Supporting Information

Rapid and Modular Assembly of Click Substrates to Assay Enzyme Activity in the Newborn Screening of Lysosomal Storage Disorders

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1) General methods

All reagents, unless otherwise noted, were purchased from commercial sources without further purification. Dichloromethane, methanol, THF and diethyl ether were dried using PURESOLV- columns (Inert Corporation, USA). Solvents used for flash column chromatography were purchased from Donau Chemie AG (Austria). Dry acetonitrile and dry DMF were obtained from Sigma-Aldrich (Germany) and ACROS Organics (Belgium), respectively, and stored under argon. All reactions were carried out under argon in air-dried glassware. Thin layer chromatography was performed using TLC plates on aluminum support (Merck, silica gel 60, fluorescent indicator 254). Column chromatography was performed using a BUCHI Sepacore Flash System (2 x BUCHI Pump Module C-605, BUCHI Pump Manager C-615, BUCHI UV Photometer C-635, and BUCHI Fraction Collector C-660) and a Reveleris® X2 Flash Chromatography/Prep Purification Systems (BUCHI). Silica gel 60 (40-63 µm) was obtained from Merck. A Kinetex® 5 µm C18 100 Å, AXIA LC column (100 x 30.0 mm, Phenomenex) was used for preparative HPLC. HPLC grade solvents were purchased from VWR (USA). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC 200 MHz, Bruker Avance UltraShield 400 MHz or Bruker Ascend 600 MHz spectrometer at 20 °C. Chemical shifts are reported in ppm (δ) relative to tetramethylsilane and calibrated using solvent residual peaks. Data are shown as follows: Chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, m = multiplet, b = broad signal), coupling constants (J, Hz) and integration. HPLC analysis for reaction monitoring was performed on a 1200 series system (Agilent Technologies, USA) using a Kinetex® (5µm C18 100 Å, 50 x 4.6 mm, Phenomenex, USA) column and water/acetonitrile gradient elution. Peak detection was enabled via DAD (Agilent Technologies) and a Bruker HCT Esquire Ion Trap MS.

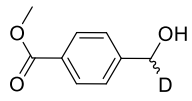
In general, no unexpected or unusually high safety hazards were encountered.

2) Synthesis

Clickable linker and click markers (CM)

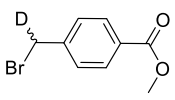
Compounds **2a**,[1] **3a**,[2] **4a**[3] and **5a**[4] have been prepared using previously published methods**.** Analytical data matched that reported.

4-(Hydroxy-d_1-methyl) benzoic acid methyl ester (2b)



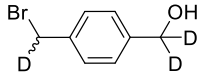
1 (2 g, 12.2 mmol) was dissolved in methanol (50 mL) and cooled to -20°C. Sodium borodeuteride (0.185 g, 3.17 mmol) was added and the reaction was stirred for 30 min. The reaction was quenched by addition of a saturated solution of sodium dihydrogen phosphate in water and washed with dichloromethane. The combined organic layer was dried over sodium sulfate, concentrated and co-evaporated with toluene to afford **2b** (1.91 g, 94%) as a colorless solid; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.04 (d, *J* = 8.2 Hz, 2H), 7.44 (d, *J* = 8.6 Hz, 2H), 4.76 (s, 1H), 3.93 (s, 3 H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 167.08 (s, 1C), 146.01 (s, 1C), 129.98 (d, 2C), 129.49 (d, 1C), 126.62 (d, 2C), 64.51 (t, $^{1}J_{CD}$ = 21.9 Hz, 1C), 52.25 (q, 1C); HR-ESI-ORBITRAP $[M+H]^+$ m/z calcd. 168.0766 for $C_9H_{10}DO_3^+$, found 168.0768.

4-(Bromo-d_1-methyl) benzoic acid methyl ester (3b)



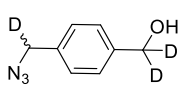
To a solution of triphenylphosphine (3.90 g, 14.85 mmol) in dichloromethane (40 mL) cooled to 0°C was added bromine (0.76 mL, 15.85 mmol) and trimethylamine (2.37 mL, 17.14 mmol). After 15 min a solution of **2b** (1.91 g, 11.43 mmol) in dichloromethane (50 mL) was added and the mixture was stirred for 10 min. The reaction was quenched by addition of aqueous HCl (100 mL, 0.1N) and phases were separated. The organic layer was washed with saturated aqueous sodium bicarbonate solution, dried over sodium sulfate and concentrated. The residue was purified by column chromatography (90 g SiO_2, 0-20% EtOAc in hexanes) to obtain **3b** (2.46 g, 93%) as a pale yellow solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (d, *J* = 8.4 Hz, 2H), 7.45 (d, *J* = 8.3 Hz, 2H), 4.48 (s, 1H), 3.91 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.64 (s, 1C), 142.68 (s, 2C), 130.20 (d, 2C), 129.14 (d, 2C), 52.35 (q, 1C), 32.14 (t, $^{1}J_{CD}$ = 23.43 Hz, 1C); HR-ESI-ORBITRAP $[M+H]^+$ m/z calcd. 229.9922 for $C_9H_9DBrO_2^+$, found 229.9924.

[4-(Bromo-d_1-methyl)phenyl]-d_2-methanol (4b)



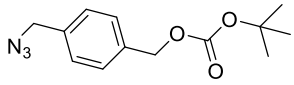
To a solution of **3b** (2.13 g, 9.25 mmol) in diethyl ether (100 mL) cooled to -60°C was added lithium aluminum deuteride (0.292 g, 6.95 mmol) in small portions and the mixture was stirred for 60 min. After addition of aqueous HCl (100 mL, 1N) the layers were separated and the aqueous layer was extracted with ether. The combined organic layer was dried over sodium sulfate and concentrated to afford **4b** (1.80 g, 95%) as a colorless solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 (d, *J* = 8.2 Hz, 2H), 7.35 (d, *J* = 8.1 Hz, 2H), 4.49 (s, 1H), 1.65 (bs, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 141.16 (s, 1C), 137.23 (s, 1C), 129.36 (d, 2C), 127.49 (d, 2C), 64.27 (quint, J_{CD} = 21.81 Hz, 1C), 33.19 (t, J_{CD} = 23.39 Hz, 1C); HR-ESI-ORBITRAP $[M+Na]^+$ m/z calcd. 225.9917 for $C_8H_6D_3BrONa^+$, found 225.9997.

4-[(Azido-d_1-methyl)phenyl]-d_2-methanol (5b)



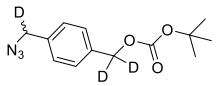
4b (1.78 g, 8.72 mmol), sodium azide (0.74 g, 11.3 mmol) and potassium iodide (0.29 g, 1.79 mmol) were dissolved in DMF (50 mL) and stirred overnight at room temperature. The mixture was diluted with water (100 mL) and extracted with diethyl ether. The combined organic layer was washed with saturated aqueous ammonium chloride solution and dried over sodium sulfate. The solvent was evaporated and the residue was purified by column chromatography (90 g SiO_2, 0-20% EtOAc in hexanes) to obtain **5b** (1.05 g, 84%) as a colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 (d, *J* = 8.2 Hz, 2H), 7.32 (d, *J* = 8.1 Hz, 2H), 4.32 (bs, 1H), 1.72 (bs, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 141.03 (s, 1C), 134.71 (s, 1C), 128.57 (d, 2C), 127.52 (d, 2C), 64.25 (quint, $^1J_{CD}$ = 21.67 Hz, 1C), 54.29 (t, $^1J_{CD}$ = 21.87 Hz, 1C); HR-ESI-ORBITRAP $[M+H]^+$ m/z calcd. 167.1007 for $C_8H_7D_3N_3O^+$, found 167.0928.

(1,1-Dimethyl)acetic acid, [4-[[acidomethyl]phenyl]methyl]ester (CM1a)



To a solution of **5a** (2 g, 12.26 mmol) and di-*tert*-butyl dicarbonate (2.94 g, 13.48 mmol) in dry dichloromethane (20 mL) was added zinc acetate (0.27 g, 1.23 mmol). The mixture was heated to reflux for 11 h, cooled to room temperature and washed with water. The organic layer was dried over sodium sulfate and concentrated. The residue was purified by column chromatography (12 g SiO_2, 0-10% EtOAc in hexanes) to afford **CM1a** (2.76 g, 86%) as a colorless liquid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 (d, *J* = 8.1 Hz, 2H), 7.31 (d, *J* = 8.1 Hz, 2H), 5.09 (s, 2H), 4.33 (s, 2H), 1.49 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 153.53 (s, 1C), 135.98 (s, 1C), 135.62 (s 1C), 128.82 (d, 2C), 128.51 (d, 2C), 82.54 (s, 1C), 68.32 (t, 1C), 54.58 (t, 1C), 27.54 (q, 3C); HR-ESI-ORBITRAP $[M+Na]^+$ m/z calcd. 286.1162 for $C_{13}H_{17}N_3O_3Na^+$, found 286.1166.

(1,1-Dimethyl)acetic acid, [4-[[acidomethyl-d_1]phenyl]methyl-d_2]ester (CM1b)

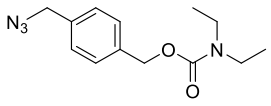


To a solution of **5b** (1 g, 6.02 mmol) and di-*tert*-butyl dicarbonate (1.44 g, 6.62 mmol) in dry dichloromethane (10 mL) was added zinc acetate (0.13 g, 0.6 mmol) were dissolved dichloromethane (10 mL). The mixture was heated to reflux for 11 h, cooled to room temperature and washed with water. The organic layer was dried over sodium sulfate and concentrated. Column chromatography (12 g SiO_2, 0-10% EtOAc in hexanes) gave **CM1b** (1.29 g, 81%) as a colorless liquid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 (d, *J* = 8.2 Hz, 2H), 7.31 (d, *J* = 7.7 Hz, 2H), 4.31 (s, 1H), 1.49 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 153.48 (s, 1C), 135.79 (s, 1C), 135.53 (s, 1C), 128.84 (d, 2C), 128.49 (d, 2C), 82.49 (s, 1C), 67.68 (quint, $^1J_{CD}$ = 22.55 Hz, 1C), 54.20 (t, $^1J_{CD}$ = 21.85 Hz, 1C), 27.84 (q, 3C); HR-ESI-ORBITRAP $[M+Na]^+$ m/z calcd. 289.135 for $C_{13}H_{14}D_3N_3O_3Na^+$, found 289.1352.

General procedure A (Carbamate formation)

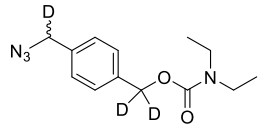
To a solution of alcohol (1 eq) in THF (1.67 mL/mmol) cooled to 0°C was added sodium carbonate (1 eq) and triphosgene (0.5 eq). The reaction mixture was stirred at room temperature overnight, filtrated and concentrated under reduced pressure. The residue was dissolved in THF (0.56 mL/mmol) and added to a solution of amine (1.1 eq) in THF (1.11 mL/mmol) and aqueous NaOH (1N, 1.67 mL/mmol). The reaction was stirred overnight and then extracted with dichloromethane. The combined organic layer was concentrated and the residue was purified by column chromatography (90 g SiO_2, 10-30 % EtOAc in hexanes).

N,N-(Diethyl)carbamic acid, [4-[[acidomethyl]phenyl]methyl]ester (CM2a)



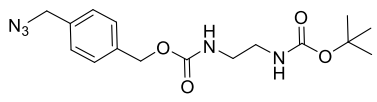
Following general procedure A, **5a** (2 g, 12.26 mmol) was reacted with diethylamine (0.99 g, 13.49 mmol) to obtain **CM2a** (1.62 g, 50%) as a colorless liquid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 (d, *J* = 8.1 Hz, 2H), 7.30 (d, *J* = 8.1 Hz, 2H), 5.13 (s, 2H), 4.34 (s, 2H), 3.30 (s, 4H), 1.13 (t, *J* = 7.0 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.84 (s, 1C), 137.49 (s, 1C), 135.09 (s, 1C), 128.43 (d, 2C), 128.30 (d, 2C), 66.41 (t, 1C), 54.66 (t, 1C), 42.05 (t, 1C), 41.39 (t, 1C), 14.25 (q, 1C), 13.60 (q, 1C); HR-ESI-ORBITRAP $[M+H]^+$ m/z calcd. 263.1503 for $C_{13}H_{19}N_4O_2^+$, found 263.1502.

N,N-(Diethyl)carbamic acid, [4-[[acidomethyl-*d*$_1$]phenyl]methyl-*d*$_2$]ester (CM2b)



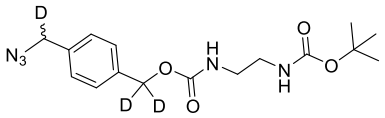
Following general procedure A, **5b** (0.875 g, 5.26 mmol) was reacted with diethylamine (0.42 g, 5.79 mmol) to obtain **CM2b** (0.99 g, 71%) as a colorless liquid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 (d, *J* = 8.2 Hz, 2H), 7.31 (d, *J*= 8.0 Hz, 2H), 4.32 (s, 1H), 3.30 (s, 4H), 1.13 (t, *J* = 7.02 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.84 (s, 1C), 137.38 (s, 1C), 135.05 (s, 1C), 128.44 (d, 2C), 128.35 (d, 2C), 65.80 (quint, $^1J_{CD}$ = 23.14 Hz, 1C), 54.34 (t, $^1J_{CD}$ = 21.87 Hz, 1C), 42.02 (t, 1C), 41.39 (t, 1C), 14.24 (q, 1C), 13.61 (q, 1C); HR-ESI-ORBITRAP $[M+H]^+$ m/z calcd. 266.1691 for $C_{13}H_{16}D_3N_4O_2^+$, found 266.1694.

N-[[[(1,1-Dimethylethoxy)carbonyl]amino]ethyl]carbamic acid, [4-[[acidomethyl]phenyl] methyl]ester (CM3a)



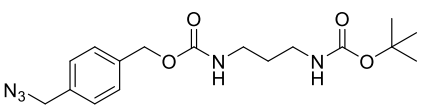
Following general procedure A, **5a** (3.2 g, 19.18 mmol) was reacted with N-boc-ethylenediamine (3.38 g, 21.1 mmol) to obtain **CM3a** (4.58 g, 67%) as a white solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 (d, *J* = 8.0 Hz, 2H), 7.30 (d, *J* = 8.0 Hz, 2H), 5.21 (bs, 1H), 5.10 (s, 2H), 4.84 (bs, 1H), 4.33 (s, 2H), 3.32-3.23 (m, 4H), 1.43 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.78 (s, 1C), 156.53 (s, 1C), 136.82 (s, 1C), 135.42 (s, 1C), 128.64 (d, 2C), 128.50 (d, 2C), 79.81 (s, 1C), 66.40 (t, 1C), 54.61(t, 1C), 41.72 (t, 1C), 40.72 (t, 1C), 28.49 (q, 3C), HR-ESI-ORBITRAP $[M+H]^+$ m/z calcd. 350.1823 for $C_{16}H_{24}N_5O_4^+$, found 350.1823.

N-[[[(1,1-Dimethylethoxy)carbonyl]amino]ethyl]carbamic acid, [4-[[acidomethyl-*d*$_1$]phenyl]methyl-*d*$_2$]ester (CM3b)



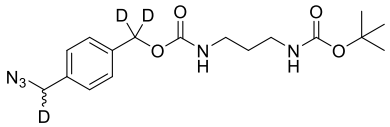
Following general procedure A, **5b** (1.3g, 7.79 mmol) was reacted with N-boc-ethylenediamine (1.37 g, 8.57 mmol) to obtain **CM3b** (1.88 g, 68%) as a white solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 (d, *J* = 8.1 Hz, 2H), 7.30 (d, *J* = 8.1 Hz, 2H), 5.21 (bs, 1H), 4.84 (bs, 1H), 4.32 (s, 1H), 3.32-3.23 (m, 4H), 1.43 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.78 (s, 1C), 156.55 (s, 1C), 136.71 (s, 1C), 135.38 (s, 1C), 128.69 (d, 2C), 128.50 (d, 2C), 79.81 (s, 1C), 54.30 (t, $^1J_{CD}$ = 21.85 Hz, 1C), 41.71 (t, 1C), 40.72 (t, 1C), 28.48 (q, 3C); HR-ESI-ORBITRAP $[M+H]^+$ m/z calcd. 353.2011 for $C_{16}H_{21}D_3N_5O_4^+$, found 353.2010.

N-[[[(1,1-Dimethylethoxy)carbonyl]amino]propyl]carbamic acid, [4-[[acidomethyl]phenyl]methyl] ester (CM4a)



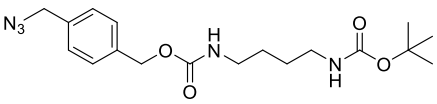
Following general procedure A, **5a** (2.4 g, 14.71 mmol) was reacted with N-boc-propanediamine (2.82 g, 16.18 mmol) to obtain **CM4a** (3.96 g, 74%) as a white solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 (d, *J* = 8.1 Hz, 2H), 7.31 (d, *J* = 8.1 Hz, 3H), 5.28 (bs, 1H), 5.10 (s, 2H), 4.80 (bs, 1H), 4.33 (s, 2H), 3.24 (q, *J* = 6.15 Hz, 2H), 3.14 (q, *J* = 5.59 Hz, 2H), 1.63 (quint, *J* = 6.24 Hz, 2H), 1.43 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.76 (s, 1C), 156.57 (s, 1C), 137.01 (s, 1C), 135.35 (s, 1C), 128.62 (d, 2C), 128.50 (d, 2C), 79.55 (s, 1C), 66.28 (t, 1C), 54.64 (t, 1C), 37.88 (t, 1C), 37.34 (t, 1C), 30.75 (t, 1C), 28.53 (s, 3C); HR-ESI-ORBITRAP $[M+H]^+$ m/z calcd. 364.1979 for $C_{17}H_{26}N_5O_4^+$, found 364.1978.

N-[[[(1,1-Dimethylethoxy)carbonyl]amino]propyl]carbamic acid, [4-[[acidomethyl-*d*$_1$]phenyl]-methyl-*d*$_2$]ester (CM4b)



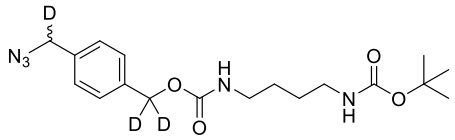
Following general procedure A, **5b** (1.05 g, 6.32 mmol) was reacted with N-boc-propanediamine (1.21 g, 6.95 mmol) to obtain **CM4b** (1.35 g, 58%) as a white solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 (d, *J* = 8.0 Hz, 2H), 7.30 (d, *J* = 8.2 Hz, 2H), 5.30 (bs, 1H), 4.81 (bs, 1H), 4.32 (s, 1H), 3.23 (q, *J* = 6.1 Hz, 2H), 3.17 (q, *J* = 5.59 Hz, 2H), 1.62 (quint, *J* = 6.34 Hz, 2H), 1.43 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.76 (s, 1C), 156.56 (s, 1C), 136.89 (s, 1C), 135.30 (s, 1C), 128.65 (d, 2C), 128.49 (d, 2C), 79.51 (s, 1C), 54.31 (t, $^1J_{CD}$ = 21.89 Hz, 1C), 37.86 (t, 1C), 37.29 (t, 1C), 30.72 (t, 1C), 28.51 (s, 3C); HR-ESI-ORBITRAP $[M+H]^+$ m/z calcd. 367.2168 for $C_{17}H_{23}D_3N_5O_4$+, found 367.2167.

N-[[[(1,1-Dimethylethoxy)carbonyl]amino]butyl]carbamic acid, [4-[[acidomethyl]phenyl]methyl] ester (CM5a)



Following general procedure A, **5a** (0.4 g, 2.45 mmol) was reacted with N-boc-butanediamine (0.51 g, 2.70 mmol) to obtain **CM5a** (0.67 g, 72%) as a white solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 (d, *J* = 8.1 Hz, 2H), 7.30 (d, *J* = 8.1 Hz, 2H), 5.09 (s, 2H), 4.85 (bs, 1H), 4.56 (bs, 1H), 4.33 (s, 2H), 3.24- 3.16 (m, 2H), 3.11 (s, 2H), 1.55- 1.47 (m, 4H), 1.43 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.45 (s, 1C), 156.13 (s, 1C), 136.96 (s, 1C), 135.37 (s, 1C), 128.65 (d, 2C), 128.49 (d, 2C), 79.38 (s, 1C), 66.27 (t, 1C), 54.61 (t, 1C), 40.86 (t, 1C), 40.32 (t, 1C), 28.54 (q, 3C), 27.52 (t, 1C), 27.38 (t, 1C); HR-ESI-ORBITRAP $[M+H]^+$ m/z calcd. 378.2136 for $C_{18}H_{28}N_5O_4^+$, found 378.2138.

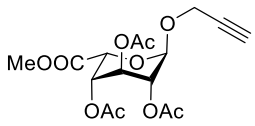
N-[[[(1,1-Dimethylethoxy)carbonyl]amino]butyl]carbamic acid, [4-[[acidomethyl-*d*$_1$]phenyl]methyl-*d*$_2$]ester (CM5b)



Following general procedure A, **5b** (0.28 g, 1.68 mmol) was reacted with N-boc-butanediamine (0.35 g, 1.85 mmol) to obtain **CM5b** (0.53 g, 83%) as a white solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 (d, *J* = 8.12 Hz, 2H), 7.30 (d, *J* = 8.04 Hz, 2H), 4.84 (bs, 1H), 4.55 (bs, 1H), 4.32 (s, 1H), 3.25-3.16 (m, 2H), 3.11 (bs, 2H), 1.54-1.46 (m, 4H), 1.43 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.45 (s, 1C), 156.12 (s, 1C), 136.84 (s, 1C), 135.32 (s, 1C), 128.69 (d, 2C), 128.49 (d, 2C), 79.36 (s, 1C), 54.29 (t, $^1J_{CD}$ = 21.86 Hz, 1C), 40.85 (t, 1C), 40.28 (t, 1C), 28.53 (q, 3C), 27.51 (t, 1C), 27.37 (t, 1C), HR-ESI-ORBITRAP $[M+H]^+$ m/z calcd. 381.2324 for $C_{18}H_{25}D_3N_5O_4^+$, found 381.2321.

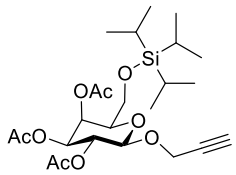
Enzyme responsive units

1-*O*-Propargyl-2,3,4-tri-*O*-acetyl-α-L-idupyranuronic acid, methyl ester (7)



To a solution of **6** (2 g, 5.95 mmol) in degassed dry acetonitrile (140 mL) was added molecular sieve (100 mg). After cooling to -20°C propargyl alcohol (3.44 mL, 59.5 mmol) was added followed by dropwise addition of TMSOTf (1.08 mL, 5.95 mmol). The mixture was allowed to reach room temperature and stirred for 24h. The mixture was diluted with CH_2Cl_2 (200 mL) and washed with saturated aqueous sodium bicarbonate solution (2 x 200 mL). The organic layer was dried over sodium sulfate and concentrated. The residue was purified by column chromatography (120 g SiO_2, 10-50% EtOAc in hexanes) to obtain **7** (0.491 g, 22%) as a colorless oil; ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.19 (bs, 1H), 5.16–5.09 (m, 1H), 5.09-5.01 (m, 1H), 4.85 (d, *J* = 2.4 Hz, 1H), 4.83–4.78 (m, 1H), 4.29 (d, *J* = 2.3 Hz, 2H), 3.78 (s, 3H), 2.44 (t, *J* = 2.4 Hz, 1H), 2.19-1.92 (m, 9H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 169.59 (s, 1C), 169.34 (s, 1C), 169.10 (s, 1C), 168.30 (s, 1C), 96.79 (d, 1C), 75.38 (s, 1C), 67.27 (d, 1C), 66.89 (d, 1C), 66.74 (d, 1C), 66.65 (d, 1C), 55.76 (t, 1C), 52.72 (q, 1C), 20.89 (q, 2C), 20.71 (q, 1C); HR-ESI-ORBITRAP $[M+Na]^+$ m/z calcd. 395.0949 for $C_{16}H_{20}O_{10}Na^+$, found 395.0948.

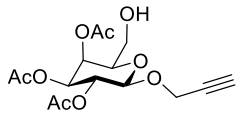
2-O-Propynyl-2,3,4-tri-*O*-acetyl-6-*O*-(triisopropylsilyl)-β,D-galactopyranoside



1-*O*-Propargyl-β,D-galactoside (**13**)[5] (1.1 g, 5 mmol, 1 eq.) was dissolved dry DMF (21 mL) and imidazole (1.0 g, 15 mmol, 3 eq) was added. Triisopropylsilyl chloride was added dropwise at 0 °C and the reaction was allowed to warm to room temperature and stirred overnight. The solvent was removed under high vacuum. The crude product (Rf: 0.83, CH_2Cl_2/MeOH = 5:1), which was used without further purification, was dissolved in pyridine (50 mL) and DMAP (18 mg, 150 µmol, 0.05 eq.) was added. Acetic anhydride (1.5 g, 15 mmol, 5 eq.) was added to the cooled, homogeneous solution. After stirring overnight, the solvent was removed under reduced pressure. The residue was dissolved in CH_2Cl_2 and washed with saturated $CuSO_4$ solution (to remove remaining pyridine), brine and dried over Na_2SO_4. Column chromatography (SiO_2, hexanes/EtOAc = 5:1) afforded the title compound as a white solid (1.2 g, 80% over 2 steps); Rf: 0.83, hexanes/EtOAc = 2:1; ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.50 (d, *J* = 3.3 Hz, 1H), 5.20 (dd, J_1 = 10.4 Hz, J_2 = 7.8 Hz, 1H), 5.06 (dd, J_1 = 10.4 Hz, J_2 = 3.3 Hz, 1H), 4.72 (d, *J* = 8.0 Hz, 1H),), 4.36 (d, *J* = 2.3 Hz, 2H), 3.87-3.68 (m, 3H), 2.44 (t, *J* = 2.3 Hz, 1H), 2.11 (s, 3H), 2.06 (s, 3H), 1.97 (s, 3H), 1.07-0.97 (m, 21H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 170.16 (s, 1C), 170.00 (s, 1C), 169.65 (s, 1C), 98.62 (d, 1C), 78.33 (s, 1C), 75.19 (d, 1C), 73.71 (d, 1C), 71.18 (d, 1C), 68.83 (d, 1C), 67.00 (d,

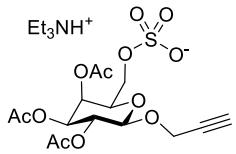
1C), 60.91 (t, 1C), 55.76 (t, 1C), 20.81 (q, 1C), 20.71 (q, 1C), 20.63 (q, 1C) 17.84 (q, 6C), 11.78 (d, 3C); HR-ESI-ORBITRAP, m/z $[M+Na]^+$ calcd. 523.2334 for $C_{24}H_{40}O_9NaSi^+$, found 523.2334.

2-O-Propynyl-2,3,4-tri-*O*-acetyl-β,D-galactopyranoside



2-Propynyl-2,3,4-tri-O-acetyl-6-*O*-(triisopropylsilyl)-β,D-galactopyranoside (5 g, 10 mmol, 1 eq) was dissolved in dry THF (150 mL). The reaction mixture was cooled to -14 °C using a cryostat and glacial acetic acid (2.3 mL, 40 mmol, 4 eq.) was added in one portion. Subsequently, tetrabutylammonium fluoride (1 M in THF, 50 mL, 50 mmol, 5 eq) was added dropwise and the reaction was kept at -10 °C overnight until complete conversion was verified by TLC. Saturated ammonium chloride solution (250 mL) was added under vigorous stirring and the layers were separated. The aqueous layer was extracted with ethyl acetate (3 x 200 mL). The combined organic layer was re-extracted with saturated sodium bicarbonate solution (2 x 100 mL), dried over Na_2SO_4 and concentrated. The residue was purified by column chromatography (SiO_2, hexanes/EtOAc = 1:1) to afford the title compound as a white solid (2.53 g, 73%); Rf: 0.4, hexanes/EtOAc = 1:1; ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.36 (d, *J* = 3.3 Hz), 5.22 (dd, J_1 = 10.4 Hz, J_2 = 7.8 Hz, 1H), 5.06 (dd, J_1 = 10.4 Hz, J_2 = 3.3 Hz, 1H), 4.73 (d, *J* = 7.8 Hz, 1H),), 4.37 (dd, J_1 = 2.3 Hz, J_2 = 0.5 Hz, 2H), 3.81-3.47 (m, 3H), 2.46 (t, *J* = 2.3 Hz, 1H), 2.15 (s, 3H), 2.05 (s, 3H), 1.98 (s, 3H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 171.12 (s, 1C), 170.05 (s, 1C), 169.61 (s, 1C), 98.98 (d, 1C), 78.35 (s, 1C), 75.35 (d, 1C), 73.64 (d, 1C), 70.88 (d, 1C), 68.81 (d, 1C), 67.81 (d, 1C), 60.53 (t, 1C), 56.12 (t, 1C), 20.75 (q, 1C), 20.66 (q, 1C), 20.58 (q, 1C); HR-ESI-ORBITRAP, m/z $[M+Na]^+$ calcd. 367.1000 for $C_{15}H_{20}O_9Na$, found 367.1005.

2-*O*-Propynyl-2,3,4-tri-*O*-acetyl-β,D-galactopyranose-6-*O*-sulfate, triethylammonium salt (14)



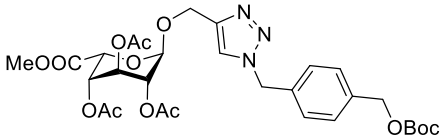
2-*O*-Propynyl-2,3,4-tri-*O*-acetyl-β,D-galactopyranoside (200 mg, 581 µmol, 1 eq) and sulfur trioxide trimethylamine complex (809 mg, 5.81 mmol, 10 eq) were dissolved in dry DMF (6 mL) and the mixture was stirred at 55 °C overnight. After completion as verified by LCMS the solvent was evaporated under high vacuum at 40 °C and the residue was dissolved/suspended in MeOH. The suspension was filtrated to remove excess SO_3-NMe_3-complex. Et_3N (1 mL) was added and the solution was concentrated. The residue was purified by column chromatography (SiO_2, MeOH in CH_2Cl_2, 0-20% gradient elution) to obtain **14** (271 mg, 90%); Rf: 0.52, $CHCl_3$/MeOH 4:1); ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.47 (d, *J* = 3.14 Hz, 1H), 5.18 (dd, J_1 = 10.4 Hz, J_2 = 7.8 Hz, 1H), 4.99 (dd, J_1 = 10.4 Hz, J_2 = 3.3 Hz, 1H), 4.67 (dd, J_1 = 16.0 Hz, J_2 = 7.9 Hz, 1H), 4.35 (d, J = 2.3 Hz, 2H), 4.27-4.16 (m, 1H), 4.15-3.95 (m, 2H) 3.14 (q, *J* = 7.37 Hz, 6H), 2.45 (t, *J* = 2.24 Hz, 1H), 2.12 (s, 3H), 2.05 (s, 3H), 1.94 (s, 3H), 1.34 (t, *J* = 7.34 Hz, 9H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 170.43 (s, 1C), 170.15 (s, 1C), 169.59 (s, 1C), 98.70 (d, 1C), 78.32 (s, 1C), 75.31 (d, 1C), 71.25 (d, 1C), 71.03 (d, 1C), 68.55 (d, 1C), 66.97 (d, 1C), 64.13 (t, 1C), 55.93 (t, 1C), 46.56 (t, 3C), 20.78 (q, 1C), 20.73 (q, 1C), 20.62 (q, 1C), 8.66 (q, 3C); HR-ESI-ORBITRAP $[M-H]^-$ m/z calcd. 423.0591731 for $C_{15}H_{19}O_{12}S^-$, found 423.06049.

IDUA Sets (Substrates, internal standards, products)

General procedure B (Click reaction, IDUA substrates)

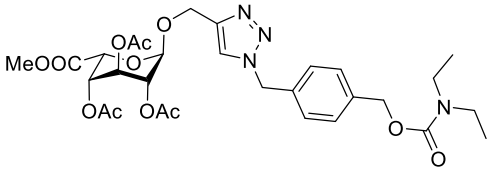
CuI (0.1 eq) was added to a solution of **7** (1 eq), **azide** (1.1 eq) and Et_3N (1.2 eq) in THF (55 mL/mmol) and the reaction mixture was stirred at room temperature overnight. The mixture was filtrated and concentrated. The residue was purified by column chromatography (40 g SiO_2, 20-100% EtOAc in hexanes)

[1-[[4-[[[[1,1-Dimethylethoxy]carbonyl]methyl]phenyl]methyl]-1*H*-1,2,3-triazol-4-yl]methyl] 3,4,6-tri-*O*-acetyl α-L-idopyranuric acid, methyl ester (8)



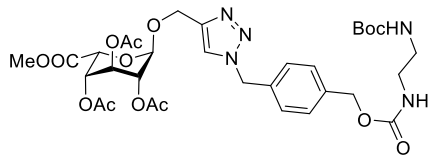
Following general procedure B, **7** (0.1 g, 0.27 mmol) was reacted with **CM1a** (0.078 g, 0.295 mmol) to afford **8** (157 mg, 92%) as a white solid; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.45 (s, 1H), 7.38 (d, *J* = 8.1 Hz, 2H), 7.28-7.25 (m, 2H), 5.51 (q, *J* = 14.5 Hz, 2H), 5.13-5.09 (m, 2H), 5.08 (s, 2H), 5.03 (td, *J* = 3.0, 0.9 Hz, 1H), 4.89-4.83 (m, 2H), 4.79-4.75 (m, 1H), 4.70 (d, *J* = 12.5 Hz, 1H), 3.77 (s, 3H), 2.06 (s, 3H), 2.04 (s, 3H), 1.96 (s, 3H), 1.48 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.46 (s, 1C), 169.26 (s, 1C), 168.96 (s, 1C), 168.27 (s, 1C), 153.36 (s, 1C), 144.34 (s, 1C), 136.56 (s, 1C), 134.63 (s, 1C), 128.87 (d, 2C), 128.33 (d, 2C), 122.70 (d, 1C), 97.44 (d, 1C), 82.50 (s, 1C), 67.93 (t, 1C), 67.19 (d, 1C), 66.68 (d, 1C), 66.66 (d, 1C), 61.96 (t, 1C), 53.85 (t, 1C), 52.58 (q, 1C), 27.77 (q, 3C), 20.76 (q, 1C), 20.68 (q, 1C), 20.59 (q, 1C); HR-ESI-ORBITRAP $[M+H]^+$ m/z calcd. 636.2399 for $C_{29}H_{38}N_3O_{13}^+$, found 636.2393.

[1-[[4-[[[[[N,N[Diethyl]amino]carbonyl]oxy]methyl]phenyl]methyl]-1*H*-1,2,3-triazol-4yl]methyl] 3,4,6-tri-*O*-acetyl α-L-idopyranuric acid, methyl ester (9)



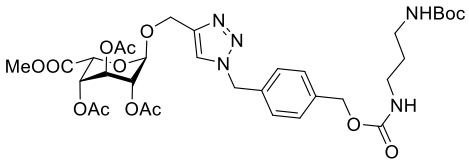
Following general procedure B, **7** (0.1 g, 0.27 mmol) was reacted with **CM2a** (0.077 g, 0.295 mmol) to obtain **9** (130 mg, 76%) as a white solid; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.47 (s, 1H), 7.36 (d, *J* = 8.1 Hz, 2H), 7.28-7.24 (m, 2H), 5.51 (q, *J* = 14.3 Hz, 2H), 5.13-5.07 (m, 4H), 5.02 (t, *J* = 4.0 Hz, 1H), 4.89-4.83 (m, 3H), 4.80-4.75 (m, 1H), 4.70 (d, *J* = 12.2 Hz, 1H), 3.77 (s, 3H), 3.29 (bs, 4H), 2.06 (s, 3H), 2.04 (s, 3H), 1.95 (s, 3H), 1.12 (bs, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.53 (s, 1C), 169.33 (s, 1C), 169.01 (s, 1C), 168.32 (s, 1C), 155.68 (s, 1C), 144.36 (s, 1C), 138.12 (s, 1C), 134.10 (s, 1C), 128.42 (d, 2C), 128.31 (d, 2C), 122.07 (d, 1C), 97.49 (d, 1C), 67.25 (d, 1C), 66.74 (d, 1C), 66.72 (d, 1C), 66.69 (d, 1C), 66.13 (t, 1C), 62.03 (t, 1C), 53.96 (t, 1C), 52.64 (q, 1C), 42.01 (t, 1C), 41.34 (t, 1C), 20.81 (q, 1C), 20.72 (q, 1C), 20.65 (q, 1C), 14.26 (q, 1C), 13.53 (q, 1C); HR-ESI-ORBITRAP $[M+H]^+$ m/z calcd. 635.2559 for $C_{29}H_{39}N_4O_{12}^+$, found 635.2558.

[1-[[4-[[[[[[[[(1,1-Dimethylethoxy)carbonyl]amino]ethyl]amino]carbonyl]oxy]methyl]phenyl] methyl]-1*H*-1,2,3-triazol-4yl]methyl] 3,4,6-tri-*O*-acetyl α-L-idopyranuric acid, methyl ester (10)



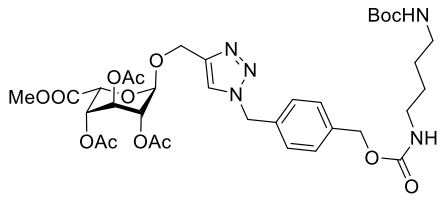
Following general procedure B, **7** (0.1 g, 0.27 mmol) was reacted with **CM3a** (0.103 g, 0.295 mmol) to obtain **10** (166 mg, 86%) as a white solid; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.45 (s, 1H), 7.36 (d, *J* = 8.0 Hz, 2H), 7.26 (d, *J* = 8.0 Hz, 2H), 5.51 (q, *J* = 15.3 Hz, 2H), 5.22 (bs, 1H), 5.11-5.07 (m, 4H), 5.02 (t, *J* = 3.4 Hz, 1H), 4.88-4.80 (m, 3H), 4.78-4.75 (m, 1H), 4.72-4.67 (m, 1H), 3.77 (s, 3H), 3.32-3.24 (m, 4H), 2.06 (s, 3H), 2.04 (s, 3H), 1.94 (s, 3H), 1.43 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.52 (s, 1C), 169.32 (s, 1C), 169.04 (s, 1C), 168.29 (s, 1C), 156.63 (s, 1C),156.52 (s, 1C), 144.37 (s, 1C), 137.49 (s, 1C), 134.39 (s, 1C), 128.74 (d, 2C), 128.32 (d, 2C), 122.73 (d, 1C), 97.45 (d, 1C), 79.58 (s, 1C), 67.20 (d, 1C), 66.70 (d, 1C), 66.65 (d, 1C), 66.02 (t, 1C), 61.97 (t, 1C), 53.88 (t, 1C), 52.61 (d, 1C), 41.67 (t, 1C), 40.58 (t, 1C), 28.40 (q, 3C), 20.78 (q, 1C), 20.70 (q, 1C), 20.62 (q, 1C), 14.23 (q, 1C); HR-ESI-ORBITRAP $[M+H]^+$ m/z calcd. 722.2880 for $C_{32}H_{44}N_5O_{14}^+$, found 722.2883.

[1-[[4-[[[[[[[[(1,1-Dimethylethoxy)carbonyl]amino]propyl]amino]carbonyl]oxy]methyl]phenyl]methyl]-1*H*-1,2,3-triazol-4yl]methyl] 3,4,6-tri-*O*-acetyl α-L-idopyranuric acid, methylester (11)



Following general procedure B, **7** (0.1 g, 0.27 mmol) was reacted with **CM4a** (0.107 g, 0.295 mmol) to give **11** (147 mg, 74%) as a white solid; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.45 (s, 1H), 7.36 (d, *J* = 8.04 Hz, 2H), 7.26 (d, *J* = 8.0 Hz, 2H), 5.51 (q, *J* = 15.0 Hz, 2H), 5.35 (bs, 1H), 5.12-5.07 (m, 3H), 5.02 (t, *J* = 3.66 Hz, 1H), 4.88-4.83 (m, 2H), 4.81-4.75 (m, 2H), 4.71-4.67 (m, 1H), 3.78 (s, 3H), 3.23 (q, *J* = 6.2 Hz, 2H), 3.18 (q, *J* = 5.9 Hz, 2H), 2.07 (s, 3H), 2.04 (s, 3H), 1.95 (s, 3H), 1.62 (quint, *J* = 6.2 Hz, 2H), 1.43 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.52 (s, 1C), 169.32 (s, 1C), 169.04 (s, 1C), 168.29 (s, 1C), 156.59 (s, 2C), 144.36 (s, 1C), 137.67 (s, 1C), 134.30 (s, 1C), 128.73 (d, 2C), 128.33 (d, 2C), 122.73 (d, 1C), 97.46 (d, 1C), 79.36 (s, 1C), 67.21 (d, 1C), 66.68 (d, 2C), 66.64 (t, 1C), 61.99 (t, 1C), 53.91 (t, 1C), 52.62 (q, 1C), 37.74 (t, 1C), 37.14 (t, 1C), 30.55 (t, 1C), 28.43 (q, 3C), 20.79 (q, 1C), 20.71 (q, 1C), 20.63 (q, 1C); HR-ESI-ORBITRAP $[M+H]^+$ m/z calcd. 736.3036 for $C_{33}H_{46}N_5O_{14}^+$, found 736.303.

[1-[[4-[[[[[[[[(1,1-Dimethylethoxy)carbonyl]amino]butyl]amino]carbonyl]oxy]methyl]phenyl] methyl]-1*H*-1,2,3-triazol-4yl]methyl] 3,4,6-tri-*O*-acetyl α-L-idopyranuric acid, methylester (12)

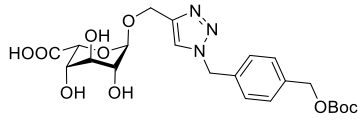


Following general procedure B, **7** (0.1 g, 0.27 mmol) was reacted with **CM5a** (0.111 g, 0.295 mmol) to obtain **12** (169 mg, 84%) as a white solid; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.45 (s, 1H), 7.36 (d, *J* = 8.0 Hz, 2H), 7.28-7.24 (m, 2H), 5.51 (q, *J* = 15.4 Hz, 2H), 5.15-5.06 (m, 4H), 5.02 (t, *J* = 3.7 Hz, 1H), 4.88-4.83 (m, 3H), 4.79-4.74 (m, 1H), 4.69 (d, *J* = 12.5 Hz, 1H), 4.57 (bs, 1H), 3.77 (s, 3H), 3.20 (q, *J* = 6.3 Hz, 2H), 3.15-3.08 (m, 2H), 2.06 (s, 3H), 2.04 (s, 3H), 1.94 (s, 3H), 1.51 (s, 4H), 1.43 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.46 (s, 1C), 169.26 (s, 1C), 168.98 (s, 1C), 168.23 (s, 1C), 156.29 (s, 1C), 156.05 (s, 1C), 144.30 (s, 1C), 137.58 (s, 1C), 134.32 (s, 1C), 128.70 (d, 2C), 128.28 (d, 2C), 122.69 (d, 1C), 97.39 (d, 1C), 79.10 (s, 1C), 67.16 (d, 1C), 66.64 (d, 2C), 66.59 (d, 1C), 65.83 (t, 1C), 61.91 (t, 1C), 53.83 (t, 1C), 52.56 (q, 1C), 40.73 (t, 1C), 40.13 (t, 1C), 28.41 (q, 3C), 27.35 (t, 1C), 27.17 (t, 1C), 20.73 (q, 1C), 20.64 (q, 1C), 20.57 (q, 1C); HR-ESI-ORBITRAP $[M+H]^+$ m/z calcd. 750.3192 for $C_{34}H_{48}N_5O_{14}^+$, found 750.3186.

General procedure C (Deprotection)

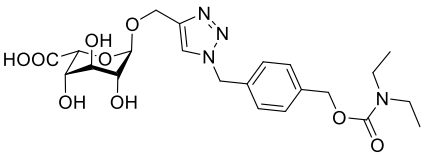
To a solution of the protected glycoside (1 eq) in THF (216 mL/mmol) and water (22 mL/mmol) cooled to 0°C was added aqueous NaOH (5 eq, 0.1M). The reaction mixture was stirred for 4h and then quenched by addition of aqueous HCl (0.1 M) to adjust the pH to 7. The reaction was concentrated and the residue was purified by preparative HPLC (C18-reversed phase, 100 x 30, 5 µm, 50 mL/min, H_2O/MeCN 5-30 %).

[1-[[4-[[[[1,1-Dimethylethoxy]carbonyl]methyl]phenyl]methyl]-1*H*-1,2,3-triazol-4yl]methyl] α-L-idopyranuric acid (CS1)



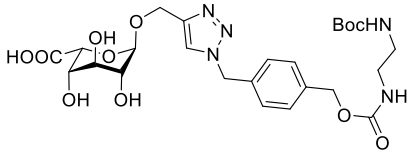
Following general procedure C, starting from **8** (70 mg, 0.11 mmol) **CS1** was obtained as a white solid (54 mg, 98%); ^{1}H NMR (600 MHz, CD_3OD) δ 8.04 (s, 1H), 7.36 (d, *J* = 8.2 Hz, 2H), 7.32 (d, *J* = 8.2 Hz, 2H), 5.58 (s, 2H), 5.05 (s, 2H), 4.85 (d, *J* = 4.7 Hz, 1H), 4.76 (d, *J* = 12.7 Hz, 1H), 4.35 (d, *J* = 4.1 Hz, 1H), 3.79 (dd, J_1 = 6.2, J_2 = 4.0 Hz, 1H), 3.64 (t, *J* = 6.2 Hz, 1H), 3.37 (dd, J_1 = 6.3, J_2 = 4.5 Hz, 1H), 1.45 (s, 9H); ^{13}C NMR (150 MHz, CD_3OD) δ 176.98 (s, 1C), 154.91 (s, 1C), 145.88 (s, 1C), 137.92 (s, 1C), 136.7 (s, 1C)5, 129.53 (d, 2C), 129.32 (d, 2C), 125.60 (d, 1C), 101.40 (d, 1C), 83.11 (s, 1C), 73.57 (d, 1C), 72.28 (d, 1C), 72.23 (d, 1C), 71.65 (d, 1C), 68.97 (t, 1C), 62.05 (t, 1C), 54.53 (t, 1C), 27.97 (q, 3C); HR-ESI-ORBITRAP $[M+H]^+$ m/z calcd. 496.1926 for $C_{22}H_{30}N_3O_{10}^+$, found 496.1921.

[1-[[4-[[[[[N,N[Diethyl]amino]carbonyl]oxy]methyl]phenyl]methyl]-1*H*-1,2,3-triazol-4yl]methyl] α-L-idopyranuric acid (CS2)



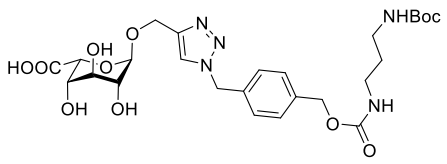
Following general procedure C, starting from **9** (70 mg, 0.11 mmol) **CS2** was obtained as a white solid (52 mg, 95%); ^{1}H NMR (600 MHz, CD_3OD) δ 8.07 (s, 1H), 7.38 (d, *J* = 8.2 Hz, 2H) 7.35 (d, *J* = 8.2 Hz, 2H), 5.60 (s, 2H), 5.11 (s, 2H), 4.87 (d, *J* = 4.4 Hz, 1H), 4.78 (d, *J* = 12.6 Hz, 1H), 4.36 (d, *J* = 4.0 Hz, 1H), 3.81 (dd, J_1 = 6.2, J_2 = 4.0 Hz, 1H), 3.66 (t, *J* = 6.2 Hz, 1H), 3.39 (dd, J_1 = 6.5, J_2 = 4.4 Hz, 1H), 3.35-3.28 (m, 4H), 1.13 (t, *J* = 7.2 Hz, 6H); ^{13}C NMR (150 MHz, CD_3OD) δ 176.97 (s, 1C), 157.40 (s, 1C), 145.86 (s, 1C), 138.72 (s, 1C), 136.52 (s, 1C), 129.34 (d, 4C), 125.60 (d, 1C), 101.39 (d, 1C), 73.58 (d, 1C), 72.29 (d, 1C), 72.22 (d, 1C), 71.65 (d, 1C), 67.50 (t, 1C), 62.05 (t, 1C), 54.54 (t, 1C), 43.08 (t, 1C), 42.56 (t, 1C), 14.37 (q, 1C), 13.71 (q, 1C); HR-ESI-ORBITRAP $[M+H]^+$ m/z calcd. 495.2086 for $C_{22}H_{31}N_4O_9^+$, found 495.2092.

[1-[[4-[[[[[[[[(1,1-Dimethylethoxy)carbonyl]amino]ethyl]amino]carbonyl]oxy]methyl]phenyl] methyl]-1*H*-1,2,3-triazol-4yl]methyl] α-L-idopyranuric acid (CS3)



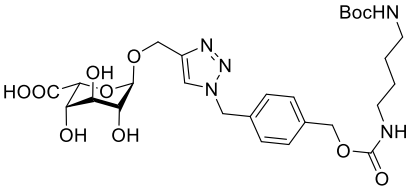
Following general procedure C, starting from **10** (100 mg, 0.139 mmol) **CS3** was obtained as a white solid (45 mg, 56%); ^{1}H NMR (600 MHz, CD_3OD) δ 8.04 (s, 1H), 7.36 (d, *J* = 8.1 Hz, 2H), 7.31 (d, *J* = 8.1 Hz, 2H), 5.58 (s, 2H), 5.06 (s, 2H), 4.82 (d, *J* = 4.7 Hz, 1H), 4.76 (d, *J* = 12.6 Hz, 1H), 4.32 (d, *J* = 4.40Hz, 1H), 3.76 (dd, J_1 = 6.8, J_2 = 4.4 Hz, 1H), 3.61 (t, *J* = 6.6 Hz, 1H), 3.34 (dd, J_1 = 6.5, J_2 = 5.3 Hz, 1H), 3.20-3.10 (m, 4H), 1.42 (s, 9H); ^{13}C NMR (150 MHz, CD_3OD) δ 176.96 (s, 1C), 158.84 (s, 1C), 158.56 (s, 1C), 145.91 (s, 1C), 138.84 (s, 1C), 136.42 (s, 1C), 129.31 (d, 2C), 129.28 (d, 2C), 125.59 (d, 1C), 101.36 (d, 1C), 80.10 (s, 1C), 73.83 (d, 1C), 72.63 (d, 1C), 72.38 (d, 1C), 71.83 (d, 1C), 66.86 (t, 1C), 62.13 (t, 1C), 54.56 (t, 1C), 41.79 (t, 1C), 41.18 (t, 1C), 28.74 (q, 3C); HR-ESI-ORBITRAP $[M+H]^+$ m/z calcd. 582.2406 for $C_{25}H_{36}N_5O_{11}^+$, found 582.2378.

[1-[[4-[[[[[[[[(1,1-Dimethylethoxy)carbonyl]amino]propyl]amino]carbonyl]oxy]methyl]phenyl] methyl]-1*H*-1,2,3-triazol-4yl]methyl] α-L-idopyranuric acid (CS4)



Following general procedure C, starting from **11** (70 mg, 0.095 mmol) **CS4** was obtained as white solid (33 mg, 58%); ^{1}H NMR (600 MHz, CD_3OD) δ 8.04 (s, 1H), 7.35 (d, *J* = 8.1 Hz, 2H), 7.32 (d, *J* = 8.1 Hz, 4H), 5.58 (s, 2H), 5.05 (s, 2H), 4.82 (d, *J* = 5.0 Hz, 1H), 4.76 (d, *J* = 12.6 Hz, 1H), 4.32 (d, *J* = 4.1 Hz, 1H), 3.76 (dd, J_1 = 6.6, J_2 = 4.2 Hz, 1H), 3.61 (t, *J* = 6.6 Hz, 1H), 3.34 (dd, J_1 = 6.3, J_2 = 4.8 Hz, 1H), 3.12 (t, *J* = 7.1 Hz, 2H), 3.05 (t, *J* = 6.8 Hz, 2H), 1.62 (quint, *J* = 6.7 Hz, 2H), 1.42 (s, 9H); ^{13}C NMR (150 MHz, CD_3OD) δ 176.96 (s, 1C), 158.78 (s, 1C), 158.54 (s, 1C), 145.91 (s, 1C), 138.90 (s, 1C), 136.42 (s, 1C), 129.29 (d, 4C), 125.58 (d, 1C), 101.36 (d, 1C), 79.96 (s, 1C), 73.80 (d, 1C), 72.59 (d, 1C), 72.36 (d, 1C), 71.81 (d, 1C), 66.82 (t, 1C), 62.12 (t, 1C), 54.56 (t, 1C), 39.15 (t, 1C), 38.60 (t, 1C), 31.13 (t, 1C), 28.76 (q, 3C); HR-ESI-ORBITRAP $[M+H]^+$ m/z calcd. 596.2562 for $C_{26}H_{38}N_5O_{11}^+$, found 596.2544.

[1-[[4-[[[[[[[[(1,1-Dimethylethoxy)carbonyl]amino]butyl]amino]carbonyl]oxy]methyl]phenyl] methyl]-1*H*-1,2,3-triazol-4yl]methyl] α-L-idopyranuric acid (CS5)

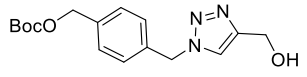


Following general procedure C, starting from **12** (70 mg, 0.093 mmol) **CS5** was obtained as white solid (37 mg, 64%); ^{1}H NMR (600 MHz, CD_3OD) δ 8.04 (s, 1H), 7.36 (d, *J* = 8.1 Hz, 2H), 7.31 (d, *J* = 8.1 Hz, 2H), 5.58 (s, 2H), 5.05 (s, 2H), 4.83 (d, *J* = 4.7 Hz, 1H), 4.76 (d, *J* = 12.6 Hz, 1H), 4.33 (d, *J* = 4.7 Hz, 1H), 3.77 (dd, J_1 = 6.6, J_2 = 4.0 Hz, 1H), 3.62 (t, *J* = 6.5 Hz, 1H), 3.35 (dd, J_1 = 6.3, J_2 = 4.8 Hz, 1H), 3.10 (t, *J* = 6.3 Hz, 2H), 3.03 (t, *J* = 6.4 Hz, 2H), 1.50-1.45 (m, 4H), 1.42 (s, 9H); ^{13}C NMR (150 MHz, CD_3OD) δ 176.96 (s, 1C), 158.75 (s, 1C), 158.53 (s, 1C), 145.90 (s, 1C), 138.95 (s, 1C), 136.39 (s, 1C), 129.27 (d, 4C), 125.58 (d, 1C), 101.37 (d, 1C), 79.83 (s, 1C), 73.76 (d, 1C), 72.55 (d, 1C), 72.34 (d, 1C), 71.78 (d, 1C), 66.75 (t, 1C), 62.11 (t, 1C), 54.56 (t, 1C), 41.45 (t, 1C), 40.94 (t, 1C), 28.78 (q, 3C), 28.17 (t, 1C), 28.13 (t, 1C); HR-ESI-ORBITRAP $[M+H]^+$ m/z calcd. 610.2719 for $C_{27}H_{40}N_5O_{11}^+$, found 610.2696.

General procedure D (Click chemistry, IDUA products and IS)

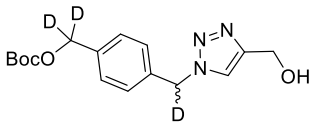
CuI (0.1 eq) was added to a solution of azide (1 eq), propargyl alcohol (1.5 eq) and Et_3N (1.2 eq) in THF (55 mL/mmol) and the reaction mixture was stirred at room temperature overnight. The mixture was filtrated and concentrated. The residue was purified by column chromatography (8 g SiO_2, 0-10% MeOH in CH_2Cl_2).

(1,1-Dimethyl)acetic acid, [4-[[[4-(hydroxymethyl)-1H-1,2,3-triazol-1-yl]methyl]phenyl]methyl] ester (P1)



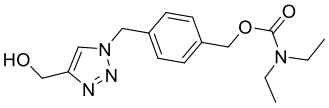
Following general procedure D, starting from **CM1a** (25 mg, 0.095 mmol) **P1** was obtained as a white solid (13 mg, 45%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 (s, 1H), 7.36 (d, *J* = 7.2 Hz, 2H), 7.24 (d, *J* = 8.0 Hz, 2H), 5.49 (s, 2H), 5.1 (s, 2H), 4.79 (bs, 2H), 1.47 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 153.45 (s, 1C), 136.62 (s, 1C), 134.64 (s, 1C), 128.95 (d, 2C), 128.46 (d, 2C), 82.65 (s, 1C), 68.06 (t, 1C), 54.24 (t, 1C), 27.87 (q, 3C); HR-ESI-ORBITRAP $[M+H]^+$ m/z calcd. 320.1605 for $C_{16}H_{22}N_3O_4^+$, found 320.1609.

(1,1-Dimethyl)acetic acid, [4-[[[4-(hydroxymethyl)-1H-1,2,3-triazol-1-yl]methyl-*d*$_1$]phenyl]methyl-*d*$_2$]ester (IS1)



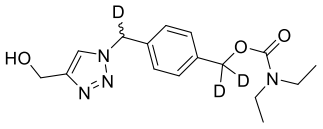
Following general procedure D, starting from **CM1b** (50 mg, 0.188 mmol) **IS1** was obtained as a white solid (47 mg, 81%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57 (s, 1H), 7.36 (d, *J* = 8.0 Hz, 2H), 7.24 (d, *J* = 8.2 Hz, 2H), 5.47 (s, 1H), 4.77 (bs, 2H), 1.46 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 153.45 (s, 1C), 136.46 (s, 1C), 134.61 (s, 1C), 128.98 (d, 2C), 128.45 (d, 2C), 82.62 (s, 1C), 67.48 (t, $^1J_{CD}$ = 22.47 Hz, 1C), 53.87 (t, $^1J_{CD}$ = 20.97 Hz, 1C), 27.86 (q, 3C); HR-ESI-ORBITRAP $[M+H]^+$ m/z calcd. 323.1793 for $C_{16}H_{19}D_3N_3O_4^+$, found 323.1798.

N,N-(Diethyl)carbamic acid, 4-[[[4-(hydroxymethyl)-1*H*-1,2,3-triazol-1-yl]methyl]phenyl]methyl ester (P2)



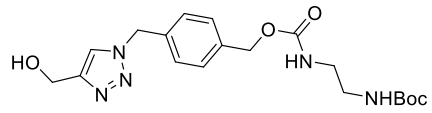
Following general procedure D, starting from **CM2a** (25 mg, 0.095 mmol, 1 eq) **P2** was obtained as a white solid (27 mg, 93%); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.72 (bs, 1H), 7.34 (d, *J* = 7.6 Hz, 2H), 7.23 (d, *J* = 7.8 Hz, 1H), 5.49 (s, 1H), 5.10 (s, 1H), 4.82 (bs, 1H), 3.28 (s, 4H), 1.11 (t, *J* = 7.0 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.76 (s, 1C), 138.10 (s, 1C), 134.09 (s, 1C), 128.42 (d, 2C), 128.40 (d, 2C), 66.19 (t, 1C), 54.40(t, 1C), 42.03(t, 1C), 41.42(t, 1C), 14.21(q, 1C), 13.56(q, 1C); HR-ESI-ORBITRAP $[M+H]^+$ m/z calcd. 319.1765 for $C_{16}H_{23}N_4O_3^+$, found 319.1763.

N,N-(Diethyl)carbamic acid, [4-[[[4-(hydroxymethyl)-1*H*-1,2,3-triazol-1-yl]methyl-*d*$_1$]phenyl] methyl-*d*$_2$]ester (IS2)



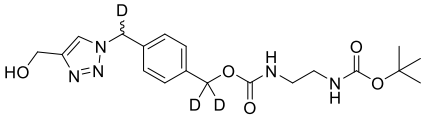
Following general procedure D, starting from **CM2b** (50 mg, 0.188 mmol) **IS2** was obtained as a white solid (50 mg, 86%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56 (bs, 1H), 7.32 (d, *J* = 8.0 Hz, 2H), 7.22 (d, *J* = 8.1 Hz, 2H), 5.45 (s, 1H), 4.76 (bs, 2H), 3.61 (s, 1H), 3.26 (s, 4H), 1.09 (t, *J* = 7.2 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.75 (s, 1C), 137.90 (s, 1C), 134.07 (s, 1C), 128.44 (d, 2C), 128.34 (d, 2C), 65.58 (quint, $^1J_{CD}$ = 22.64 Hz, 1C), 53.86 (t, $^1J_{CD}$ = 21.32 Hz, 1C), 41.99 (t, 1C), 41.37 (t, 1C), 14.18 (q, 1C), 13.54 (q, 1C); HR-ESI-ORBITRAP $[M+H]^+$ m/z calcd. 322.1953 for $C_{16}H_{20}D_3N_4O_3^+$, found 322.1953.

N-[[[(1,1-Dimethylethoxy)carbonyl]amino]ethyl]carbamic acid, 4-[[[4-(hydroxymethyl)-1*H*-1,2,3-triazol-1-yl]methyl]phenyl]methyl ester (P3)



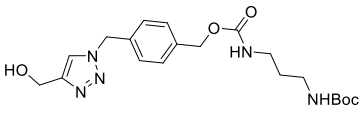
Following general procedure D, starting from **CM3a** (25 mg, 0.072 mmol) **P3** was obtained as a white solid (24 mg, 83%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 (s, 1H), 7.32 (d, *J* = 7.9 Hz, 2H), 7.23 (d, *J* = 7.9 Hz, 2H), 5.48 (s, 2H), 5.38 (bs, 1H), 5.06 (s, 2H), 4.95 (bs, 1H), 4.73 (s, 2H), 3.30-3.20 (m, 4H), 1.41 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.74 (s, 1C), 156.63 (s, 1C), 148.36 (s, 1C), 137.52 (s, 1C), 134.45 (s, 1C), 128.81 (d, 2C), 128.42 (d, 2C), 121.79 (d, 1C), 77.48 (s, 1C), 66.15 (t, 1C), 56.59 (t, 1C), 53.99 (t, 1C), 41.71 (t, 1C), 40.65 (t, 1C), 28.49 (q, 3C); HR-ESI-ORBITRAP $[M+H]^+$ m/z calcd. 406.2085 for $C_{19}H_{28}N_5O_5^+$, found 406.2084.

N-[[[(1,1-Dimethylethoxy)carbonyl]amino]ethyl]carbamic acid, [4-[[[4-(hydroxymethyl)-1*H*-1,2,3-triazol-1-yl]methyl-*d*$_1$]phenyl]methyl-*d*$_2$] ester (IS3)



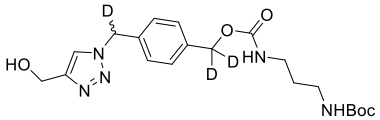
Following general procedure D, starting from **CM3b** (50 mg, 0.142 mmol) **IS3** was obtained as a white solid (40 mg, 69%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 (s, 1H), 7.33 (d, *J* = 8.0 Hz, 2H), 7.23 (d, *J* = 8.0 Hz, 2H), 5.47 (s, 1H), 5.36 (bs, 1H), 4.93 (bs, 1H), 4.73 (s, 2H), 3.33-3.18 (m, 4H), 1.41 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.76 (s, 1C), 156.61 (s, 1C) 148.33 (s, 1C), 137.38 (s, 1C), 134.42 (s, 1C), 128.86 (d, 2C), 128.42 (d, 2C), 121.73 (d, 1C), 79.79 (s, 1C), 56.62 (t, 1C), 53.71 (t, $^{1}J_{CD}$ = 21.1 Hz, 1C), 41.71 (t, 1C), 40.63 (t, 1C), 28.48 (q, 3C); HR-ESI-ORBITRAP $[M+H]^+$ m/z calcd. 409.2273 for $C_{19}H_{25}D_3N_5O_5^+$, found 409.2251.

N-[[[(1,1-Dimethylethoxy)carbonyl]amino]propyl]carbamic acid, 4-[[[4-(hydroxymethyl)-1*H*-1,2,3-triazol-1-yl]methyl]phenyl]methyl ester (P4)



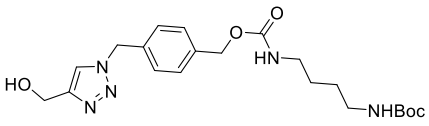
Following general procedure D, starting from **CM4a** (25 mg, 0.069 mmol) **P4** was obtained as a white solid (17 mg, 59%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 (s, 1H), 7.35 (d, *J* = 8.1 Hz, 2H), 7.24 (d, *J* = 8.1 Hz, 2H), 5.49 (s, 2H), 5.41 (bs, 1H), 5.07 (s, 2H), 4.85 (bs, 1H), 4.75 (s, 2H), 3.21 (q, *J* = 6.2 Hz, 2H), 3.14 (q, *J* = 5.9 Hz, 2H), 1.60 (quint, *J* = 6.4 Hz, 2H), 1.42 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.69 (s, 2C), 148.31 (s, 1C), 137.72 (s, 1C), 134.35 (s, 1C), 128.82 (d, 2C), 128.44 (d, 2C), 121.73 (d, 1C), 79.54 (s, 1C), 66.04 (t, 1C), 56.69 (t, 1C), 54.05 (t, 1C), 37.80 (t, 1C), 37.17 (t, 1C), 30.64 (t, 1C), 28.52 (q, 3C); HR-ESI-ORBITRAP $[M+H]^+$ m/z calcd. 420.2242 for $C_{20}H_{30}N_5O_5^+$, found 420.2237.

N-[[[(1,1-Dimethylethoxy)carbonyl]amino]propyl]carbamic acid, [4-[[[4-(hydroxymethyl)-1*H*-1,2,3-triazol-1-yl]methyl-*d*$_1$]phenyl]methyl-*d*$_2$] ester (IS4)



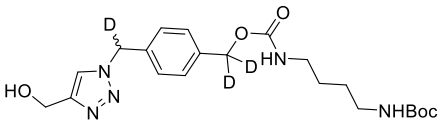
Following general procedure D, starting from **CM4b** (50 mg, 0.136 mmol) **IS4** was obtained as a white solid (32 mg, 55%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 (s, 1H), 7.33 (d, *J* = 8.0 Hz, 2H), 7.23 (d, *J* = 8.0 Hz, 2H), 5.52-5.42 (m, 2H), 4.90 (s, 1H), 4.72 (s, 2H), 3.19 (q, *J* = 6.1 Hz, 2H), 3.12 (q, *J* = 5.8 Hz, 2H), 2.70 (s, 6H), 1.59 (quint, *J* = 6.1 Hz, 2H), 1.41 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.72 (s, 1C), 156.62 (s, 1C), 148.40 (s, 1C), 137.56 (s, 1C), 134.34 (s, 1C), 128.82 (d, 2C), 128.41 (d, 2C), 121.76 (d, 1C), 79.49 (s, 1C), 56.56 (t, 1C), 53.73 (t, $^{1}J_{CD}$ = 21.7 Hz, 1C), 37.78 (s, 1C), 37.18 (t, 1C), 30.57 (t, 1C), 28.50 (q, 3C); HR-ESI-ORBITRAP $[M+H]^+$ m/z calcd. 423.2430 for $C_{20}H_{27}D_3N_5O_5^+$, found 423.2430.

N-[[[(1,1-Dimethylethoxy)carbonyl]amino]butyl]carbamic acid, 4-[[[4-(hydroxymethyl)-1*H*-1,2,3-triazol-1-yl]methyl]phenyl]methyl ester (P5)



Following general procedure D, starting from **CM5a** (25 mg, 0.066 mmol) **P5** was obtained as a white solid (16 mg, 55%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 (s, 1H), 7.33 (d, *J* = 8.4 Hz, 2H), 7.23 (d, *J* = 7.9 Hz, 2H), 5.48 (s, 2H), 5.06 (s, 2H), 4.99 (bs, 1H), 4.74 (s, 2H), 4.63 (bs, 1H), 3.21-3.13 (m, 2H), 3.13-3.06 (m, 2H), 1.55-1.45 (m, 4H), 1.42 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.40 (s, 1C), 156.17 (s, 1C), 148.35 (s, 1C), 137.60 (s, 1C), 134.41 (s, 1C), 128.81 (d, 2C), 128.41 (d, 2C), 121.73 (d, 1C), 79.37 (s, 1C), 66.01 (t, 1C), 56.61 (t, 1C), 53.97 (t, 1C), 40.81 (t, 1C), 40.22 (t, 1C), 28.52 (q, 3C), 27.43 (t, 1C), 27.26 (t, 1C); HR-ESI-ORBITRAP $[M+H]^+$ m/z calcd. 434.2398 for $C_{21}H_{32}N_5O_5^+$, found 434.2422.

N-[[[(1,1-Dimethylethoxy)carbonyl]amino]butyl]carbamic acid, [4-[[[4-(hydroxymethyl)-1*H*-1,2,3-triazol-1-yl]methyl-*d_1*]phenyl]methyl-*d_2*] ester (IS5)



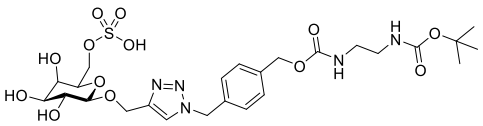
Following general procedure D, starting from **CM5b** (50 mg, 0.131 mmol) **IS5** was obtained as a white solid (42 mg, 72%); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.43 (s, 1H), 7.34 (d, *J* = 8.1 Hz, 2H), 7.24 (d, *J* = 8.0 Hz, 2H), 5.47 (s, 1H), 4.97 (bs, 1H), 4.74 (s, 2H), 4.62 (bs, 1H), 3.22-3.06 (m, 4H), 1.55-1.45 (m, 4H), 1.42 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 156.43 (s, 1C), 156.19 (s, 1C), 148.38 (s, 1C), 137.54 (s, 1C), 134.39 (s, 1C), 128.86 (d, 12C), 128.43 (d, 2C), 121.72 (d, 1C), 79.38 (s, 1C), 56.64 (t, 1C), 53.72 (t, $^{1}J_{CD}$ = 21.5 Hz, 1C), 40.83 (t, 1C), 40.25 (t, 1C), 28.54 (q, 3C), 27.45 (t, 1C), 27.29 (t, 1C); HR-ESI-ORBITRAP $[M+H]^+$ m/z calcd. 437.2586 for $C_{21}H_{29}D_3N_5O_5^+$, found 437.2570.

GALNS Sets (Substrates, internal standards, products)

General procedure E (click chemistry & deprotection, GALNS substrates)

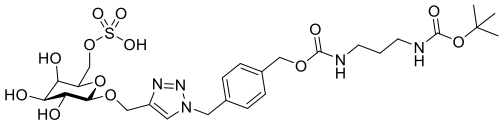
Azide (1 eq) and alkyne **14** (1 eq) were dissolved in dry THF (19 mL/mmol alkyne) under argon and Et_3N (1.2 eq) was added in one portion at room temperature. The reaction was initiated by the addition of CuI (0.1 eq). After 10 minutes the formation of a bright, yellow complex was observed. The reaction was stirred at room temperature for 4 hours. After completion, the mixture was concentrated and directly subjected to Zemplén conditions by re-suspending the crude mixture in dry methanol (30 mmol/mL) and addition of sodium methoxide in methanol (4.88 M, 2 eq). The reaction was stirred overnight and completion was verified by LCMS. Sodium bicarbonate (20 eq) was added in one portion and the mixture was stirred for 30 minutes. The suspension was filtered and the residue was washed three times with methanol. The combined filtrate was concentrated, re-dissolved in water containing 3% MeCN and subjected to prep-HPLC (AXIA 30x100 mm, 5µm coreshell C18XB on a Reveleris X2, 50 mL/min, 5-70% MeCN in H_2O).

N-[[[[[[4-[[4-[[6-O-Sulfato-β-D-galactopyranosyl]methyl]-1*H*-1,2,3-triazol-1-yl]methyl]phenyl]methyl]oxy]carbonyl]amino]ethyl]carbamic acid, 1,1-dimethylethyl ester (CS6)



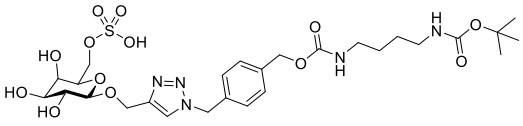
Following general procedure E, **14** (40 mg, 83 µmol) was reacted with **CM3a** (29 mg, 83 µmol) to obtain **CS6** (40 mg, 75%) as a white solid; ^{1}H NMR (400 MHz, CD_3OD): δ 8.14 (s, 1H), 7.43-7.35 (m, 4H), 5.62 (s, 2H), 5.08 (s, 2H), 4.31 (d, *J* = 7.4, 1H), 4.19 (d, *J* = 6.1, 2H), 3.86 (d, *J* = 3.1, 1H), 3.78 (t, *J* = 6.1, 1H), 3.57-3.50 (m, 1H), 3.47 (dd, J_1 = 9.7, J_2 = 3.3, 1H), 3.18 (d, *J* = 5.0, 2H), 3.14 (d, *J* = 4.9, 2H), 1.44 (s, 9H); ^{13}C NMR (100 MHz, CD_3OD): δ 157.49 (s, 1C), 157.20 (s, 1C), 144.68 (s, 1C), 137.35 (s, 1C), 135.23 (s, 1C), 128.09 (d, 2C), 127.88 (d, 2C), 124.86 (d, 1C), 103.30 (d, 1C), 78.72 (s, 1C), 73.24 (d, 1C), 73.16 (d, 1C), 70.94 (d, 1C), 68.78 (d, 1C), 66.73 (t, 1C), 65.54 (t, 1C), 62.16 (t, 1C), 53.16 (t, 1C), 40.41 (t, 1C), 39.83 (t, 1C), 27.34 (q, 3C); HR-ESI-ORBITRAP $[M-H]^-$ m/z calcd. 646.20248 for $C_{25}H_{36}N_5O_{13}S^-$, found 646.20271.

N-[[[[[[4-[[4-[[6-O-Sulfato-*β*-D-galactopyranosyl]methyl]-1H-1,2,3-triazol-1-yl]methyl]phenyl]methyl] oxy]carbonyl]amino]propyl]carbamic acid, 1,1-dimethylethyl ester (CS7)



Following general procedure E, **14** (40 mg, 83 µmol) was reacted with **CM4a** (30 mg, 83 µmol) to obtain **CS7** (44 mg, 80%) as a white solid; ^{1}H NMR (600 MHz, CD_3OD): δ 8.14 (s, 1H), 7.39-7.36 (m, 4H), 5.62 (s, 2H), 5.07 (s, 2H), 4.90 (d, *J* = 12.7, 1H), 4.85 (d, *J* = 12.8, 1H), 4.32 (d, *J* = 7.9, 1H), 4.19 (d, *J* = 6.2, 2H), 3.87 (dd, J_1 = 3.4, J_2 = 1.1, 1H), 3.79 (t, *J* = 6.1, 1H), 3.54 (dd, J_1 = 9.7, J_2 = 7.7, 1H), 3.49 (dd, J_1 = 9.72, J_2 = 3.3, 1H), 3.14 (t, J = 6.8, 2H), 3.07 (t, *J* = 6.7, 2H), 1.64 (quint, *J* = 6.8, 2H), 1.45 (s, 9H); ^{13}C NMR (150 MHz, CD_3OD): δ 157.43 (s, 1C), 157.17 (s, 1C), 144.66 (s, 1C), 137.42 (s, 1C), 135.20 (s, 1C), 128.09 (d, 2C), 127.86 (d, 2C), 124.78 (d, 1C), 103.23 (d, 1C), 78.59 (s, 1C), 73.20 (d, 1C), 73.13 (d, 1C), 70.93 (d, 1C), 68.76 (d, 1C), 66.67 (t, 1C), 65.50 (t, 1C), 62.14 (t, 1C), 53.18 (t, 1C), 37.78 (t, 1C), 37.25 (t, 1C), 29.75 (t, 1C), 27.39 (q, 3C); HR-ESI-ORBITRAP $[M-H]^-$ m/z calcd. 660.21813 for $C_{26}H_{38}N_5O_{13}S^-$, found 660.21842.

N-[[[[[[4-[[4-[[6-O-Sulfato-*β*-D-galactopyranosyl]methyl]-1H-1,2,3-triazol-1-yl]methyl]phenyl]methyl] oxy]carbonyl]amino]butyl]carbamic acid, 1,1-dimethylethyl ester (CS8)

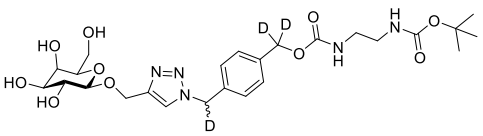


Following general procedure E, **14** (40, 83 µmol) was reacted with **CM5a** (31 mg, 83 µmol) to obtain **CS8** (44 mg, 78%) as a white solid; ^{1}H NMR (600 MHz, CD_3OD): δ 8.15 (s, 1H), 7.41-7.36 (d, 4H), 5.62 (s, 2H), 5.07 (s, 2H), 4.90-4.84 (m, 2H), 4.32 (d, *J* = 7.6, 1H), 4.19 (d, *J* = 6.2, 2H), 3.87-3.85 (m, 1H), 3.79 (t, *J* = 6.1, 1H), 3.53 (dd, J_1 = 9.8, J_2 = 7.5, 1H), 3.48 (dd, J_1 = 9.7, J_2 = 3.6, 1H), 3.12 (t, *J* = 6.4, 2H), 3.05 (t, *J* = 6.4, 2H), 1.53-1.47 (m, 4H), 1.44 (s, 9H); ^{13}C NMR (150 MHz, CD_3OD): δ 157.41 (s, 1C), 157.16 (s, 1C), 144.67 (s, 1C), 137.47 (s, 1C), 135.20 (s, 1C), 128.09 (d, 2C), 127.83 (d, 2C), 124.85 (d, 1C), 103.30 (d, 1C), 78.45 (s, 1C), 73.22 (d, 1C), 73.14 (d, 1C), 70.92 (d, 1C), 68.78 (d, 1C), 66.73 (t, 1C), 65.42 (t, 1C), 62.16 (t, 1C), 53.16 (t, 1C), 40.07 (t, 1C), 39.57 (t, 1C), 27.39 (q, 3C), 26.79 (t, 1C), 26.75 (t, 1C);HR-ESI-ORBITRAP $[M-H]^-$ m/z calcd. 674.23378 for $C_{27}H_{40}N_5O_{13}S^-$, found 674.2338.

General procedure F (Click chemistry, GALNS products and IS)

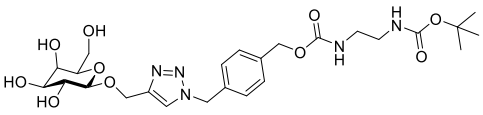
Azide (1 eq) and alkyne **13** (1 eq) were dissolved in dry THF (19 mL/mmol alkyne) under argon and Et_3N (1.2 eq) was added in one portion at room temperature. The reaction was initiated by the addition of CuI (0.1 eq). After 10 minutes the formation of a bright, yellow complex was observed. The reaction was stirred at room temperature for 4 hours. After completion, the mixture was concentrated and directly subjected to column chromatography (SiO_2, MeOH in CH_2Cl_2, 0-20% gradient elution).

N-[[[[[[4-[[4-[[β-D-Galactopyranosyl]methyl]-*1H*-1,2,3-triazol-1-yl]methyl-*d_1*]phenyl]methyl-*d_2*]oxy]carbonyl] amino]ethyl]carbamic acid, 1,1-dimethylethyl ester (IS6)



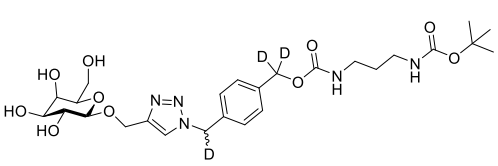
Following general procedure F, **13** (10 mg, 46 µmol) was reacted with **CM3b** (16 mg, 46 µmol) to obtain **IS6** (20 mg, 76%) as a white solid; ^{1}H NMR (200 MHz, CD_3OD): δ 8.00 (s, 1H), 7.38 (d, *J* = 8.1, 2H), 7.31 (d, *J* = 8.4, 2H), 5.57 (s, 1H), 4.96 (d, *J* = 12.5, 1H), 4.76 (d, *J* = 12.5, 1H), 4.32 (d, *J* = 7.0, 1H), 3.85-3.80 (m, 1H), 3.77-3.70 (m, 2H), 3.56-3.45 (m, 3H), 3.19-3.10 (m, 4H), 1.42 (s, 9H); ^{13}C NMR (50 MHz, CD_3OD): δ 158.85 (s, 1C), 158.57 (s, 1C), 146.27 (s, 1C), 138.89 (s, 1C), 136.39 (s, 1C), 129.38 (d, 2C), 129.31 (d, 2C), 125.34 (d, 1C), 104.30 (d, 1C), 80.13 (s, 1C), 76.80 (d, 1C), 74.87 (d, 1C), 72.42 (d, 1C), 70.29 (d, 1C), 63.06 (t, 1C), 62.55 (t, 1C), 41.84 (t, 1C), 41.22 (t, 1C), 28.75 (q, 3C); HR-ESI-ORBITRAP $[M+H]^+$ m/z calcd. 571.28015 for $C_{25}H_{35}D_3N_5O_{10}^+$, found 571.27863.

N-[[[[[[4-[[4-[[β-D-Galactopyranosyl]methyl]-*1H*-1,2,3-triazol-1-yl]methyl]phenyl]methyl]oxy]carbonyl] amino]ethyl]carbamic acid, 1,1-dimethylethyl ester (P6)



Following general procedure F, **13** (30 mg, 138 µmol) was reacted with **CM3a** (48 mg, 138 µmol) to obtain **P6** (62 mg, 79%) as a white solid; ^{1}H NMR (200 MHz, CD_3OD): δ 7.99 (s, 1H), 7.37 (d, *J* = 8.3, 2H), 7.30 (d, *J* = 8.3, 2H), 5.58 (s, 2H), 5.06 (s, 2H), 4.95 (d, *J* = 12.5, 1H), 4.76 (d, *J* = 12.5, 1H), 4.33 (d, *J* = 7.3, 1H), 3.85-3.80 (m, 1H), 3.78-3.69 (m, 2H), 3.57-3.45 (m, 3H), 3.22-3.08 (m, 4H), 1.42 (s, 9H); ^{13}C NMR (50 MHz, CD_3OD): δ 158.84 (s, 1C), 158.56 (s, 1C), 146.26 (s, 1C), 138.92 (s, 1C), 136.42 (s, 1C), 129.33 (m, 4C), 125.40 (d, 1C), 104.31 (d, 1C), 80.14 (s, 1C), 76.80 (d, 1C), 74.87 (d, 1C), 72.43 (d, 1C), 70.30 (d, 1C), 66.88 (t, 1C), 63.07 (t, 1C), 62.57 (t, 1C), 54.63 (t, 1C), 41.86 (t, 1C), 41.24 (t, 1C), 28.77 (q, 3C); HR-ESI-ORBITRAP $[M+H]^+$ m/z calcd. 568.26132 for $C_{25}H_{38}N_5O_{10}^+$, found 568.2601.

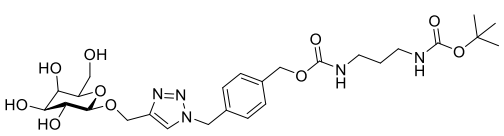
N-[[[[[[4-[[4-[[β-D-Galactopyranosyl]methyl]-*1H*-1,2,3-triazol-1-yl]methyl-*d_1*]phenyl]methyl-*d_2*]oxy]carbonyl] amino]propyl]carbamic acid, 1,1-dimethylethyl ester (IS7)



Following general procedure F, **13** (10 mg, 46 µmol) was reacted with **CM4b** (17 mg, 46 µmol) to give **IS7** (19 mg, 70%) as a white solid; ^{1}H NMR (600 MHz, CD_3OD): δ 8.00 (s, 1H), 7.38 (d, *J* = 8.1, 2H), 7.33 (d, *J* = 8.1, 2H), 5.58 (d, *J* = 2.9, 1H), 4.97 (d, *J* = 12.1, 2H), 4.78 (d, *J* = 12.5, 1H), 4.34 (d, *J* = 7.6, 1H), 3.85-3.83 (m, 1H), 3.77 (dd, J_1 = 11.4, J_2 = 7.1, 1H), 3.72 (dd, J_1 = 11.3, J_2 = 5.2, 1H), 3.57-3.51 (m, 2H), 3.47 (dd, J_1 = 9.7, J_2 = 3.4, 1H), 3.14 (t, *J* = 6.8, 2H), 3.07 (t, *J* = 6.6, 2H), 1.63 (quint, *J* = 6.8, 2H), 1.44 (s, 9H); ^{13}C NMR (150 MHz, CD_3OD): δ 157.41 (s, 1C), 157.17 (s, 1C), 144.88 (s, 1C), 137.49 (s, 1C), 134.98 (s, 1C), 127.98 (d, 2C), 127.93 (d, 2C), 123.96 (d, 1C), 102.91 (d, 1C), 78.60 (s, 1C), 75.41 (d, 1C), 73.48 (d, 1C), 71.03 (d, 1C), 68.91 (d, 1C), 64.86 (quint, 1CD_2), 61.67 (t, 1C), 61.17 (t, 1C), 52.98 (t,

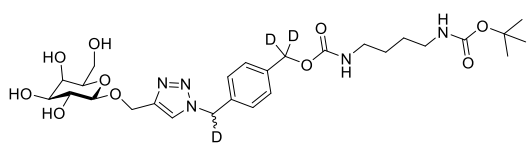
1CD), 37.78 (t, 1C), 37.23 (t, 1C), 29.76 (t, 1C), 27.39 (q, 3C); HR-ESI-ORBITRAP $[M+H]^+$ m/z calcd. 585.295799 for $C_{26}H_{37}D_3N_5O_{10}^+$, found. 585.29448.

N-[[[[[[4-[[4-[[β-D-Galactopyranosyl]methyl]-*1H*-1,2,3-triazol-1-yl]methyl]phenyl]methyl] oxy]carbonyl]amino]propyl]carbamic acid, 1,1-dimethylethyl ester (P7)



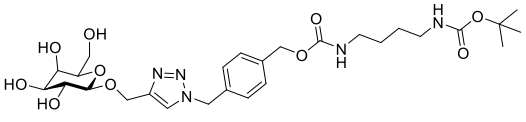
Following general procedure F, **13** (30 mg, 138 µmol) was reacted with **CM4a** (50 mg, 138 µmol) to give **P7** (66 mg, 82%) as a white solid; ^{1}H NMR (600 MHz, CD_3OD): δ 8.01 (s, 1H), 7.39 (d, *J* = 8.0, 2H), 7.34 (d, *J* = 8.1, 2H), 5.61 (s, 2H), 5.08 (s, 2H), 4.97 (d, *J* = 12.5, 1H), 4.79 (d, *J* = 12.5, 1H), 4.33 (d, *J* = 7.7, 1H), 3.84 (dd, J_1 = 3.4, J_2 = 0.8, 1H), 3.77 (dd, J_1 = 11.3, J_2 = 7.0, 1H), 3.72 (dd, J_1 = 11.3, J_2 = 5.1, 1H), 3.56-3.51 (m, 2H), 3.47 (dd, J_1 = 9.7, J_2 = 3.4, 1H), 3.14 (t, *J* = 6.8, 2H), 3.07 (t, *J* = 6.7, 2H), 1.64 (quint, *J* = 6.8, 2H), 1.45 (s, 9H); ^{13}C NMR (150 MHz, CD_3OD): δ 157.40 (s, 1C), 157.17 (s, 1C), 144.95 (s, 1C), 137.62 (s, 1C), 135.01 (s, 1C), 127.90 (d, 4C), 123.98 (d, 1C), 102.91 (d, 1C), 78.58 (s, 1C), 75.41 (d, 1C), 73.48 (d, 1C), 71.01 (d, 1C), 68.90 (d, 1C), 65.43 (t, 1C), 61.66 (t, 1C), 61.16 (t, 1C), 53.23 (t, 1C), 37.76 (t, 1C), 37.21 (t, 1C), 29.75 (t, 1C), 27.36 (q, 3C); HR-ESI-ORBITRAP $[M+H]^+$ m/z calcd. 582.2770 for $C_{26}H_{40}N_5O_{10}^+$, found 582.2760.

N-[[[[[[4-[[4-[β-D-Galactopyranosyl]methyl]-*1H*-1,2,3-triazol-1-yl]methyl-d_1]phenyl]methyl-d_2]oxy]carbonyl] amino]butyl]carbamic acid, 1,1-dimethylethyl ester (IS8)



Following general procedure A, **13** (10 mg, 46 µmol) was reacted with **CM5b** (18 mg, 46 µmol) to give **IS8** (20 mg, 72%) as a white solid; ^{1}H NMR (400 MHz, CD_3OD): δ 8.03 (s, 1H), 7.38 (d, *J* = 8.8, 2H), 7.33 (d, *J* = 9.0, 2H), 5.59 (s, 1H), 4.97 (d, *J* = 12.5, 1H), 4.79 (d, *J* = 12.4, 1H), 4.46-4.44 (m, 1H), 4.35 (d, *J* = 7.5, 1H), 3.88-3.84 (m, 1H), 3.79-3.72 (m, 1H), 3.56-3.52 (m, 1H), 3.49 (dd, J_1 = 9.8, J_2 = 3.3, 1H), 3.12 (t, *J* = 6.4, 2H), 3.04 (t, *J* = 6.1, 2H), 1.52-1.47 (m, 4H), 1.44 (s, 9H); ^{13}C NMR (100 MHz, CD_3OD): δ 157.40 (s, 1C), 157.15 (s, 1C), 144.82 (s, 1C), 137.54 (s, 1C), 134.96 (s, 1C), 127.94 (d, 4C), 124.02 (d, 1C), 102.87 (d, 1C), 78.79 (s, 1C), 75.35 (d, 1C), 73.45 (d, 1C), 71.04 (d, 1C), 70.89 (d, 1C), 68.92 (d, 1C), 61.13 (t, 1C), 55.16 (t, 1C), 40.11 (t, 1C), 39.60 (t, 1C), 27.44 (q, 3C), 26.82 (t, 1C), 26.76 (t, 1C); HR-ESI-ORBITRAP $[M+H]^+$ m/z calcd. 599.311449 for $C_{27}H_{39}D_3N_5O_{10}^+$, found 599.31016.

N-[[[[[[4-[[4-[[β-D-Galactopyranosyl]methyl]-*1H*-1,2,3-triazol-1-yl]methyl]phenyl]methyl] oxy]carbonyl]amino]butyl]carbamic acid, 1,1-dimethylethyl ester (P8)



Following general procedure E, **13** (30 mg, 138 µmol) was reacted with **CM4a** (52 mg, 138 µmol) to give **P8** (72 mg, 87%) as a white solid; ^{1}H NMR (400 MHz, CD_3OD): δ 8.02 (s, 1H), 7.39 (d, *J* = 8.0, 2H), 7.34 (d, *J* = 8.0, 2H), 5.61 (s, 2H), 5.08 (s, 2H), 4.97 (d, *J* = 12.5, 1H), 4.79 (d, *J* = 12.4, 1H), 4.44 (dd, J_1 = 5.0, J_2 = 2.3, 1H), 4.34 (d, *J* = 7.6, 1H), 3.87-3.83 (m, 1H), 3.79-3.70 (m, 1H), 3.56-3.50 (m, 1H), 3.47 (dd, J_1 = 9.8, J_2 = 3.1, 1H), 3.12 (t, *J* = 5.9, 2H), 3.05 (t, *J* = 5.7, 2H), 1.52-1.47 (m, 4H), 1.44 (s, 9H); ^{13}C NMR (100 MHz, CD_3OD): δ 157.38 (s, 1C), 157.16 (s, 1C), 144.86 (s, 1C), 137.67 (s, 1C), 134.99 (s, 1C), 127.90 (d, 2C), 127.87 (d, 2C), 123.97 (d, 1C), 102.89 (d, 1C), 78.46 (s, 1C), 75.38 (d, 1C), 71.01 (d, 1C), 70.86 (d, 1C), 68.92 (d, 1C), 61.67 (t, 1C), 61.11 (t, 1C), 55.09 (t, 1C), 53.23 (t, 1C), 40.08 (t, 1C), 39.58 (t, 1C), 27.39 (q, 3C), 26.80 (t, 1C), 26.75 (t, 1C); HR-ESI-ORBITRAP $[M+H]^+$ m/z calcd. 596.2926 for $C_{27}H_{42}N_5O_{10}^+$, found 596.2918.

3) Analytical methods and materials used for enzyme assays and DBS analysis

Materials and reagents

Acetonitrile (LC-MS Grade), methanol (Ultra LC-MS Grade) and water (Ultra LC-MS Grade) were obtained from Carl Roth GmbH (Austria). α-L-Iduronidase (IDUA) and all other chemicals were obtained from Sigma-Aldrich (Germany). Equipment used to perform the enzyme assays: MT-plate shaker TiMix 5 control equipped with incubation hood TH15 (Edmund Bühler GmbH, Germany), Centrifuge 5810 from Eppendorf (Germany), DBS Puncher® Instrument from Perkin Elmer (USA), balance (PCB 200-2) from Kern&Sohn GmbH (Germany), pH-meter (seven compact) from Mettler Toledo (USA). DBS quality control cards were kindly provided by the CDC (Center for Disease Control and Prevention, USA), including permission for their use in this and related studies. DBS samples of newborns were provided by the Medical University of Vienna and the Vienna General Hospital (Newborn Screening Program) and anonymized DBS samples of affected patients (non-newborns) were kindly provided by the University Medical Center of the Johannes Gutenberg-University Mainz (Germany). The institutional ethics committee approved the study (EK 478/2009 and EK 1687/2014).

Assaying IDUA

IDUA assay buffer

Sodium formate (1.9 g) was dissolved in water (250 mL). The pH was adjusted to 4.04 by addition of formic acid. The buffer solution was partitioned to give 14 vials, each containing 17.5 mL. The buffer was stored at -20 °C. A D-saccharolactone inhibitor solution (3 mmol/L) was prepared by dissolving D-saccharolactone (CAS 61278-30-6, 31.5 mg) in water (40 mL) followed by vortexing for 2 min. Water was added to reach an end volume of 50 mL. 500 µL of the inhibitor solution (3 mmol/L) were added to 17.5 mL assay buffer and the final solution was used immediately. Saccharolactone is used to inhibit β-glucuronidase (GUSB) and thus prevent unwanted enzymatic cleavage of IDUA substrates by GUSB and has been included in assay buffers of previously developed methods.[6-9]

Preparation of IDUA assay cocktail

A 2 mg/mL substrate stock solution in assay buffer and a 13.2 µg/mL IS stock solution in assay buffer were freshly prepared and mixed in a 50:50 ratio to result in an assay cocktail containing 1 mg/mL substrate and 6.6 µg/mL internal standard (IS).

Sample preparation using recombinant human IDUA

10UG enzyme formulation was diluted in 200 µL assay buffer. 16 µL of the resulting solution were added to 1984 µL assay buffer and vortexed for 2 minutes to reach a 400 ng/mL stock solution. This stock was further diluted in assay buffer to reach 200 ng/mL (500µL + 500 µL buffer), 100 ng/mL (250 µL + 750 µL buffer) and 50 ng/mL (125 µL + 875 µL buffer) concentrations. The respective assay cocktail (see above) was mixed in equal amounts with enzyme solutions (13.5 µL enzyme solution + 13.5 µL assay cocktail) in duplicates and incubated in a well plate for 20 min at 22 °C and 250 rpm orbital shake. The well plate was centrifuged for 3 minutes at 3000 rpm and then quenched by adding 75 µL MeCN to each well. The plate was covered with aluminum foil and subjected to orbital shaking at 350 rpm and room temperature for 2 min. The plate was then centrifuged for 15 min at 3200 rpm and subsequently cooled to 4 °C for 10 min to complete precipitation. Finally, 25 µL of supernatant were transferred into a new plate and diluted with 175 µL water for analysis.

Sample preparation – DBS assay (IDUA)

DBS cards were punched as 3.2 mm spots into a well plate and each spot was extracted with 70 µL of PBS buffer for 1 h at 37 °C. DBS cards without blood were used as controls (blank). 15 µL of the assay cocktail and 10 µL of the sample extracts were mixed in a new well plate, covered with adhesive aluminum foil and incubated for 22 h at 37 °C at 500 rpm orbital shake. The plate was centrifuged for 3 minutes at 3000 rpm and then quenched by addition of 75 µL MeCN to each well. The plate was covered with aluminum foil and subjected to orbital shaking at 350 rpm and room temperature for 2 min. The plate was centrifuged for 15 min at 3200 rpm and subsequently cooled to 4 °C for 10 min to complete precipitation. Finally, 25 µL of supernatant were transferred into a new plate and diluted with 175 µL water for analysis.

Sample preparation – Triplex DBS assay (IDUA)

DBS cards were punched as 3.2 mm spots into a well plate and each spot was extracted with 70 µL of PBS Buffer for 1 h at 37 °C. DBS cards without blood were used as controls (blank). 15 µL of assay cocktail and 10 µL of the sample extracts were mixed in a new well plate, covered with adhesive aluminum foil and incubated for 22 h at 37 °C at 500 rpm orbital shake. The plate was centrifuged for 3 minutes at 3000 rpm and then quenched by addition of 75 µL MeCN to each well. The plate was covered with aluminum foil and subjected to orbital shaking at 350 rpm and room temperature for 2 min. The plate was centrifuged for 15 min at 3200 rpm and subsequently cooled to 4 °C for 10 min to complete precipitation. For multiplex measurements 25 µL supernatant of three individually incubated plates that contain different assay cocktail sets (3, 4 and 5) were combined into the same position on a new well plate resulting in 75 µL of mixed sets that were subsequently diluted with 125 µL water for analysis.

Liquid chromatography, mass spectrometry, data analysis (IDUA)

Chromatography was performed on a Shimadzu Nexera X2 system (Japan). Samples (1 µL) were injected onto a Waters (ACQUITY UPLC Peptide BEH C18 Column, 130 Å, 1.7 µm, 2.1 mm x 50 mm) column maintained at 60 °C in reversed phase mode. Solvent A contained 0.1 % formic acid in water. Solvent B contained 0.1 % formic acid in MeCN/MeOH = 1:1 (v/v). The flow rate was set to 0.7 mL/min with the following gradient conditions: 0–2.5 min, 19 % B (isocratic); 2.5–2.53 min, 19–95% B (linear gradient); 2.53–3.5 min, 95 % B (isocratic); 3.5–3.53 min, 95–19 % B (linear gradient); 3.53–4.9 min, 19 % B (isocratic). The total run time was 4.9 min with 1 µL injection. Autosampler temperature was set to 5 °C. Mass spectrometric detection was performed using a Shimadzu 8050 spectrometer (Japan) operated in positive ion electrospray ionization (ESI) mode. The source parameters of the mass spectrometer were optimized and maintained as follows: nebulizing gas flow, 3 L/min; heating gas flow, 10 L/min; interface temperature, 400 °C; DL temperature, 200 °C; ion spray voltage, 4000 V; heating block temperature, 300 °C; drying gas flow, 10 L/min; collision pressure (argon), 270 kPa. Nitrogen was used as nebulizer, auxiliary and curtain gas. Data was processed using LabSolutions 5.8 (Shimadzu) and analyzed in Prism 7 (GraphPad). Results are summarized in Table S1. Means of different groups of patients were compared using the Mann-Whitney U test. For MRM see Table S2.

Table S1. Results of DBS assays using IDUA sets 3-5; shown as blank-corrected "mean values (SD)" [µM/h]

IDUA	Set 3	Set 4	Set 5
CDC QCL	0.151 (0.037)	0.152 (0.019)	0.169 (0.008)
CDC QCM	0.277 (0.076)	0.298 (0.052)	0.305 (0.029)
CDC QCH	0.473 (0.065)	0.468 (0.095)	0.481 (0.031)
DBS of confirmed MPS I patients (n=9, anonymized)	0.170 (0.032)	0.116 (0.053)	0.084 (0.039)
DBS of random patients (n=88, anonymized)	0.404 (0.056)	0.431 (0.069)	0.368 (0.091)

Table S2. IDUA Multiple reaction monitoring (MRM).

	Precursor [m/z]	Product 1 [m/z]	Product 2 [m/z]	Product 3 [m/z]
Set 1 P	320.20	264.10	165.10	202.20
Set 1 IS	323.20	267.10	168.10	205.10
Set 2 P	319.20	100.05	220.20	202.10
Set 2 IS	322.20	100.15	223.15	205.20
Set 3 P	406.30	306.10	202.20	146.10
Set 3 IS	409.30	309.20	205.15	166.25
Set 4 P	420.30	320.15	105.15	202.10
Set 4 IS	423.30	323.20	205.15	107.15
Set 5 P	434.30	334.25	202.15	174.20
Set 5 IS	437.30	337.25	205.15	177.15

Assaying GALNS

GALNS assay buffer

Sodium formate (1.7 g) was dissolved in water (200 mL). To the stirred solution were added 430 µL formic acid and lead (II) formate (2.23 g) in small portions. The mixture was stirred for approximately 20 minutes, until it formed a clear, homogenous solution. The pH was adjusted to 4.04 by addition of 0.1 M NaOH solution. Water was added to reach an end volume of 250 mL. The buffer was freshly prepared once a month and stored at 0-5 °C.

Preparation of GALNS assay cocktail

A 2 mg/mL substrate stock solution in assay buffer and a 20 µg/mL IS stock solution in assay buffer were freshly prepared and mixed in a 1:1 ratio to obtain an assay cocktail containing 1 mg/mL substrate and 10 µg/mL internal standard.

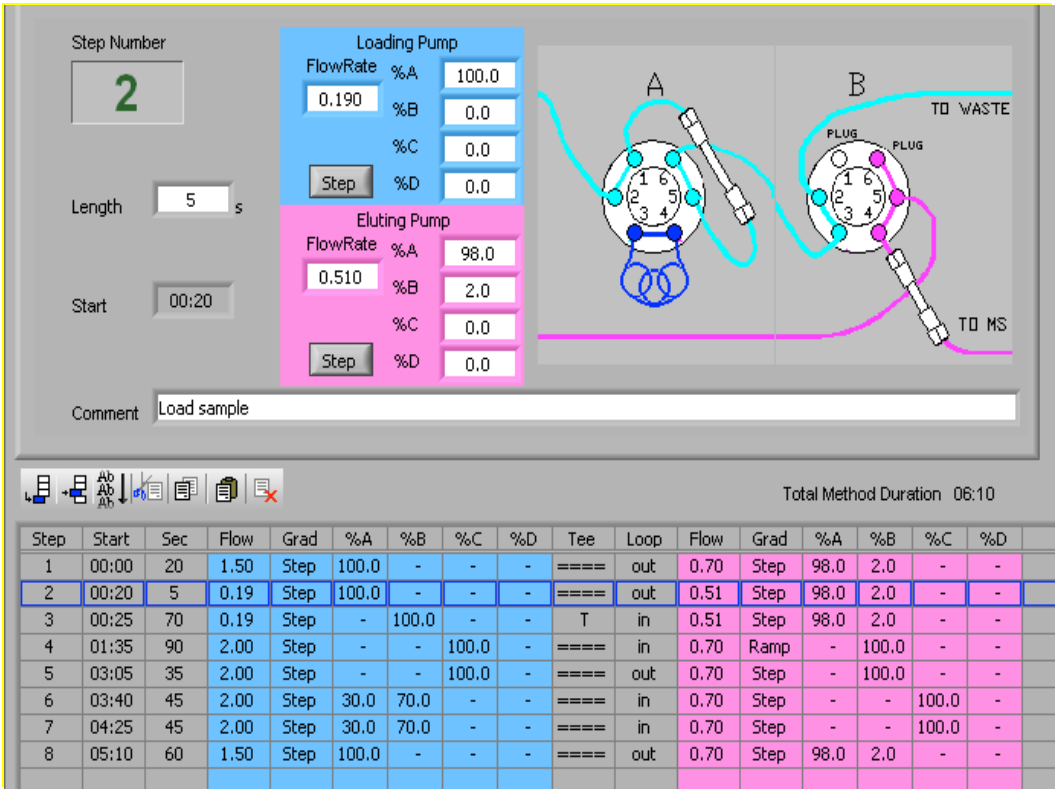
Sample preparation – DBS assay (GALNS)

DBS cards were punched as 3.2 mm spots (4 spots each for blank, QCL, QCM, QCH; 2 spots each for affected patient DBS, and 1 spot each for random newborn DBS) into a 96 well plate. DBS cards without blood were used as controls (blank). Then 30 µL of assay cocktail were added, the plate was covered with adhesive aluminum foil and incubated for 22 h at 37 °C at 500 rpm orbital shake. The plate was centrifuged for 3 minutes at 3000 rpm and the assays quenched by adding 100 µL MeCN to each well.

The plate was again covered with aluminum foil and subjected to orbital shaking at 350 rpm and room temperature for 2 min. The plate was centrifuged for 15 min at 3200 rpm and subsequently cooled to 4 °C for 10 min to complete precipitation. A volume of 50 µL of supernatant was transferred to a new plate and diluted with 100 µL water for analysis.

Liquid chromatography, mass spectrometry, data analysis (GALNS)

Chromatography was performed on a TLX2 system with Accela pumps and a PAL RTC autosampler (Switzerland). Samples (1 µL) were analyzed using a Waters ACQUITY CSH column (C18, 130 Å, 1.7 µm, 2.1 x 50 mm) and a Turboflow HTC column (Thermo Cyclom P, 0.5 x 50 mm) maintained at 40 °C. Solvent A contained 0.1 % formic acid in water. Solvent B contained 0.1 % formic acid in MeCN/MeOH = 1:1 (v/v). A mixture of MeCN/2-propanol/acetone = 45:45:10 (v/v) was used as solvent C. The autosampler was run at room temperature. The total flow rate during separation was 0.7 mL/min with an injection volume of 1 µL, using a turboflow® online sample pre-treatment according to following gradient conditions:



Step	Start	Sec	Flow	Grad	%A	%B	%C	%D	Tee	Loop	Flow	Grad	%A	%B	%C	%D
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2	00:20	5	0.19	Step	100.0	-	-	-	====	out	0.51	Step	98.0	2.0	-	-
3	00:25	70	0.19	Step	-	100.0	-	-	T	in	0.51	Step	98.0	2.0	-	-
4	01:35	90	2.00	Step	-	-	100.0	-	====	in	0.70	Ramp	-	100.0	-	-
5	03:05	35	2.00	Step	-	-	100.0	-	====	out	0.70	Step	-	100.0	-	-
6	03:40	45	2.00	Step	30.0	70.0	-	-	====	in	0.70	Step	-	-	100.0	-
7	04:25	45	2.00	Step	30.0	70.0	-	-	====	in	0.70	Step	-	-	100.0	-
8	05:10	60	1.50	Step	100.0	-	-	-	====	out	0.70	Step	98.0	2.0	-	-

Mass spectrometric detection was performed with a Thermo Scientific TSQ Quantum Ultra (USA) operated in positive ion electrospray ionization (ESI) mode. The source parameters of the mass spectrometer were optimized and used as follows: ion spray voltage, 4500 V; vaporizer temperature, 289°C; sheath gas pressure, 35 psi; ion sweep gas pressure, 2.0 psi, aux gas pressure, 35 psi; capillary temperature, 289 °C, skimmer offset, 0; argon was used as collision gas; nitrogen was used as nebulizer, auxiliary and curtain gas. Data was processed using LCquan™ 2.7 (Thermo Scientific, USA) and analyzed in Prism 7 (GraphPad). Results are summarized in Table S3. Means of different groups of patients were compared using the Mann-Whitney U test. For MRM see Table S4.

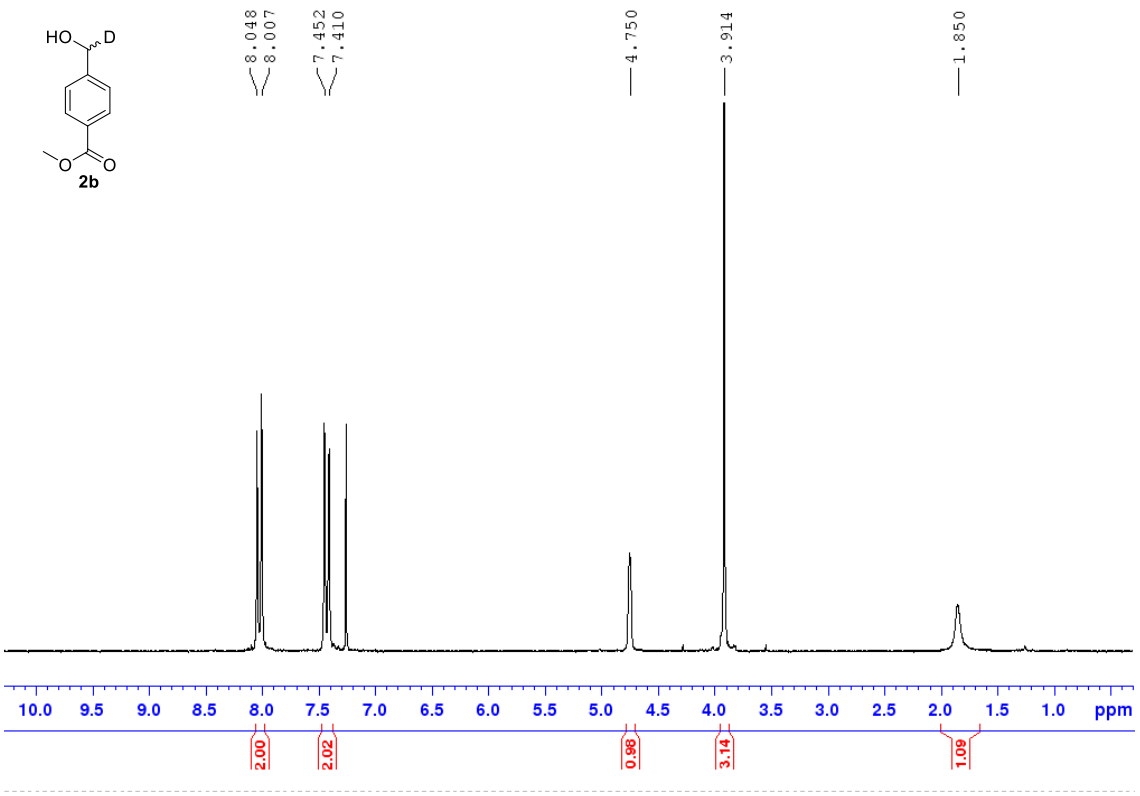
Table S3. Results of DBS assays using GALNS sets 6-8; shown as blank-corrected "mean values (SD)" [µM/h]

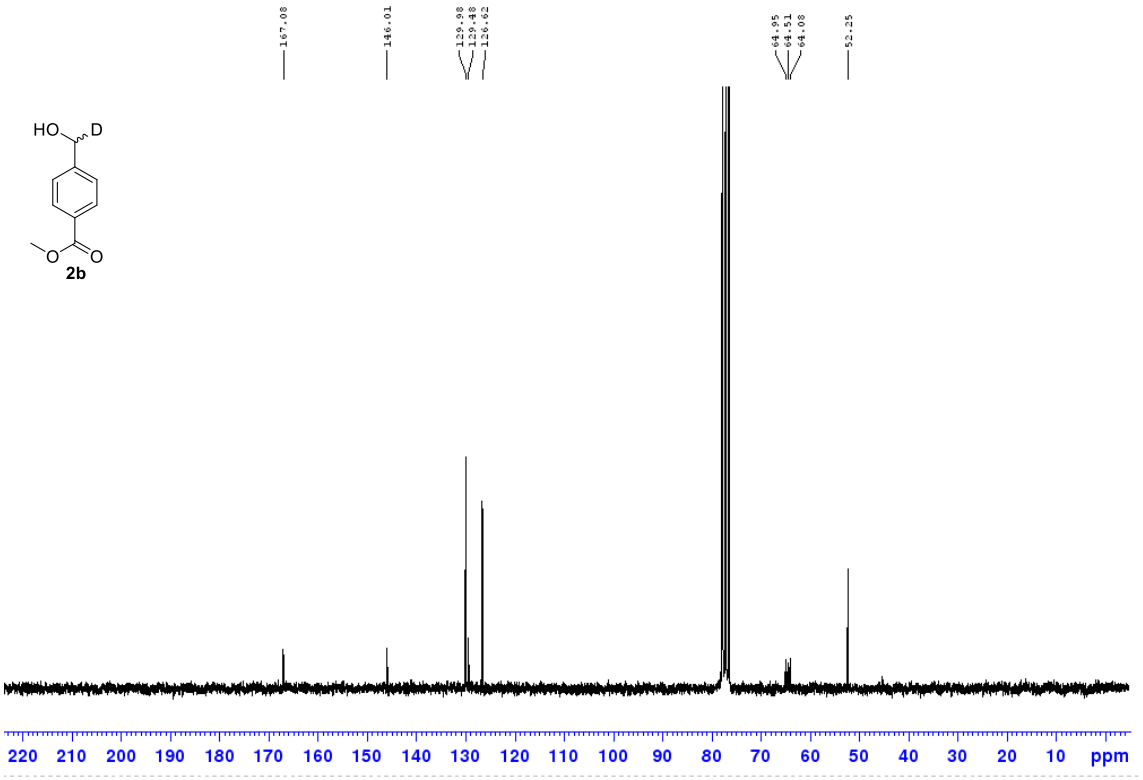
GALNS	Set 6	Set 7	Set 8
CDC QCL	1.26 (0.13)	1.21 (0.14)	1.51 (0.09)
CDC QCM	7.30 (0.03)	8.27 (0.09)	7.08 (0.02)
CDC QCH	12.50 (0.75)	13.11 (0.06)	12.11 (0.46)
DBS of confirmed MPS IVa patients (n=9, anonymized)	0.756 (0.722)	-	-
Random patients (n=116, anonymized)	13.50 (5.87)	-	-

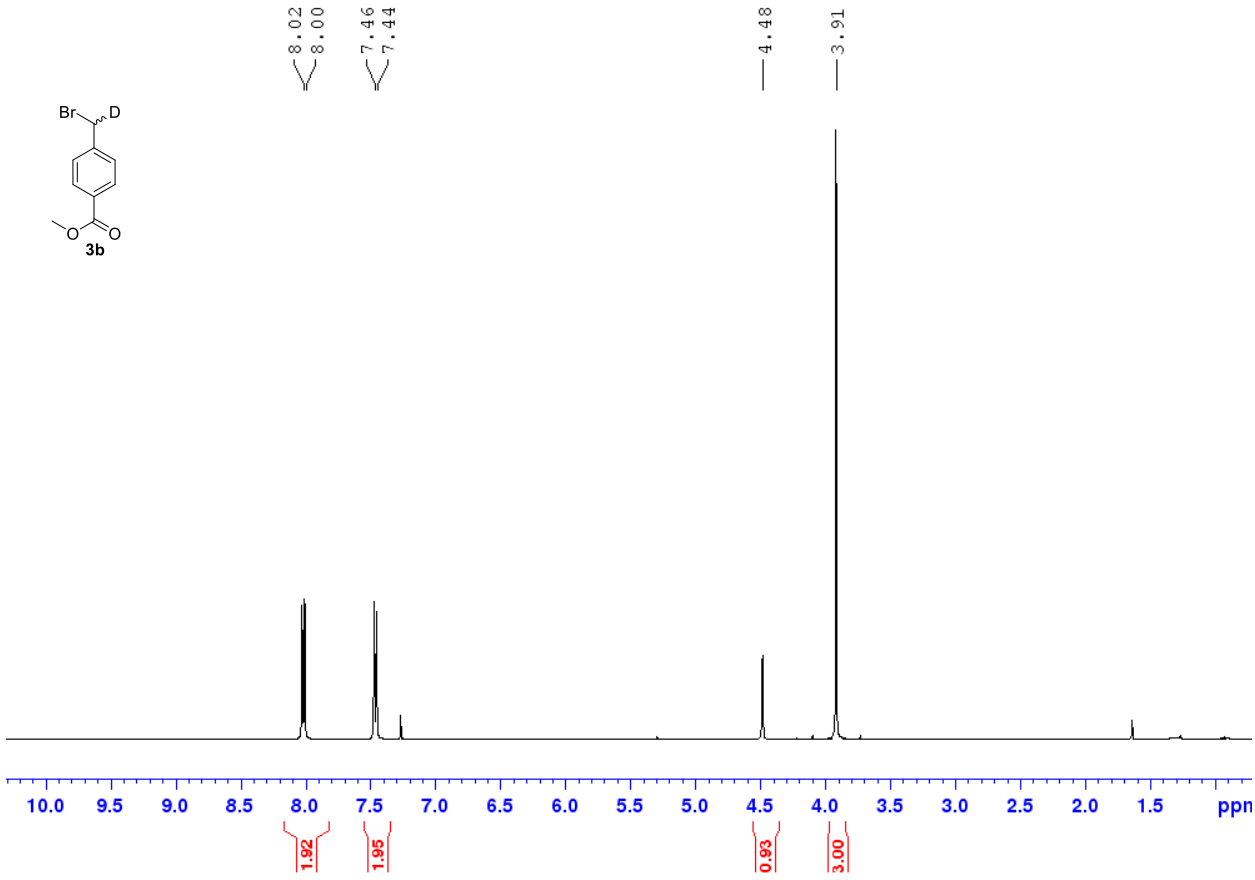
Table S4. GALNS Multiple reaction monitoring.

	Precursor [m/z]	Product 1 [m/z]	Product 2 [m/z]	Product 3 [m/z]
Set 6 P	568.3	306.15	468.1	202.15
Set 6 IS	571.323	471.150	-	-
Set 7 P	582.3	320.2	482.2	105.05
Set 7 IS	585.313	485.12	323.15	-
Set 8 P	596.3	496.15	334.2	174.1
Set 8 IS	599.327	499.05	327.09	-

4) NMR spectra



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8.0
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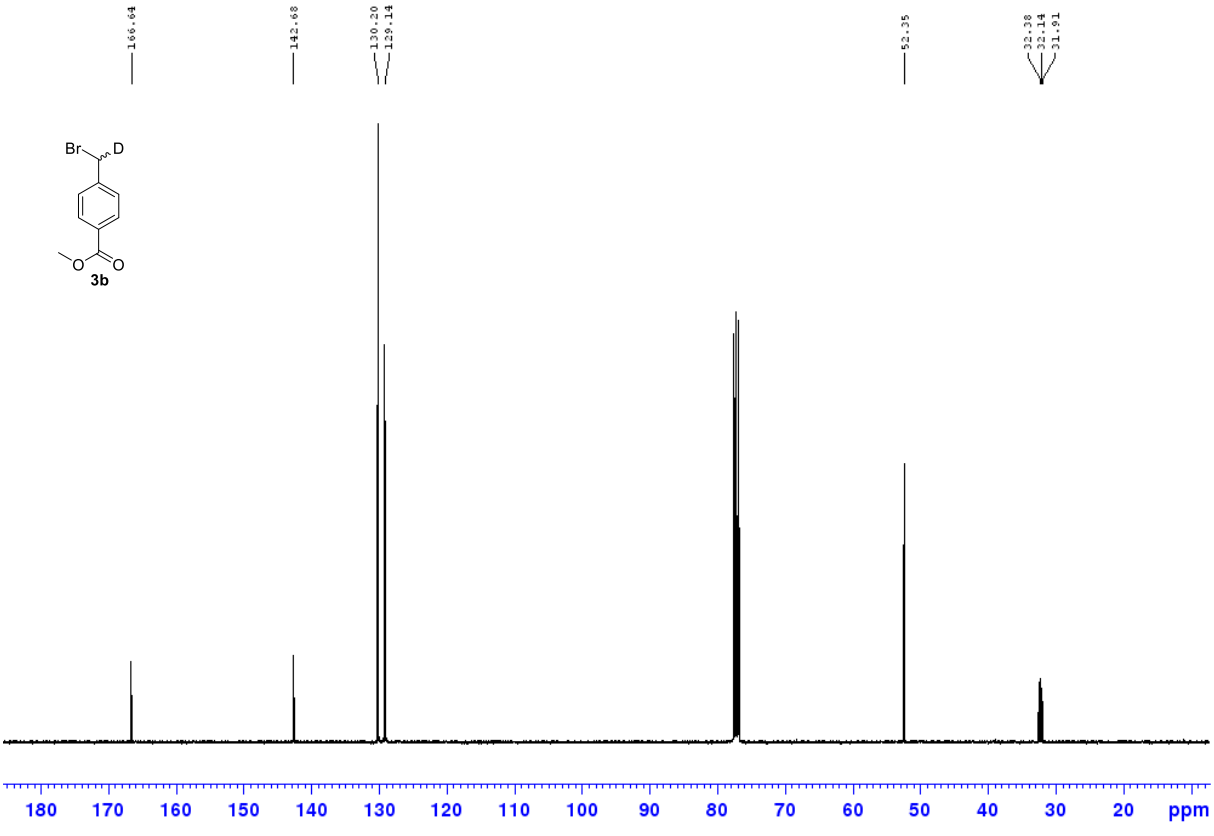
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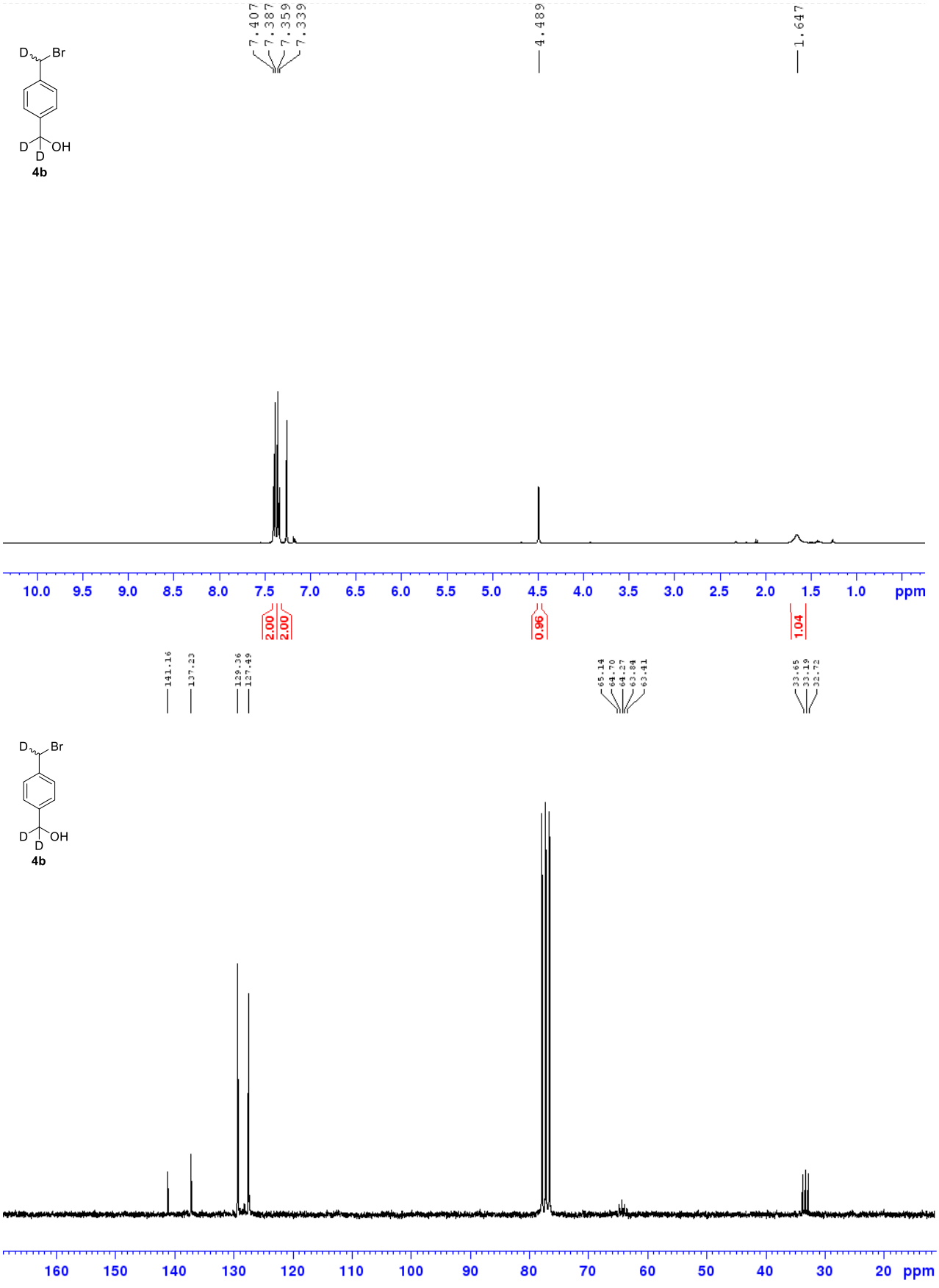
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2.00
2.00
0.96
1.04
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137.23
129.36
127.49
65.14
64.70
64.27
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4b
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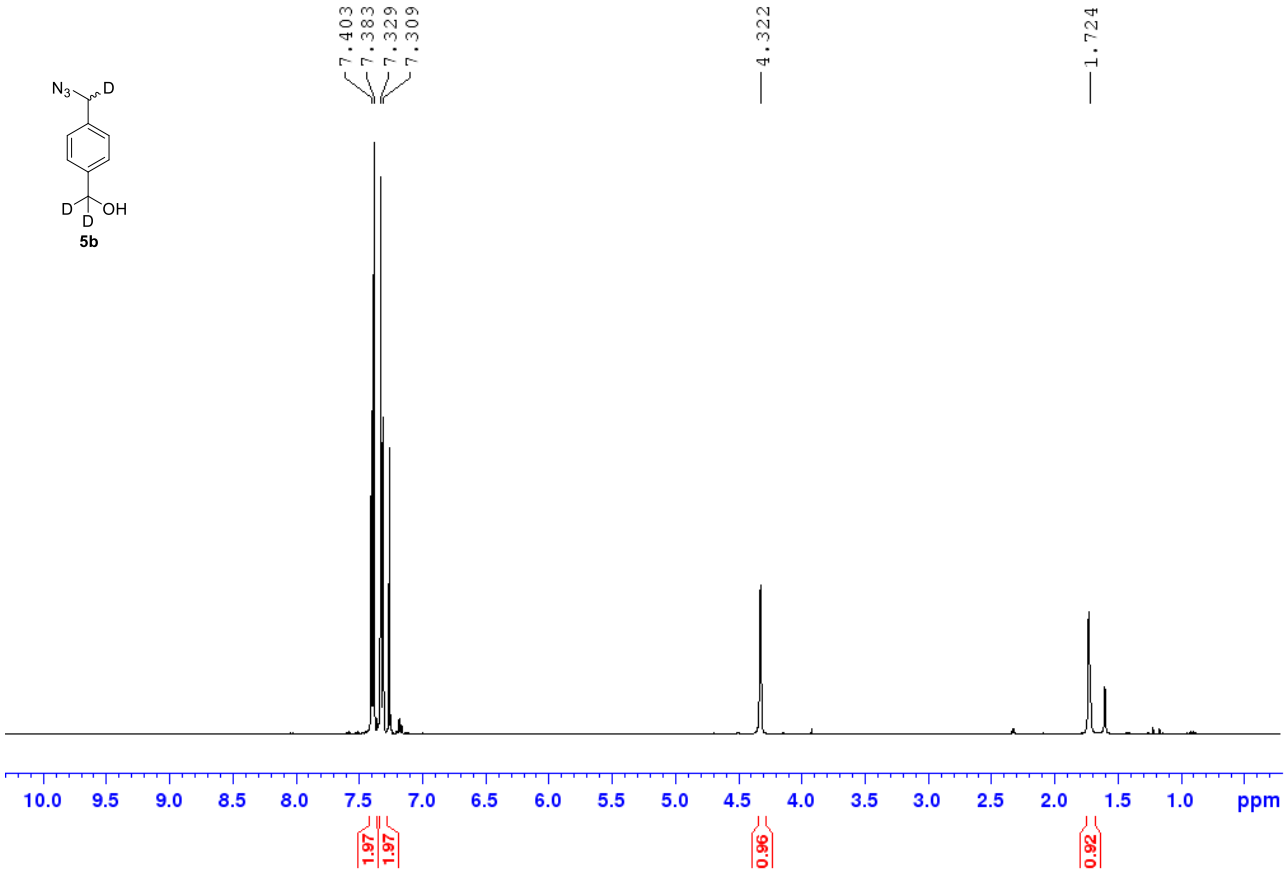
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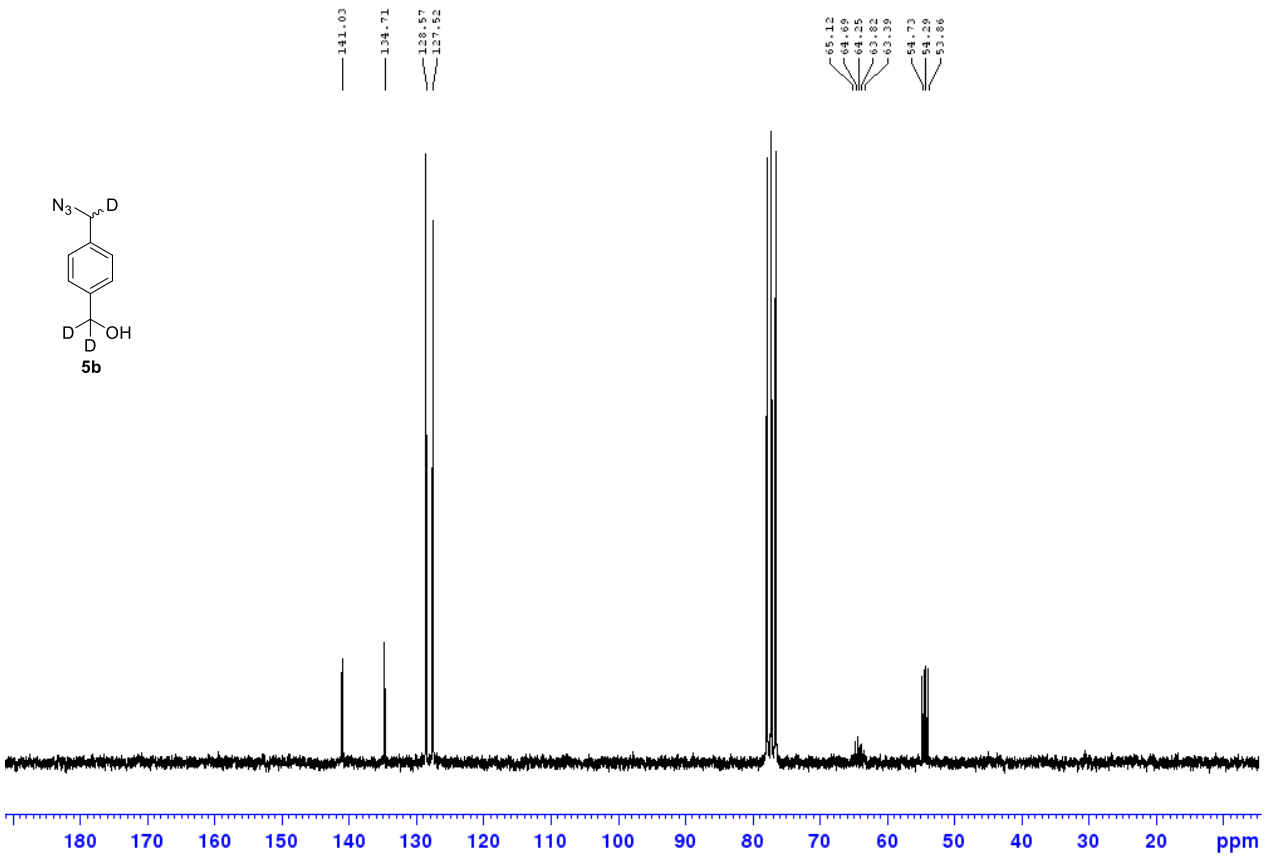
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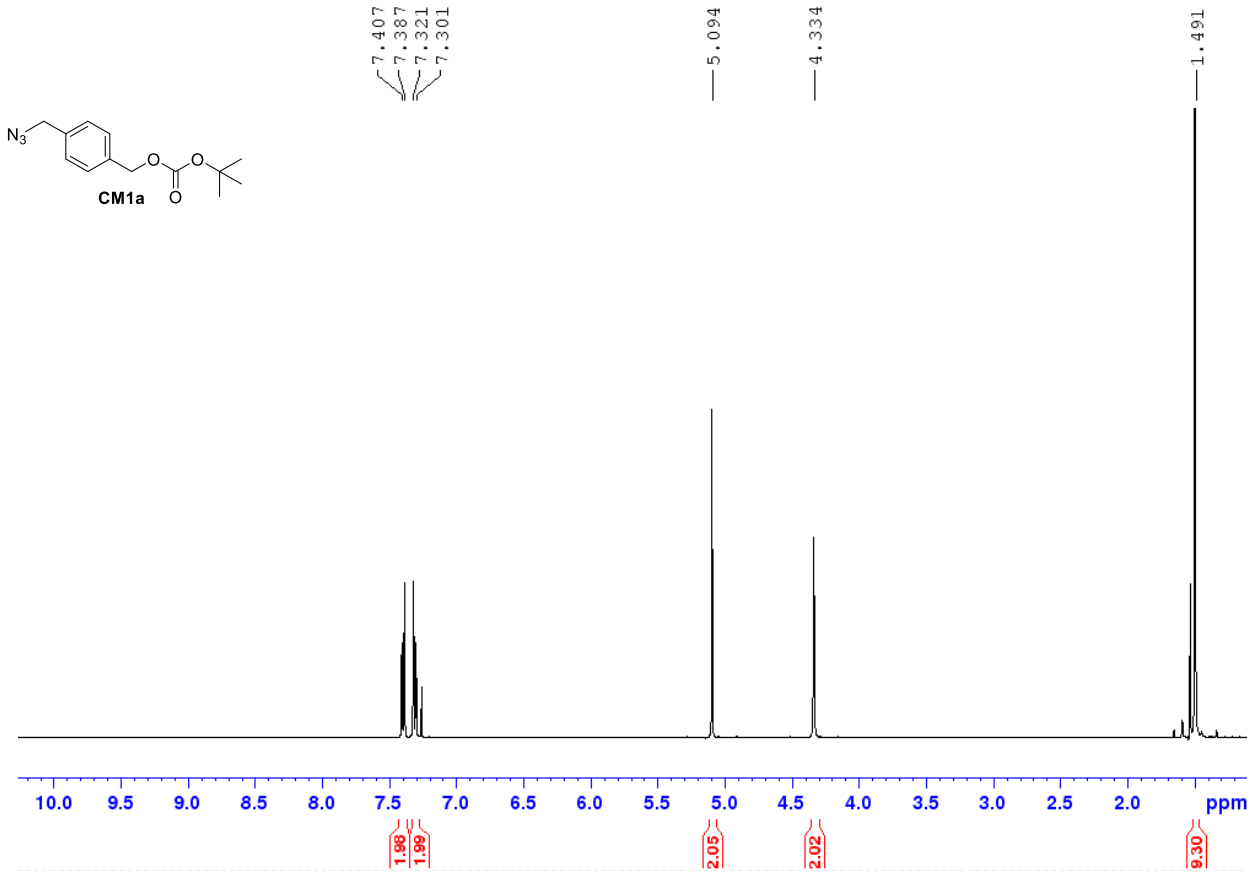
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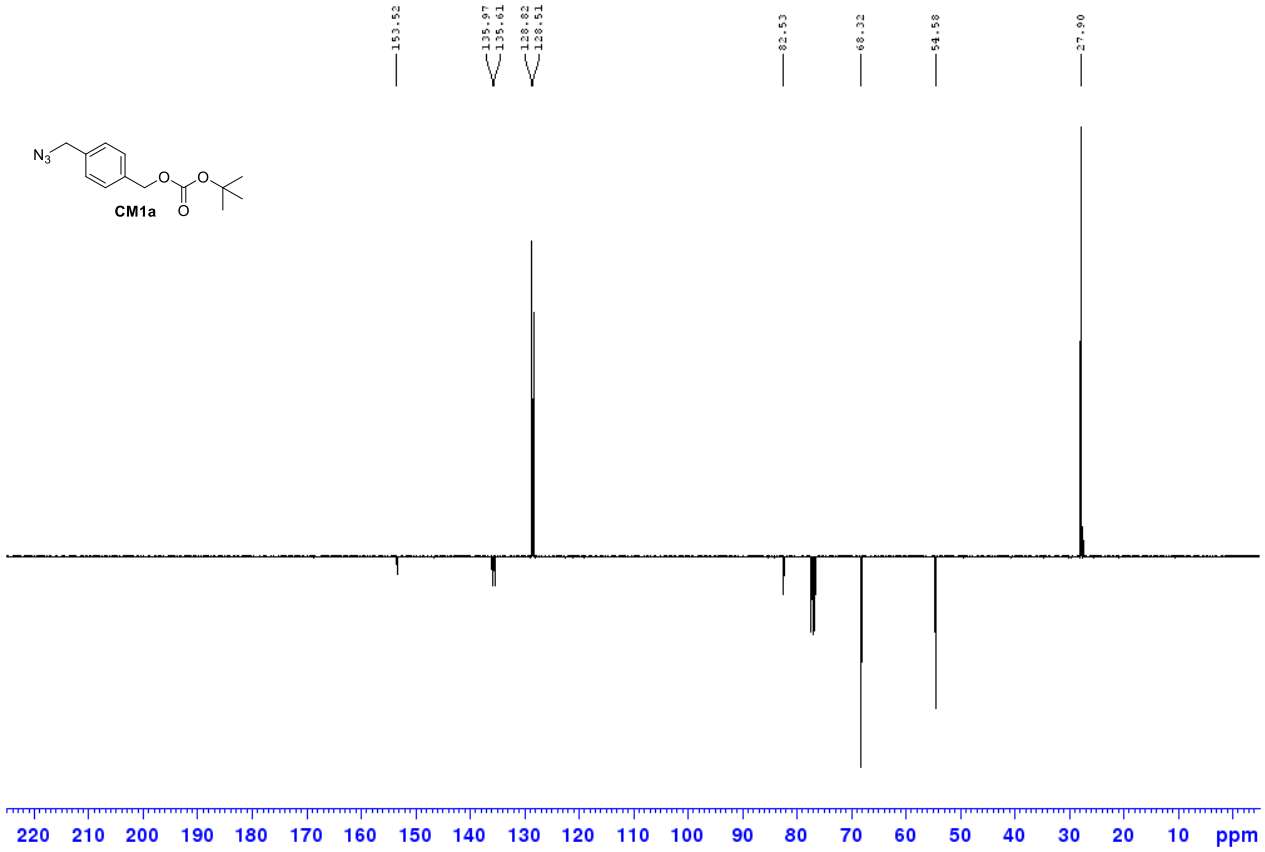
2.02
9.30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CM1a
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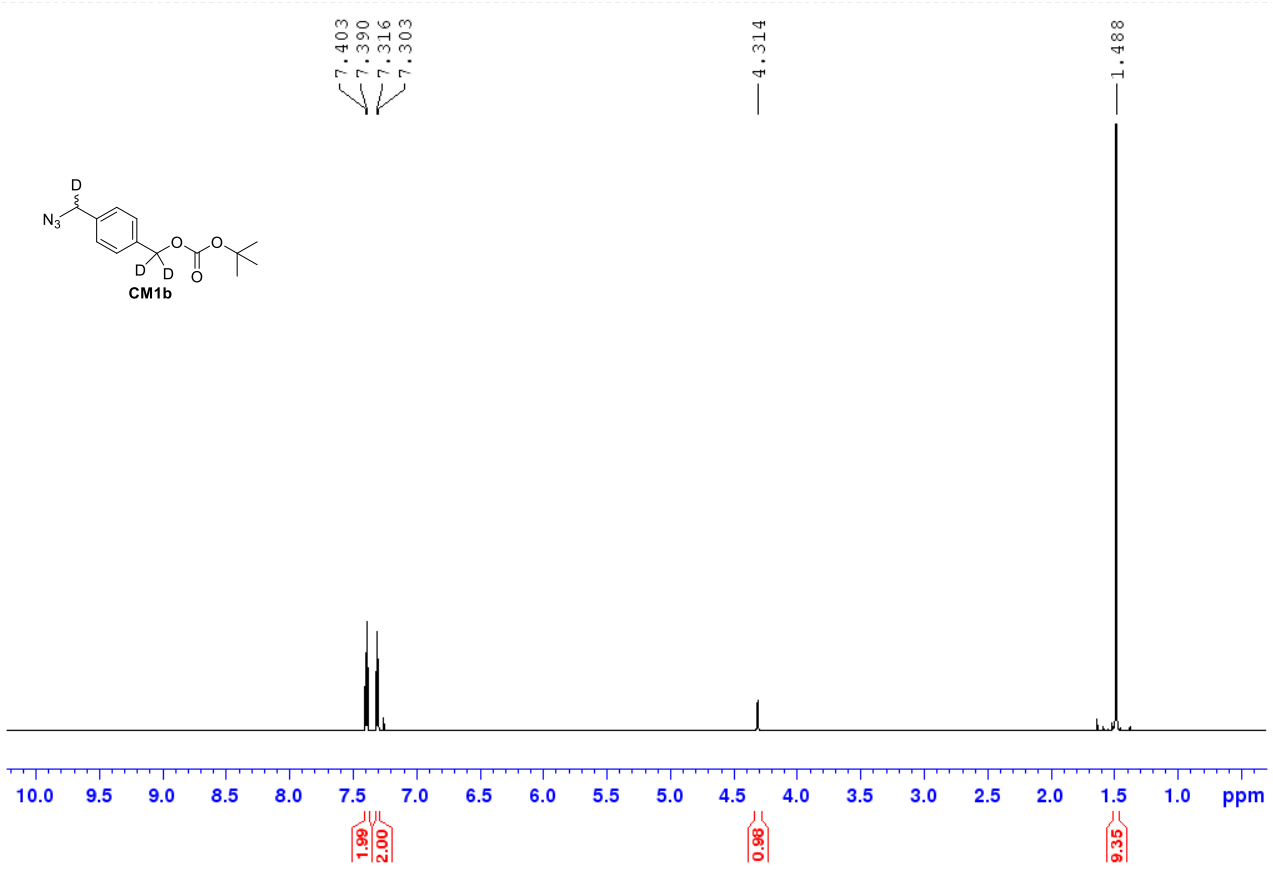
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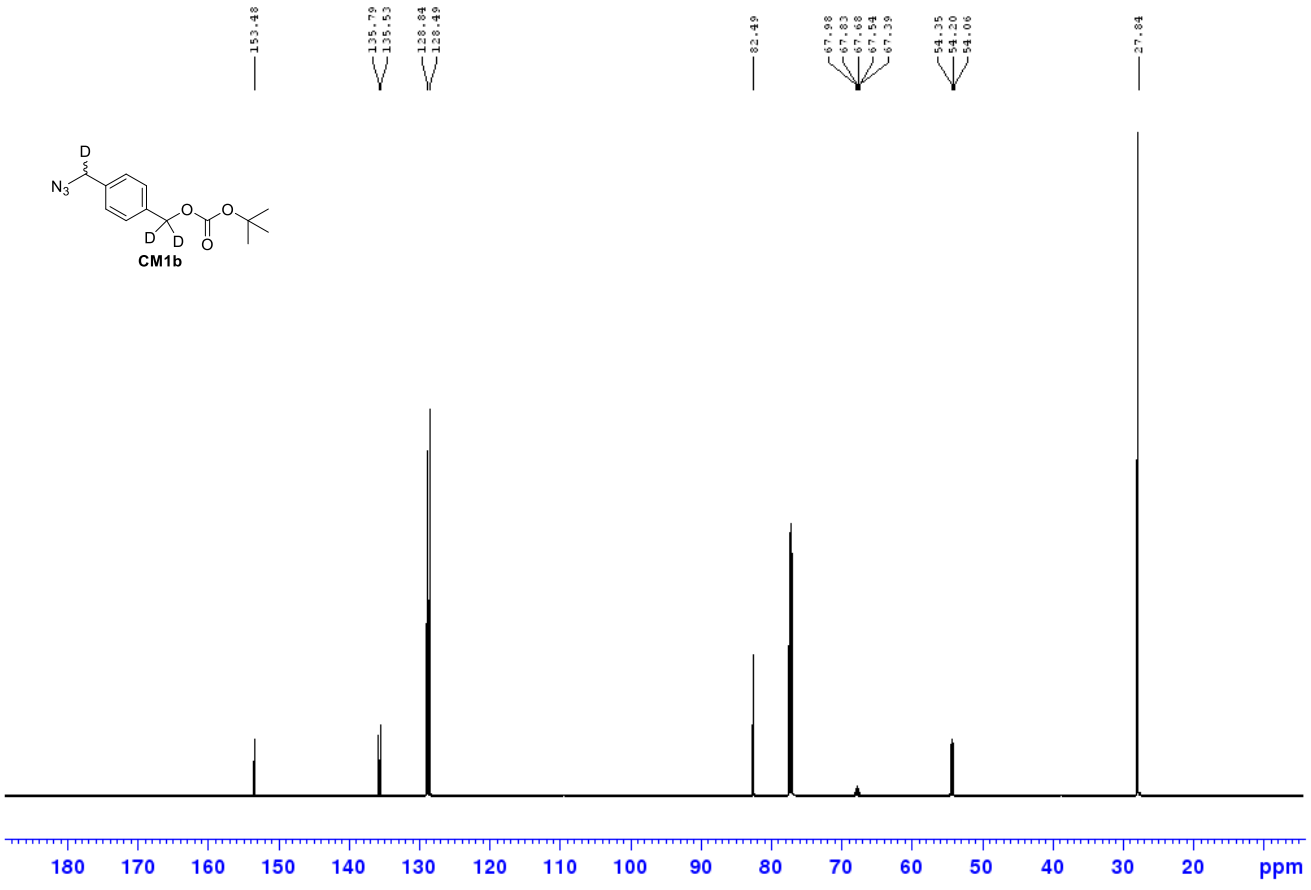
2.00
0.98
9.35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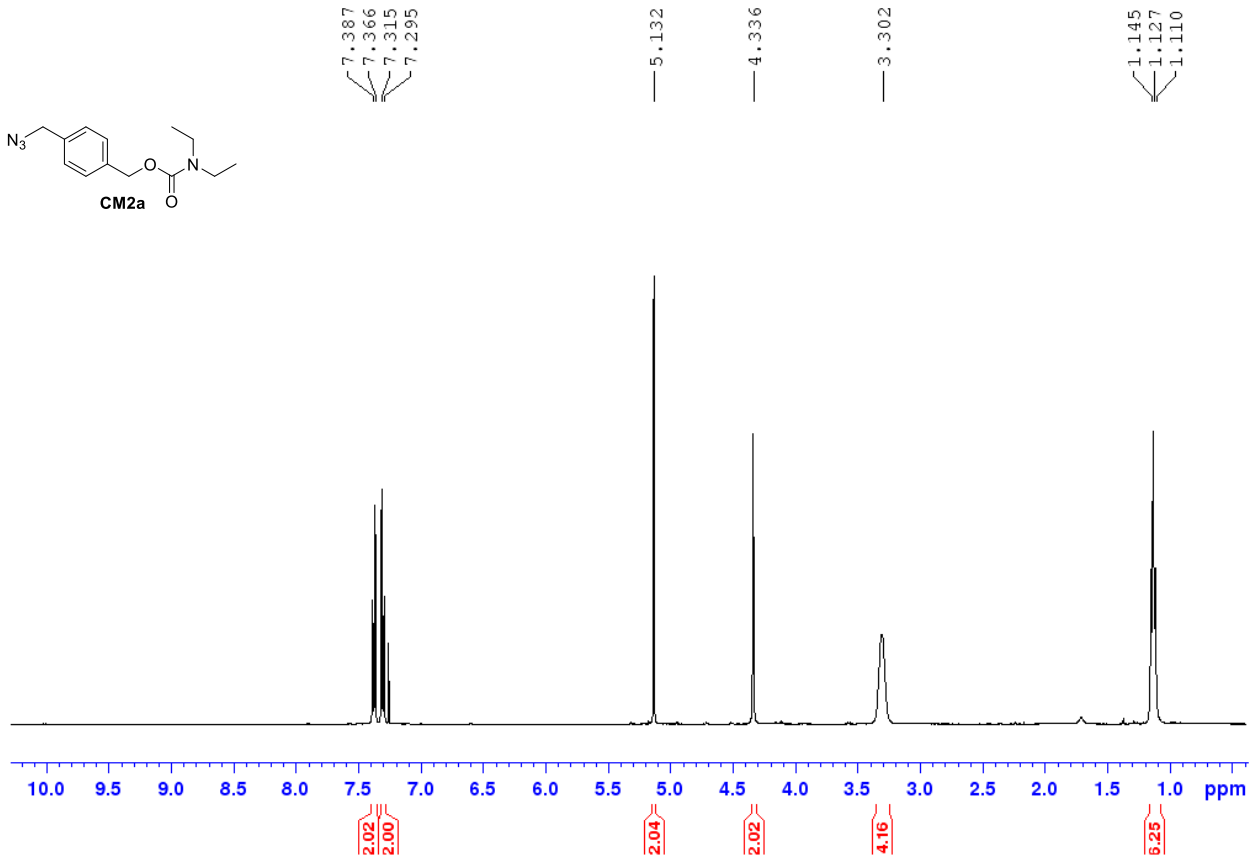
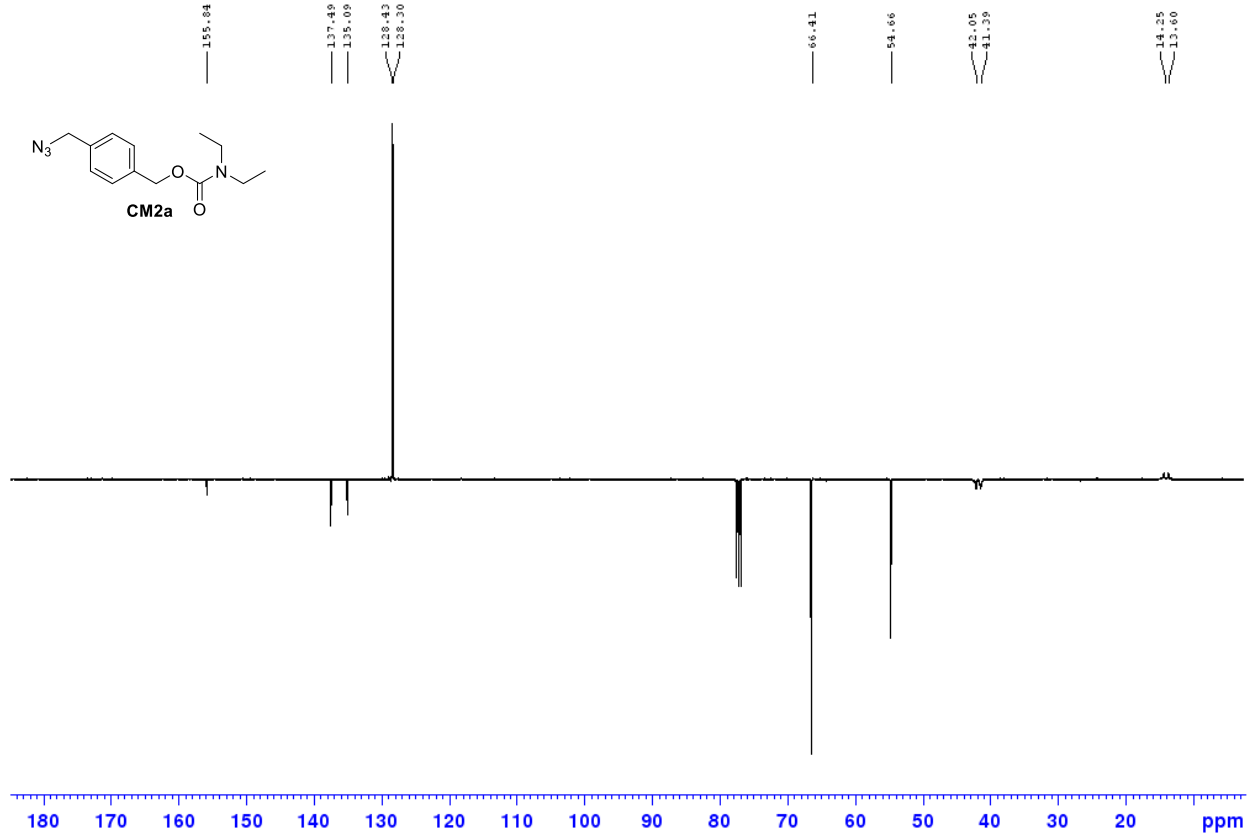
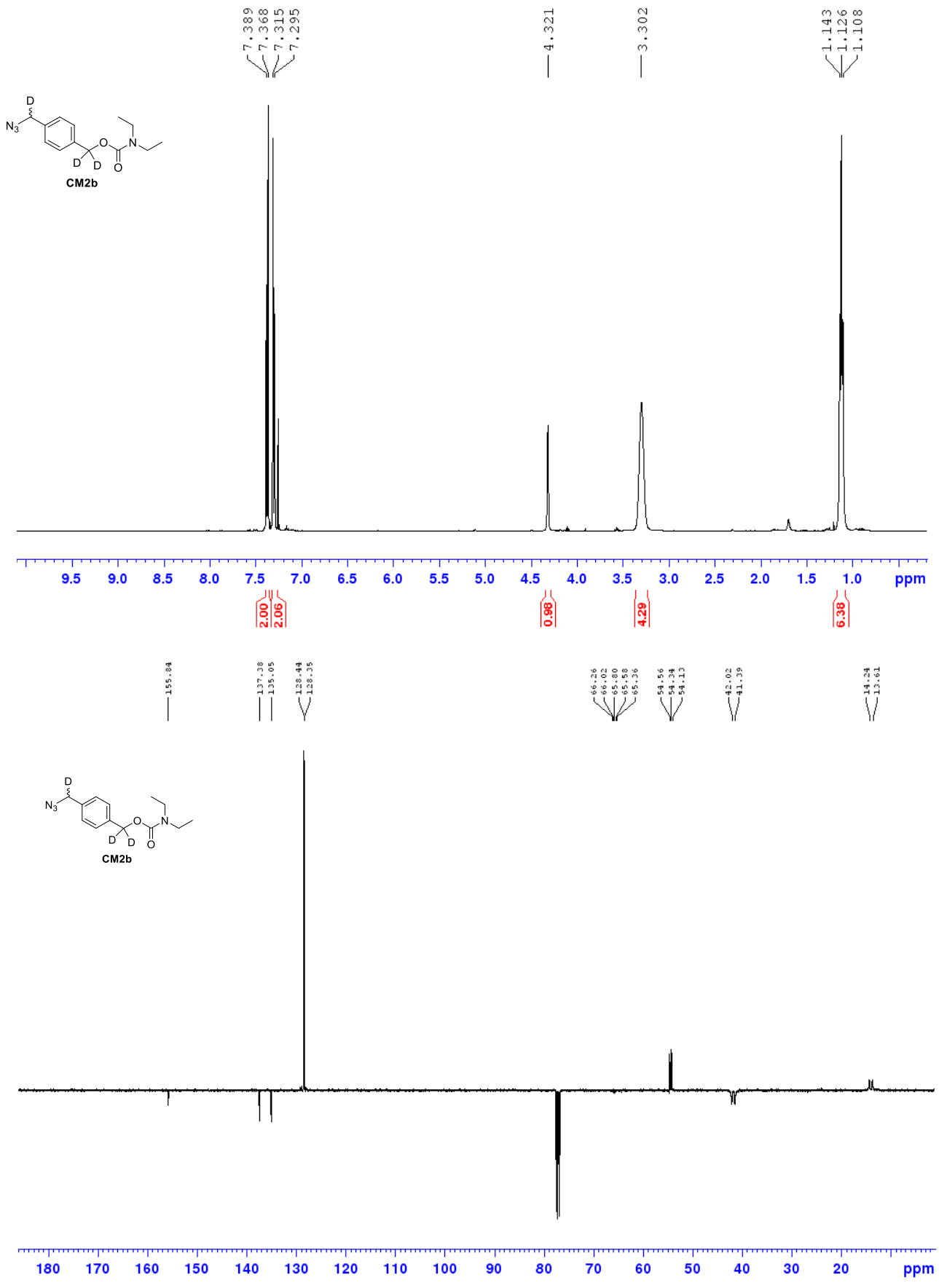
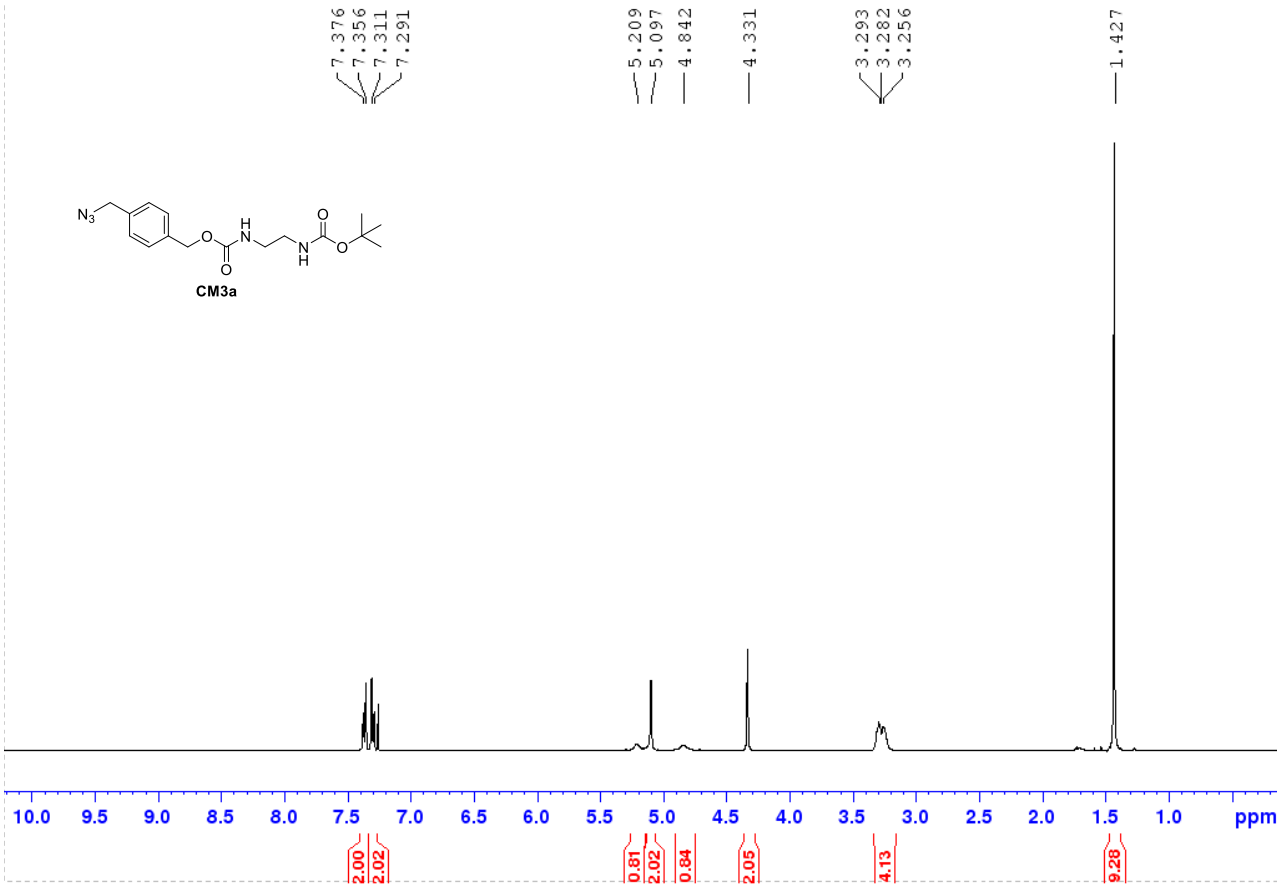
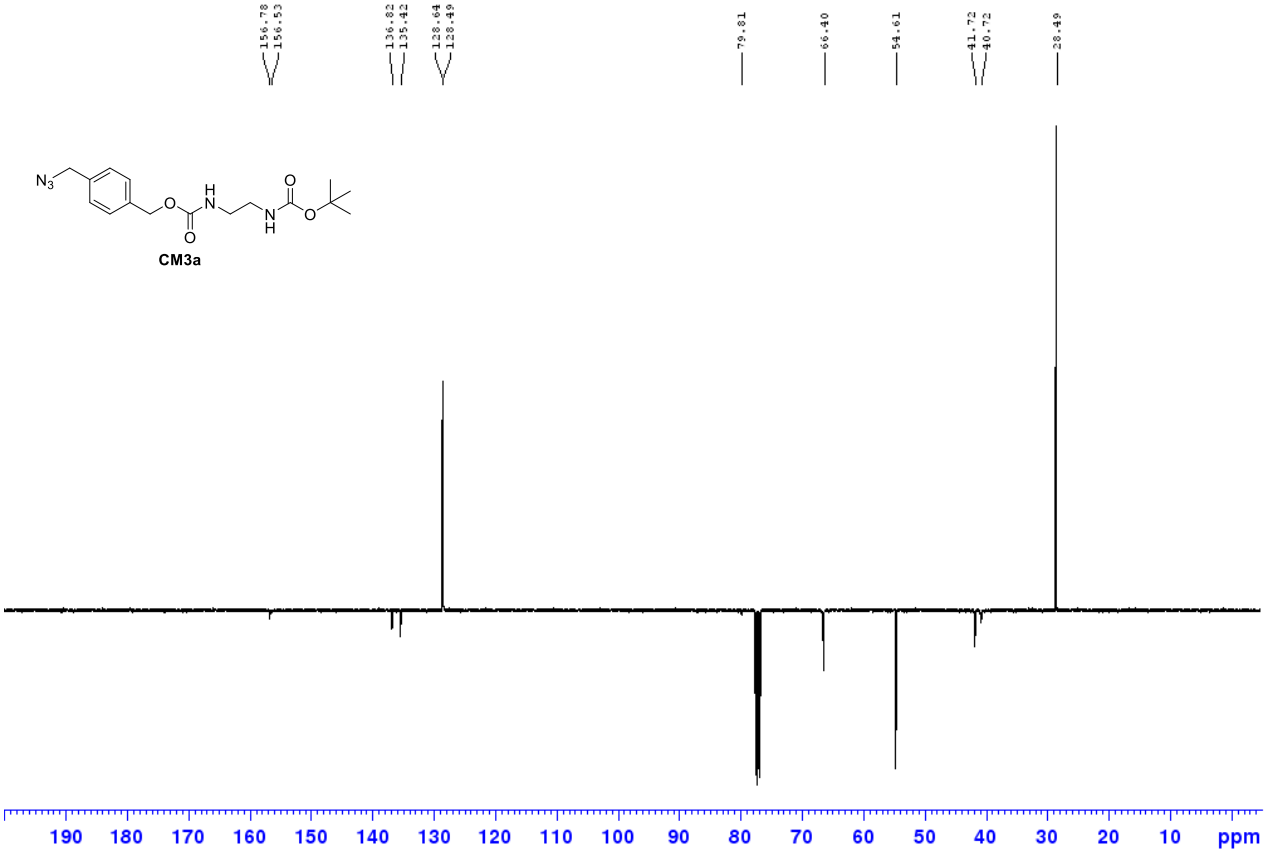
54.06
27.84
CM1b
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm

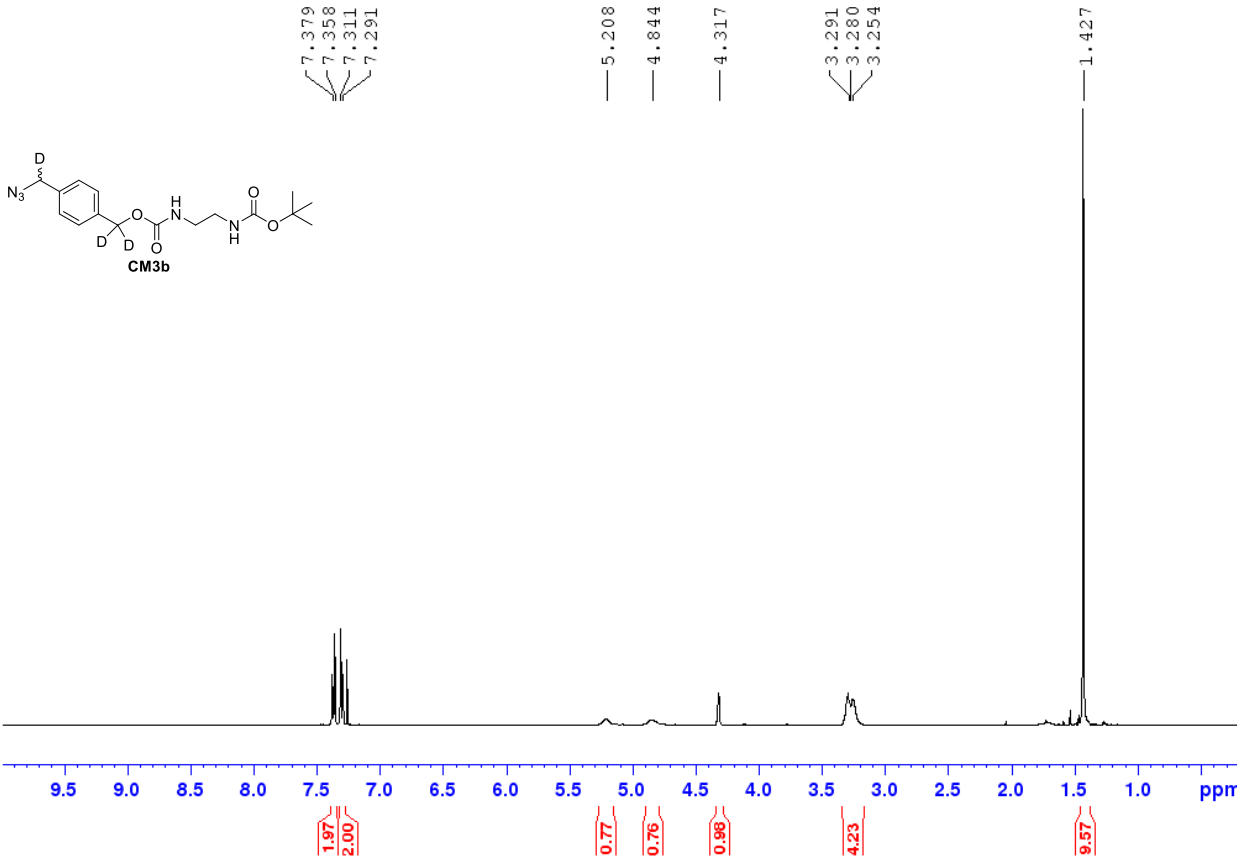

7.387
7.366
7.315
7.295
5.132
4.336
3.302
1.145
1.127
1.110
CM2a
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 ppm
2.02
2.00
2.04
2.02
4.16
6.25

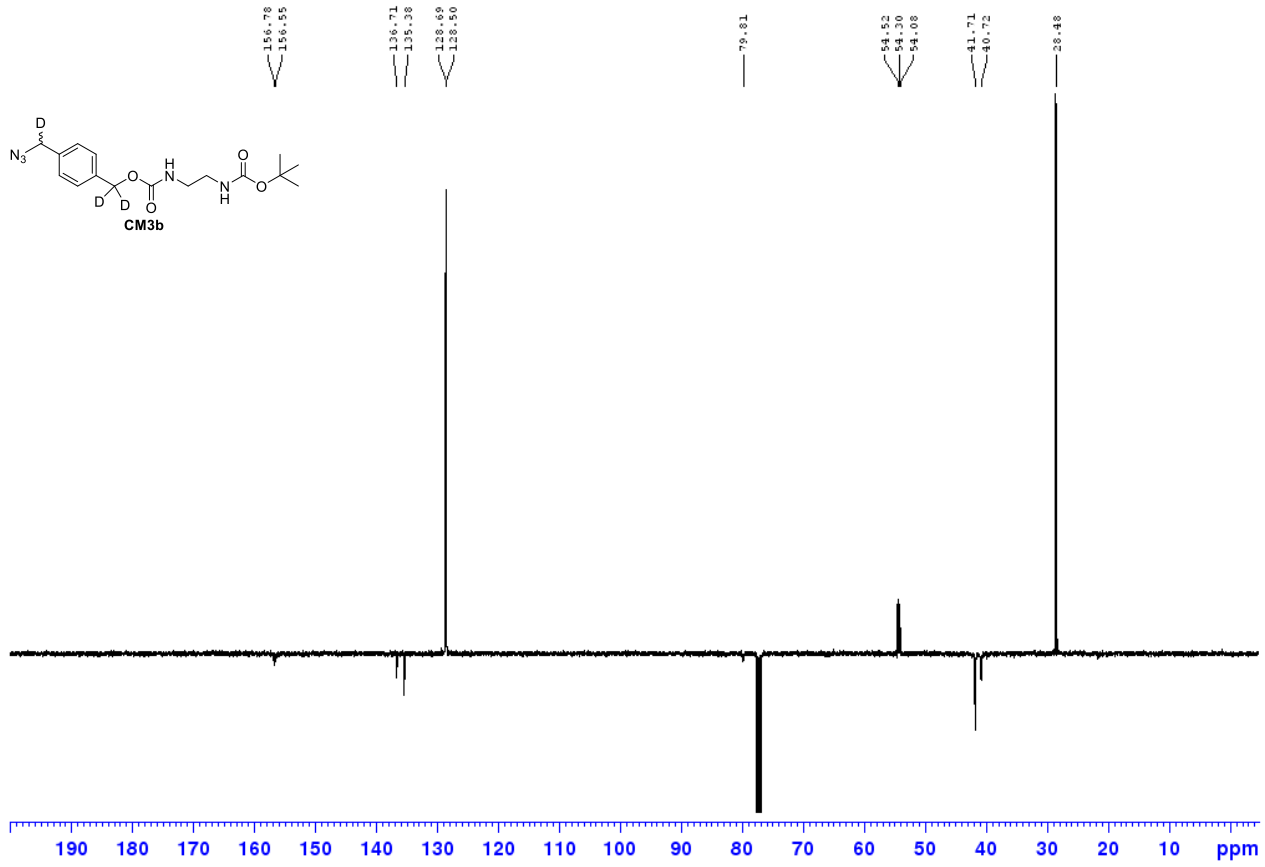

155.84
137.49
135.09
128.43
128.30
66.41
54.66
42.05
41.39
14.25
13.60
CM2a
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm

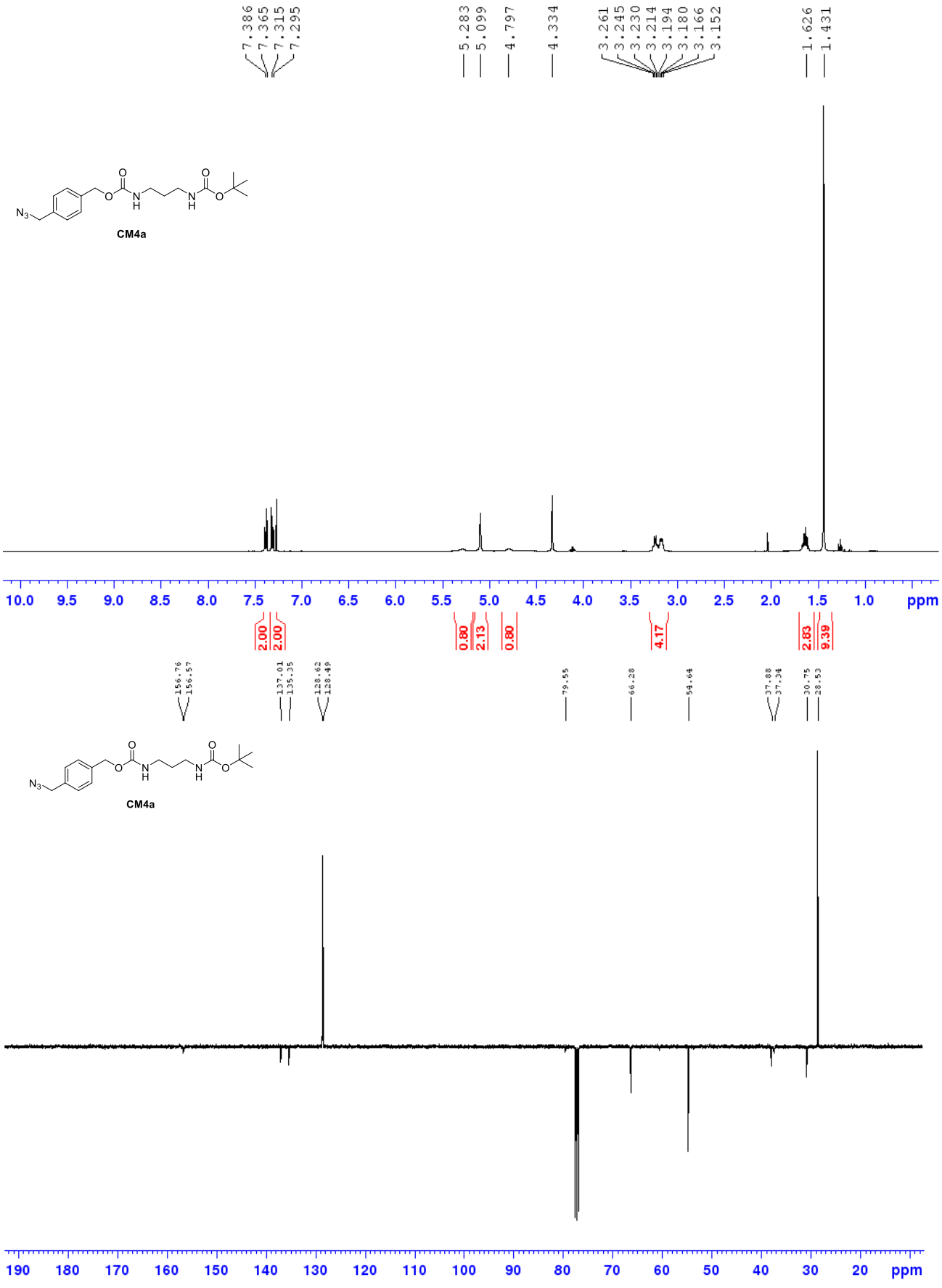

7.389
7.368
7.315
7.295
4.321
3.302
1.143
1.126
1.108
CM2b
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 ppm
2.00
2.06
0.98
4.29
6.38
155.84
137.38
135.05
128.44
128.35
66.26
66.02
65.80
65.58
65.36
54.56
54.34
54.13
42.02
41.39
14.24
13.61
CM2b
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm

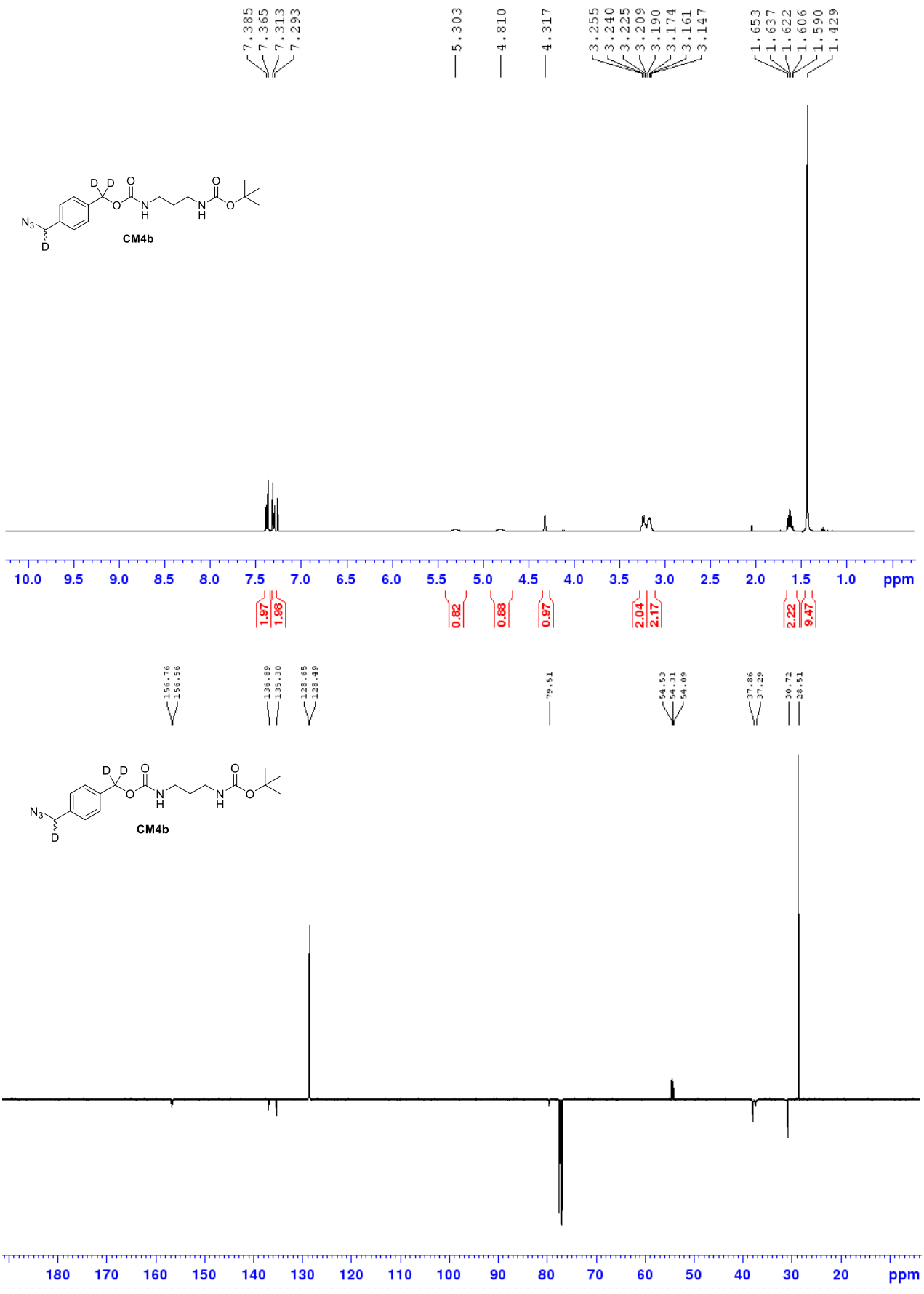

7.376
7.356
7.311
7.291
5.209
5.097
4.842
4.331
3.293
3.282
3.256
1.427
CM3a
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 ppm
2.00
2.02
0.81
2.02
0.84
2.05
4.13
9.28

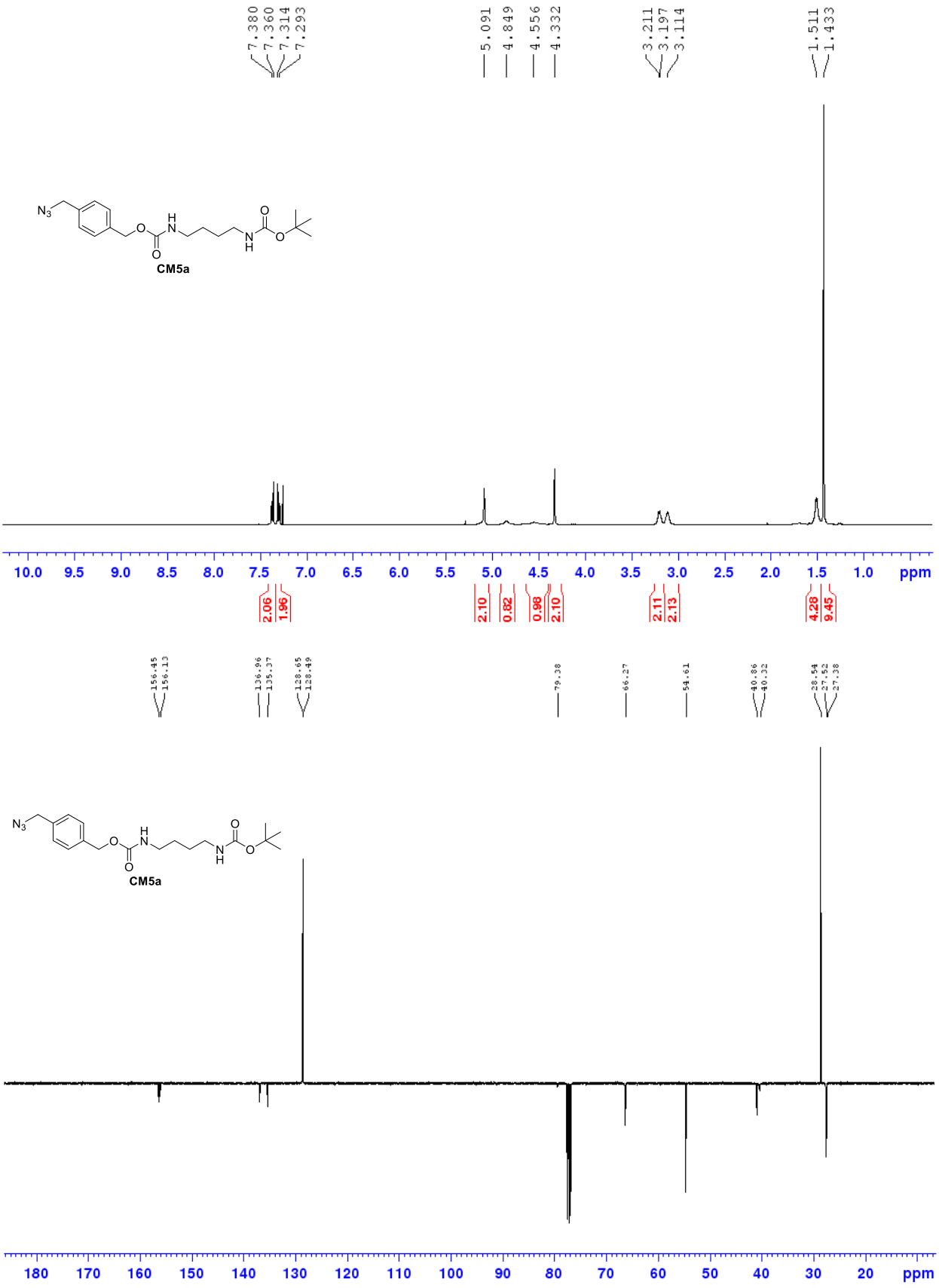

156.78
156.53
136.82
135.42
128.64
128.49
79.81
66.40
54.61
41.72
40.72
28.49
CM3a
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

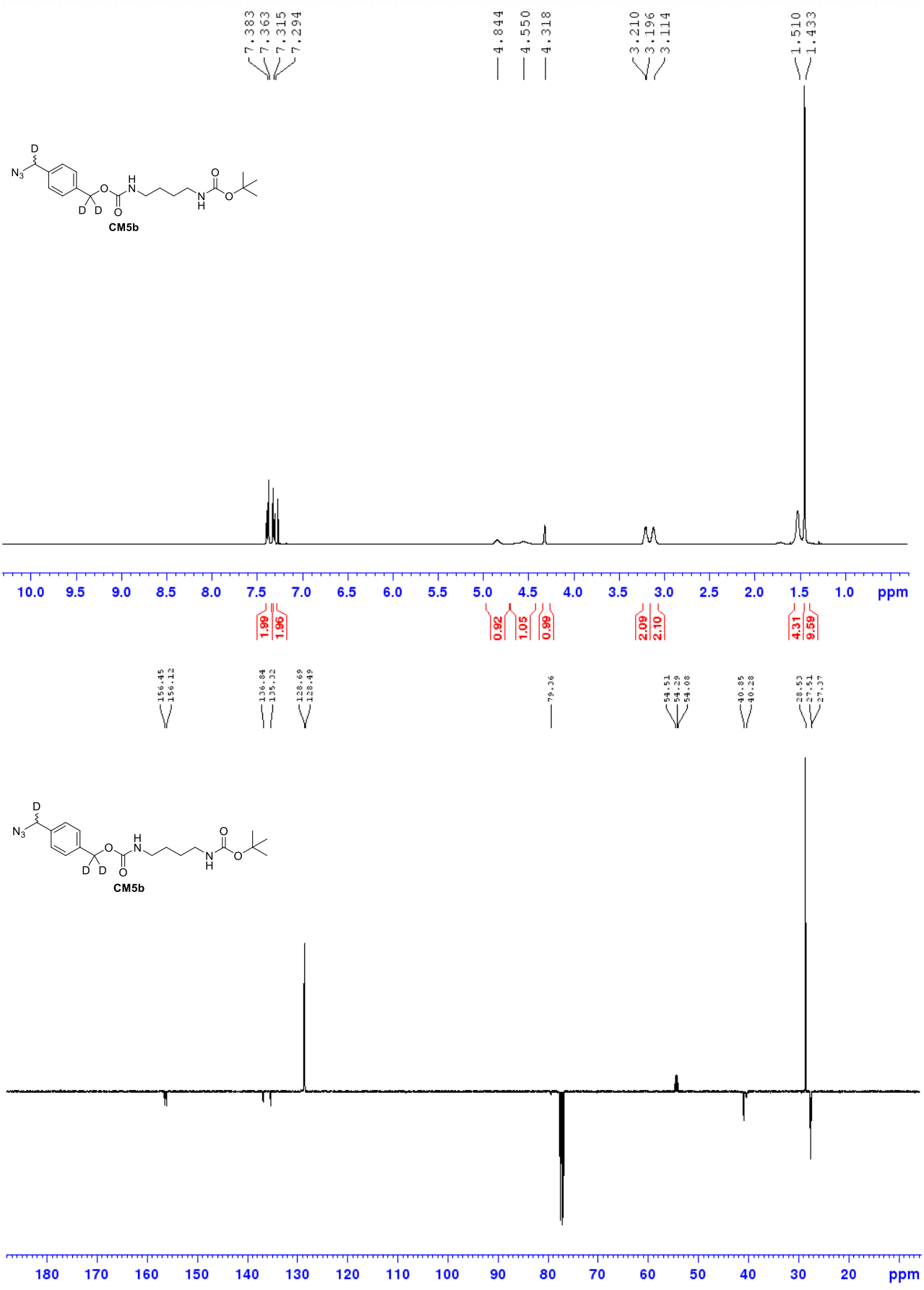

7.379
7.358
7.311
7.291
5.208
4.844
4.317
3.291
3.280
3.254
1.427
CM3b
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 ppm
1.97
2.00
0.77
0.76
0.98
4.23
9.57

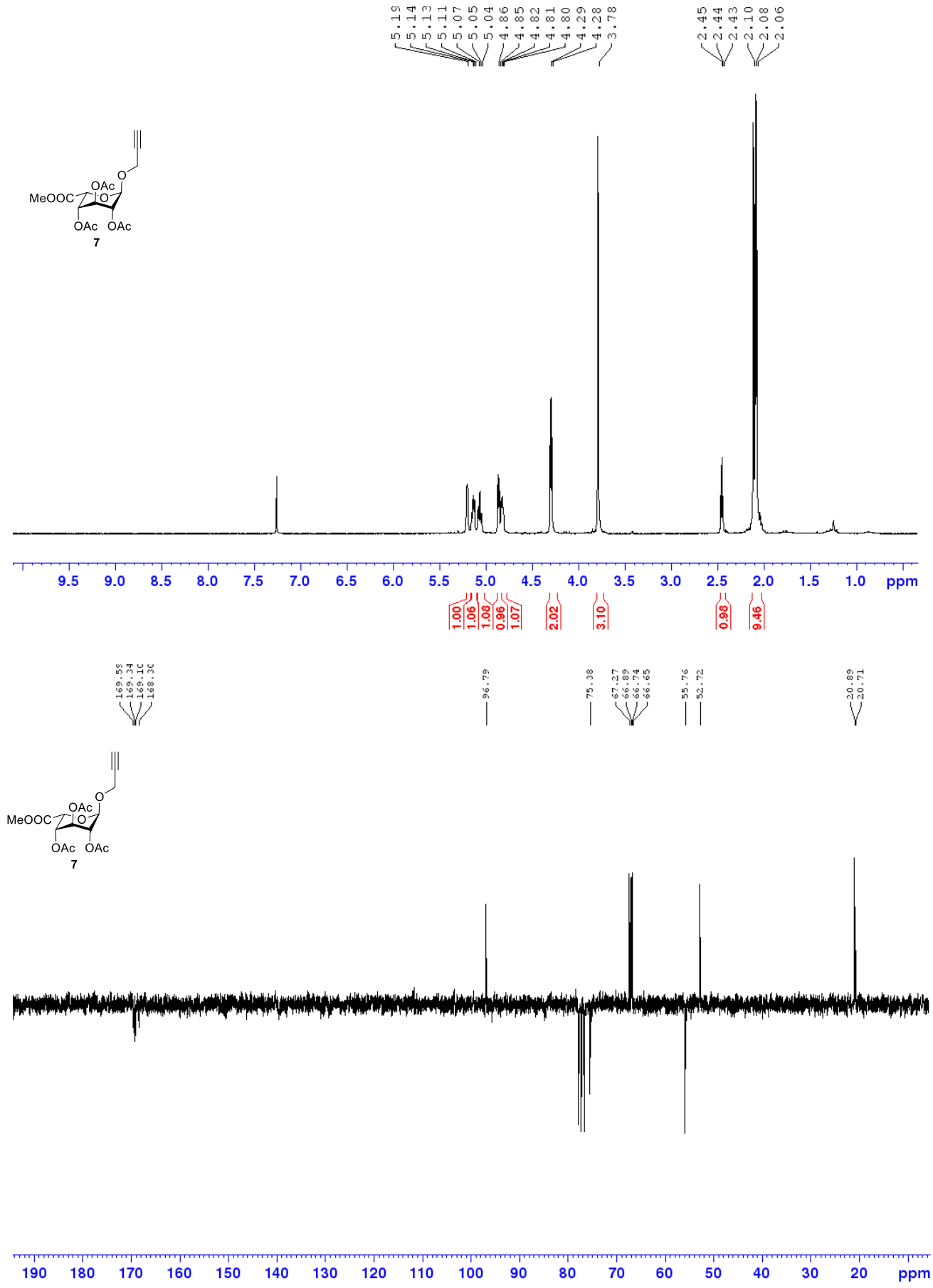

156.78
156.55
136.71
135.38
128.69
128.50
79.81
54.52
54.30
54.08
41.71
40.72
28.48
CM3b
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm


7.386
7.365
7.315
7.295
5.283
5.099
4.797
4.334
3.261
3.245
3.230
3.214
3.194
3.180
3.166
3.152
1.626
1.431
CM4a
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 ppm
2.00
2.00
0.80
2.13
0.80
4.17
2.83
9.39
156.76
156.57
137.01
135.35
128.62
128.49
79.55
66.28
54.64
37.88
37.34
30.75
28.53
CM4a
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm

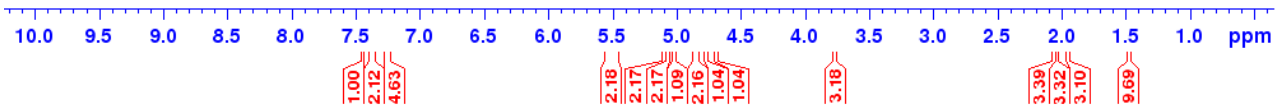

7.385
7.365
7.313
7.293
5.303
4.810
4.317
3.255
3.240
3.225
3.209
3.190
3.174
3.161
3.147
1.653
1.637
1.622
1.606
1.590
1.429
CM4b
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 ppm
1.97
1.98
0.82
0.88
0.97
2.04
2.17
2.22
9.47
156.76
156.56
136.89
135.30
128.65
128.49
79.51
54.53
54.31
54.09
37.86
37.29
30.72
28.51
CM4b
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm

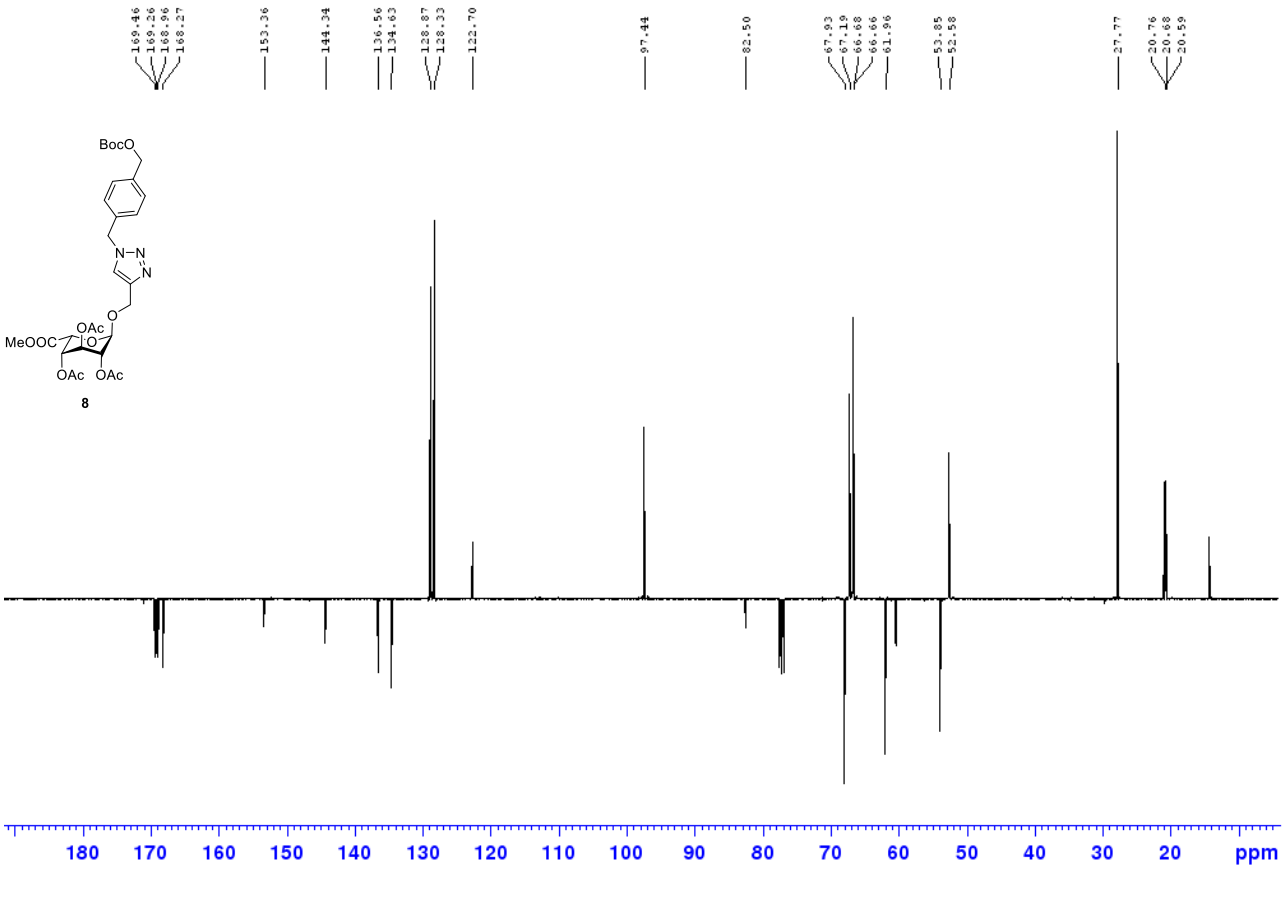

7.380
7.360
7.314
7.293
5.091
4.849
4.556
4.332
3.211
3.197
3.114
1.511
1.433
CM5a
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 ppm
2.06 1.96 2.10 0.82 0.98 2.10 2.11 2.13 4.28 9.45
156.45
156.13
136.96
135.37
128.65
128.49
79.38
66.27
54.61
40.86
40.32
28.54
27.52
27.38
CM5a
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm

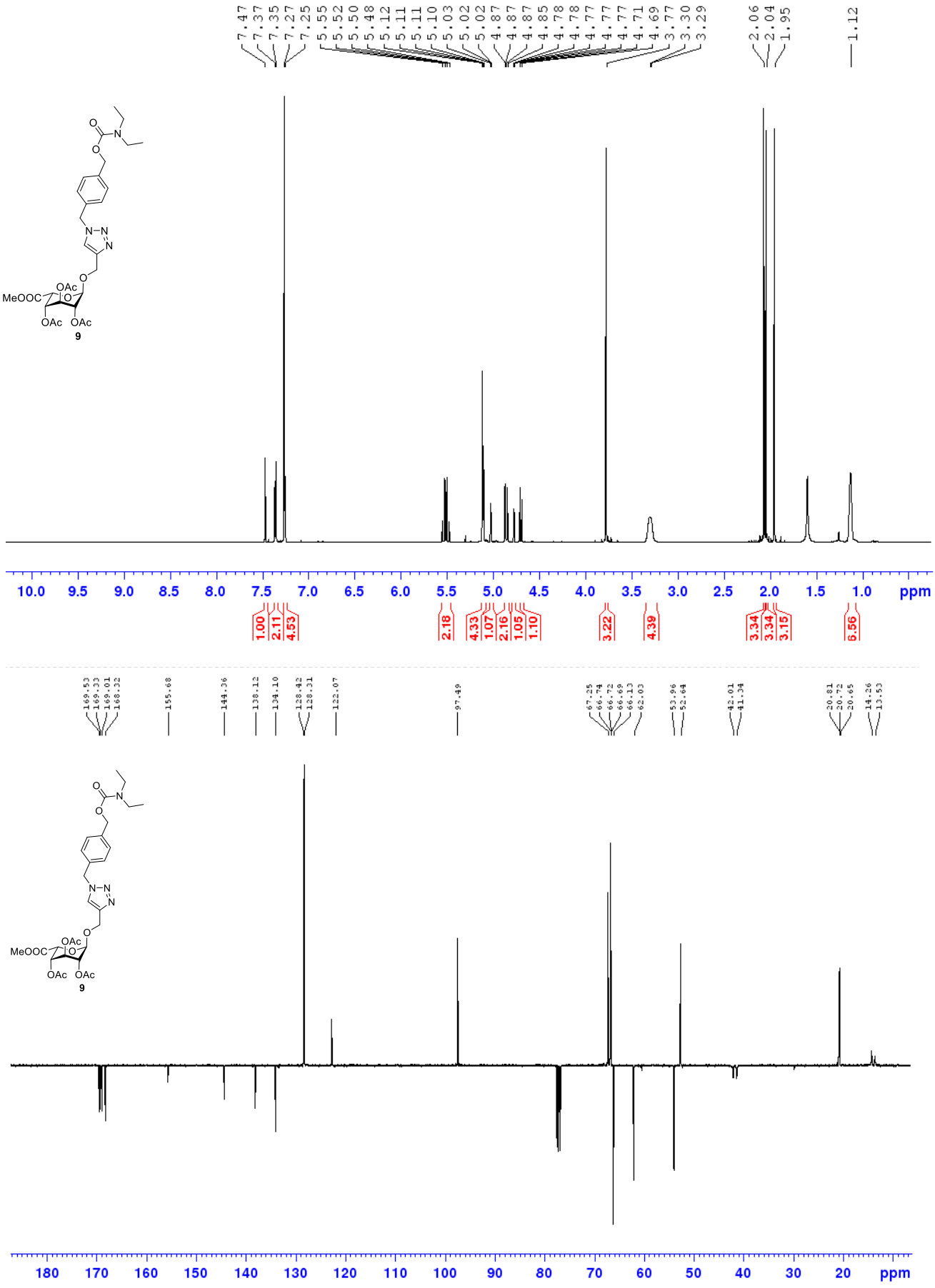

7.383
7.363
7.315
7.294
4.844
4.550
4.318
3.210
3.196
3.114
1.510
1.433
CM5b
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 ppm
1.99
1.96
0.92
1.05
0.99
2.09
2.10
4.31
9.59
156.45
156.12
136.84
135.32
128.69
128.49
79.36
54.51
54.29
54.08
40.85
40.28
28.53
27.51
27.37
CM5b
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm

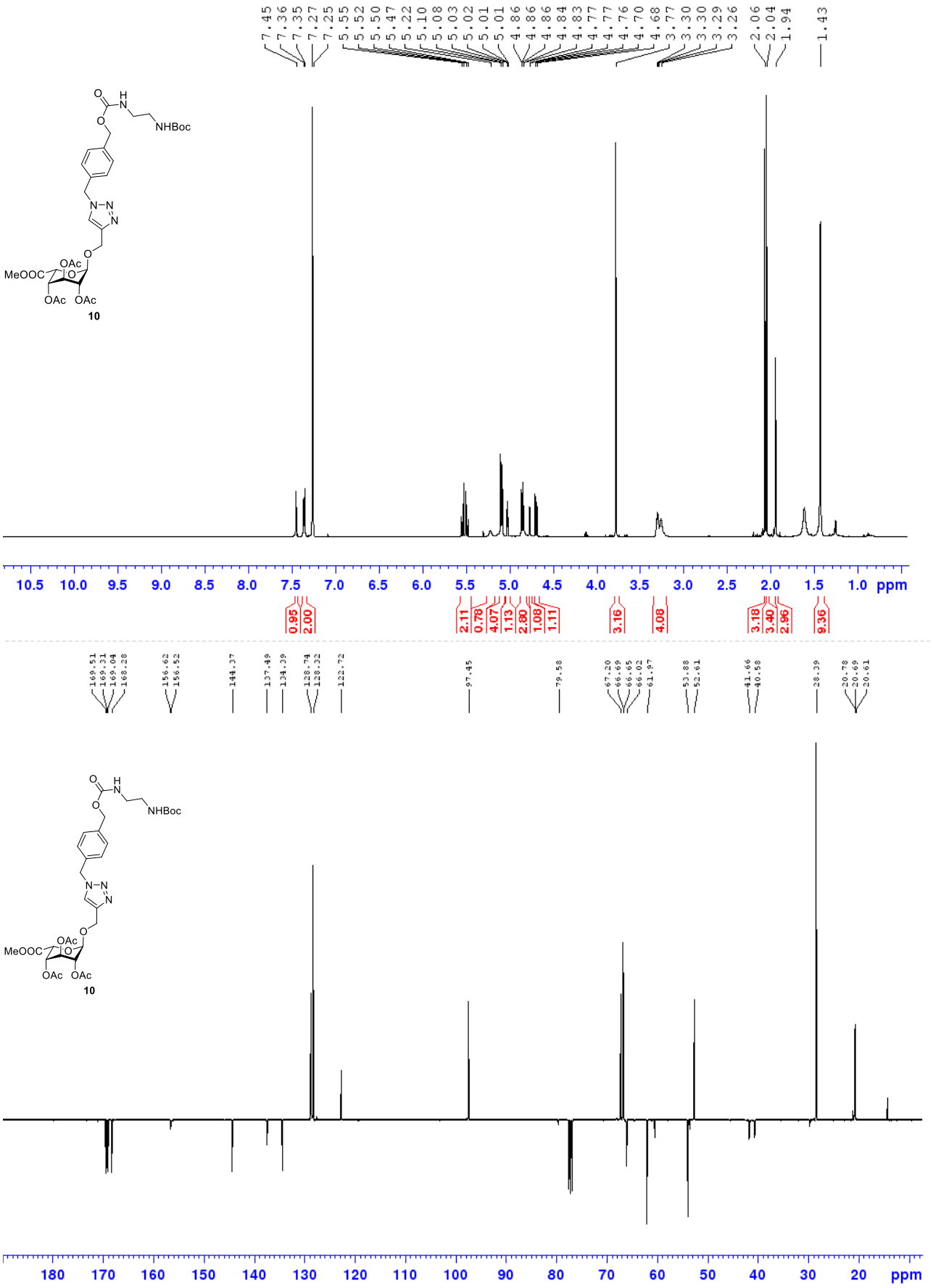

5.19
5.14
5.13
5.11
5.07
5.05
5.04
4.86
4.85
4.82
4.81
4.80
4.29
4.28
3.78
2.45
2.44
2.43
2.10
2.08
2.06
MeOOC
OAc
OAc
OAc
O
O
7
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 ppm
1.00
1.06
1.08
0.96
1.07
2.02
3.10
0.98
9.46
169.58
169.34
169.10
168.30
96.79
75.38
67.27
66.89
66.74
66.65
55.76
52.72
20.89
20.71
MeOOC
OAc
OAc
OAc
O
O
7
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm

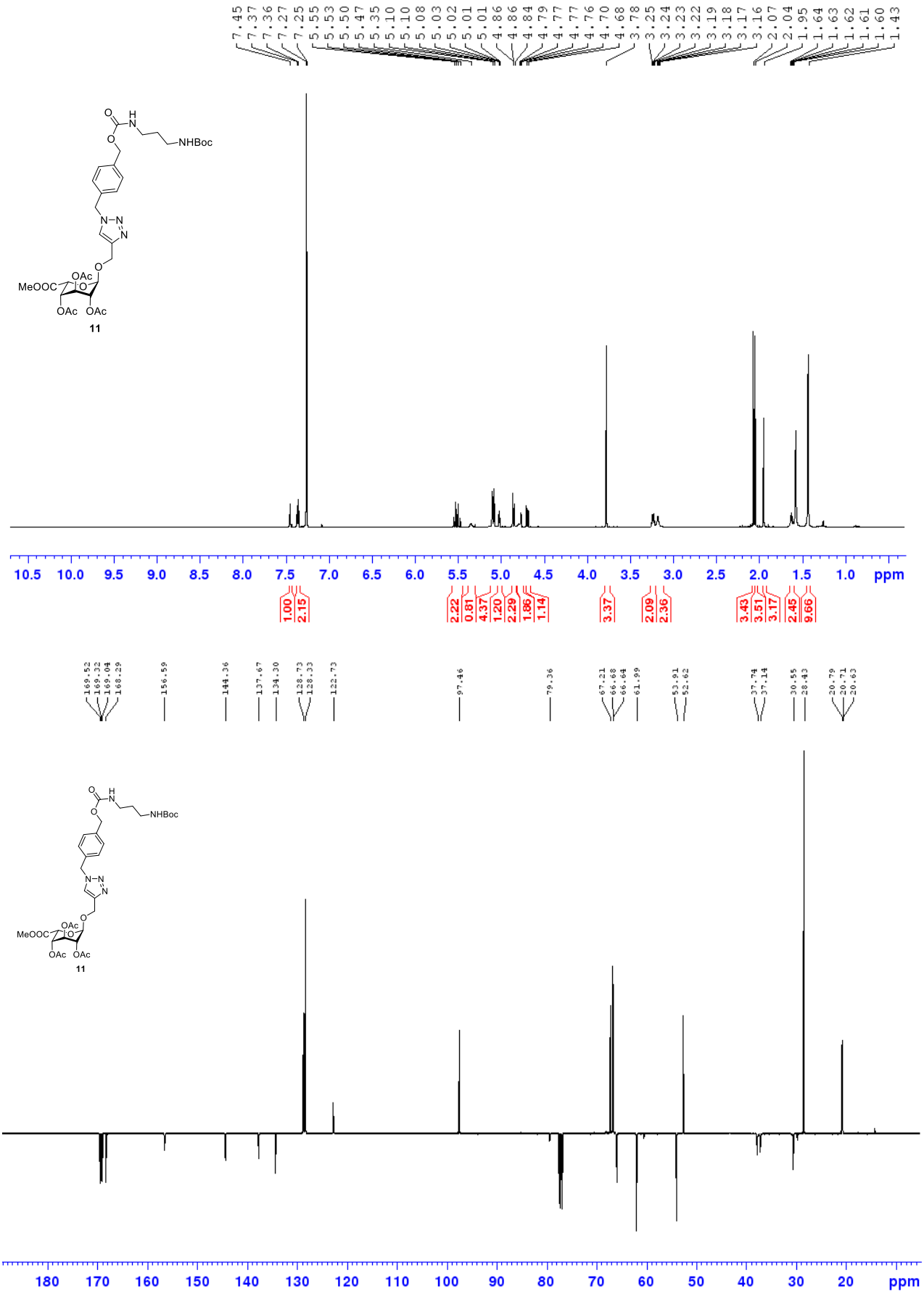

7.45
7.39
7.37
7.27
5.55
5.53
5.50
5.48
5.11
5.11
5.10
5.08
5.03
5.03
5.03
5.02
5.02
5.02
4.87
4.87
4.86
4.84
4.78
4.78
4.77
4.77
4.77
4.77
4.71
4.69
3.77
2.06
2.04
1.96
1.48
BocO
N-N
N
O
OAc
MeOOC
O
OAc
OAc
OAc
8


10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm
1.00
2.12
4.63
2.18
2.17
2.17
1.09
2.16
1.04
1.04
3.18
3.39
3.32
3.10
9.69


169.46
169.26
168.96
168.27
153.36
144.34
136.56
134.63
128.87
128.33
122.70
97.44
82.50
67.93
67.19
66.68
66.66
61.96
53.85
52.58
27.77
20.76
20.68
20.59
BocO
N-N
N
O
OAc
MeOOC
O
OAc
OAc
OAc
8
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm


7.47
7.37
7.35
7.27
7.25
5.55
5.52
5.50
5.48
5.12
5.11
5.11
5.10
5.03
5.02
5.02
4.87
4.87
4.85
4.78
4.78
4.77
4.77
4.77
4.71
4.69
3.77
3.30
3.29
2.06
2.04
1.95
1.12
MeOOC
OAc
OAc
OAc
9
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm
1.00
2.11
4.53
2.18
4.33
1.07
2.16
1.05
1.10
3.22
4.39
3.34
3.34
3.15
6.56
169.53
169.33
169.01
168.32
155.68
144.36
138.12
134.10
128.42
128.31
122.07
97.49
67.25
66.74
66.72
66.69
66.13
62.03
53.96
52.64
42.01
41.34
20.81
20.72
20.65
14.26
13.53
MeOOC
OAc
OAc
OAc
9
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm


7.45
7.36
7.35
7.27
7.25
5.55
5.52
5.50
5.47
5.22
5.10
5.08
5.03
5.02
5.01
5.01
4.86
4.86
4.86
4.84
4.83
4.77
4.77
4.76
4.70
4.68
3.77
3.30
3.30
3.29
3.26
2.06
2.04
1.94
1.43
NHBoc
MeOOC
OAc
OAc
OAc
10
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 ppm
0.95
2.00
2.11
0.78
4.07
1.13
2.80
1.08
1.11
3.16
4.08
3.18
3.40
2.96
9.36
169.51
169.31
169.04
168.28
156.62
156.52
144.37
137.49
134.39
128.74
128.32
122.72
97.45
79.58
67.20
66.69
66.65
66.02
61.97
53.88
52.61
41.66
40.58
28.39
20.78
20.69
20.61
NHBoc
MeOOC
OAc
OAc
OAc
10
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm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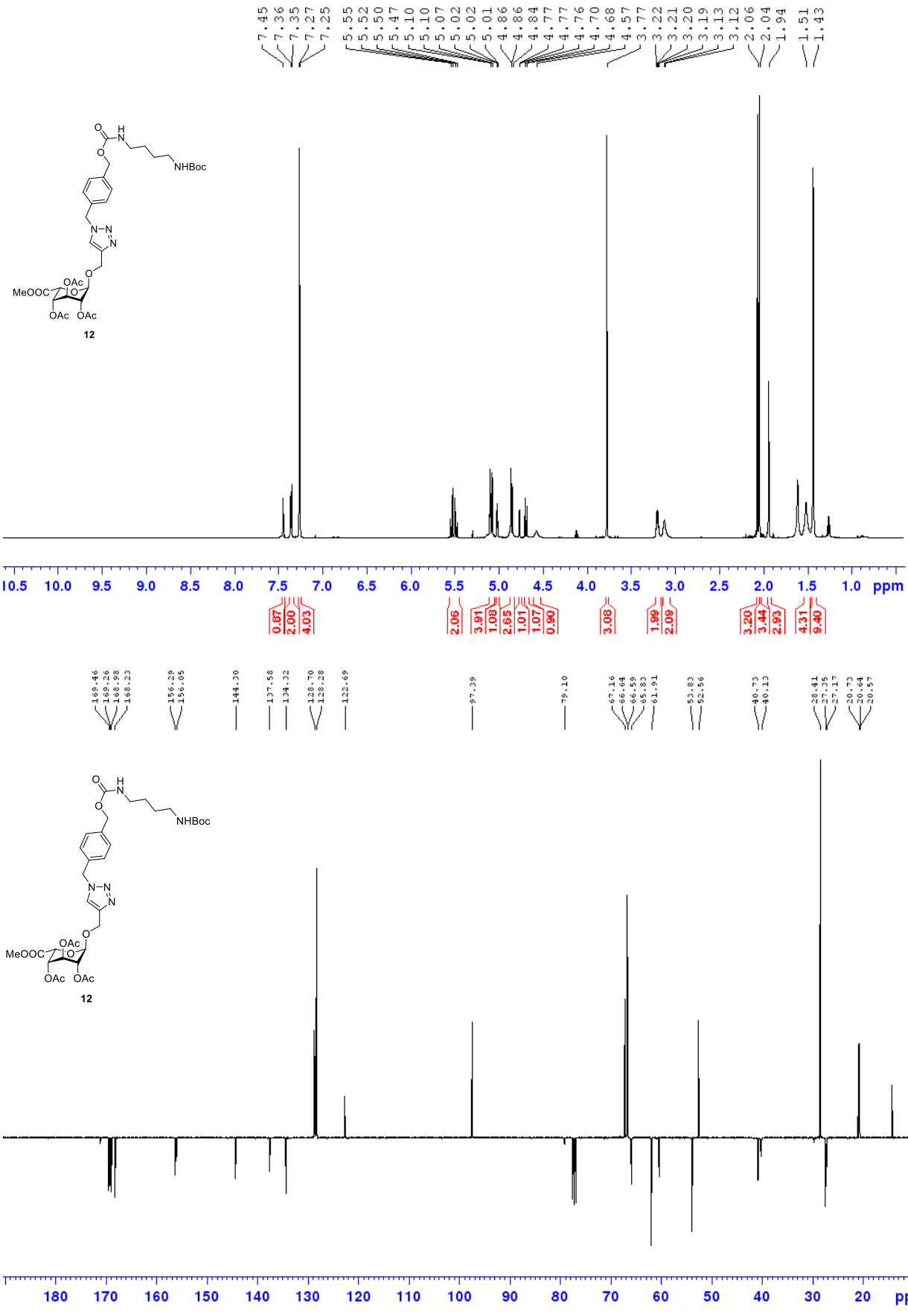
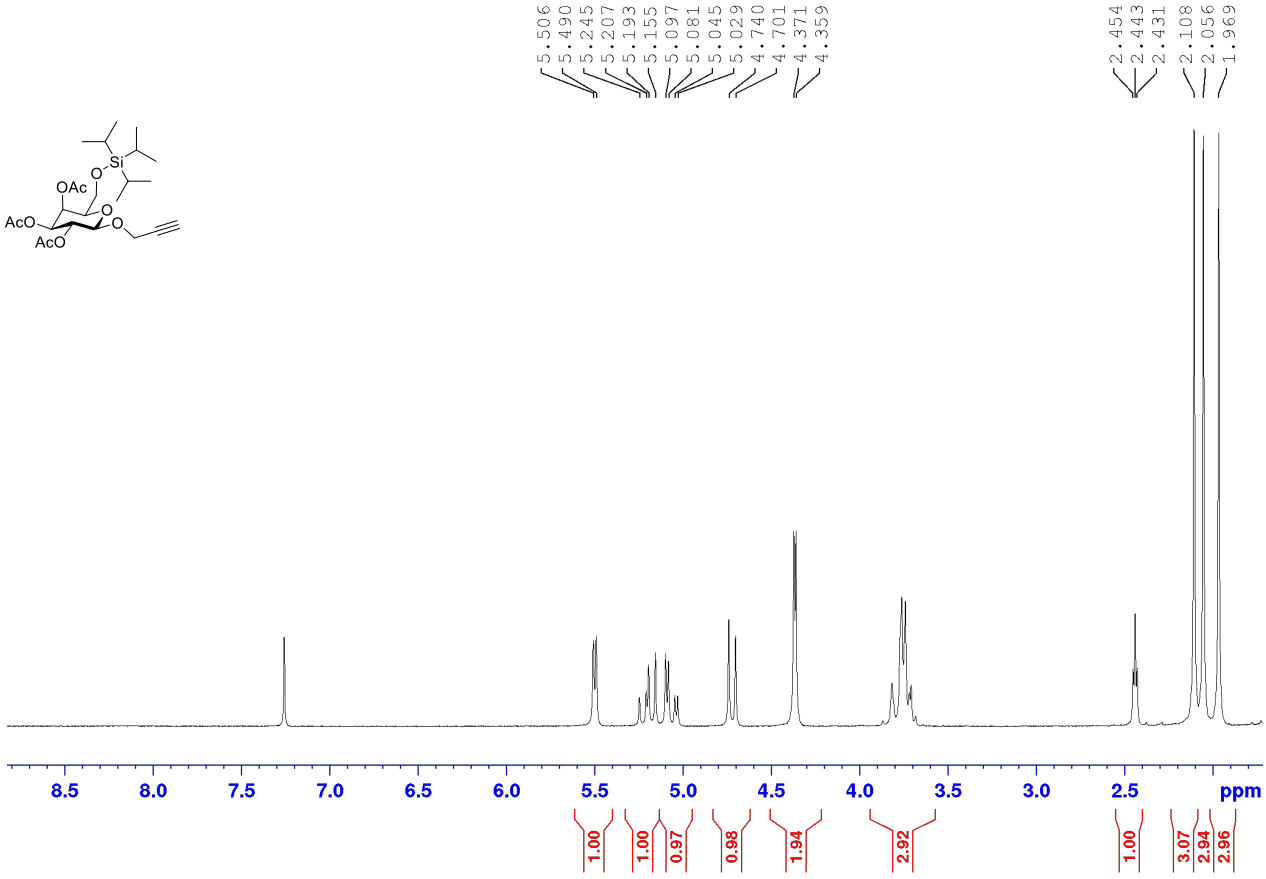
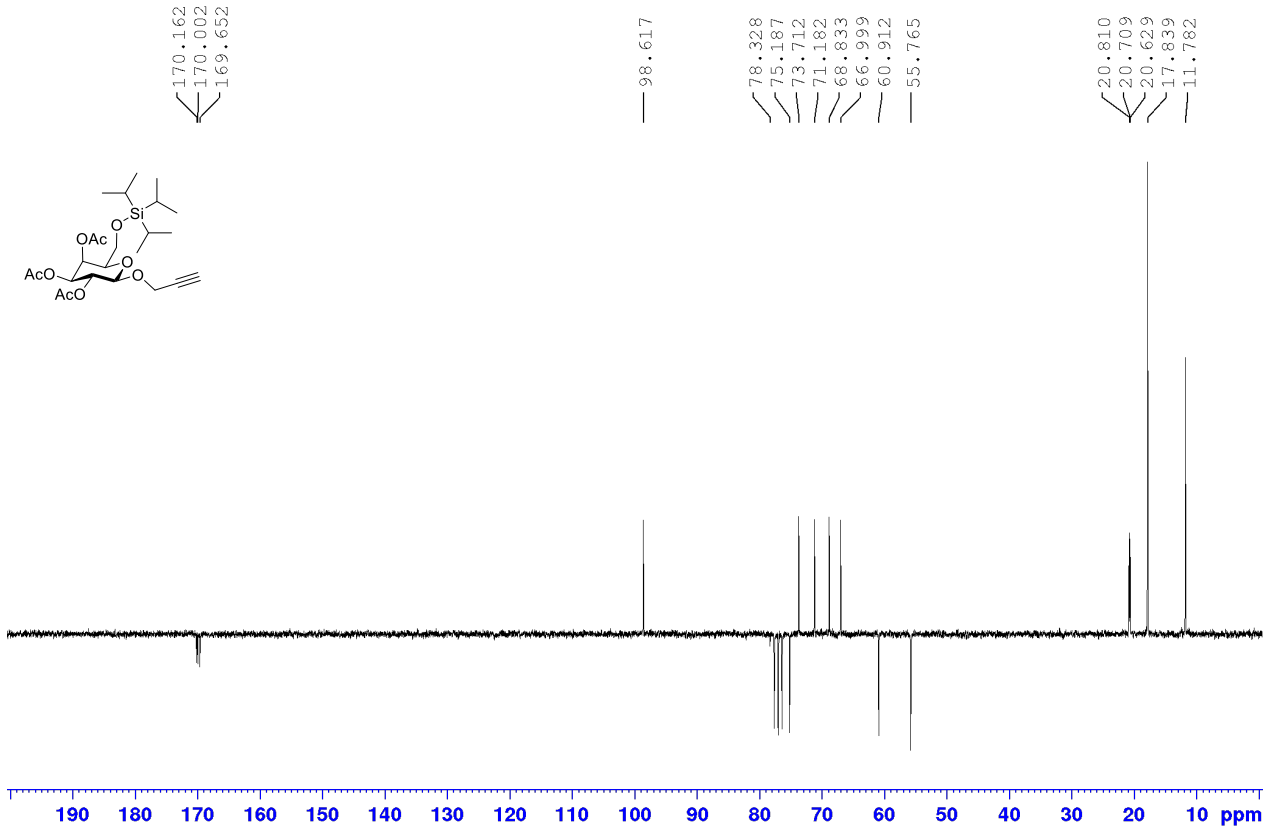
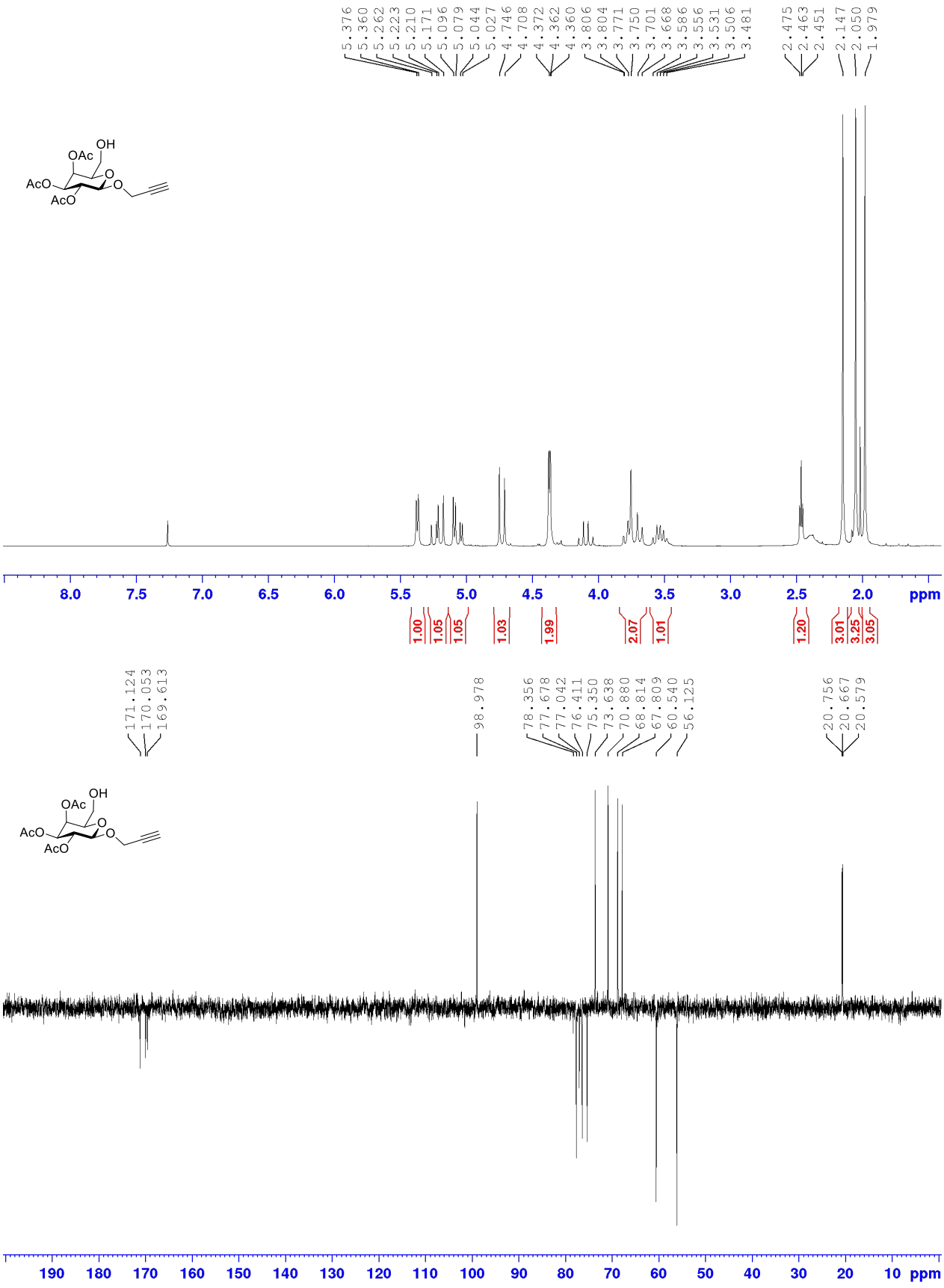
7.37
7.36
7.27
7.25
5.55
5.53
5.50
5.47
5.35
5.10
5.10
5.08
5.03
5.02
5.01
5.01
4.86
4.86
4.84
4.79
4.77
4.77
4.76
4.70
4.68
3.78
3.25
3.24
3.23
3.22
3.19
3.18
3.17
3.16
2.07
2.04
1.95
1.64
1.63
1.62
1.61
1.60
1.43
NHBoc
MeOOC
OAc
OAc
OAc
11
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 ppm
1.00 2.15 2.22 0.81 4.37 1.20 2.29 1.86 1.14 3.37 2.09 2.36 3.43 3.51 3.17 2.45 9.66
169.52
169.32
169.04
168.29
156.59
144.36
137.67
134.30
128.73
128.33
122.73
97.46
79.36
67.21
66.68
66.64
61.99
53.91
52.62
37.74
37.14
30.55
28.43
20.79
20.71
20.63
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm


7.45
7.36
7.35
7.27
7.25
5.55
5.52
5.50
5.47
5.10
5.07
5.02
5.01
4.86
4.84
4.77
4.76
4.70
4.68
4.57
3.77
3.22
3.21
3.20
3.19
3.13
3.12
2.06
2.04
1.94
1.51
1.43
NHBoc
MeOOC
OAc
OAc
OAc
12
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 ppm
0.87
2.00
4.03
2.06
3.91
1.08
2.65
1.01
1.07
0.90
3.08
1.99
2.09
3.20
3.44
2.93
4.31
9.40
169.46
169.26
168.98
168.23
156.29
156.05
144.30
137.58
134.32
128.70
128.28
122.69
97.39
79.10
67.16
66.64
66.59
65.83
61.91
53.83
52.56
40.73
40.13
28.41
27.35
27.17
20.73
20.64
20.57
NHBoc
MeOOC
OAc
OAc
OAc
12
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm


5.506
5.490
5.245
5.207
5.193
5.155
5.097
5.081
5.045
5.029
4.740
4.701
4.371
4.359
2.454
2.443
2.431
2.108
2.056
1.969
OAc
AcO
AcO
Si
O
O
O
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
ppm
1.00
1.00
0.97
0.98
1.94
2.92
1.00
3.07
2.94
2.96


170.162
170.002
169.652
98.617
78.328
75.187
73.712
71.182
68.833
66.999
60.912
55.765
20.810
20.709
20.629
17.839
11.782
OAc
AcO
AcO
Si
O
O
O
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm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5.262
5.223
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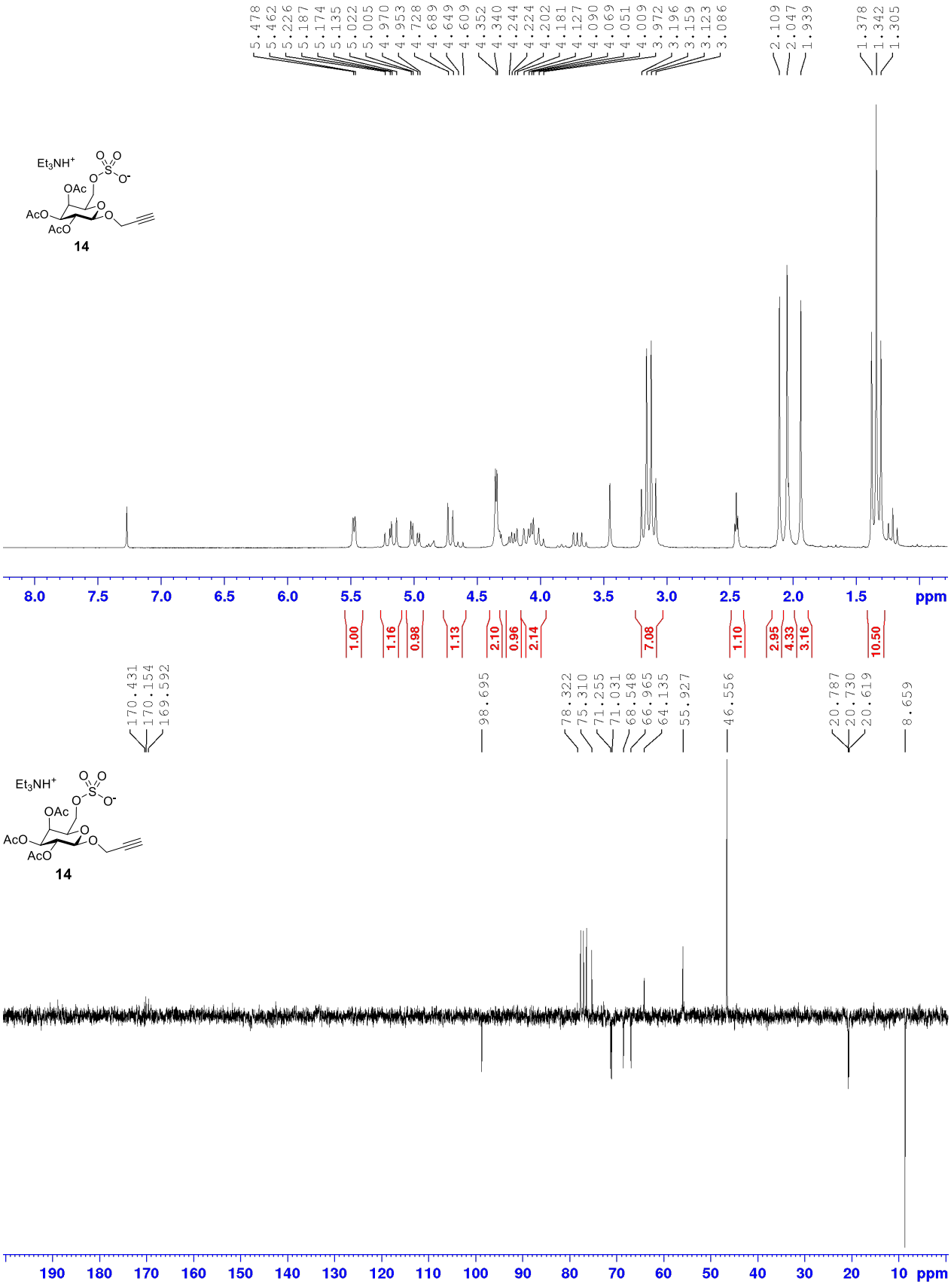
5.079
5.044
5.027
4.746
4.708
4.372
4.362
4.360
3.806
3.804
3.771
3.750
3.701
3.668
3.586
3.556
3.531
3.506
3.481
2.475
2.463
2.451
2.147
2.050
1.979
OH
OAc
O
AcO
O
AcO
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
ppm
1.00
1.05
1.05
1.03
1.99
2.07
1.01
1.20
3.01
3.25
3.05
171.124
170.053
169.613
98.978
78.356
77.678
77.042
76.411
75.350
73.638
70.880
68.814
67.809
60.540
56.125
20.756
20.667
20.579
OH
OAc
O
AcO
O
AcO
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm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5.226
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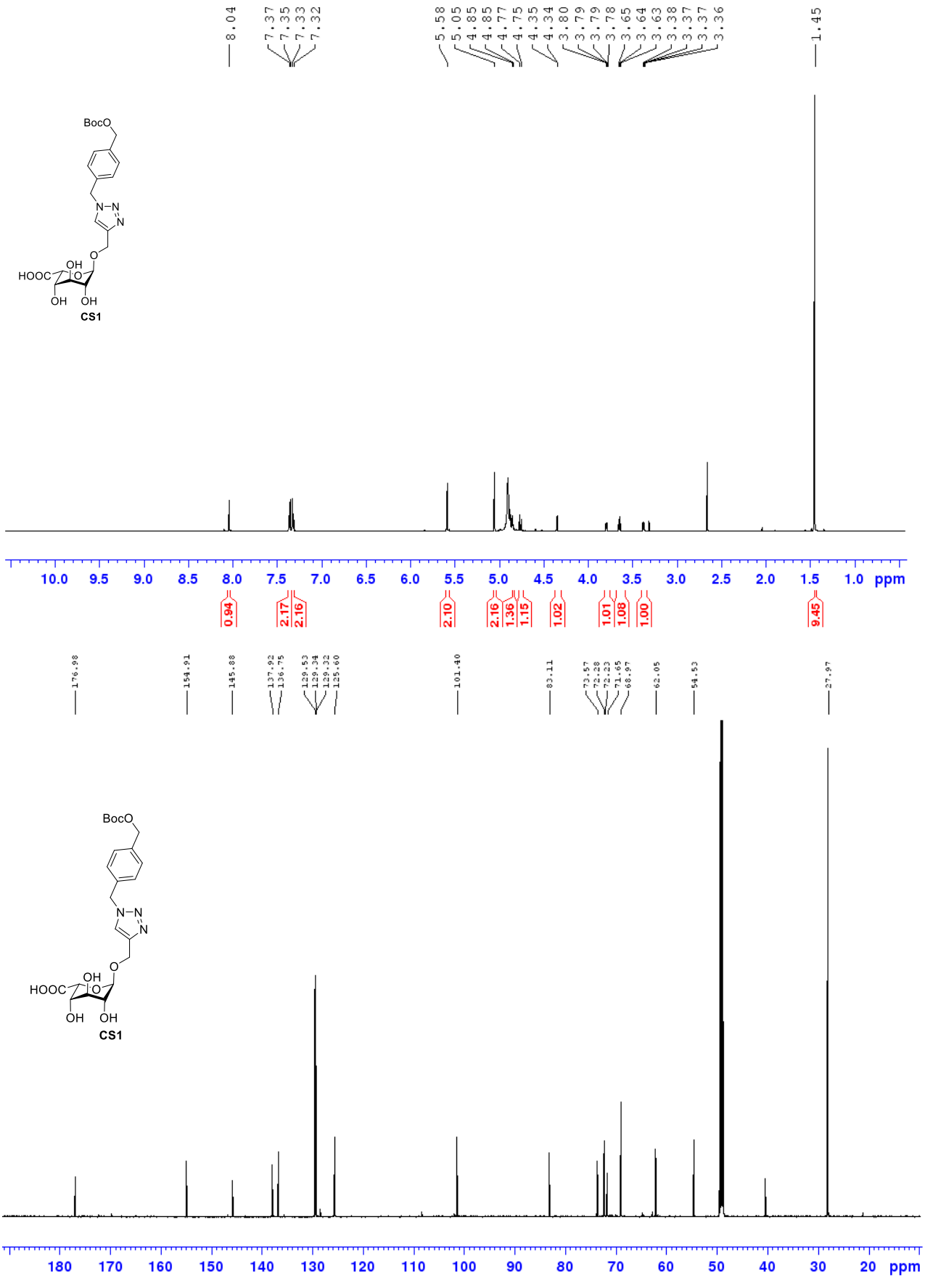
5.005
4.970
4.953
4.728
4.689
4.649
4.609
4.352
4.340
4.244
4.224
4.202
4.181
4.127
4.090
4.069
4.051
4.009
3.972
3.196
3.159
3.123
3.086
2.109
2.047
1.939
1.378
1.342
1.305
Et3NH+
OAc
AcO
AcO
14
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
ppm
1.00
1.16
0.98
1.13
2.10
0.96
2.14
7.08
1.10
2.95
4.33
3.16
10.50
170.431
170.154
169.592
98.695
78.322
75.310
71.255
71.031
68.548
66.965
64.135
55.927
46.556
20.787
20.730
20.619
8.659
Et3NH+
OAc
AcO
AcO
14
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm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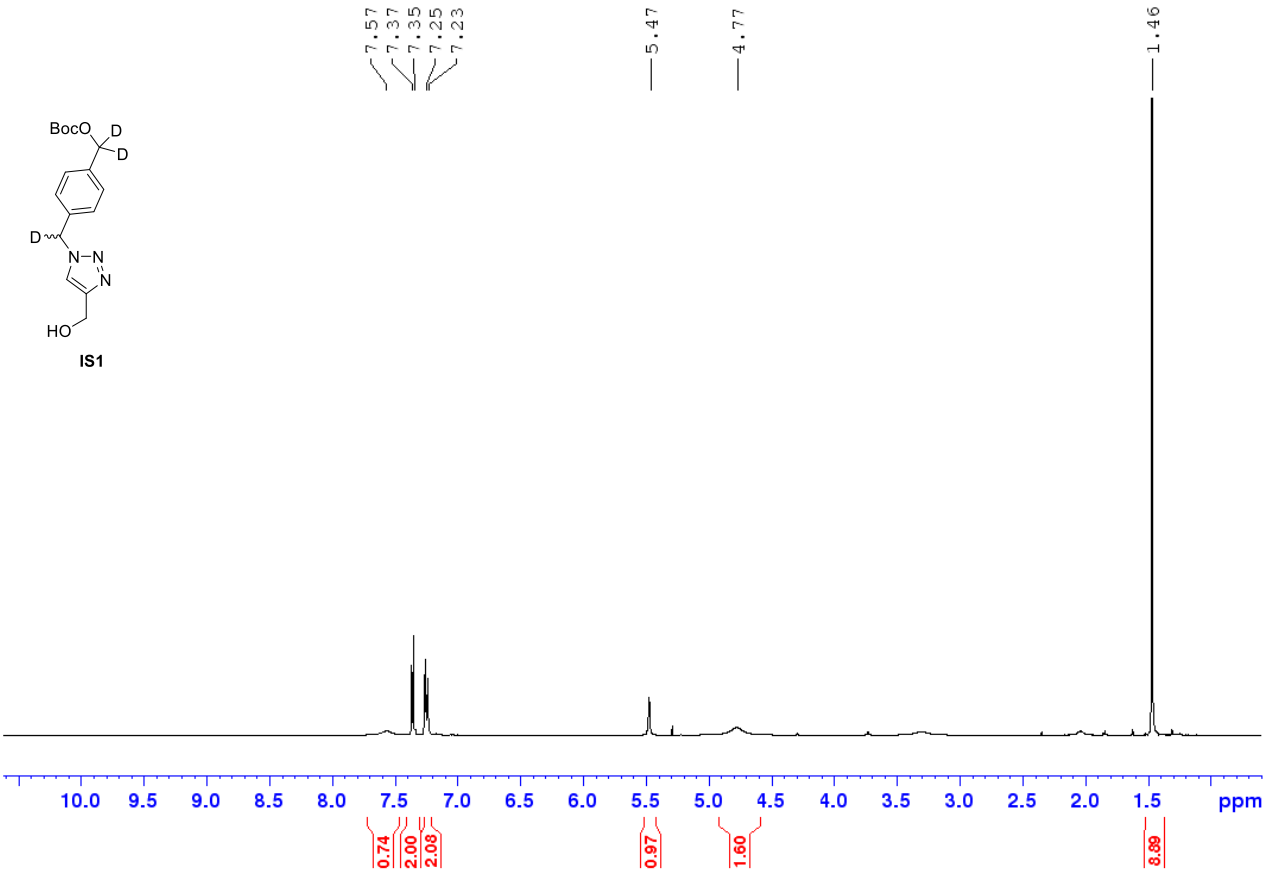
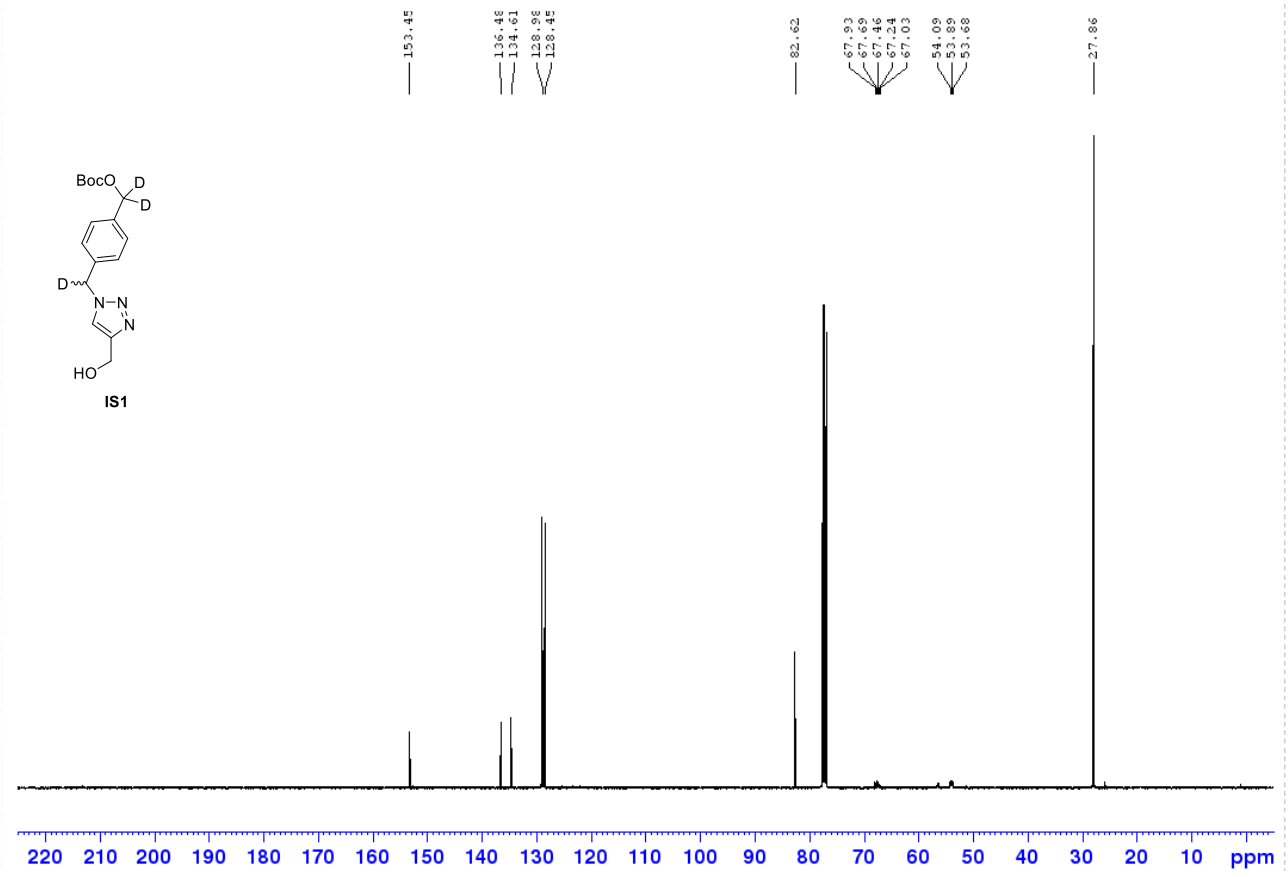
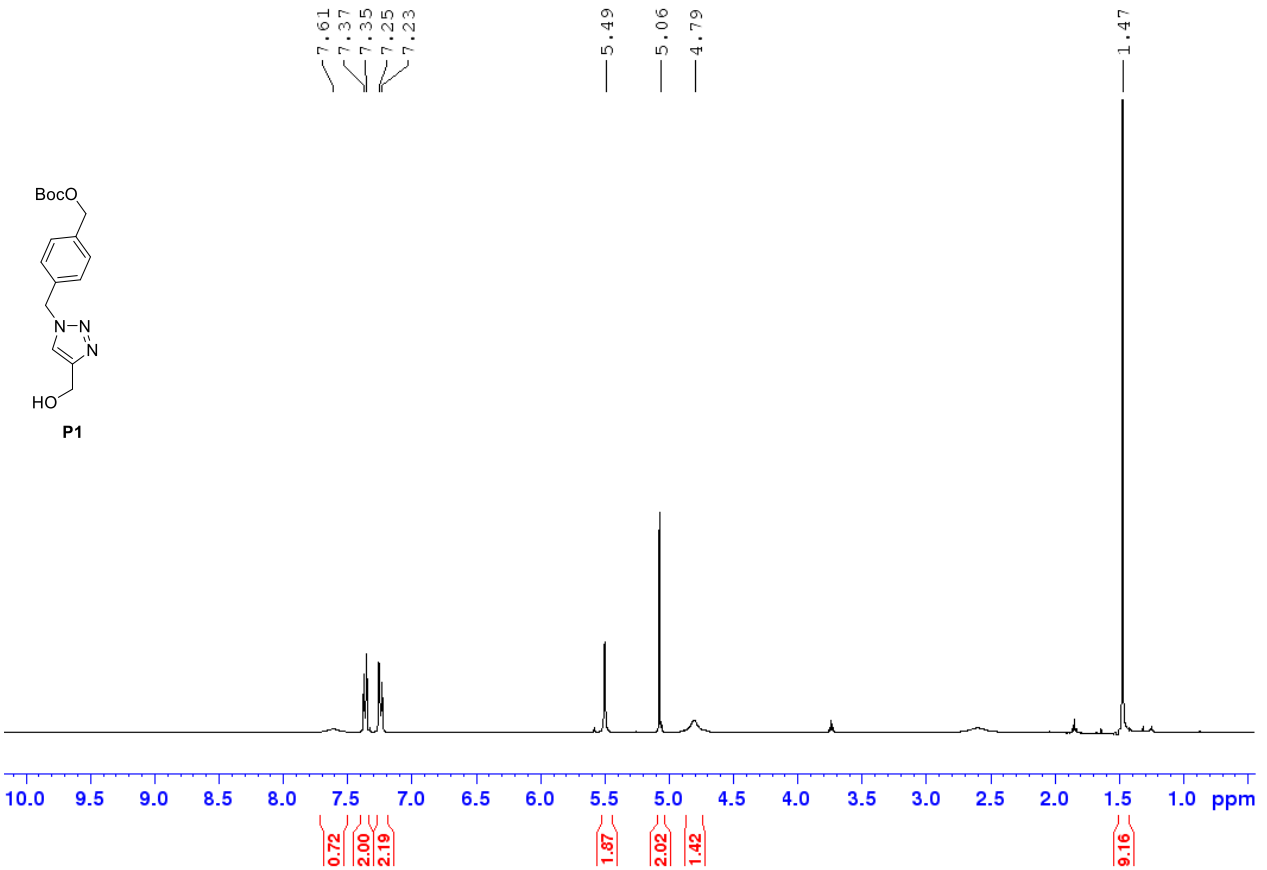
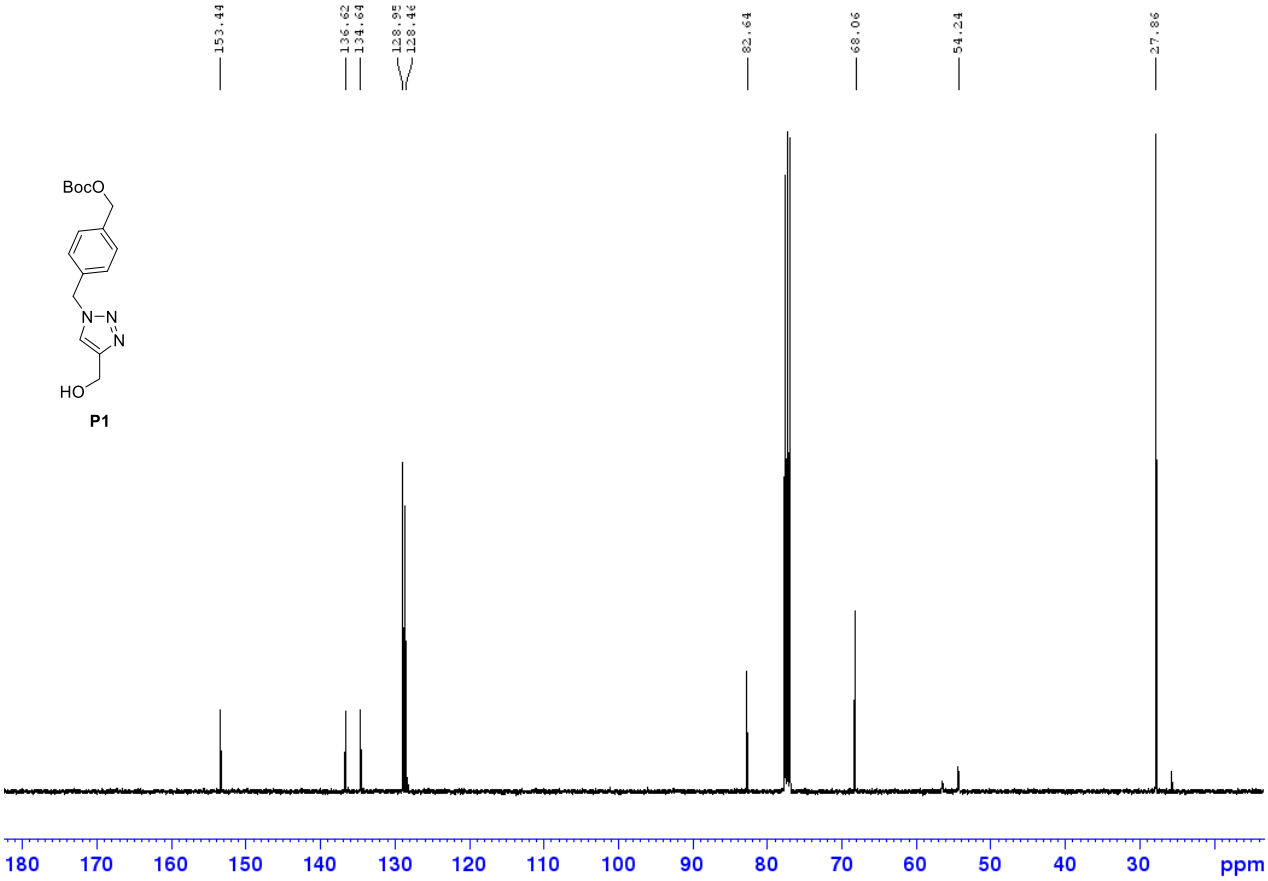
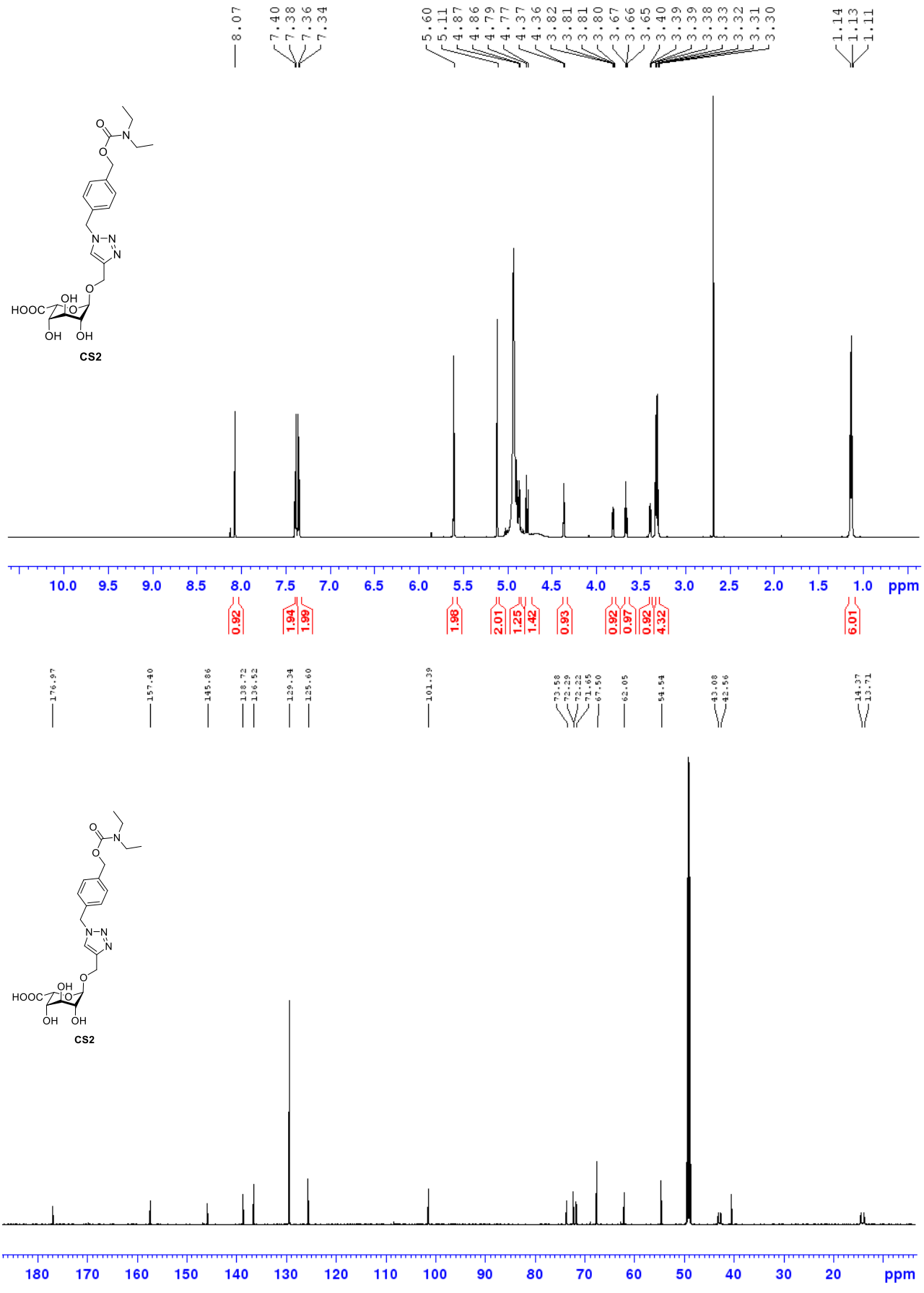
7.37
7.35
7.33
7.32
5.58
5.05
4.85
4.85
4.77
4.75
4.35
4.34
3.80
3.79
3.79
3.78
3.65
3.64
3.63
3.38
3.37
3.37
3.36
1.45
BocO
N–N
N
O
OH
HOOC
O
OH
OH
CS1
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 ppm
0.94
2.17
2.16
2.10
2.16
1.36
1.15
1.02
1.01
1.08
1.00
9.45
176.98
154.91
145.88
137.92
136.75
129.53
129.34
129.32
125.60
101.40
83.11
73.57
72.28
72.23
71.65
68.97
62.05
54.53
27.97
BocO
N–N
N
O
OH
HOOC
O
OH
OH
CS1
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm

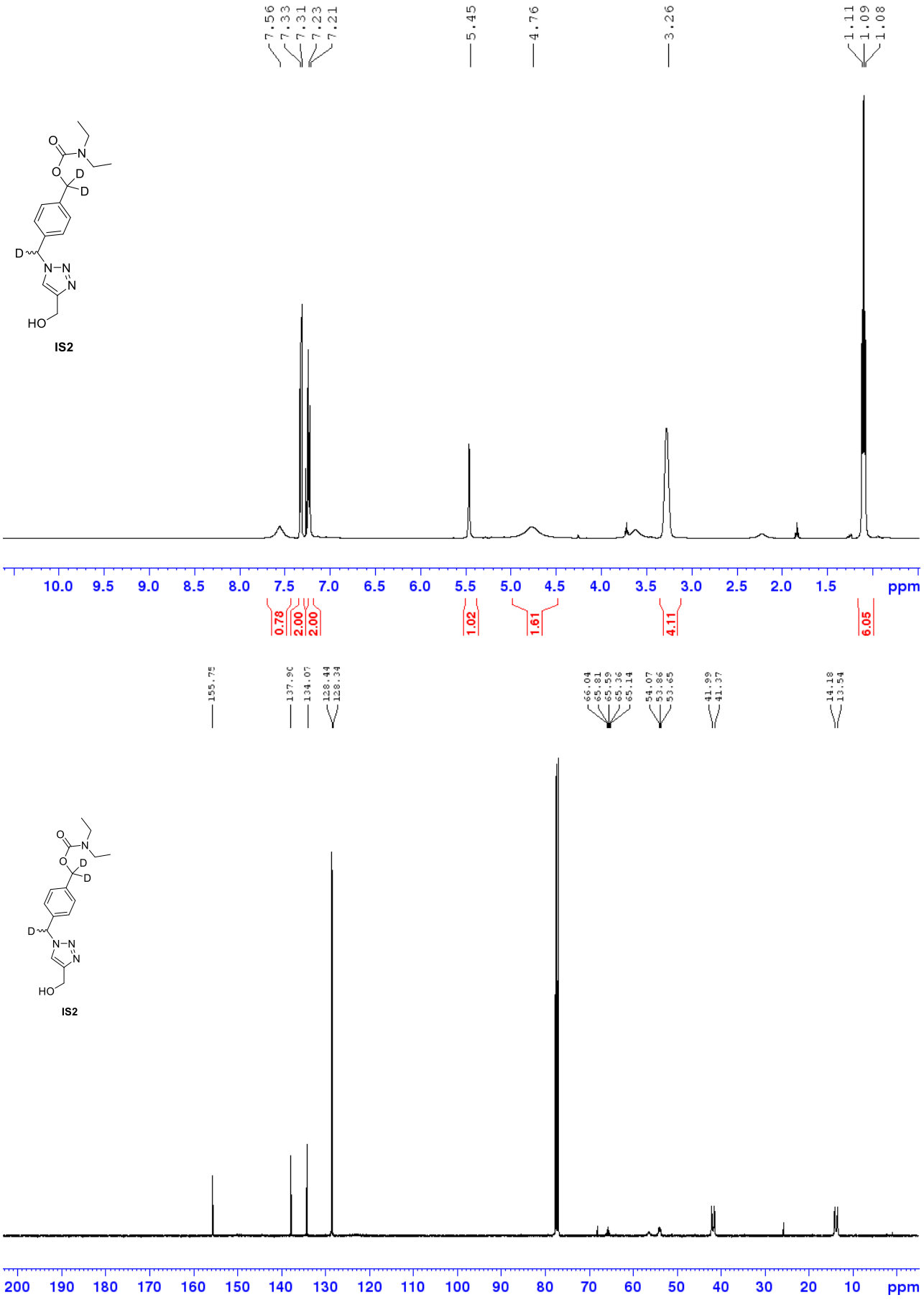

7.57
7.37
7.35
7.25
7.23
5.47
4.77
1.46
BocO
D
D
D
N–N
N
HO
IS1
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 ppm
0.74
2.00
2.08
0.97
1.60
3.89


153.45
136.48
134.61
128.98
128.45
82.62
67.93
67.69
67.46
67.24
67.03
54.09
53.89
53.68
27.86
BocO
D
D
D
N–N
N
HO
IS1
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm


7.61
7.37
7.35
7.25
7.23
5.49
5.06
4.79
1.47
BocO
N–N
N
HO
P1
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 ppm
0.72
2.00
2.19
1.87
2.02
1.42
9.16


153.44
136.62
134.64
128.95
128.46
82.64
68.06
54.24
27.86
BocO
N–N
N
HO
P1
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 ppm


8.07
7.40
7.38
7.36
7.34
5.60
5.11
4.87
4.86
4.79
4.77
4.37
4.36
3.82
3.81
3.81
3.80
3.67
3.66
3.65
3.40
3.39
3.39
3.38
3.33
3.32
3.31
3.30
1.14
1.13
1.11
HOOC
OH
OH
OH
CS2
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 ppm
0.92
1.94
1.99
1.98
2.01
1.25
1.42
0.93
0.92
0.97
0.92
4.32
6.01
176.97
157.40
145.86
138.72
136.52
129.34
125.60
101.39
73.58
73.29
72.22
71.65
67.50
62.05
54.54
43.08
42.56
14.37
13.71
HOOC
OH
OH
OH
CS2
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm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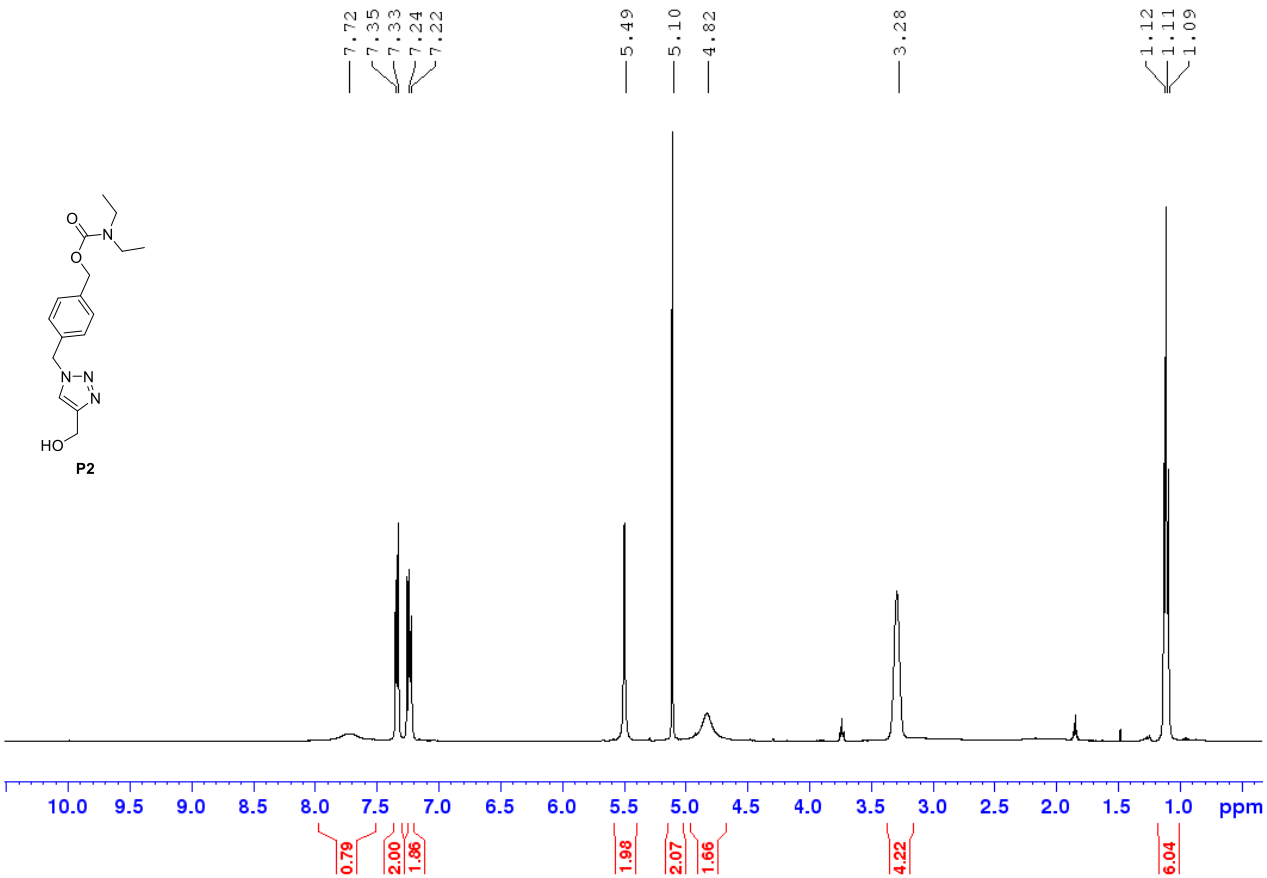
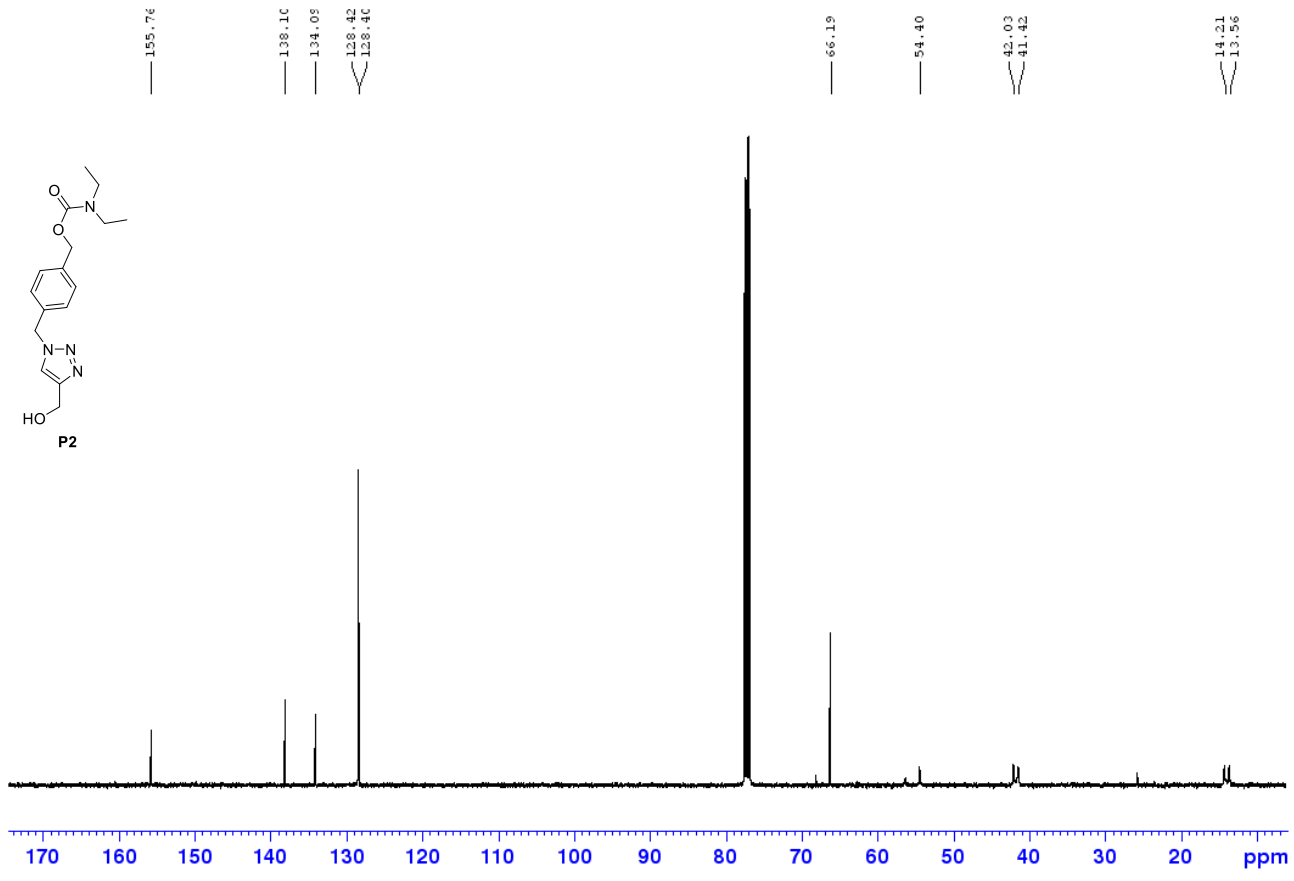
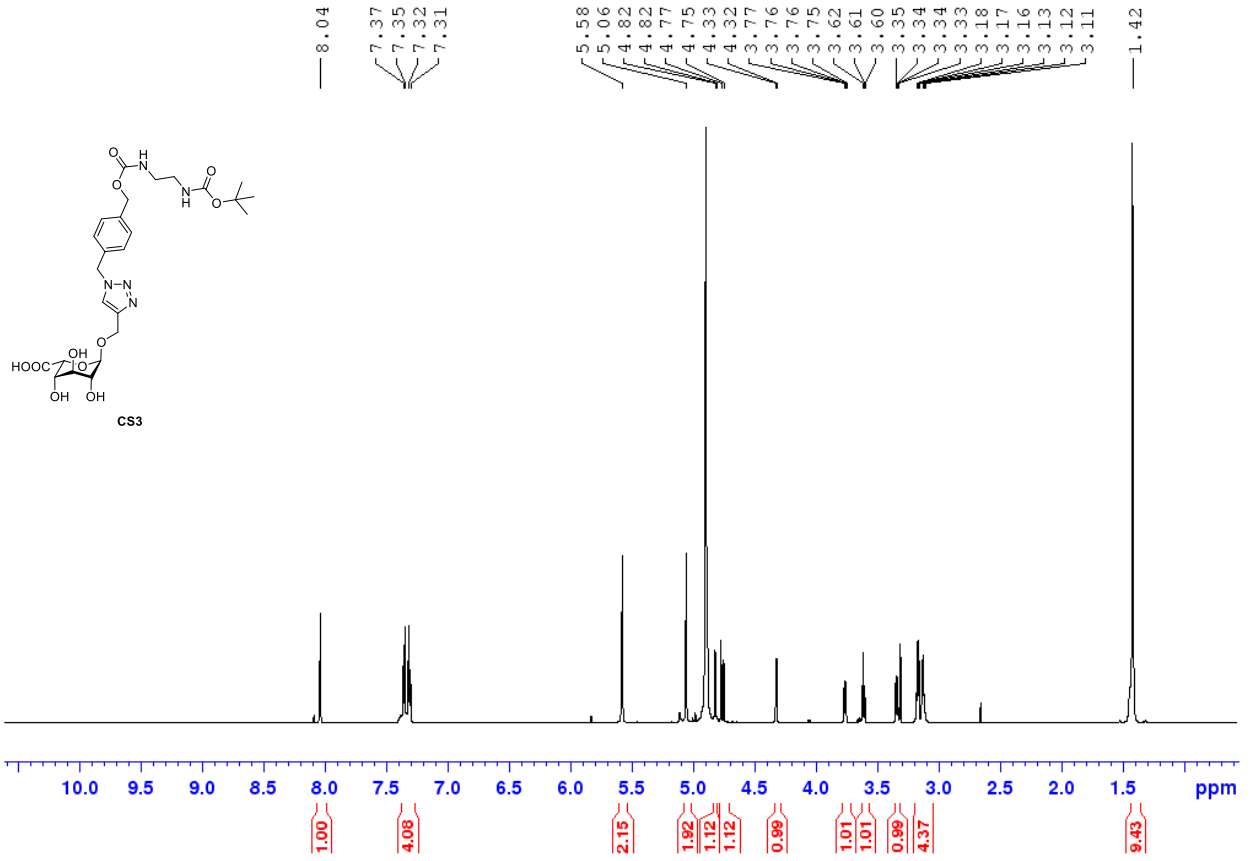
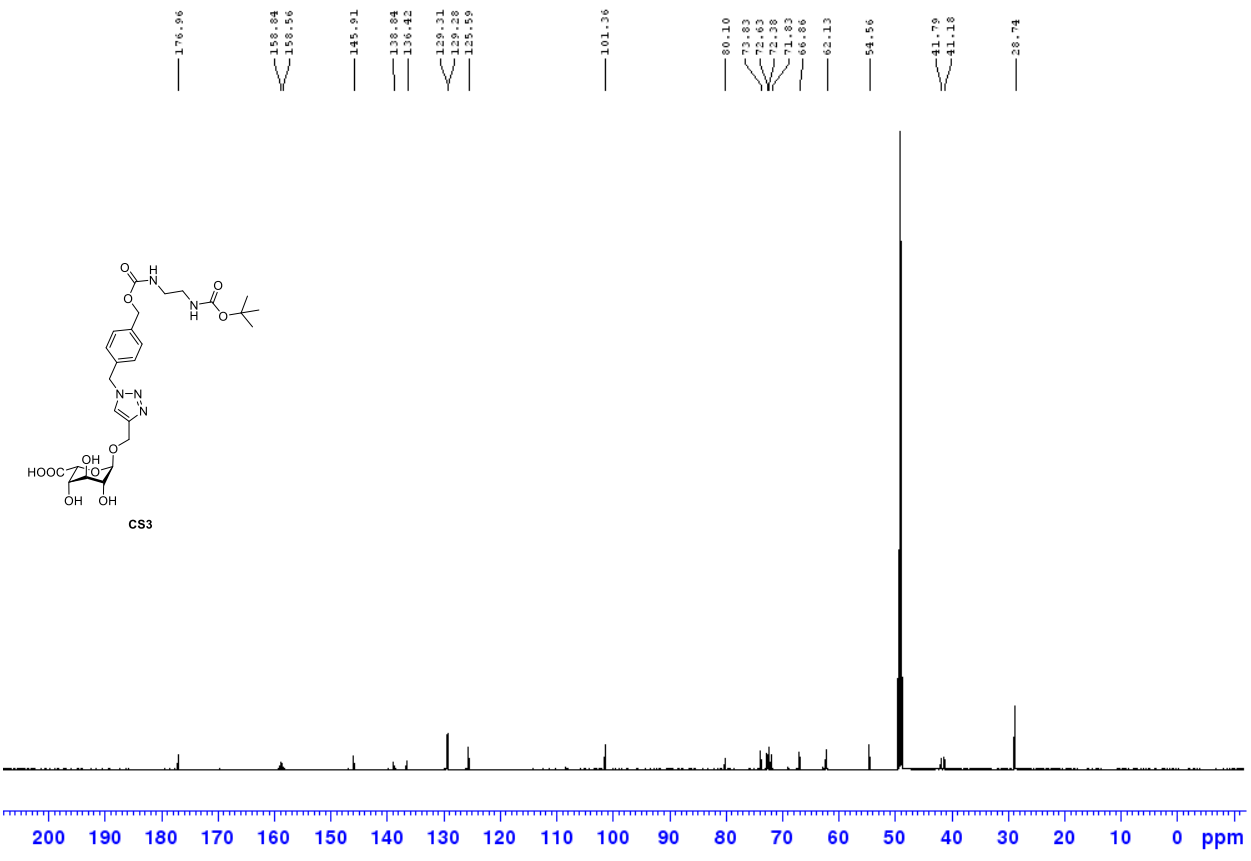
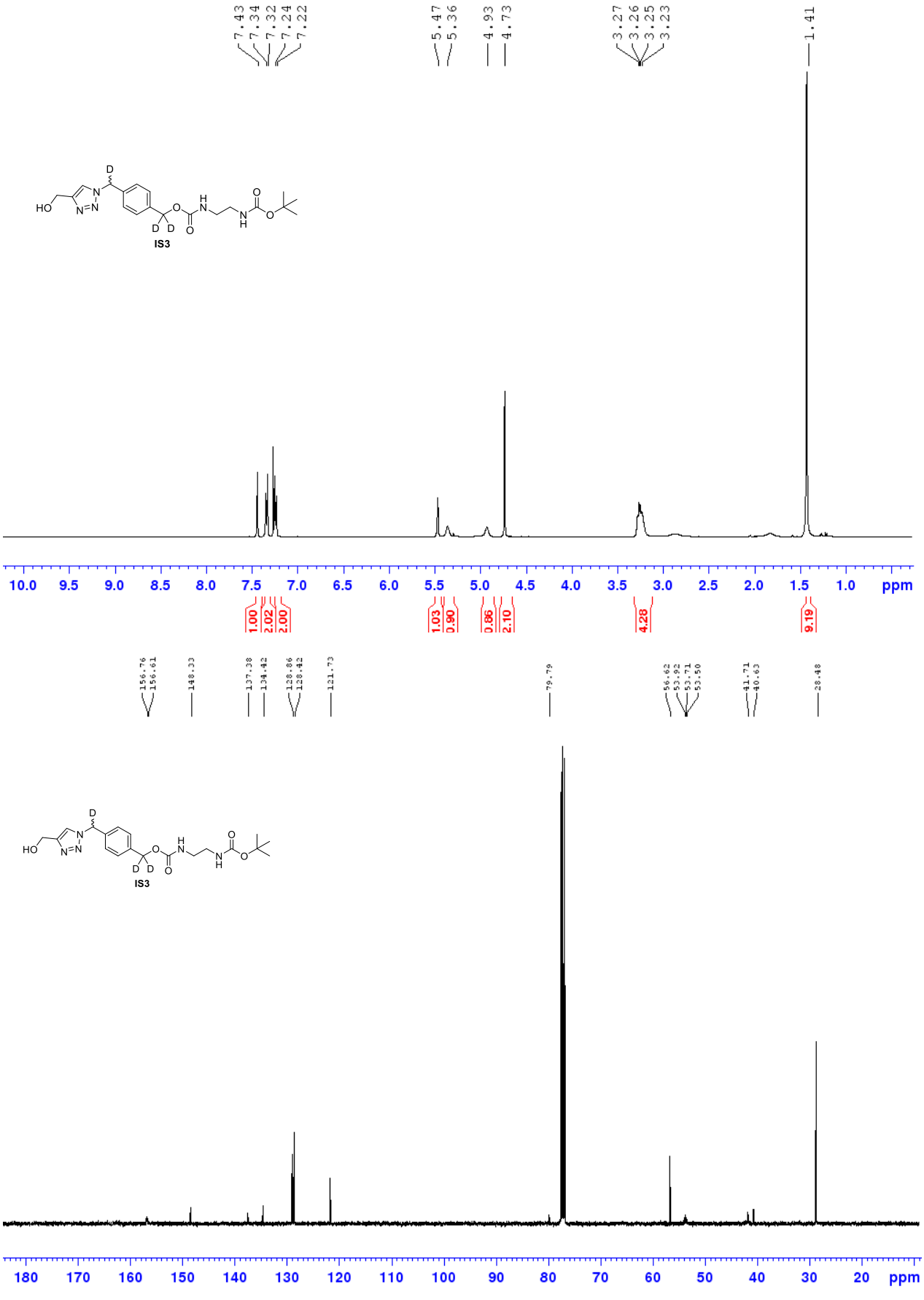
7.33
7.31
7.23
7.21
5.45
4.76
3.26
1.11
1.09
1.08
IS2
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 ppm
0.78
2.00
2.00
1.02
1.61
4.11
6.05
155.75
137.90
134.07
128.44
128.34
66.04
65.81
65.59
65.36
65.14
54.07
53.86
53.65
41.99
41.37
14.18
13.54
IS2
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

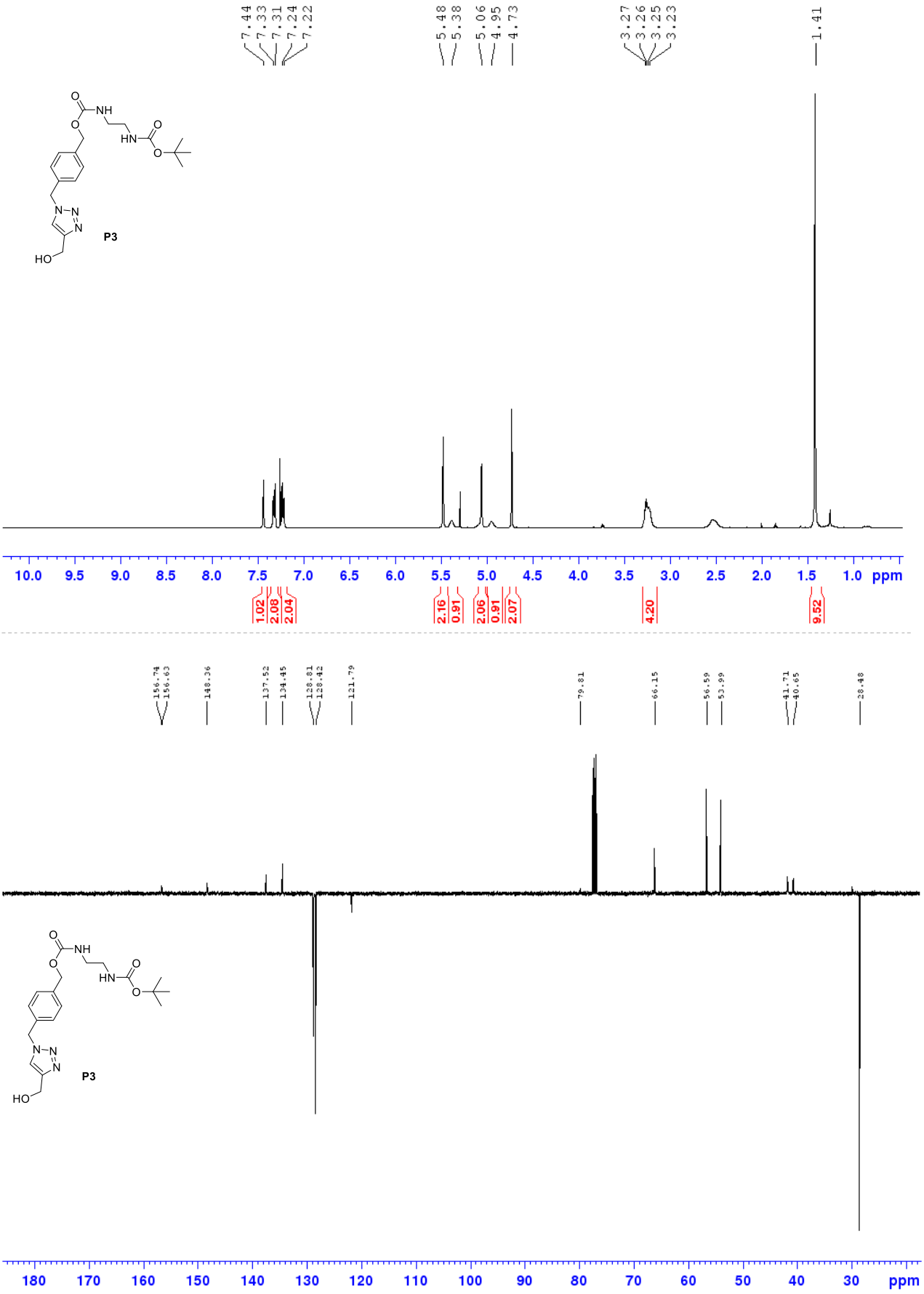

7.72
7.35
7.33
7.24
7.22
5.49
5.10
4.82
3.28
1.12
1.11
1.09
HO
P2
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 ppm
0.79
2.00
1.86
1.98
2.07
1.66
4.22
6.04


155.76
138.10
134.09
128.42
128.40
66.19
54.40
42.03
41.42
14.21
13.56
HO
P2
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm


8.04
7.37
7.35
7.32
7.31
5.58
5.06
4.82
4.82
4.77
4.75
4.33
4.32
3.77
3.76
3.75
3.62
3.61
3.60
3.35
3.34
3.34
3.33
3.18
3.17
3.16
3.13
3.12
3.11
1.42
CS3
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 ppm
1.00
4.08
2.15
1.92
1.12
1.12
0.99
1.01
1.01
0.99
4.37
9.43


176.96
158.84
158.56
145.91
138.84
136.42
129.31
129.28
125.59
101.36
80.10
73.83
72.63
72.38
71.83
66.86
62.13
54.56
41.79
41.18
28.74
CS3
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm


7.43
7.34
7.32
7.24
7.22
5.47
5.36
4.93
4.73
3.27
3.26
3.25
3.23
1.41
HO
D
N
N=N
O
H
N
O
O
D D
O
IS3
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 ppm
1.00
2.02
2.00
1.03
0.90
0.86
2.10
4.28
9.19
156.76
156.61
148.33
137.38
134.42
128.86
128.42
121.73
79.79
56.62
53.92
53.71
53.50
41.71
40.63
28.48
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm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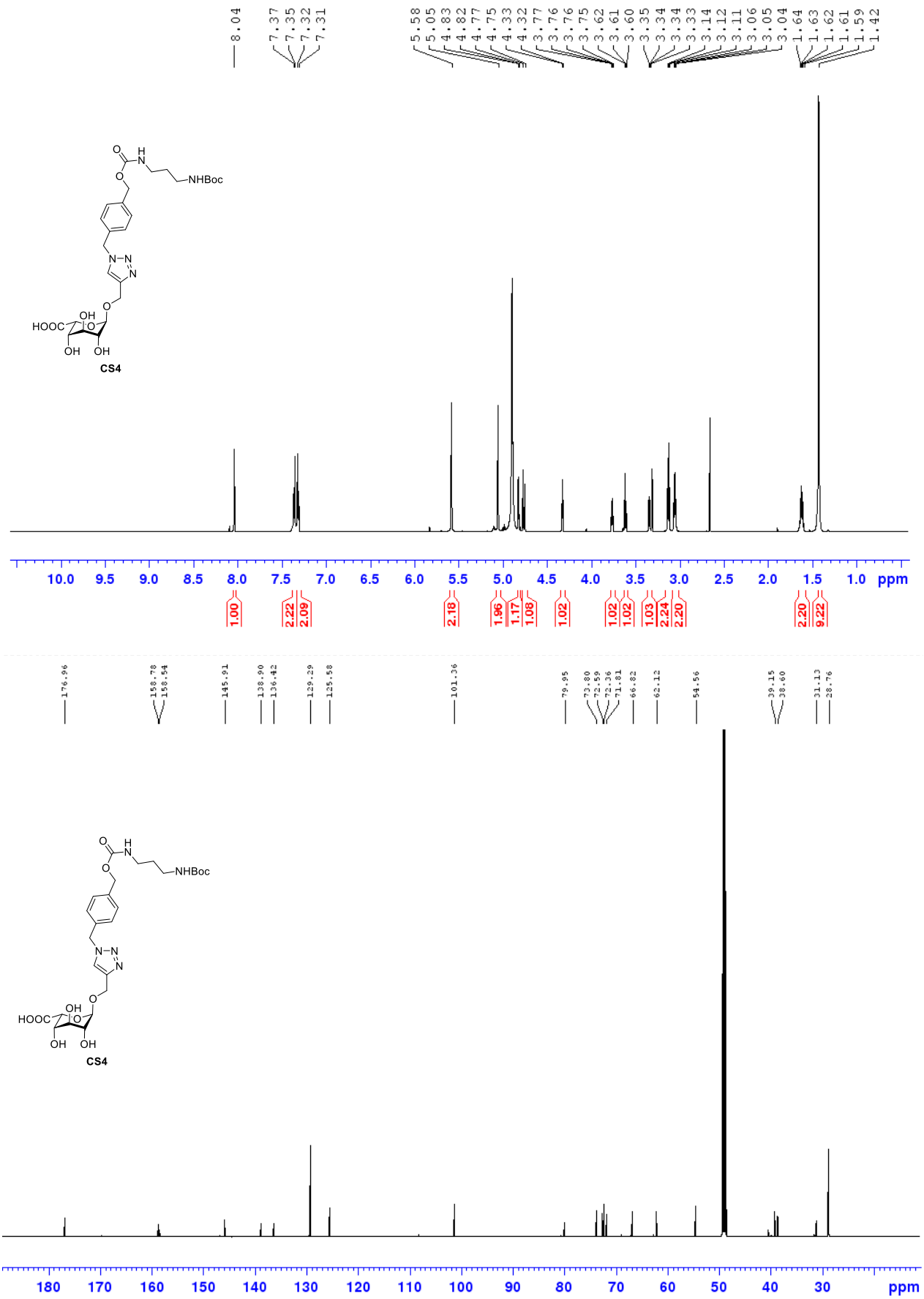
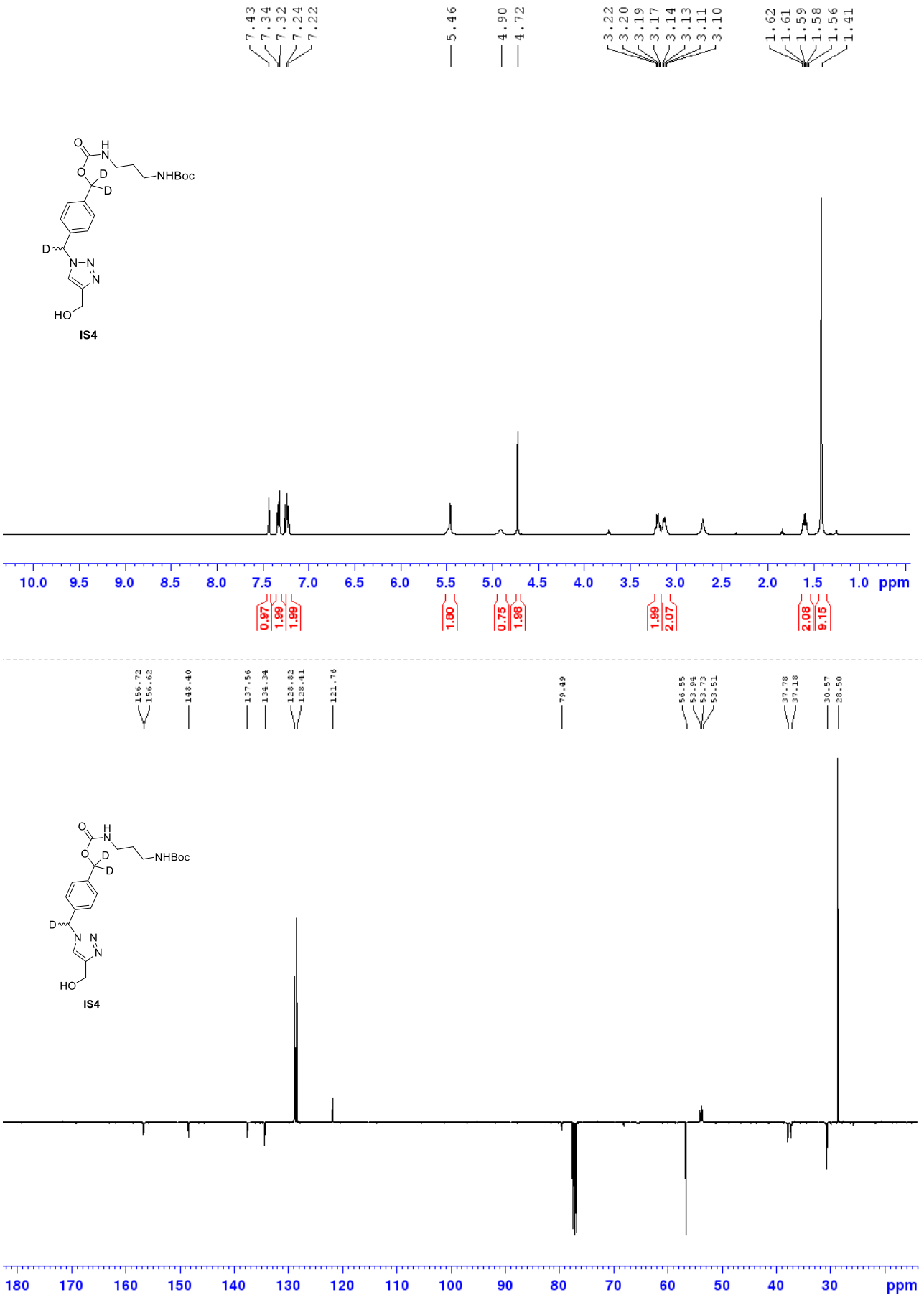
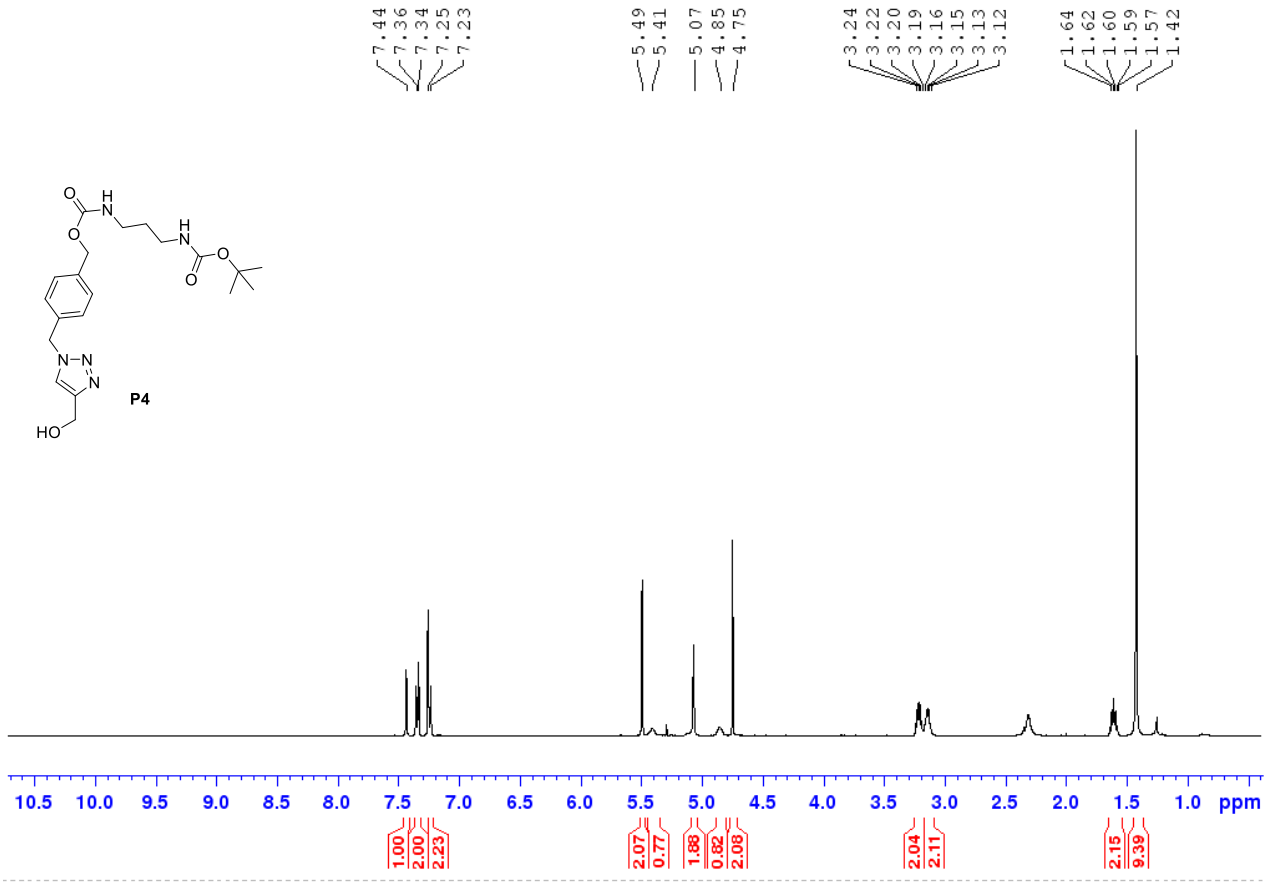
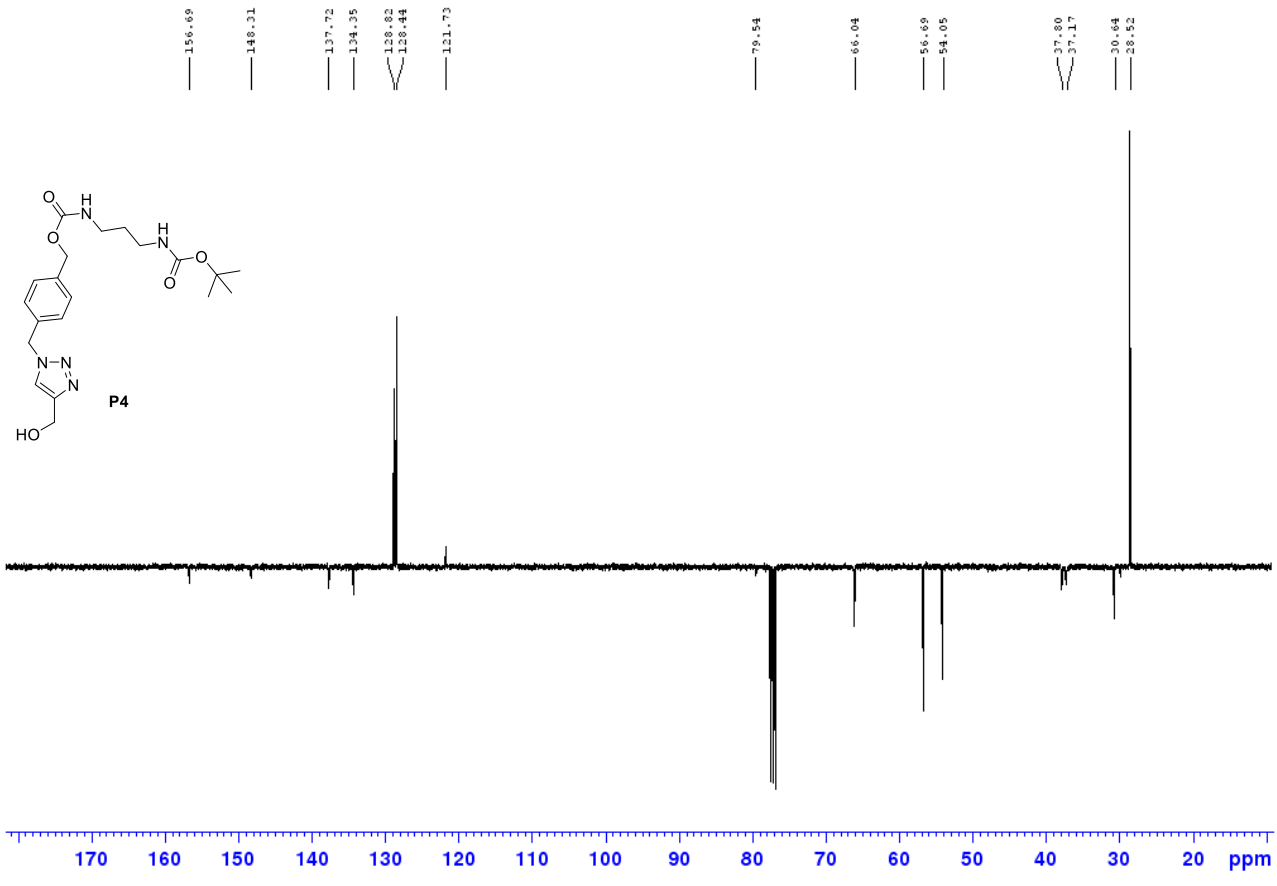
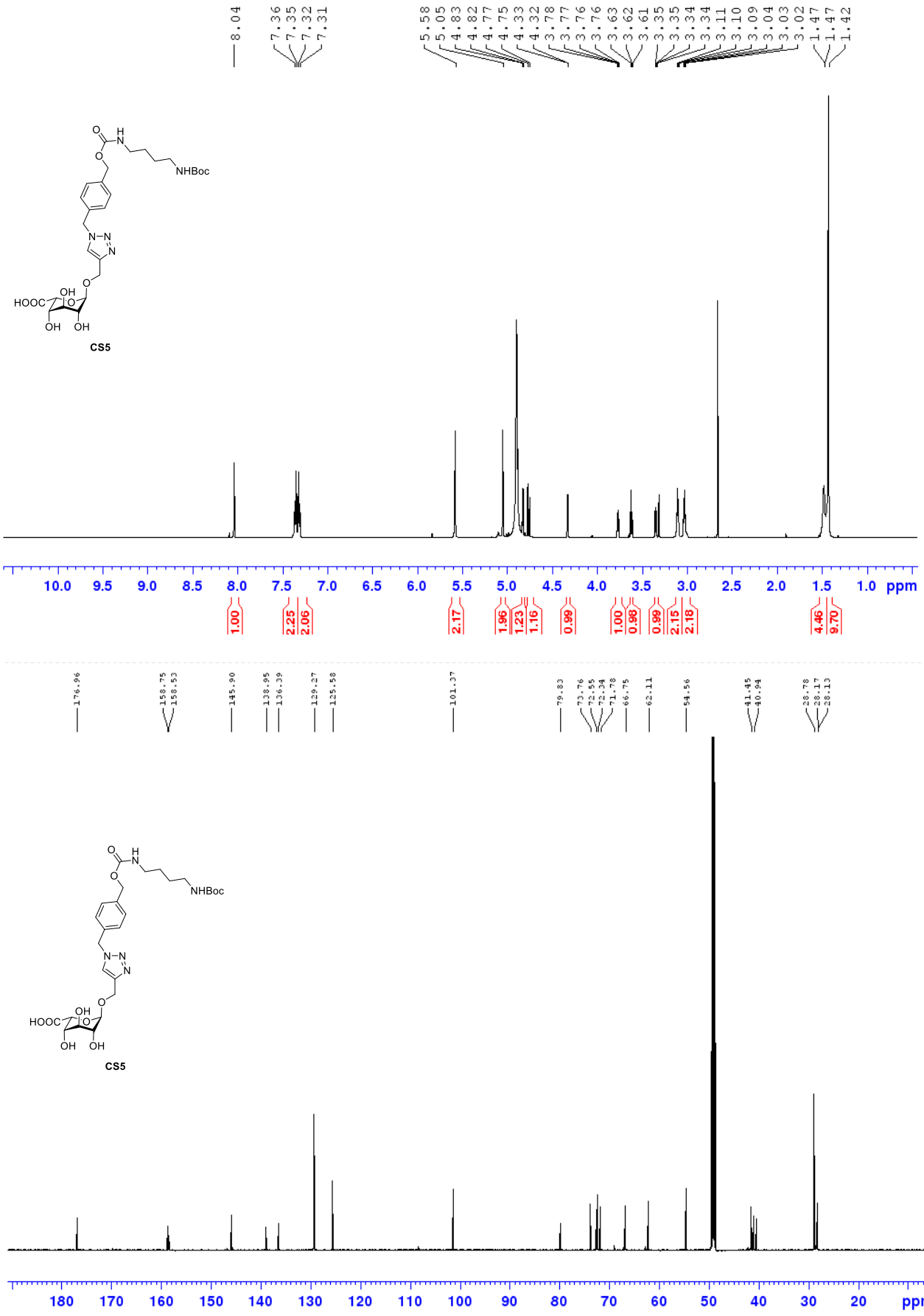
7.33
7.31
7.24
7.22
5.48
5.38
5.06
4.95
4.73
3.27
3.26
3.25
3.23
1.41
P3
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 ppm
1.02
2.08
2.04
2.16
0.91
2.06
0.91
2.07
4.20
9.52
156.74
156.63
148.36
137.52
134.45
128.81
128.42
121.79
79.81
66.15
56.59
53.99
41.71
40.65
28.48
P3
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 ppm


8.04
7.37
7.35
7.32
7.31
5.58
5.05
4.83
4.82
4.77
4.75
4.33
4.32
3.77
3.76
3.76
3.75
3.62
3.61
3.60
3.35
3.34
3.34
3.33
3.14
3.12
3.11
3.06
3.05
3.04
1.64
1.63
1.62
1.61
1.59
1.42
NHBoc
HOOC
OH
OH
OH
CS4
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 ppm
1.00
2.22
2.09
2.18
1.96
1.17
1.08
1.02
1.02
1.02
1.03
2.24
2.20
2.20
9.22
176.96
158.78
158.54
145.91
138.90
136.42
129.29
125.58
101.36
79.95
73.80
72.59
72.36
71.81
66.82
62.12
54.56
39.15
38.60
31.13
28.76
NHBoc
HOOC
OH
OH
OH
CS4
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 ppm


7.43
7.34
7.32
7.24
7.22
5.46
4.90
4.72
3.22
3.20
3.19
3.17
3.14
3.13
3.11
3.10
1.62
1.61
1.59
1.58
1.56
1.41
NHBoc
HO
IS4
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 ppm
0.97
1.99
1.99
1.80
0.75
1.98
1.99
2.07
2.08
9.15
156.72
156.62
148.40
137.56
134.34
128.82
128.41
121.76
79.49
56.55
53.94
53.73
53.51
37.78
37.18
30.57
28.50
NHBoc
HO
IS4
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 ppm


7.44
7.36
7.34
7.25
7.23
5.49
5.41
5.07
4.85
4.75
3.24
3.22
3.20
3.19
3.16
3.15
3.13
3.12
1.64
1.62
1.60
1.59
1.57
1.42
P4
HO
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 ppm
1.00
2.00
2.23
2.07
0.77
1.88
0.82
2.08
2.04
2.11
2.15
9.39


156.69
148.31
137.72
134.35
128.82
128.44
121.73
79.54
66.04
56.69
54.05
37.80
37.17
30.64
28.52
P4
HO
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm


8.04
7.36
7.35
7.32
7.31
5.58
5.05
4.83
4.82
4.77
4.75
4.33
4.32
3.78
3.77
3.76
3.76
3.63
3.62
3.61
3.35
3.35
3.34
3.34
3.11
3.10
3.09
3.04
3.03
3.02
1.47
1.47
1.42
NHBoc
HOOC
OH
OH
OH
CS5
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 ppm
1.00
2.25
2.06
2.17
1.96
1.23
1.16
0.99
1.00
0.98
0.99
2.15
2.18
4.46
9.70
176.96
158.75
158.53
145.90
138.95
136.39
129.27
125.58
101.37
79.83
73.76
72.55
72.34
71.78
66.75
62.11
54.56
41.45
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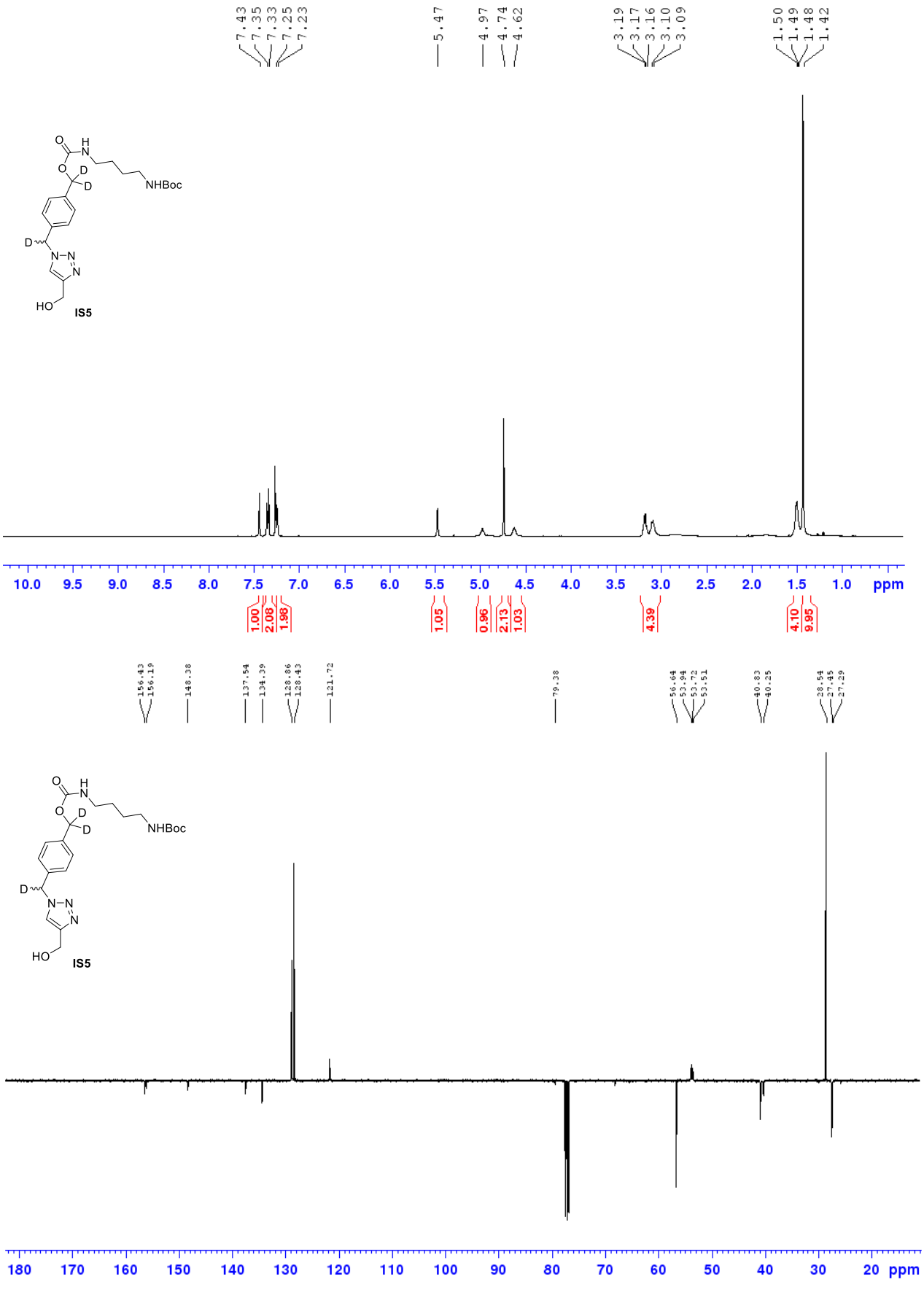
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3.10
3.09
1.50
1.49
1.48
1.42
NHBoc
IS5
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1.00
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1.05
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2.13
1.03
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4.10
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134.39
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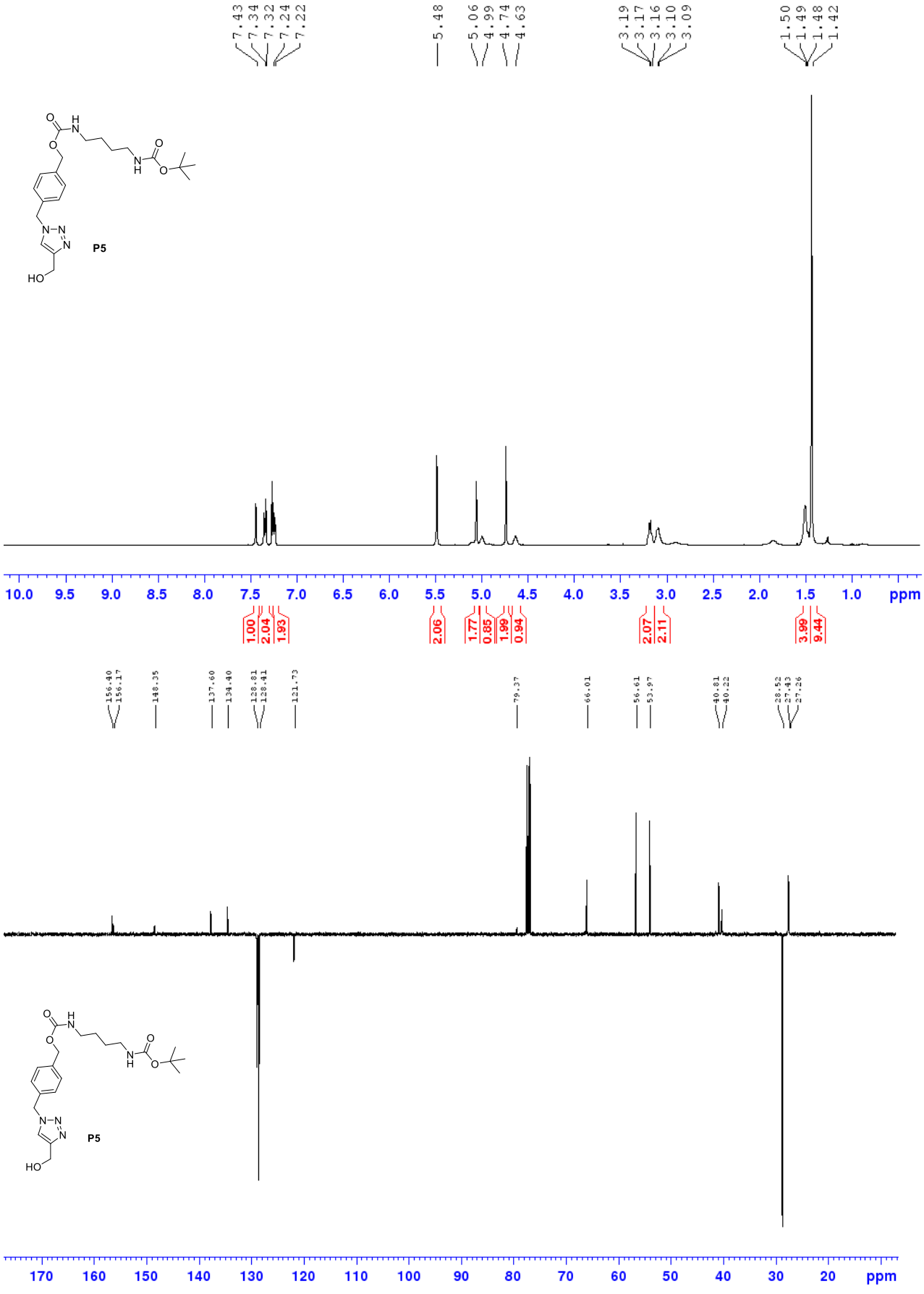
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3.10
3.09
1.50
1.49
1.48
1.42
P5
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1.00 2.04 1.93 2.06 1.77 0.85 1.99 0.94 2.07 2.11 3.99 9.44
156.40
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148.35
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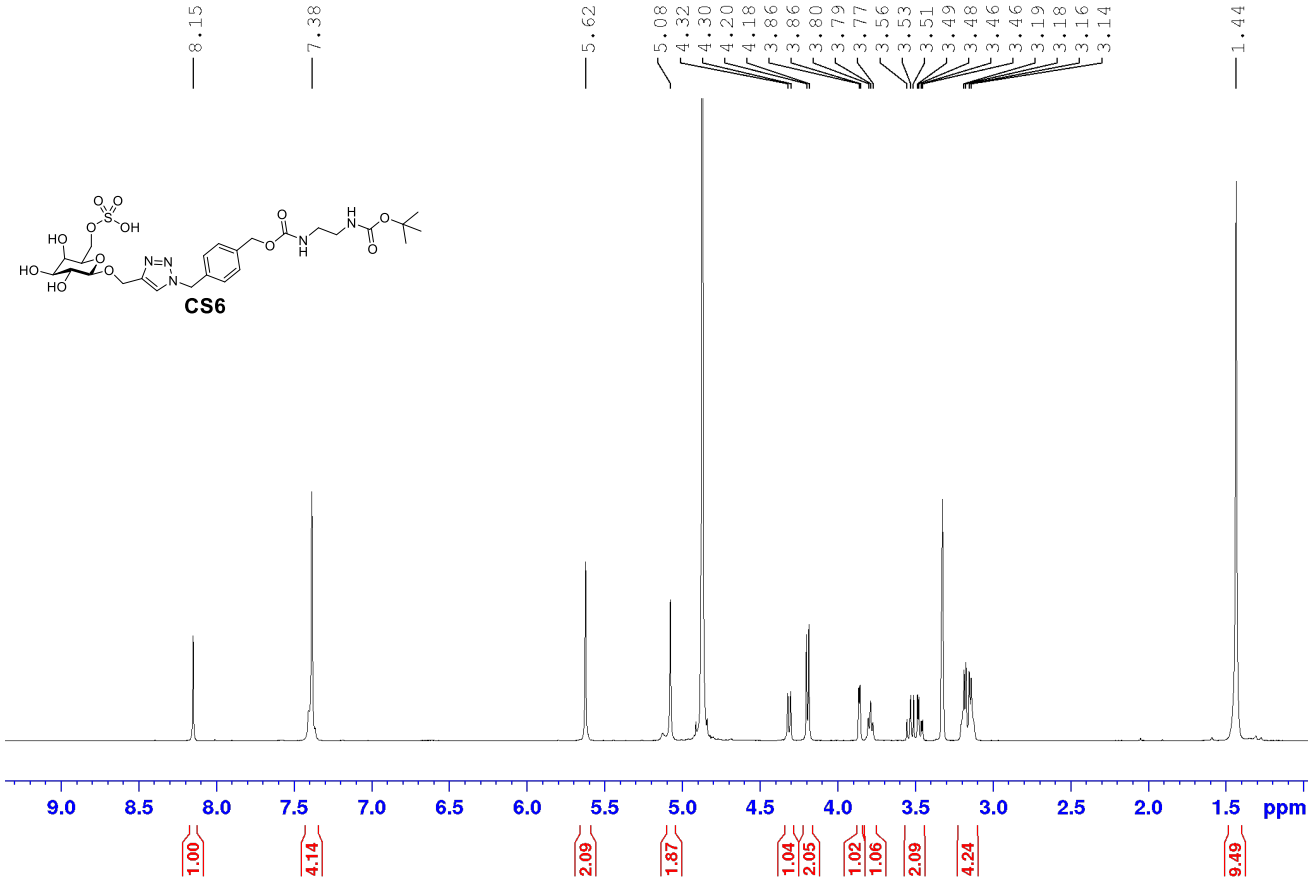
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3.46
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3.14
1.44
CS6
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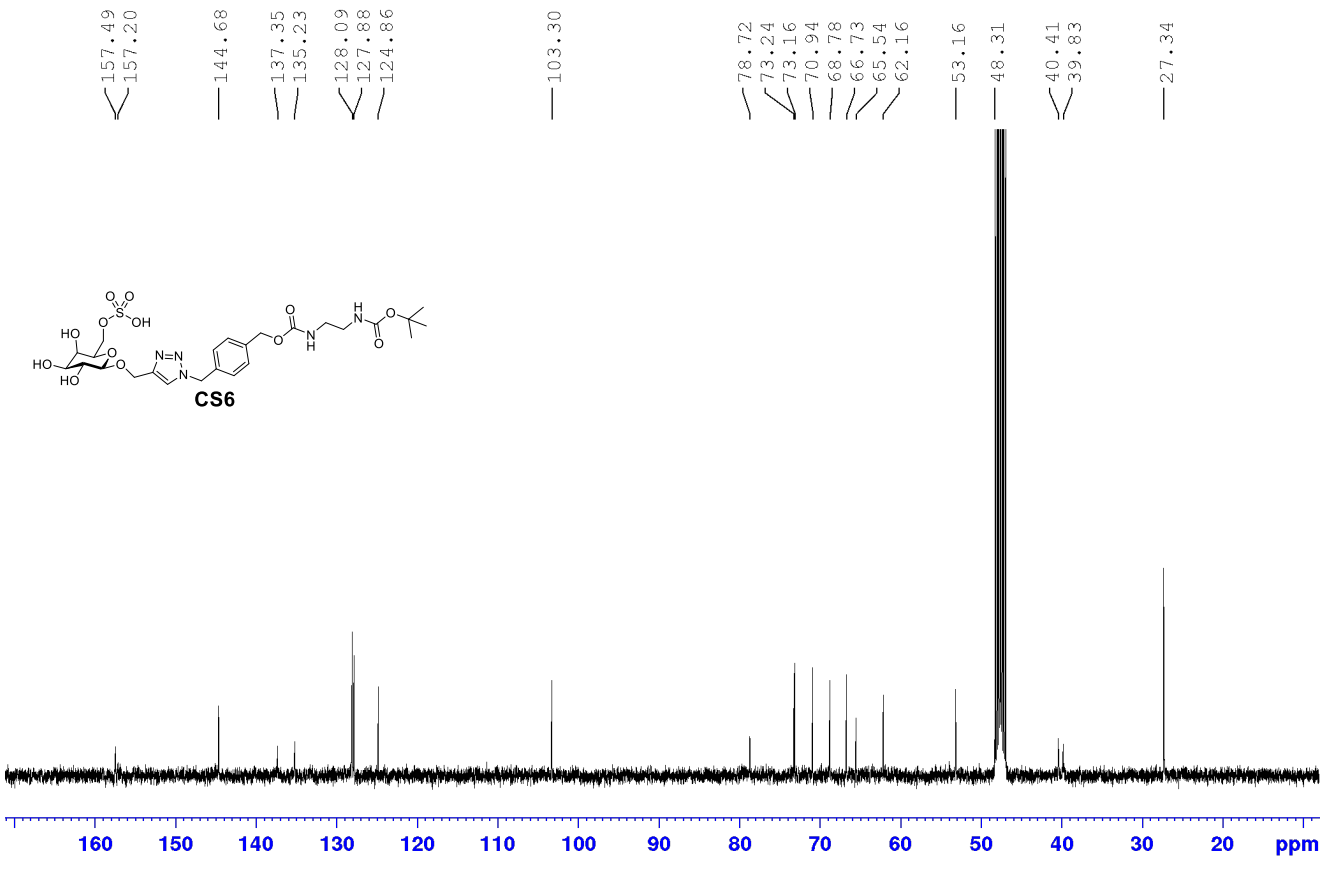
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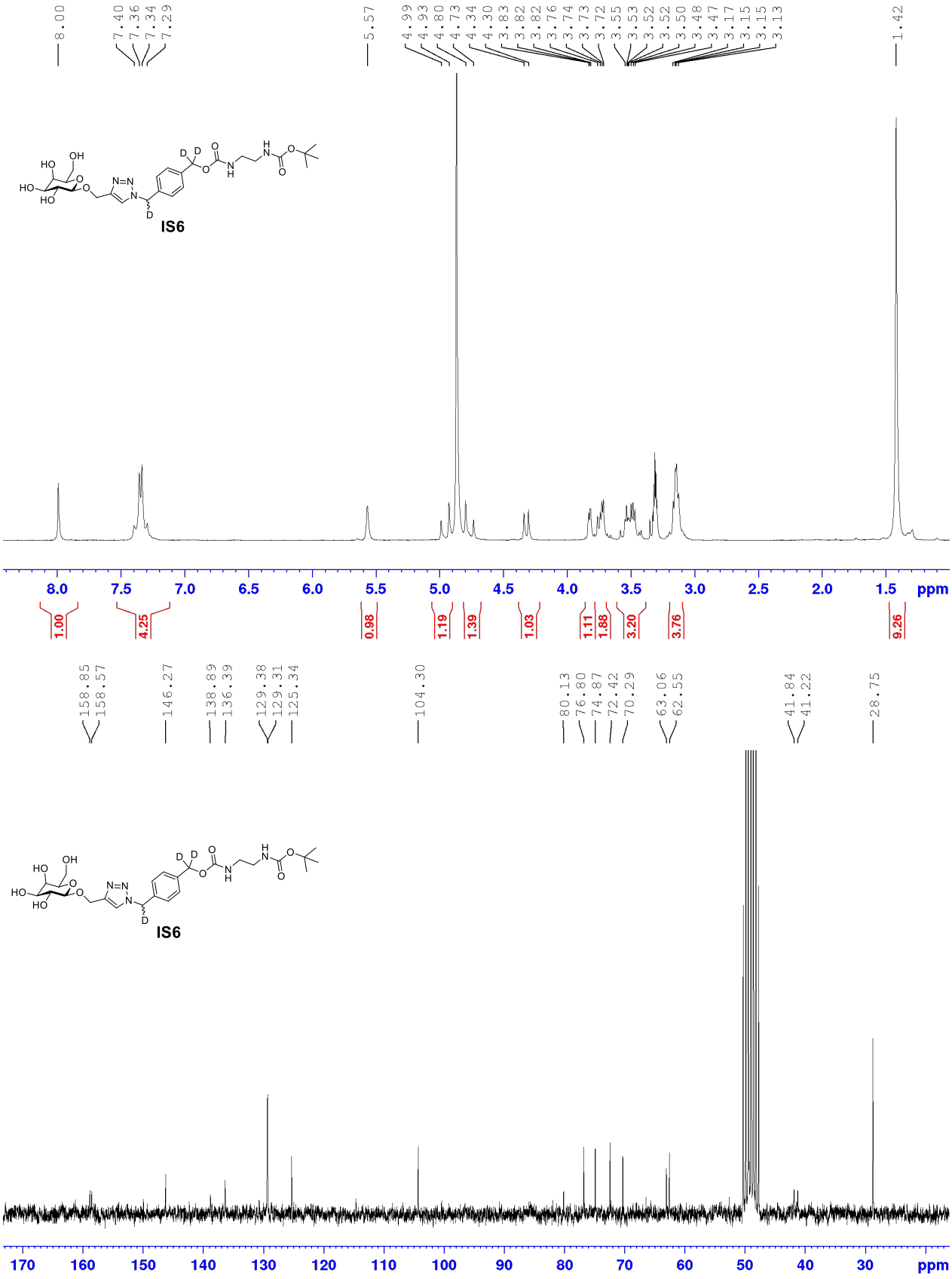
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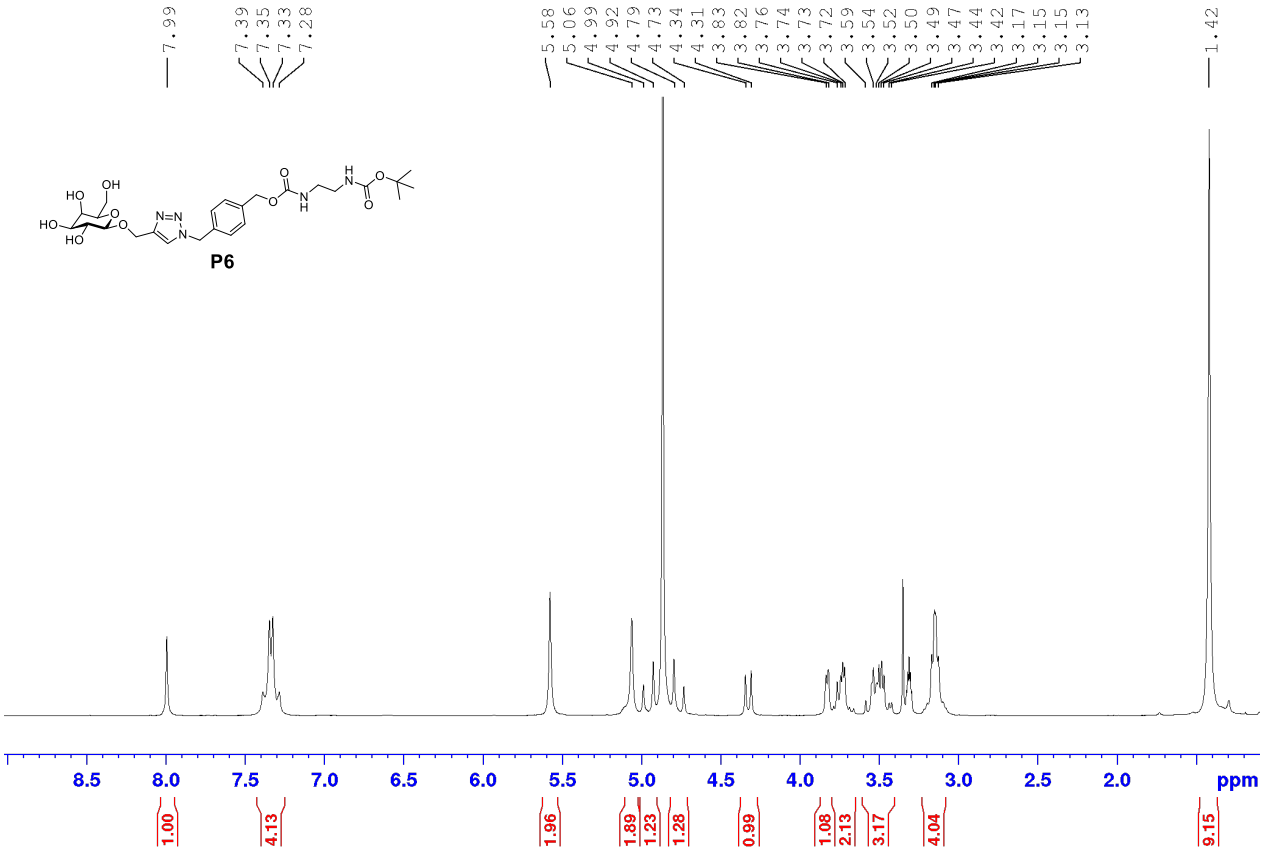
ppm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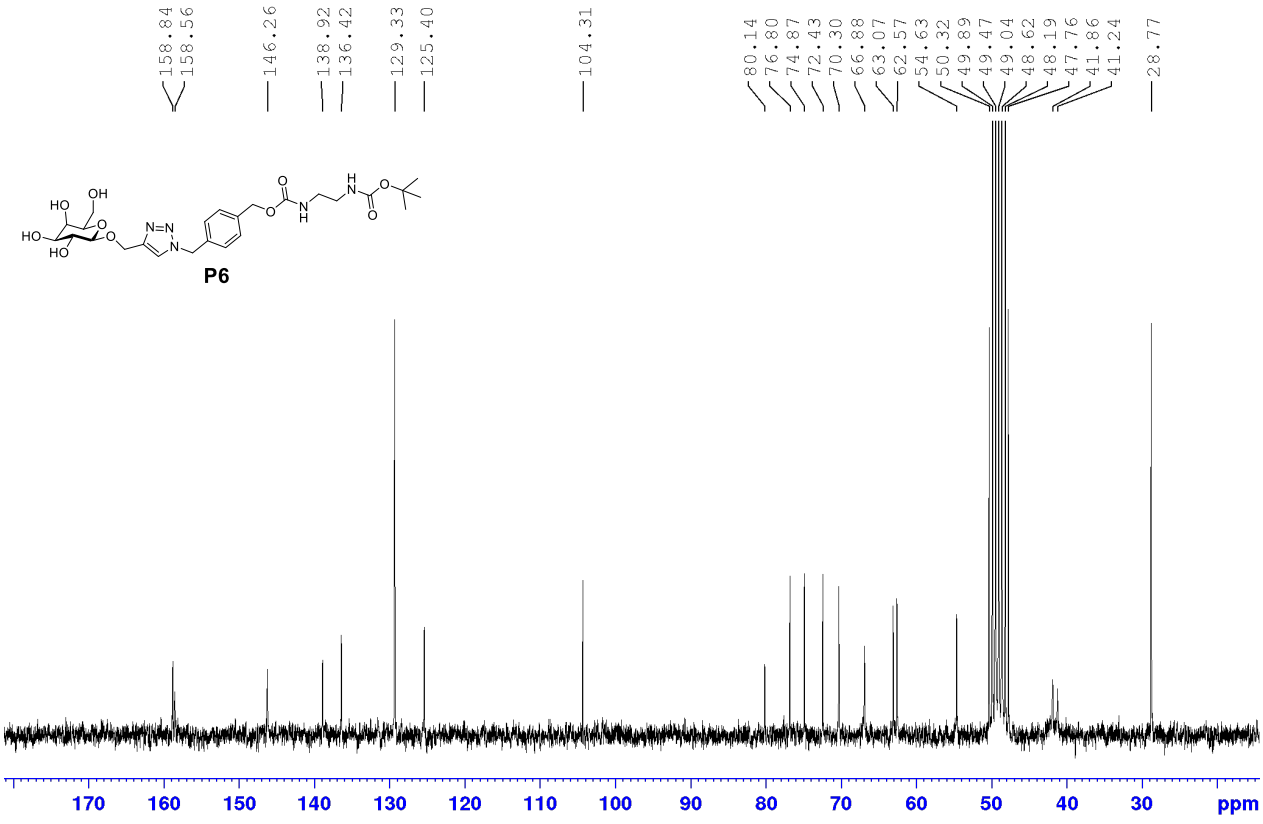
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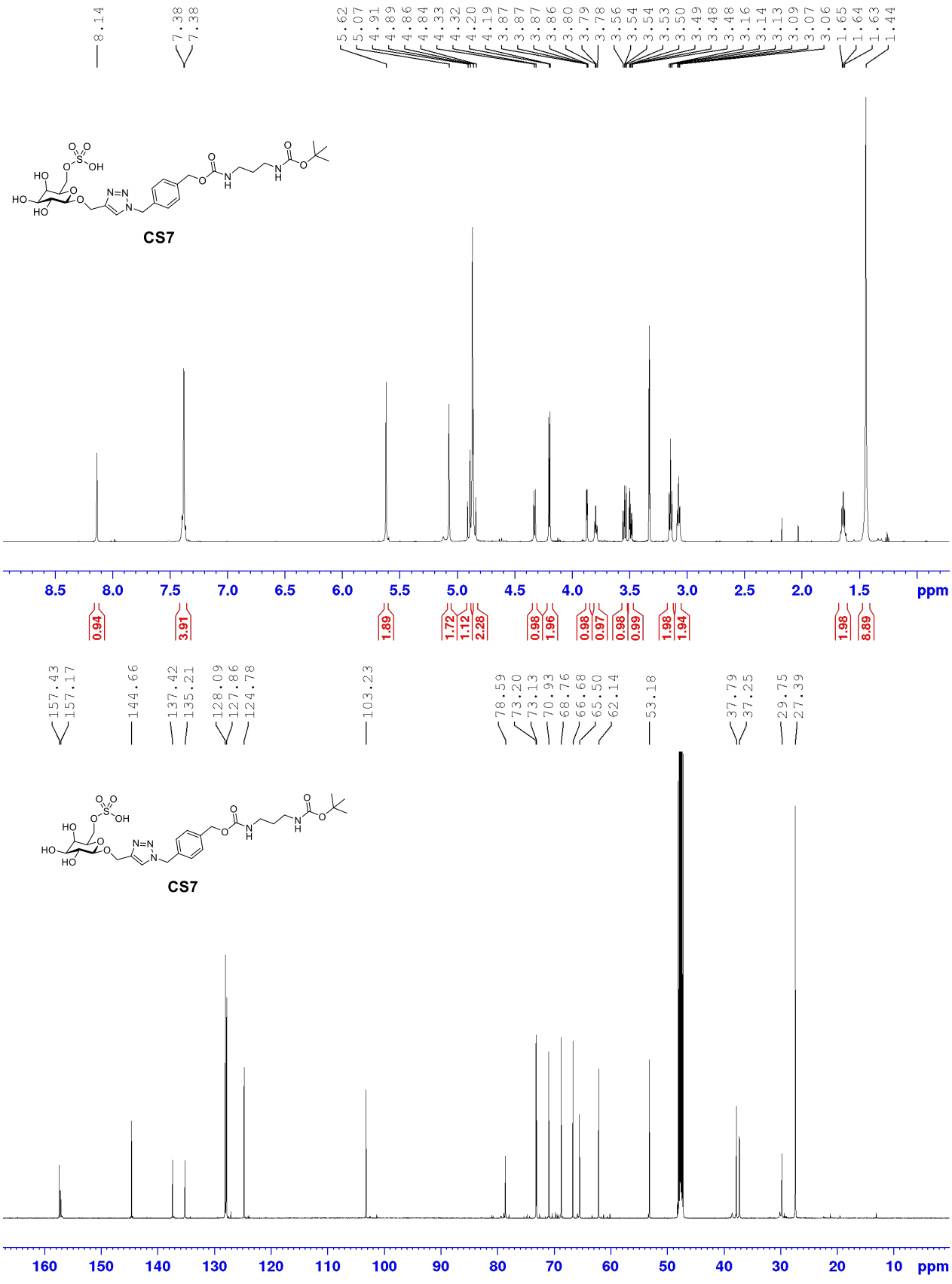
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3.50
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3.48
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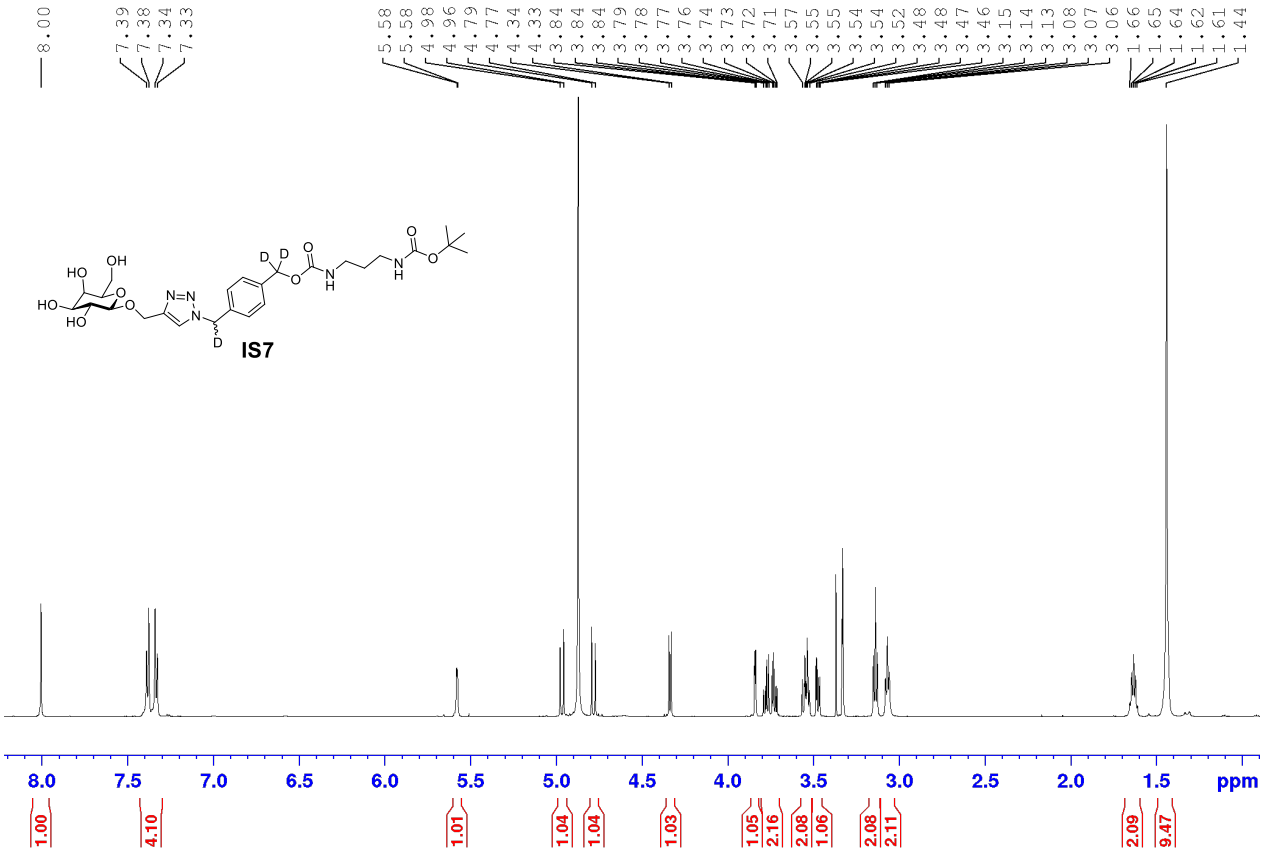
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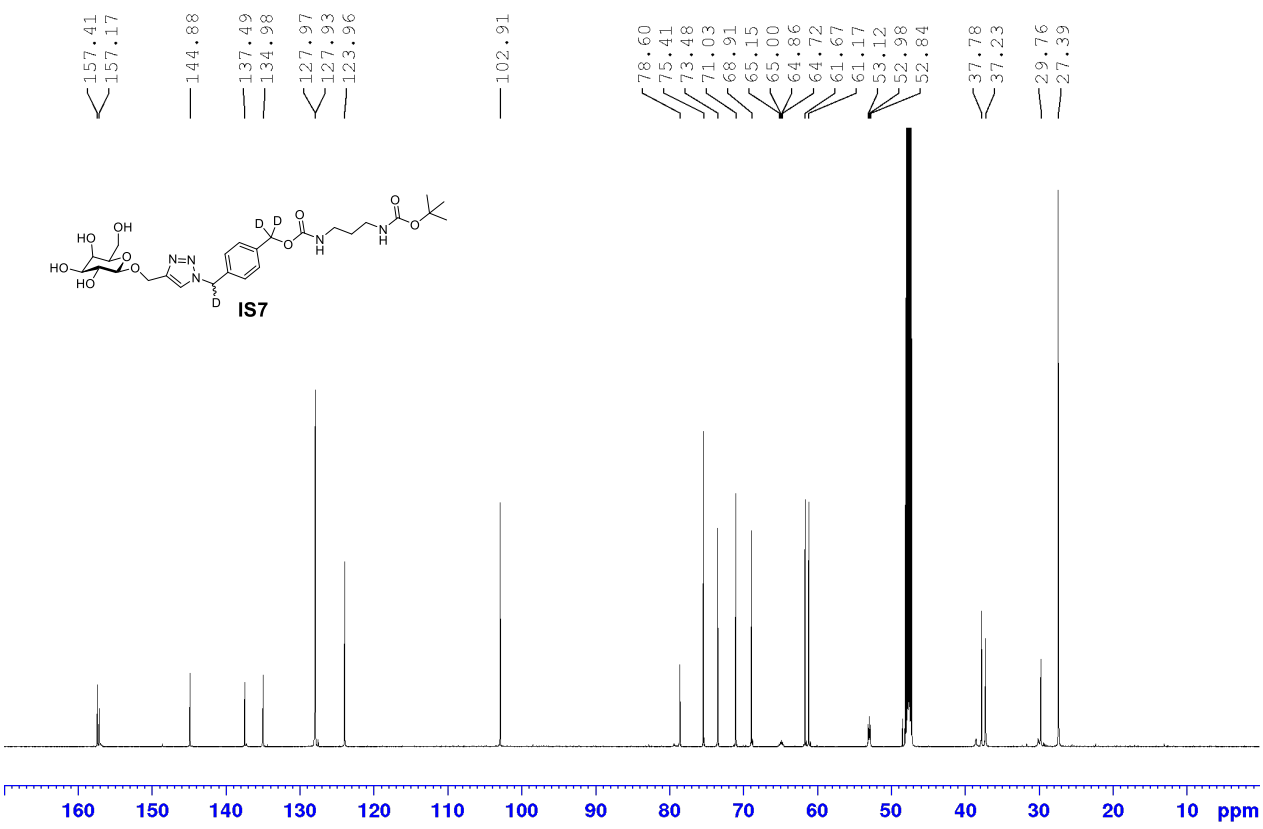
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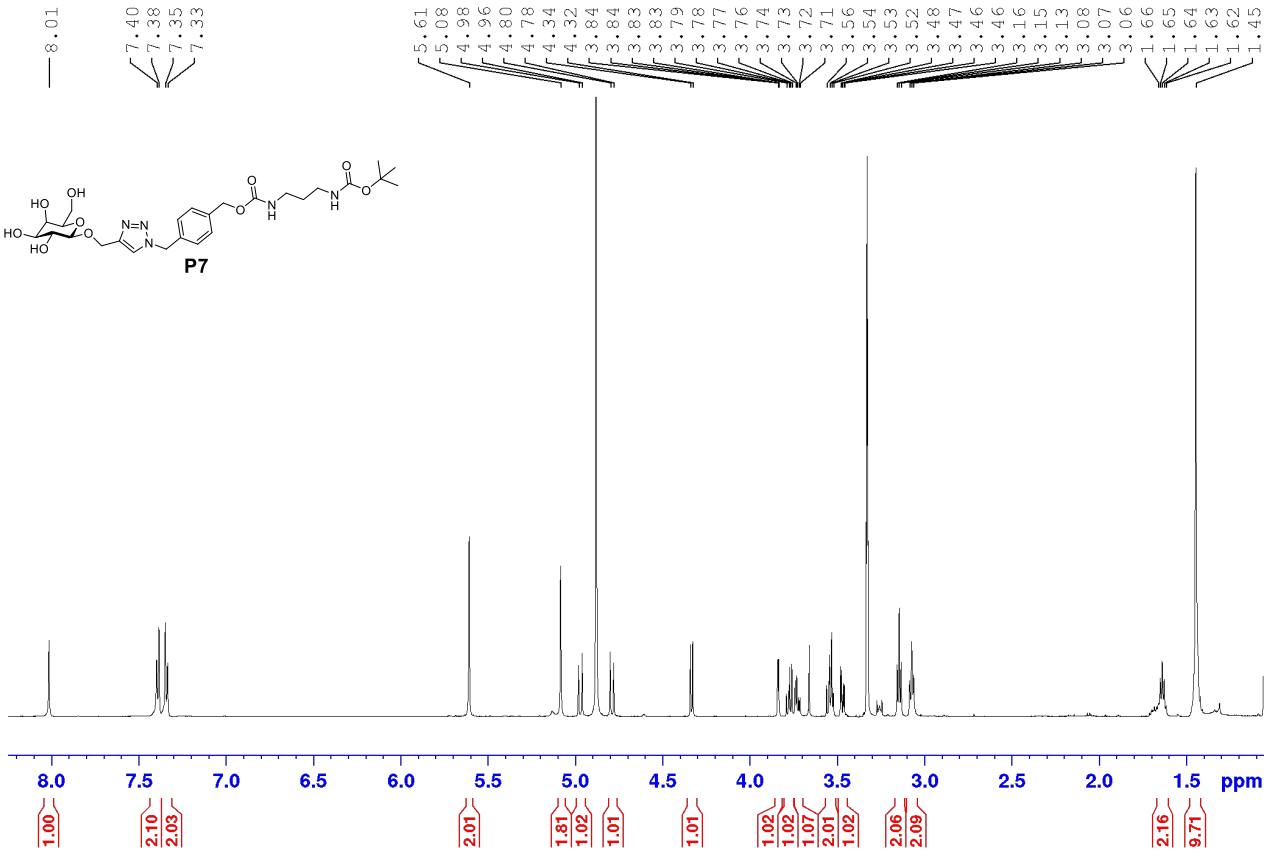
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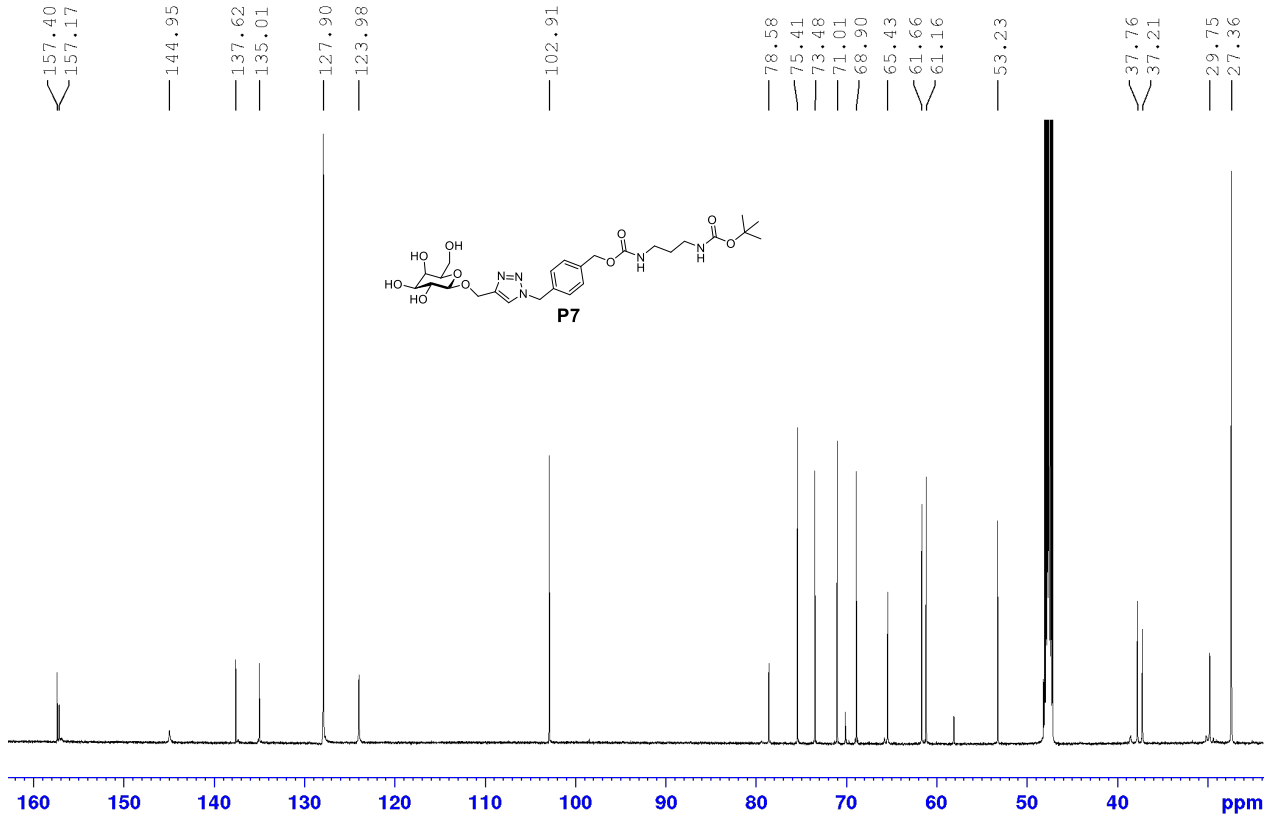
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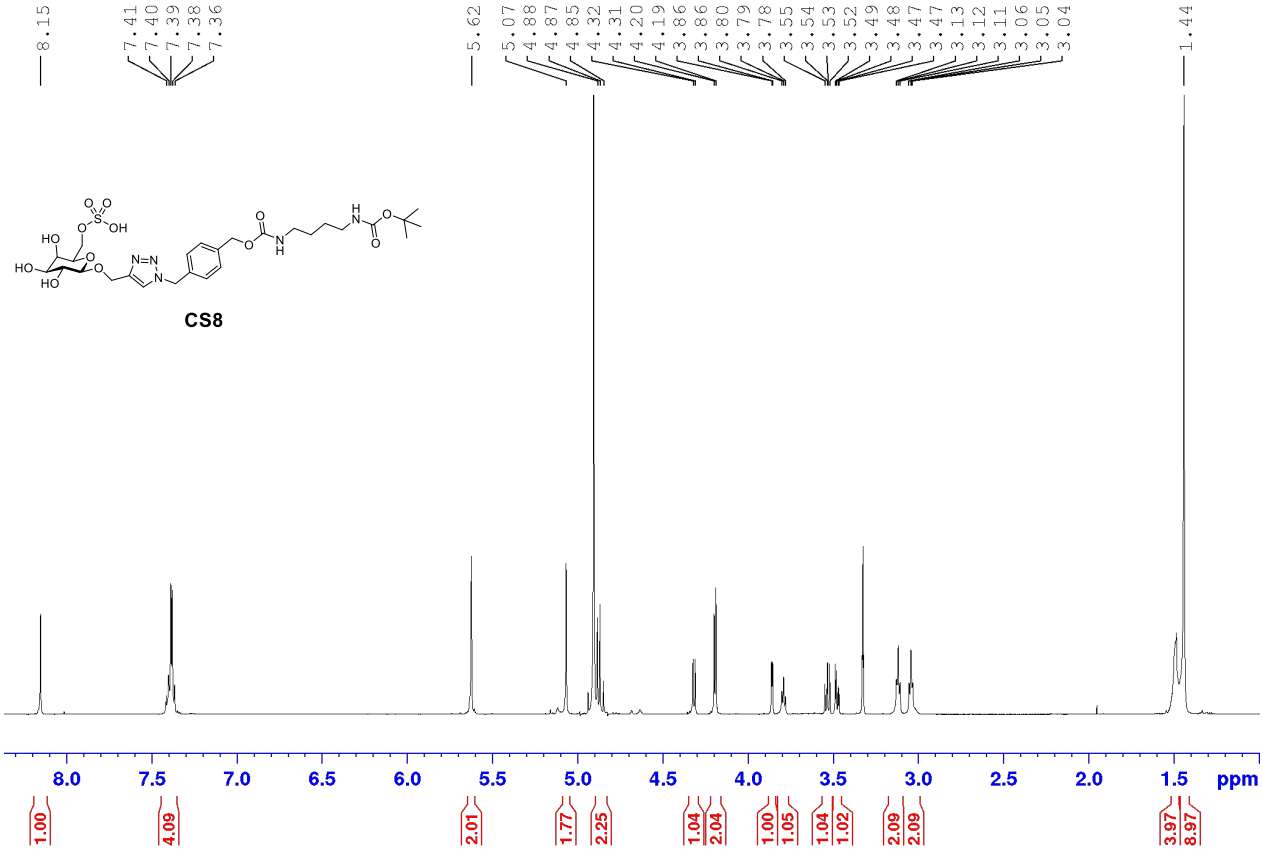
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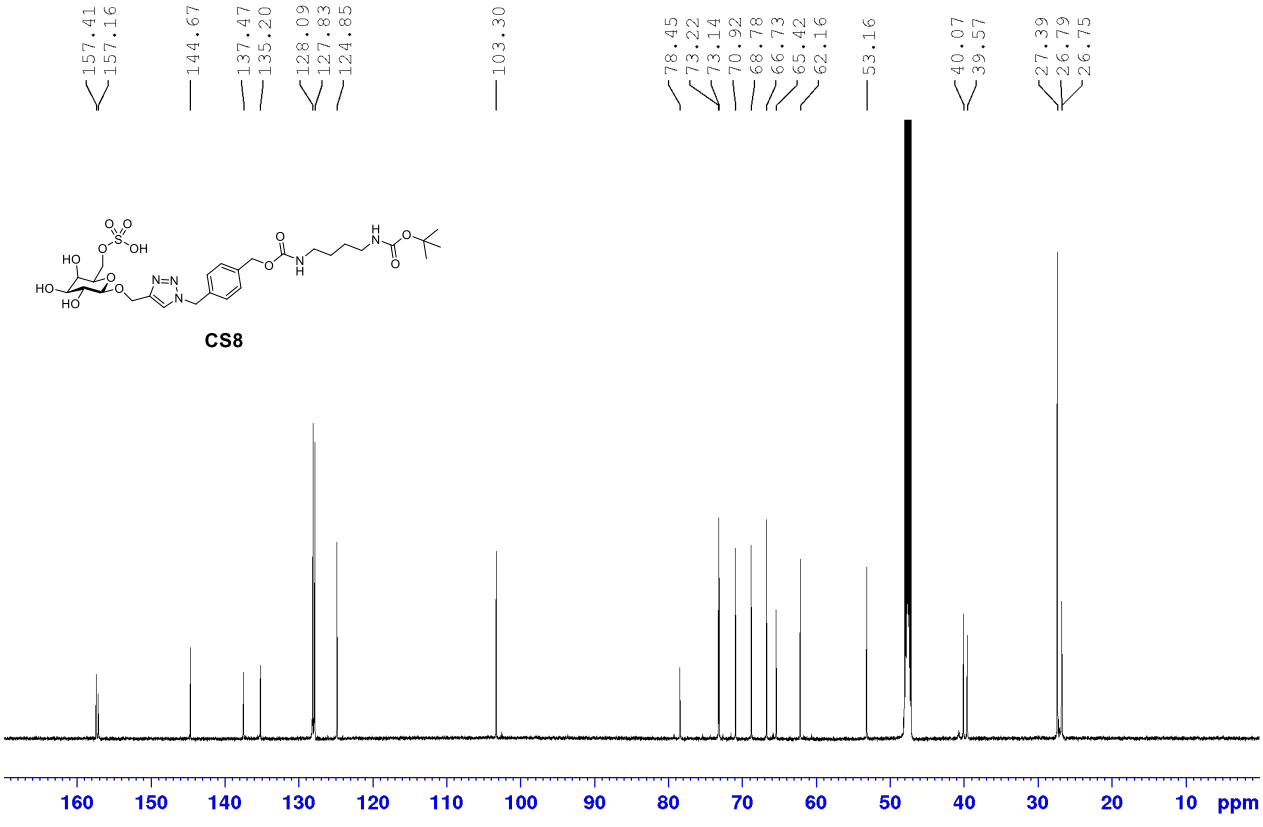
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40.07
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26.79
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10
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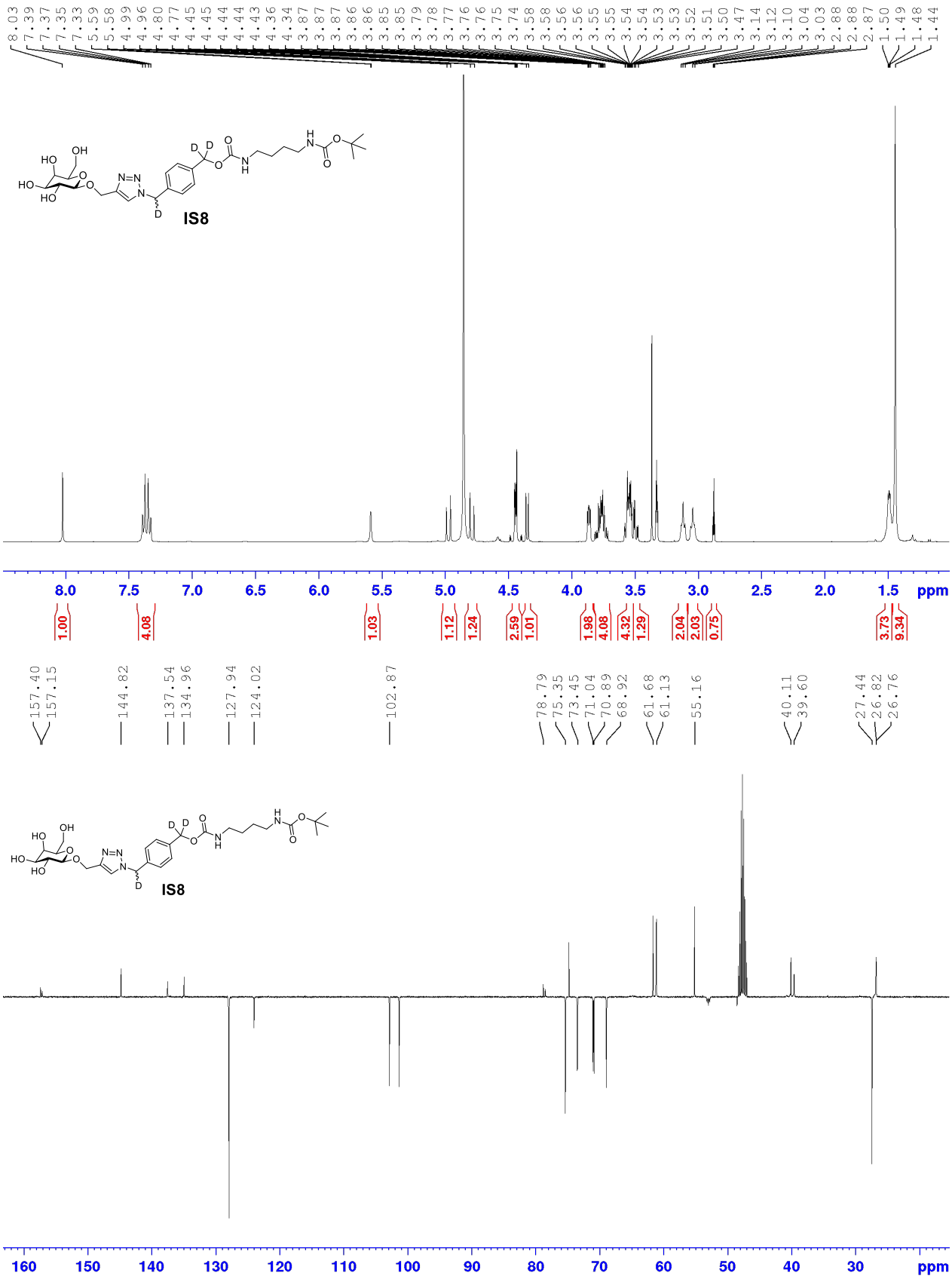
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IS8
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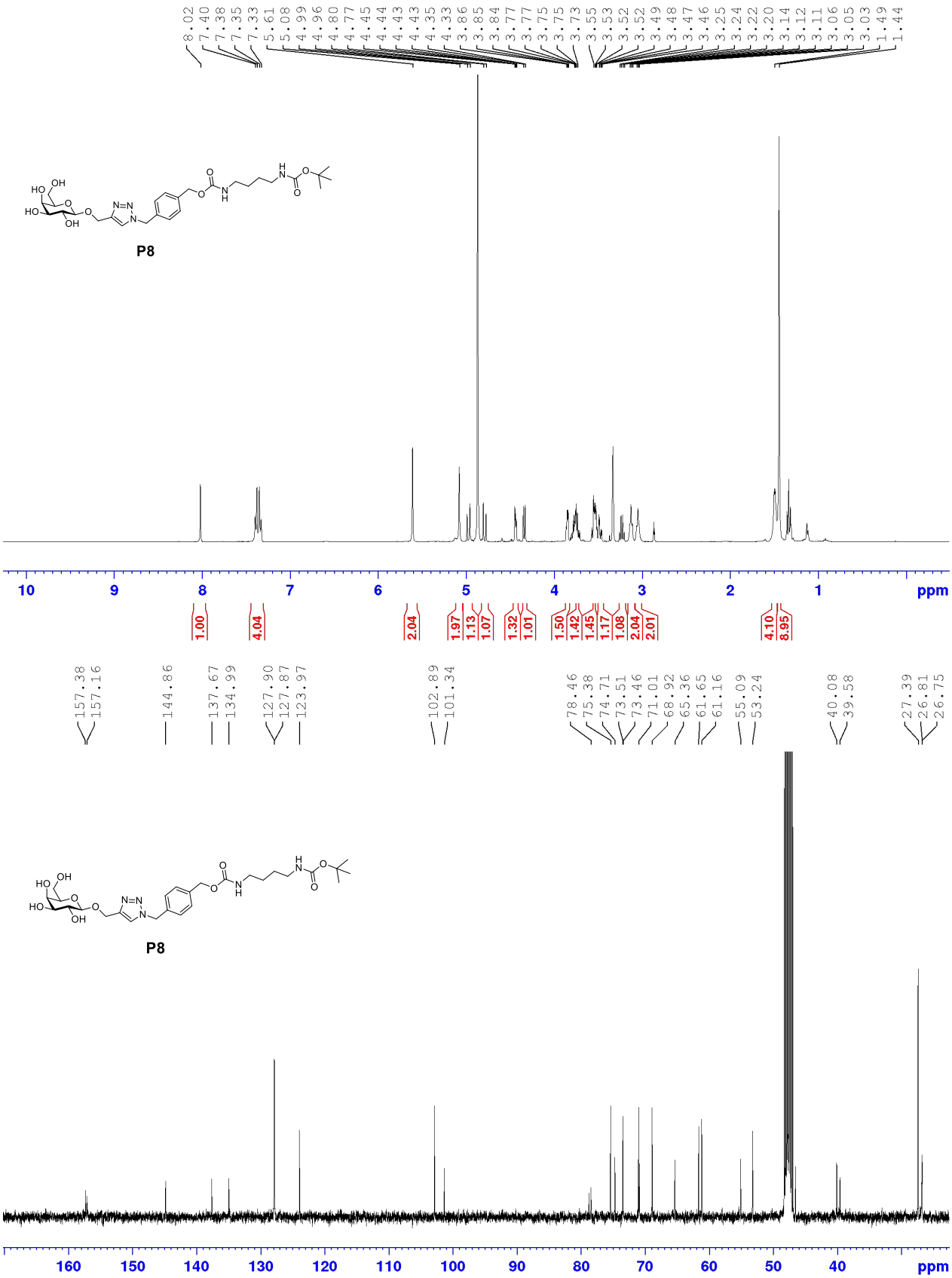
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ppm

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