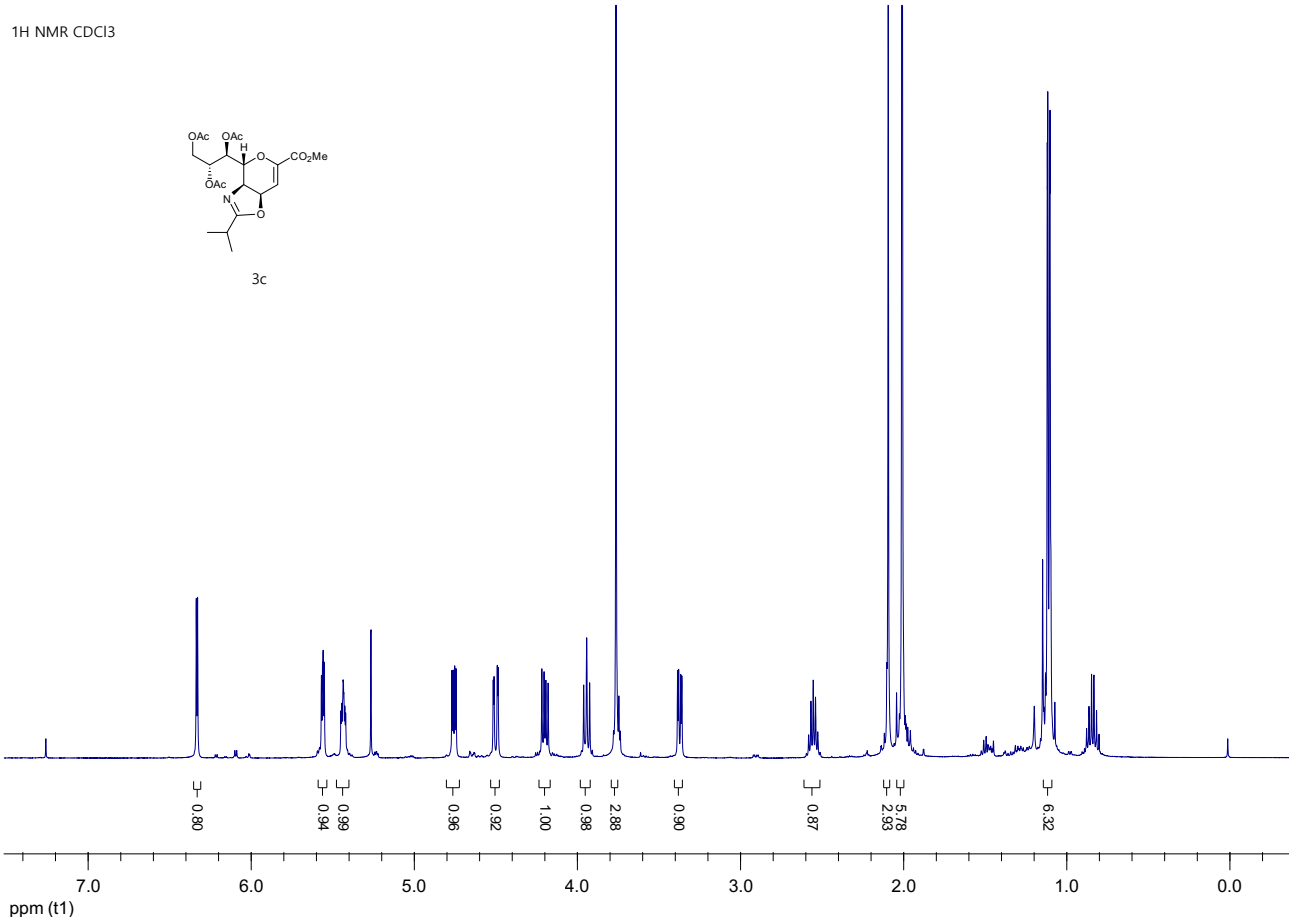
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0.90
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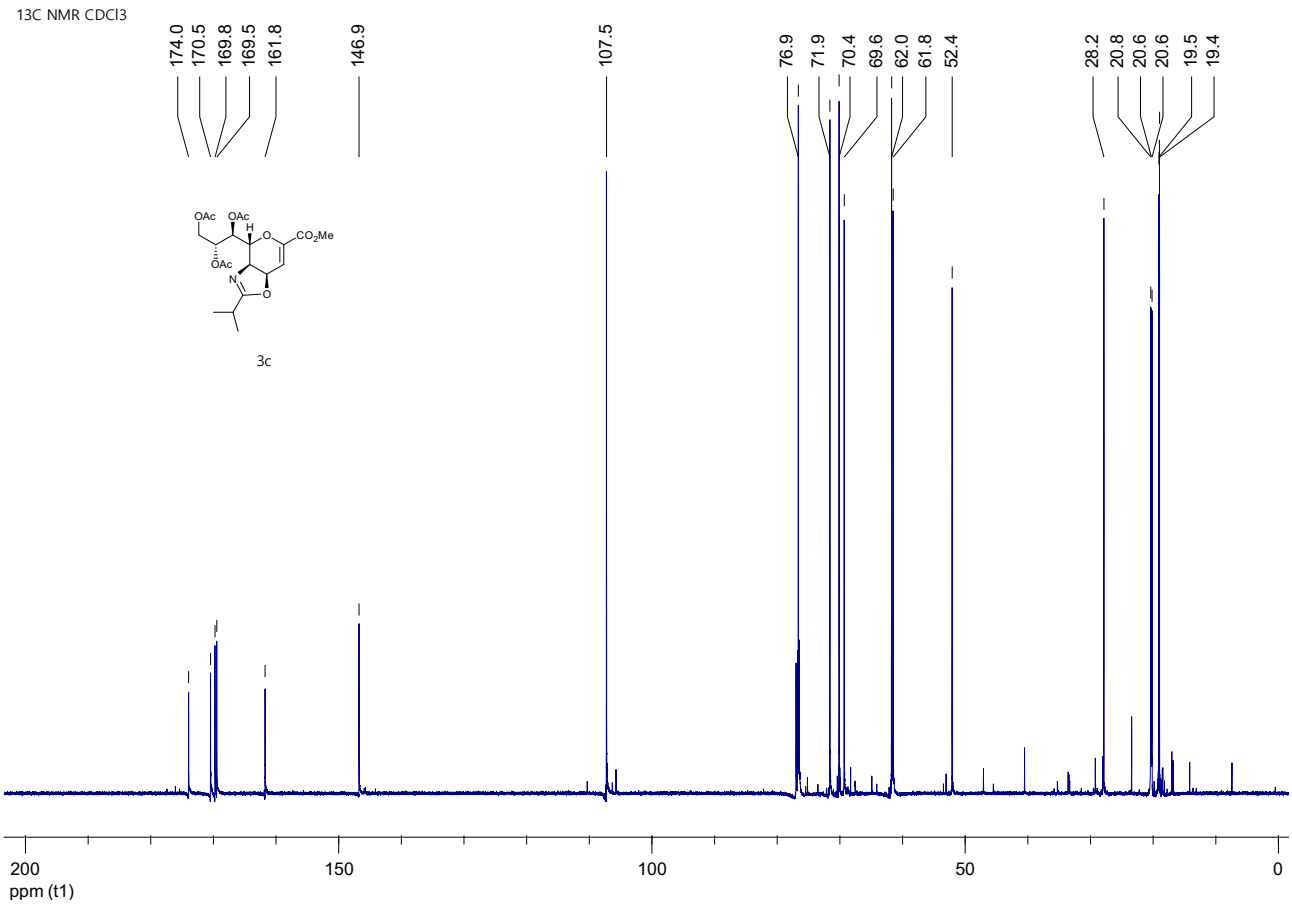
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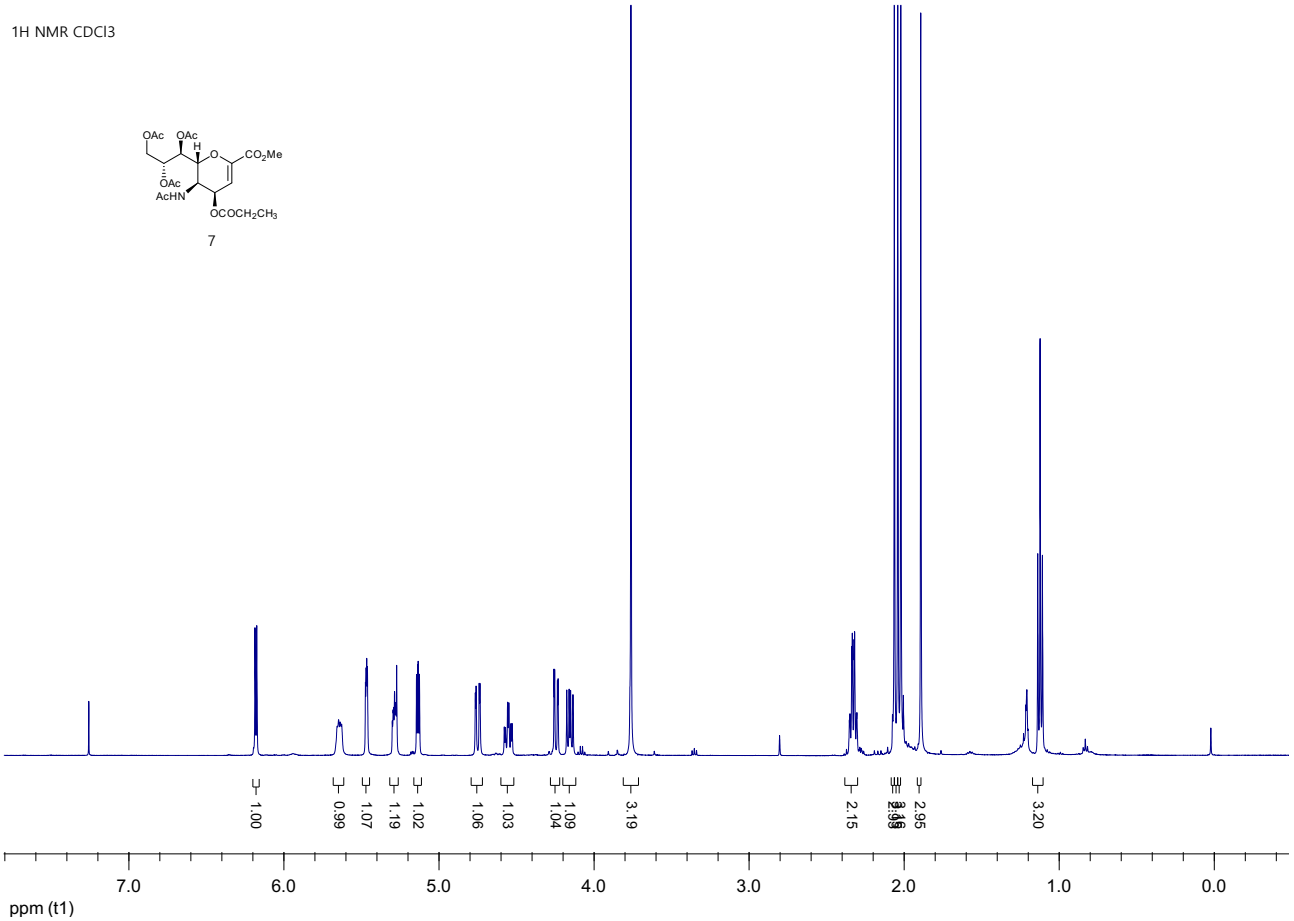
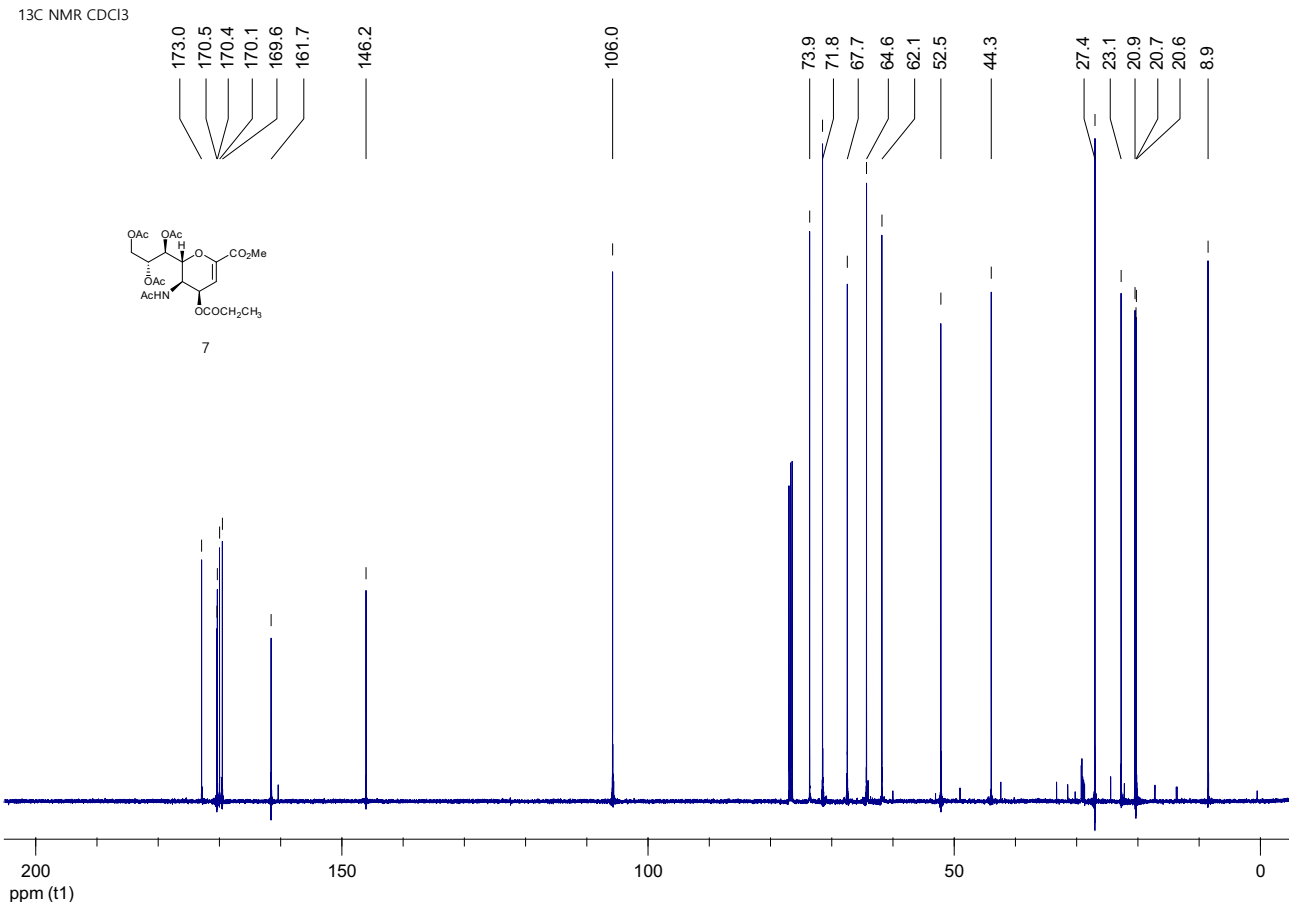
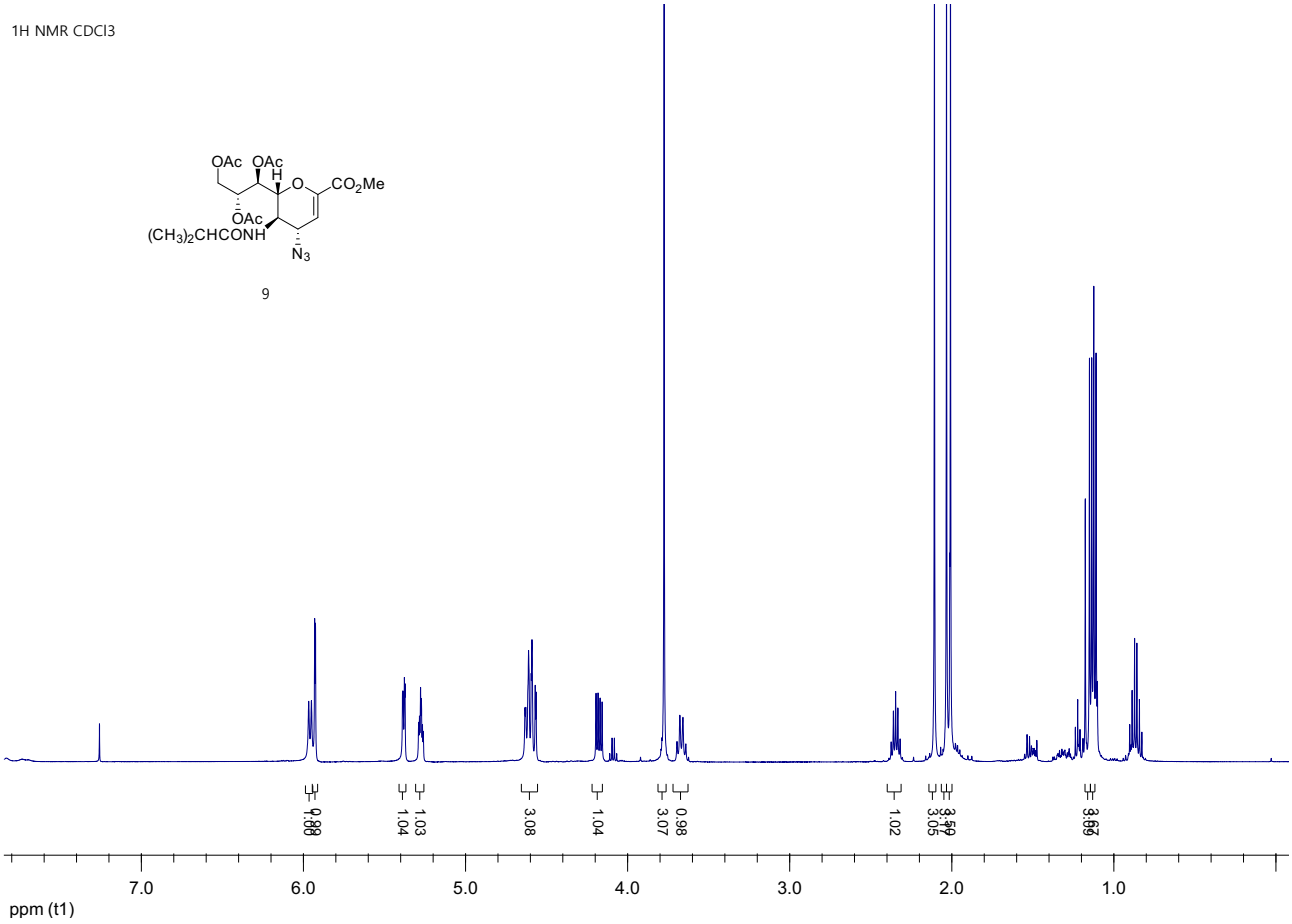
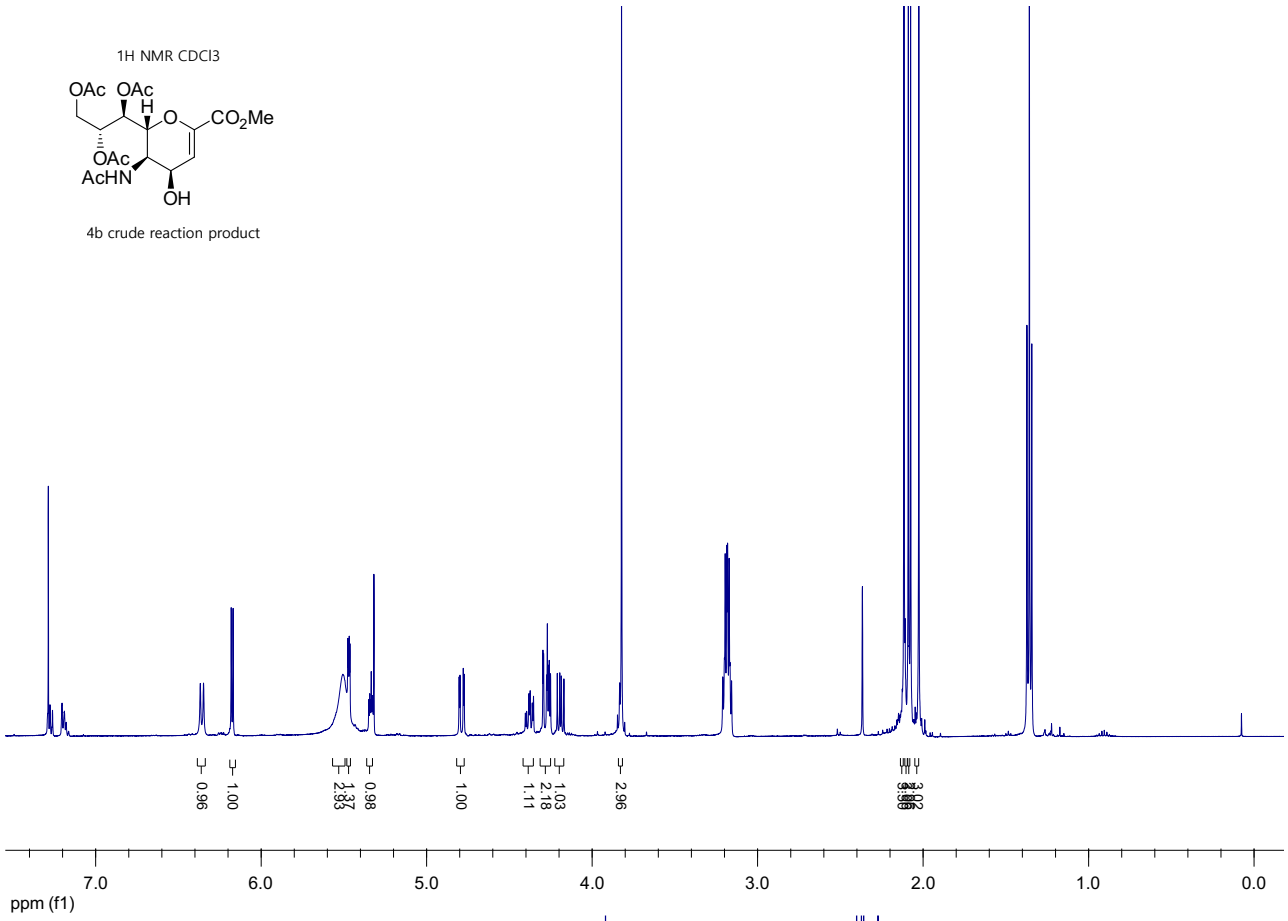
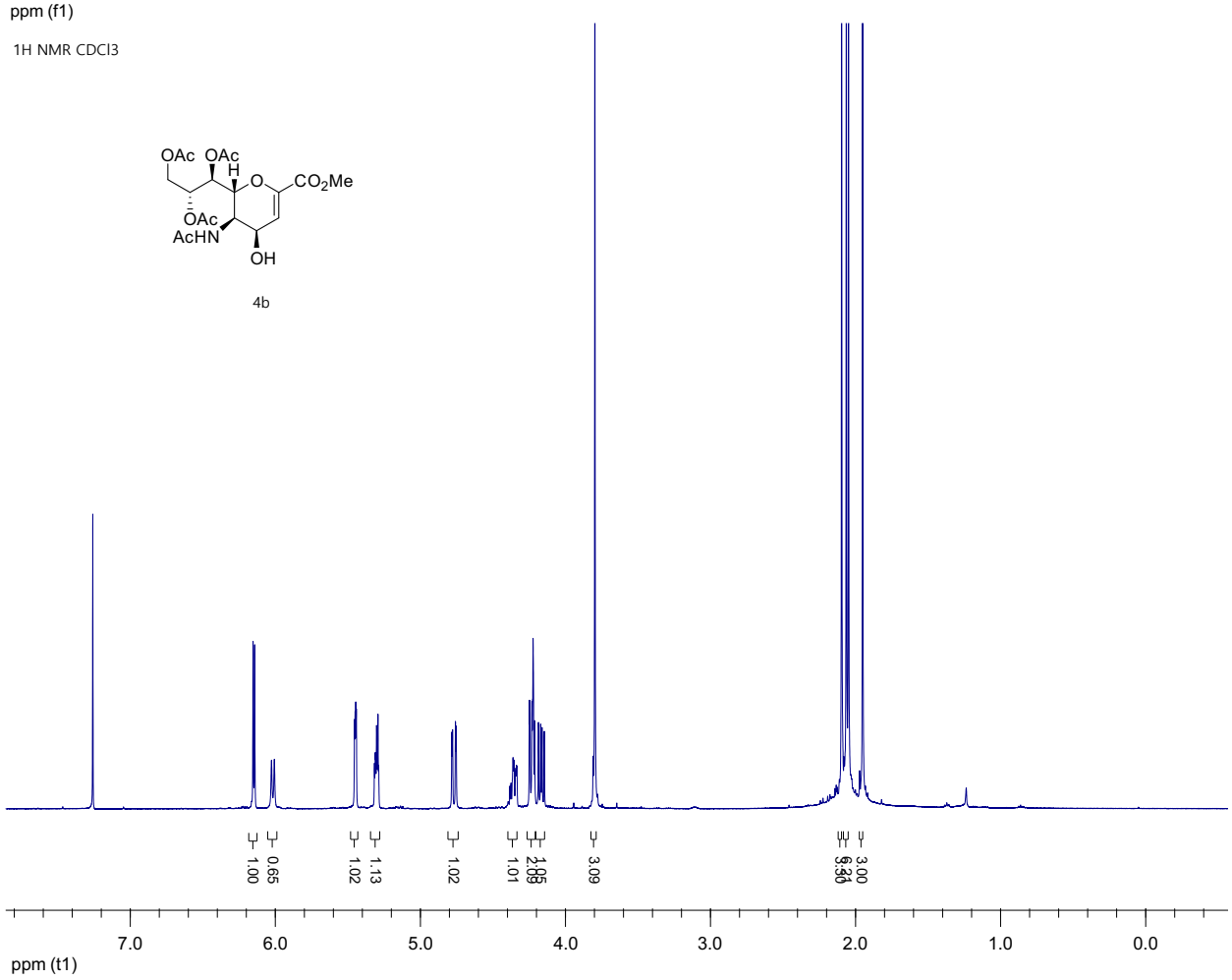
0
ppm (t1)

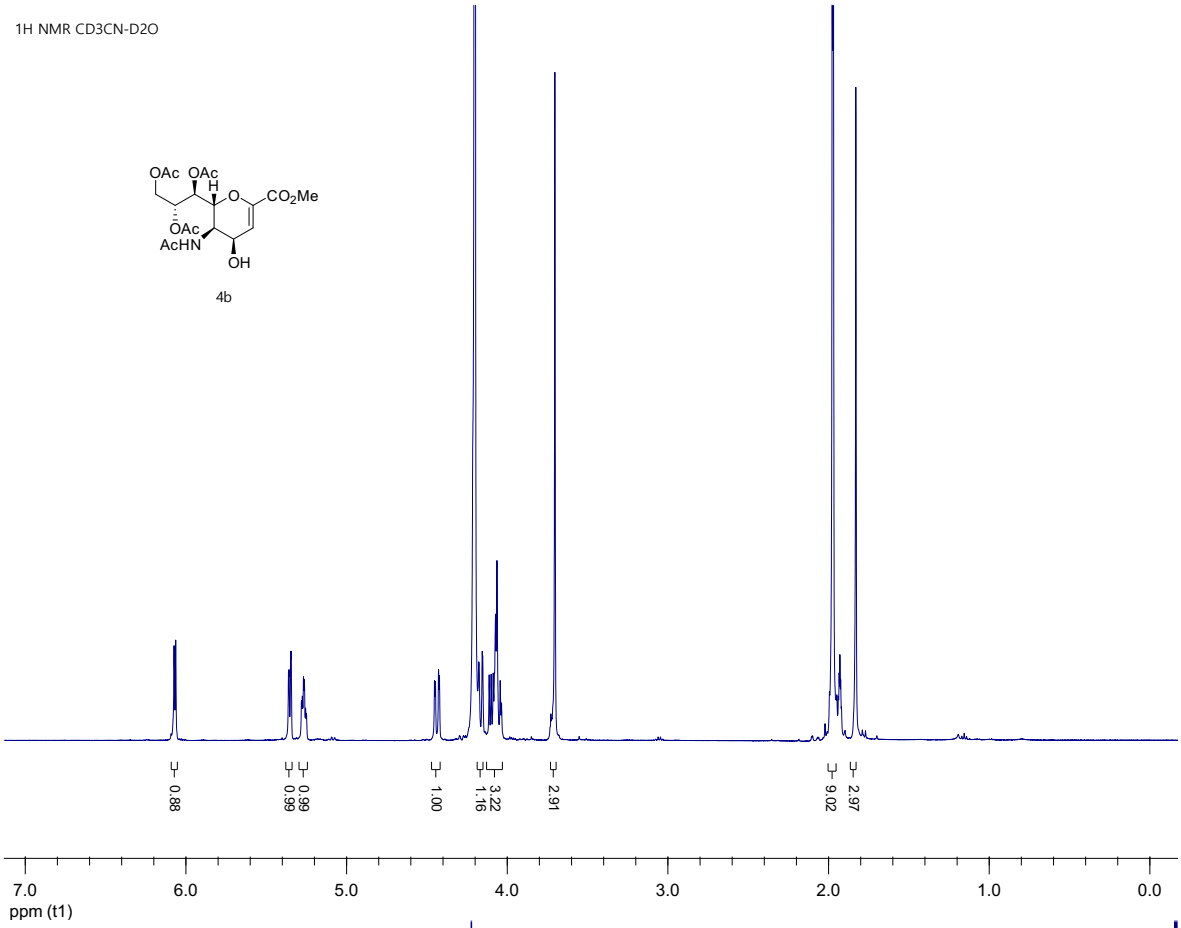

1H NMR CDCl3
OAc
OAc
H
O
CO2Me
OAc
AcHN
OCOCH2CH3
7
1.00
0.99
1.07
1.19
1.02
1.06
1.03
1.04
1.09
3.19
2.15
2.95
3.20
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (t1)

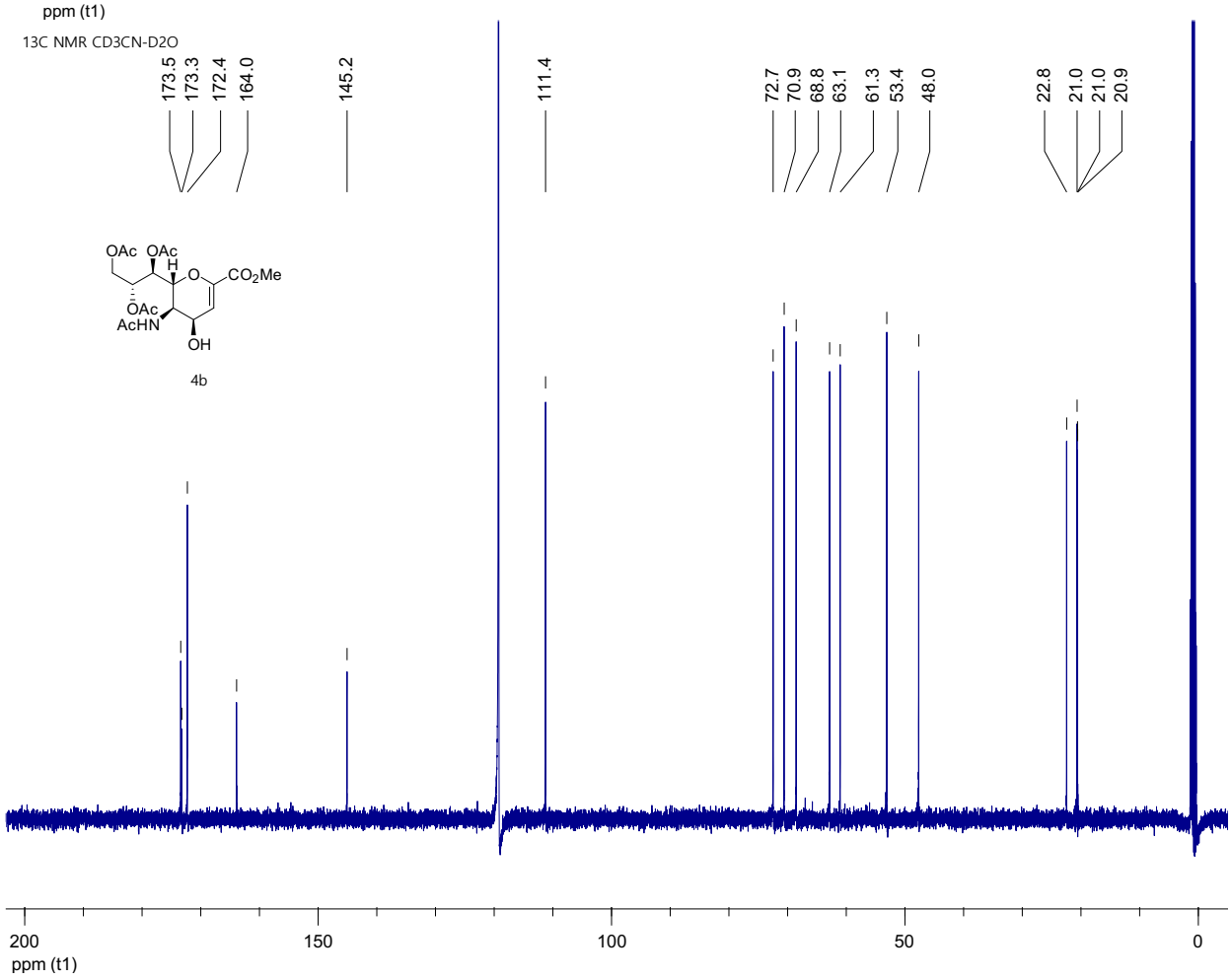

13C NMR CDCl3
173.0
170.5
170.4
170.1
169.6
161.7
146.2
106.0
73.9
71.8
67.7
64.6
62.1
52.5
44.3
27.4
23.1
20.9
20.7
20.6
8.9
OAc
OAc
H
O
CO2Me
OAc
AcHN
OCOCH2CH3
7
200
150
100
50
0
ppm (t1)

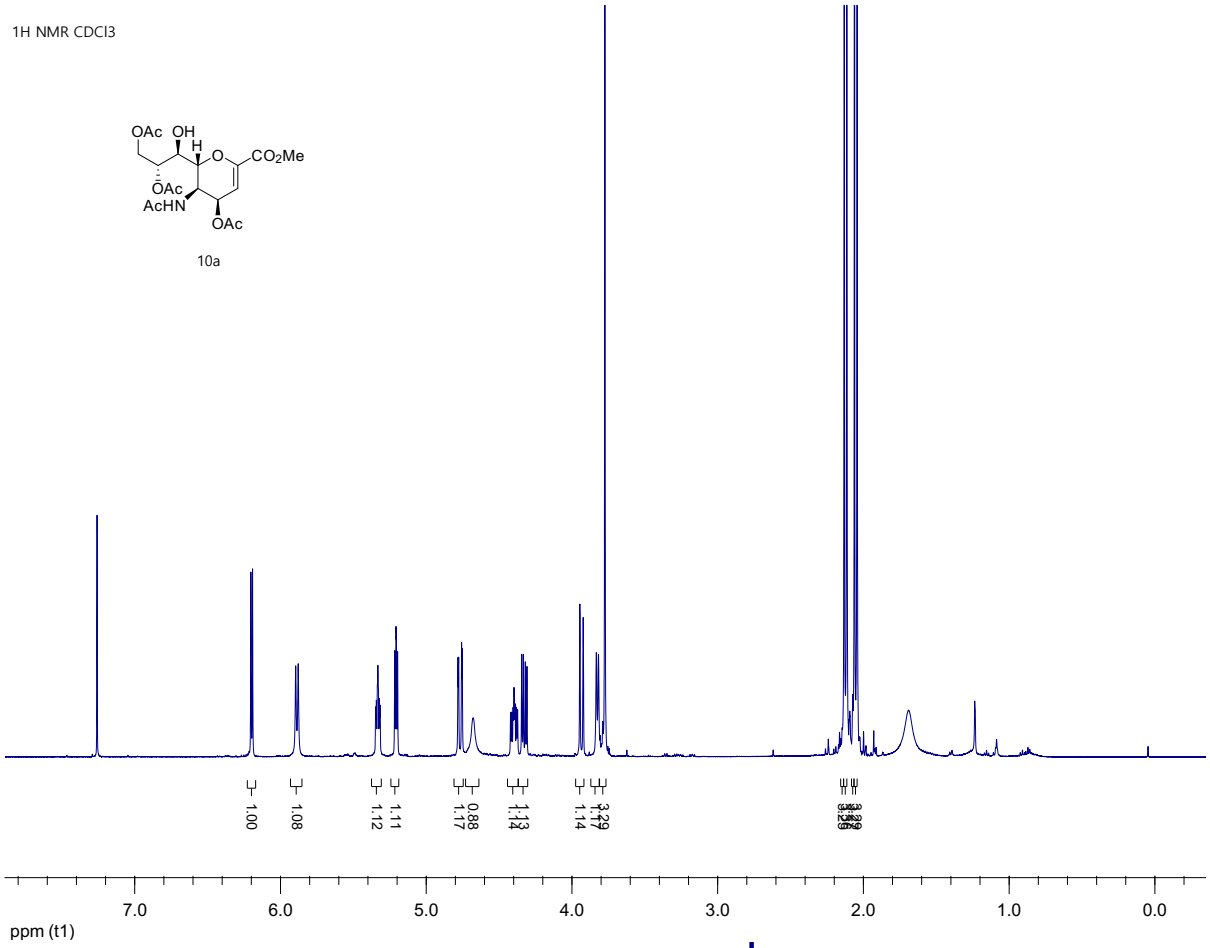

1H NMR CDCl3
OAc
OAc
H
O
CO2Me
OAc
(CH3)2CHCONH
N3
9
1.00
1.04
1.03
3.08
1.04
3.07
0.98
1.02
3.05
3.00
3.66
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (t1)

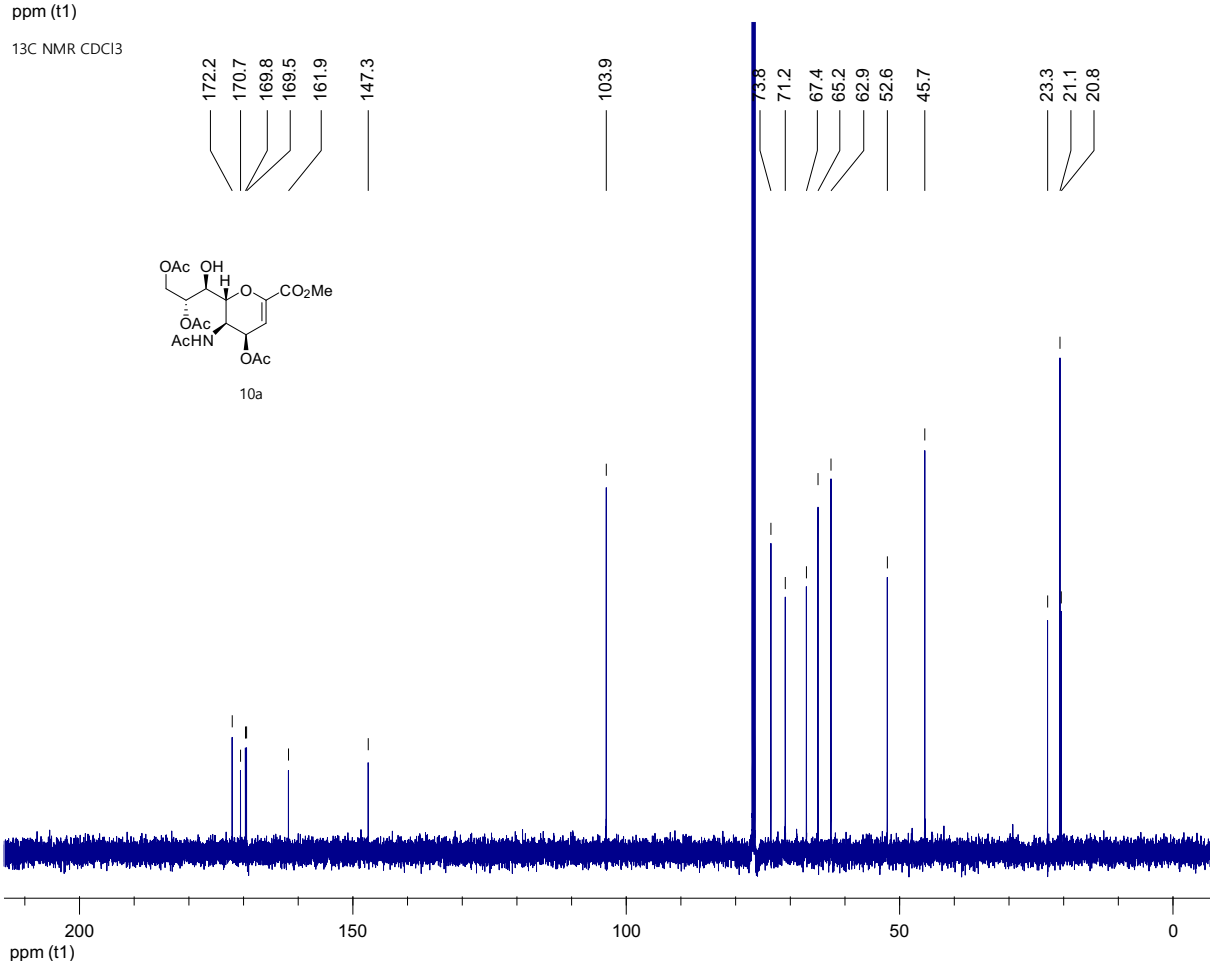

1H NMR CDCl3
OAc OAc
H
O
CO2Me
OAc
AcHN
OH
4b crude reaction product
0.96
1.00
2.93
2.97
0.98
1.00
1.11
2.18
1.03
2.96
3.02
3.06
3.09
3.09
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (f1)

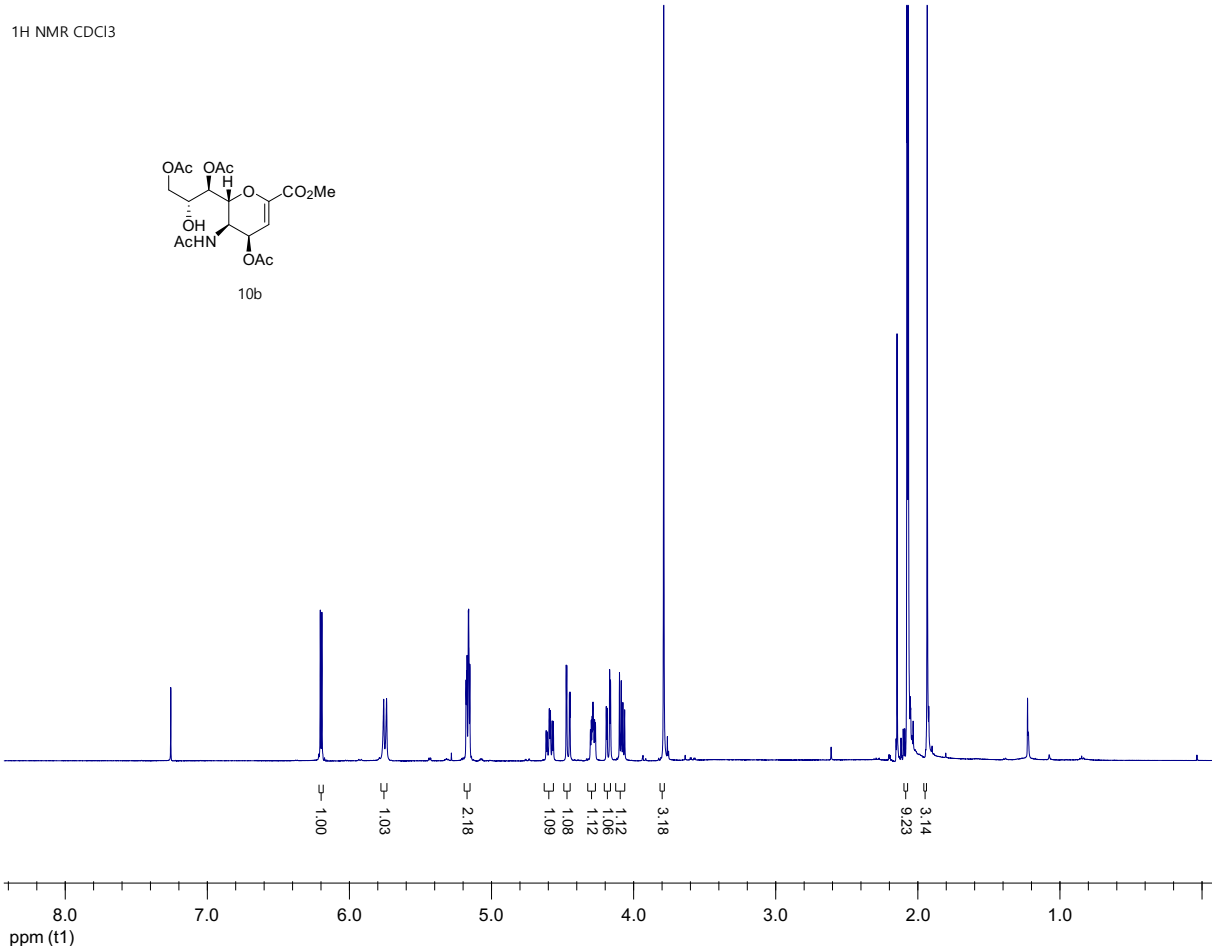

1H NMR CDCl3
OAc OAc
H
O
CO2Me
OAc
AcHN
OH
4b
1.00
0.65
1.02
1.13
1.02
1.01
2.05
2.05
3.09
9.30
9.30
3.00
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (t1)

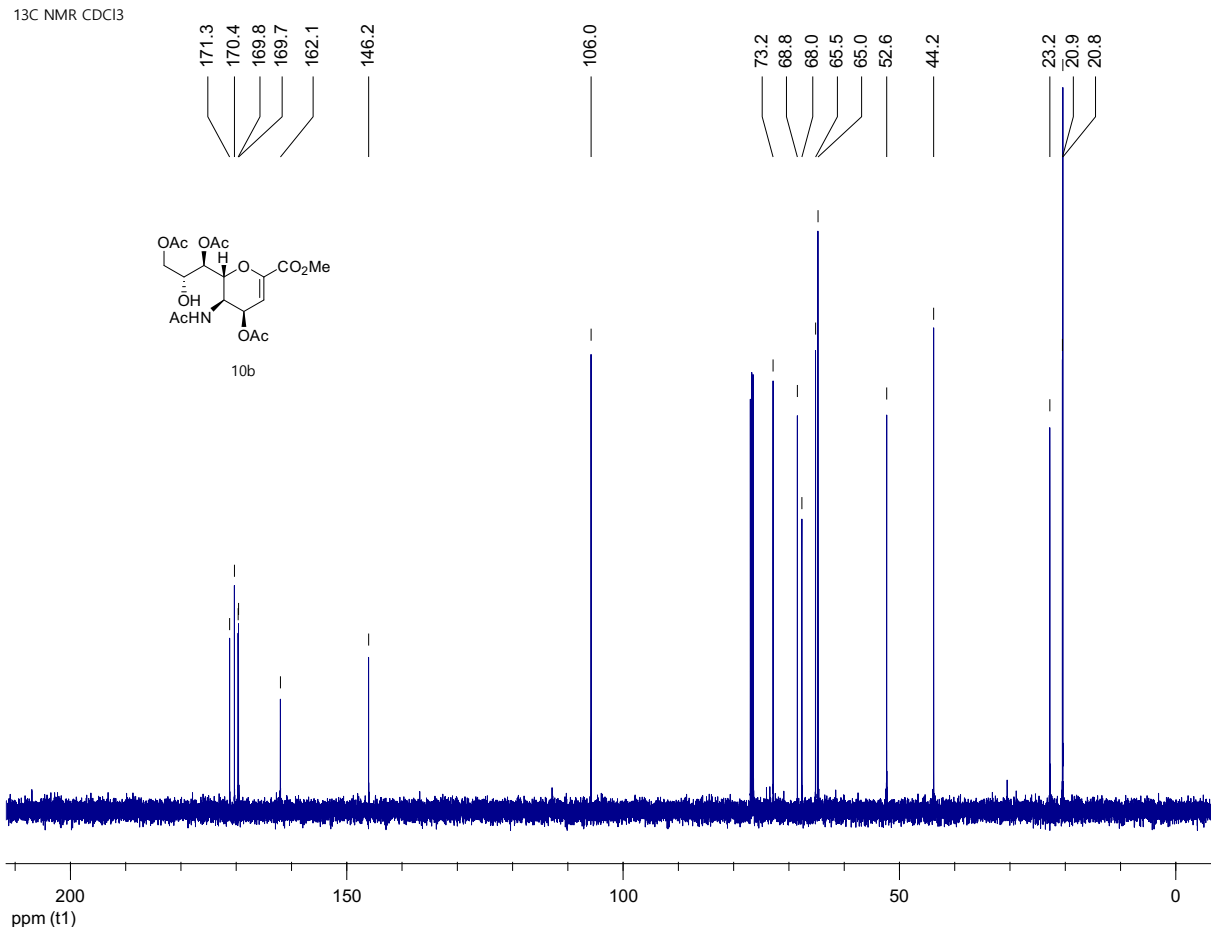

1H NMR CD3CN-D2O
OAc OAc
H
O
CO2Me
OAc
AcHN
OH
4b
0.88
0.99
0.99
1.00
1.16
3.22
2.91
6.02
2.97
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (t1)

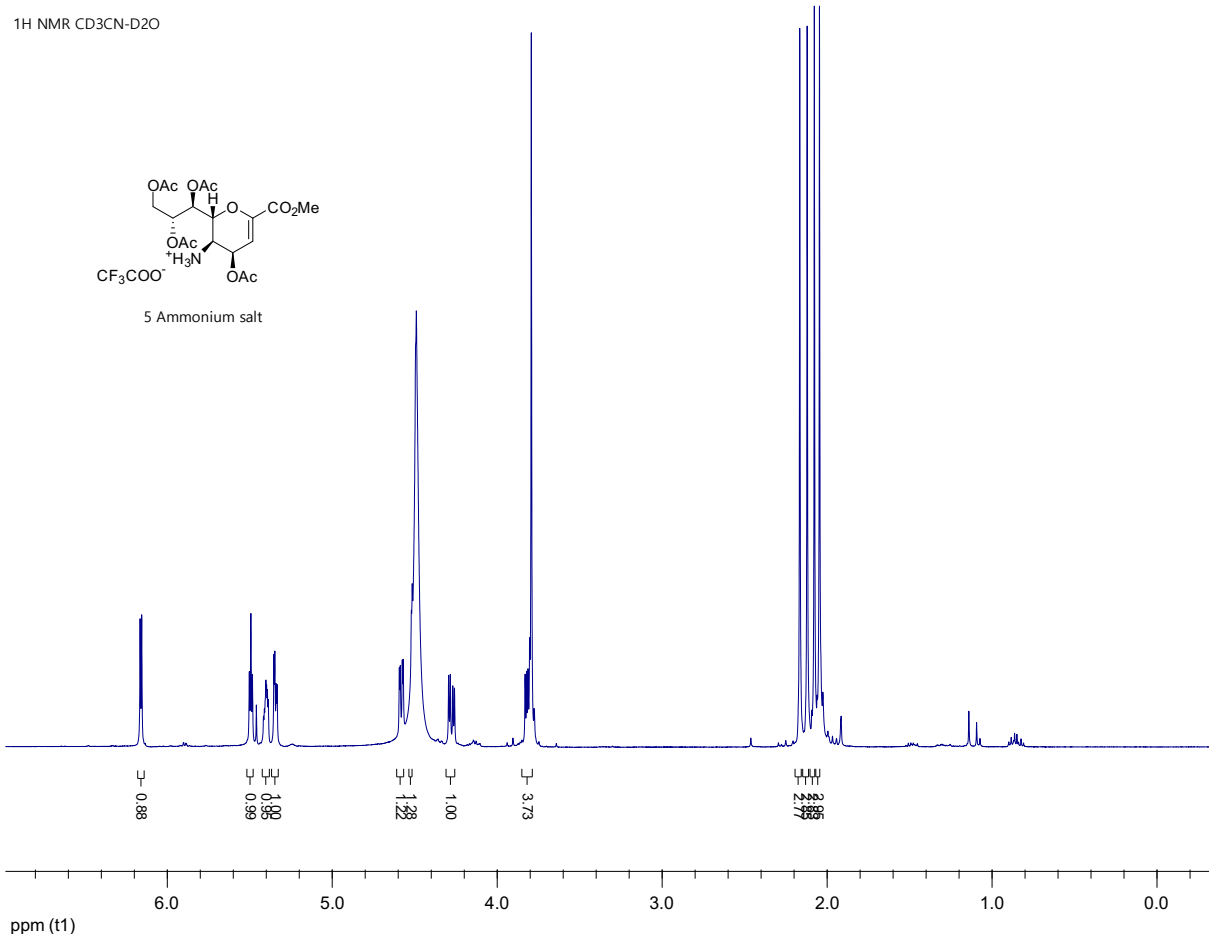

13C NMR CD3CN-D2O
173.5
173.3
172.4
164.0
145.2
111.4
72.7
70.9
68.8
63.1
61.3
53.4
48.0
22.8
21.0
21.0
20.9
OAc OAc
H
O
CO2Me
OAc
AcHN
OH
4b
200
150
100
50
0
ppm (t1)

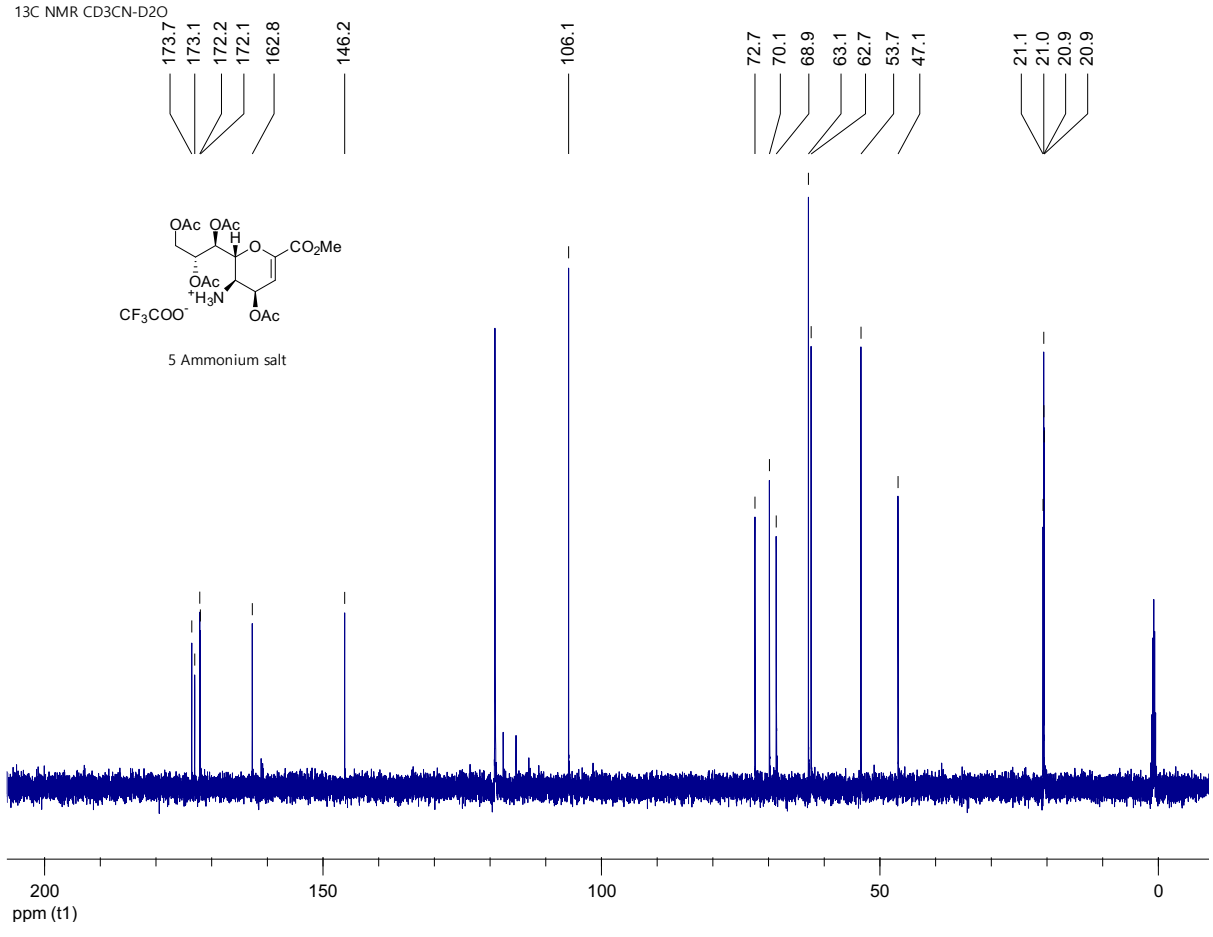

1H NMR CDCl3
OAc OH
H
O
CO2Me
OAc
AcHN
OAc
10a
1.00
1.08
1.12
1.11
1.17
0.88
1.14
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (t1)

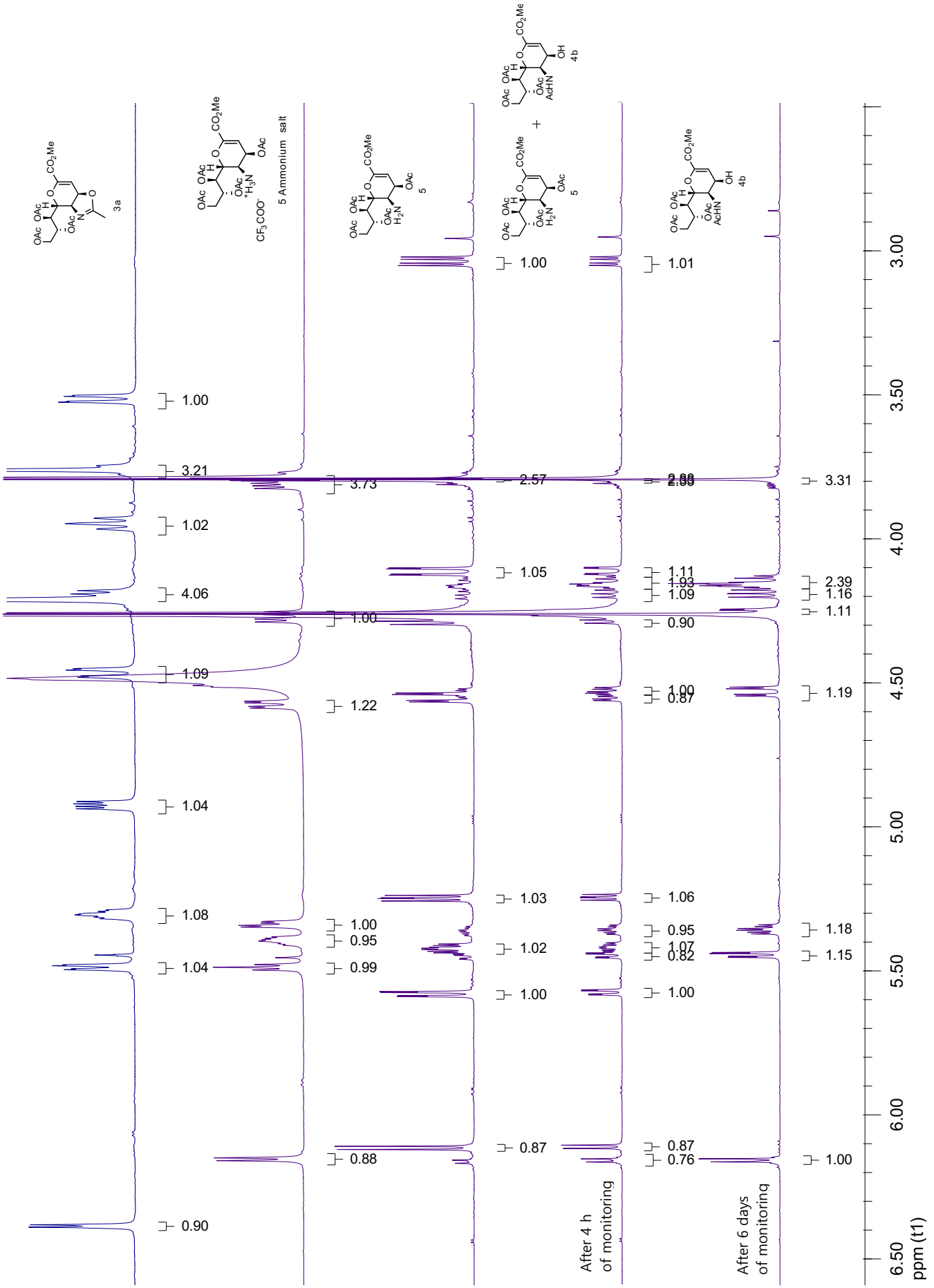

13C NMR CDCl3
172.2
170.7
169.8
169.5
161.9
147.3
103.9
73.8
71.2
67.4
65.2
62.9
52.6
45.7
23.3
21.1
20.8
OAc OH
H
O
CO2Me
OAc
AcHN
OAc
10a
200
150
100
50
0
ppm (t1)


1H NMR CDCl3
OAc
OAc
H
O
CO2Me
OH
AcHN
OAc
10b
1.00
1.03
2.18
1.09
1.08
1.12
1.06
1.12
3.18
9.23
3.14
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (t1)


13C NMR CDCl3
171.3
170.4
169.8
169.7
162.1
146.2
106.0
73.2
68.8
68.0
65.5
65.0
52.6
44.2
23.2
20.9
20.8
OAc
OAc
H
O
CO2Me
OH
AcHN
OAc
10b
200
150
100
50
0
ppm (t1)


1H NMR CD3CN-D2O
OAc OAc
H
O
CO2Me
OAc
+H3N
CF3COO-
OAc
5 Ammonium salt
0.88
0.99
1.00
1.22
1.00
3.73
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (t1)


13C NMR CD3CN-D2O
173.7
173.1
172.2
172.1
162.8
146.2
106.1
72.7
70.1
68.9
63.1
62.7
53.7
47.1
21.1
21.0
20.9
20.9
OAc OAc
H
O
CO2Me
OAc
+H3N
CF3COO-
OAc
5 Ammonium salt
200
150
100
50
0
ppm (t1)


3a
5 Ammonium salt
5
4b
After 4 h of monitoring
After 6 days of monitoring
ppm (t1)