Electronic Supplementary Material (ESI) for MedChemComm.

Supporting Information File

Exploring inhibitor structural features required to engage the 216-loop of human parainfluenza virus type-3 Hemagglutinin-Neuraminidase

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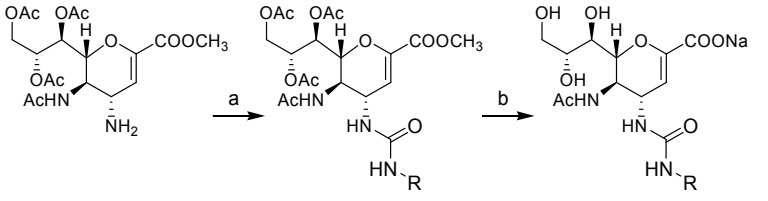
Chemistry

General Synthetic Methods

Reagents and dry solvents purchased from commercial sources were used without further purification. Anhydrous reactions were carried out under an atmosphere of argon, using oven-dried glassware. Reactions were monitored using thin layer chromatography (TLC) on aluminium plates pre-coated with Silica Gel 60 F254 (E. Merck). Developed plates were observed under UV light at 254 nm and then visualized after application of a solution of H_2SO_4 in EtOH (5% v/v) and heating. Flash chromatography was performed on Silica Gel 60 (0.040-0.063mm) using distilled solvents. ^1H and ^{13}C NMR spectra were recorded either at 300 and 75.5 MHz respectively on a BrukerAvance 300 MHz spectrometer or at 400 and 100 MHz respectively on a BrukerAvance 400 MHz spectrometer. Chemical shifts (δ) are reported in parts per million, relative to the residual solvent peak as internal reference [$CDCl_3$: 7.26 (s) for ^1H, 77.0 (t) for ^{13}C; D_2O: 4.79 (s) for ^1H]. 2D COSY and HSQC experiments were run to support assignments. Low-resolution mass spectra (LRMS) were recorded, in electrospray ionization mode, on a BrukerDaltonics Esquire 3000 ESI spectrometer, using positive mode. High-resolution mass spectra (HRMS) were recorded for either the protected or deprotected final derivatives, and were carried out by the University of Queensland FTMS Facility on a BrukerDaltonics Apex III 4.7e Fourier Transform micrOTOF-Q70 MS or by Griffith University SmartWater Research Centre facility using an Agilent 1290 HPLC coupled to an Agilent 6530 QTOF fitted with a Jet Stream ESI source. Final deprotected sialic acid derivatives were purified by running through GracePure™ SPE C18-Aq 5000mg/20 mL using 2% acetonitrile/H_2O. The purities of all synthetic intermediates after chromatographic purification were judged to be >90% by ^1H and ^{13}C NMR, while the purity of the reference compound **5** synthesised for screening purposes and of the new final products **9a-g, 11a-c, 13a-d** and **16** was judged to be ≥95%. Analytical HPLC was performed using U-XR-ODS-036 column, eluted at a flow rate of 0.26 mL/min. An isocratic elution was performed using 80% aq. MeOH containing 0.1% formic acid, and measurements were made at the wavelength corresponding to the maximum absorbance by each compound. The synthesis of reference inhibitor **5**[1] and the intermediates **7** and **14**[2] followed typical procedures to that mentioned in literature. The details of synthetic methods used and full characterization data of key intermediates and novel finals are reported here in the supplementary material.

Synthesis of Compounds 9a-g



7

8a: R = 4-OCH_3-Bn
8b: R = 2-Cl-Ph
8c: R = 2-OCH_3-Ph
8d: R = 3-OCH_3-Ph
8e: R = 4-OCH_3-Ph
8f: R = 2-OCF_3-Ph
8g: R = 3-(benzo[*d*][1,3] dioxol-6-yl)

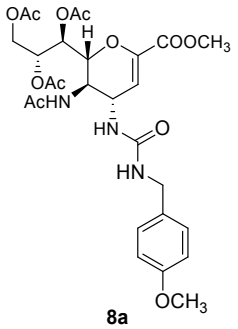
9a: R = 4-OCH_3-Bn
9b: R = 2-Cl-Ph
9c: R = 2-OCH_3-Ph
9d: R = 3-OCH_3-Ph
9e: R = 4-OCH_3-Ph
9f: R = 2-OCF_3-Ph
9g: R = 3-(benzo[*d*][1,3] dioxol-6-yl)

[a]Reagents and conditions: (a) R-NCO, DMAP, DCM, rt, o/n, (**8a**, 87%, **8b**, 96%; **8c**, 90%; **8d**, 84%; **8e**, 89%; **8f**, 91%; **8g**, 82%) (b) NaOH, MeOH/H_2O (1:1), rt, o/n, (**9a**, 80%, **9b**, 91%; **9c**, 78%; **9d**, 87%; **9e**, 77%; **9f**, 88%; **9g**, 85%)

General Procedure for the synthesis of compounds 8a-g

To a solution of the amine **7** (60 mg, 0.14 mmol) and DMAP (cat.) in DCM (3 mL) was added the corresponding isocyanate (0.21 mmol) while stirring. The reaction mixture was stirred at rt o/n, then the solvent was removed under vacuum and the reside was purified by silica gel chromatography using the proper solvent system to yield the pure urea derivative **8a-g**.

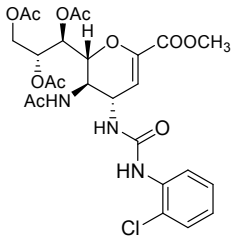
Methyl 5-acetamido-7,8,9-tri-*O*-acetyl-2,6-anhydro-3,4,5-trideoxy-4-(3-(4-methoxybenzyl)ureido)-D-*glycero*-D-*galacto*-non-2-enonate (8a).



8a

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.56 (s, 3H, NAc), 1.99 (s, 3H, OAc), 2.00 (s, 3H, OAc), 2.04 (s, 3H, OAc), 3.72 (s, 3H, OCH_3), 3.79 (s, 3H, $COOCH_3$), 3.96 (m, 1H, H-5), 4.12-4.22 (m, 3H, H-9, CH_2), 4.36 (dd, *J* = 9.8, 2.4 Hz, 1H, H-6), 4.63 (dd, *J* = 12.5, 2.6 Hz, 1H, H-9'), 4.79 (t, *J* = 9.7 Hz, 1H, H-4), 5.28 (ddd, *J* = 7.6, 5.0, 2.6 Hz, 1H, H-8), 5.51 (dd, *J* = 5.3, 2.3 Hz, 1H, H-7), 5.65 (d, *J* = 9.2 Hz, 1H, Urea-N1-NH), 5.90 (d, *J* = 1.2 Hz, 1H, H-3), 6.19 (t, *J* = 5.1 Hz, 1H, Urea-N2-NH), 6.77 (d, *J* = 8.6 Hz, 2H, Ph-H-3', Ph-H-5'), 7.10 (d, *J* = 8.6 Hz, 2H, Ph-H-2', Ph-H-6'), 7.29 (d, *J* = 10.5 Hz, 1H, NHAc); ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.73, 20.83 (3 OCO<u>CH_3</u>), 22.85 (NHCO<u>CH_3</u>), 43.53 (Ph-<u>CH_2</u>), 48.12 (C-5), 49.05 (C-4), 52.44 (COO<u>CH_3</u>), 55.22 (Ph-O<u>CH_3</u>), 62.04 (C-9), 67.78 (C-7), 71.06 (C-8), 77.49 (C-6), 112.06 (C-3), 113.96 (Ph), 128.23 (Ph), 131.12 (Ph q carbon), 144.59 (C-2), 158.54 (Urea-CO), 158.77 (Ph q carbon), 161.73 (<u>CO</u>OCH_3), 169.92, 170.10, 170.57, 171.65 (NH<u>CO</u>CH_3, 3 O<u>CO</u>CH_3); LRMS [$C_{27}H_{35}N_3O_{12}$] (*m/z*): (+ve ion mode) 616.2 $[M+Na]^+$.

Methyl 5-acetamido-7,8,9-tri-*O*-acetyl-2,6-anhydro-4-(3-(2-chlorophenyl)ureido)-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enonate (8b).

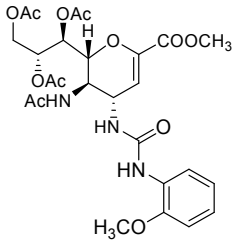


8b

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.59 (s, 3H, NAc), 2.00 (s, 3H, OAc), 2.03 (s, 3H, OAc), 2.12 (s, 3H, OAc), 3.83 (s, 3H, $COOCH_3$), 4.00–4.22 (m, 2H, H-5, H-9), 4.39 (dd, *J* = 9.9, 2.4 Hz, 1H, H-6), 4.64 (dd, *J* = 12.4, 2.8 Hz, 1H, H-

9'), 4.91 (ddd, *J* = 9.7, 2.5, 1.1 Hz, 1H, H-4), 5.33 (ddd, *J* = 6.8, 5.3, 2.7 Hz, 1H, H-8), 5.54 (dd, *J* = 5.4, 2.4 Hz, 1H, H-7), 6.00 (d, *J* = 2.4 Hz, 1H, H-3), 6.53 (d, *J* = 9.7 Hz, 1H, Urea-N1-NH), 6.94 (ddd, *J* = 8.1, 7.4, 1.5 Hz, 1H, Ph-H-4'), 7.21 (dd, *J* = 8.1, 1.5 Hz, 1H, Ph-H-3'), 7.25–7.40 (m, 2H, NHAc, Ph-H-5'), 7.79 (dd, *J* = 8.2, 1.5 Hz, 1H, Ph-H-6'), 8.02 (s, 1H, Urea-N2-NH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.81 (3 OCO$\underline{CH_3}$), 22.71 (NHCO$\underline{CH_3}$), 48.05 (C-5), 49.16 (C-4), 52.55 (COO$\underline{CH_3}$), 62.09 (C-9), 67.96 (C-7), 70.89 (C-8), 77.73 (C-6), 111.42 (C-3), 123.22 (Ph), 124.26 (Ph), 124.40 (Ph q carbon), 127.63 (Ph), 129.25 (Ph), 135.27 (Ph q carbon), 145.03 (C-2), 156.12 (Urea-CO), 161.60 ($\underline{CO}OCH_3$), 169.91, 169.99, 170.57, 172.13 (NH$\underline{CO}CH_3$, 3 O$\underline{CO}CH_3$); LRMS [$C_{25}H_{30}ClN_3O_{11}$] (*m/z*): (+ve ion mode) 606.1 $[M+Na]^+$.

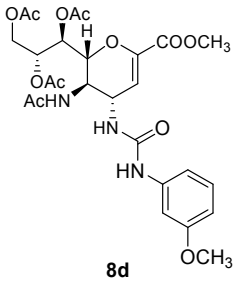
Methyl 5-acetamido-7,8,9-tri-*O*-acetyl-2,6-anhydro-3,4,5-trideoxy-4-(3-(2-methoxybenzyl)ureido)-D-*glycero*-D-*galacto*-non-2-enonate (8c).[3]



8c

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.75 (s, 3H, NAc), 2.02 (s, 3H, OAc), 2.03 (s, 3H, OAc), 2.10 (s, 3H, OAc), 3.64 (s, 3H, OCH_3), 3.80 (s, 3H, $COOCH_3$), 4.08–4.27 (m, 2H, H-5, H-9), 4.39 (dd, *J* = 9.9, 2.5 Hz, 1H, H-6), 4.65 (dd, *J* = 12.4, 2.7 Hz, 1H, H-9'), 4.86 (ddd, *J* = 9.4, 2.5, 1.1 Hz, 1H, H-4), 5.33 (ddd, *J* = 6.2, 5.2, 2.7 Hz, 1H, H-8), 5.58 (dd, *J* = 5.3, 2.5 Hz, 1H, H-7), 5.93 (d, *J* = 9.3 Hz, 1H, Urea-N1-NH), 5.99 (d, *J* = 2.4 Hz, 1H, H-3) 6.72 (m, 1H, Ph-H-5'), 6.82–7.06 (m, 3H, NHAc, Ph-H-3', Ph-H-4'), 7.47 (s, 1H, Urea-N2-NH), 7.93 (m, 1H, Ph-H-6'); ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.74, 20.79, 20.85 (3 OCO$\underline{CH_3}$), 22.86 (NHCO$\underline{CH_3}$), 47.67 (C-5), 49.17 (C-4), 52.45 (COO$\underline{CH_3}$), 55.40 (Ph-O$\underline{CH_3}$), 62.07 (C-9), 67.86 (C-7), 71.00 (C-8), 77.57 (C-6), 110.15 (Ph), 111.62 (C-3), 119.84 (Ph), 121.11 (Ph), 122.88 (Ph), 127.99 (Ph q carbon), 144.66 (C-2), 148.35 (Ph q carbon), 155.86 (Urea-CO), 161.78 ($\underline{CO}OCH_3$), 169.98, 170.17, 170.61, 171.75 (NH$\underline{CO}CH_3$, 3 O$\underline{CO}CH_3$); LRMS [$C_{26}H_{33}N_3O_{12}$] (*m/z*): (+ve ion mode) 602.2 $[M+Na]^+$.

Methyl 5-acetamido-7,8,9-tri-*O*-acetyl-2,6-anhydro-3,4,5-trideoxy-4-(3-(3-methoxyphenyl)ureido)-D-*glycero*-D-*galacto*-non-2-enonate (8d).[3]

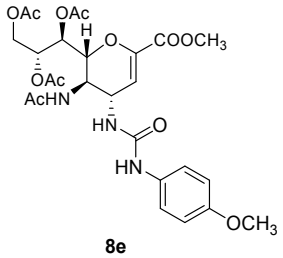


8d

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.77 (s, 3H, NAc), 2.00 (s, 3H, OAc), 2.03 (s, 3H, OAc), 2.09 (s, 3H, OAc), 3.66 (s, 3H, Ph-OCH_3), 3.82 (s, 3H, $COOCH_3$), 4.05 (q, *J* = 10.1 Hz, 1H, H-5), 4.17 (dd, *J* = 12.4, 7.2 Hz, 1H, H-9), 4.42 (dd, *J* = 10.2, 2.2 Hz, 1H, H-6), 4.66 (dd, *J* = 12.4, 2.6 Hz, 1H, H-9'), 4.91 (td, *J* = 9.8, 2.4 Hz, 1H, H-4), 5.32 (ddd, *J* = 7.4,

4.9, 2.6 Hz, 1H, H-8), 5.57 (dd, *J* = 5.2, 2.1 Hz, 1H, H-7), 5.95 (d, *J* = 2.2 Hz, 1H, H-3), 6.02 (d, *J* = 9.7 Hz, 1H, Urea-N1-NH), 6.52 (dd, *J* = 8.3, 2.4 Hz, 1H, Ph-H-4'), 6.74 (t, *J* = 2.2 Hz, 1H, Ph-H-2'), 6.85 (dd, *J* = 8.0, 2.1 Hz, 1H, Ph-H-6'), 7.15 (t, *J* = 8.1 Hz, 1H, Ph-H-5'), 7.51 (d, *J* = 10.0 Hz, 1H, NHAc), 8.25 (s, 1H, Urea-N2-NH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.73, 20.77, 20.82 (3 OCO$\underline{CH_3}$), 23.08 (NHCO$\underline{CH_3}$), 48.19 (C-5), 49.04 (C-4), 52.55 (COO$\underline{CH_3}$), 55.04 (Ph-O$\underline{CH_3}$), 62.15 (C-9), 68.02 (C-7), 71.10 (C-8), 77.61 (C-6), 105.59 (Ph), 108.62 (Ph), 111.44 (C-3), 112.10 (Ph), 130.02 (Ph), 139.77 (Ph q carbon), 145.01 (C-2), 156.32 (Urea-CO), 160.20 (Ph q carbon), 161.58 ($\underline{CO}OCH_3$), 169.83, 170.15, 170.60, 172.18 (NH$\underline{CO}CH_3$, 3 O$\underline{CO}CH_3$); LRMS [$C_{26}H_{33}N_3O_{12}$] (*m/z*): (+ve ion mode) 602.4 $[M+Na]^+$; HRMS (API) (*m/z*): $[M+1]^+$ calcd for $C_{26}H_{34}N_3O_{12}$ $[M+1]^+$ 580.21370; found, 580.21378.

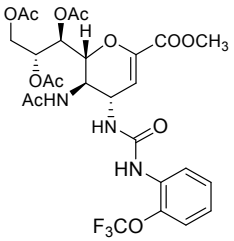
Methyl 5-acetamido-7,8,9-tri-*O*-acetyl-2,6-anhydro-3,4,5-trideoxy-4-(3-(4-methoxyphenyl)ureido)-D-*glycero*-D-*galacto*-non-2-enonate (8e).[3]



8e

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.61 (s, 3H, NAc), 2.00 (s, 3H, OAc), 2.03 (s, 3H, OAc), 2.08 (s, 3H, OAc), 3.73 (s, 3H, OCH_3), 3.81 (s, 3H, $COOCH_3$), 4.03 (q, *J* = 10.0 Hz, 1H, H-5), 4.16 (dd, *J* = 12.4, 7.2 Hz, 1H, H-9), 4.42 (dd, *J* = 10.2, 2.1 Hz, 1H, H-6), 4.68 (dd, *J* = 12.4, 2.7 Hz, 1H, H-9'), 4.90 (t, *J* = 9.9 Hz, 1H, H-4), 5.35 (ddd, *J* = 7.4, 5.0, 2.6 Hz, 1H, H-8), 5.57 (dd, *J* = 5.1, 2.1 Hz, 1H, H-7), 5.81 (d, *J* = 10.1 Hz, 1H, Urea-N1-NH), 5.94 (d, *J* = 2.3 Hz, 1H, H-3), 6.79 (d, *J* = 8.8 Hz, 2H, Ph-H-3', Ph-H-5'), 7.06 (d, *J* = 8.9 Hz, 2H, Ph-H-2', Ph-H-6'), 7.69 (d, *J* = 9.9 Hz, 1H, NHAc), 8.03 (s, 1H, Urea-N2-NH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.73, 20.79, 20.86 (3 OCO$\underline{CH_3}$), 22.89 (NHCO$\underline{CH_3}$), 47.97 (C-5), 49.12 (C-4), 52.49 (COO$\underline{CH_3}$), 55.43 (Ph-O$\underline{CH_3}$), 62.20 (C-9), 68.07 (C-7), 71.17 (C-8), 77.50 (C-6), 111.67 (C-3), 114.49 (Ph), 122.64 (Ph), 131.12 (Ph q carbon), 144.76 (C-2), 156.25 (Ph), 156.90 (Urea-CO), 161.72 ($\underline{CO}OCH_3$), 169.84, 170.19, 170.64, 171.99 (NH$\underline{CO}CH_3$, 3 O$\underline{CO}CH_3$); LRMS [$C_{26}H_{33}N_3O_{12}$] (*m/z*): (+ve ion mode) 601.7 $[M+Na]^+$; HRMS (API) (*m/z*): $[M+1]^+$ calcd for $C_{26}H_{34}N_3O_{12}$ $[M+1]^+$ 580.2137; found, 580.214037.

Methyl 5-acetamido-7,8,9-tri-*O*-acetyl-2,6-anhydro-3,4,5-trideoxy-4-(3-(2-(trifluoromethoxy)phenyl)ureido)-D-*glycero*-D-*galacto*-non-2-enonate (8f).

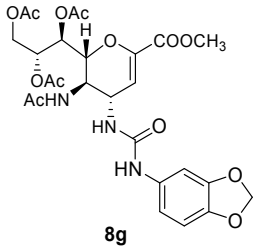


8f

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.72 (s, 3H, NAc), 2.01 (s, 3H, OAc), 2.03 (s, 3H, OAc), 2.13 (s, 3H, OAc), 3.83 (s, 3H, $COOCH_3$), 4.02–4.23 (m, 2H, H-5, H-9), 4.40 (dd, *J* = 9.1, 3.2 Hz, 1H, H-6), 4.63 (dd, *J* = 12.4, 2.8 Hz, 1H, H-9'), 4.86 (ddd, *J* = 9.2, 2.7, 1.1 Hz, 1H, H-4), 5.29 (ddd, *J* = 7.5, 4.9, 2.8 Hz, 1H, H-8), 5.56 (dd, *J* = 5.1, 3.2 Hz, 1H, H-7), 6.01 (d, *J* = 2.6 Hz, 1H, H-3), 6.48 (d, *J* = 9.5 Hz, 1H, Urea-N1-NH), 6.91–7.06 (m, 2H, NHAc, Ph-H-4'), 7.15

(d, *J* = 8.7, 1.3 Hz, 1H, Ph-H-3'), 7.31 (ddd, J = 8.5, 8.0, 1.5 Hz, 1H, Ph-H-5'), 8.00 (dd, *J* = 8.3, 1.6 Hz, 1H, Ph-H-6'), 8.08 (s, 1H, Urea-N2-NH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.71, 20.80 (3 OCO<u>CH_3</u>), 22.43 (NHCO<u>CH_3</u>), 48.22 (C-5), 48.52 (C-4), 52.62 (COO<u>CH_3</u>), 61.91 (C-9), 67.72 (C-7), 71.03 (C-8), 77.65 (C-6), 111.22 (C-3), 118.70 (OCF_3), 121.23 (Ph), 122.27 (Ph), 123.16 (Ph), 127.73 (Ph), 131.80 (Ph q carbon), 138.55 (Ph q carbon), 144.88 (C-2), 155.68 (Urea-CO), 161.68 (<u>CO</u>OCH_3), 169.94, 170.36, 170.63, 172.27 (NH<u>CO</u>CH_3, 3 O<u>CO</u>CH_3); LRMS [$C_{26}H_{30}F_3N_3O_{12}$] (*m/z*): (+ve ion mode) 656.2 $[M+Na]^+$.

Methyl 5-acetamido-7,8,9-tri-*O*-acetyl-2,6-anhydro-4-(3-(benzo[d][1,3]dioxol-5-yl)ureido)-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enonate (8g).



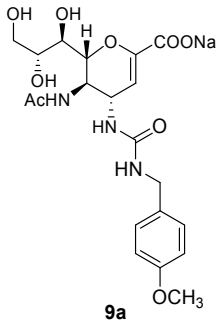
8g

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.68 (s, 3H, NAc), 2.02 (s, 3H, OAc), 2.03 (s, 3H, OAc), 2.06 (s, 3H, OAc), 3.80 (s, 3H, $COOCH_3$), 4.00 (m, 1H, H-5), 4.17 (dd, *J* = 12.4, 7.3 Hz, 1H, H-9), 4.39 (dd, *J* = 10.0, 2.1 Hz, 1H, H-6), 4.64 (dd, *J* = 12.4, 2.7 Hz, 1H, H-9'), 4.87 (td, *J* = 9.9, 2.4 Hz, 1H, H-4), 5.34 (m, 1H, H-8), 5.52 (dd, *J* = 5.3, 2.1 Hz, 1H, H-7), 5.75–5.99 (m, 4H, CH_2, Urea-N1-NH, H-3), 6.57 (dd, *J* = 8.4, 2.0 Hz, 1H, Ph-H-5'), 6.62–6.74 (m, 2H, Ph-H-2', Ph-H-6'), 7.67 (d, *J* = 10.0 Hz, 1H, NHAc), 8.13 (s, 1H, Urea-N2-NH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.71, 20.79, 20.93 (3 OCO<u>CH_3</u>), 23.11 (NHCO<u>CH_3</u>), 48.04 (C-5), 49.00 (C-4), 52.51 (COO<u>CH_3</u>), 62.15 (C-9), 67.92 (C-7), 71.33 (C-8), 77.47 (C-6), 101.26 (CH_2), 103.59 (Ph), 108.32 (Ph), 111.37, 114.17 (Ph), 132.21 (Ph q carbon), 144.22 (Ph q carbon), 144.73 (C-2), 148.00 (Ph q carbon), 156.53 (Urea-CO), 161.67 (<u>CO</u>OCH_3), 169.90, 170.40, 170.62, 171.88 (NH<u>CO</u>CH_3, 3 O<u>CO</u>CH_3); LRMS [$C_{26}H_{31}N_3O_{13}$] (*m/z*): (+ve ion mode) 615.7 $[M+Na]^+$; HRMS (API) (*m/z*): $[M+1]^+$ calcd for $C_{26}H_{32}N_3O_{13}$ $[M+1]^+$ 594.192964; found, 594.190122.

General Procedure for the synthesis of compounds 9a-g

To a suspension of compound **8a-g** (0.10 mmol) in a (1:1) mixture of MeOH and water (2 mL) at 0 ºC was added NaOH solution (1.0 M) dropwise until the pH reaches 13-14. The temperature was raised gradually to rt and the mixture was stirred at rt overnight. The compound was then purified by passing through C18-GracePure™ cartridge, using 2% acetonitrile/water, to yield the pure deprotected urea derivative **9a-g** as fluffy white powder after freeze drying.

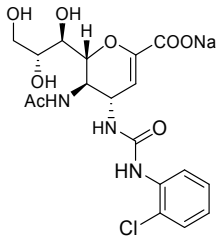
Sodium 5-acetamido-2,6-anhydro-3,4,5-trideoxy-4-(3-(4-methoxybenzyl)ureido)-D-*glycero*-D-*galacto*-non-2-enonate (9a).



9a

^{1}H NMR (300 MHz, D_2O): δ 1.92 (s, 3H, NAc), 3.59–3.71 (m, 2H, H-7, H-9), 3.85 (s, 3H, OCH_3), 3.87–4.00 (m, 2H, H-8, H-9'), 4.08 (dd, *J* = 10.7, 9.7 Hz, 1H, H-5), 4.16–4.35 (m, 3H, H-6, CH_2), 4.60 (dd, *J* = 9.7, 2.3 Hz, 1H, H-4), 5.59 (d, *J* = 2.3 Hz, 1H, H-3), 7.01 (d, *J* = 8.8 Hz, 2H, Ph-H-3', Ph-H-5'), 7.27 (d, *J* = 8.8 Hz, 2H, Ph-H-2', Ph-H-6'); ^{13}C NMR (75 MHz, D_2O): δ 24.52 (NHCO<u>CH_3</u>), 45.26 (CH_2), 50.92 (C-5), 51.36(C-4), 57.92 (Ph-O<u>CH_3</u>), 65.66 (C-9), 70.79 (C-7), 72.34 (C-8), 78.17 (C-6), 109.57 (C-3), 116.65 (Ph), 130.67 (Ph), 134.79 (Ph q carbon), 150.72 (C-2), 160.34 (Ph q carbon), 162.44 (Urea-CO), 172.18 (COONa), 176.76 (NH<u>CO</u>CH_3); LRMS [$C_{20}H_{26}N_3NaO_9$] (*m/z*): (+ve ion mode) 498.2 $[M+Na]^+$; HRMS (API) (*m/z*): $[M+Na]^+$ calcd for $C_{20}H_{26}N_3Na_2O_9$ $[M+Na]^+$ 498.1459; found, 498.1455. Purity by analytical HPLC (197 nm) = 100%, t_R= 2.95 min.

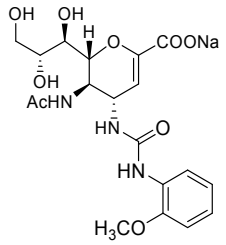
Sodium 5-acetamido-2,6-anhydro-4-(3-(2-chlorophenyl)ureido)-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enonate (9b).



9b

^{1}H NMR (300 MHz, D_2O): δ 2.06 (s, 3H, NAc), 3.63–3.74 (m, 2H, H-7, H-9), 3.89–4.03 (m, 2H, H-8, H-9'), 4.17 (dd, *J* = 10.7, 9.7 Hz, 1H, H-5), 4.37 (dd, *J* = 10.7, 1.2 Hz, 1H, H-6), 4.71 (dd, *J* = 9.7, 2.3 Hz, 1H, H-4), 5.68 (d, *J* = 2.3 Hz, 1H, H-3), 7.27 (ddd, *J* = 8.0, 7.5, 1.7 Hz, 1H, Ph-H-4'), 7.27 (ddd, *J* = 7.9, 7.6, 1.6 Hz, 1H, Ph-H-5'), 7.50-7.56 (m, 2H, Ph-H-3', Ph-H-6'); ^{13}C NMR (75 MHz, D_2O): δ 22.15 (NHCO<u>CH_3</u>), 48.25 (C-5), 48.97 (C-4), 63.15 (C-9), 68.24 (C-7), 69.87 (C-8), 75.66 (C-6), 106.68 (C-3), 126.80 (Ph), 126.98 (Ph), 127.70 (Ph), 128.69 (Ph q carbon), 129.71 (Ph), 134.13 (Ph q carbon), 148.40 (C-2), 158.08 (Urea-CO), 169.54 (COONa), 174.36 (NH<u>CO</u>CH_3); LRMS [$C_{18}H_{21}ClN_3NaO_8$] (*m/z*): (+ve ion mode) 488.1 $[M+Na]^+$; HRMS (API) (*m/z*): $[M+Na]^+$ calcd for $C_{18}H_{21}ClN_3Na_2O_8$ $[M+Na]^+$ 488.0807; found, 488.0812. Purity by analytical HPLC (244 nm) = 100%, t_R= 4.54 min.

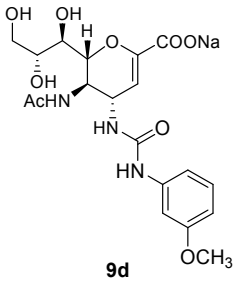
Sodium 5-acetamido-2,6-anhydro-3,4,5-trideoxy-4-(3-(2-methoxybenzyl)ureido)-D-*glycero*-D-*galacto*-non-2-enonate (9c).[3]



9c

^{1}H NMR (300 MHz, D_2O): δ 2.05 (s, 3H, NAc), 3.62–3.74 (m, 2H, H-7, H-9), 3.90 (s, 3H, OCH_3), 3.91–4.02 (m, 2H, H-8, H-9'), 4.16 (m, 1H, H-5), 4.37 (dd, *J* = 10.7, 1.2 Hz, 1H, H-6), 4.70 (dd, *J* = 9.8, 2.3 Hz, 1H, H-4), 5.67 (d, *J* = 2.3 Hz, 1H, H-3), 7.05 (ddd, *J* = 8.2, 7.6, 1.4 Hz, 1H, Ph-H-5'), 7.13 (dd, *J* = 8.3, 1.4 Hz, 1H, Ph-H-3'), 7.25 (ddd, *J* = 8.2, 7.4, 1.7 Hz, 1H, Ph-H-4'), 7.52 (dd, *J* = 7.8, 1.6 Hz, 1H, Ph-H-6'); ^{13}C NMR (75 MHz, D_2O): δ 22.08 (NHCO<u>CH_3</u>), 48.18 (C-5), 48.91 (C-4), 55.96 (Ph-O<u>CH_3</u>), 63.15 (C-9), 68.25 (C-7), 69.86 (C-8), 75.68 (C-6), 106.85 (C-3), 112.10 (Ph), 121.10 (Ph), 123.96 (Ph), 125.87 (Ph), 126.40 (Ph q carbon), 148.33 (C-2), 151.48 (Ph q carbon), 158.22 (Urea-CO), 169.59 (COONa), 174.42 (NH<u>CO</u>CH_3); LRMS [$C_{19}H_{24}N_3NaO_9$] (*m/z*): (+ve ion mode) 484.2 $[M+Na]^+$; HRMS (API) (*m/z*): $[M+Na]^+$ calcd for $C_{19}H_{24}N_3Na_2O_9$ $[M+Na]^+$ 484.1302; found, 484.1311. Purity by analytical HPLC (244 nm) = 100%, t_R= 3.82 min.

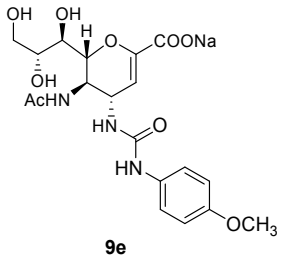
Sodium 5-acetamido-2,6-anhydro-3,4,5-trideoxy-4-(3-(3-methoxyphenyl)ureido)-D-*glycero*-D-*galacto*-non-2-enonate (9d).[3]



9d

^{1}H NMR (300 MHz, D_2O): δ 2.05 (s, 3H, NAc), 3.67-3.73 (m, 2H, H-7, H-9), 3.87 (s, 3H, Ph-OCH_3), 3.91–4.06 (m, 2H, H-9', H-8), 4.20 (m, 1H, H-5), 4.39 (d, *J* = 10.7 Hz, 1H, H-6), 4.68–4.76 (m, 1H, H-4), 5.67 (d, *J* = 2.8 Hz, 1H, H-3), 6.81 (dd, *J* = 8.4, 2.4 Hz, 1H, Ph-H-4'), 6.93 (dd, *J* = 8.1, 1.4 Hz, 1H, Ph-H-6'), 7.02 (d, t, *J* = 2.4 Hz, 1H, Ph-H-2'), 7.35 (t, *J* = 8.2 Hz, 1H, Ph-H-5'); ^{13}C NMR (75 MHz, D_2O): δ 22.02 (NHCO<u>CH_3</u>), 48.16 (C-5), 48.90 (C-4), 55.32 (OCH_3), 63.13 (C-9), 68.25 (C-7), 69.79 (C-8), 75.59 (C-6), 106.72 (Ph), 106.89 (C-3), 109.56 (Ph), 113.91 (Ph), 130.09 (Ph), 139.36 (Ph q carbon), 148.32 (C-2), 157.71 (Ph q carbon), 159.36 (Urea-CO), 169.61 (COONa), 174.37 (NH<u>CO</u>CH_3); LRMS [$C_{19}H_{24}N_3NaO_9$] (*m/z*): (+ve ion mode) 484.1 $[M+Na]^+$. Purity by analytical HPLC (209 nm) = 100%, t_R= 6.01 min.

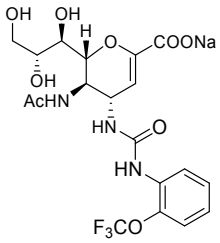
Sodium 5-acetamido-2,6-anhydro-3,4,5-trideoxy-4-(3-(4-methoxyphenyl)ureido)-D-*glycero*-D-*galacto*-non-2-enonate (9e).[3]



9e

^{1}H NMR (300 MHz, D_2O): δ 2.06 (s, 3H, NAc), 3.64–3.74 (m, 2H, H-7, H-9), 3.86 (s, 3H, OCH_3), 3.90–4.04 (m, 2H, H-8, H-9'), 4.17 (dd, *J* = 10.7, 9.8 Hz, 1H, H-5), 4.37 (dd, *J* = 10.7, 1.2 Hz, 1H, H-6), 4.69 (dd, *J* = 9.7, 2.3 Hz, 1H, H-4), 5.67 (d, *J* = 2.3 Hz, 1H, H-3), 7.02 (d, *J* = 9.0 Hz, 2H, Ph-H-3', Ph-H-5'), 7.24 (d, *J* = 9.1 Hz, 2H, Ph-H-2', Ph-H-6'); ^{13}C NMR (75 MHz, D_2O): δ 22.06 (NHCO<u>CH_3</u>), 48.15 (C-5), 48.95 (C-4), 55.57 (OCH_3), 63.11 (C-9), 68.21 (C-7), 69.84 (C-8), 75.61 (C-6), 106.93 (C-3), 114.50 (Ph), 124.84 (Ph), 130.63 (Ph q carbon), 148.52 (C-2), 156.04 (Ph q carbon), 158.52 (Urea-CO), 169.57 (COONa), 174.35 (NH<u>CO</u>CH_3); LRMS [$C_{19}H_{24}N_3NaO_9$] (*m/z*): (+ve ion mode) 484.1 $[M+Na]^+$. Purity by analytical HPLC (198 nm) = 97.7%, t_R= 4.21 min.

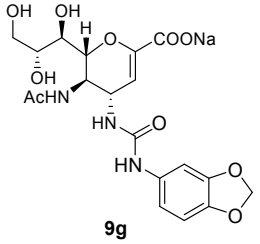
Sodium 5-acetamido-2,6-anhydro-3,4,5-trideoxy-4-(3-(2-(trifluoromethoxy)phenyl)ureido)-D-*glycero*-D-*galacto*-non-2-enonate (9f).



9f

^{1}H NMR (300 MHz, D_2O): δ 2.04 (s, 3H, NAc), 3.66-3.72 (m, 2H, H-7, H-9), 3.89–4.04 (m, 2H, H-8, H-9'), 4.17 (t, *J* = 10.2 Hz, 1H, H-5), 4.37 (d, *J* = 10.7 Hz, 1H, H-6), 4.71 (d, *J* = 2.4 Hz, 1H, H-4), 5.70 (d, *J* = 2.3 Hz, 1H, H-3), 7.31 (m, 1H, Ph-H-4'), 7.38–7.50 (m, 2H, Ph-H-3', Ph-H-5'), 7.59 (dd, *J* = 7.9, 1.7 Hz, 1H, Ph-H-6'); ^{13}C NMR (75 MHz, D_2O): δ 21.97 (NHCO<u>CH_3</u>), 48.09 (C-5), 48.83 (C-4), 63.14 (C-9), 68.22 (C-7), 69.86 (C-8), 75.75 (C-6), 107.06 (C-3), 122.13 (Ph), 126.23 (Ph), 127.86 (Ph), 130.37 (Ph q carbon), 141.74 (Ph q carbon), 147.96 (C-2), 157.88 (Urea-CO), 169.23 (COONa), 174.34 (NH<u>CO</u>CH_3); LRMS [$C_{19}H_{21}F_3N_3NaO_9$] (*m/z*): (+ve ion mode) 538.1 $[M+Na]^+$; HRMS (API) (*m/z*): $[M+Na]^+$ calcd for $C_{19}H_{21}F_3N_3Na_2O_9$ $[M+Na]^+$ 538.1020; found, 538.1008. Purity by analytical HPLC (202 nm) = 96.1%, t_R= 4.86 min.

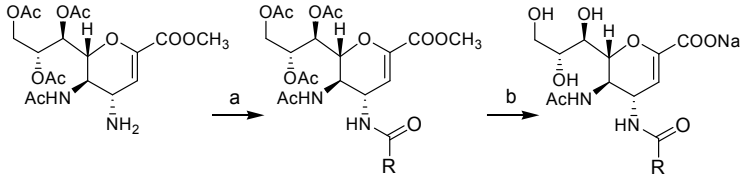
Sodium 5-acetamido-2,6-anhydro-4-(3-(benzo[d][1,3]dioxol-5-yl)ureido)-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enonate (9g).



9g

^{1}H NMR (300 MHz, D_2O): δ 2.03 (s, 3H, NAc), 3.60–3.72 (m, 2H, H-7, H-9), 3.87–4.01 (m, 2H, H-8, H-9'), 4.14 (dd, *J* = 10.7, 9.7 Hz, 1H, H-5), 4.34 (dd, *J* = 10.7, 1.2 Hz, 1H, H-6), 4.66 (dd, *J* = 9.7, 2.4 Hz, 1H, H-4), 5.63 (d, *J* = 2.3 Hz, 1H, H-3), 5.98 (m, 2H, CH_2), 6.71 (dd, *J* = 8.4, 2.1 Hz, 1H, Ph-H-5'), 6.82–6.92 (m, 2H, Ph-H-2', Ph-H-6'); ^{13}C NMR (75 MHz, D_2O): δ 22.04 (NHCO<u>CH_3</u>), 48.18 (C-5), 48.98 (C-4), 63.13 (C-9), 68.25 (C-7), 69.79 (C-8), 75.58 (C-6), 101.33 (CH_2), 105.26 (Ph), 106.77 (C-3), 108.33 (Ph), 116.53 (Ph), 131.50 (Ph q carbon), 144.27 (Ph q carbon), 147.33 (Ph q carbon), 148.27 (C-2), 158.45 (Urea-CO), 169.60 (COONa), 174.32 (NH<u>CO</u>CH_3); LRMS [$C_{19}H_{22}N_3NaO_{10}$] (*m/z*): (+ve ion mode) 498.0 $[M+Na]^+$. Purity by analytical HPLC (249 nm) = 100%, t_R= 2.82 min.

Synthesis of Compounds 11a-c



7

10a: R = Ph
10b: R = 4-Cl-Ph
10c: R = 4-OCH_3-Ph

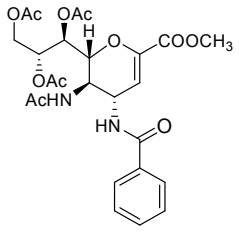
11a: R = Ph
11b: R = 4-Cl-Ph
11c: R = 4-OCH_3-Ph

[a]Reagents and conditions: (a) R-COCl, Et_3N, DCM, argon, rt, o/n, (**10a**, 80%; **10b**, 77%, **10c**, 89%) (b) NaOH, MeOH/H_2O (1:1), rt, o/n, (**11a**, 89%; **11b**, 85%, **11c**, 91%).

General Procedure for the synthesis of compounds 10a-c

To a solution of the amine **7** (60 mg, 0.14 mmol) and triethylamine (60 µL, 0.42 mmol) in DCM (3 mL) was added the corresponding acid chloride (0.28 mmol) while stirring. The reaction mixture was stirred at rt o/n, then the solvent was removed under vacuum and the reside was purified by silica gel chromatography using the proper solvent system to yield the pure urea derivative **10a-c**.

Methyl 5-acetamido-7,8,9-tri-*O*-acetyl-2,6-anhydro-4-benzamido-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enonate (10a).

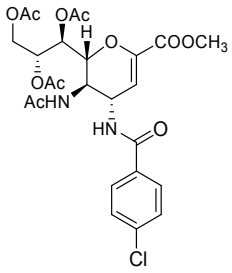


10a

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.72 (s, 3H, NAc), 2.06 (s, 3H, OAc), 2.07 (s, 3H, OAc), 2.09 (s, 3H, OAc), 3.78 (s, 3H, $COOCH_3$), 4.20 (dd, *J* = 12.4, 7.4 Hz, 1H, H-9), 4.30–4.49 (m, 2H, H-5, H-6), 4.74 (dd, *J* = 12.5, 2.5 Hz, 1H, H-

9'), 5.05 (t, *J* = 8.4 Hz, 1H, H-4), 5.35 (ddd, *J* = 7.2, 4.5, 2.5 Hz, 1H, H-8), 5.59 (d, *J* = 4.1 Hz, 1H, H-7), 6.02 (d, *J* = 1.9 Hz, 1H, H-3), 6.90 (d, *J* = 7.9 Hz, 1H, C4-NH), 7.02 (d, *J* = 9.1 Hz, 1H, NHAc), 7.40 (t, *J* = 7.4 Hz, 2H, Ph-2H), 7.47 (t, *J* = 7.3 Hz, 1H, Ph-H), 7.73 (d, *J* = 7.3 Hz, 2H, Ph-2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.62, 20.82, 20.94 (3 OCO<u>CH_3</u>), 22.86 (NHCO<u>CH_3</u>), 46.58 (C-5), 49.86 (C-4), 52.47 (COO<u>CH_3</u>), 62.26 (C-9), 68.07 (C-7), 71.59 (C-8), 77.15 (C-6), 110.70 (C-3), 127.06 (Ph), 128.82 (Ph), 132.10 (Ph), 133.39 (Ph q carbon), 144.46 (C-2), 161.82 (<u>CO</u>OCH_3), 168.60 (Ph-<u>CO</u>), 169.96, 170.48, 170.69, 171.83; (NH<u>CO</u>CH_3, 3 O<u>CO</u>CH_3); LRMS [$C_{25}H_{30}N_2O_{11}$] (*m/z*): (+ve ion mode) 557.1 $[M+Na]^+$.

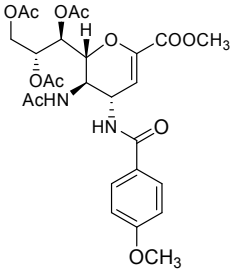
Methyl 5-acetamido-7,8,9-tri-*O*-acetyl-2,6-anhydro-4-(4-chlorobenzoyl)-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enonate (10b).



10b

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.72 (s, 3H, NAc), 2.05 (s, 3H, OAc), 2.06 (s, 3H, OAc), 2.08 (s, 3H, OAc), 3.76 (s, 3H, $COOCH_3$), 4.19 (dd, *J* = 12.4, 7.7 Hz, 1H, H-9), 4.35 (q, *J* = 9.9 Hz, 1H, H-5), 4.43 (dd, *J* = 10.4, 2.0 Hz, 1H, H-6), 4.76 (dd, *J* = 12.4, 2.5 Hz, 1H, H-9'), 5.00 (ddd, *J* = 10.4, 8.3, 2.4 Hz, 1H, H-4), 5.33 (ddd, *J* = 7.2, 4.3, 2.5 Hz, 1H, H-8), 5.59 (dd, *J* = 4.2, 1.9 Hz, 1H, H-7), 6.00 (d, *J* = 2.2 Hz, 1H, H-3), 7.13 (d, *J* = 8.3 Hz, 1H, C4-NH), 7.20 (d, *J* = 9.6 Hz, 1H, NHAc), 7.35 (d, *J* = 8.5 Hz, 2H, Ph-2H), 7.68 (d, *J* = 8.5 Hz, 2H, Ph-2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.56, 20.81, 20.92 (3 OCO<u>CH_3</u>), 22.88 (NHCO<u>CH_3</u>), 46.49 (C-5), 50.00 (C-4), 52.49 (COO<u>CH_3</u>), 62.30 (C-9), 68.12 (C-7), 71.79 (C-8), 77.08 (C-6), 110.57 (C-3), 128.58 (Ph), 129.03 (Ph), 131.73 (Ph q carbon), 138.39 (Ph q carbon), 144.40 (C-2), 161.77 (<u>CO</u>OCH_3), 167.53 (Ph-<u>CO</u>), 169.92, 170.59, 170.72, 171.90 (NH<u>CO</u>CH_3, 3 O<u>CO</u>CH_3); LRMS [$C_{25}H_{29}ClN_2O_{11}$] (*m/z*): (+ve ion mode) 591.3 $[M+Na]^+$.

Methyl 5-acetamido-7,8,9-tri-*O*-acetyl-2,6-anhydro-3,4,5-trideoxy-4-(4-methoxybenzoyl)-D-*glycero*-D-*galacto*-non-2-enonate (10c).



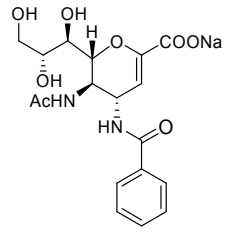
10c

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.66 (s, 3H, NAc), 2.05 (s, 6H, 2OAc), 2.08 (s, 3H, OAc), 3.76 (s, 3H, $COOCH_3$), 3.79 (s, 3H, Ph-O$\underline{CH_3}$), 4.20 (dd, *J* = 12.4, 7.5 Hz, 1H, H-9), 4.28–4.45 (m, 2H, H-5, H-6), 4.74 (dd, *J* = 12.5, 2.6 Hz, 1H, H-9'), 5.02 (ddd, *J* = 10.8, 8.4, 2.4 Hz, 1H, H-4), 5.35 (ddd, *J* = 7.3, 4.5, 2.5 Hz, 1H, H-8), 5.59 (dd, *J* = 4.5, 1.9 Hz, 1H, H-7), 6.00 (d, *J* = 2.3 Hz, 1H, H-3), 6.85–6.89 (m, 3H, C4-NH, Ph-2H), 7.29 (d, *J* = 9.5 Hz, 1H, N$\underline{H}$Ac), 7.69 (d, *J* = 8.8 Hz, 2H, Ph-2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.61, 20.81, 20.90 (3 OCO$\underline{CH_3}$), 22.80 (NHCO$\underline{CH_3}$), 46.53 (C-5), 49.75 (C-4), 52.40 (COO$\underline{CH_3}$), 55.38 (Ph-$\underline{OCH_3}$), 62.33 (C-9), 68.18 (C-7), 71.59 (C-8), 77.11 (C-6), 111.04 (C-3), 113.97 (Ph), 125.59 (Ph q carbon), 128.98 (Ph), 144.27 (C-2), 161.87 ($\underline{CO}OCH_3$), 162.61 (Ph q carbon), 168.05 (Ph-$\underline{CO}$), 169.91, 170.41, 170.67, 171.78 (NH$\underline{CO}CH_3$, 3 O$\underline{CO}CH_3$); LRMS [$C_{26}H_{32}N_2O_{12}$] (*m/z*): (+ve ion mode) 587.3 $[M+Na]^+$.

General Procedure for the synthesis of compounds 11a-c

To a suspension of compound **10a-c** (0.10 mmol) in a (1:1) mixture of MeOH and water (2 mL) at 0 ºC was added NaOH solution (1.0 M) dropwise until the pH reaches 13-14. The temperature was raised gradually to rt and the mixture was stirred at rt overnight. The compound was then purified by passing through C18-GracePure™ cartridge, using 2% acetonitrile/water, to yield the pure deprotected amide derivative **11a-c** as fluffy white powder after freeze drying.

Sodium 5-acetamido-2,6-anhydro-4-benzamido-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enonate (11a).

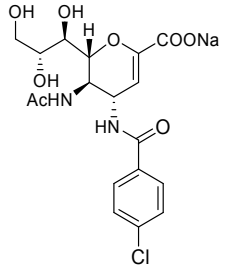


11a

1H NMR (300 MHz, D_2O): δ 1.96 (s, 3H, NAc), 3.65–3.72 (m, 2H, H-7, H-9), 3.86–4.07 (m, 2H, H-8, H-9'), 4.32 (t, *J* = 10.2 Hz, 1H, H-5), 4.44 (d, *J* = 10.7 Hz, 1H, H-6), 5.05 (dd, *J* = 9.6, 2.6 Hz, 1H, H-4), 5.67 (d, *J* = 2.3 Hz, 1H, H-3), 7.52–7.74 (m, 5H, Ph-H); ^{13}C NMR (75 MHz, D_2O): δ 21.91 (NHCO$\underline{CH_3}$), 47.93 (C-5), 48.93 (C-4), 63.13 (C-9), 68.20 (C-7), 69.82 (C-8), 75.46 (C-6), 105.50 (C-3), 127.08 (Ph), 128.76 (Ph), 132.18 (Ph), 133.57 (Ph q carbon), 148.82 (C-2), 169.49 (COONa), 171.38 (Ph-$\underline{CO}$), 174.20 (NH$\underline{CO}CH_3$); LRMS [$C_{18}H_{21}N_2NaO_8$] (*m/z*): (+ve ion mode)

439.1 $[M+Na]^+$; HRMS (API) (*m/z*): $[M+1]^+$ calcd for $C_{18}H_{23}N_2O_8$ $[M+1]^+$ 395.1449; found, 395.1461. Purity by analytical HPLC (237 nm) = 100%, t_R= 6.49 min.

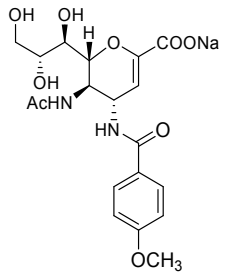
Sodium 5-acetamido-2,6-anhydro-4-(4-chlorobenzoyl)-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enonate (11b).



11b

^{1}H NMR (400 MHz, D_2O): δ 1.95 (s, 3H, NAc), 3.62–3.73 (m, 2H, H-7, H-9), 3.92 (dd, *J* = 11.9, 2.7 Hz, 1H, H-9'), 3.99 (ddd, *J* = 9.3, 6.4, 2.7 Hz, 1H, H-8), 4.30 (t, *J* = 10.2 Hz, 1H, H-5), 4.43 (dd, *J* = 10.9, 1.3 Hz, 1H, H-6), 5.03 (dd, *J* = 9.7, 2.3 Hz, 1H. H-4), 5.65 (d, *J* = 2.3 Hz, 1H, H-3), 7.53 (d, *J* = 8.6 Hz, 2H, Ph-2H), 7.68 (d, *J* = 8.6 Hz, 2H, Ph-2H); ^{13}C NMR (100 MHz, D_2O): δ 21.92 (NHCO<u>CH$_3$</u>), 47.91 (C-5), 49.01 (C-4), 63.14 (C-9), 68.19 (C-7), 69.80 (C-8), 75.44 (C-6), 105.38 (C-3), 128.68 (Ph), 128.83 (Ph), 132.10 (Ph q carbon), 137.64 (Ph q carbon), 148.86 (C-2), 169.47 (COONa), 170.29 (Ph-<u>CO</u>), 174.20 (NH<u>CO</u>CH$_3$); LRMS [$C_{18}H_{20}ClN_2NaO_8$] (*m/z*): (+ve ion mode) 473.0 $[M+Na]^+$; HRMS (API) (*m/z*): $[M+1]^+$ calcd for $C_{18}H_{22}ClN_2O_8$ $[M+1]^+$ 429.1059; found, 429.1074. Purity by analytical HPLC (243 nm) = 98.6%, t_R= 6.57 min.

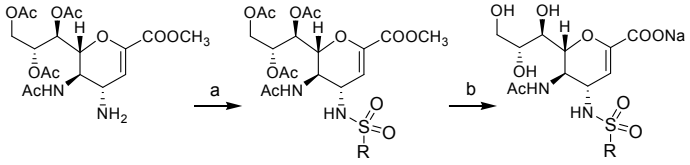
Sodium 5-acetamido-2,6-anhydro-3,4,5-trideoxy-4-(4-methoxybenzoyl)-D-*glycero*-D-*galacto*-non-2-enonate (11c).



11c

^{1}H NMR (400 MHz, D_2O): δ 1.94 (s, 3H, NAc), 3.62–3.72 (m, 2H, H-7, H-9), 3.89 (s, 3H, OCH_3), 3.92 (dd, *J* = 12.0, 2.7 Hz, 1H, H-9'), 3.99 (ddd, *J* = 9.3, 6.3, 2.6 Hz, 1H, H-8), 4.30 (t, *J* = 10.2 Hz, 1H, H-5), 4.42 (d, *J* = 10.7 Hz, 1H, H-6), 5.02 (dd, *J* = 9.7, 2.3 Hz, 1H, H-4), 5.65 (d, *J* = 2.2 Hz, 1H, H-3), 7.07 (d, *J* = 8.8 Hz, 2H, Ph-2H), 7.71 (d, *J* = 8.8 Hz, 2H, Ph-2H); ^{13}C NMR (100 MHz, D_2O): δ 21.91 (NHCO<u>CH$_3$</u>), 47.92 (C-5), 48.88 (C-4), 55.47 (OCH_3), 63.14 (C-9), 68.22 (C-7), 69.81 (C-8), 75.48 (C-6), 105.68 (C-3), 114.02 (Ph), 125.94 (Ph q carbon), 129.18 (Ph), 148.75 (C-2), 162.03 (Ph q carbon), 169.51 (COONa), 170.60 (Ph-<u>CO</u>), 174.19 (NH<u>CO</u>CH$_3$); LRMS [$C_{19}H_{23}N_2NaO_9$] (*m/z*): (+ve ion mode) 469.1 $[M+Na]^+$; HRMS (API) (*m/z*): $[M+1]^+$ calcd for $C_{19}H_{25}N_2O_9$ $[M+1]^+$ 425.1555; found, 425.1570. Purity by analytical HPLC (240 nm) = 100%, t_R= 2.23 min.

Synthesis of Compounds 13a-d



7

12a: R = Ph
12b: R = 4-Cl-Ph
12c: R = 4-OCH_3-Ph
12d: R = 4-CH_3-Ph

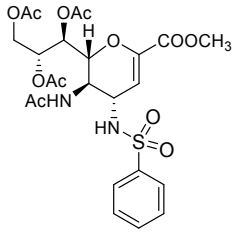
13a: R = Ph
13b: R = 4-Cl-Ph
13c: R = 4-OCH_3-Ph
13d: R = 4-CH_3-Ph

[a]Reagents and conditions: (a) R-SO_2Cl, Et_3N, DCM, argon, rt, o/n, (**12a**, 83%; **12b**, 89%, **12c**, 82%; **12d**, 74%) (b) NaOH, MeOH/H_2O (1:1), rt, o/n, (**13a**, 85%; **13b**, 92%, **13c**, 89%; **13d**, 88%).

General Procedure for the synthesis of compounds 12a-d

To a solution of the amine **7** (60 mg, 0.14 mmol) and triethylamine (60 µL, 0.42 mmol) in DCM (3 mL) was added the corresponding arylsulfonyl chloride (0.28 mmol) while stirring. The reaction mixture was stirred at rt o/n, then the solvent was removed under vacuum and the reside was purified by silica gel chromatography using the proper solvent system to yield the pure urea derivative **12a-d**.

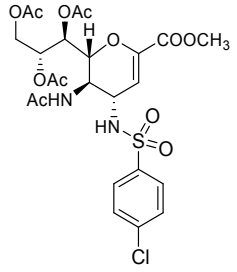
Methyl 5-acetamido-7,8,9-tri-*O*-acetyl-2,6-anhydro-3,4,5-trideoxy-4-phenylsulfonamido-D-*glycero*-D-*galacto*-non-2-enonate (12a).



12a

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.75 (s, 3H, NAc), 2.01 (s, 9H, 3OAc), 3.71 (s, 3H, $COOCH_3$), 4.03–4.19 (m, 3H, H-4, H-5, H-9), 4.34 (dd, *J* = 10.3, 2.2 Hz, 1H, H-6), 4.66 (dd, *J* = 12.3, 2.7 Hz, 1H, H-9'), 5.23 (m, 1H, H-8), 5.42 (dd, *J* = 4.4, 2.1 Hz, 1H, H-7), 5.71 (d, *J* = 2.3 Hz, 1H, H-3), 6.30–6.36 (m, 2H, NHAc, C4-NH), 7.42–7.61 (m, 3H, Ph-3H), 7.82 (d, *J* = 7.3 Hz, 2H, Ph-2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.60, 20.78, 20.88 (3 OCO$\underline{CH_3}$), 23.02 (NHCO$\underline{CH_3}$), 47.01 (C-5), 52.43 (COO$\underline{CH_3}$), 52.73 (C-4), 62.14 (C-9), 68.04 (C-7), 71.49 (C-8), 77.00 (C-6), 110.97 (C-3), 126.79 (Ph), 129.30 (Ph), 132.75 (Ph), 140.97 (Ph q carbon), 144.20 (C-2), 161.61 ($\underline{CO}OCH_3$), 169.99, 170.58, 170.68, 172.06 (NH$\underline{CO}CH_3$, 3 O$\underline{CO}CH_3$); LRMS [$C_{24}H_{30}N_2O_{12}S$] (*m/z*): (+ve ion mode) 593.2 $[M+Na]^+$.

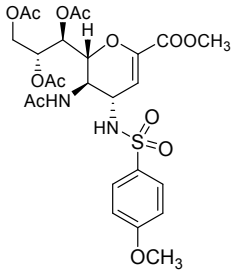
Methyl 5-acetamido-7,8,9-tri-*O*-acetyl-2,6-anhydro-4-(4-chlorophenylsulfonamido)-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enonate (12b).



12b

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.79 (s, 3H, NAc), 2.02 (s, 9H, 3OAc), 3.74 (s, 3H, $COOCH_3$), 4.02–4.20 (m, 3H, H-4, H-5, H-9), 4.33 (d, *J* = 8.6 Hz, 1H, H-6), 4.68 (dd, *J* = 12.5, 2.7 Hz, 1H, H-9'), 5.24 (m, 1H, H-8), 5.43 (dd, *J* = 4.3, 2.1 Hz, 1H, H-7), 5.71 (d, *J* = 1.9 Hz, 1H, H-3), 6.34 (d, *J* = 8.2 Hz, 1H, C4-NH), 6.45 (d, *J* = 5.9 Hz, 1H, NHAc), 7.46 (d, *J* = 8.3 Hz, 2H, Ph-2H), 7.77 (d, *J* = 8.3 Hz, 2H, Ph-2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.58, 20.79, 20.88 (3 $OCOCH_3$), 23.02 ($NHCOCH_3$), 47.01 (C-5), 52.52 ($COOCH_3$), 52.78 (C-4), 62.11 (C-9), 68.06 (C-7), 71.52 (C-8), 77.01 (C-6), 110.71 (C-3), 128.35 (Ph), 129.53 (Ph), 139.21 (Ph q carbon), 139.51 (Ph q carbon), 144.30 (C-2), 161.54 ($COOCH_3$), 169.94, 170.60, 170.72, 172.08 ($NHCOCH_3$, 3 $OCOCH_3$); LRMS [$C_{24}H_{29}ClN_2O_{12}S$] (*m/z*): (+ve ion mode) 627.2 $[M+Na]^+$.

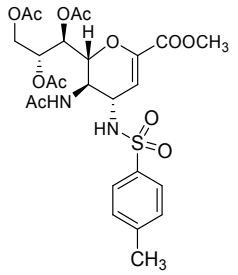
Methyl 5-acetamido-7,8,9-tri-*O*-acetyl-2,6-anhydro-3,4,5-trideoxy-4-(4-methoxyphenylsulfonamido)-D-*glycero*-D-*galacto*-non-2-enonate (12c).



12c

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.79 (s, 3H, NAc), 2.02 (s, 9H, 3OAc), 3.72 (s, 3H, $COOCH_3$), 3.84 (s, 3H, Ph-OCH_3), 4.04 (q, *J* = 9.8 Hz, 1H, H-5), 4.13 (m, 2H, H-4, H-9), 4.35 (dd, *J* = 10.3, 2.1 Hz, 1H, H-6), 4.65 (dd, *J* = 12.5, 2.6 Hz, 1H, H-9'), 5.24 (ddd, *J* = 7.4, 4.7, 2.6 Hz, 1H, H-8), 5.43 (dd, *J* = 4.7, 2.1 Hz, 1H, H-7), 5.72 (d, *J* = 2.3 Hz, 1H, H-3), 6.01 (d, *J* = 8.3 Hz, 1H, C4-NH), 6.24 (d, *J* = 9.3 Hz, 1H, NHAc), 6.95 (d, *J* = 8.9 Hz, 2H, Ph-2H), 7.75 (d, *J* = 8.8 Hz, 2H, Ph-2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.64, 20.80, 20.90 (3 $OCOCH_3$), 23.10 ($NHCOCH_3$), 47.19 (C-5), 52.46 (C-4, $COOCH_3$), 55.68 (Ph-OCH_3), 62.12 (C-9), 68.00 (C-7), 71.38 (C-8), 76.93 (C-6), 111.02 (C-3), 114.43 (Ph), 129.05 (Ph), 132.28 (Ph q carbon), 144.19 (C-2), 161.65 ($COOCH_3$), 162.98 (Ph q carbon), 170.01, 170.53, 170.69, 171.98 ($NHCOCH_3$, 3 $OCOCH_3$); LRMS [$C_{25}H_{32}N_2O_{13}S$] (*m/z*): (+ve ion mode) 623.3 $[M+Na]^+$.

Methyl 5-acetamido-7,8,9-tri-*O*-acetyl-2,6-anhydro-3,4,5-trideoxy-4-(4-methylphenylsulfonamido)-D-*glycero*-D-*galacto*-non-2-enonate (12d).



12d

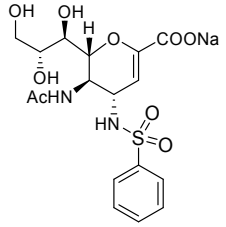
^{1}H NMR (300 MHz, $CDCl_3$): δ 1.78 (s, 3H, NAc), 2.03 (s, 9H, 3OAc), 2.40 (s, 3H, Ph-CH_3), 3.73 (s, 3H, $COOCH_3$), 3.95–4.21 (m, 3H, H-4, H-5, H-9), 4.35 (dd, *J* = 10.1, 2.1 Hz, 1H, H-6), 4.64 (dd, *J* = 12.5, 2.7 Hz, 1H, H-9'), 5.25 (td, *J* = 6.0, 4.7, 2.7 Hz, 1H, H-8), 5.43 (dd, *J* = 4.7, 2.1 Hz, 1H, H-7), 5.73 (d, *J* = 2.3 Hz, 1H, H-3), 5.88 (d, *J* = 8.2 Hz, 1H, C4-NH), 6.00 (d, *J* = 9.2 Hz, 1H, NHAc), 7.29 (d, *J* = 8.1 Hz, 2H, PH-2H), 7.71 (d, *J* = 8.1 Hz, 2H, Ph-2H); ^{13}C

NMR (75 MHz, $CDCl_3$): δ 20.61, 20.77, 20.87 (3 OCO<u>CH_3</u>), 21.51 (Ph-CH_3), 23.04 (NHCO<u>CH_3</u>), 47.33 (C-5), 52.43 (COO<u>CH_3</u>), 52.55 (C-4), 62.09 (C-9), 67.96 (C-7), 71.27 (C-8), 76.90 (C-6), 110.86 (C-3), 126.90 (Ph), 129.87 (Ph), 137.82 (Ph q carbon), 143.67 (Ph q carbon), 144.27 (C-2), 161.61 (<u>CO</u>OCH_3), 169.97, 170.42, 170.62, 171.85 (NH<u>CO</u>CH_3, 3 O<u>CO</u>CH_3); LRMS [$C_{25}H_{32}N_2O_{12}S$] (*m/z*): (+ve ion mode) 607.2 $[M+Na]^+$.

General Procedure for the synthesis of compounds 13a-d

To a suspension of compound **12a-d** (0.10 mmol) in a (1:1) mixture of MeOH and water (2 mL) at 0 ºC was added NaOH solution (1.0 M) dropwise until the pH reaches 13-14. The temperature was raised gradually to rt and the mixture was stirred at rt overnight. The compound was then purified by passing through C18-GracePure™ cartridge, using 2% acetonitrile/water, to yield the pure deprotected sulfonamide derivative **13a-d** as fluffy white powder after freeze drying.

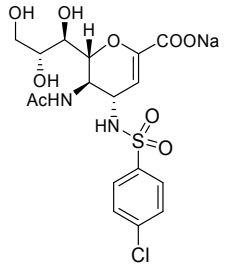
Sodium 5-acetamido-2,6-anhydro-3,4,5-trideoxy-4-phenylsulfonamido-D-*glycero*-D-*galacto*-non-2-enonate (13a).



13a

^{1}H NMR (400 MHz, D_2O): δ 1.76 (s, 3H, NAc), 3.54 (dd, *J* = 9.2, 1.0 Hz, 1H, H-7), 3.62 (dd, *J* = 11.8, 6.1 Hz, 1H, H-9), 3.82–3.96 (m, 2H, H-8, H-9'), 4.02 (t, *J* = 10.2 Hz, 1H, H-5), 4.14–4.29 (m, 2H, H-4, H-6), 5.37 (d, *J* = 2.2 Hz, 1H, H-3), 7.63–7.71 (m, 2H, Ph-2H), 7.75 (m, 1H, Ph-H), 7.88–7.96 (m, 2H, Ph-2H); ^{13}C NMR (100 MHz, D_2O): δ 22.03 (NHCO<u>CH_3</u>), 47.78 (C-5), 52.02 (C-4), 63.04 (C-9), 68.14 (C-7), 69.66 (C-8), 75.64 (C-6), 105.71 (C-3), 126.43 (Ph), 129.67 (Ph), 133.57 (Ph), 139.64 (Ph q carbon), 148.62 (C-2), 169.21 (COONa), 174.28 (NH<u>CO</u>CH_3); LRMS [$C_{17}H_{21}N_2NaO_9S$] (*m/z*): (+ve ion mode) 475.2 $[M+Na]^+$; HRMS (API) (*m/z*): $[M+1]^+$ calcd for $C_{17}H_{23}N_2O_9S$ $[M+1]^+$ 431.1119; found, 431.1124. Purity by analytical HPLC (197 nm) = 98.8%, t_R= 5.68 min.

Sodium 5-acetamido-2,6-anhydro-4-(4-chlorophenylsulfonamido)-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enonate (13b).

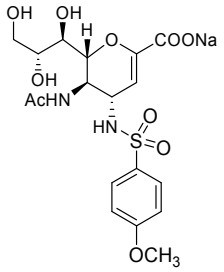


13b

^{1}H NMR (400 MHz, D_2O): δ 1.79 (s, 3H, NAc), 3.54 (d, *J* = 9.6 Hz, 1H, H-7), 3.62 (dd, *J* = 11.8, 6.2 Hz, 1H, H-9), 3.81–3.95 (m, 2H, H-8, H-9'), 4.02 (t, *J* = 10.2 Hz, 1H, H-5), 4.22 (dd, *J* = 10.2, 7.6 Hz, 2H, H-4, H-6), 5.39 (d, *J* = 1.7 Hz, 1H, H-3), 7.67 (d, *J* = 8.2 Hz, 2H, Ph-2H), 7.87 (d, *J* = 8.5 Hz, 2H, Ph-2H); ^{13}C NMR (100 MHz, D_2O): δ

21.97 (NHCO<u>CH$_3$</u>), 47.83 (C-5), 52.06 (C-4), 63.03 (C-9), 68.14 (C-7), 69.66 (C-8), 75.64 (C-6), 105.67 (C-3), 128.09 (Ph), 129.78 (Ph), 138.49 (Ph q carbon), 139.22 (Ph q carbon), 148.66 (C-2), 169.20 (COONa), 174.16 (NH<u>CO</u>CH$_3$); LRMS [$C_{17}H_{20}ClN_2NaO_9S$] (*m/z*): (+ve ion mode) 509.0 [M+Na]$^+$; HRMS (API) (*m/z*): [M+Na]$^+$ calcd for $C_{17}H_{22}ClN_2O_9S$ [M+1]$^+$ 465.0729; found, 465.0743. Purity by analytical HPLC (197 nm) = 98.3%, t_R= 5.58 min.

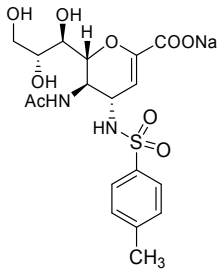
Sodium 5-acetamido-2,6-anhydro-3,4,5-trideoxy-4-(4-methoxyphenylsulfonamido)-D-*glycero*-D-*galacto*-non-2-enonate (13c).



13c

^{1}H NMR (400 MHz, D_2O): δ 1.80 (s, 3H, NAc), 3.55 (d, *J* = 9.4 Hz, 1H, H-7), 3.62 (dd, *J* = 11.8, 6.1 Hz, 1H, H-9), 3.83–3.96 (m, 5H, H-8, H-9', OCH_3), 4.01 (t, *J* = 10.2 Hz, 1H, H-5), 4.15 (dd, *J* = 9.6, 2.3 Hz, 1H, H-4), 4.22 (d, *J* = 10.7 Hz, 1H, H-6), 5.38 (d, *J* = 2.2 Hz, 1H, H-3), 7.17 (d, *J* = 8.8 Hz, 2H, Ph-2H), 7.84 (d, *J* = 8.9 Hz, 2H, Ph-2H); ^{13}C NMR (100 MHz, D_2O): δ 22.07 (NHCO<u>CH$_3$</u>), 47.80 (C-5), 51.88 (C-4), 55.75 (OCH_3), 63.04 (C-9), 68.14 (C-7), 69.70 (C-8), 75.64 (C-6), 105.81 (C-3), 114.85 (Ph), 128.87 (Ph), 131.33 (Ph q carbon), 148.60 (C-2), 162.87 (Ph q carbon), 169.17 (COONa), 174.22 (NH<u>CO</u>CH$_3$); LRMS [$C_{18}H_{23}N_2NaO_{10}S$] (*m/z*): (+ve ion mode) 504.2 [M+Na]$^+$; HRMS (API) (*m/z*): [M+1]$^+$ calcd for $C_{18}H_{25}N_2O_{10}S$ [M+1]$^+$ 461.1224; found, 461.1235. Purity by analytical HPLC (197 nm) = 98%, t_R= 6.25 min.

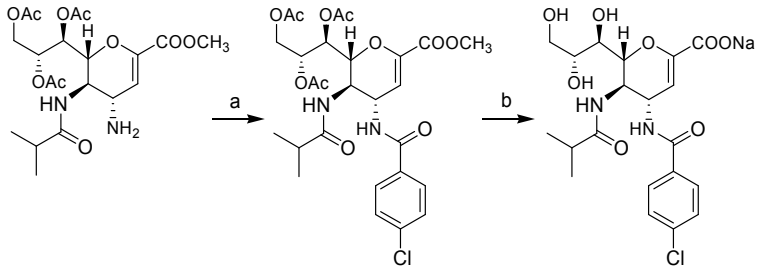
Sodium 5-acetamido-2,6-anhydro-3,4,5-trideoxy-4-(4-methylphenylsulfonamido)-D-*glycero*-D-*galacto*-non-2-enonate (13d).



13d

^{1}H NMR (400 MHz, D_2O): δ 1.75 (s, 3H, NAc), 2.45 (s, 3H, Ph-CH_3), 3.53 (d, *J* = 9.4 Hz, 1H, H-7), 3.62 (dd, *J* = 11.8, 6.1 Hz, 1H, H-9), 3.82–3.95 (m, 2H, H-8, H-9'), 4.01 (t, *J* = 10.2 Hz, 1H, H-5), 4.11–4.28 (m, 2H, H-4, H-6), 5.38 (d, *J* = 2.4 Hz, 1H, H-3), 7.48 (d, *J* = 8.1 Hz, 2H, Ph-2H), 7.78 (d, *J* = 8.0 Hz, 2H, Ph-2H); ^{13}C NMR (100 MHz, D_2O): δ 20.61 (Ph-<u>CH$_3$</u>), 21.97 (NHCO<u>CH$_3$</u>), 47.82 (C-5), 51.95 (C-4), 63.03 (C-9), 68.14 (C-7), 69.66 (C-8), 75.63 (C-6), 105.88 (C-3), 126.49 (Ph), 130.15 (Ph), 136.62 (Ph q carbon), 144.91 (Ph q carbon), 148.55 (C-2), 169.25 (COONa), 174.23 (NH<u>CO</u>CH$_3$); LRMS [$C_{18}H_{23}N_2NaO_9S$] (*m/z*): (+ve ion mode) 489.0 [M+Na]$^+$; HRMS (API) (*m/z*): [M+Na]$^+$ calcd for $C_{18}H_{25}N_2O_9S$ [M+1]$^+$ 445.1275; found, 445.1285. Purity by analytical HPLC (200 nm) = 98.6%, t_R= 4.16 min.

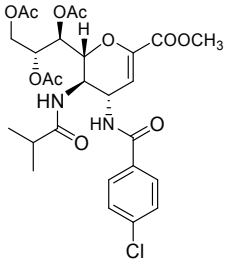
Synthesis of Compound 16



14 **15** **16**

[a]Reagents and conditions: (a) 4-Cl-Ph-COCl, Et_3N, DCM, argon, rt, o/n, 85%; (b) NaOH, MeOH/H_2O (1:1), rt, o/n, 94%.

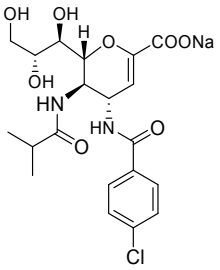
Methyl 7,8,9-tri-*O*-acetyl-2,6-anhydro-4-(4-chlorobenzamido)-3,4,5-trideoxy-5-isobutyramido-D-*glycero*-D-*galacto*-non-2-enonate (15).



15

To a solution of the amine **14** (60 mg, 0.13 mmol) and triethylamine (56 µL, 0.39 mmol) in DCM (3 mL) was added 4-chlorobenzoyl chloride (33 µL, 0.26 mmol) while stirring. The reaction mixture was stirred at rt o/n, then the solvent was removed under vacuum and the reside was purified by silica gel chromatography using ethylacetate/hexane (3:2) to yield 66 mg of pure **15** (85%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.83 (d, *J* = 6.6 Hz, 3H, isobut-CH_3), 0.96 (d, *J* = 6.5 Hz, 3H, isobut-CH_3), 2.05 (s, 3H, OAc), 2.07 (s, 3H, OAc), 2.09 (s, 3H, OAc), 2.51 (m, 1H, isobut-CH), 3.78 (s, 3H, $COOCH_3$), 4.20 (dd, *J* = 12.4, 7.6 Hz, 1H, H-9), 4.42 (t, *J* = 9.4 Hz, 1H, H-5), 4.50 (d, *J* = 10.5 Hz, 1H, H-6), 4.78 (dd, *J* = 12.4, 2.4 Hz, 1H, H-9'), 5.09 (t, *J* = 9.2 Hz, 1H, H-4), 5.31 (ddd, *J* = 7.6, 4.5, 2.7 Hz, 1H, H-8), 5.58 (d, *J* = 4.1 Hz, 1H, H-7), 5.99 (s, 1H, H-3), 6.75 (d, *J* = 10.0 Hz, 1H, 5-NH), 6.81 (d, *J* = 7.2 Hz, 1H, 4-NH), 7.38 (d, *J* = 8.1 Hz, 2H, Ph-2H), 7.67 (d, *J* = 8.2 Hz, 2H, Ph-2H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 18.80 (isobut-CH_3), 19.36 (isobut-CH_3), 20.57, 20.82, 20.97 (3 OCO$\underline{CH_3}$), 35.55 (isobut-CH), 46.16 (C-5), 49.95 (C-4), 52.51 (COO$\underline{CH_3}$), 62.27 (C-9), 67.99 (C-7), 71.92 (C-8), 77.20 (C-6), 110.43 (C-3), 128.61 (Ph), 129.03 (Ph), 131.74 (Ph q carbon), 138.38 (Ph q carbon), 144.56 (C-2), 161.79 ($\underline{CO}OCH_3$), 167.47 (Ph-$\underline{CO}$), 169.79, 170.64, 170.67 (3 O$\underline{CO}CH_3$), 178.79 (isobut-$\underline{CO}$); LRMS [$C_{27}H_{33}ClN_2O_{11}$] (*m/z*): (+ve ion mode) 619.3 $[M+Na]^+$.

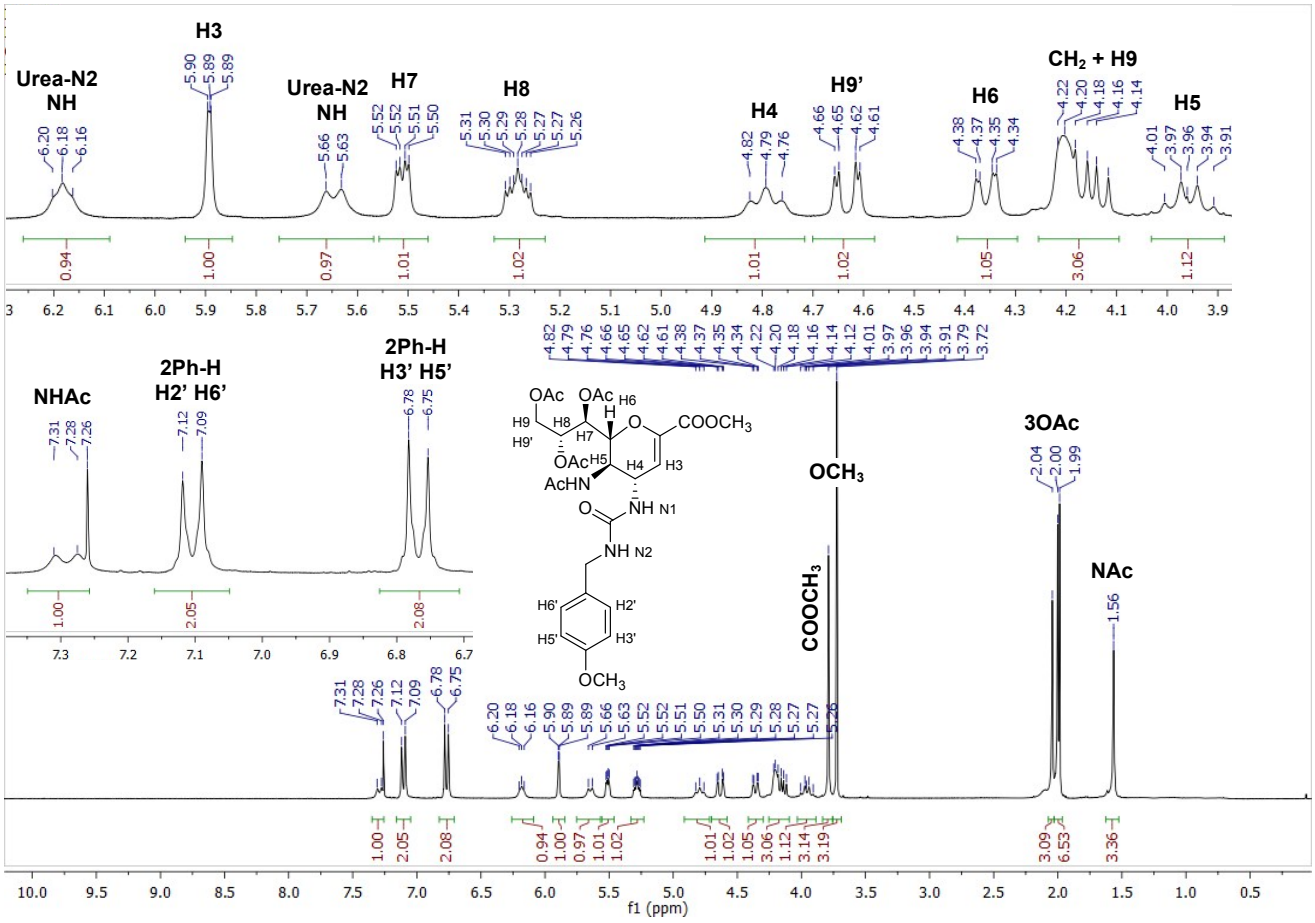
Sodium 2,6-anhydro-4-(4-chlorobenzamido)-3,4,5-trideoxy-5-isobutyramido-D-*glycero*-D-*galacto*-non-2-enonate (16).



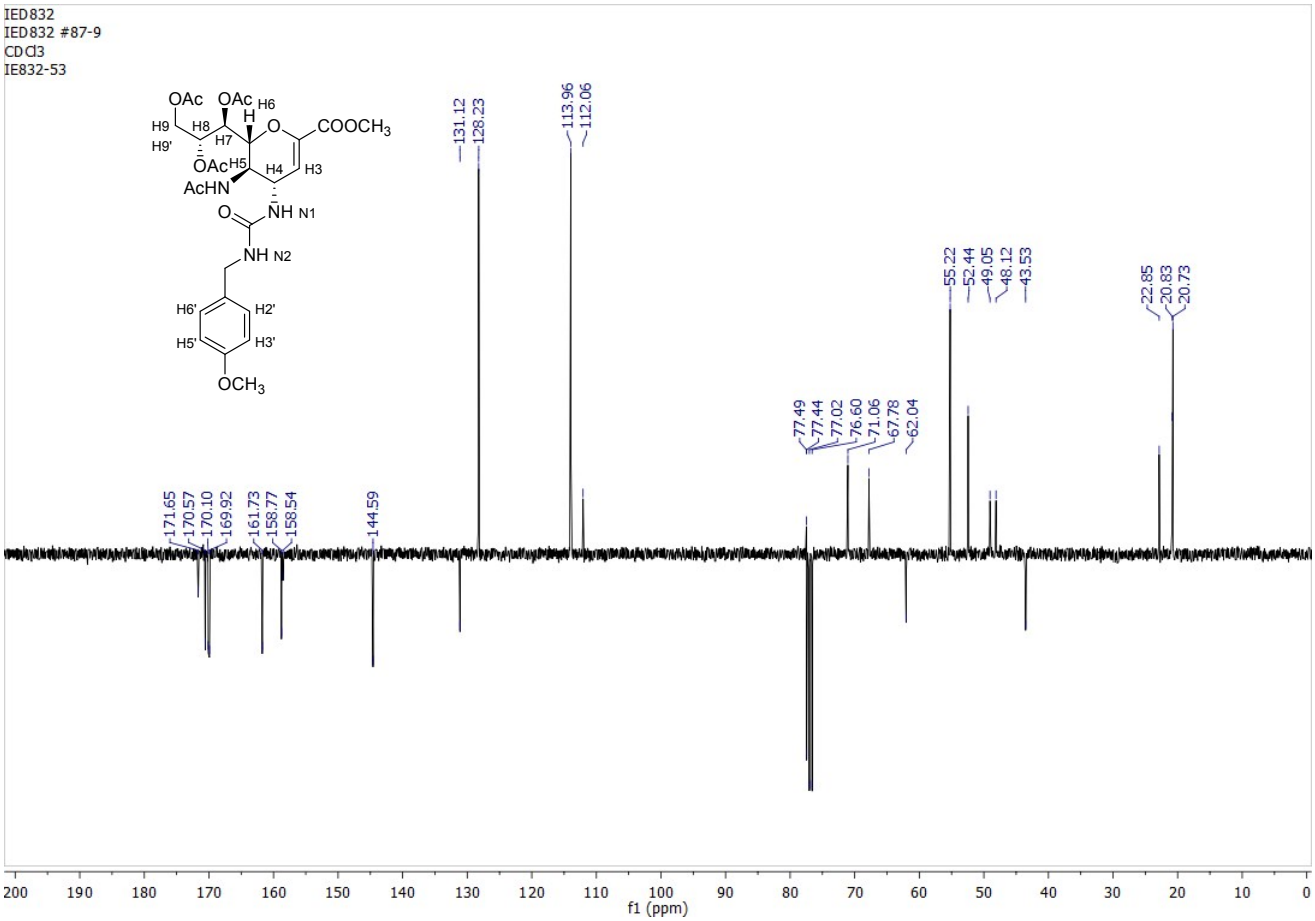
16

To a suspension of compound **15** (50 mg, 0.084 mmol) in a (1:1) mixture of MeOH and water (2 mL) at 0 ºC was added NaOH solution (1.0 M) dropwise until the pH reaches 13-14. The temperature was raised gradually to rt and the mixture was stirred at rt overnight. The solution was then acidified with Amberlite® IR-120 (H^+) resin (to pH = 5), filtered and washed with MeOH (10 mL) and H_2O (10 mL). The compound was then purified by passing through C18-GracePure™ cartridge, using 2% acetonitrile/water, to yield the pure deprotected amide **16** as fluffy white powder after freeze drying (38 mg, 94%). ^{1}H NMR (400 MHz, D_2O): δ 0.92 (d, *J* = 6.9 Hz, 3H, isobut-CH_3), 1.03 (d, *J* = 6.9 Hz, 3H, isobut-CH_3), 2.46 (m, 1H, isobut-CH), 3.59–3.72 (m, 2H, H-7, H-9), 3.91 (dd, *J* = 11.9, 2.7 Hz, 1H, H-9'), 3.99 (ddd, *J* = 9.3, 6.3, 2.6 Hz, 1H, H-8), 4.33 (t, *J* = 10.3 Hz, 1H, H-5), 4.45 (d, *J* = 10.8 Hz, 1H, H-6), 5.04 (dd, *J* = 9.8, 2.3 Hz, 1H, H-4), 5.64 (d, *J* = 2.2 Hz, 1H, H-3), 7.52 (d, *J* = 8.6 Hz, 2H, Ph-2H), 7.66 (d, *J* = 8.6 Hz, 2H, Ph-2H); ^{13}C NMR (101 MHz, D_2O): δ 18.35 (isobut-CH_3), 18.67 (isobut-CH_3), 35.22 (isobut-CH), 47.37 (C-5), 48.91 (C-4), 63.12 (C-9), 68.26 (C-7), 69.84 (C-8), 75.46 (C-6), 105.46 (C-3), 128.76 (Ph), 128.83 (Ph), 132.02 (Ph q carbon), 137.69 (Ph q carbon), 148.80 (C-2), 169.49 (COONa), 170.07 (Ph-CO), 181.23 (isobut-CO); LRMS [$C_{20}H_{24}ClN_2NaO_8$] (*m/z*): (+ve ion mode) 501.0 $[M+Na]^+$; HRMS (API) (*m/z*): $[M+1]^+$ calcd for $C_{20}H_{24}ClN_2NaO_8$ $[M+1]^+$ 479.1192; found, 479.1194.

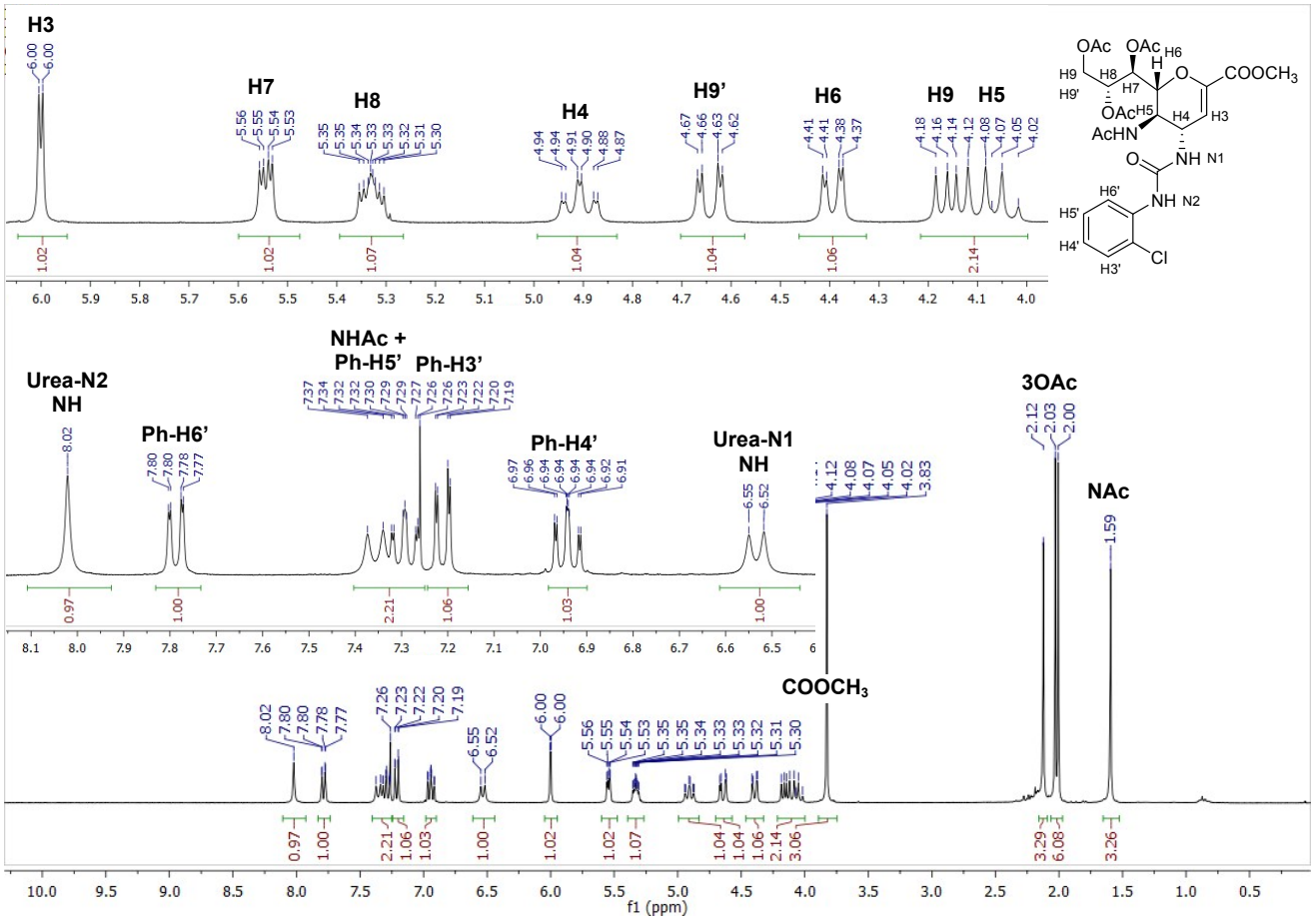
^{1}H and ^{13}C NMR spectra of new compounds



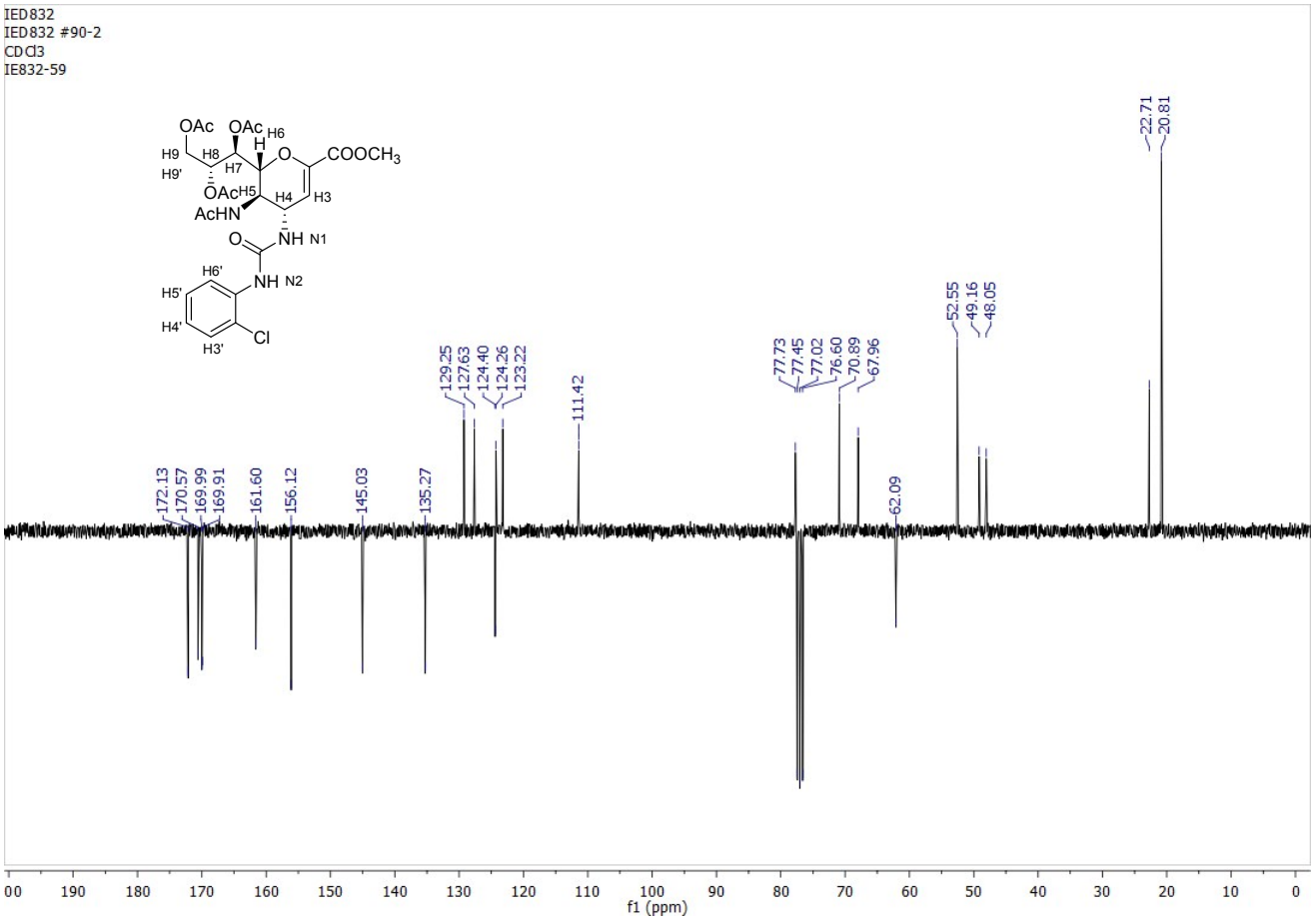
^{1}H NMR spectrum of **8a** (300 MHz, $CDCl_3$)



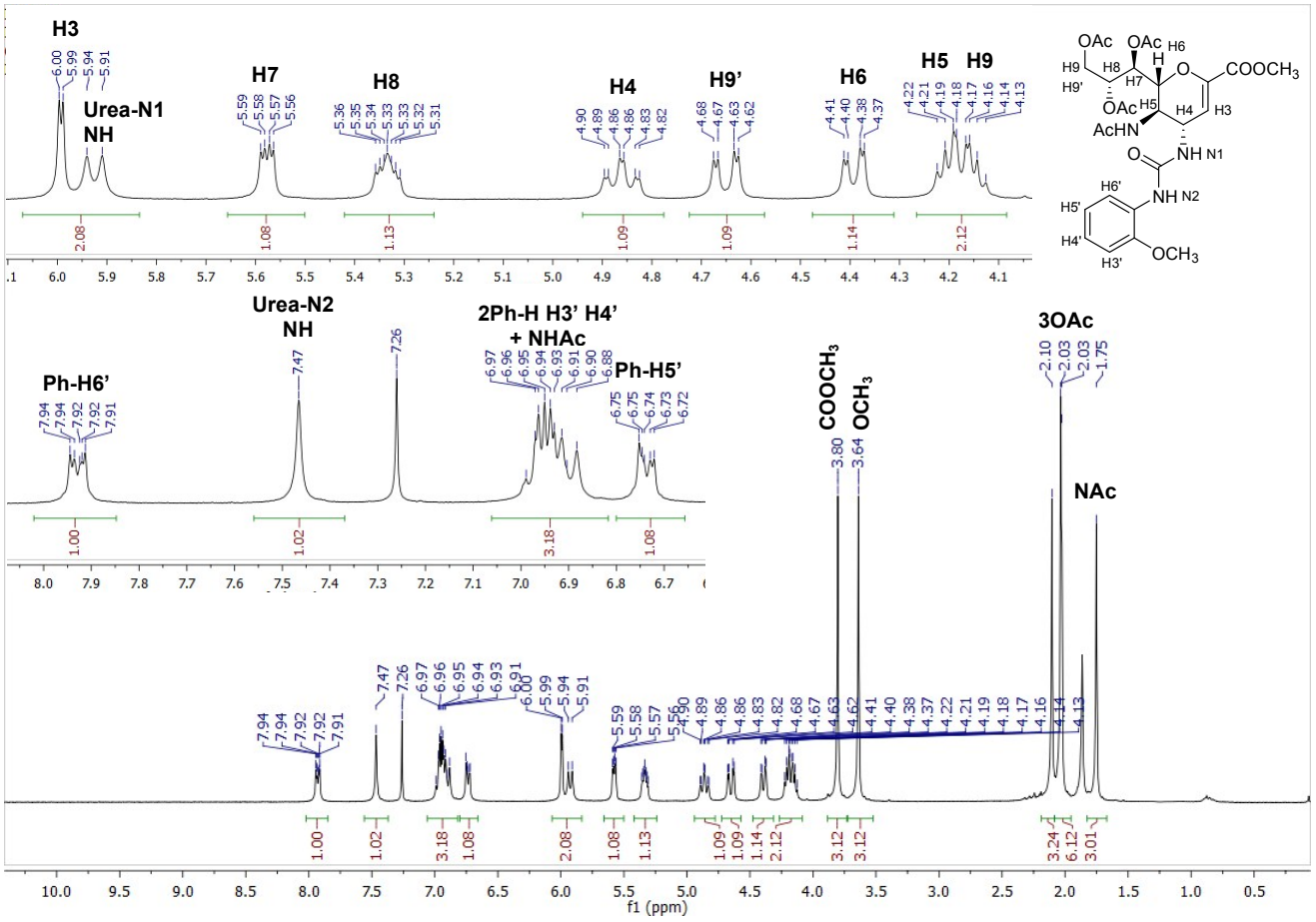
^{13}C NMR spectrum of **8a** (75 MHz, $CDCl_3$)



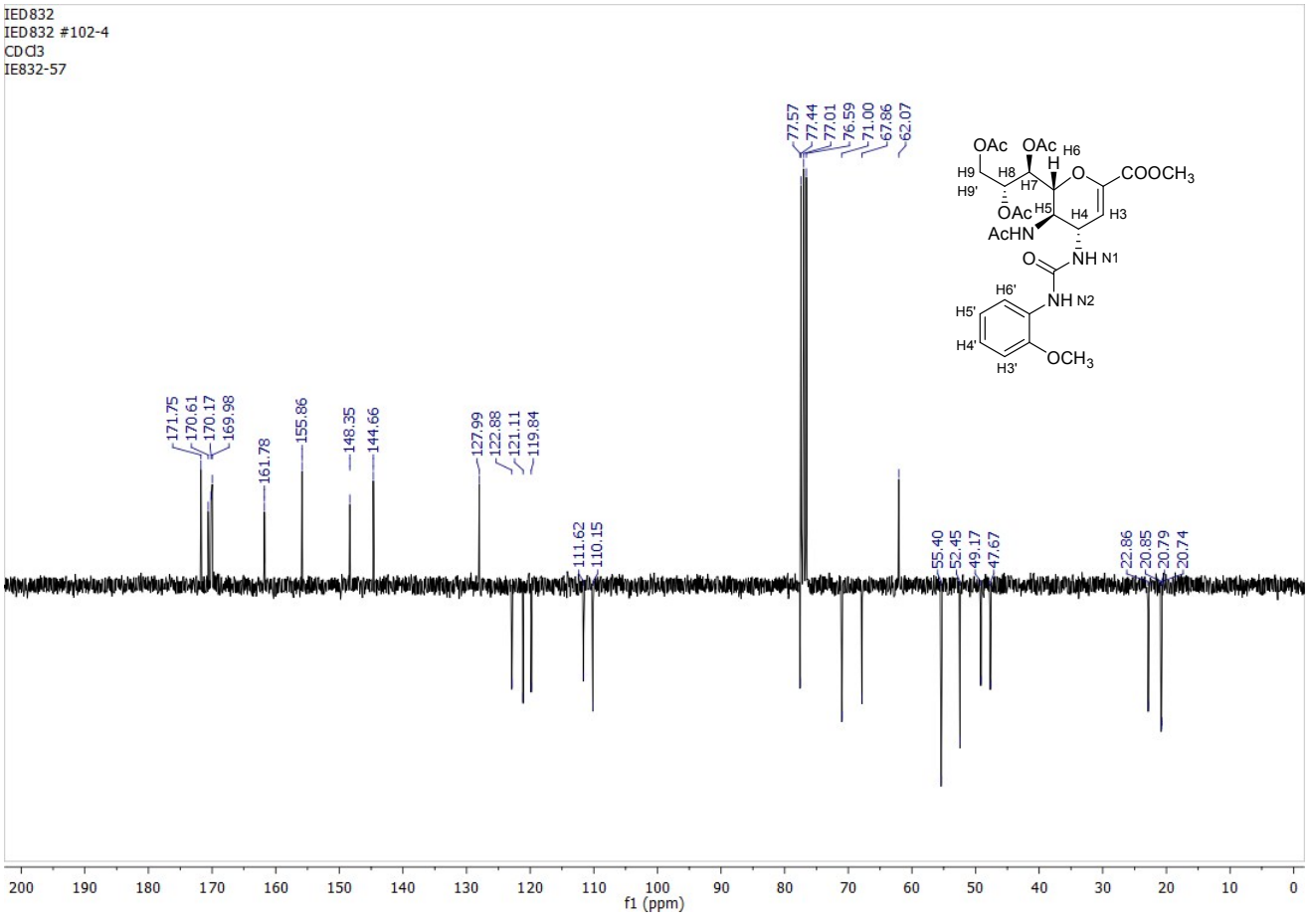
^{1}H NMR spectrum of **8b** (300 MHz, $CDCl_3$)



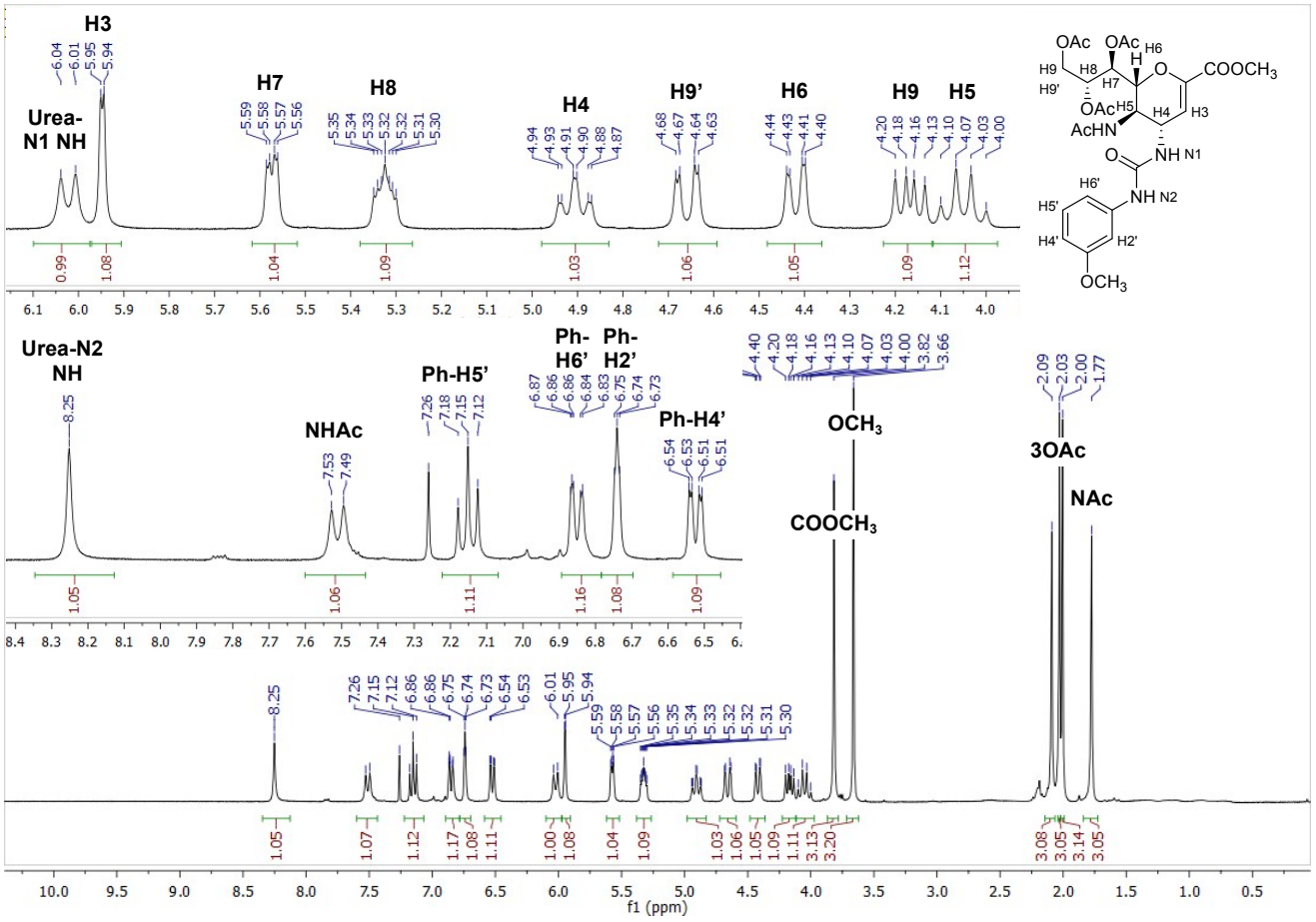
^{13}C NMR spectrum of **8b** (75 MHz, $CDCl_3$)



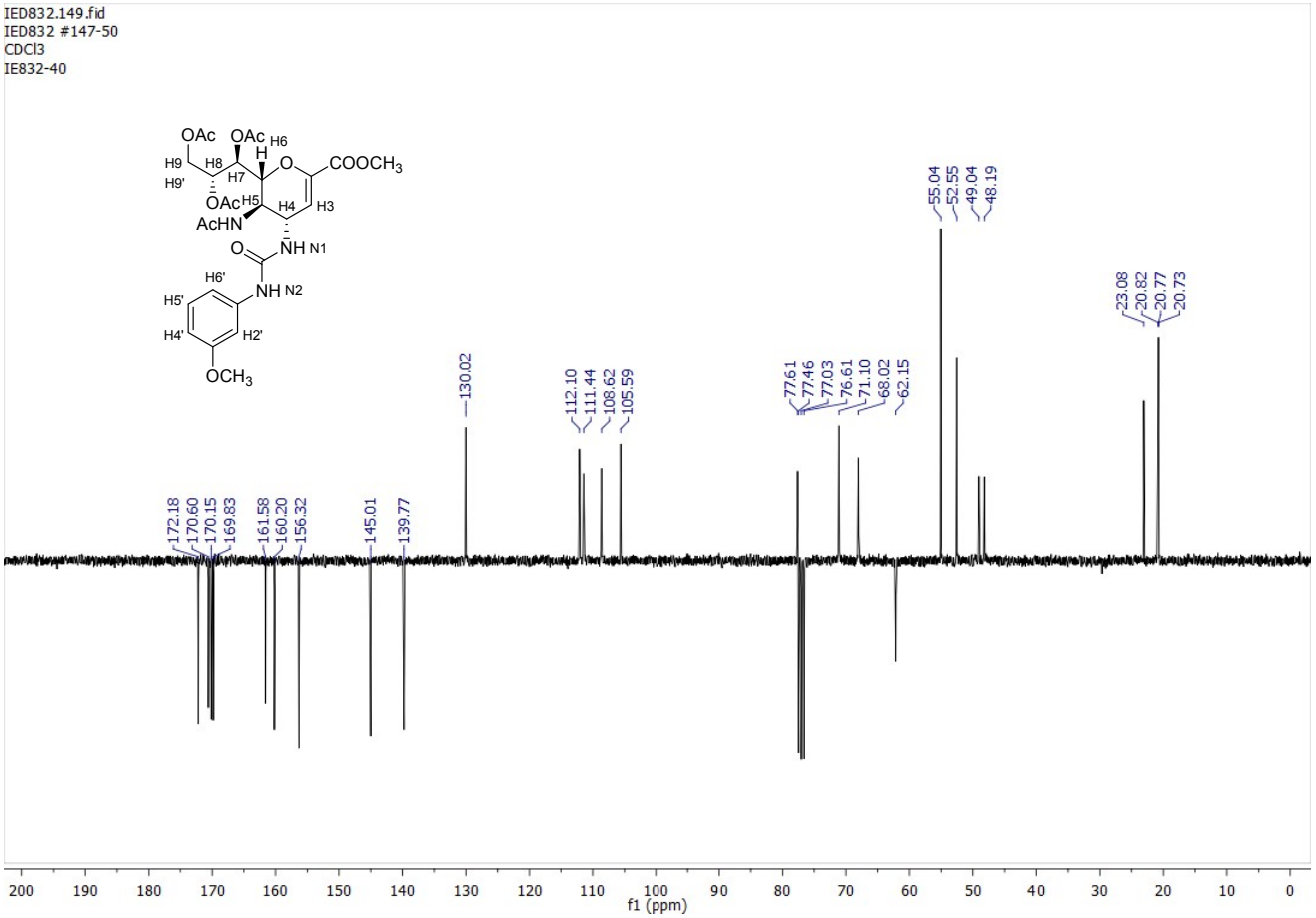
^{1}H NMR spectrum of **8c** (300 MHz, $CDCl_3$)



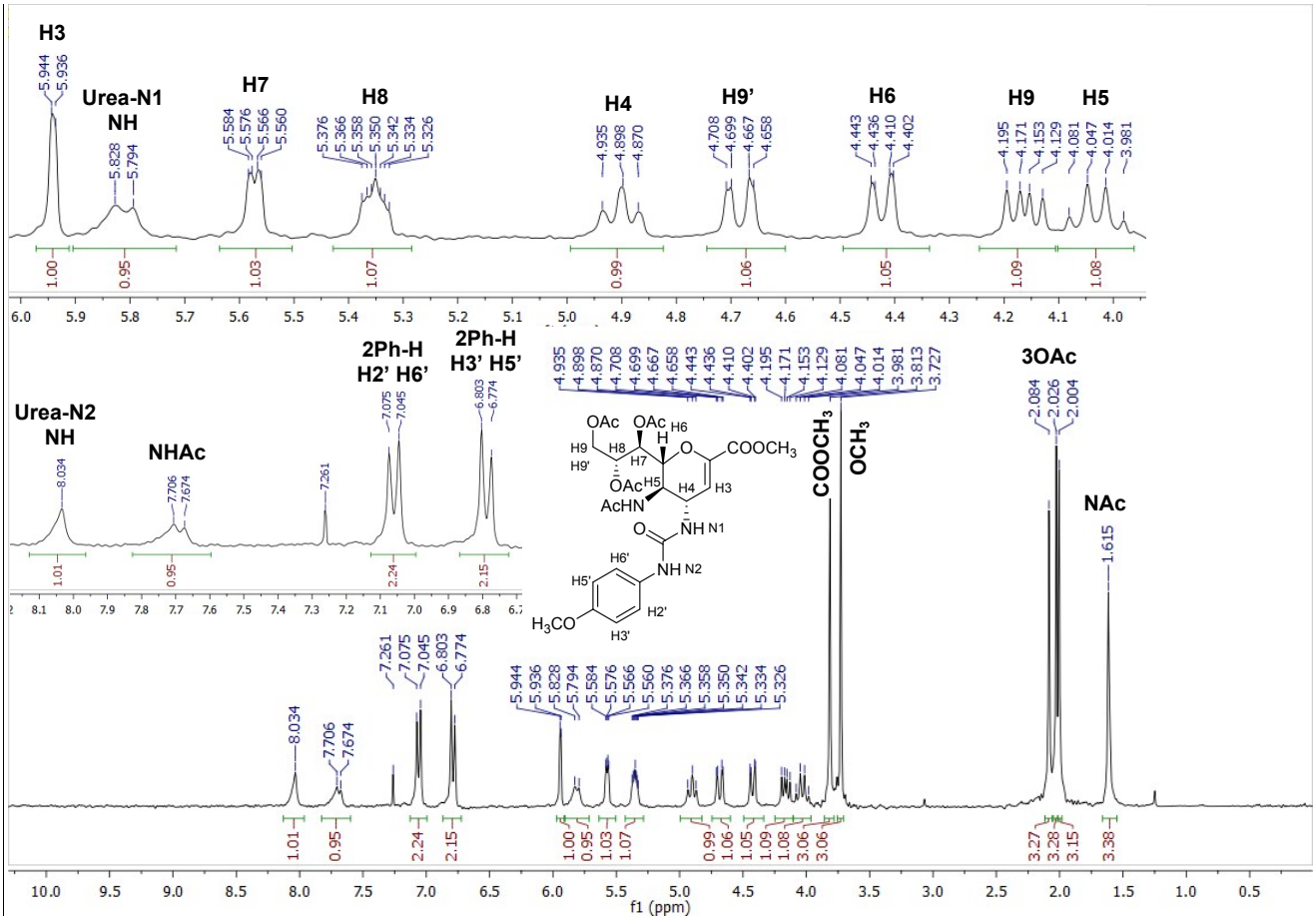
^{13}C NMR spectrum of **8c** (75 MHz, $CDCl_3$)



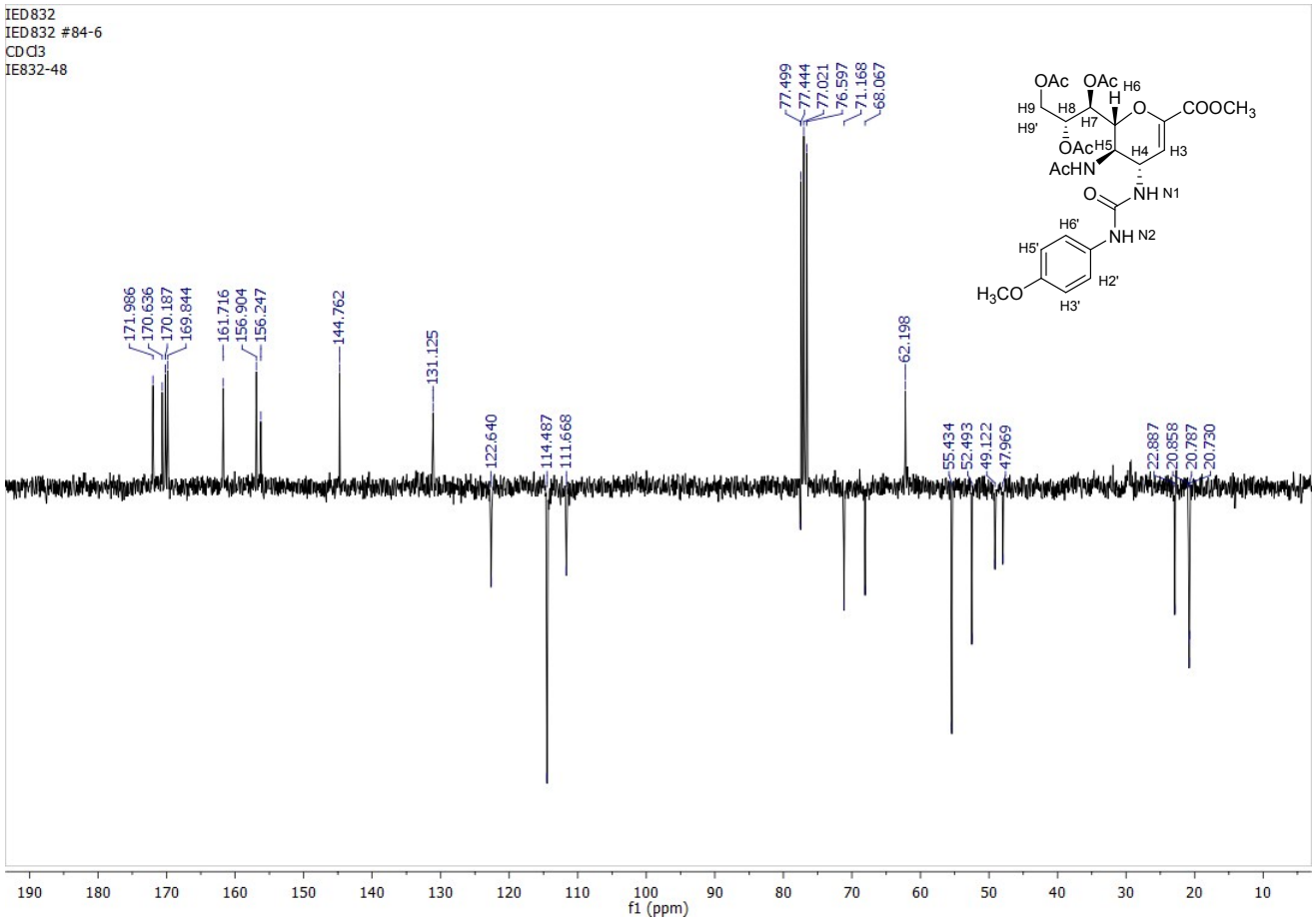
^{1}H NMR spectrum of **8d** (300 MHz, $CDCl_3$)



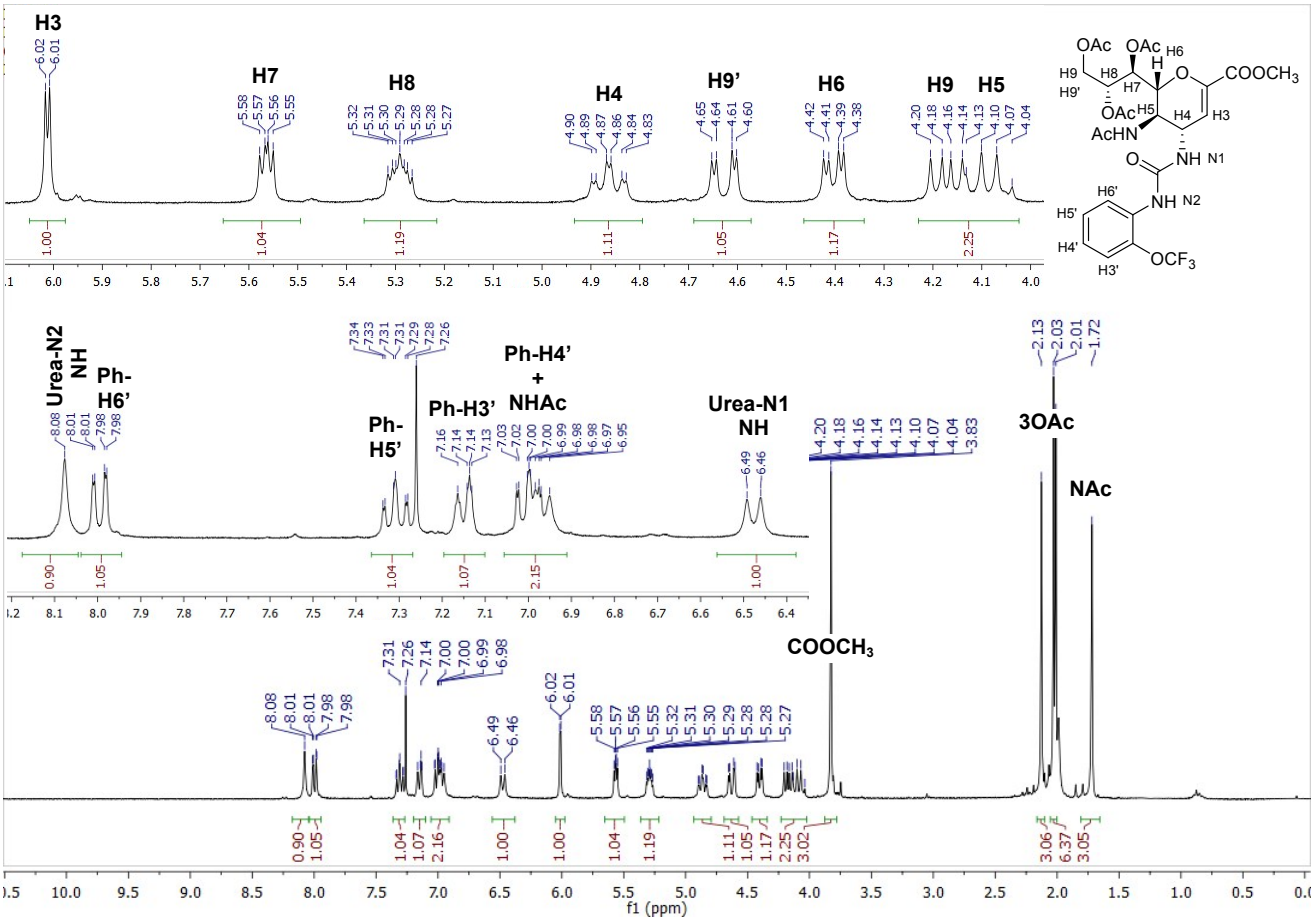
^{13}C NMR spectrum of **8d** (75 MHz, $CDCl_3$)



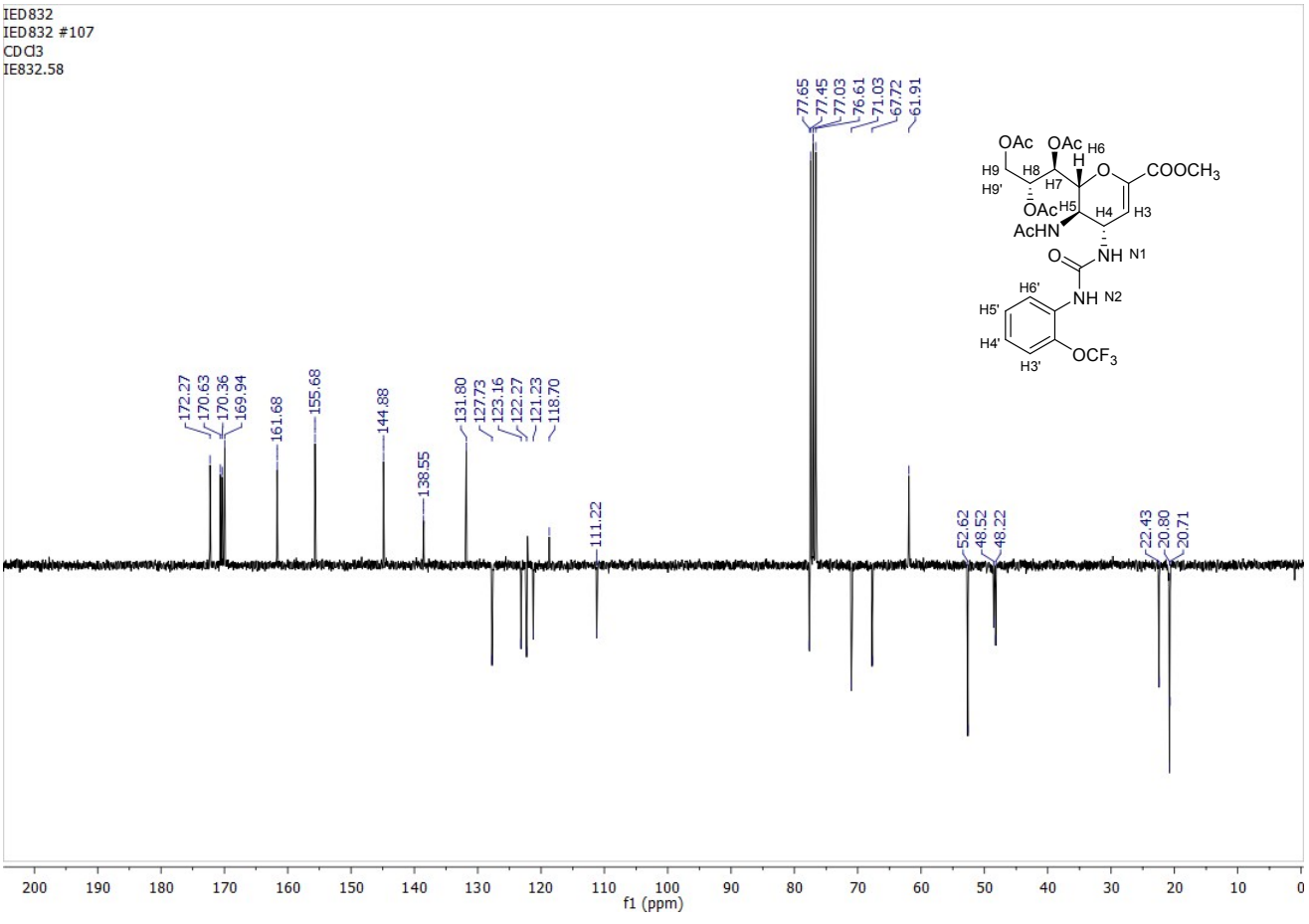
^{1}H NMR spectrum of **8e** (300 MHz, $CDCl_3$)



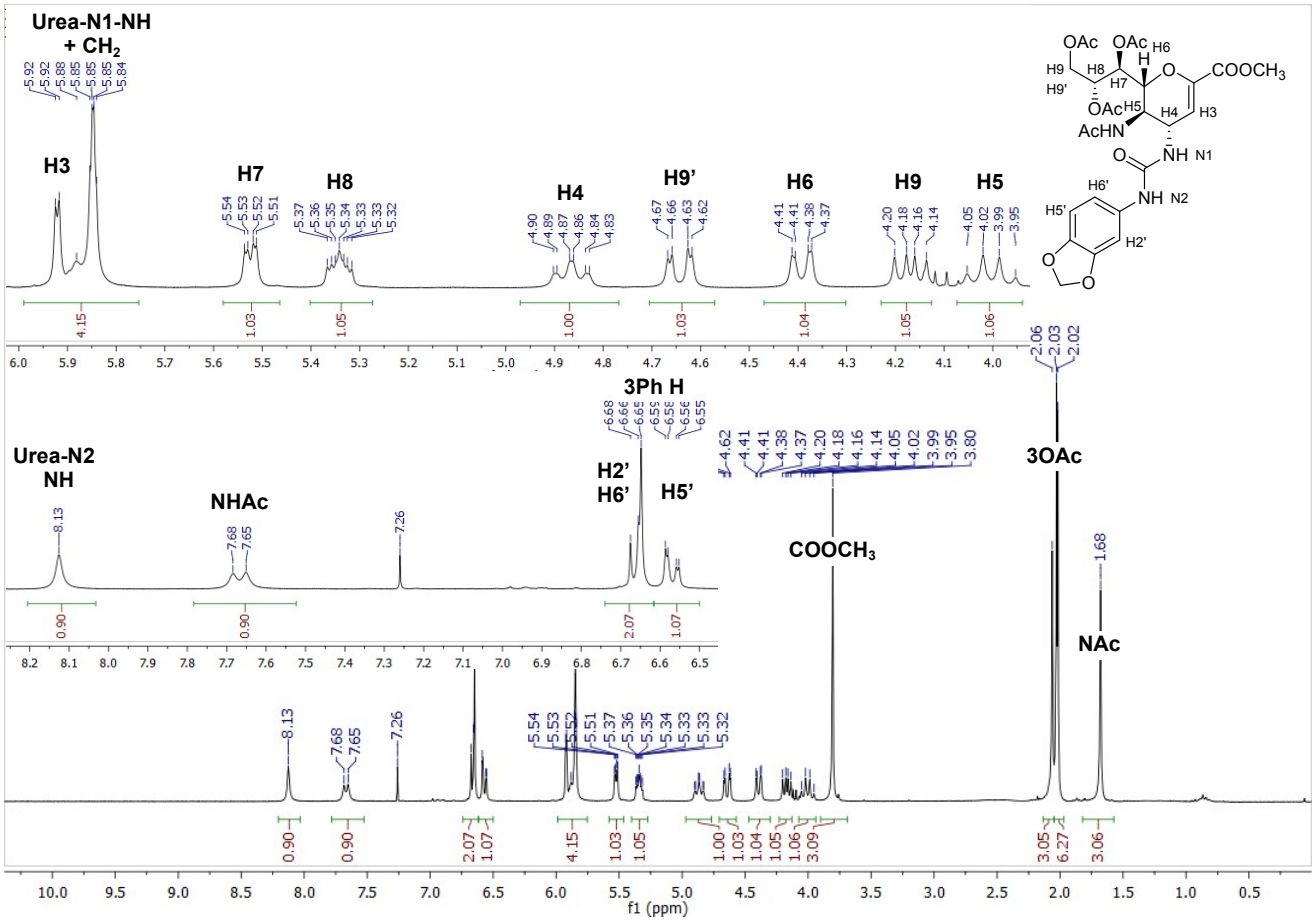
^{13}C NMR spectrum of **8e** (75 MHz, $CDCl_3$)



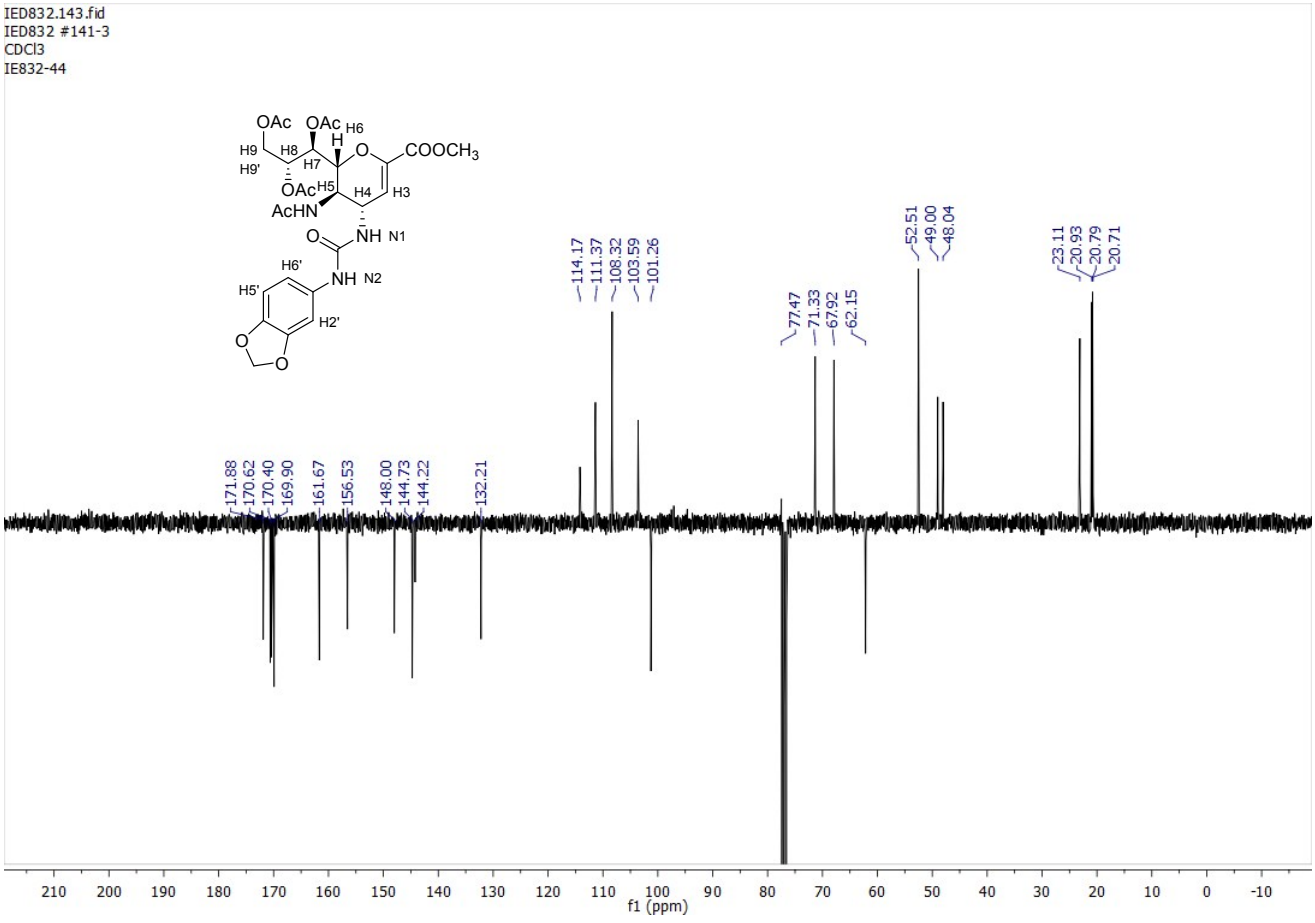
^{1}H NMR spectrum of **8f** (300 MHz, $CDCl_3$)



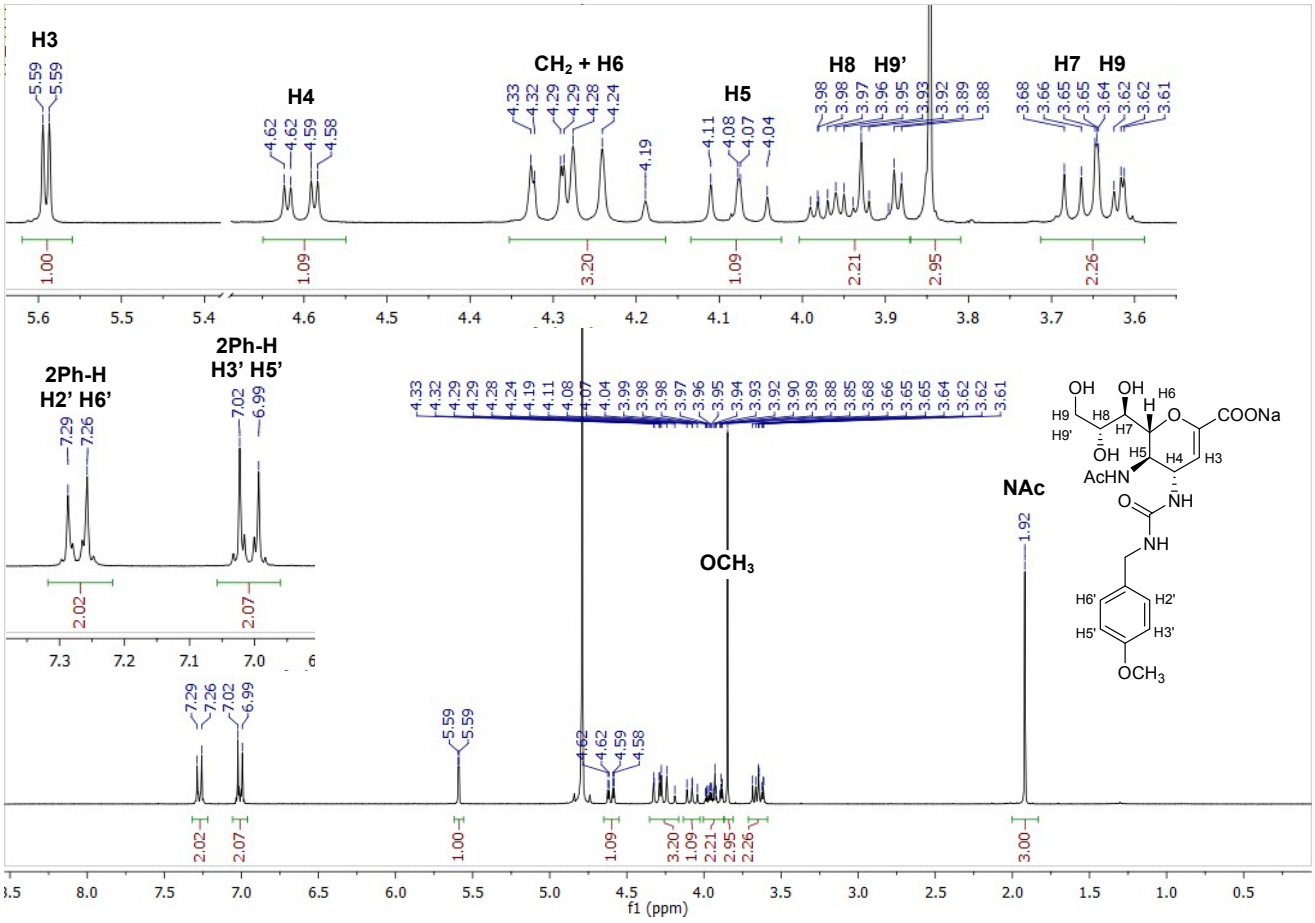
^{13}C NMR spectrum of **8f** (75 MHz, $CDCl_3$)



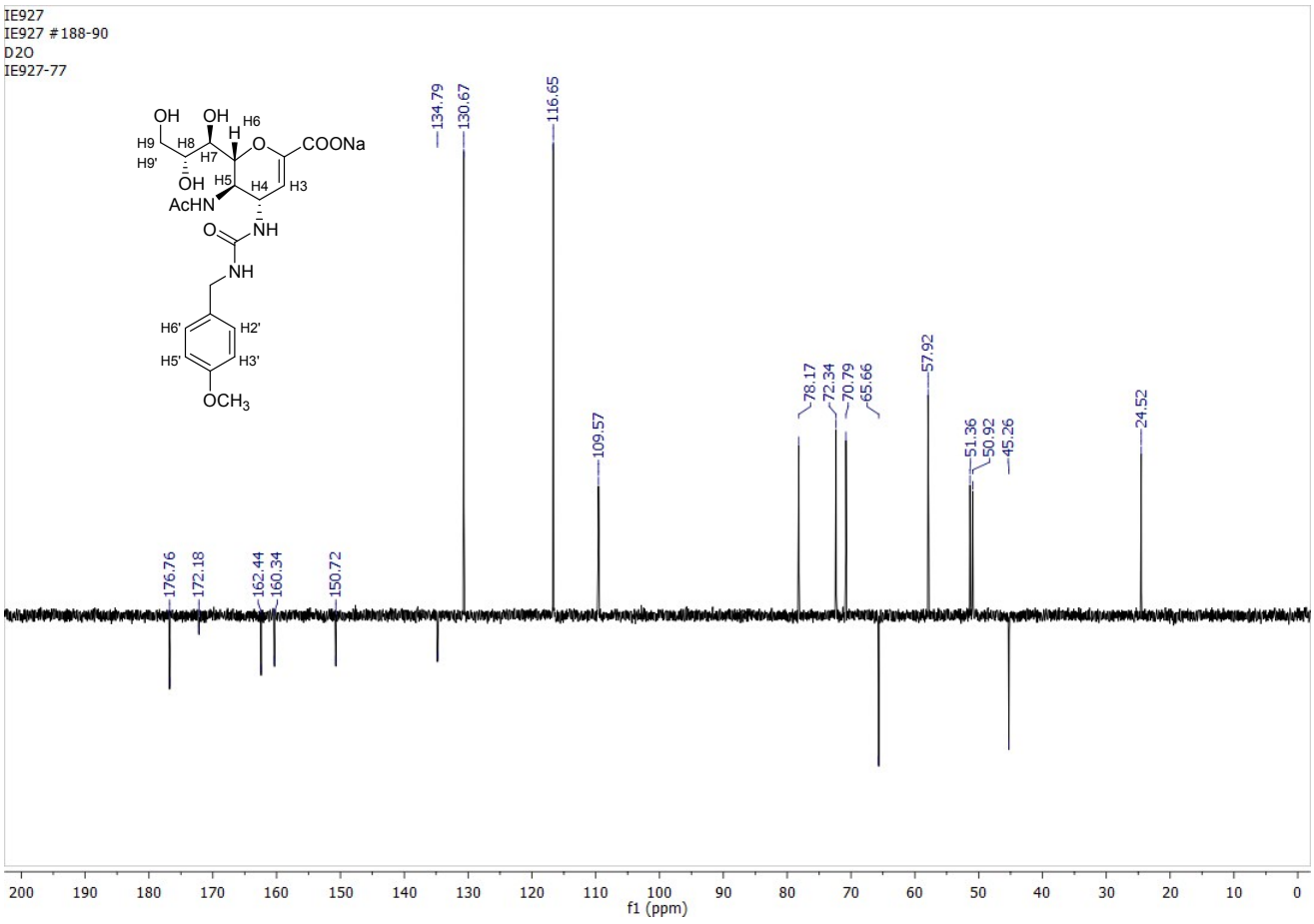
^{1}H NMR spectrum of **8g** (300 MHz, $CDCl_3$)



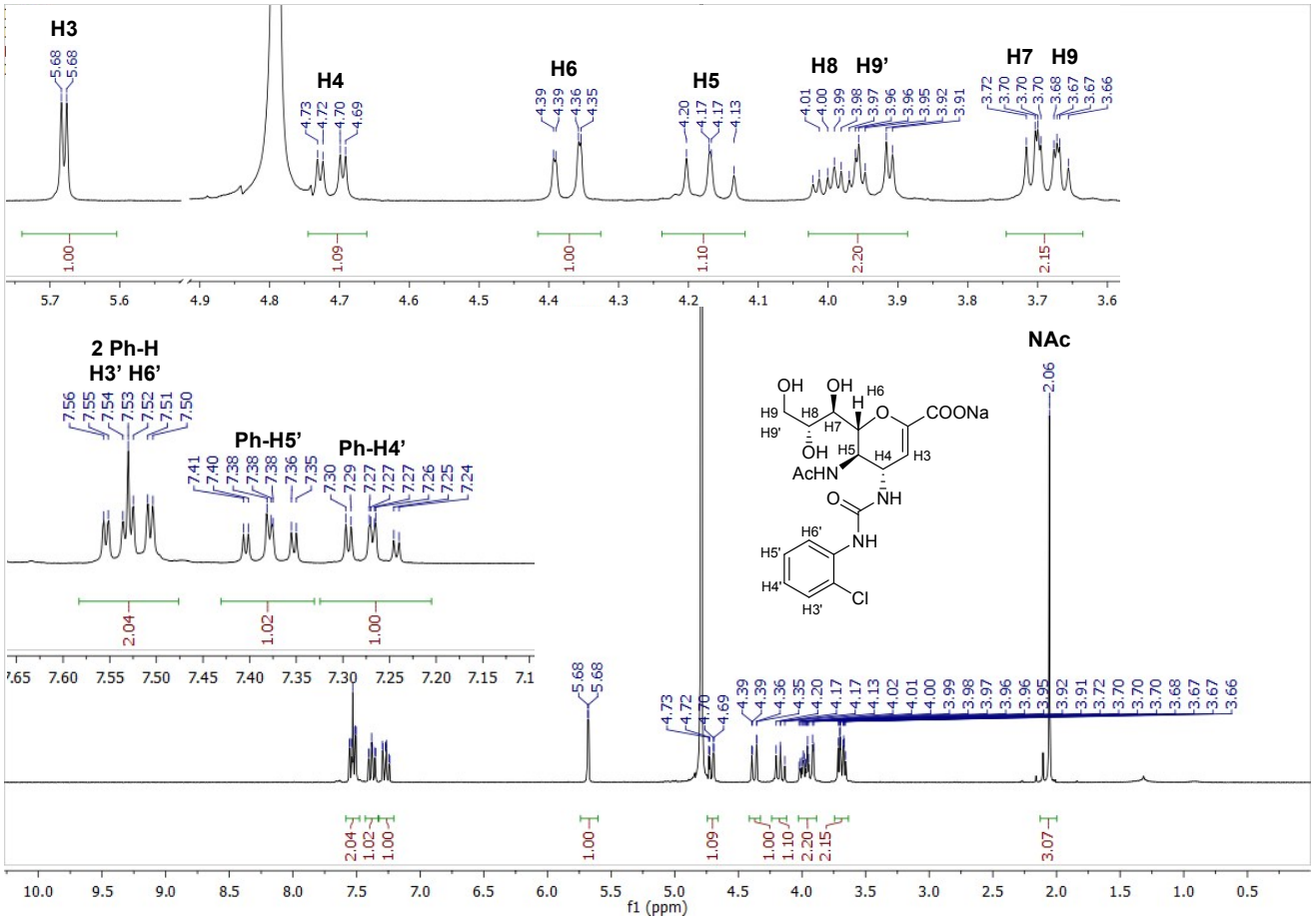
^{13}C NMR spectrum of **8g** (75 MHz, $CDCl_3$)



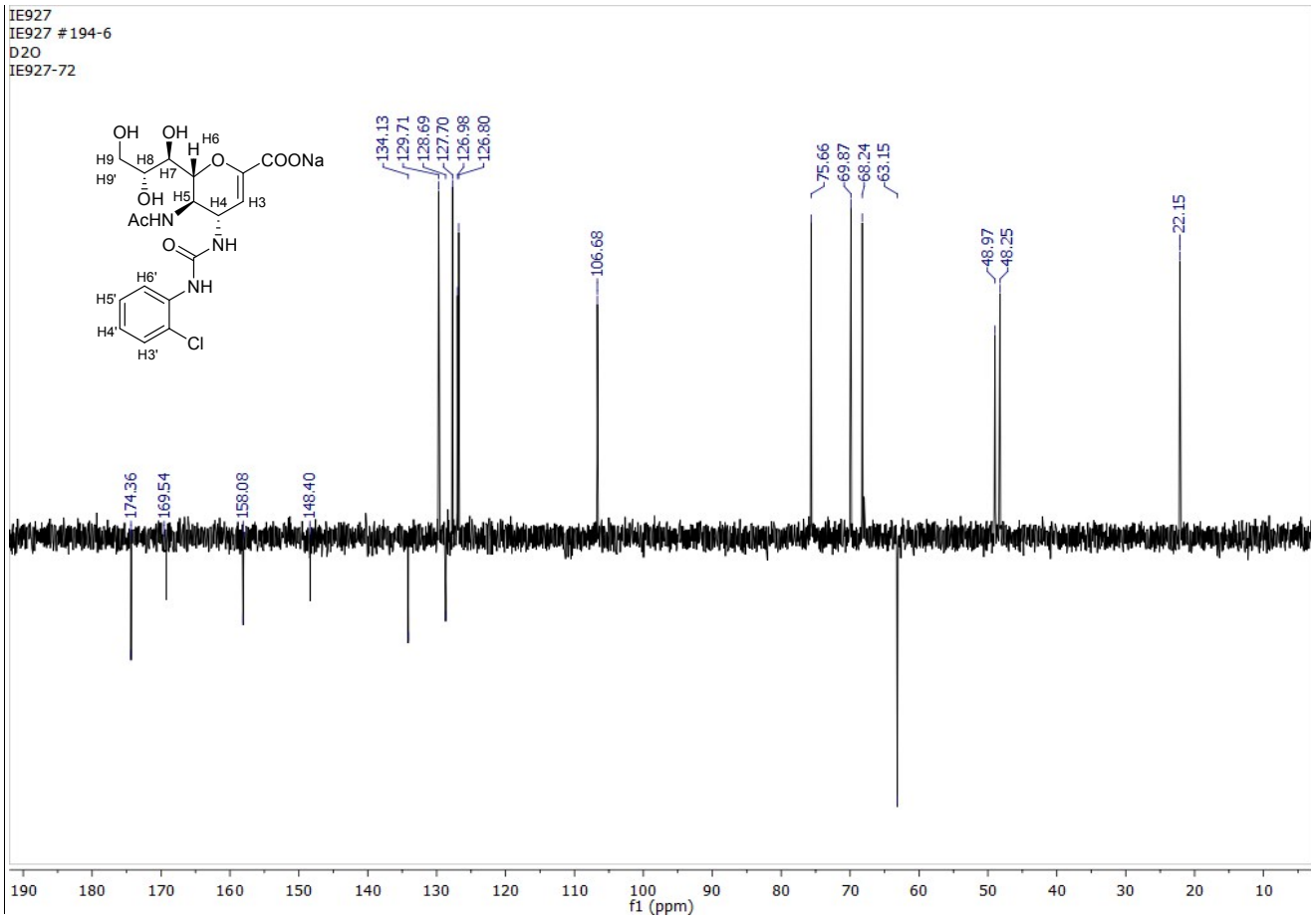
^{1}H NMR spectrum of **9a** (300 MHz, D_2O)



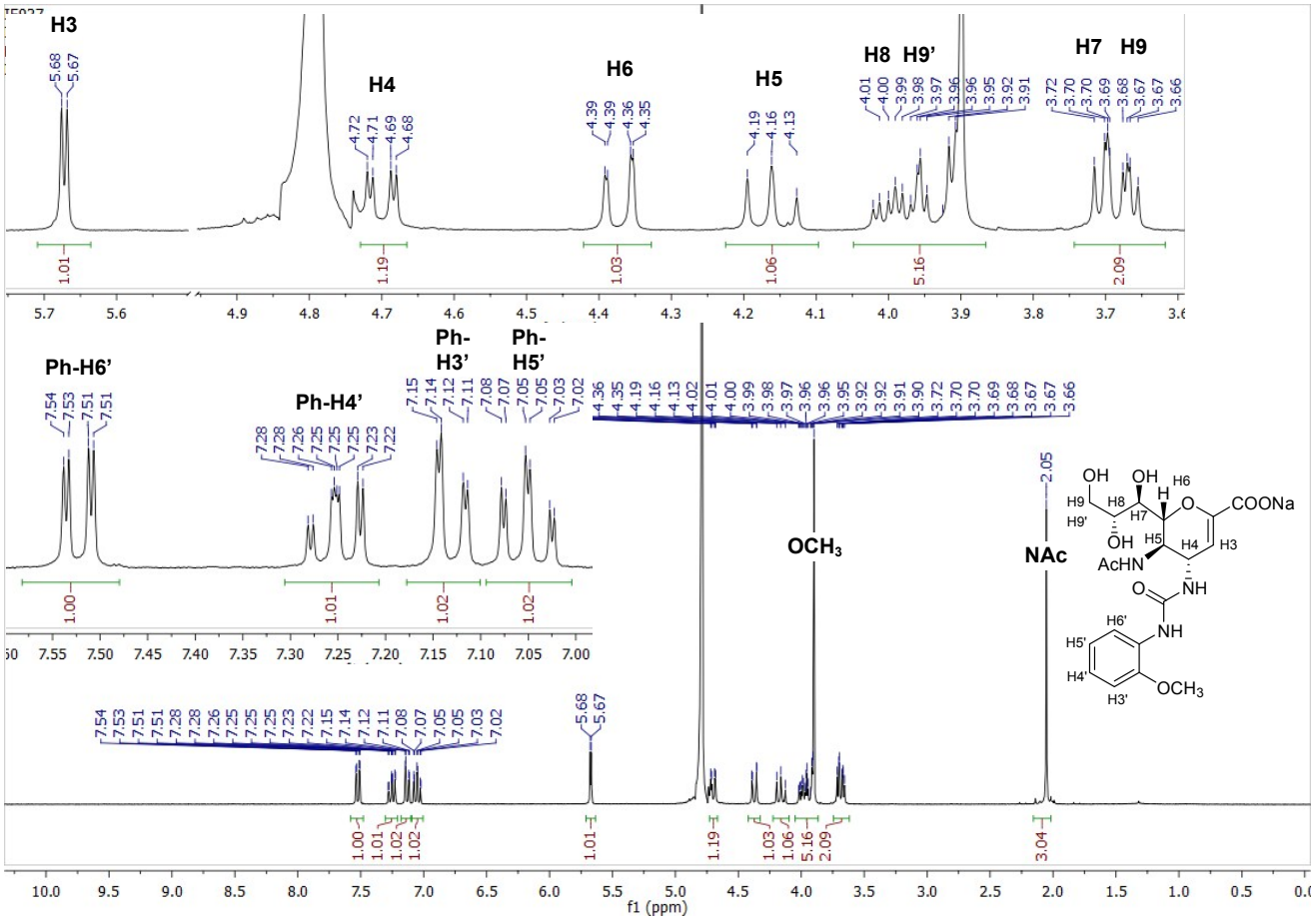
^{13}C NMR spectrum of **9a** (75 MHz, D_2O)



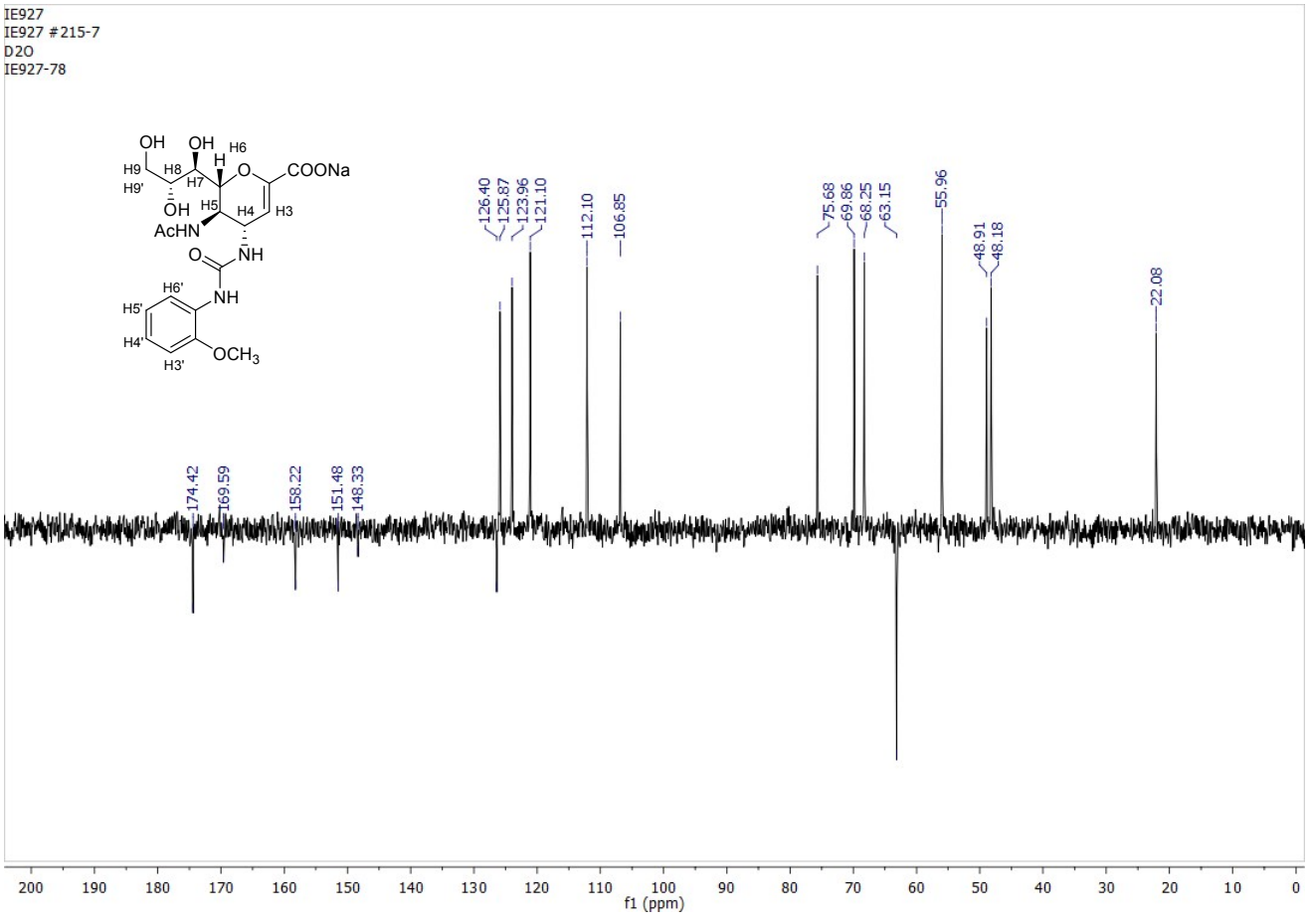
^{1}H NMR spectrum of **9b** (300 MHz, D_2O)



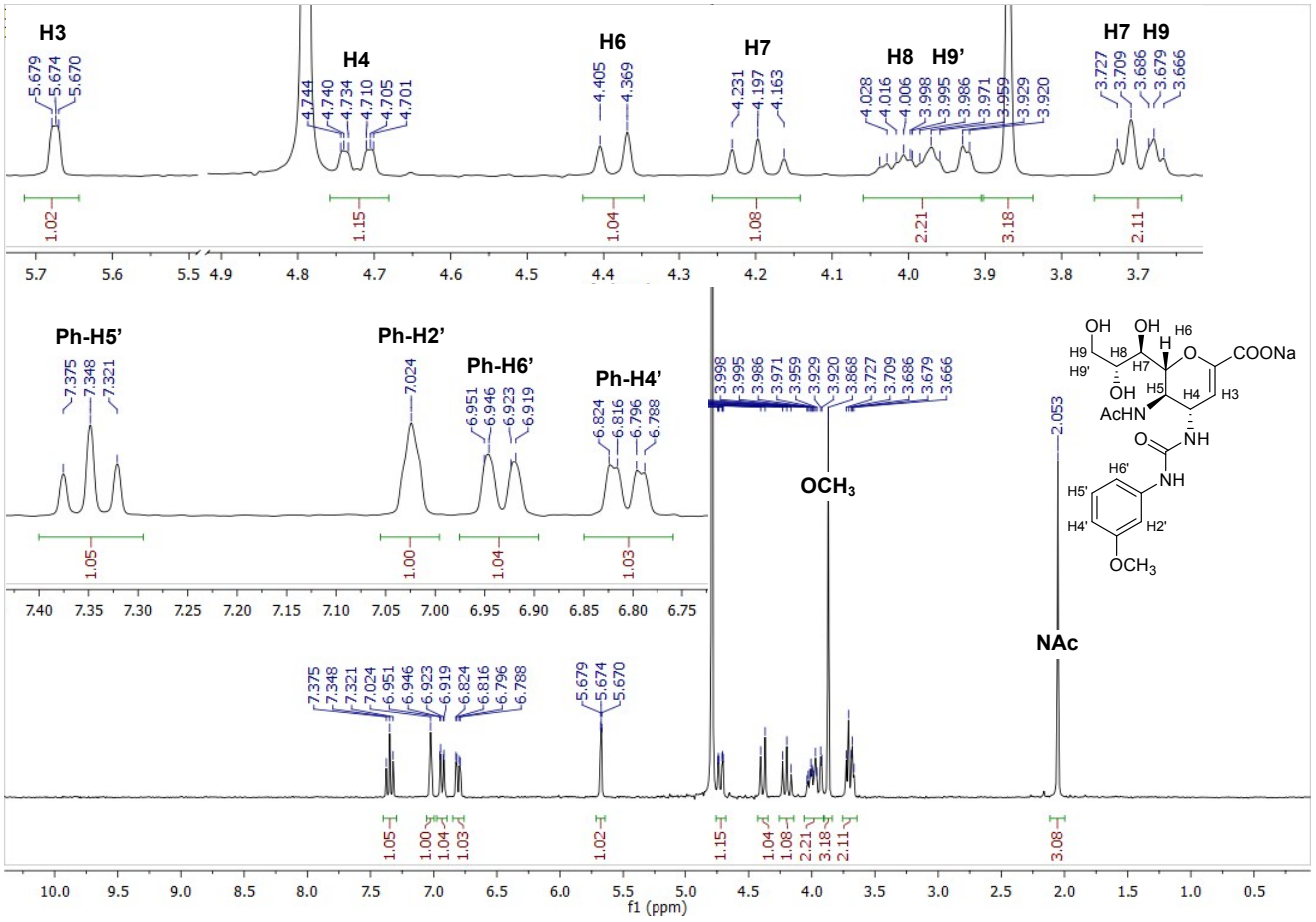
^{13}C NMR spectrum of **9b** (75 MHz, D_2O)



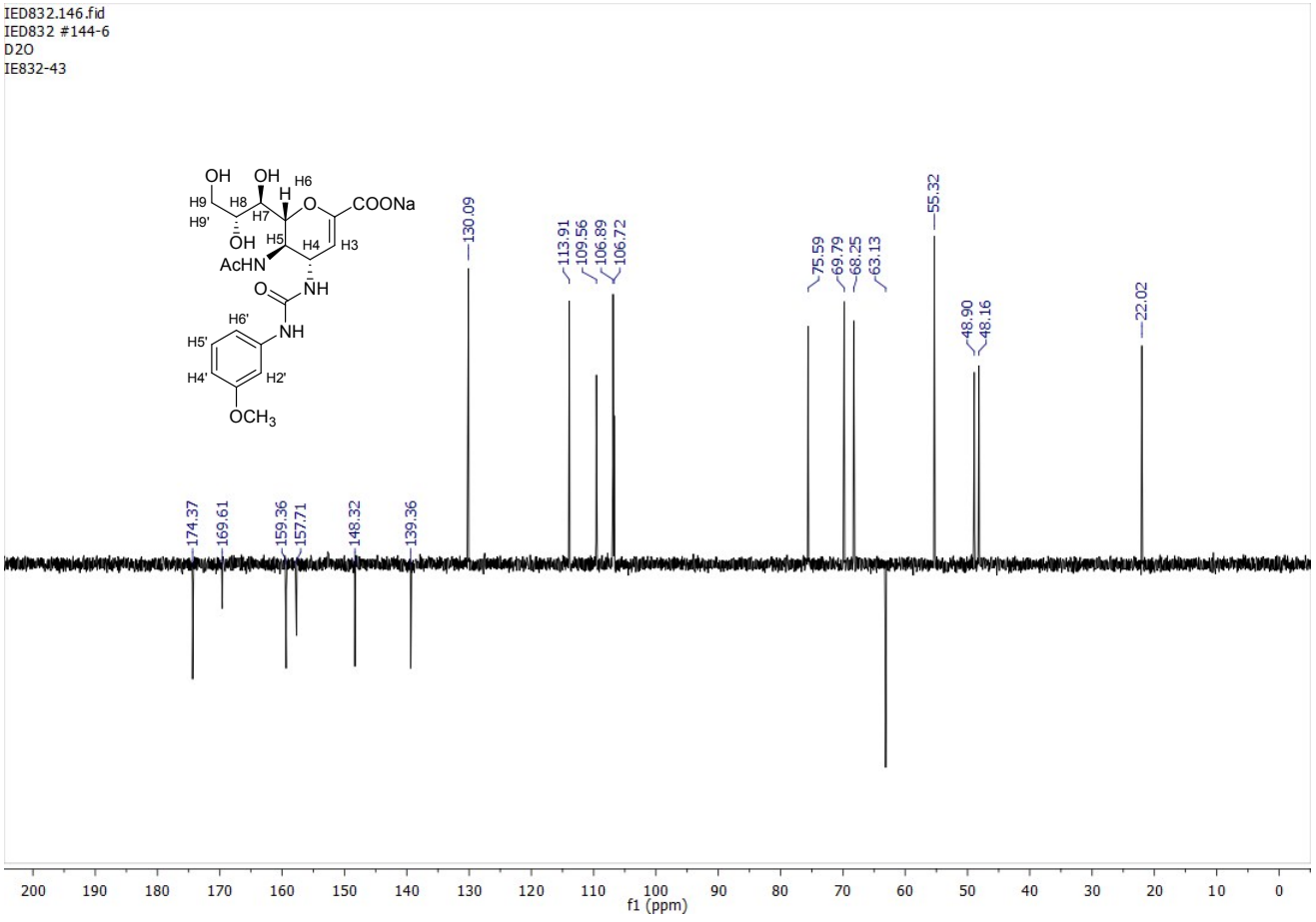
^{1}H NMR spectrum of **9c** (300 MHz, D_2O)



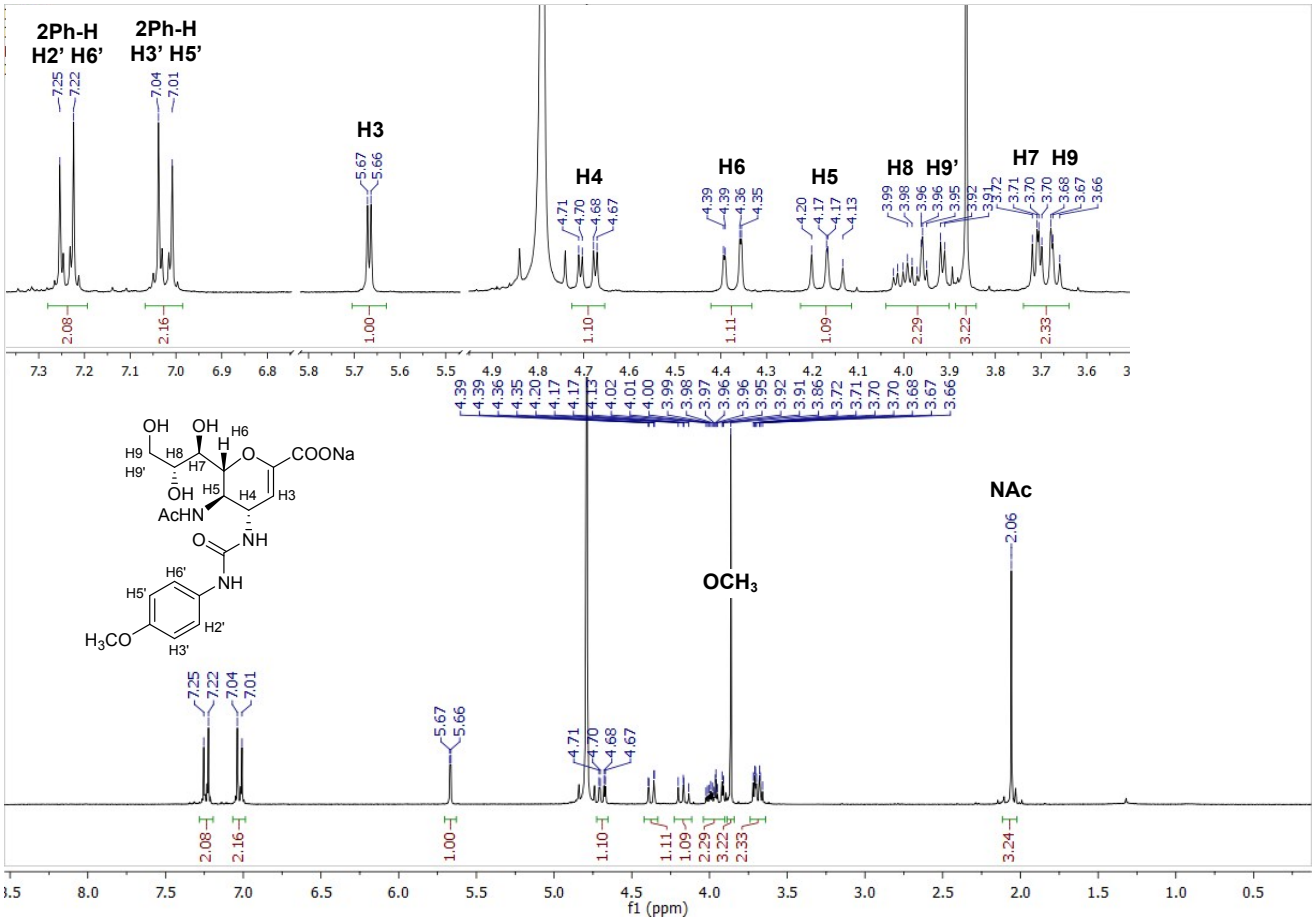
^{13}C NMR spectrum of **9c** (75 MHz, D_2O)



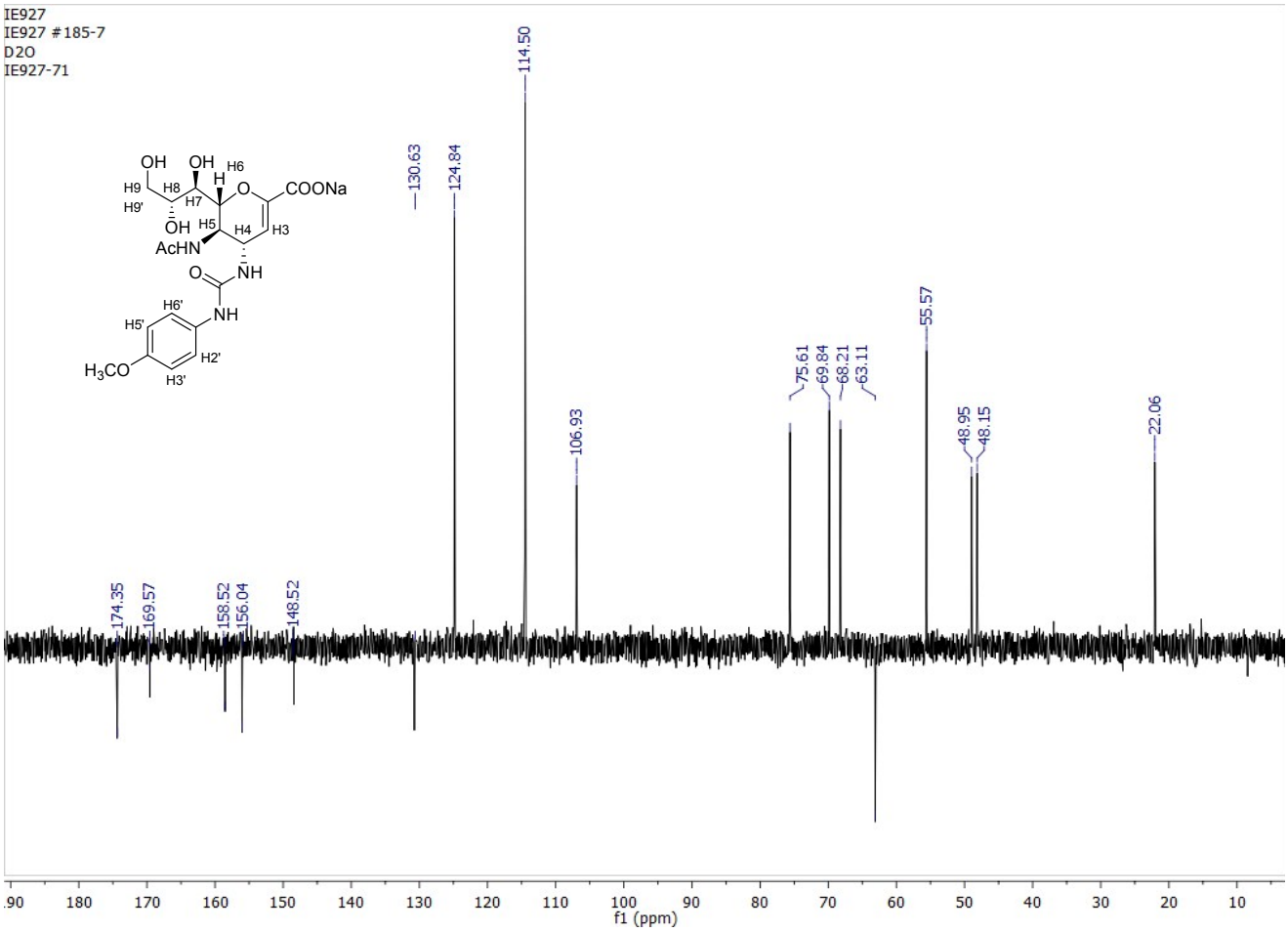
^{1}H NMR spectrum of **9d** (300 MHz, D_2O)



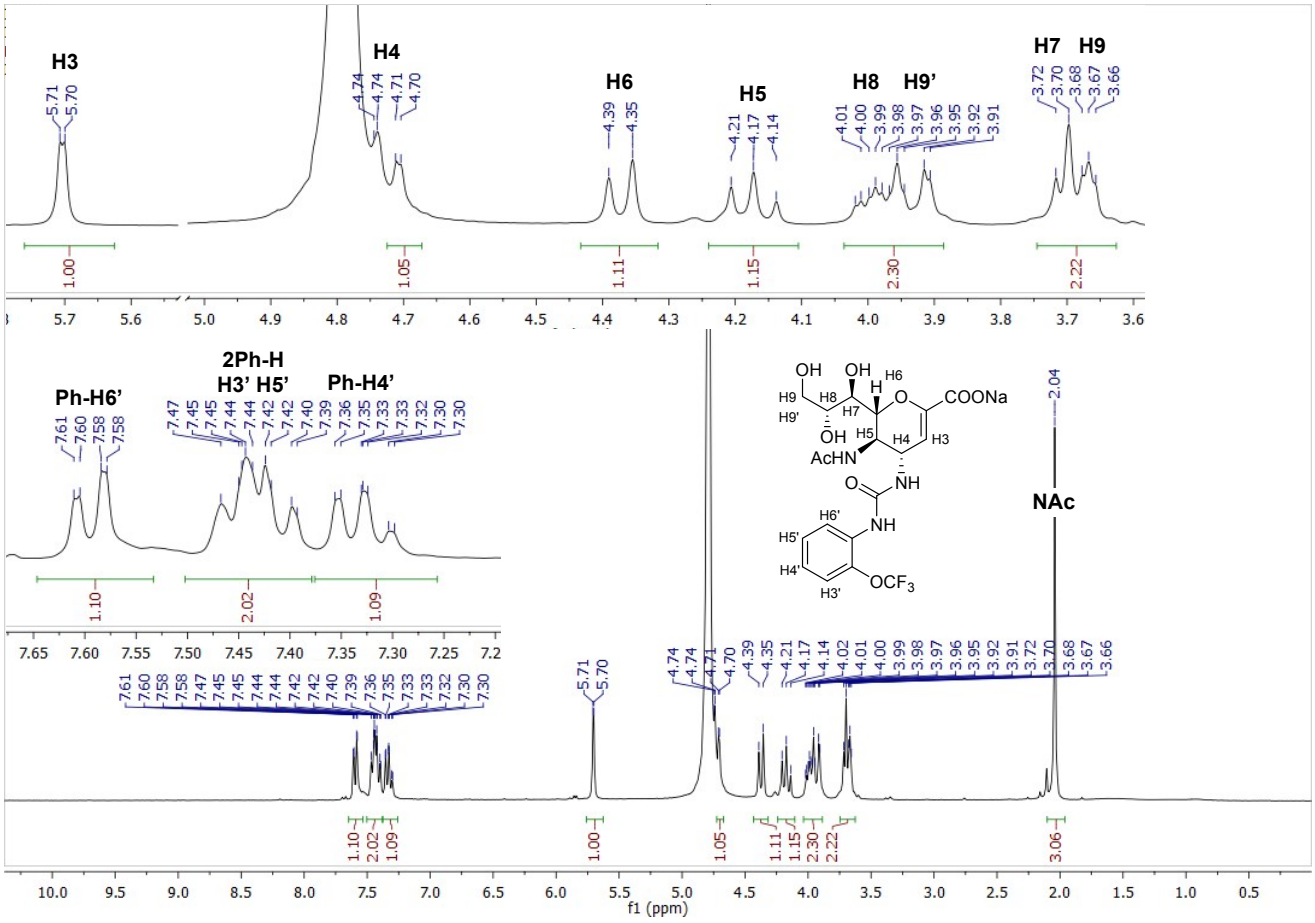
^{13}C NMR spectrum of **9d** (75 MHz, D_2O)



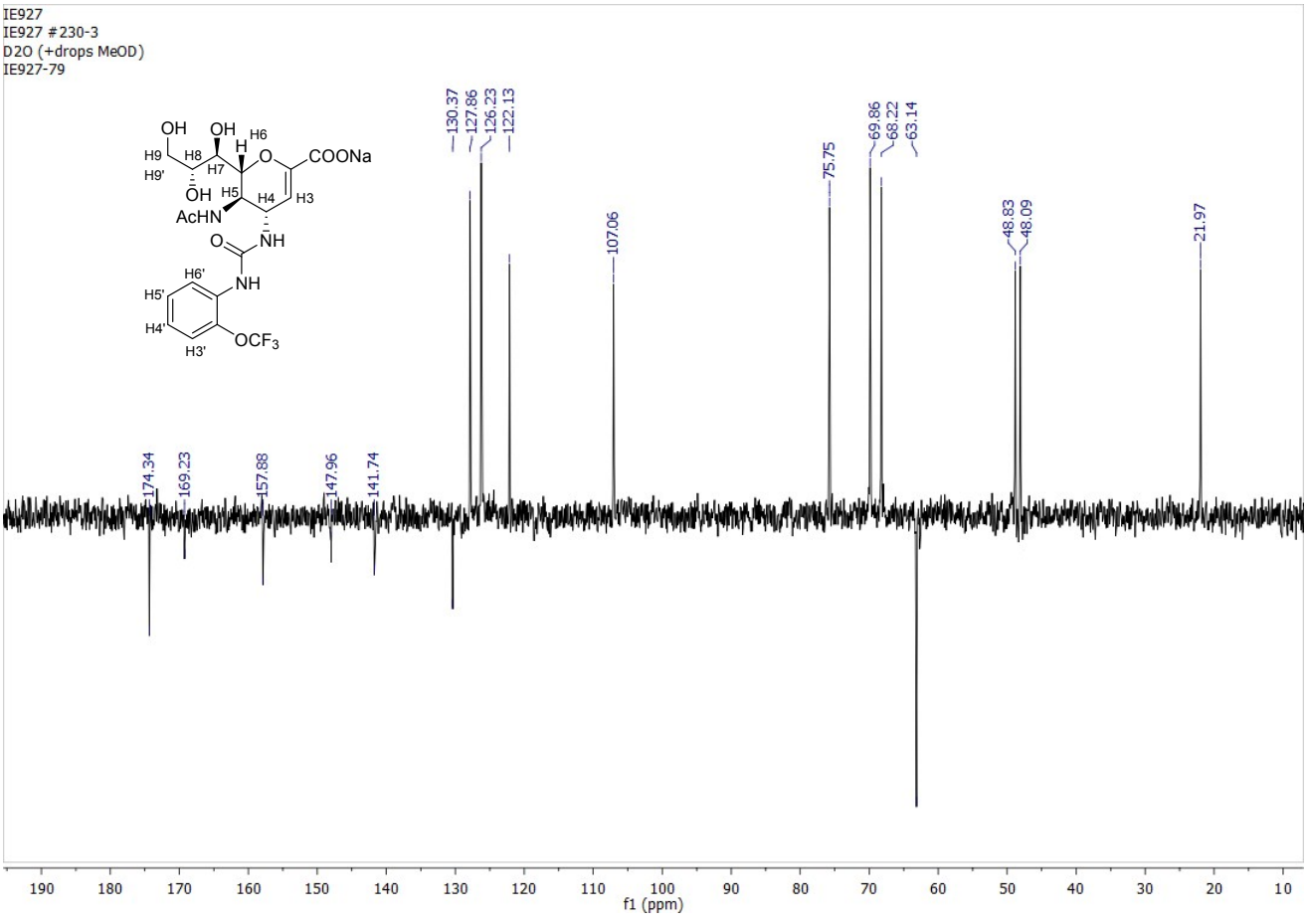
^{1}H NMR spectrum of **9e** (300 MHz, D_2O)



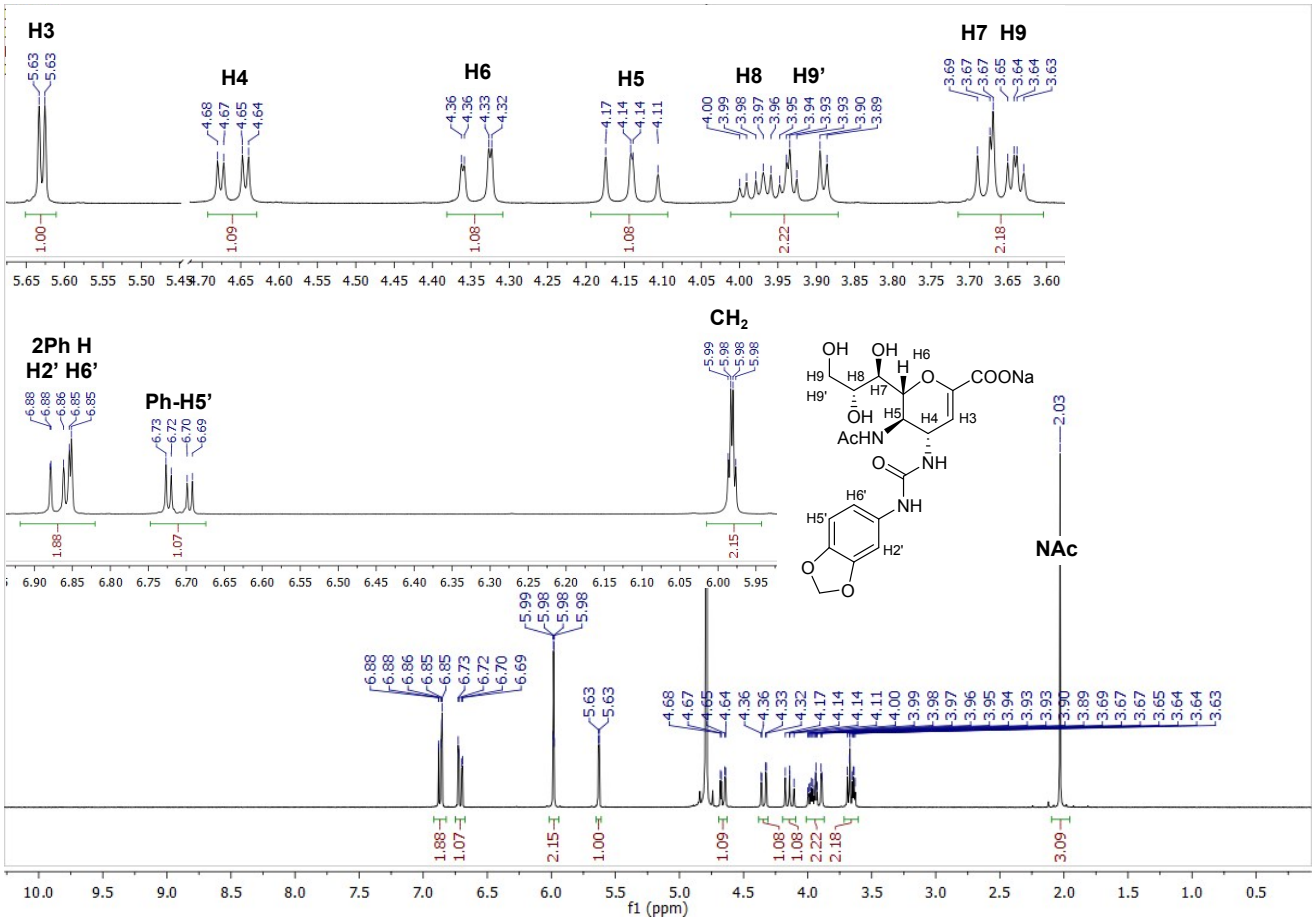
^{13}C NMR spectrum of **9e** (75 MHz, D_2O)



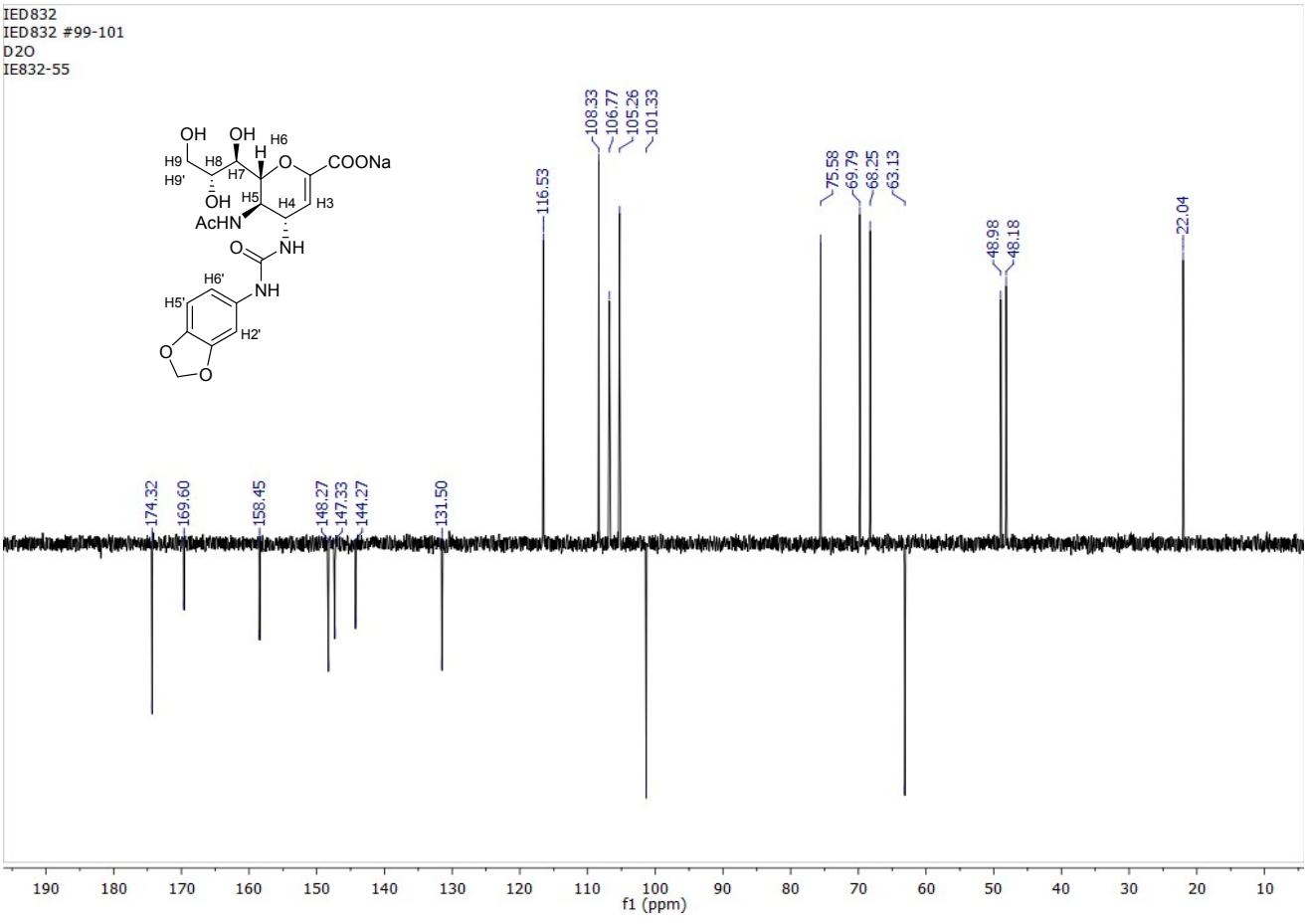
^{1}H NMR spectrum of **9f** (300 MHz, D_2O)



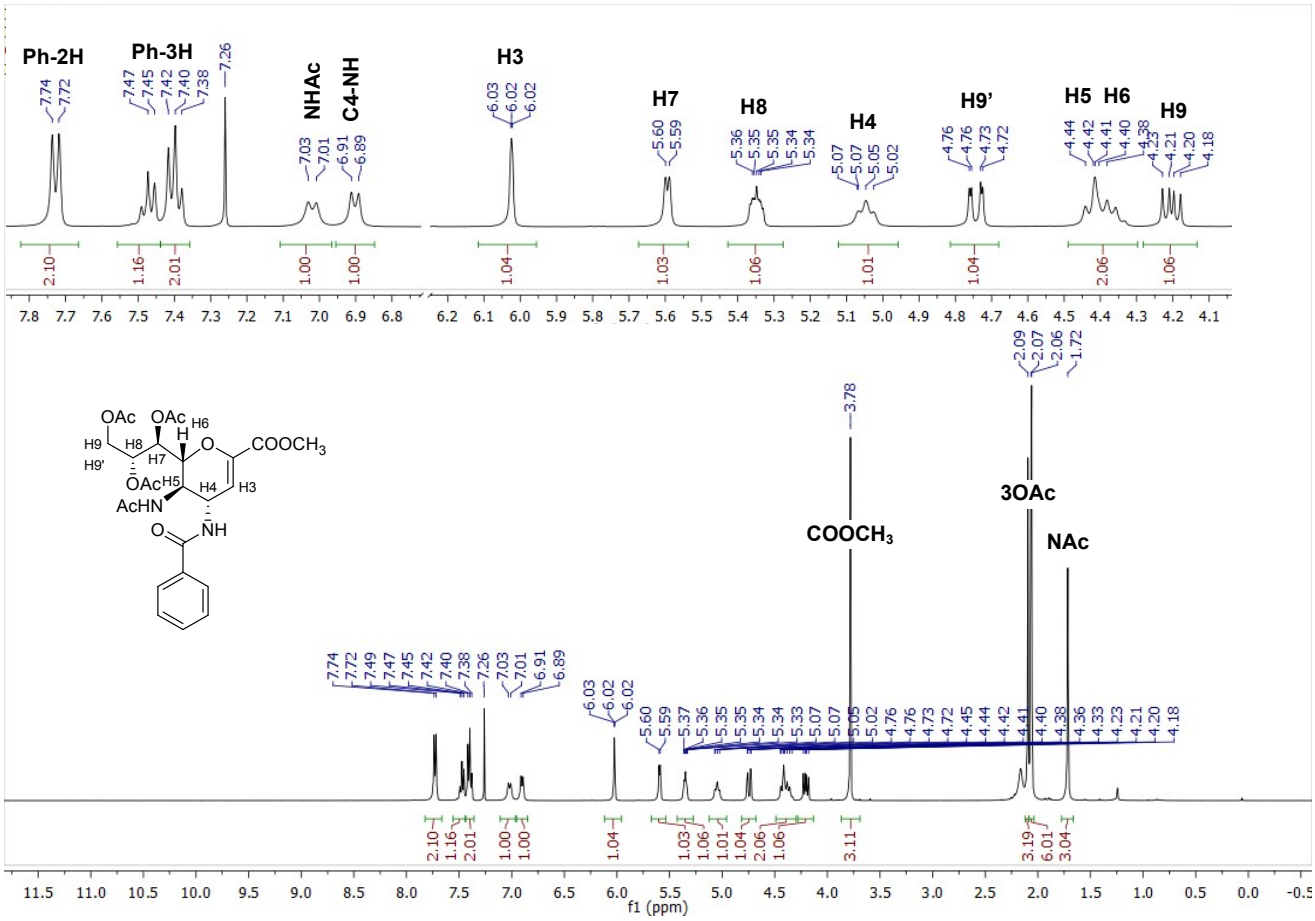
^{13}C NMR spectrum of **9f** (75 MHz, D_2O)



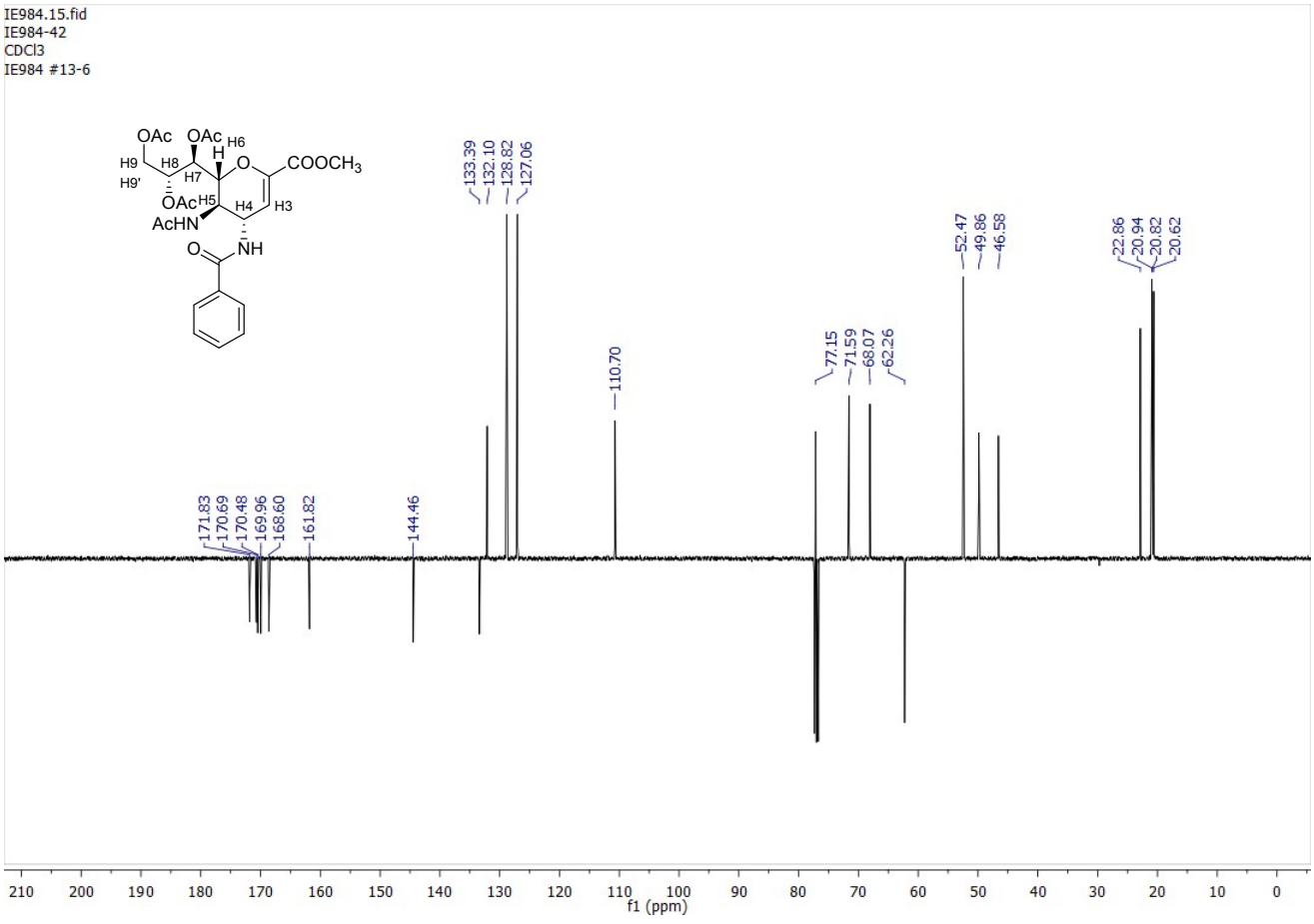
^{1}H NMR spectrum of **9g** (300 MHz, D_2O)



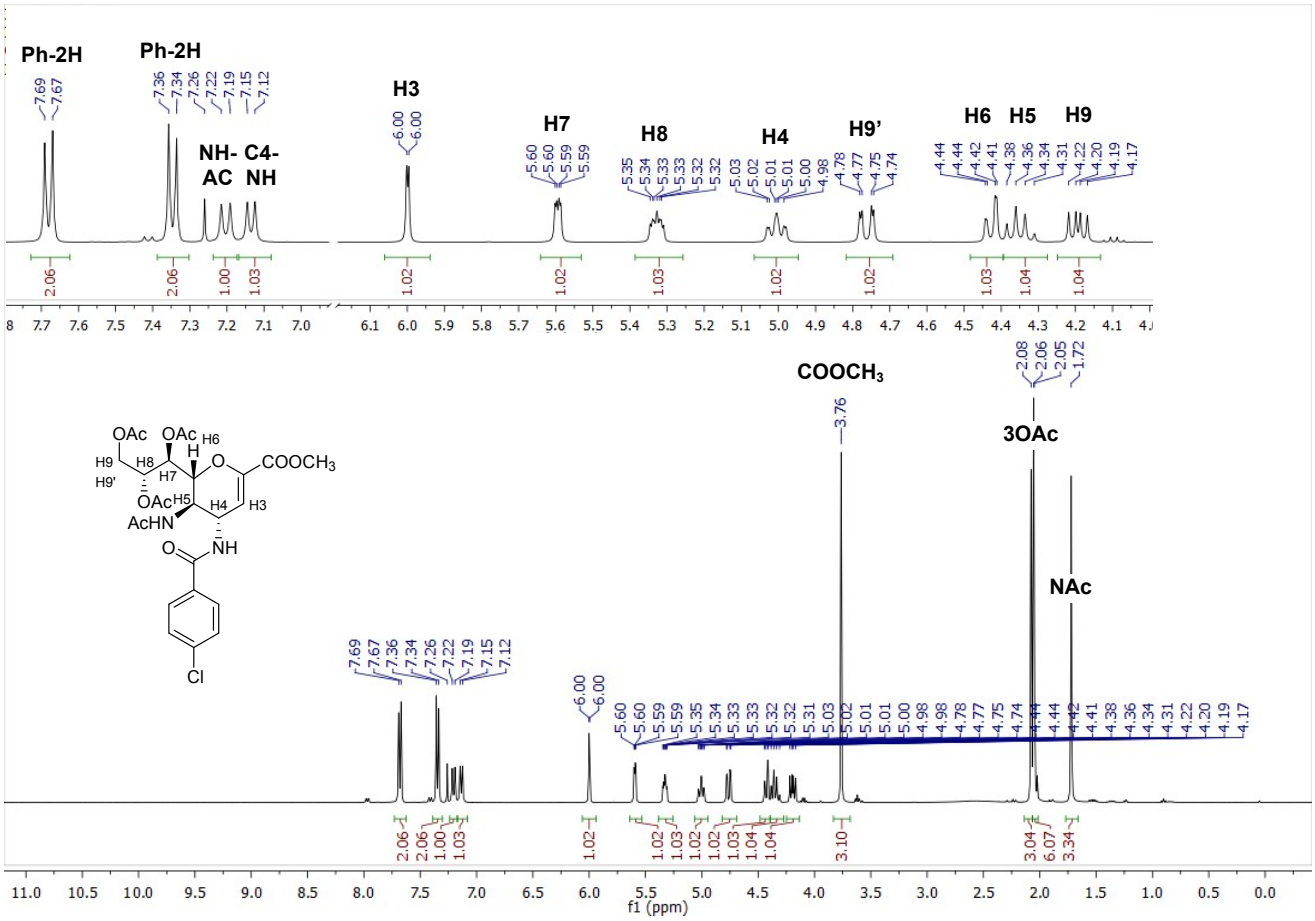
^{13}C NMR spectrum of **9g** (75 MHz, D_2O)



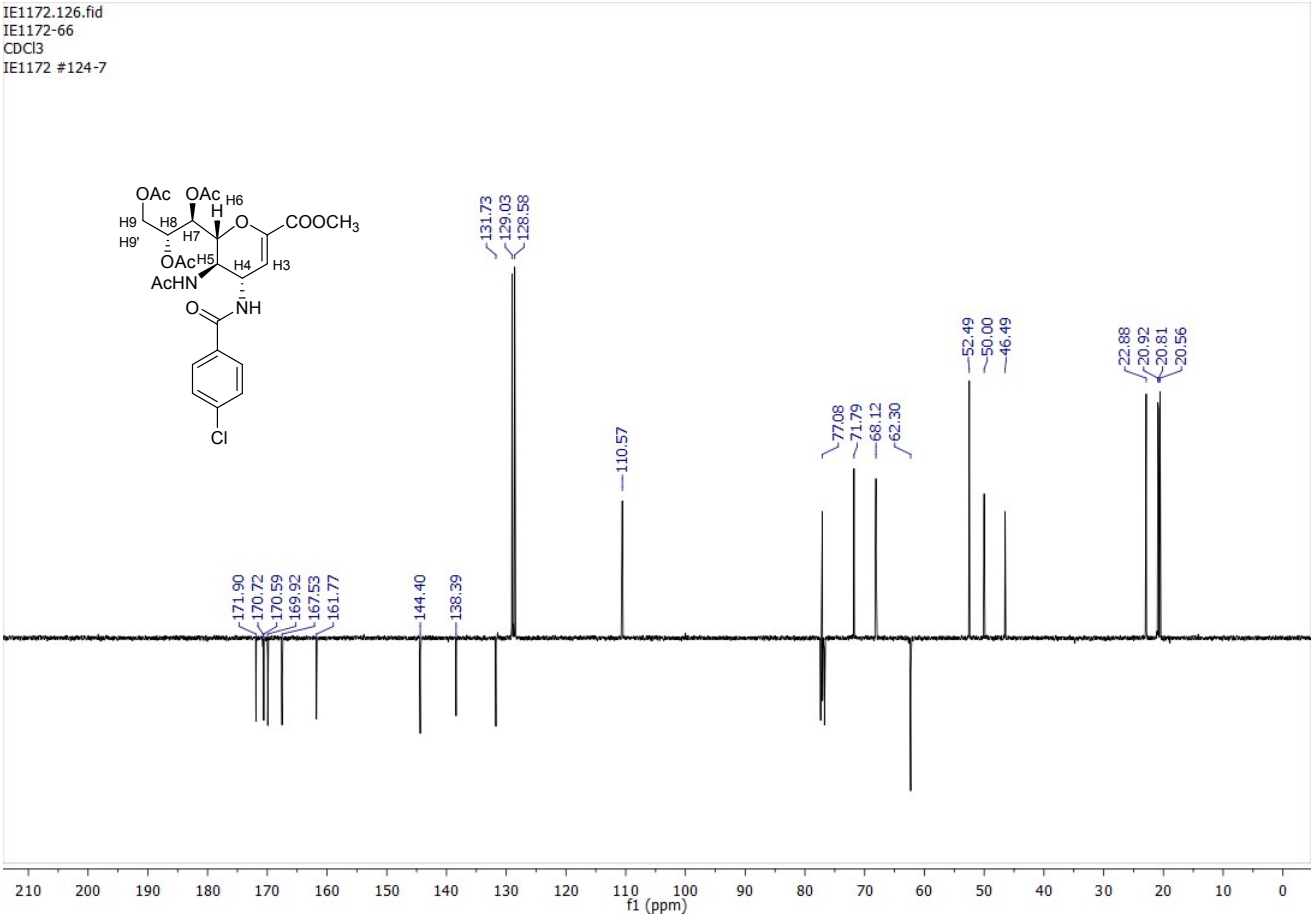
^{1}H NMR spectrum of **10a** (400 MHz, $CDCl_3$)



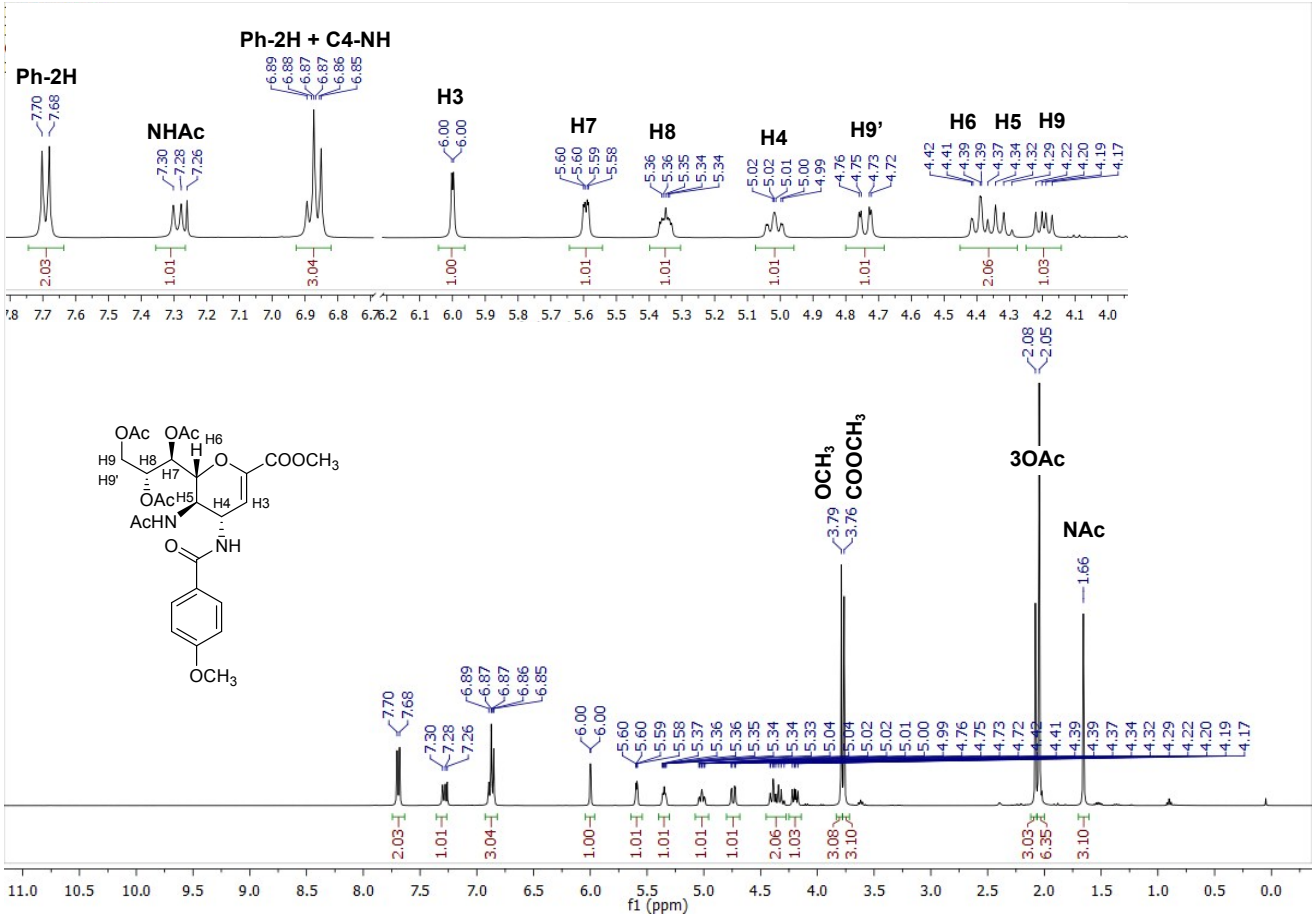
^{13}C NMR spectrum of **10a** (100 MHz, $CDCl_3$)



^{1}H NMR spectrum of **10b** (400 MHz, $CDCl_3$)



^{13}C NMR spectrum of **10b** (100 MHz, $CDCl_3$)



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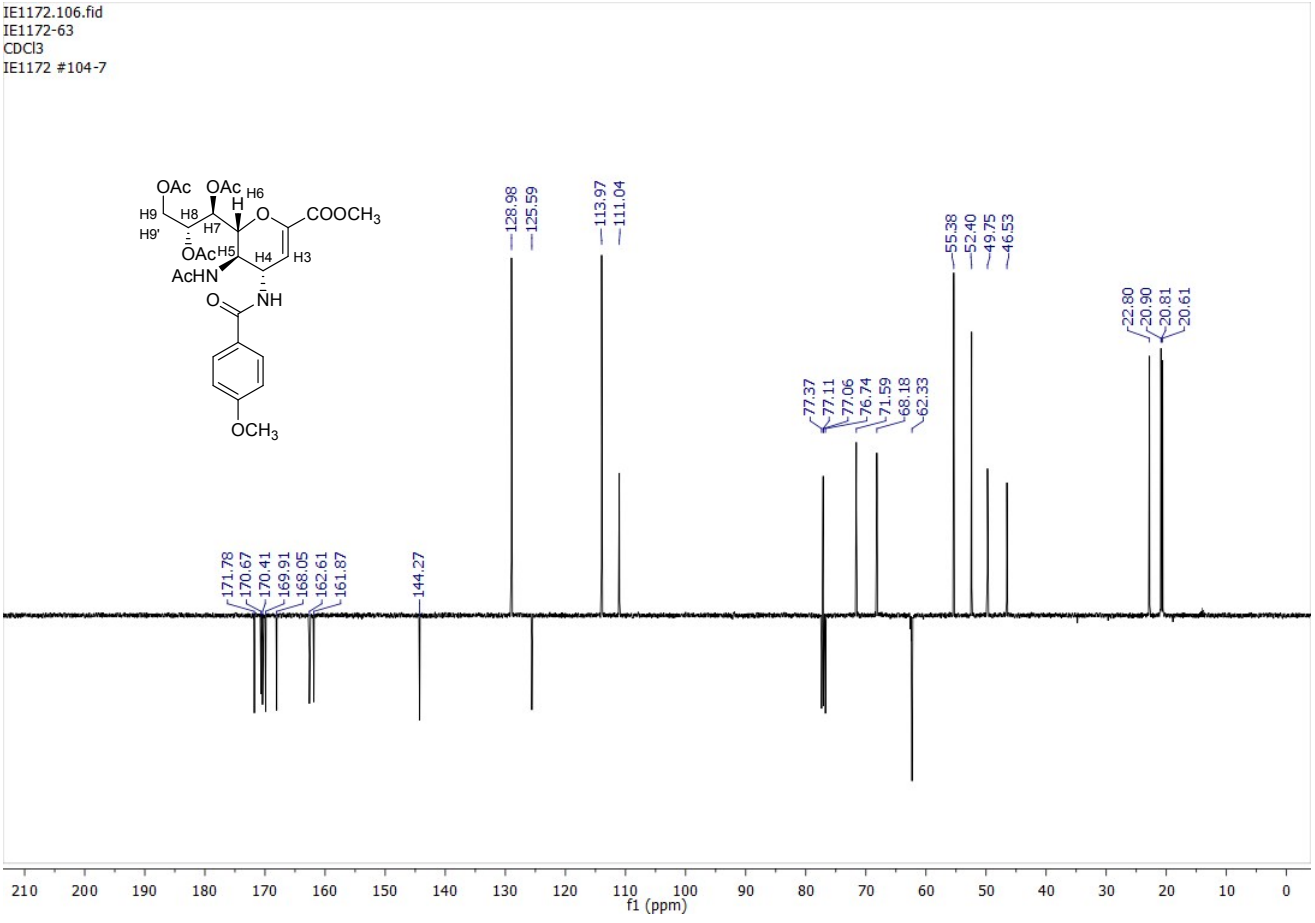
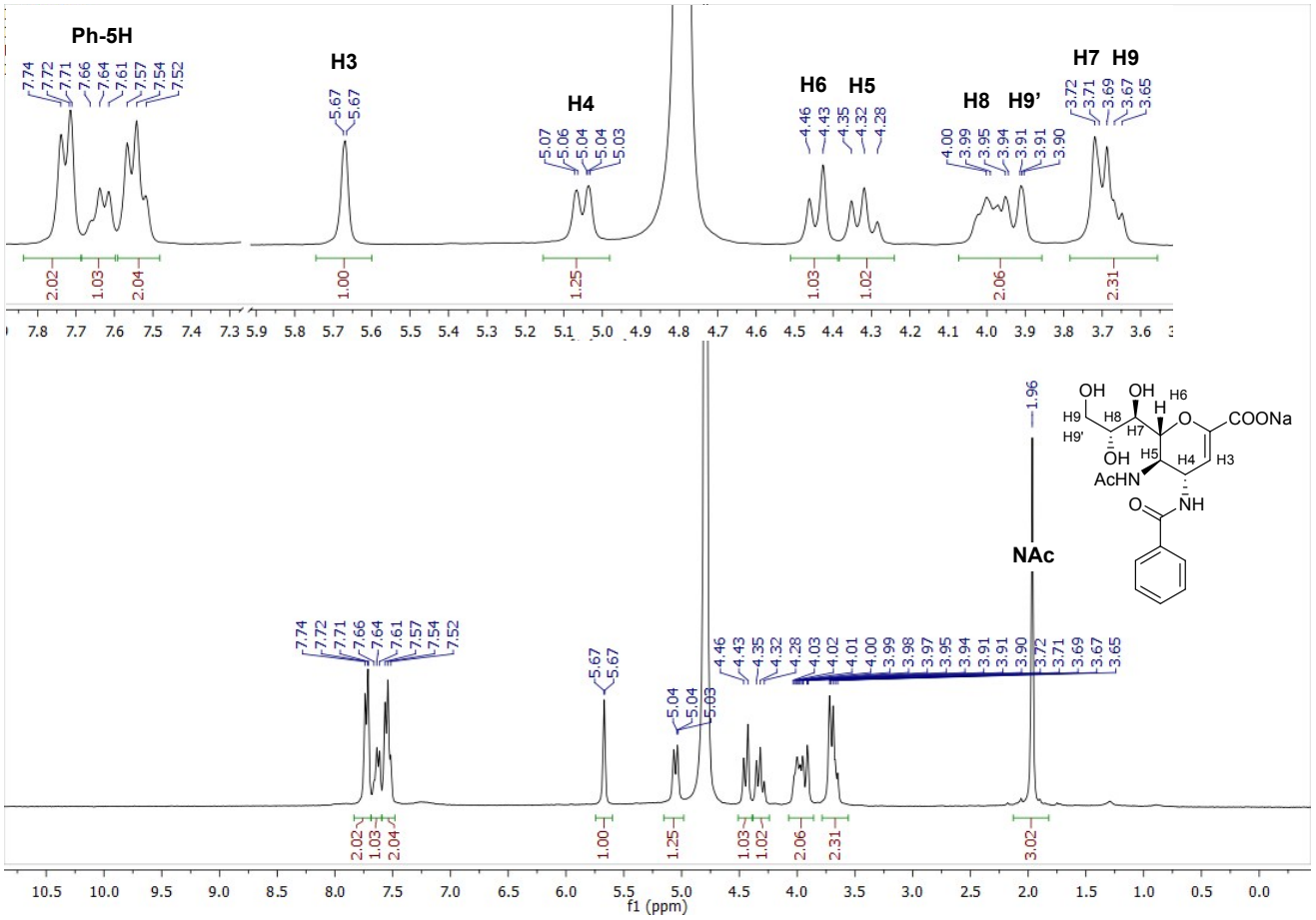
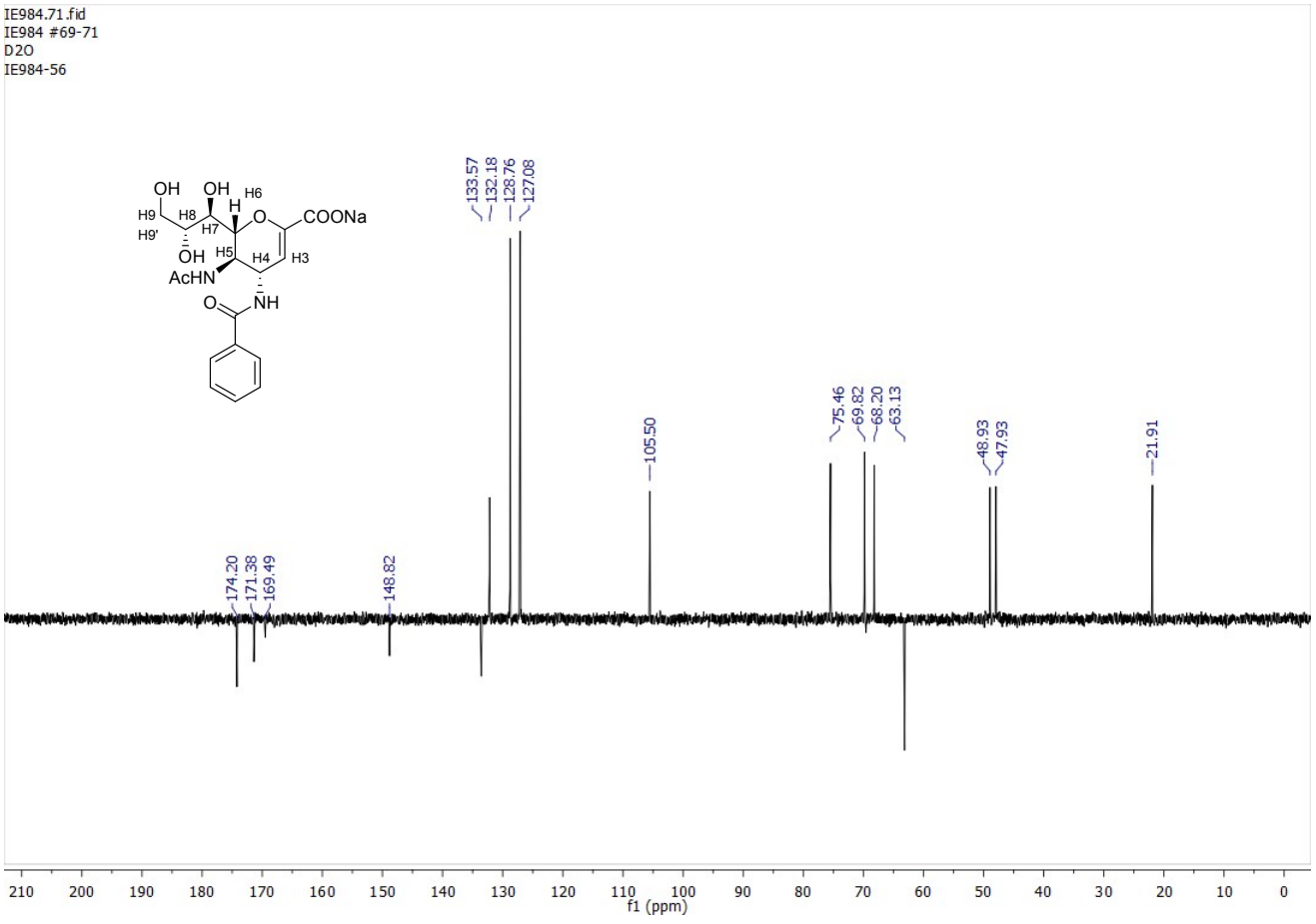


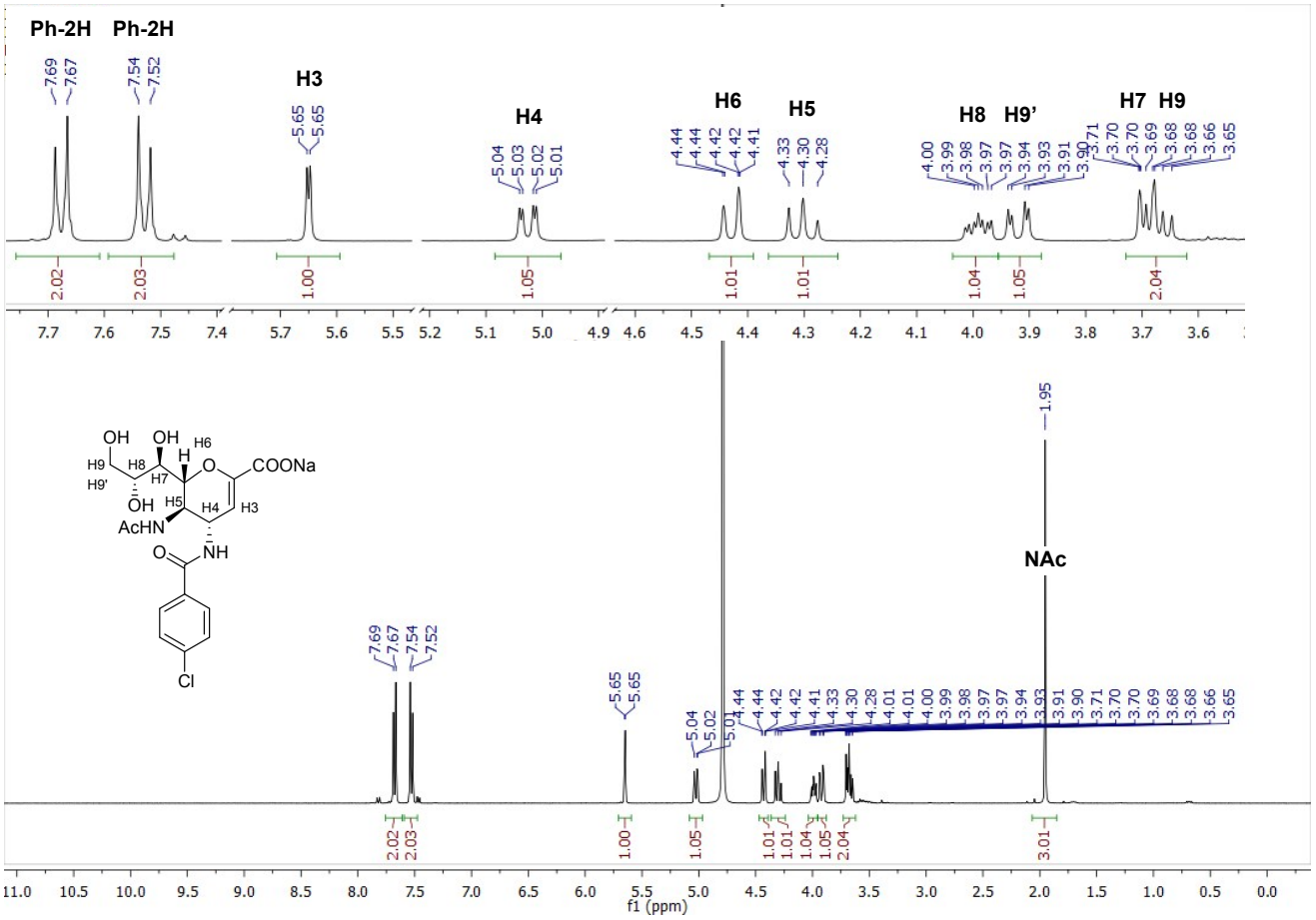
^{13}C NMR spectrum of **10c** (100 MHz, $CDCl_3$)



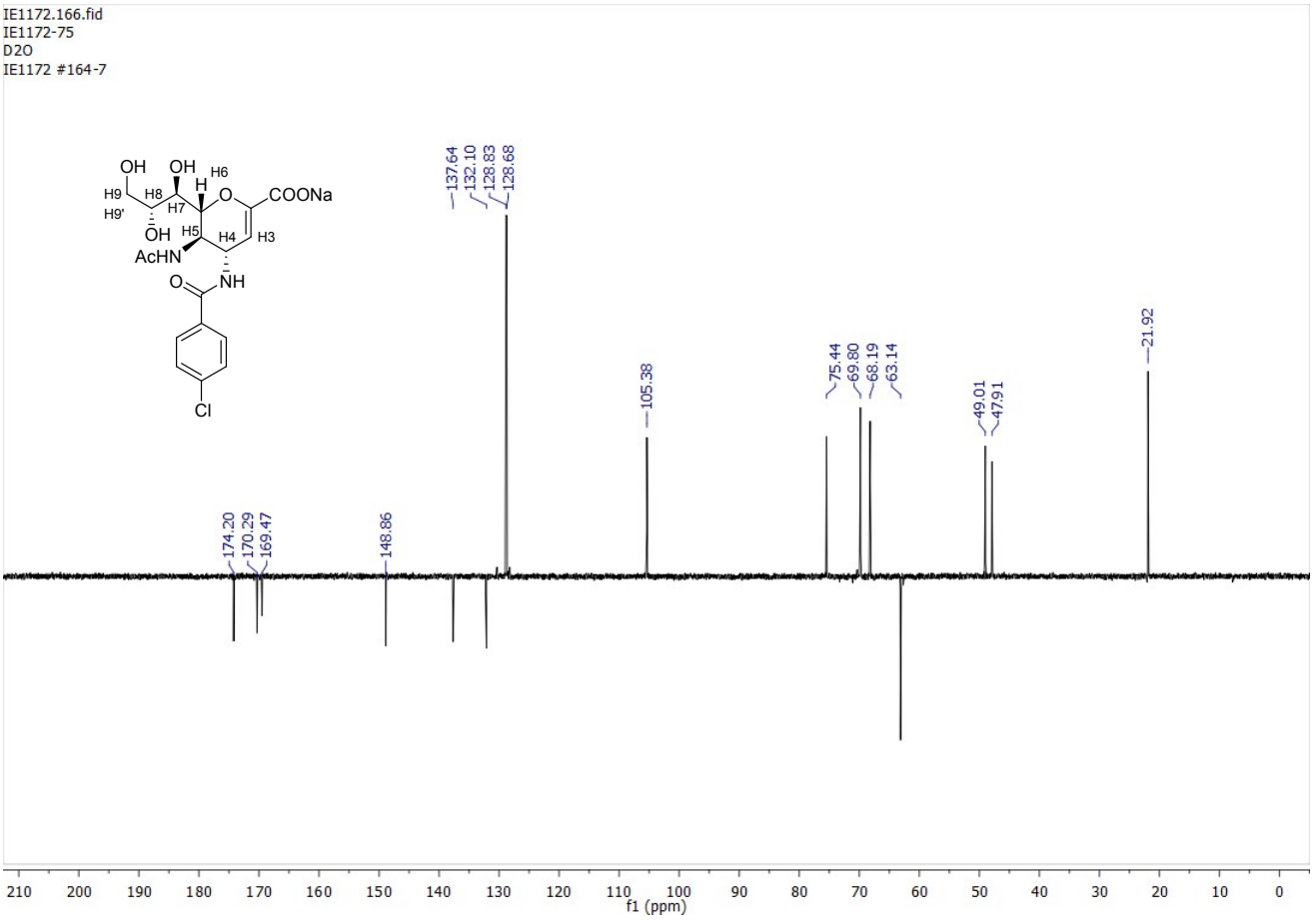
^{1}H NMR spectrum of **11a** (400 MHz, D_2O)



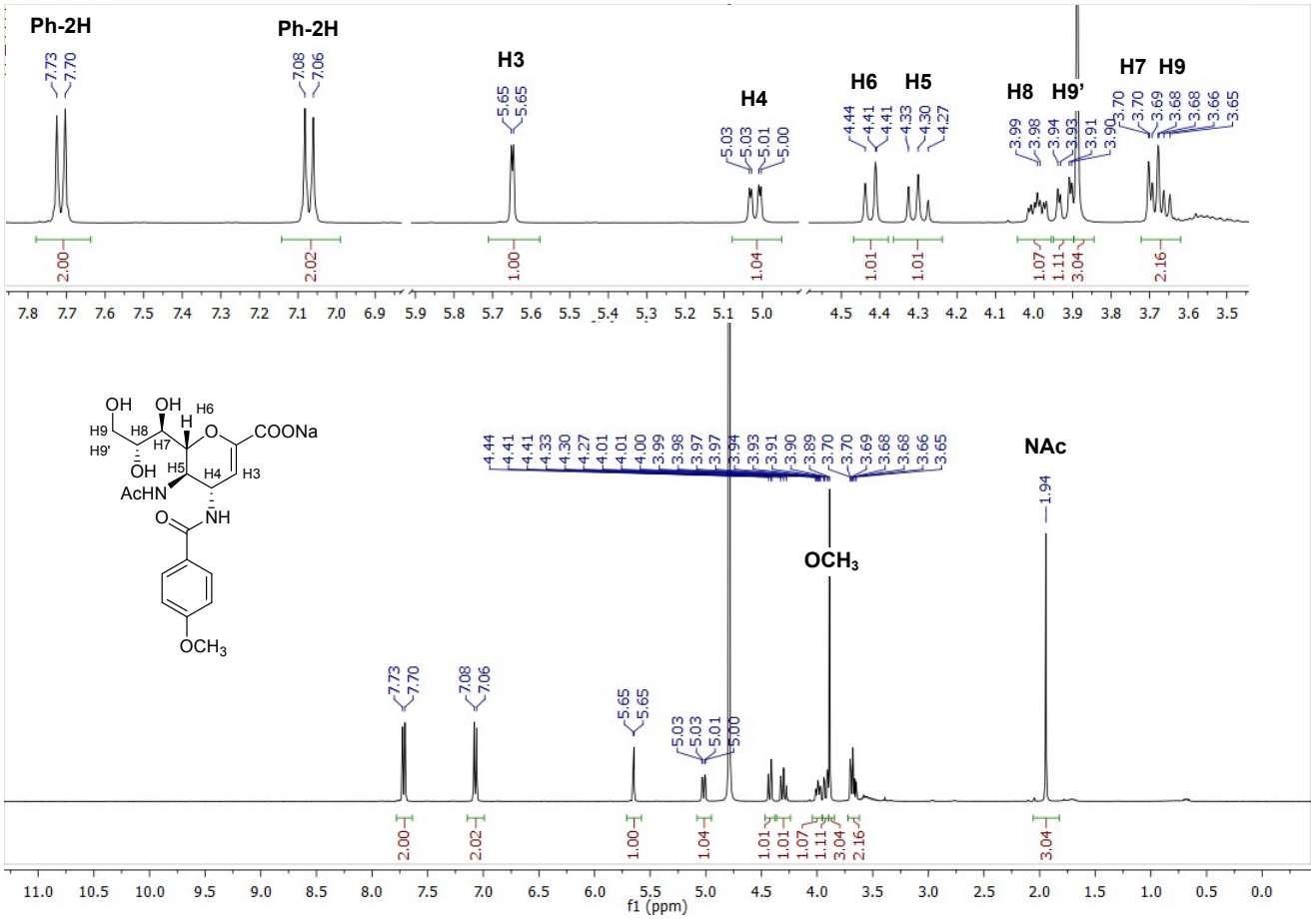
^{13}C NMR spectrum of **11a** (100 MHz, D_2O)



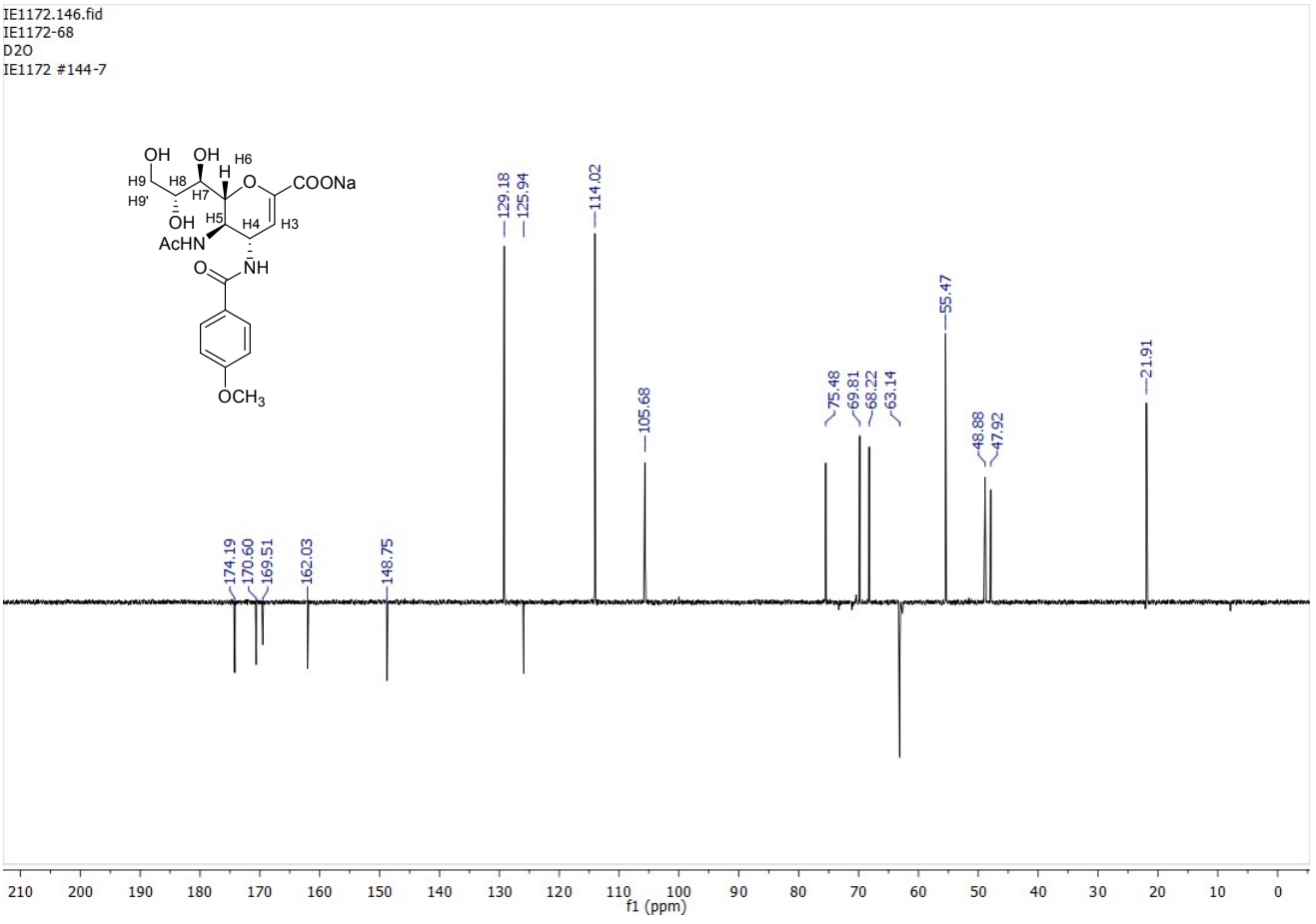
^{1}H NMR spectrum of **11b** (400 MHz, D_2O)



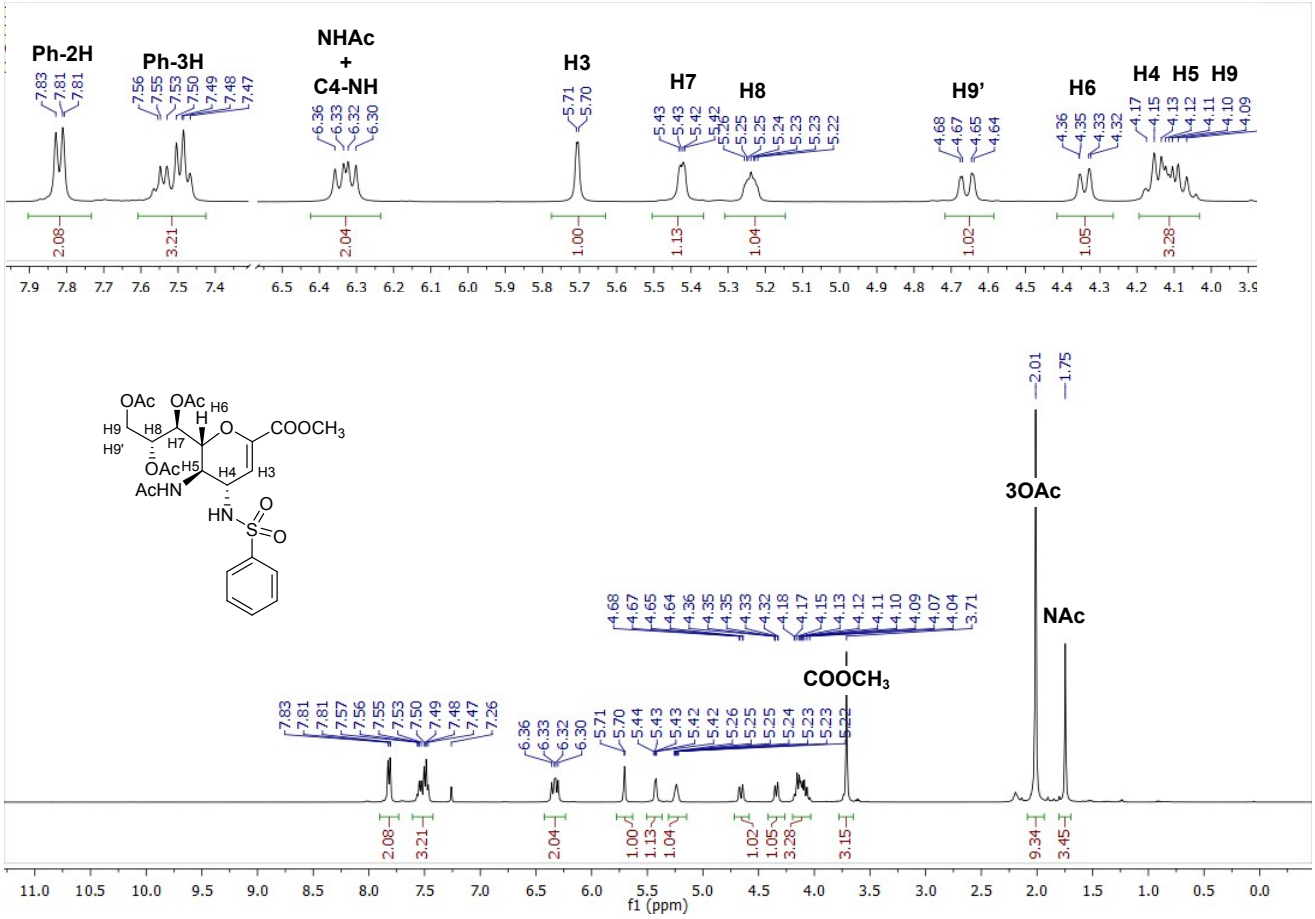
^{13}C NMR spectrum of **11b** (100 MHz, D_2O)



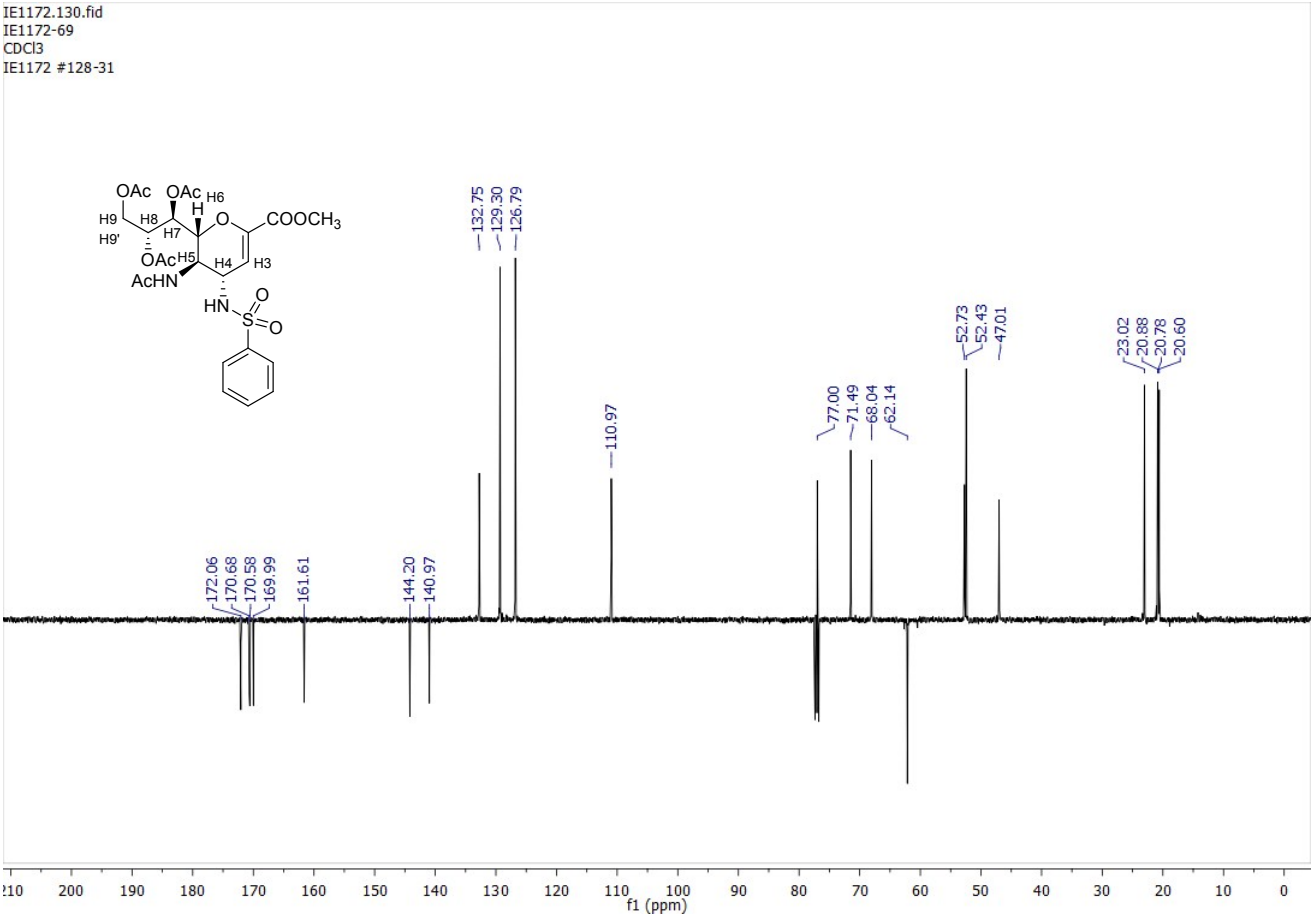
^{1}H NMR spectrum of **11c** (400 MHz, D_2O)



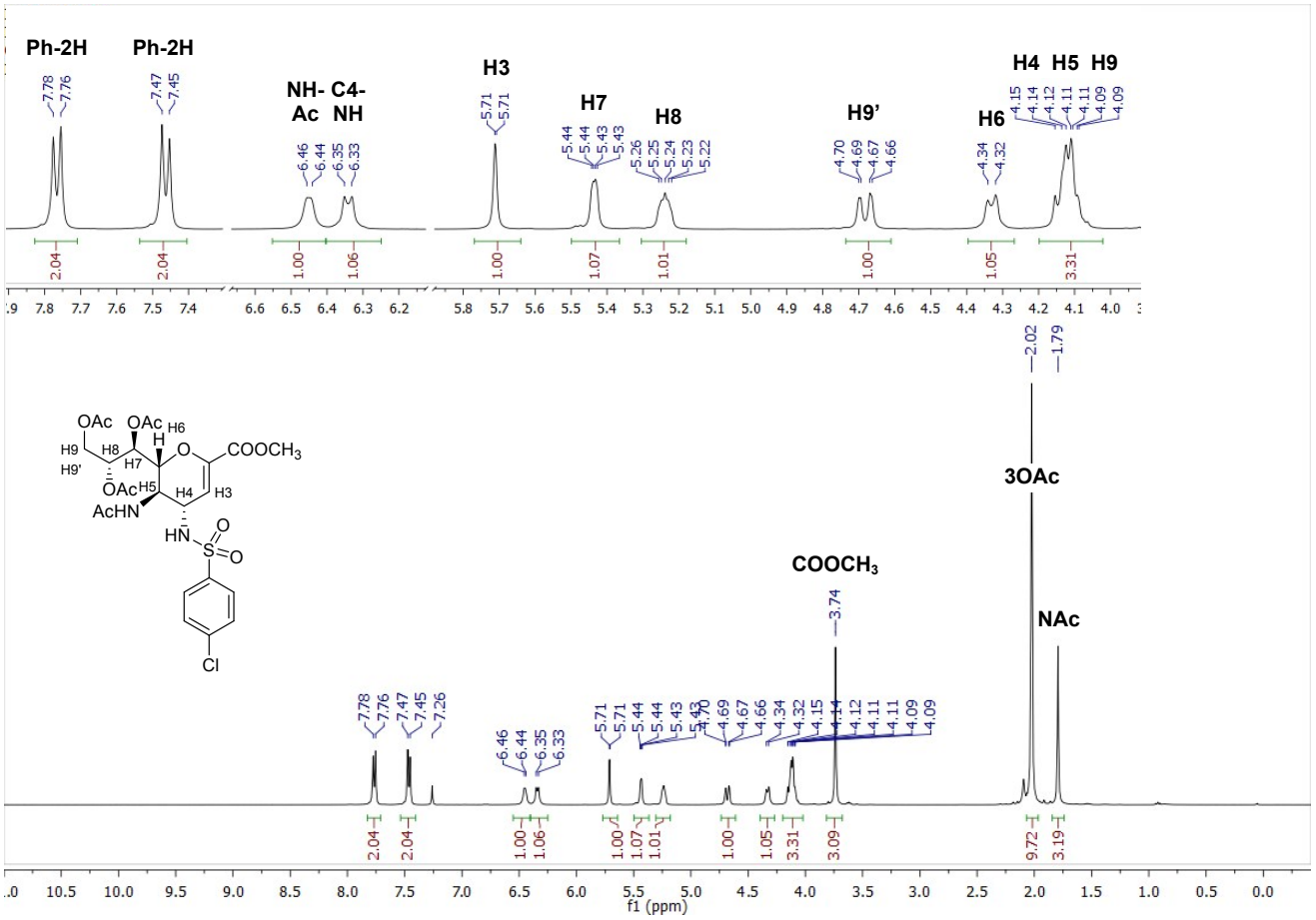
^{13}C NMR spectrum of **11c** (100 MHz, D_2O)



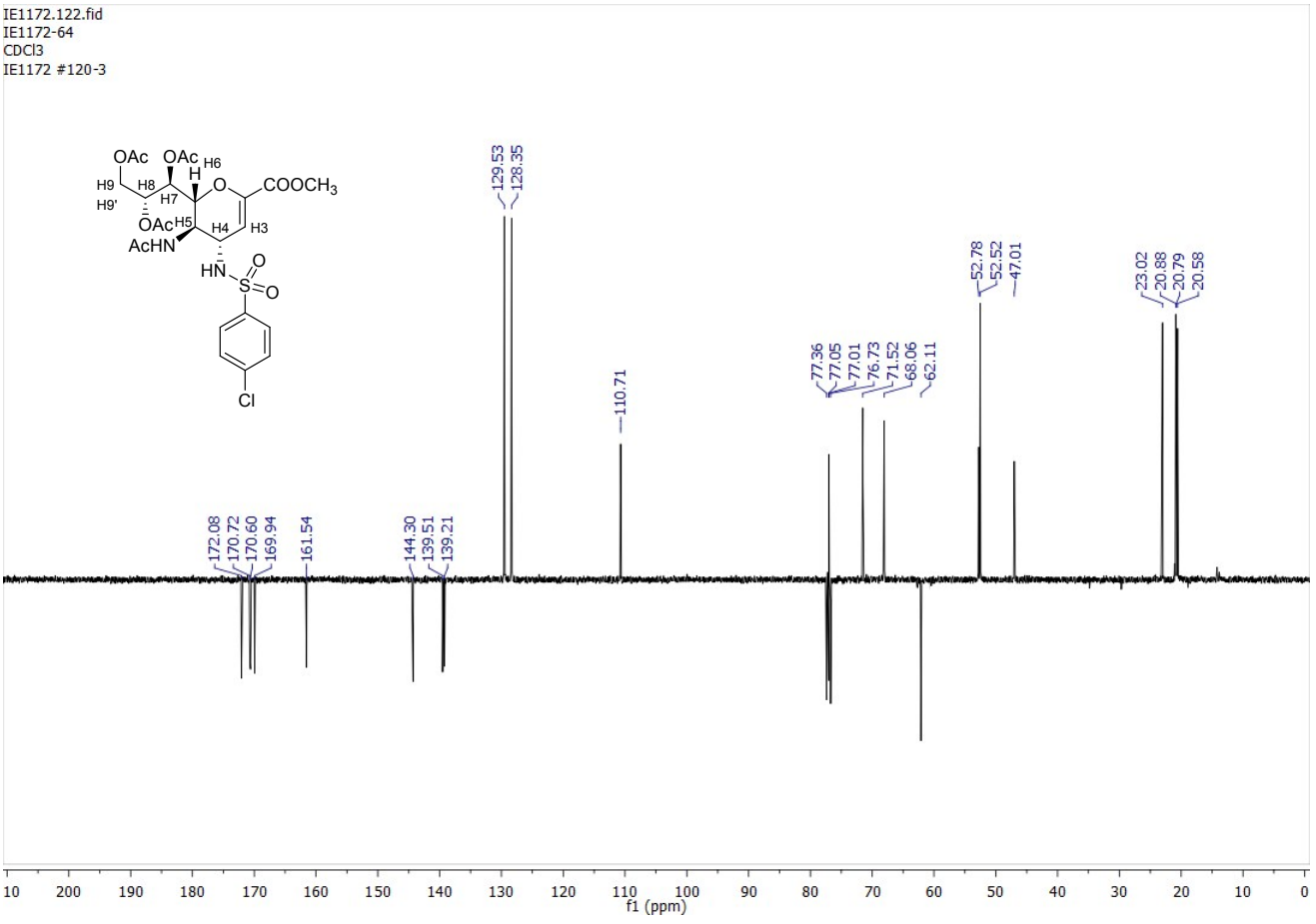
^{1}H NMR spectrum of **12a** (400 MHz, $CDCl_3$)



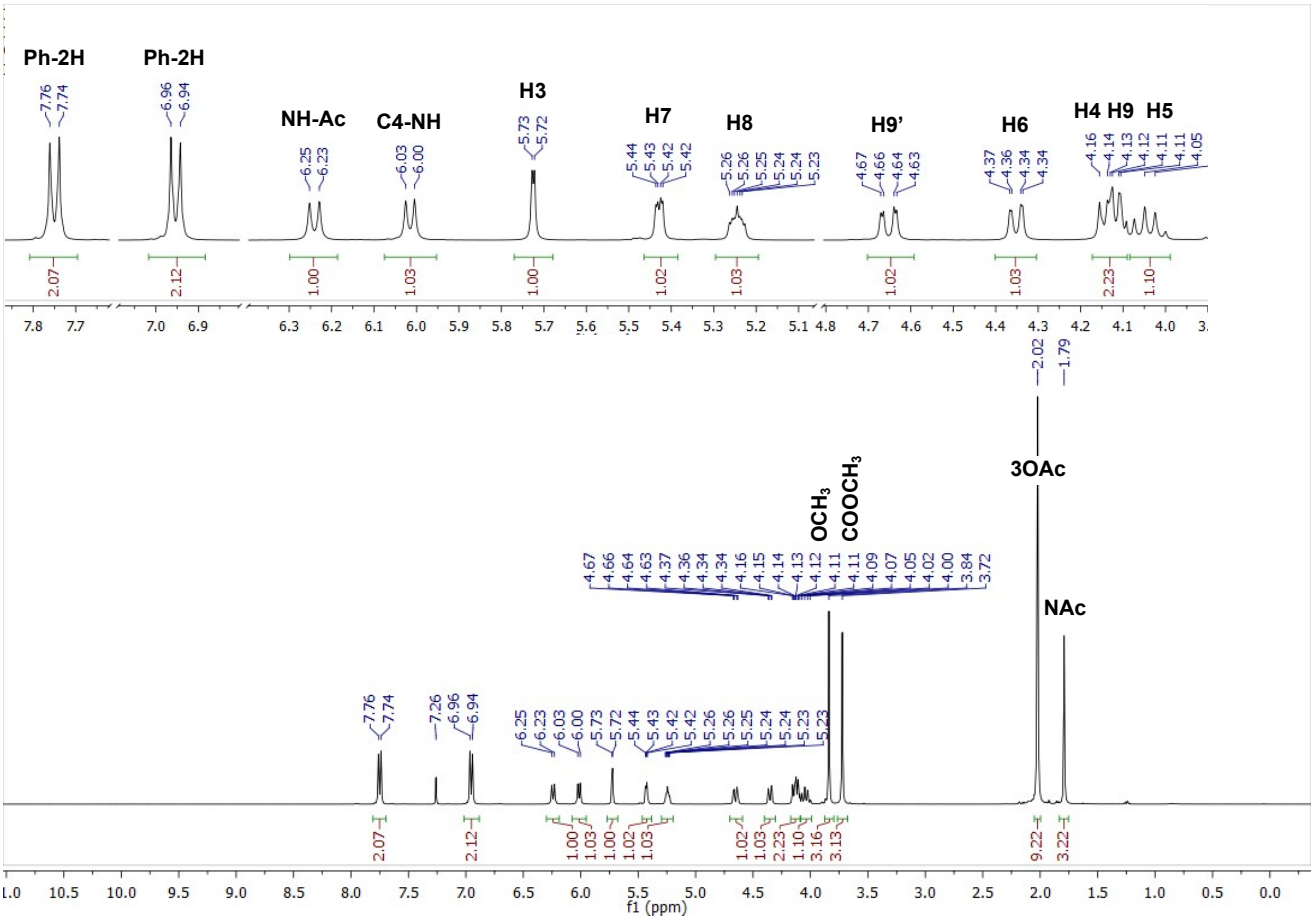
^{13}C NMR spectrum of **12a** (100 MHz, $CDCl_3$)



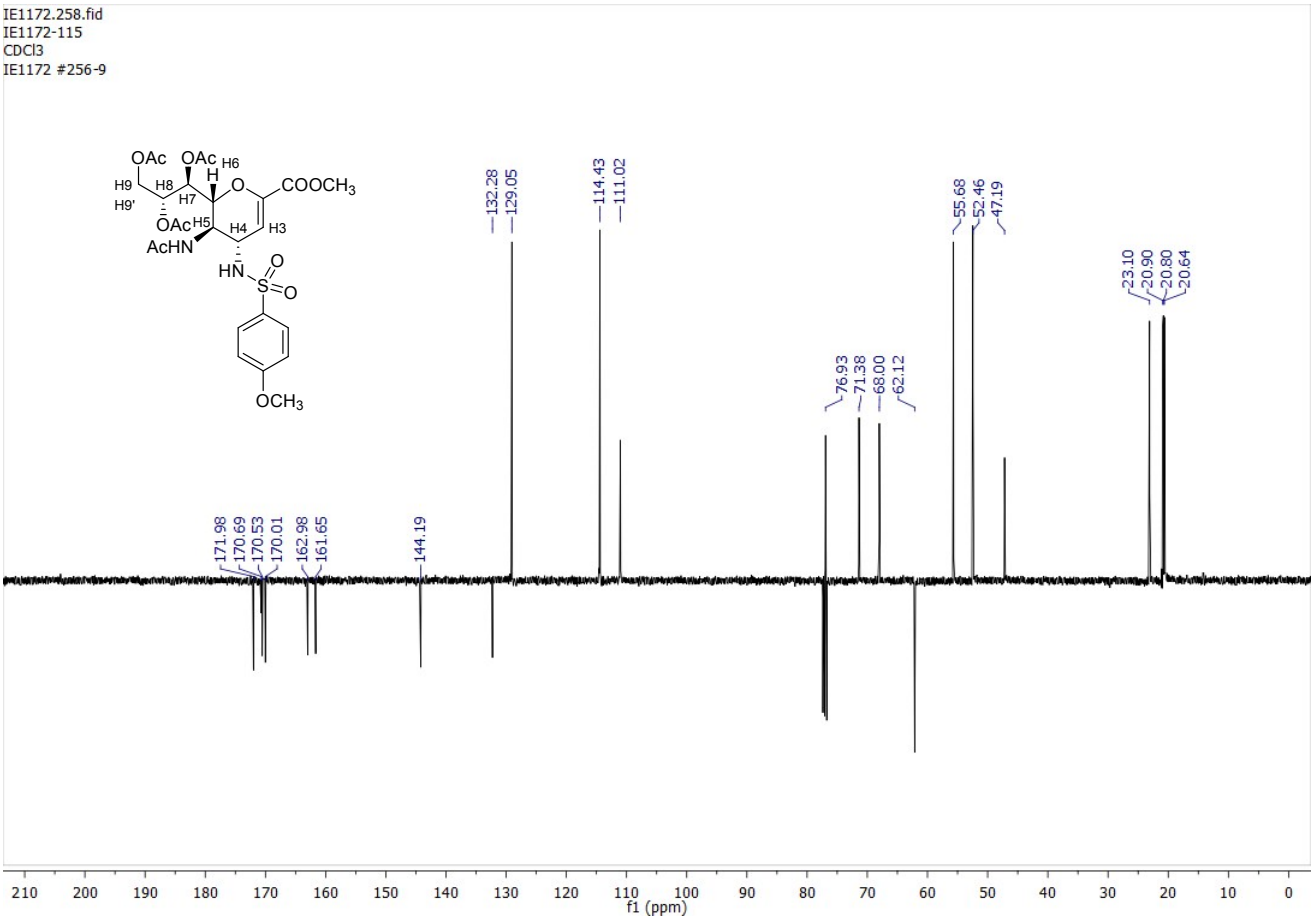
^{1}H NMR spectrum of **12b** (400 MHz, $CDCl_3$)



^{13}C NMR spectrum of **12b** (100 MHz, $CDCl_3$)



^{1}H NMR spectrum of **12c** (400 MHz, $CDCl_3$)



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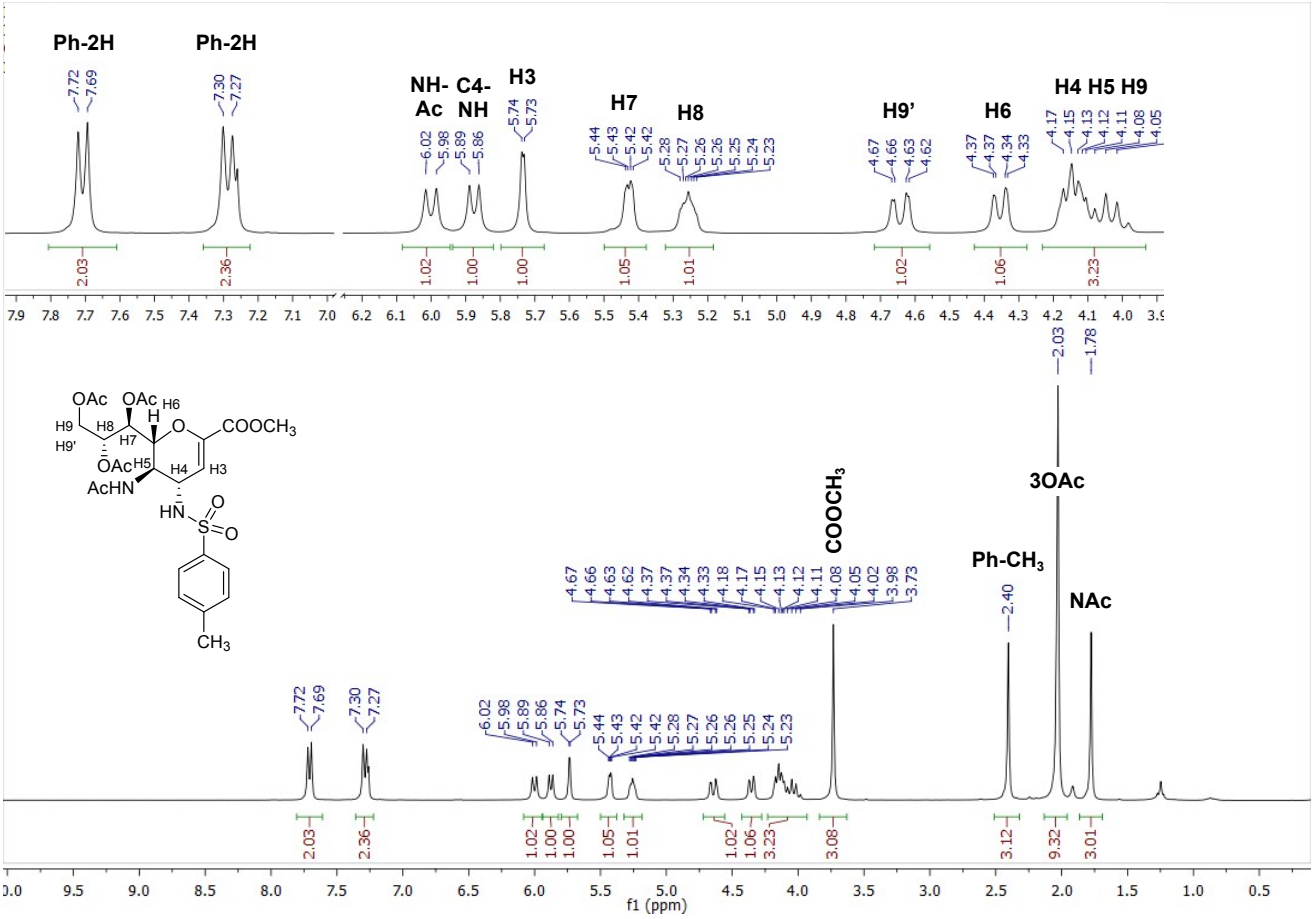

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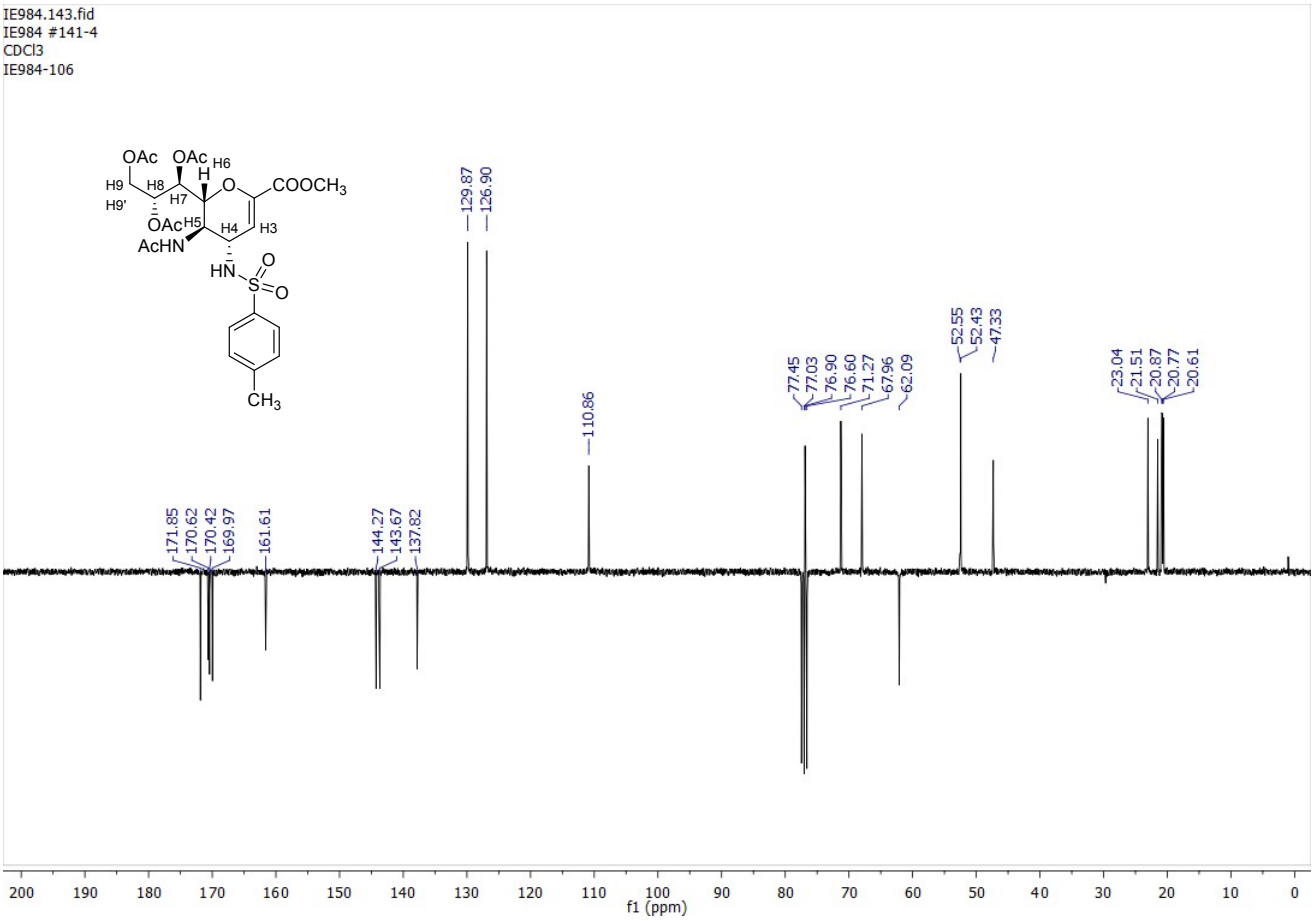
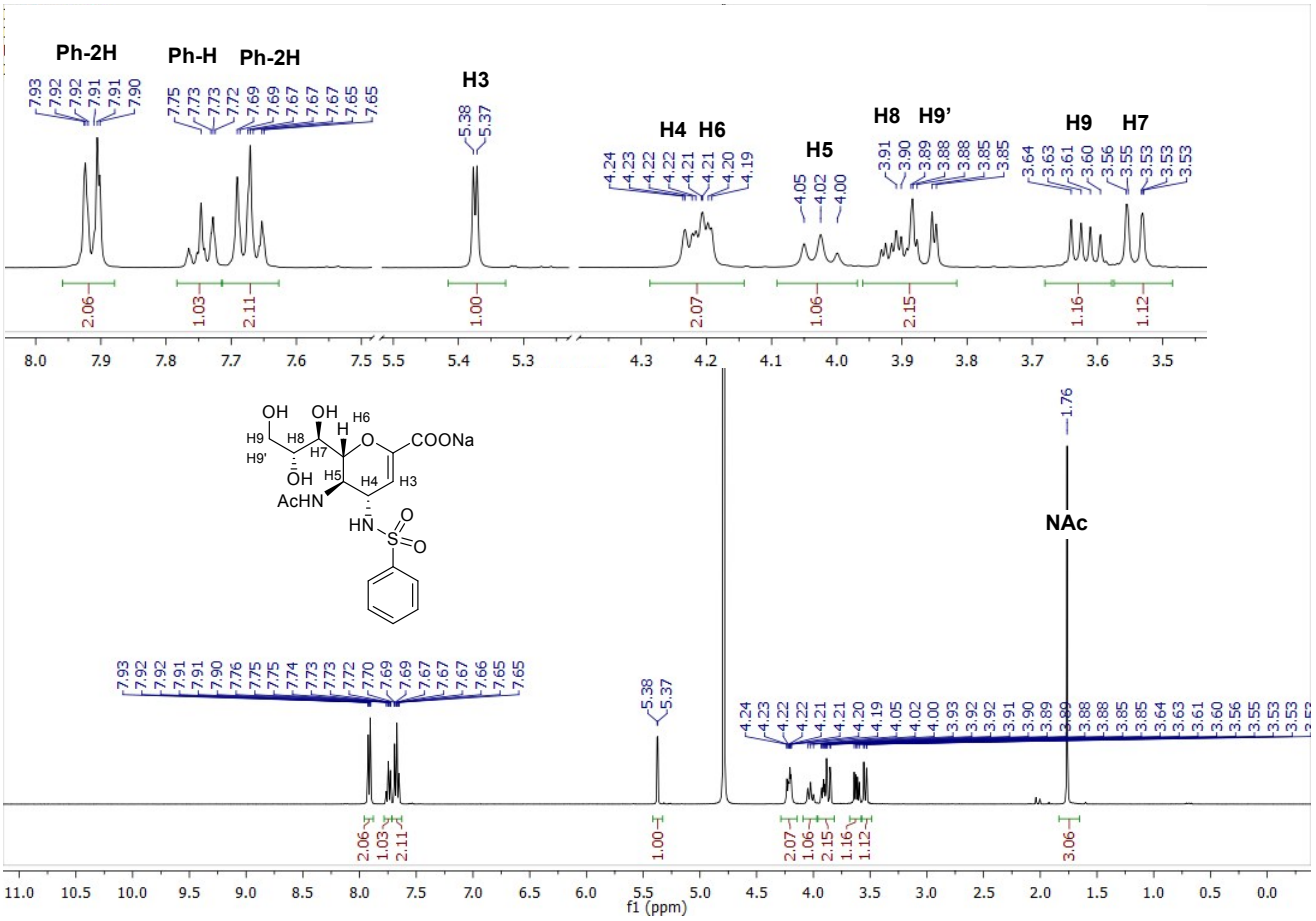
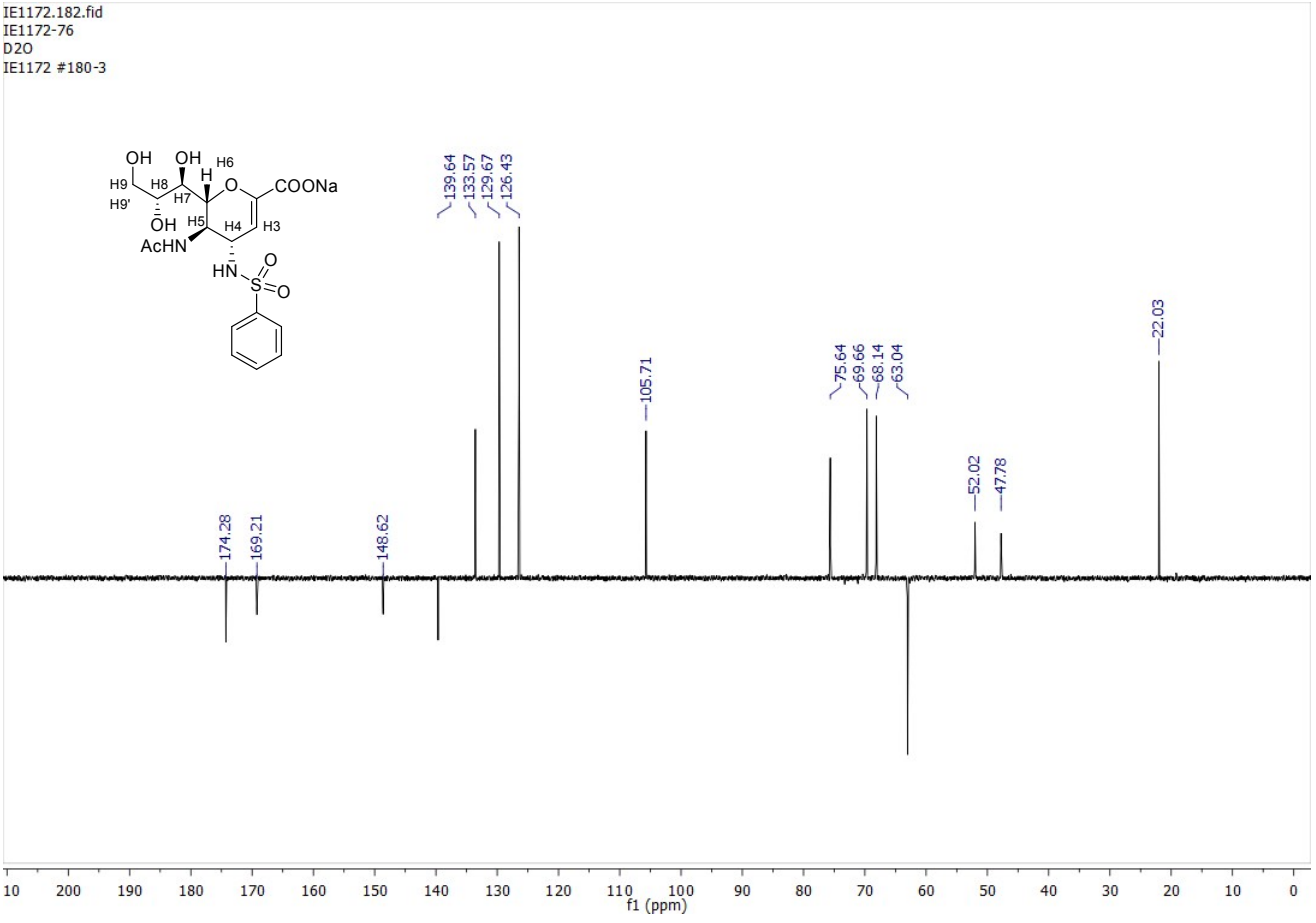


^{13}C NMR spectrum of **12d** (100 MHz, $CDCl_3$)



^{1}H NMR spectrum of **13a** (400 MHz, D_2O)



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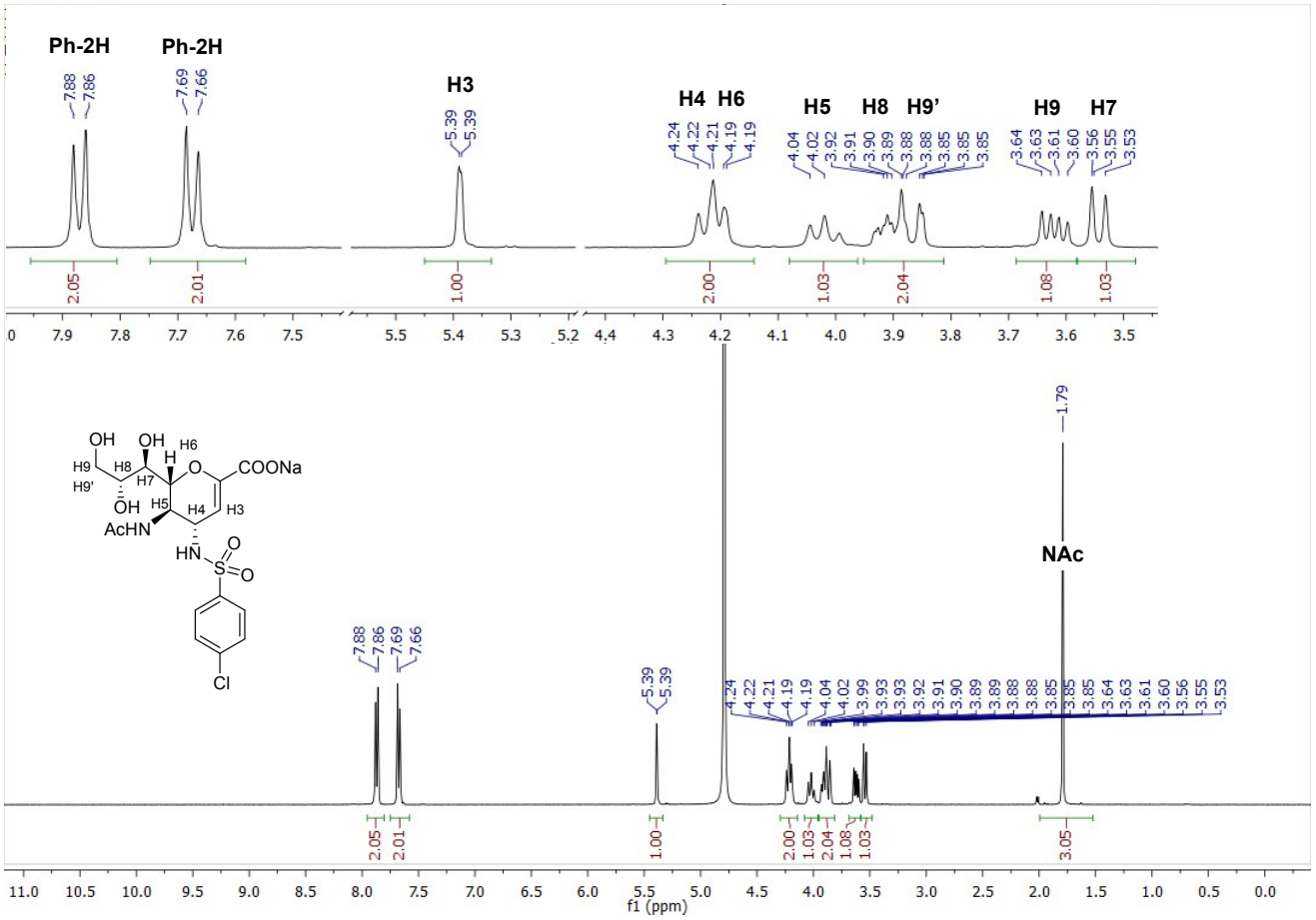
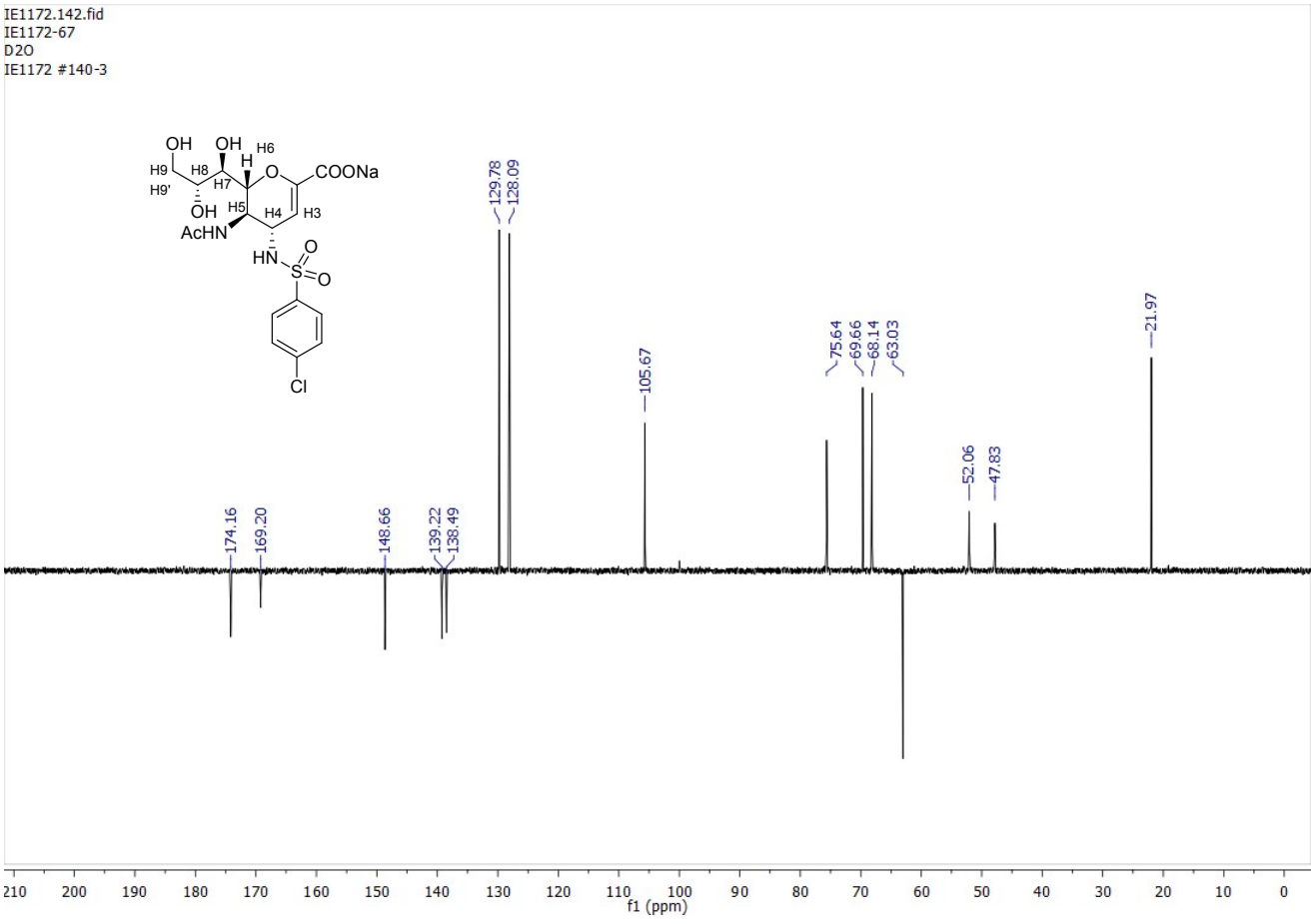
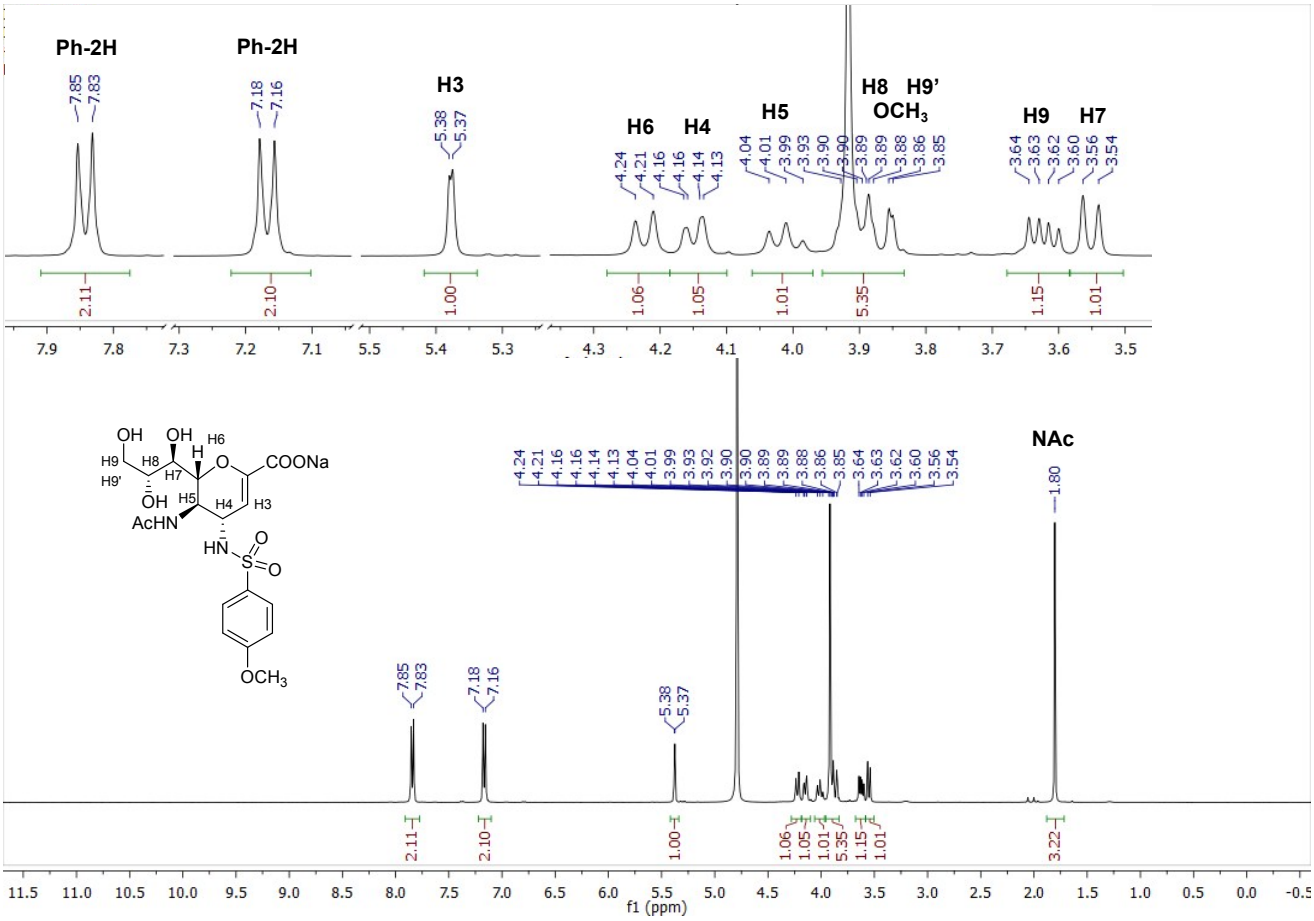


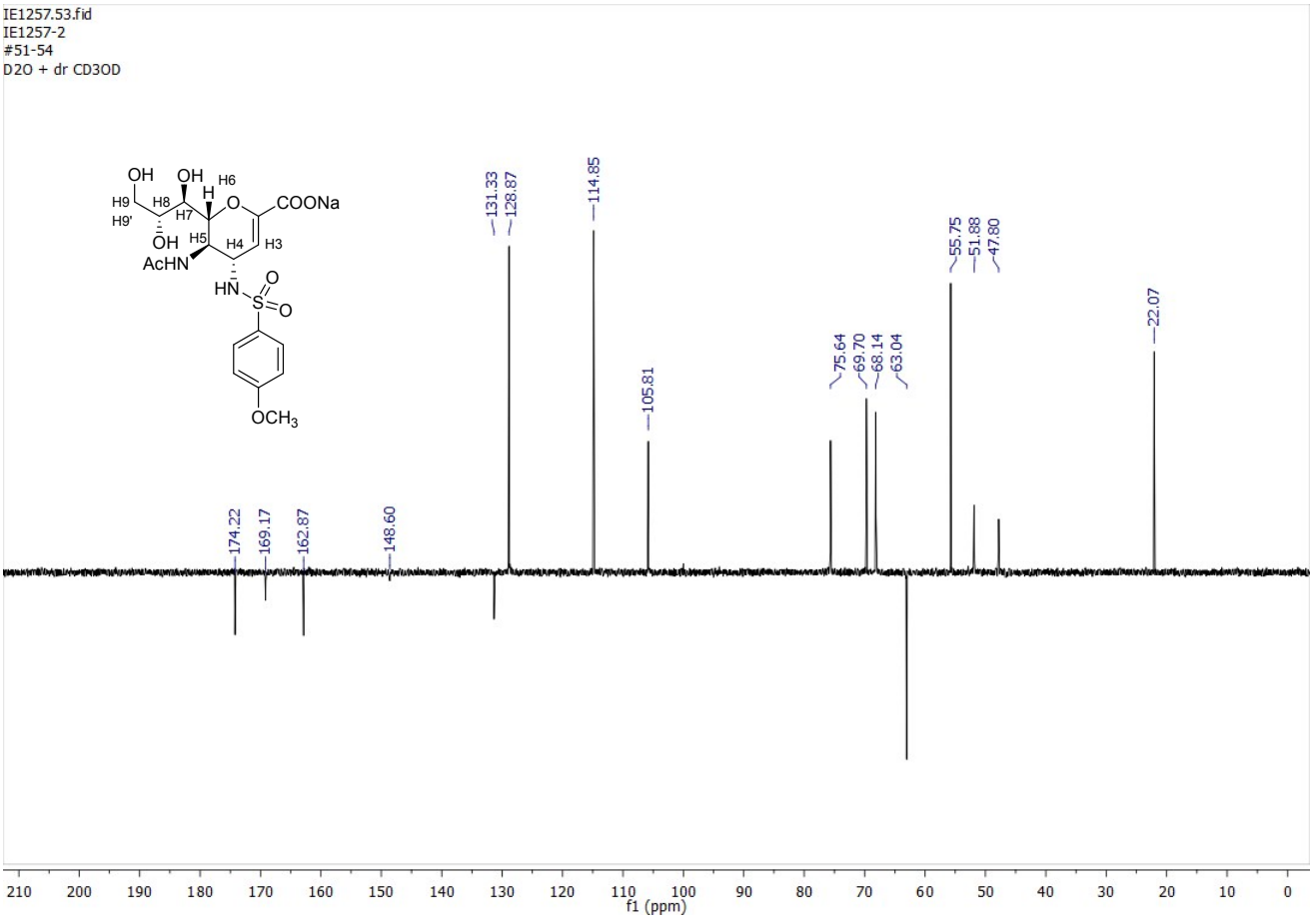
^{1}H NMR spectrum of **13b** (400 MHz, D_2O)



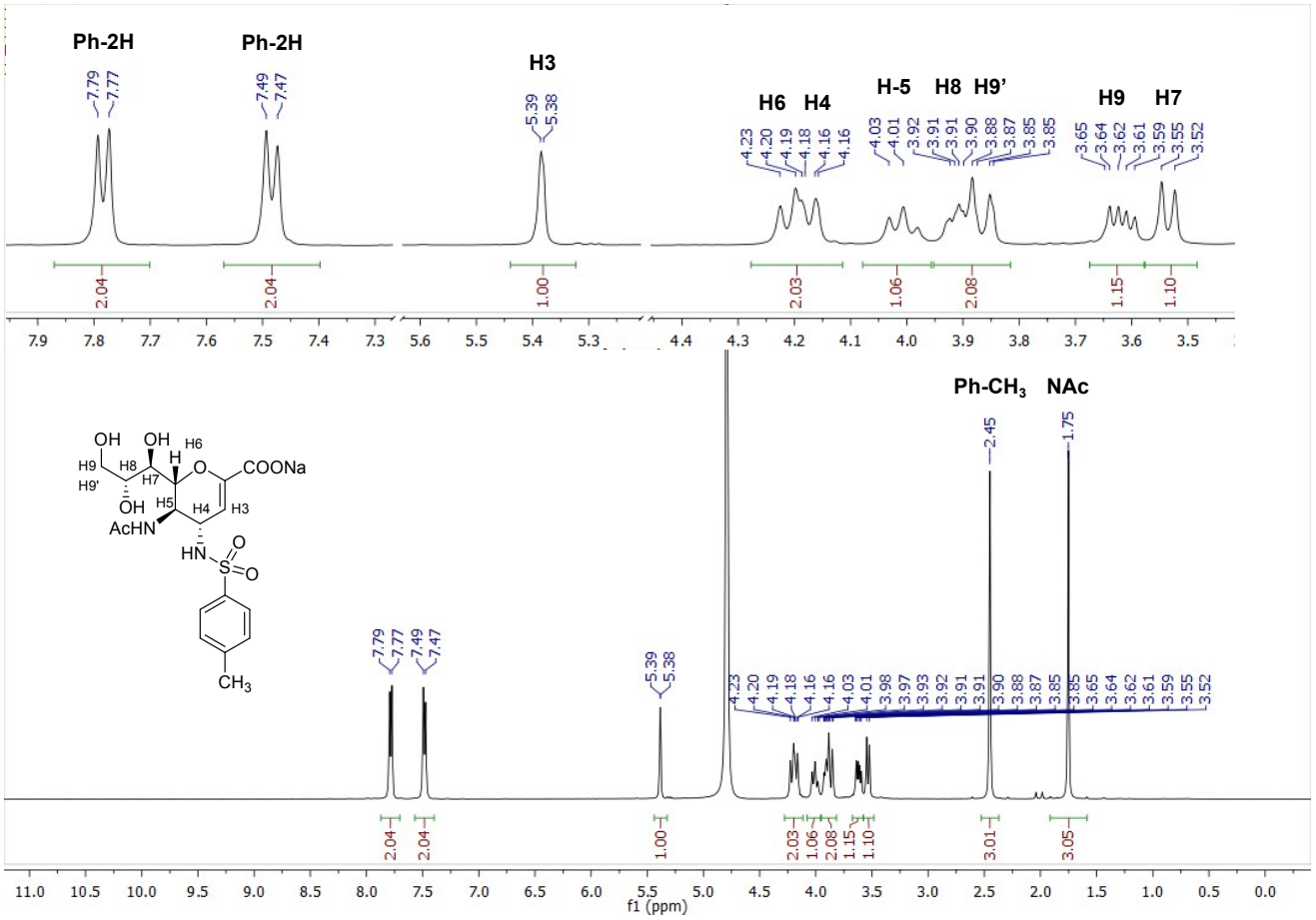
^{13}C NMR spectrum of **13b** (100 MHz, D_2O)



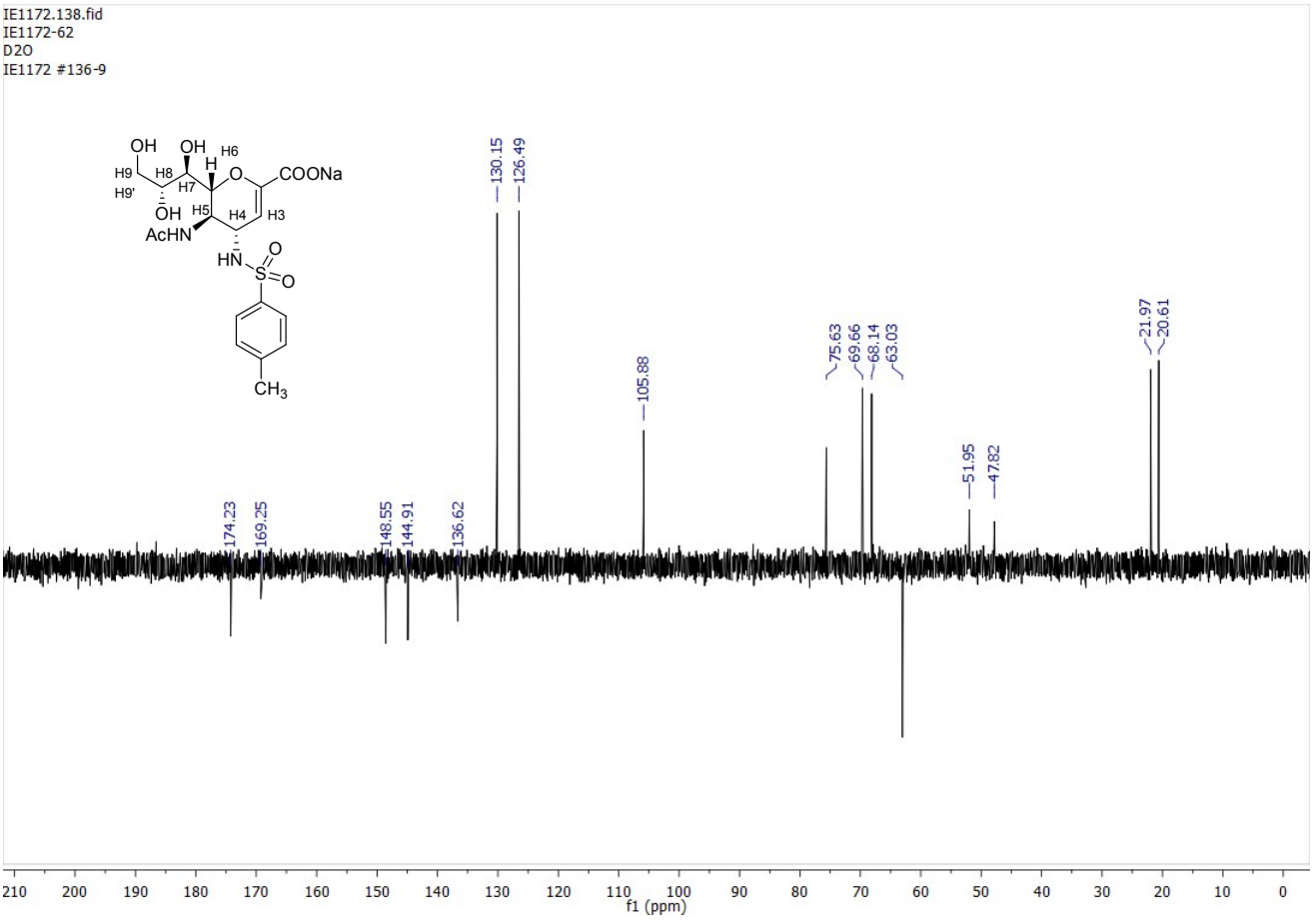
^{1}H NMR spectrum of **13c** (400 MHz, D_2O)



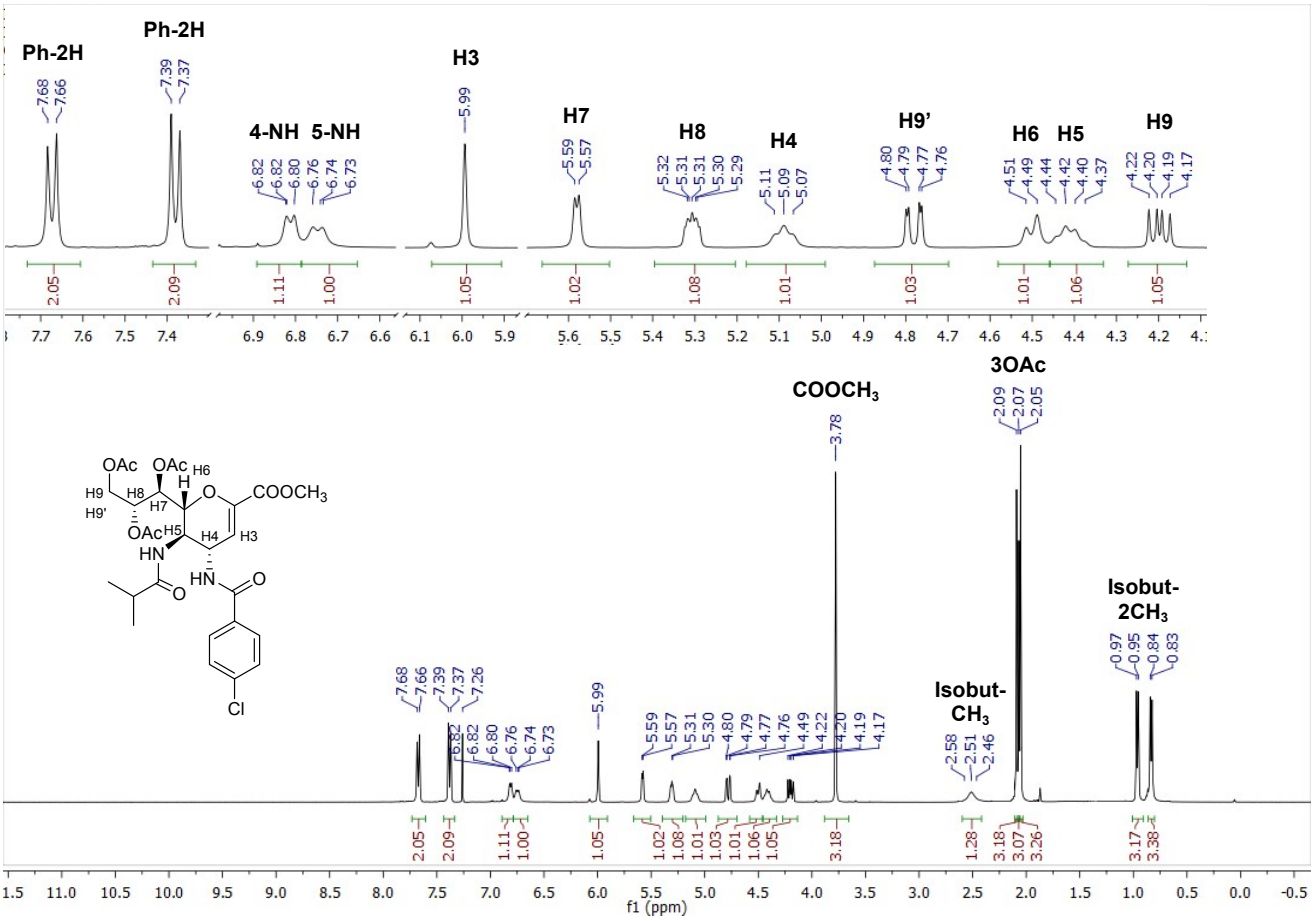
^{13}C NMR spectrum of **13c** (100 MHz, D_2O)



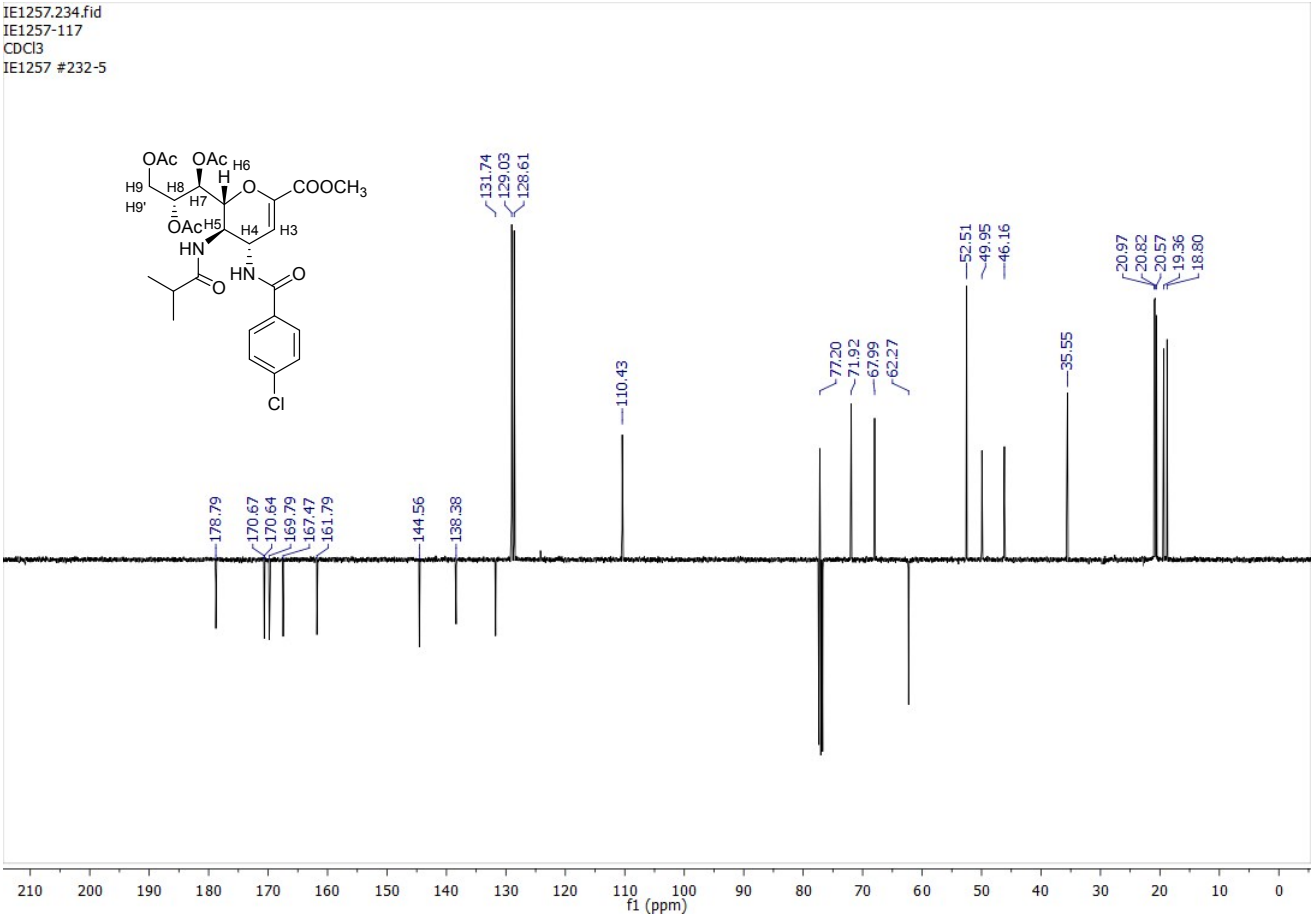
^{1}H NMR spectrum of **13d** (400 MHz, D_2O)



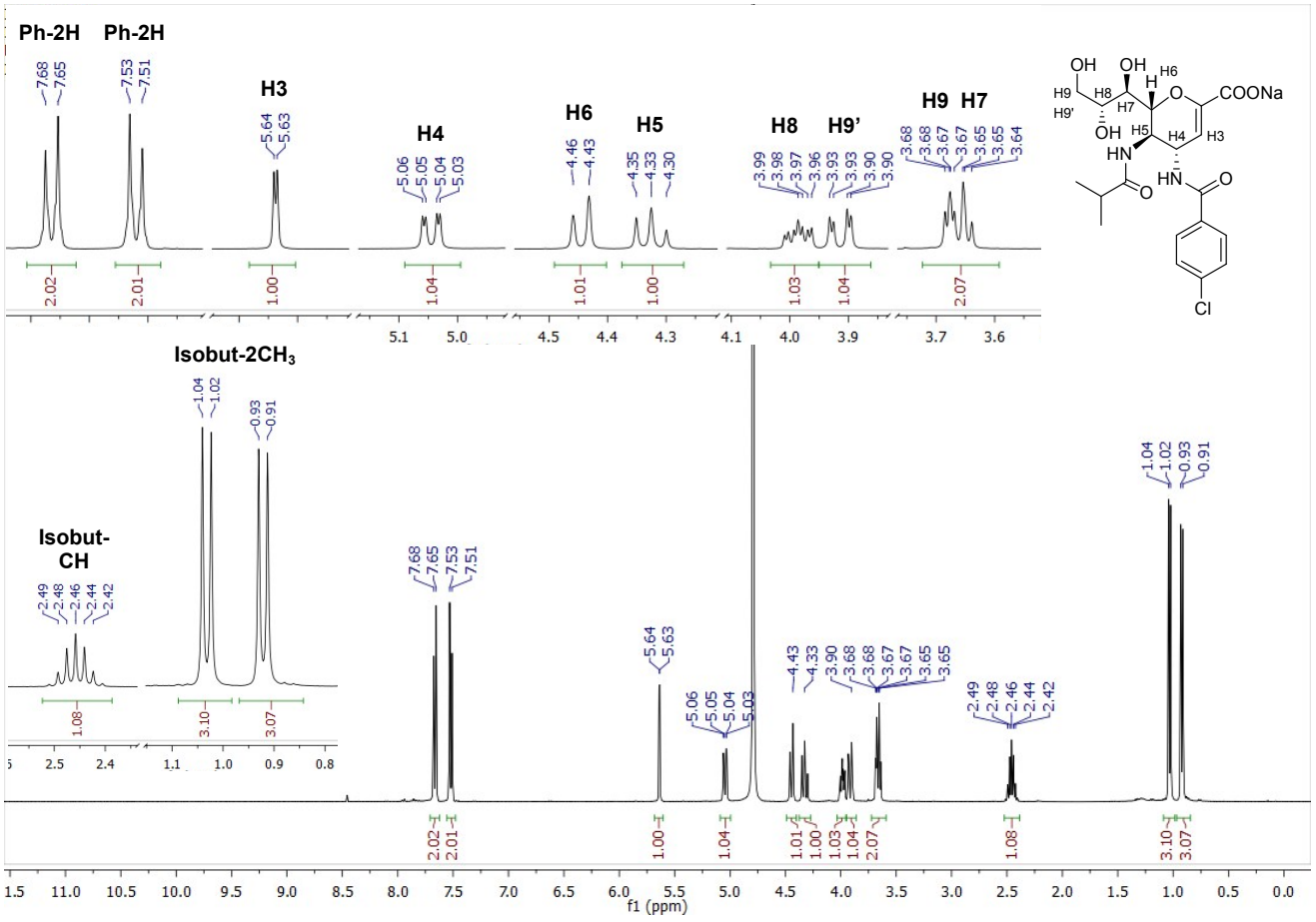
^{13}C NMR spectrum of **13d** (100 MHz, D_2O)



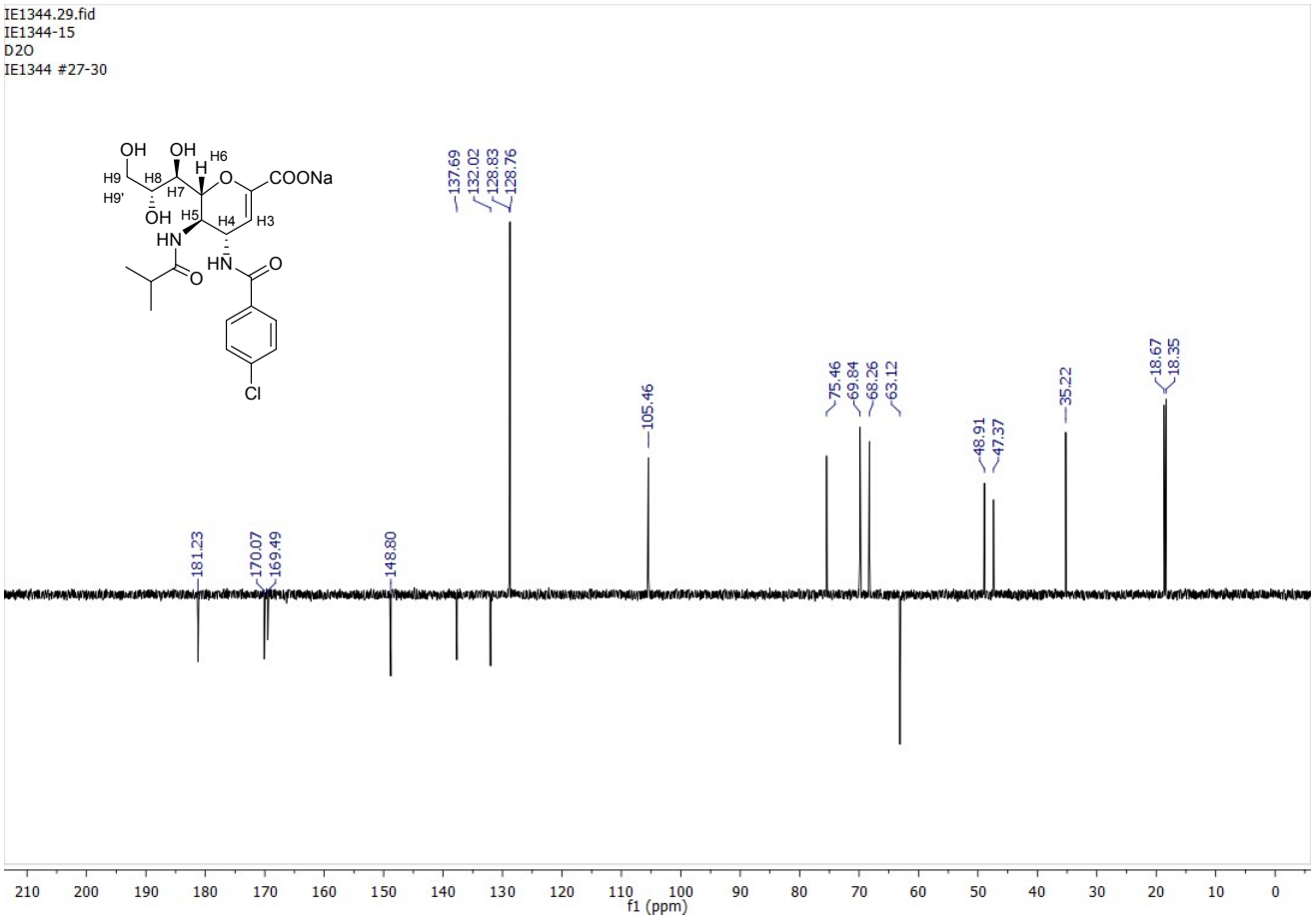
^{1}H NMR spectrum of **15** (400 MHz, $CDCl_3$)



^{13}C NMR spectrum of **15** (100 MHz, $CDCl_3$)



^{1}H NMR spectrum of **16** (400 MHz, $CDCl_3$)



^{13}C NMR spectrum of **16** (100 MHz, $CDCl_3$)

Computational Chemistry

Molecular Modeling studies were performed using "Molecular Operating Environment (MOE) version 2008.10", Chemical Computing Group Inc., 1010 Sherbrooke Street West, Suite 910, Montreal, H3A 2R7, Canada. The simulated compounds were built using the builder interface of the MOE program and subjected to energy minimization using the standard Forcefield MMFF94x calculations. The distance between the triazole N-1 and C-1 of the phenyl ring in reference inhibitors **5** and **6**; or between C4-nitrogen and C1 of the phenyl ring in the new derivatives was measured using the standard "atom to atom" distance measurement tool in the modeling software. Structures alignments of the built compounds were performed using the flexible alignment function in the software, setting the parameters to the following: Iteration limit = 200, failure limit = 20, energy cut-off = 10, enabling both "forcefield charges calculation prior to search" and "Stochastic conformational search". The top scoring aligned structures were always picked from the output database.

Biological Screening

The neuraminidase activity and neuraminidase inhibition of purified hPIV-3 was assayed using a method adapted from Potier et al[4] and based on a previously described[1, 2] end-point measurement of the relative fluorescence of 4-methylumbelliferone, the product of the hPIV-3 HN enzymatic hydrolysis of MUN (Sigma-Aldrich, St Louis, MO).

Briefly, purified hPIV-3, inhibitors and MUN were diluted in neuraminidase reaction buffer (NaOAc 50 mM, $CaCl_2$ 5 mM, pH 4.6). Neuraminidase activity of the virus was initially measured to determine the lowest virus concentration to be used in the assays to obtain a maximal fluorescence signal at least 5 times higher than the background for the experiment. Neuraminidase inhibition (NI) assays were done in duplicate. For each concentration tested, 2 µL of purified hPIV-3 and 4 µL of 2.5X inhibitor solution (1X final) was added to each well. The plate was kept at room temperature for 20 min before 4 µL of 5 mM MUN (2 mM final) was added to each well. The plate was then incubated at 37 ºC for 30 min with agitation (1000 rpm) and the enzymatic reaction was stopped by the addition of 190 µL of glycine buffer (glycine 0.25 M, pH 10.4) to each well. A negative control was included by the addition of MUN only to virus and then the enzymatic reaction stopped at t = 0. Relative fluorescence (RF) was measured with a Victor 3 multilabel reader (PerkinElmer, Waltham, MA). Data were processed by background subtraction (negative control RF) and then analysed with GraphPadPrism 4 (GraphPad Software Inc., La Jolla, CA) to calculate IC_{50} values (nonlinear regression (curve fit), Dose-response - inhibition, 3 parameter logistic). For each inhibitor, the concentration that reduced the maximal neuraminidase activity (RF) by 50% was considered to be the NI IC_{50} value.

References

1. P. Guillon, L. Dirr, I. M. El-Deeb, M. Winger, B. Bailly, T. Haselhorst, J. C. Dyason and M. von Itzstein, *Nature communications*, 2014, **5**, 5268.
2. I. M. El-Deeb, P. Guillon, M. Winger, T. Eveno, T. Haselhorst, J. C. Dyason and M. von Itzstein, *Journal of medicinal chemistry*, 2014, **57**, 7613-7623.
3. D. Ye, W. J. Shin, N. Li, W. Tang, E. Feng, J. Li, P. L. He, J. P. Zuo, H. Kim, K. Y. Nam, W. Zhu, B. L. Seong, K. T. No, H. Jiang and H. Liu, *Eur J Med Chem*, 2012, **54**, 764-770.
4. M. Potier, L. Mameli, M. Belisle, L. Dallaire and S. B. Melancon, *Analytical biochemistry*, 1979, **94**, 287-296.