Supporting Information

for

Chemical Synthesis of GPI Glycan-Peptide Conjugates by Traceless Staudinger Ligation

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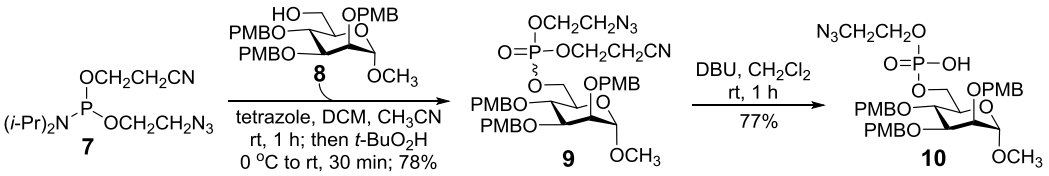
I. Experimental

General methods. Commercial chemicals and materials were used as received without additional purification unless noted otherwise. Molecular sieves 4Å were flame-dried under vacuum and then cooled to rt under an N_2 atmosphere immediately before use. TLC was carried out on Silica Gel 60Å F254 plates with detection by a UV detector and/or by charring with 10% H_2SO_4 in EtOH (v/v). Mass spectrometry (MS) was recorded either on a high resolution ESI-TOF machine or on a normal resolution MALD-TOF machine. NMR spectra were recorded on a 400, 500 or 600 MHz machine with chemical shifts reported in ppm (δ) downfield from internal tetramethylsilane (TMS) or DHO references and with signals described as s (singlet), d (doublet), t (triplet) or m (multiplet) and coupling constants reported in Hz.

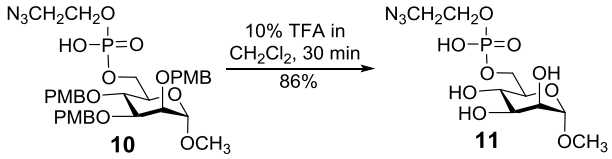
Methyl 6-*O*-[(2-azidoethoxy)-phosphono]-2,3,4-tri-*O*-(*p*-methoxybenzyl)-α-D-manno-pyranoside (10).



To a solution of **8** (560 mg, 1.01 mmol) and freshly prepared **7** (870 mg, 3.03 mmol) in CH_2Cl_2/CH_3CN (v/v, 2/1, 10 mL) was added slowly an acetonitrile solution of tetrazole (~0.45 M, 11.22 mL, 5.05 mmol) at rt under an N_2 atmosphere. After the mixture was stirred at rt for 1 h, it was cooled to 0 °C and mixed with *tert*-butyl hydroperoxide (5.5 M solution in decane, 0.92 mL, 5.05 mmol). The mixture was stirred at rt for another 30 min and quench with aqueous $Na_2S_2O_3$. The aqueous layer was extracted with CH_2Cl_2 three times. The combined organic layer was washed with aqueous $NaHCO_3$ and brine, dried, and concentrated under vacuum. The residue was purified by column chromatography (EtOAc/hexane 2:1) to give **9** (595 mg, 78% yield) as a diastereomeric mixture (R/S 1:1). It was dissolved in CH_2Cl_2 (10 mL). After adding DBU (2 drops) and stirring at rt for 1 h, the solvent was evaporated, and the residue was purified by flash silica gel column chromatography (CH_3OH/CH_2Cl_2 1:10) to give **10** (426.6 mg, 77%) as colorless syrup. R_f = 0.45 (CH_3OH/CH_2Cl_2 1:4); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.17 – 7.13 (m, 6H, Ph), 6.78 – 6.74 (m, 6H, Ph), 4.72 (d, J = 10.5 Hz, 1H, Ph-CH_2), 4.55 (d, J = 12 Hz, 1H, Ph-CH_2), 4.53 (d, J = 1.5 Hz, 1H, H-1), 4.50 (d, J = 10.5 Hz, 1H, Ph-CH_2), 4.48 (d, J = 10.0 Hz, 1H, Ph-CH_2), 4.44 (d, J = 12.0 Hz, 1H, Ph-CH_2), 4.41 (d, J = 11.5 Hz, 1H, Ph-CH_2), 4.08 (dd, J =

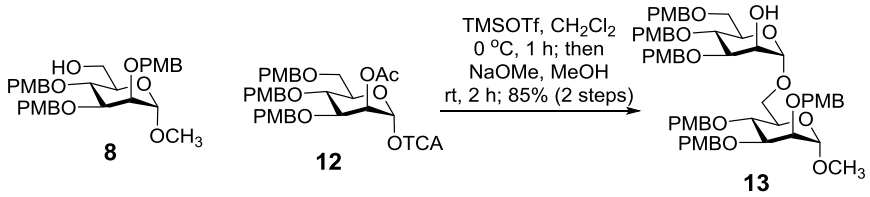
12.0, 5.0 Hz, 1H, H-6a), 4.02 (dd, *J* = 11.0, 5.0 Hz, 1H, H-6b), 3.92 – 3.88 (m, 2H, -OC*H*$_2$CH$_2$), 3.72 (s, 3H, Ph-OC*H*$_3$), 3.71 (s, 3H, Ph-OC*H*$_3$), 3.70 (s, 3H, Ph-OC*H*$_3$), 3.66 – 3.64 (m, 3H, H-2, H-3, H-4), 3.58 – 3.56 (m, 1H, H-5), 3.26 – 3.24 (m, 2H, -CH$_2$C*H*$_2$-N$_3$), 3.19 (s, 3H, -OCH$_3$). ^{13}C NMR (125 MHz, CDCl$_3$) δ: 159.25, 159.13, 159.08, 130.53, 130.48, 130.06, 129.62, 129.50, 129.24, 113.71, 99.13(C-1), 79.67, 74.62, 74.27, 74.14, 72.49, 71.78, 71.28, 64.80, 64.17, 55.20, 54.62, 51.29. ^{31}P NMR (160 MHz, CDCl$_3$) δ: -2.49. HR ESI-TOF MS (m/z): calcd for $C_{33}H_{41}N_3O_{12}P$ [M - H]$^-$, 702.2433; found, 702.2446.

Methyl 6-*O*-[(2-azidoethoxy)-phosphono]-α-D-mannopyranoside (11).



To a solution of **10** (330 mg, 0.47 mmol) in CH_2Cl_2 (4.5 mL) was added TFA (0.5 mL). After stirring at rt for 30 min, the reaction mixture was co-evaporated with toluene three times. The residue was subjected to silica gel column chromatography (CH_3OH/EtOAc 1:1) to produce **11** (138 mg, 86% yield) as colorless syrup. R_f = 0.42 (CH_3OH/EtOAc 1:1); ^{1}H NMR (600 MHz, CD_3OD) δ: 4.61 (d, *J* = 1.8 Hz, 1H, H-1), 4.14 – 4.06 (m, 2H, H-6a, H-6b), 4.03 – 4.00 (m, 2H, -OC*H*$_2$CH$_2$-), 3.76 (dd, *J* = 3.6, 1.8 Hz, 1H, H-2), 3.70 (dd, *J* = 9.6, 5.4 Hz, 1H, H-4), 3.66 (dd, *J* = 9.6, 3.6 Hz, 1H, H-3), 3.57 – 3.55 (m, 1H, H-5), 3.46 (t, *J* = 5.4 Hz, 2H, -CH$_2$C*H*$_2$N$_3$), 3.35 (s, 3H, -OCH$_3$). ^{13}C NMR (150 MHz, CD_3OD) δ: 102.79 (C-1), 73.44 (C-5), 72.47 (C-3), 72.05 (C-2), 68.17 (C-4), 66.08 (C-6), 65.42 (-O*C*H$_2$CH$_2$-), 55.26 (-OCH$_3$), 52.60 (-CH$_2$*C*H$_2$N$_3$). ^{31}P NMR (160 MHz, CD_3OD_3) δ: 0.61. HR ESI-TOF MS (m/z): calcd for $C_9H_{17}N_3O_9P$ [M - H]$^-$, 342.0708; found, 342.0717.

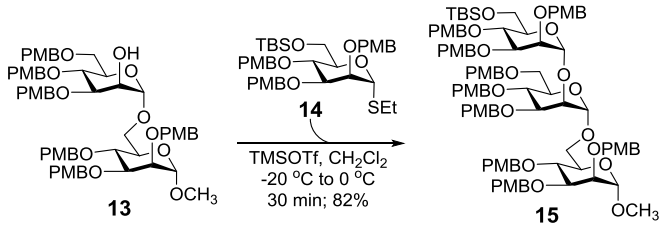
Methyl 3,4,6-tri-*O*-(*p*-methoxybenzyl)-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-(*p*-methoxybenzyl)-α-D-mannopyranoside (13).



To a stirred mixture of **8** (118 mg, 0.213 mmol), **12** (196 mg, 0.319 mmol) and molecular sieves 4Å in anhydrous CH_2Cl_2 (5 mL) was added slowly TMSOTf (5.8 μL, 0.032 mmol) under an N_2 atmosphere at 0 °C. After the mixture was stirred at 0 °C for 1 h, it was neutralized with Et_3N,

filtered, and concentrated under vacuum. The residue was purified by silica gel column chromatography (EtOAc/hexane 1:2) to give the disaccharide as colorless syrup. This product was then dissolved in CH_3OH (5 mL) and mixed with CH_3ONa (11.5 mg, 0.213 mmol) at rt. After the mixture was stirred at rt for 2 h, it was concentrated under vacuum. The residue was purified by silica gel column chromatography (EtOAc/hexane 3:1) to afford **13** (195 mg, 85% for two steps) as colorless syrup. R_f = 0.30 (EtOAc/hexane 3:1); ^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.30 – 7.24 (m, 8H, Ph), 7.16 (d, J = 8.4 Hz, 2H, Ph), 7.05 (d, J = 8.8 Hz, 2H, Ph), 6.87 – 6.78 (m, 12H, Ph), 5.05 (d, J = 1.2 Hz, 1H, H-1$^{Man\text{-}II}$), 4.82 (d, J = 10.8 Hz, 1H, Ph-CH_2), 4.72 (d, J = 10.2 Hz, 1H, Ph-CH_2), 4.67 (d, J = 12.0 Hz, 1H, Ph-CH_2), 4.66 (d, J = 1.2 Hz, 1H, H-1$^{Man\text{-}I}$), 4.63 – 4.48 (m, 6H, Ph-CH_2), 4.42 (d, J = 10.2 Hz, 1H, Ph-CH_2), 4.41 (d, J = 12.0 Hz, 1H, Ph-CH_2), 4.37 (d, J = 10.2 Hz, 1H, Ph-CH_2), 4.08 (dd, J = 3.0, 4.8 Hz, 1H), 3.88 – 3.70 (m, 8H), 3.81 (s, 3H, Ph-OCH_3), 3.79 (s, 3H, Ph-OCH_3), 3.77 (s, 3H, Ph-OCH_3), 3.76 (s, 3H, Ph-OCH_3), 3.75 (s, 3H, Ph-OCH_3), 3.72 (s, 3H, Ph-OCH_3), 3.67 (dd, J = 10.8, 4.2 Hz, 1H), 3.62 (dd, J = 8.4, 4.8 Hz, 1H), 3.59 (dd, J = 10.8, 1.8 Hz, 1H), 3.25 (s, 3H, -OCH_3), 2.35 (d, J = 3.0 Hz, 1H, -OH). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 159.43 (Ph-OCH_3), 159.33 (Ph-OCH_3), 159.26 (Ph-OCH_3), 159.23 (3C, Ph-OCH_3), 130.86, 130.82, 130.53, 130.11, 129.78, 129.70, 129.65, 129.53, 129.40, 113.99, 113.88, 113.86, 113.81, 113.76, 99.78(C-1$^{Man\text{-}II}$), 98.88(C-1$^{Man\text{-}I}$), 80.07, 79.18, 74.84, 74.77, 74.49, 74.45, 74.03, 73.08, 72.44, 71.83, 71.50, 71.14, 68.56, 68.16, 66.48, 55.41 (Ph-OCH_3), 55.39 (Ph-OCH_3), 55.37 (2C, Ph-OCH_3), 55.34 (Ph-OCH_3), 55.31 (Ph-OCH_3), 54.75 (-OCH_3). HR ESI-TOF MS (m/z): calcd for $C_{61}H_{72}O_{17}Na$ $[M + Na]^+$, 1099.4667; found, 1099.4663.

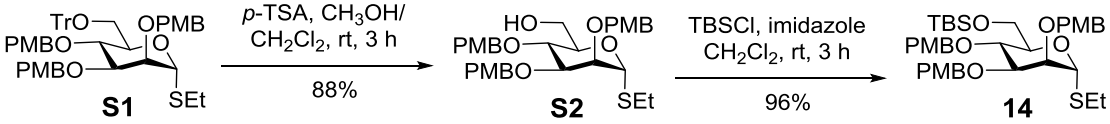
Methyl 6-*O*-*tert*-butyldimethylsilyl-2,3,4-tri-*O*-(*p*-methoxybenzyl)-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-(*p*-methoxybenzyl)-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-(*p*-methoxybenzyl)-α-D-mannopyranoside (15).



After the mixture of **13** (688 mg, 0.64 mmol), **14** (537 mg, 0.77 mmol), and molecular sieves 4Å in anhydrous diethyl ether (10 mL) was stirred at rt for 10 min, it was cooled to -20 °C, which was followed by the addition of NIS (346 mg, 1.54 mmol) and AgOTf (19.8 mg, 0.077 mmol). The mixture was stirred at 0 °C for 30 min and then quenched with Et_3N, filtered, and

concentrated under vacuum. The residue was purified by silica gel column chromatography (EtOAc/hexane 1:2) to produce **15** (899mg, 82%) as colorless syrup. R_f = 0.60 (EtOAc/hexane 1:1); ^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.30 (d, J = 8.4 Hz, 2H, Ph), 7.26 – 7.18 (m, 12H, Ph), 7.15 (d, J = 8.4 Hz, 2H, Ph), 7.11 (d, J = 8.4 Hz, 2H, Ph), 6.86 – 6.73 (m, 18H, Ph), 5.24 (s, 1H, H-1$^{Man\text{-}II}$), 4.86 (s, 1H, H-1$^{Man\text{-}III}$), 4.83 (d, J = 10.8 Hz, 2H, Ph-CH_2), 4.75 (d, J = 10.8 Hz, 1H, Ph-CH_2), 4.68 (s, 1H, H-1$^{Man\text{-}I}$), 4.67 (d, J = 12.0 Hz, 1H, Ph-CH_2), 4.63 (d, J = 12.0 Hz, 1H, Ph-CH_2), 4.60 (d, J = 12.0 Hz, 1H, Ph-CH_2), 4.56 (d, J = 10.8 Hz, 1H, Ph-CH_2), 4.51 – 4.45 (m, 4H, Ph-CH_2), 4.44 – 4.35 (m, 7H, Ph-CH_2), 4.15 (d, J = 2.4 Hz, 1H), 3.96 – 3.85 (m, 5H), 3.83 – 3.59 (m, 11H), 3.81 (s, 3H, Ph-OCH_3), 3.80 (s, 3H, Ph-OCH_3), 3.78 (s, 3H, Ph-OCH_3), 3.76 (s, 3H, Ph-OCH_3), 3.74 (s, 6H, Ph-OCH_3 x 2), 3.73 (s, 3H, Ph-OCH_3), 3.68 (s, 3H, Ph-OCH_3), 3.66 (s, 3H, Ph-OCH_3), 3.57 (d, J = 10.8 Hz, 1H), 3.25 (s, 3H, -OCH_3), 0.90 (s, 9H, -*t*Bu), 0.08 (s, 3H, -SiCH_3), 0.07 (s, 3H, -SiCH_3). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 159.30 (Ph-OCH_3), 159.23 (2C, Ph-OCH_3), 159.18 (Ph-OCH_3), 159.15 (Ph-OCH_3), 159.14 (Ph-OCH_3), 159.08 (Ph-OCH_3), 159.03 (2C, Ph-OCH_3), 131.42, 131.13, 131.04, 130.89, 130.78, 130.47, 129.76, 129.57, 129.43, 129.37, 129.22, 113.89, 113.85, 113.81, 113.79, 113.74, 113.73, 113.71, 113.59, 99.33(C-1$^{Man\text{-}II}$), 98.88(C-1$^{Man\text{-}III}$), 98.72(C-1$^{Man\text{-}I}$), 80.28, 79.59, 74.72, 74.60, 74.57, 74.50, 74.27, 73.67, 73.07, 72.96, 72.32, 72.05, 71.84, 71.79, 71.54, 71.42, 71.08, 69.07, 66.69, 62.87, 60.53, 55.39 (2C, Ph-O*C*H$_3$), 55.36 (Ph-O*C*H$_3$), 55.32 (3C, Ph-O*C*H$_3$), 55.29 (Ph-O*C*H$_3$), 55.25 (Ph-O*C*H$_3$), 55.18 (Ph-O*C*H$_3$), 54.64 (-OCH$_3$), 26.14, 18.51, -4.83, -5.06. HR ESI-TOF MS (m/z): calcd for $C_{97}H_{120}O_{25}SiNa$ $[M + Na]^+$, 1735.7786; found, 1735.7780.

Ethyl 6-*O*-*tert*-butyldimethyl- silyl-2,3,4-tri-*O*-(*p*-methoxybenzyl)-1-thio-α-D-mannopyranoside (14)

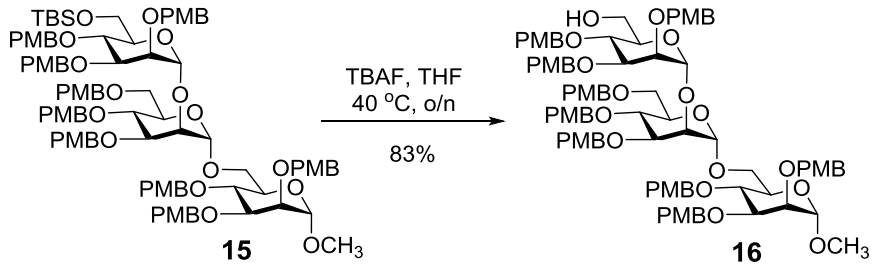


To a solution of **S1** (836 mg, 1.0 mmol) in CH_3OH/CH_2Cl_2 (1/1, v/v, 10 mL) was added *p*-TSA (19 mg, 0.1 mmol). After stirring at rt for 3 h, the reaction was quenched with Et_3N. The solvent was removed under vacuum, and the residue was purified by silica gel column chromatography (EtOAc/hexane 1:2) to give **S2** (513.9 mg, 88%) as colorless syrup. R_f = 0.45 (EtOAc/hexane 1:1); ^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.28 – 7.22 (m, 6H, Ph), 6.86 – 6.84 (m, 6H, Ph), 5.24 (s, 1H, H-1), 4.85 (d, J = 10.8 Hz, 1H, Ph-CH_2), 4.64 (d, J = 12.6 Hz, 1H, Ph-CH_2), 4.61 (d, J = 12.0 Hz,

1H, Ph-CH_2), 4.55 – 4.48 (m, 3H, Ph-CH_2), 3.95 (dd, J = 9.6, 3.6 Hz, 1H, H-3), 3.92 (t, J = 9.6 Hz, 1H, H-4), 3.80 – 3.73 (m, 4H, H-2, H-5, H-6a, H-6b), 3.80 (s, 3H, Ph-OCH_3), 3.79 (s, 3H, Ph-OCH_3), 3.78 (s, 3H, Ph-OCH_3), 2.58 – 2.50 (m, 2H, -SCH_2CH$_3$), 1.94 (dd, J = 7.2, 6.0 Hz, 1H, -OH), 1.22 (t, J = 7.2 Hz, 1H, -SCH$_2$CH_3). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 159.41 (Ph-OCH$_3$), 159.37 (Ph-OCH$_3$), 159.32 (Ph-OCH$_3$), 130.74, 130.50, 130.24, 129.80, 129.65, 129.50, 113.94, 82.26(C-1), 80.14, 76.16, 74.96, 74.89, 72.48, 72.07, 71.87, 62.57, 55.40 (3C, Ph-OCH$_3$), 25.41 (-SCH$_2$CH$_3$), 15.01 (-SCH$_2$$CH_3$). HR ESI-TOF MS (m/z): calcd for $C_{32}H_{40}O_8SNa$ $[M + Na]^+$, 607.2342; found, 607.2332.

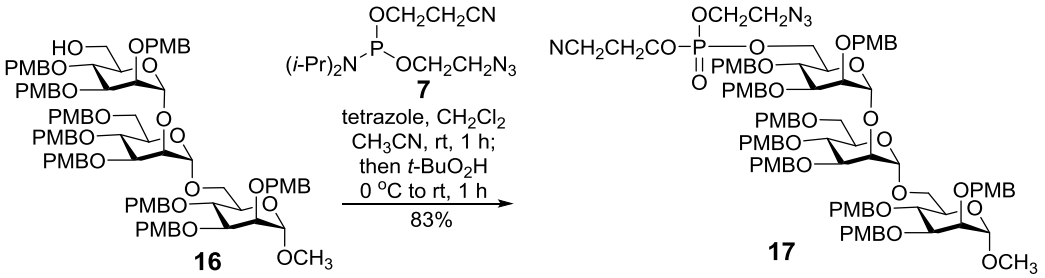
To a solution of **S2** (368 mg, 0.63 mmol) in CH_2Cl_2 (5 mL) was added imidazole (85.6 mg, 1.26 mmol). After stirring at rt for 10 min, TBSCl (142.7 mg, 0.95 mmol) was added, and the mixture was stirred at rt for another 3 h. The mixture was concentrated under vacuum, and the residue was purified by silica gel column chromatography (EtOAc/hexane 1:10) to give **14** (422.7 mg, 96%) as colorless syrup. R_f = 0.50 (EtOAc/hexane 1:5); ^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.31 – 7.24 (m, 6H, Ph), 6.87 (d, J = 8.4 Hz, 6H, Ph), 5.31 (s, 1H, H-1), 4.85 (d, J = 10.8 Hz, 1H, Ph-CH_2), 4.63 (d, J = 12.6 Hz, 1H, Ph-CH_2), 4.61 (d, J = 12.0 Hz, 1H, Ph-CH_2), 4.55 – 4.49 (m, 3H, Ph-CH_2), 3.94 (dd, J = 9.6, 3.6 Hz, 1H, H-3), 3.88 (t, J = 9.6 Hz, 1H, H-4), 3.83 – 3.80 (m, 3H, H-5, H-6a, H-6b), 3.80 (s, 6H, Ph-OCH_3 x 2), 3.79 (s, 3H, Ph-OCH_3), 3.76 (dd, J = 3.0, 1.8 Hz, 1H, H-2), 2.64 – 2.50 (m, 2H, -SCH_2CH$_3$), 1.25 (t, J = 7.8 Hz, 1H, -SCH$_2$CH_3), 0.90 (s, 9H, -tBu), 0.07 (s, 3H, -SiCH$_3$), 0.06 (s, 3H, -SiCH$_3$). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 159.23 (Ph-OCH$_3$), 131.06, 130.62, 130.36, 129.65, 129.49, 113.77, 81.36(C-1), 80.07, 76.15, 74.86, 74.78, 73.50, 71.75, 71.50, 62.86, 55.33 (3C, Ph-OCH$_3$), 26.00 (-tBu), 25.02 (-SCH$_2$CH$_3$), 18.37 (-SiC(CH$_3$)$_3$), 14.98 (-SCH$_2$$CH_3$), -5.06 (-Si$CH_3$), -5.22 (-Si$CH_3$). HR ESI-TOF MS (m/z): calcd for $C_{38}H_{54}O_8SSiNa$ $[M + Na]^+$, 721.3206; found, 721.3231.

Methyl 2,3,4-tri-*O*-(*p*-methoxybenzyl)-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-(*p*-methoxybenzyl)-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-(*p*-methoxybenzyl)-α-D-mannopyranoside (16).



To a solution of **15** (678 mg, 0.395 mmol) in THF (4.0 mL) was added TBAF (0.60 mL, 1.0 M in THF, 0.60 mmol). The mixture was stirred at 40 °C overnight. After the solvent was removed under vacuum, the residue was purified by silca gel column chromatography (EtOAc/hexane 1:1) to afford **16** as colorless syrup (545 mg, 86%). R_f = 0.50 (EtOAc/hexane 2:1); ^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.30 – 7.11 (m, 18H, Ph), 6.87 – 6.76 (m, 18H, Ph), 5.15 (d, J = 1.2 Hz, 1H, H-1$^{Man\text{-}II}$), 5.01 (d, J = 1.8 Hz, 1H, H-1$^{Man\text{-}III}$), 4.83 (d, J = 10.8 Hz, 1H, Ph-CH_2), 4.81 (d, J = 10.8 Hz, 1H, Ph-CH_2), 4.77 (d, J = 10.2 Hz, 1H, Ph-CH_2), 4.69 (d, J = 1.2 Hz, 1H, H-1$^{Man\text{-}I}$), 4.66 (d, J = 13.2 Hz, 2H, Ph-CH_2), 4.62 (d, J = 12.0 Hz, 1H, Ph-CH_2), 4.54 – 4.41 (m, 12H, Ph-CH_2), 4.12 (t, J = 1.8 Hz, 1H), 3.90 – 3.58 (m, 17H), 3.81 (s, 3H, Ph-OCH_3), 3.80 (s, 3H, Ph-OCH_3), 3.79 (s, 3H, Ph-OCH_3), 3.76 (s, 3H, Ph-OCH_3), 3.74 (s, 3H, Ph-OCH_3), 3.73 (s, 6H, Ph-OCH_3 x 2), 3.67 (s, 6H, Ph-OCH_3 x 2), 3.26 (s, 3H, -OCH_3). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 159.30 (Ph-OCH_3), 159.21 (Ph-OCH_3), 159.17 (Ph-OCH_3), 159.11 (Ph-OCH_3), 159.03 (Ph-OCH_3), 130.89, 130.85, 130.83, 130.80, 130.77, 130.54, 130.39, 130.31, 129.63, 129.52, 129.34, 129.19, 113.91, 113.85, 113.82, 113.74, 113.72, 113.68, 99.71(C-1$^{Man\text{-}II}$), 99.61(C-1$^{Man\text{-}III}$), 99.00(C-1$^{Man\text{-}I}$), 80.06, 79.65, 78.84, 74.96, 74.77, 74.65, 74.56, 74.23, 74.18, 74.10, 72.97, 72.85, 72.30, 72.06, 71.94, 71.83, 71.60, 71.33, 68.99, 66.46, 62.78, 55.35 (Ph-OCH$_3$), 55.34 (2C, Ph-OCH$_3$), 55.30 (Ph-OCH$_3$), 55.28 (2C, Ph-OCH$_3$), 55.26 (Ph-OCH$_3$), 55.20 (Ph-OCH$_3$), 55.16 (Ph-OCH$_3$), 54.78 (-OCH_3). HR ESI-TOF MS (m/z): calcd for $C_{91}H_{106}O_{25}Na$ $[M + Na]^+$, 1621.6921; found, 1621.6911.

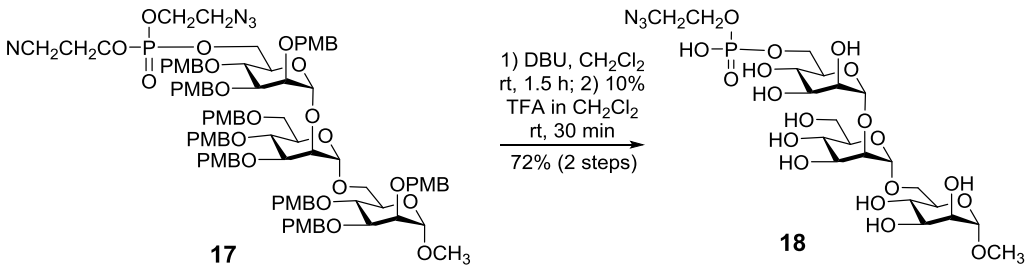
Methyl 6-*O*-[(2-azidoethoxy)-phosphono]-2,3,4-tri-*O*-(*p*-methoxybenzyl)-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-(*p*-methoxybenzyl)-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-(*p*-methoxybenzyl)-α-D-mannopyranoside (17).



To a solution of **16** (500 mg, 0.313 mmol) and freshly prepared **7** (269mg, 0.938 mmol) in anhydrous CH_2Cl_2/CH_3CN (2:1, v/v, 8 mL) was added slowly an acetonitrile solution of tetrazole (~0.45 M, 6.96 mL, 3.13 mmol) under an N_2 atmosphere. After the mixture was stirred at rt for 1 h, it was cooled to 0 °C, and then *tert*-butyl hydroperoxide (5.5 M solution in decane, 0.57 mL,

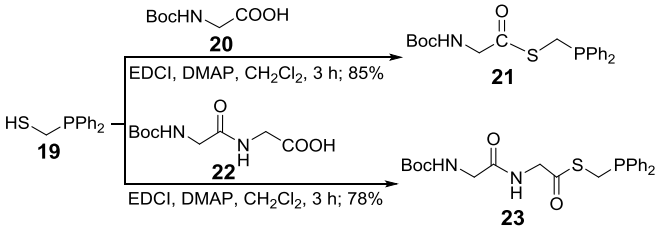
3.13 mmol) was added. The mixture was stirred at rt for 1 h and quenched with aqueous $Na_2S_2O_3$. The aqueous layer was extracted with CH_2Cl_2 three times. The combined organic layer was washed with aqueous $NaHCO_3$ and brine, dried, and concentrated under vacuum. The residue was purified by column chromatography (EtOAc/hexane 2:1) to give the phosphorylated product as a diastereomeric mixture (R/S ≈ 1/1). This product was then dissolved in CH_2Cl_2 (8 mL) and mixed with DBU (2 drops). After stirring at rt for 1.5 h, the solution was concentrated, and the residue was subjected to silica gel column chromatography (CH_3OH/CH_2Cl_2 1:10) to produce **17** (393.5 mg, 72% for 2 steps) as colorless syrup. R_f = 0.55 (CH_3OH/CH_2Cl_2 1:5); ^{1}H NMR (600 MHz, CD_3OD) δ: 7.35 (d, J = 8.4 Hz, 2H, Ph), 7.25 – 7.11 (m, 12H, Ph), 7.06 (d, J = 8.4 Hz, 2H, Ph), 7.02 (d, J = 8.4 Hz, 2H, Ph), 6.86 – 6.75 (m, 14H, Ph), 6.71 (d, J = 8.4 Hz, 2H, Ph), 6.68 (d, J = 8.4 Hz, 2H, Ph), 5.09 (s, 1H, H-1$^{\text{Man-II}}$), 4.92 (s, 1H, H-1$^{\text{Man-III}}$), 4.82 (s, 1H, H-1$^{\text{Man-I}}$), 4.77 (d, J = 10.8 Hz, 1H, Ph-CH_2), 4.71 – 4.64 (m, 4H, Ph-CH_2), 4.61 (d, J = 10.8 Hz, 1H, Ph-CH_2), 4.51 (d, J = 10.8 Hz, 1H, Ph-CH_2), 4.45 – 4.32 (m, 10H, Ph-CH_2), 4.27 (d, J = 10.8 Hz, 1H, Ph-CH_2), 4.20 – 4.15 (m, 2H), 4.02 – 3.92 (m, 3H), 3.89 – 3.40 (m, 17H), 3.75 (s, 3H, Ph-OCH_3), 3.73 (s, 3H, Ph-OCH_3), 3.71 (s, 3H, Ph-OCH_3), 3.69 (s, 3H, Ph-OCH_3), 3.68 (s, 6H, Ph-OCH_3 x 2), 3.62 (s, 3H, Ph-OCH_3), 3.61 (s, 3H, Ph-OCH_3), 3.57 (s, 3H, Ph-OCH_3), 3.32 (s, 3H, -OCH_3). ^{13}C NMR (150 MHz, CD_3OD) δ: 160.84 (Ph-OCH_3), 160.80 (Ph-OCH_3), 160.76 (Ph-OCH_3), 160.67 (Ph-OCH_3), 160.65 (Ph-OCH_3), 160.58 (Ph-OCH_3), 160.52 (Ph-OCH_3), 132.43, 132.08, 132.01, 131.97, 131.91, 131.75, 131.65, 131.38, 131.27, 130.97, 130.90, 130.81, 130.69, 130.66, 130.49, 130.43, 114.99, 114.81, 114.76, 114.71, 114.60, 114.57, 101.02(C-1$^{\text{Man-II}}$), 100.64(C-1$^{\text{Man-III}}$), 100.19(C-1$^{\text{Man-I}}$), 81.18, 80.32, 79.38, 76.05, 75.57, 75.34, 75.19, 75.12, 73.86, 73.37, 73.31, 73.16, 72.83, 72.72, 72.56, 72.46, 71.61, 69.86, 67.91, 65.47, 63.51, 55.70, 55.65, 55.58, 55.45, 53.58, 52.59. MALDI-TOF MS (m/z): calcd for $C_{93}H_{109}N_3O_{28}PNa$ $[M + 2Na - H]^+$, 1792.673; found, 1792.610.

Methyl 6-*O*-[(2-azidoethoxy)-phosphono]-α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→6)-α-D-mannopyranoside (18).



To a solution of **17** (286 mg, 0.164 mmol) in CH_2Cl_2 (3.6mL) was added TFA (0.4 mL). After stirring at rt for 30 min, the reaction mixture was co-evaporated with toluene three times. The residue was subjected to flash silica gel column chromatography (CH_3OH/EtOAc 1:1) to afford **18** (96.3 mg, 88% yield) as a white foamy solid. R_f = 0.45 (CH_3OH/EtOAc 2:1); ^{1}H NMR (600 MHz, D_2O) δ: 5.13 (d, J = 1.2 Hz, 1H, H-1$^{Man\text{-}II}$), 5.02 (d, J = 1.2 Hz, 1H, H-1$^{Man\text{-}III}$), 4.74 (d, J = 1.2 Hz, 1H, H-1$^{Man\text{-}I}$), 4.15 (ddd, J = 11.4, 5.4, 1.8 Hz, 1H), 4.09 (t, J = 5.4 Hz, 1H), 4.07 – 4.03 (m, 3H, H-2$^{Man\text{-}III}$, -O*CH*$_2$CH$_2$-), 3.99 (dd, J = 3.6, 1.2 Hz, 1H, H-2$^{Man\text{-}II}$), 3.96 – 3.94 (m, 2H), 3.92 (dd, J = 3.6, 1.2 Hz, 1H, H-2$^{Man\text{-}I}$), 3.82 (dd, J = 9.6, 1.2 Hz, 1H), 3.76 – 3.68 (m, 7H), 3.52 (t, J = 4.8 Hz, 2H, -CH$_2$*CH*$_2$N$_3$), 3.39 (s, 3H, -OCH$_3$). ^{13}C NMR (150 MHz, D_2O) δ: 102.33 (C-1$^{Man\text{-}II}$), 100.87 (C-1$^{Man\text{-}III}$), 97.81 (C-1$^{Man\text{-}I}$), 78.87, 72.64, 71.93, 70.62, 70.11, 69.81, 66.76, 66.54, 66.21, 65.75, 64.63, 60.79, 54.69 (-OCH$_3$), 50.89 (-CH$_2$*CH*$_2$N$_3$). ^{31}P NMR (160 MHz, D_2O) δ: 0.30. HR ESI-TOF MS (m/z): calcd for $C_{21}H_{37}N_3O_{19}P$ [M - H]$^-$, 666.1764; found, 666.1764.

General procedure for the preparation of peptide phosphinothioesters.



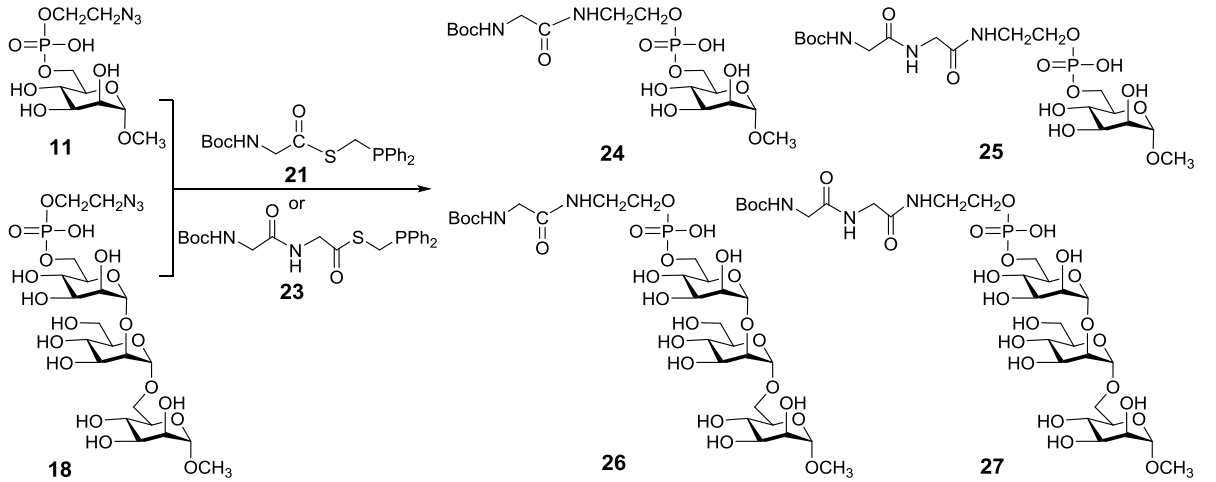
To a stirred solution of freshly prepared **19** (232.3 mg, 1.0 mmol) and amino acid **20** (175.2 mg, 1.0 mmol) or peptide **21** (232.2 mg, 1.0 mmol) in anhydrous CH_2Cl_2 (10 mL) were added EDCI (465.7 mg, 3.0 mmol) and DMAP (24.4 mg, 0.2 mmol). After stirring at rt for 3 h, the mixture was diluted with CH_2Cl_2, washed with water and brine, dried, and concentrated under vacuum. The residue was purified by silica gel column chromatography to produce **21** (350.2 mg, 85%) and **23** (348.7 mg, 78%).

21: a white solid. R_f = 0.65 (EtOAc/hexane 1:3); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.45 – 7.34 (m, 10H, Ph), 5.16 (s, 1H, -*NH*-), 3.99 (d, J = 6.4 Hz, 2H, -NH*CH*$_2$CO-), 3.52 (d, J = 3.6 Hz, 2H, -S*CH*$_2$PPh$_2$), 1.43 (s, 9H, -*t*Bu). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 197.55 (-*C*OS-), 155.52 (-O*C*ONH-), 136.76, 132.69, 129.24, 128.60, 80.42 (-O*t*Bu), 50.25 (-NH*CH*$_2$CO-), 28.36 (-*t*Bu), 25.23 (-S*CH*$_2$PPh$_2$). HR ESI-TOF MS (m/z): calcd for $C_{20}H_{24}NO_3SPNa$ [M + Na]$^+$, 412.1112; found, 412.1116.

23: a white solid. R_f = 0.35 (EtOAc/hexane 1:1); ^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.42 – 7.35 (m, 10H, Ph), 6.86 (s, 1H, -*NH*-), 5.20 (s, 1H, -*NH*-), 4.17 (d, J = 6.0 Hz, 2H, -BocNH*CH*$_2$CO-), 3.83

(d, J = 4.8 Hz, 2H, -NH*CH*$_2$CO-), 3.52 (d, J = 3.6 Hz, 2H, -S*CH*$_2$PPh$_2$), 1.44 (s, 9H, -*t*Bu). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 195.71 (-*C*OS-), 169.80 (-O*C*ONH-), 156.11 (-O*C*ONHBoc-), 136.50, 132.76, 129.24, 128.58, 80.47 (-O*t*Bu), 48.78 (-BocNH*CH*$_2$CO-), 44.28 (-NH*CH*$_2$CO-), 28.28 (-*t*Bu), 25.47 (-S*CH*$_2$PPh$_2$). HR ESI-TOF MS (m/z): calcd for $C_{22}H_{28}N_2O_4PS$ $[M + H]^+$, 447.1507; found, 447.1503.

General procedure for peptide-GPI ligation.



entry	azide	thioester	temp (^{o}C)	solvent[a]	time (day)	product	yield[b] (%)
1	**11**	**21**	rt	DMF	2	**24**	55
2	**11**	**21**	40	DMF	1	**24**	96
3	**11**	**21**	40	DMF/H_2O	1	**24**	93
4	**11**	**23**	40	DMF	1	**25**	93
5	**11**	**23**	40	DMF/H_2O	1	**25**	92
6	**18**	**21**	40	DMF	1	**26**	93
7	**18**	**21**	40	DMF/H_2O	1	**26**	91
8	**18**	**23**	40	DMF	1	**27**	92
9	**18**	**23**	40	DMF/H_2O	1	**27**	89

An azide, **11** (5.15 mg, 0.015 mmol) or **18** (10.2 mg, 0.015 mmol) and a thioester, **21** (8.8 mg, 0.023 mmol) or **23** (10.1 mg, 0.023 mmol) were dissolved in DMF (0.5 mL) or DMF/H_2O (1/1, v/v, 0.5 mL). After stirring at 40 °C for 1 day, the solution was concentrated under vacuum. The residue was purified by silica gel column chromatography to give **24**, **25**, **26**, and **27**, respectively (their yields are shown in Table 1)

24: a white foamy solid (6.83 mg, 96%, entry 2). R_f = 0.50 (EtOAc/CH_3OH 1:1); ^{1}H NMR (600 MHz, D_2O) δ: 4.74 (s, 1H, H-1), 4.12 (dd, J = 11.4, 5.4 Hz, 1H, H-6a), 4.05 (dd, J = 10.8, 5.4 Hz, 1H, H-6b), 3.94 – 3.91 (m, 3H, -OC*H*$_2$CH$_2$, H-2), 3.77 – 3.69 (m, 5H, -C*H*$_2$NHBoc, H-3, H-4, H-5), 3.46 (s, 2H, -OCH$_2$C*H*$_2$), 3.39 (s, 3H, -OCH_3), 1.42 (s, 9H, -*t*Bu). ^{13}C NMR (150 MHz, D_2O) δ:

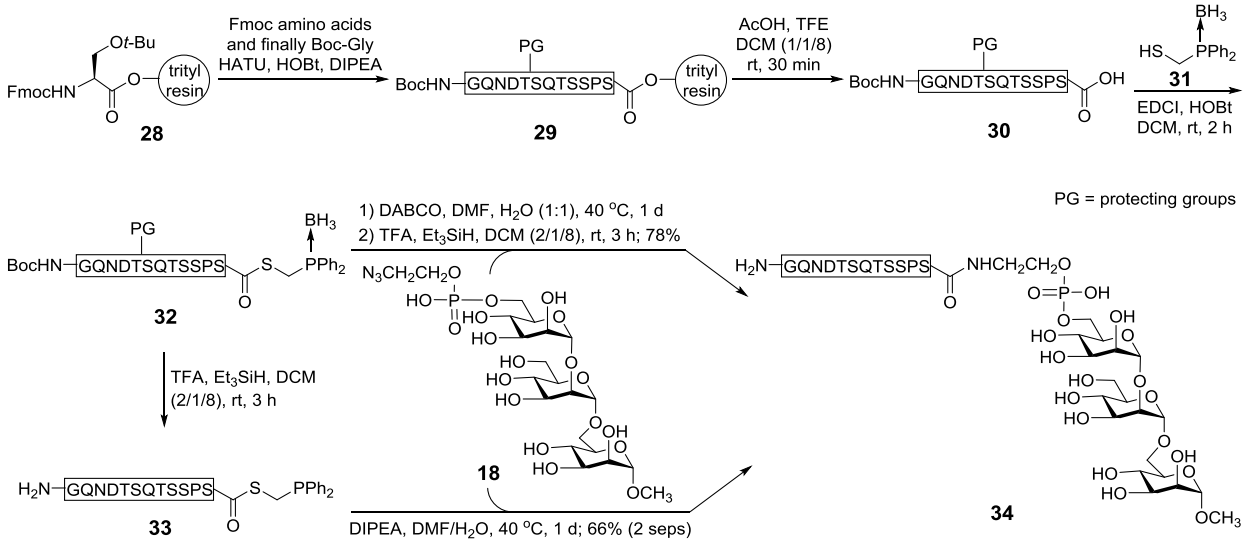
172.56 (-*C*ONH), 158.00 (-*t*BuO*C*ONH-), 100.83 (C-1), 81.56 (-O*t*Bu), 71.31, 70.35, 69.74, 66.15, 64.39, 63.96, 54.68 (-OCH_3), 43.31 (-CO*C*H_2NHBoc), 39.79 (-OCH_2*C*H_2NH), 27.49 (-*t*Bu). ^{31}P NMR (160 MHz, D_2O) δ: 0.29. HR ESI-TOF MS (m/z): calcd for $C_{16}H_{30}N_2O_{12}P$ [M - H]$^-$, 473.1542; found, 473.1521.

25: a white foamy solid (7.33 mg, 92%, entry 5). R_f = 0.40 (EtOAc/CH_3OH 1:1); ^{1}H NMR (600 MHz, D_2O) δ: 4.74 (s, 1H, H-1), 4.12 (dd, J = 11.4, 5.4 Hz, 1H, H-6a), 4.05 (dd, J = 10.8, 5.4 Hz, 1H, H-6b), 3.93 – 3.91 (m, 5H, -OC*H*$_2$CH$_2$, -COC*H*$_2$NH-, H-2), 3.81 (s, 2H, -C*H*$_2$NHBoc), 3.76 (dd, J = 9.0, 3.6 Hz, 1H, H-3), 3.70 – 3.69 (m, 2H, H-4, H-5), 3.46 (t, J = 5.4 Hz, 2H, -OCH_2C*H*$_2$), 3.39 (s, 3H, -OCH_3), 1.42 (s, 9H, -*t*Bu). ^{13}C NMR (150 MHz, D_2O) δ: 173.18 (-*C*ONH), 173.18 (-*C*ONH), 156.94 (-*t*BuO*C*ONH-), 100.81 (C-1), 81.65 (-O*t*Bu), 71.29, 70.35, 69.74, 66.14, 64.37, 63.91, 54.66 (-OCH_3), 43.32 (-CO*C*H_2NHBoc), 42.23 (-CO*C*H_2NH), 39.88 (-OCH_2*C*H_2NH), 27.47 (-*t*Bu). HR ESI-TOF MS (m/z): calcd for $C_{18}H_{33}N_3O_{13}P$ [M - H]$^-$, 530.1756; found, 530.1728.

26: a white foamy solid (11.80 mg, 92%, entry 8). R_f = 0.50 (EtOAc/CH_3OH 1:2); ^{1}H NMR (600 MHz, D_2O) δ: 5.12 (s, 1H, H-1$^{Man\text{-}III}$), 5.02 (s, 1H, H-1$^{Man\text{-}II}$), 4.74 (s, 1H, H-1$^{Man\text{-}I}$), 4.11 – 4.02 (m, 3H), 3.98 – 3.92 (m, 6H), 3.86 – 3.84 (m, 3H), 3.82 (d, J = 11.4 Hz, 1H), 3.77 – 3.64 (m, 9H), 3.48 (t, J = 3.6 Hz, 2H, -OCH_2C*H*$_2$), 3.39 (s, 3H, -OCH_3), 1.43 (s, 9H, -*t*Bu). ^{13}C NMR (150 MHz, D_2O) δ: 172.56 (-*C*ONH), 157.99 (-*t*BuO*C*ONH-), 102.32 (C-1$^{Man\text{-}III}$), 100.88 (C-1$^{Man\text{-}I}$), 97.80 (C-1$^{Man\text{-}II}$), 81.58 (-O*t*Bu), 78.81, 72.65, 71.98, 70.65, 70.12, 69.81, 66.76, 66.54, 66.24, 65.75, 64.65, 64.01, 60.80, 60.03, 54.69 (-OCH_3), 43.34 (-CO*C*H_2NHBoc), 39.88 (-OCH_2*C*H_2NH), 27.53 (-*t*Bu). ^{31}P NMR (160 MHz, D_2O) δ: 0.50. HR ESI-TOF MS (m/z): calcd for $C_{28}H_{50}N_2O_{22}P$ [M - H]$^-$, 797.2598; found, 797.2605.

27: a white foamy solid (6.83 mg, 96%, entry 2). R_f = 0.45 (EtOAc/CH_3OH 1:2); ^{1}H NMR (600 MHz, D_2O) δ: 5.12 (s, 1H, H-1$^{Man\text{-}III}$), 5.02 (s, 1H, H-1$^{Man\text{-}II}$), 4.74 (s, 1H, H-1$^{Man\text{-}I}$), 4.11 – 4.03 (m, 3H), 3.99 – 3.92 (m, 7H), 3.87 – 3.79 (m, 6H), 3.77 – 3.63 (m, 8H), 3.48 (t, J = 5.4 Hz, 2H, -OCH_2C*H*$_2$), 3.39 (s, 3H, -OCH_3), 1.43 (s, 9H, -*t*Bu). ^{13}C NMR (150 MHz, D_2O) δ: 173.16 (-*C*ONH), 171.34 (-*C*ONH), 159.88 (-*t*BuO*C*ONH-), 102.31 (C-1$^{Man\text{-}III}$), 100.88 (C-1$^{Man\text{-}I}$), 97.79 (C-1$^{Man\text{-}II}$), 81.69 (-O*t*Bu), 78.80, 72.64, 72.02, 70.64, 70.12, 69.81, 66.76, 66.54, 66.24, 65.73, 64.57, 63.91, 60.79, 60.03, 54.69 (-OCH_3), 43.36 (-CO*C*H_2NHBoc), 42.28 (-CO*C*H_2NH), 39.93 (-OCH_2*C*H_2NH), 27.52 (-*t*Bu). ^{31}P NMR (160 MHz, D_2O) δ: 0.56. HR ESI-TOF MS (m/z): calcd for $C_{30}H_{53}N_3O_{23}P$ [M - H]$^-$, 854.2813; found, 854.2810.

Synthesis of CD52 peptide phosphinothioesters 32 and 33.



CD52 peptide **30** was prepared by solid-phase synthesis on a peptide synthesizer using standard Fmoc chemistry. The synthesis started from commercial serine-modified 2-chlorotrityl resin **28**, and the completed peptide **30** was released from the resin with 10% AcOH in TFE and DCM (1/1/8 v/v/v). The product was purified by silica gel column chromatography (CH_3OH/CH_2Cl_2 1:5) and characterized with MALDI-TOF MS (calcd for $C_{135}H_{179}N_{15}O_{26}Na$ $[M + Na]^+$, 2449.3; found, 2449.8). Peptide **30** (86 mg, 0.035 mmol) was then dissolved in CH_2Cl_2 (2 mL), and to the solution were added mercaptan **31** (13.08 mg, 0.053 mmol), EDCI (20.16 mg, 0.106 mmol), and HOBt (9.45 mg, 0.07 mmol) at rt. After stirring at rt for 2 h, the mixture was concentrated, and the residue was purified by silica gel column chromatography (CH_3OH/CH_2Cl_2 1:10) to produce **32** (87.5 mg, 93%) as a white solid. MALDI-TOF MS: calcd for $C_{135}H_{179}N_{15}O_{26}Na$ $[M – BH_3 + Na]^+$, 2663.3; found, 2663.5.

To a solution of **32** (87.5 mg, 0.033 mmol) in CH_2Cl_2 (1.6 mL) were added TFA (0.4 mL) and Et_3SiH (0.2 mL). After stirring at rt for 3 h, the mixture was poured into cold ethyl ether (20 mL). The precipitated product **33** was filtered, dried under vacuum, and applied directly to the next step. MALDI-TOF MS: calcd for $C_{58}H_{83}N_{15}O_{23}PS$ $[M – H]^-$, 1420.5; found, 1420.7.

Staudinger ligation between 32 and 18. To a solution of **32** (2.3 mg, 0.87 nmol) and **18** (0.85 mg, 1.30 nmol) in DMF and H_2O (1:1, v/v, 300 μL) was added DABCO (0.3 mg, 2.60 nmol. After stirring at 40 °C for 1 day and MS indication of completed reaction, the mixture was concentrated under vacuum. The residue was dissolved in 20% TEA in Et_3SiH and DCM (2/1/8, v/v/v, 0.5 mL). After stirring at rt for 3 h, 5 mL of cold ether was added to the mixture to precipitate the

reaction product, which was finally purified by reversed-phase HPLC to afford **34** (1.24 mg, 78%) as a white solid. HPLC conditions: C18 column (5 μm, 250 x 4.6 mm), gradient elution with 10~60% CH_3CN in H_2O, 1.5 mL/min flow rate, UV detection at 220 nm, retention time 9.5 min. MALDI-TOF MS: calcd for $C_{66}H_{110}N_{16}O_{42}P$ $[M - H]^-$, 1829.7; found, 1829.9.

Staudinger ligation between 33 and 18. To a solution of **33** (1.5 mg, 1.05 nmol) and **18** (1.1 mg, 1.60 nmol) in DMF and H_2O (1:1, v/v, 0.5 mL) was added DIPEA (1.84 μL, 10.5 nmol). After stirring at 40 °C for 1 day, the mixture was concentrated and the product was purified by HPLC as described above to give **34** (1.24 mg, 66%) as a white solid (identical to the product obtained from the above procedure).

II. MS spectra and HPLC diagrams of CD52 peptide, its phosphinothioesters, and its GPI conjugate

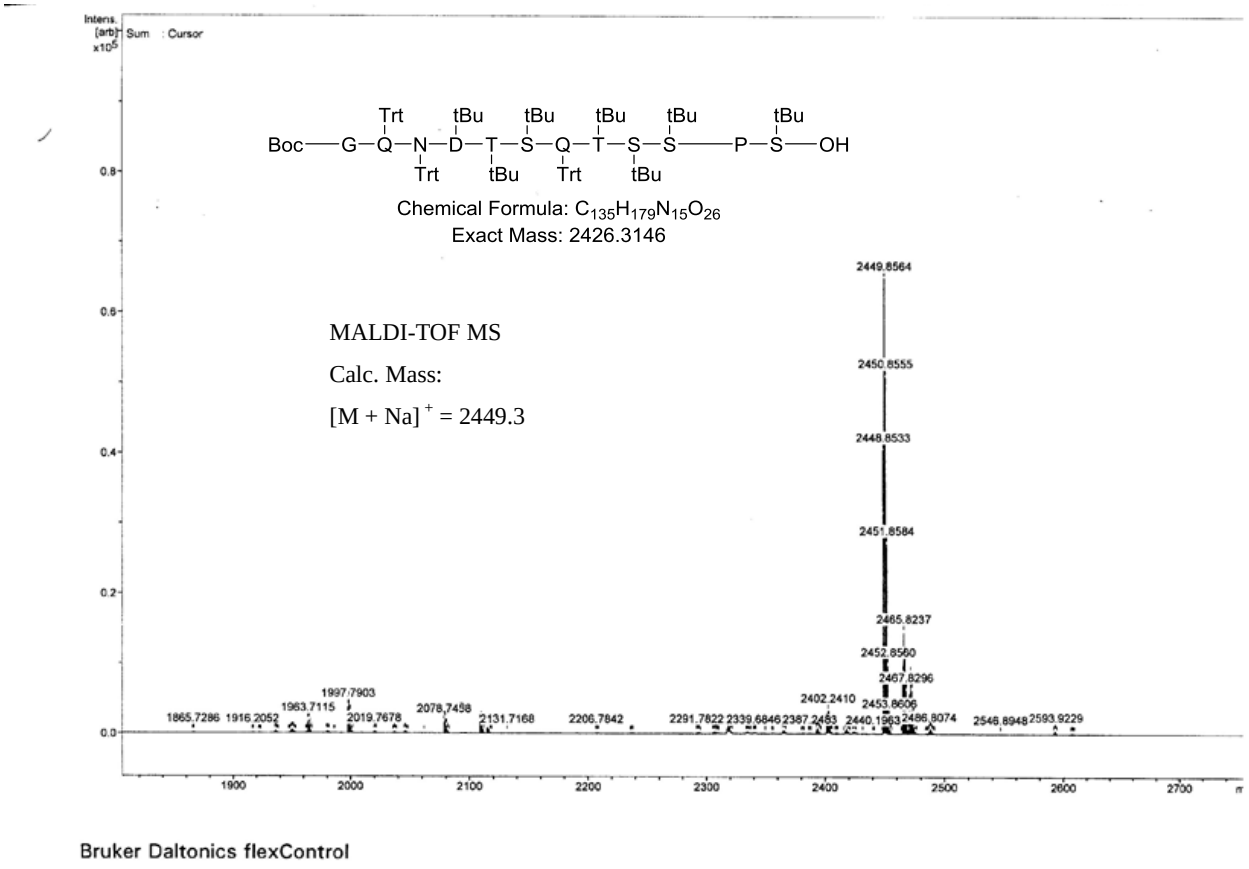


Figure S1. MALDI-TOF MS spectrum of the peptide **30** obtained from solid-phase synthesis

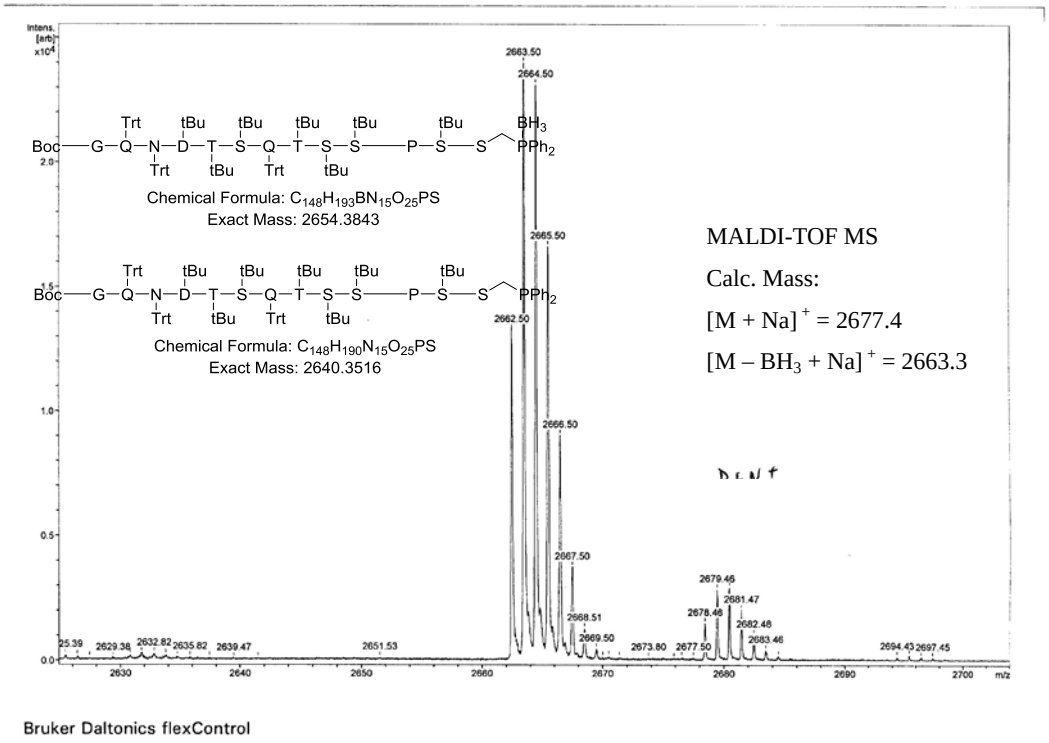


Figure S2. MALDI-TOF MS spectrum of peptide phosphinothioester **32**

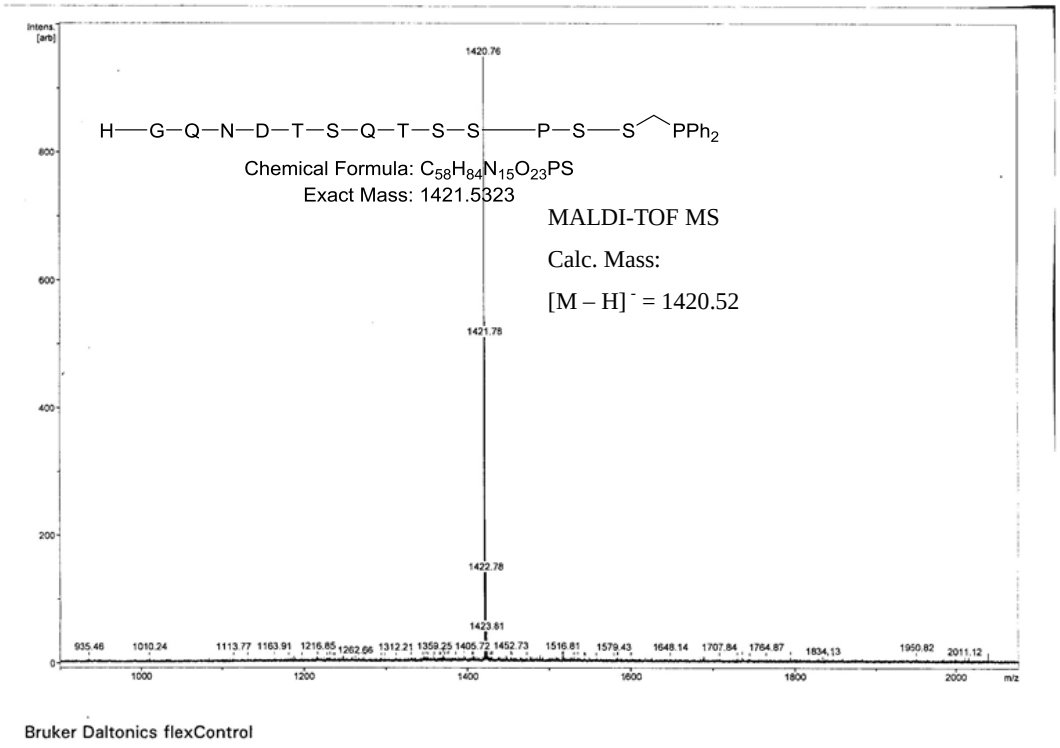


Figure S3. MALDI-TOF MS spectrum of peptide phosphinothioester **33**

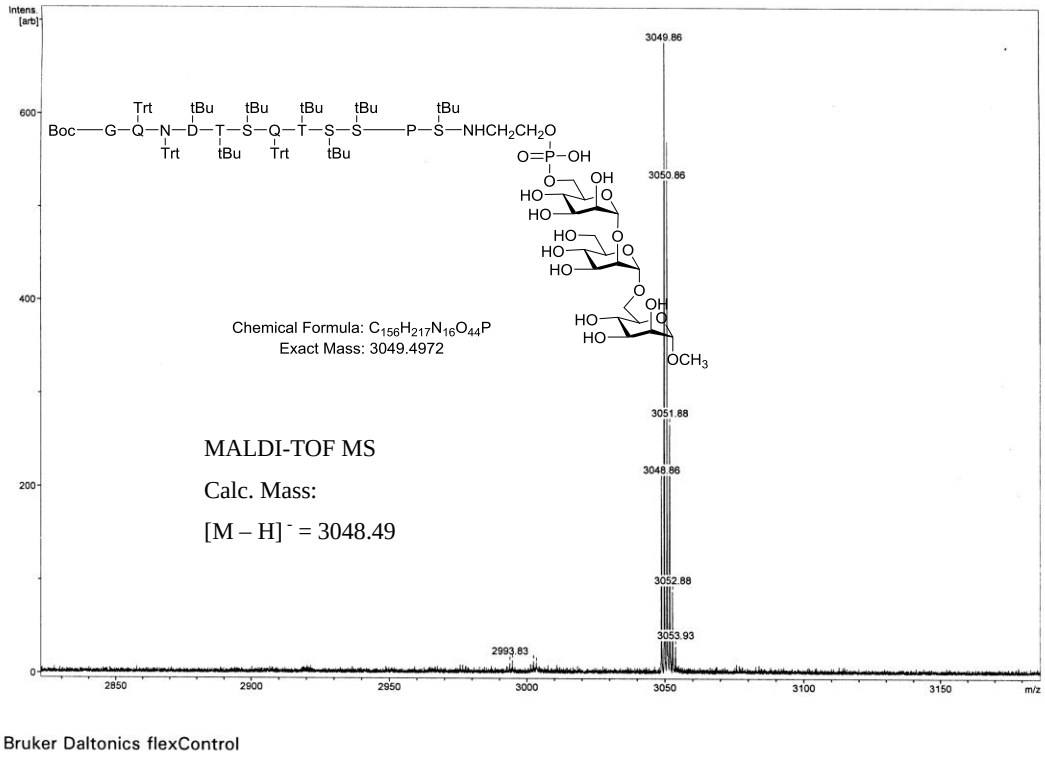


Figure S4. MALDI-TOF MS spectrum of the reaction product of **18** and **32** (i.e., protected **34**)

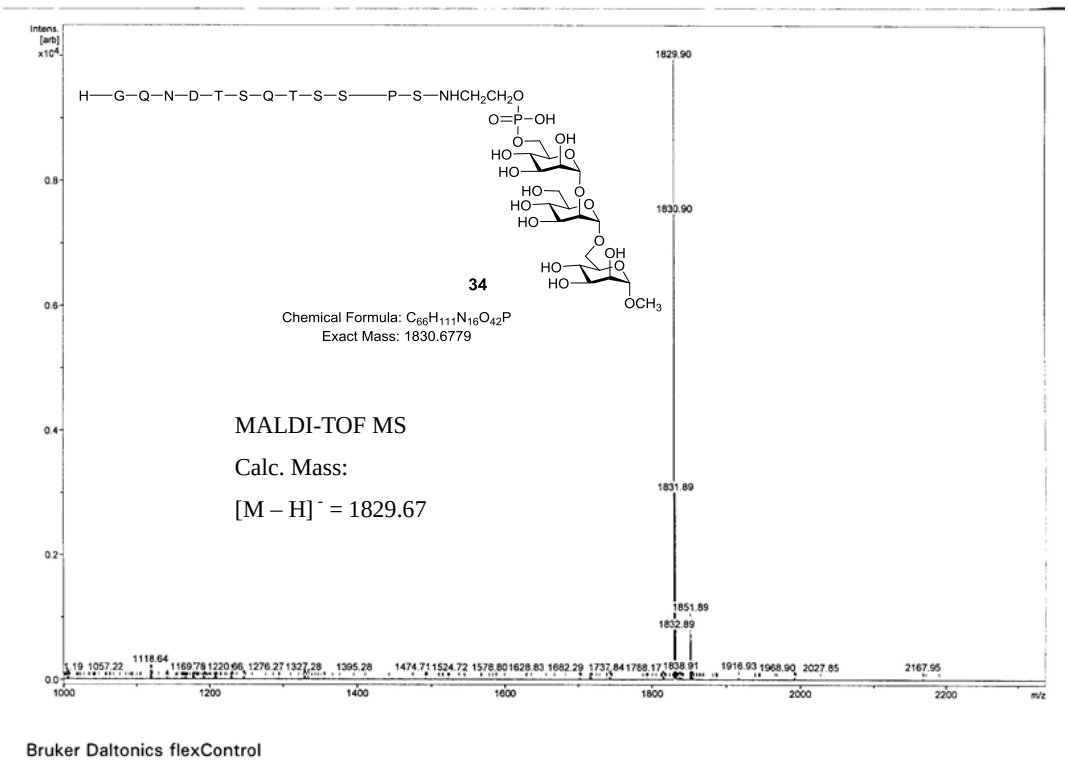


Figure S5. MALDI-TOF MS spectrum of CD52 analog **34**

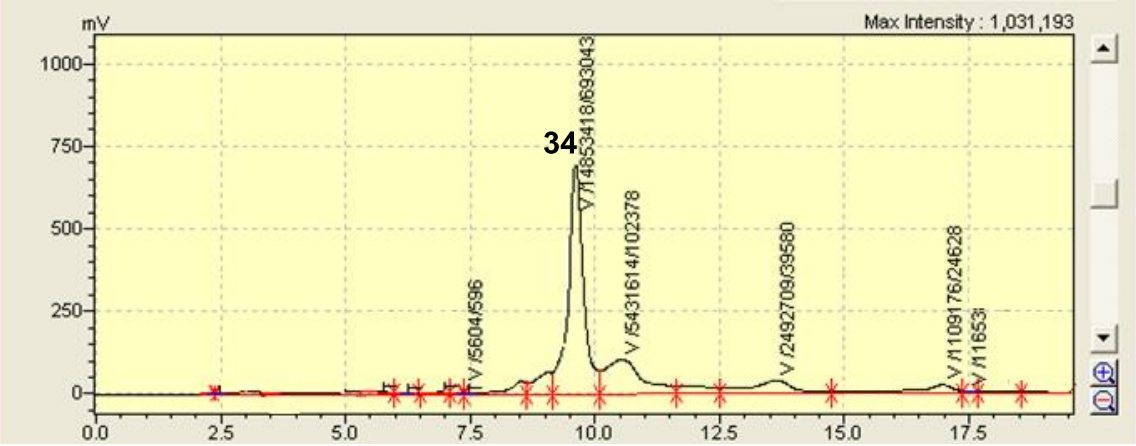


Figure S6. HPLC diagram of **34** derived from **18** and **32** (after the deprotection step). C18 column (5 µm, 250 x 4.6 mm); gradient eluent: 10-60% CH_3CN in H_2O; flow rate: 1.5 mL/min, UV detection at 220 nm.

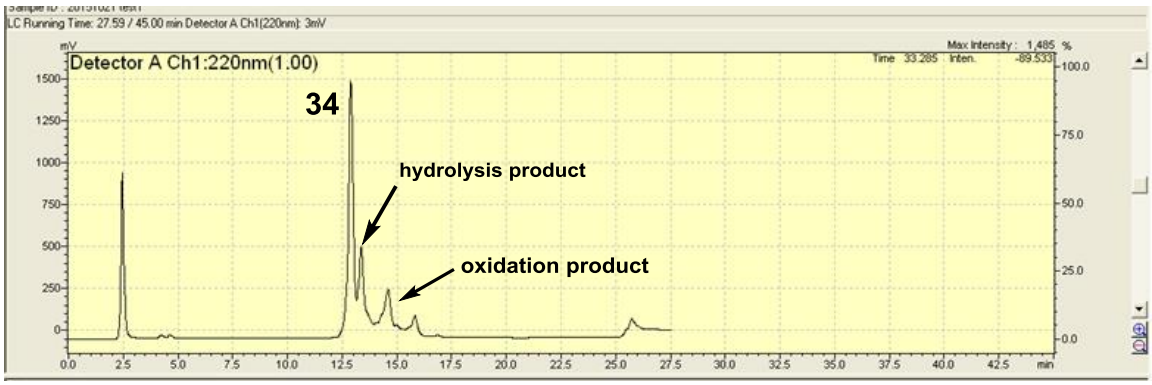


Figure S7. HPLC diagram of **34** derived from **18** and **33**. C18 column (5 µm, 250 x 4.6 mm); gradient eluent: 5-60% CH_3CN in H_2O; flow rate: 1.5 mL/min, UV detection at 220 nm.

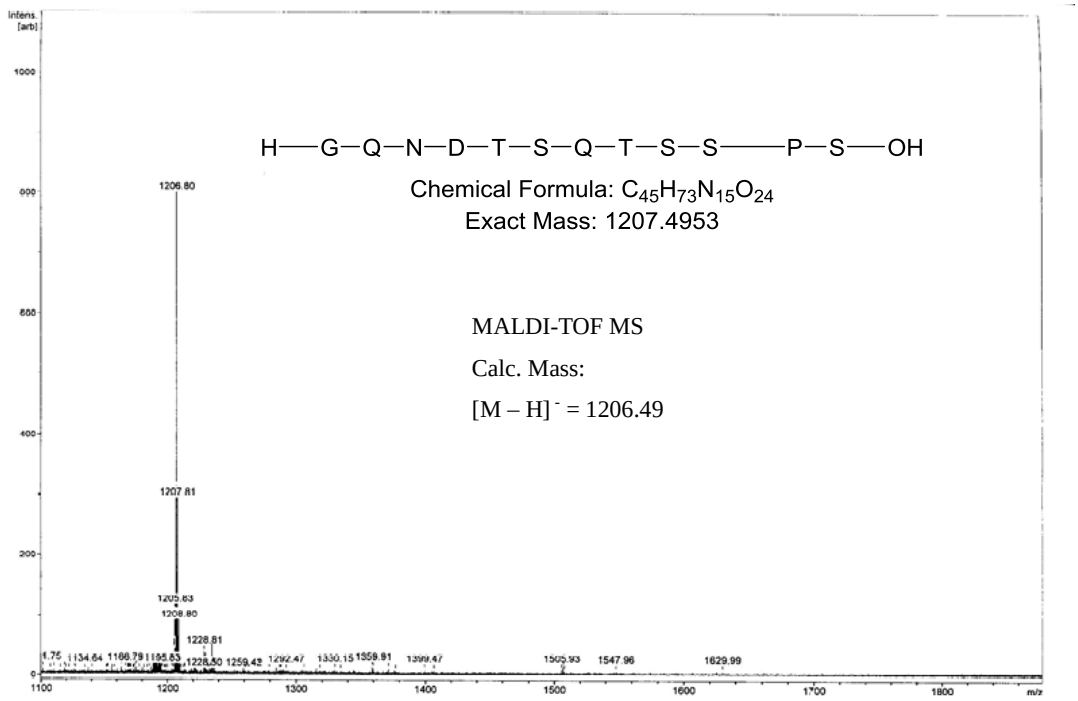


Figure S8. MALDI-TOF MS spectrum of the hydrolysis product of **33**

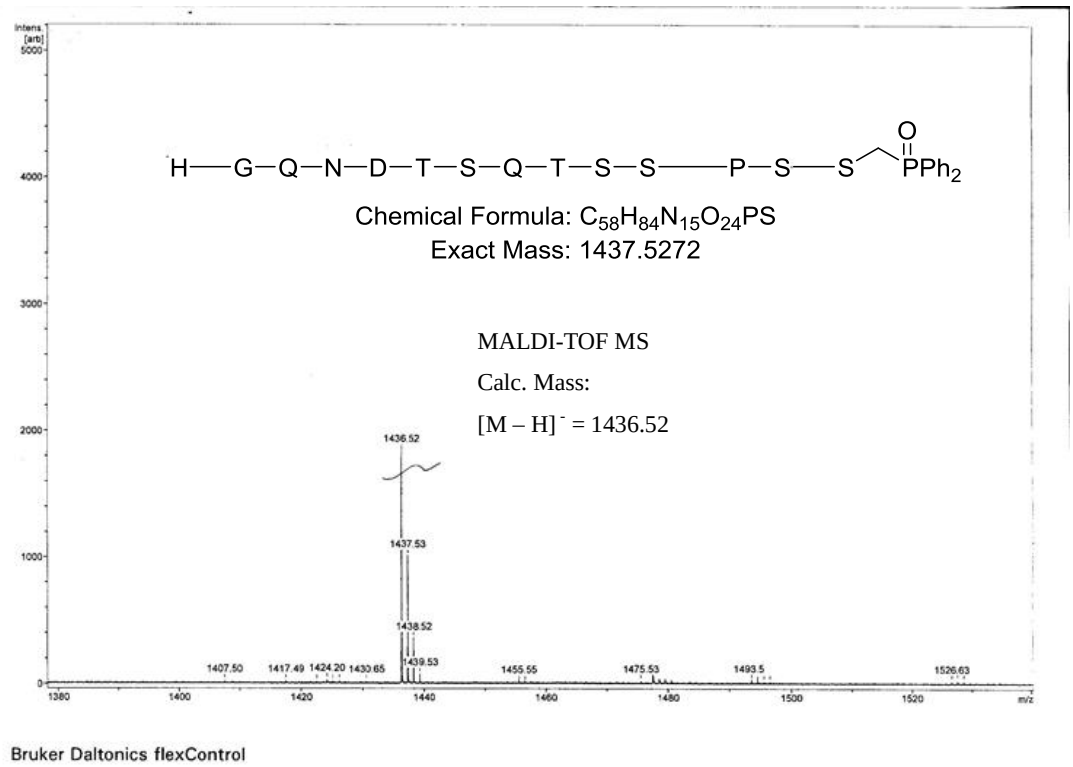
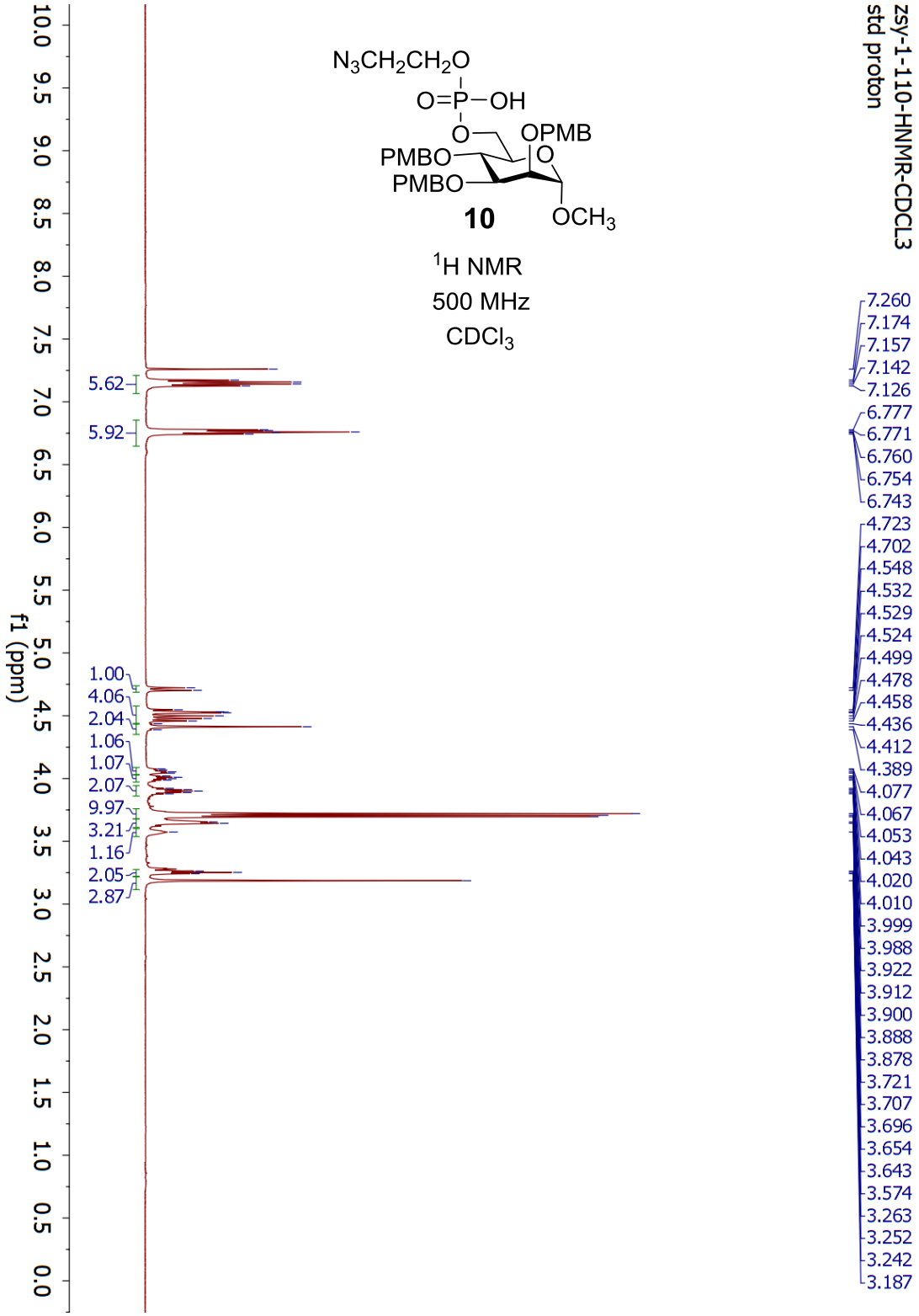
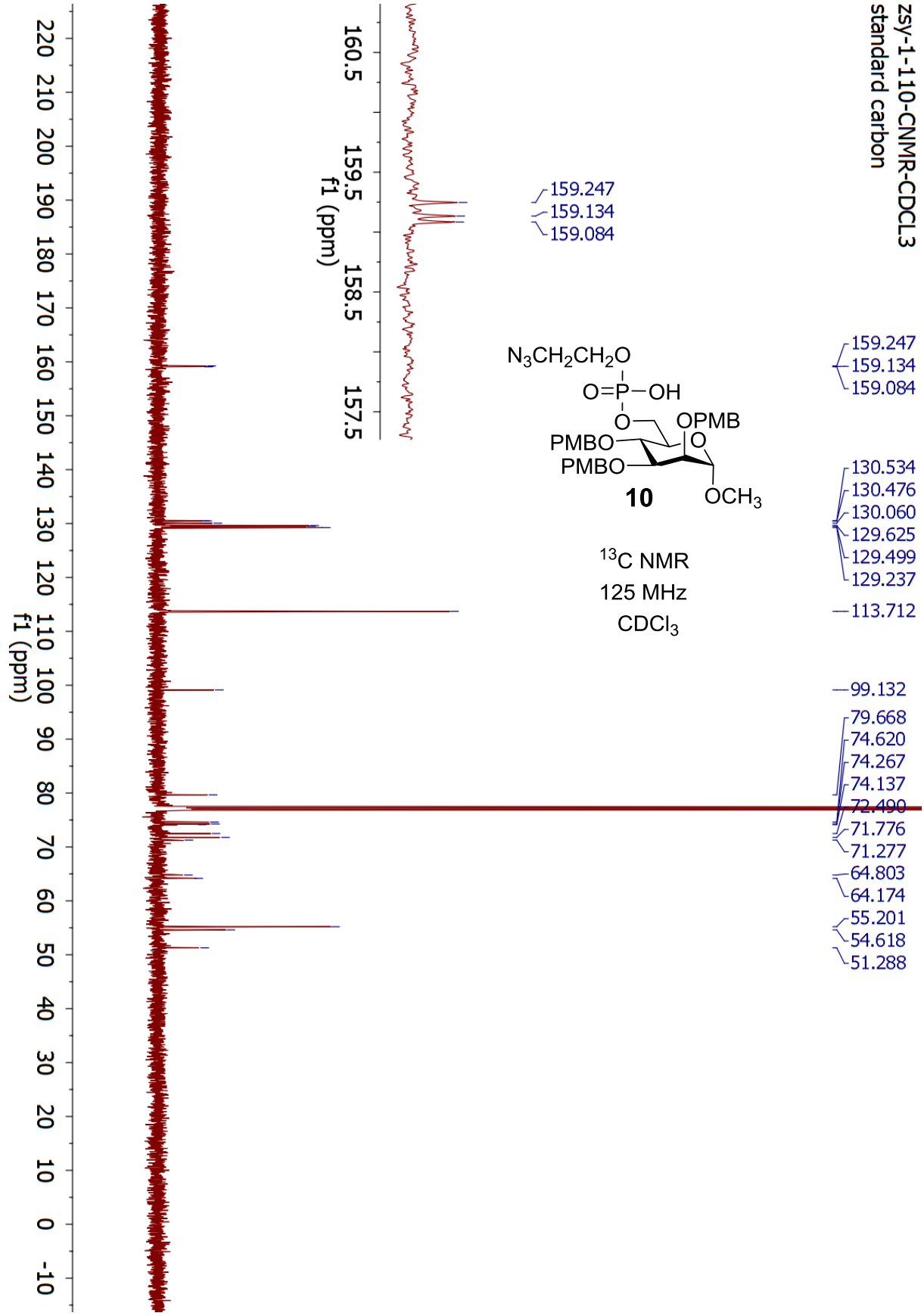
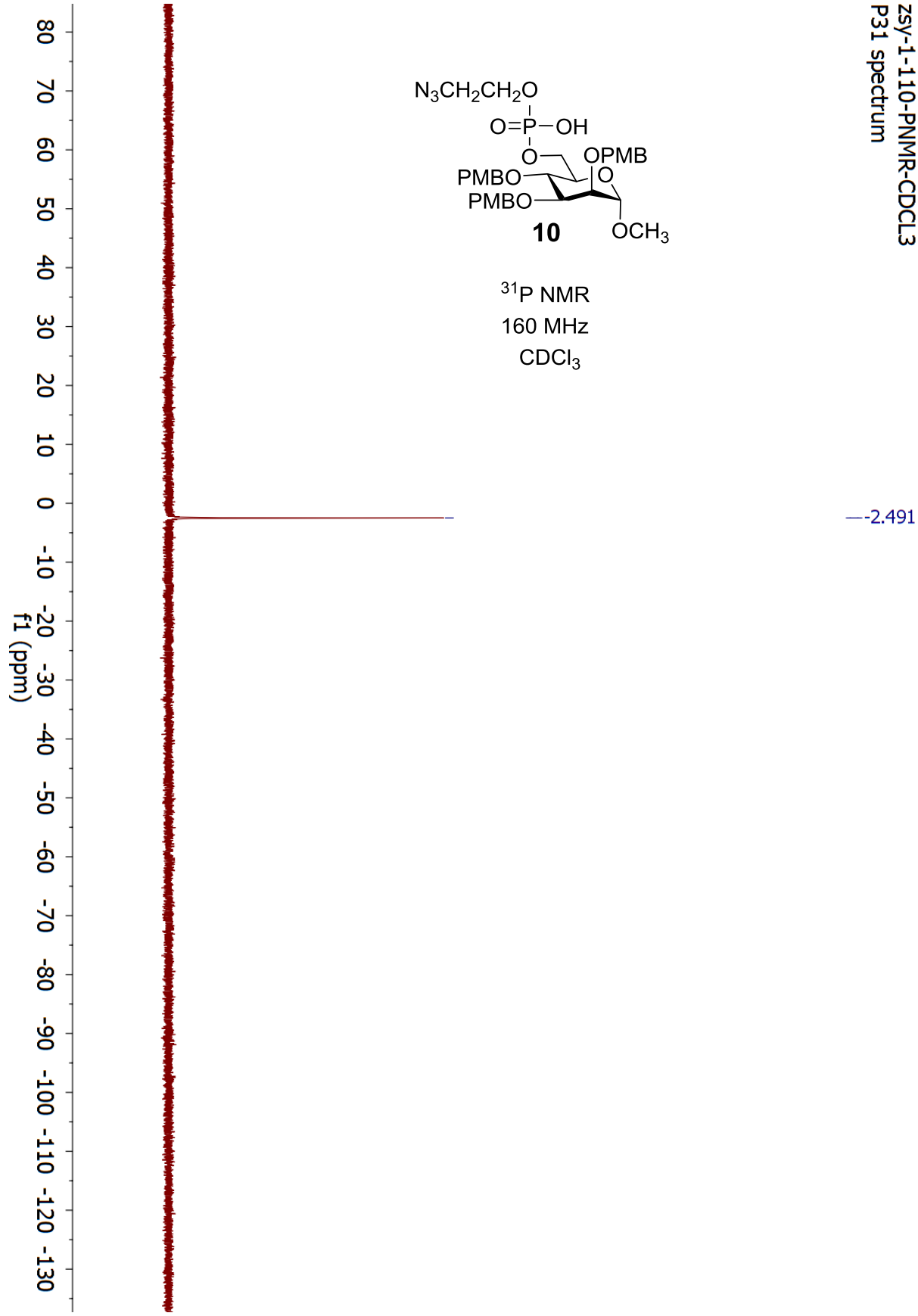


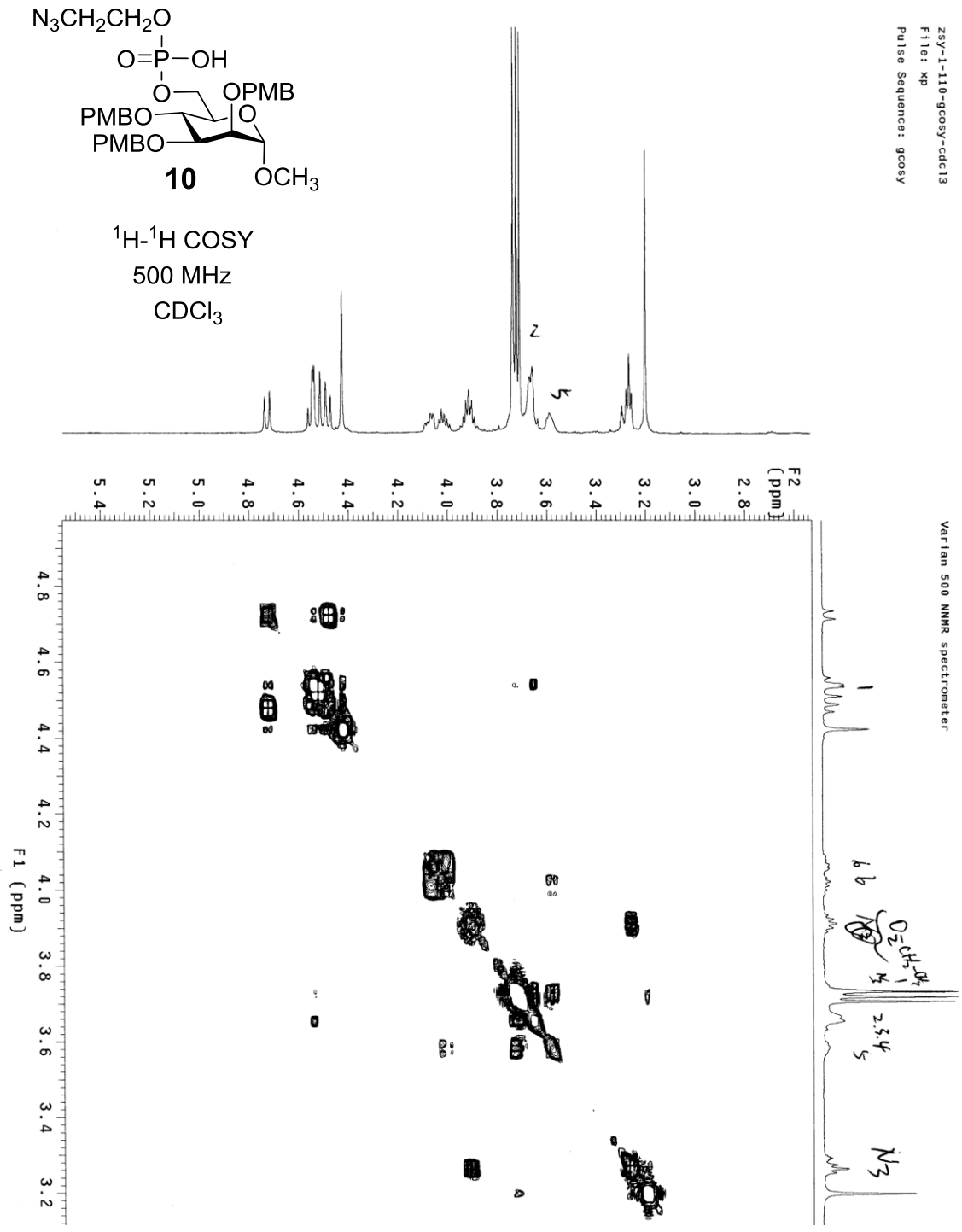
Figure S9. MALDI-TOF MS spectrum of the oxidation product of **33**

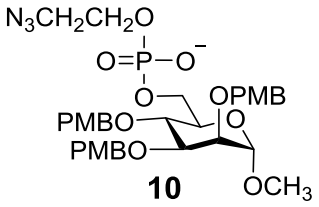
III. NMR and MS spectra of synthetic intermediates and other GPI-peptide conjugates




zsy-1-110-CNMR-CDCL3
standard carbon
159.247
159.134
159.084
159.247
159.134
159.084
130.534
130.476
130.060
129.625
129.499
129.237
113.712
99.132
79.668
74.620
74.267
74.137
72.490
71.776
71.277
64.803
64.174
55.201
54.618
51.288
N3CH2CH2O
O=P–OH
O
OPMB
PMBO
PMBO
O
OCH3
10
13C NMR
125 MHz
CDCl3
f1 (ppm)
160.5
159.5
158.5
157.5
f1 (ppm)
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10


zsy-1-110-PNMR-CDCL3
P31 spectrum
$N_3CH_2CH_2O$
O=P–OH
O
OPMB
PMBO
O
PMBO
OCH3
10
31P NMR
160 MHz
CDCl3
-2.491
80 70 60 50 40 30 20 10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130
f1 (ppm)


N3CH2CH2O
O=P–OH
O
OPMB
PMBO
O
PMBO
10
OCH3
1H-1H COSY
500 MHz
CDCl3
zsy-1-110-gcosy-cdcl3
File: xp
Pulse Sequence: gcosy
Varian 500 NMR spectrometer
F2 (ppm)
F1 (ppm)



Exact Mass: 702.2433

HR ESI-TOF MS

Calc. Mass:

$[M - H]^-$ = 702.2433

Elemental Composition Report

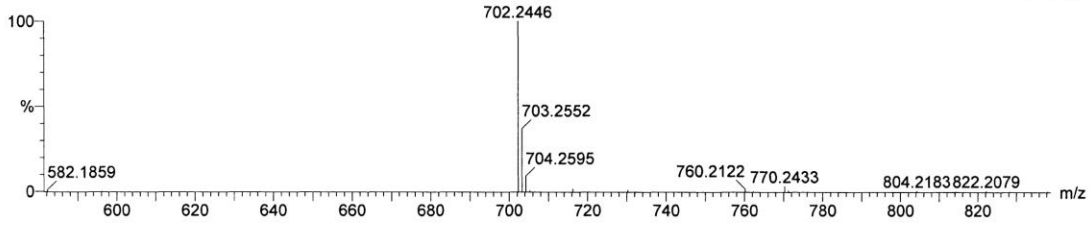
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 6

Monoisotopic Mass, Even Electron Ions
225 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 33-33 H: 0-50 N: 0-5 O: 0-20 31P: 0-1

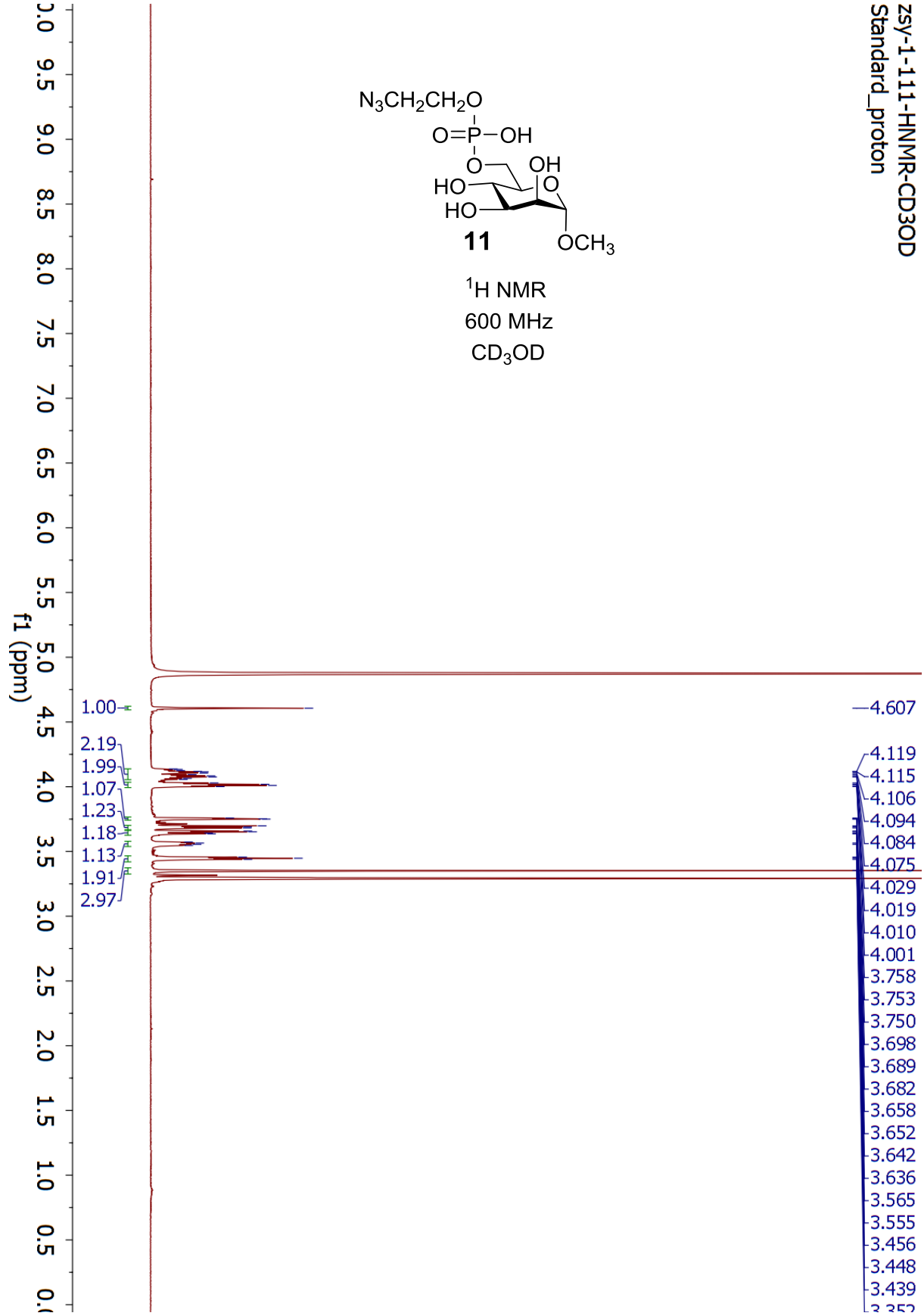
S.Zhu ZSY-1-110 inMeOH Cone(V)50

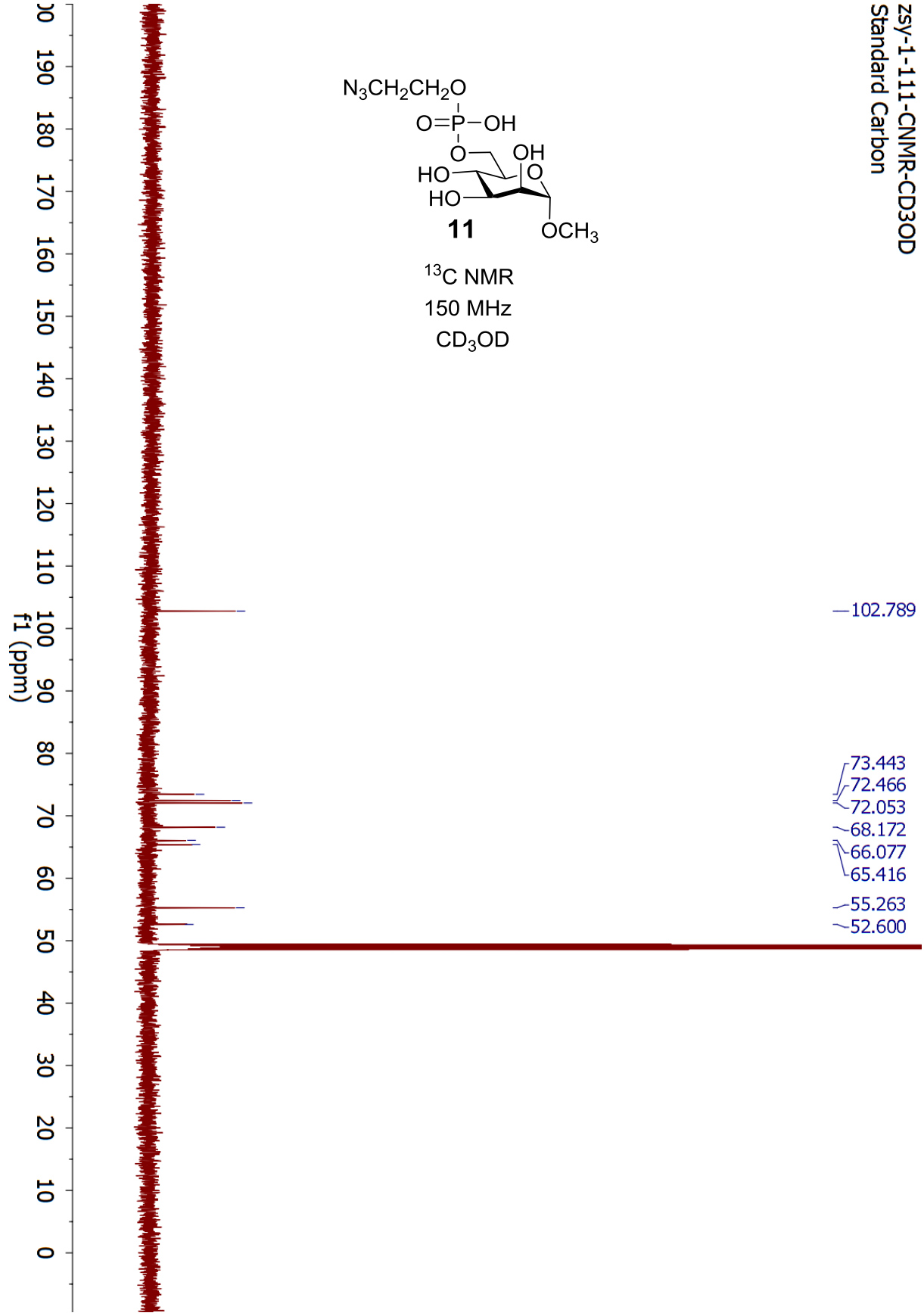
LCT Premier KD128
TOF MS ES-

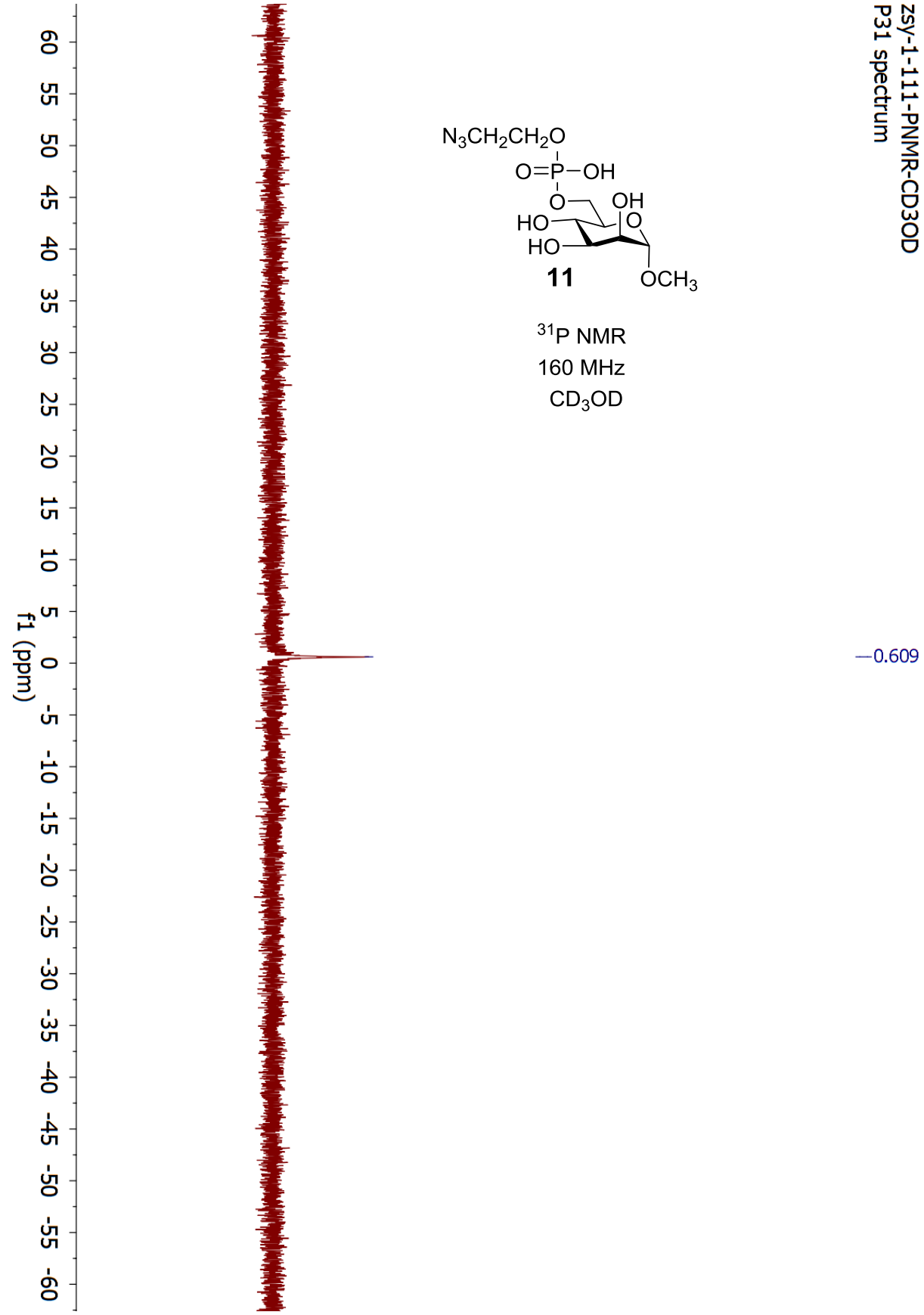
7.24e+004

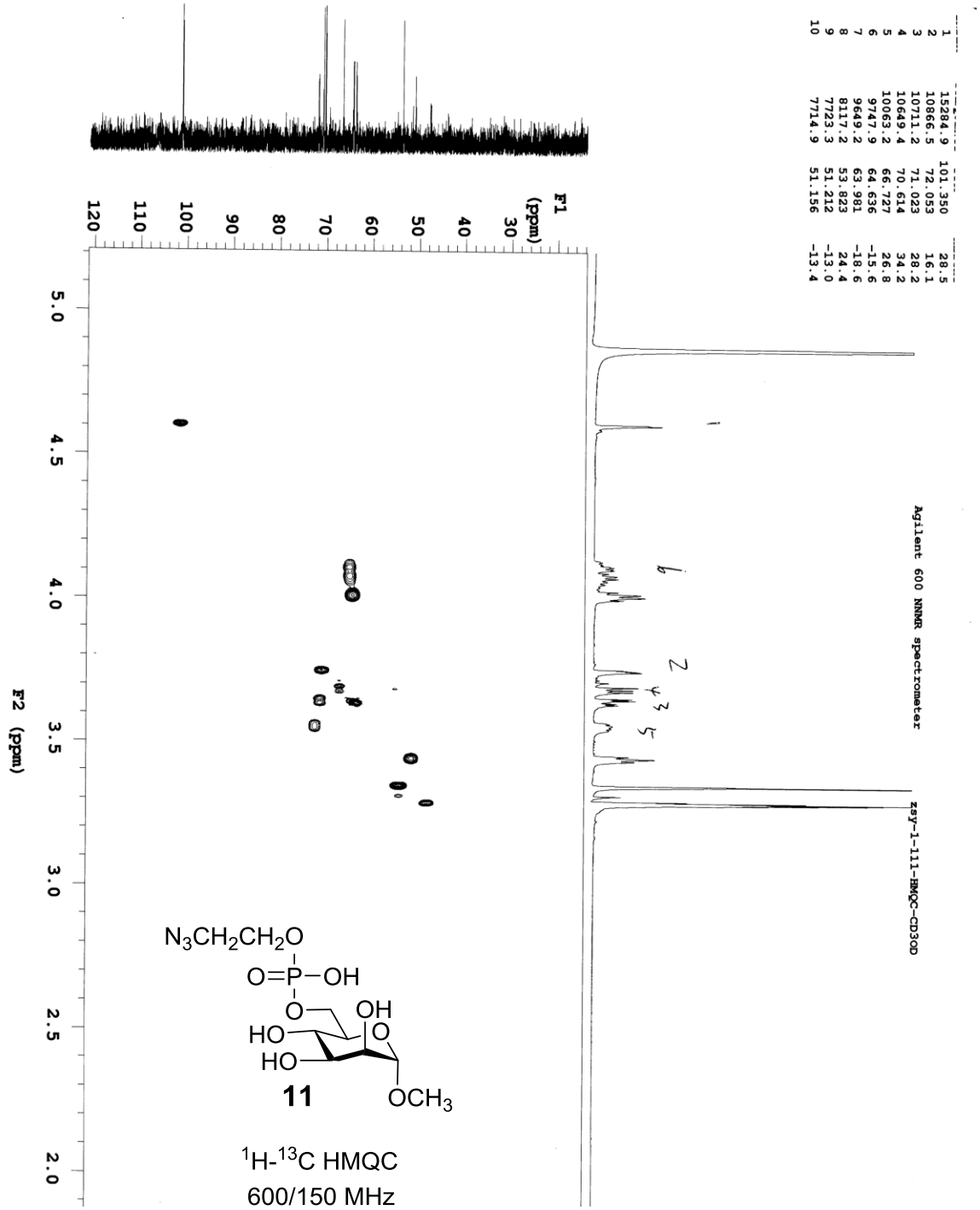


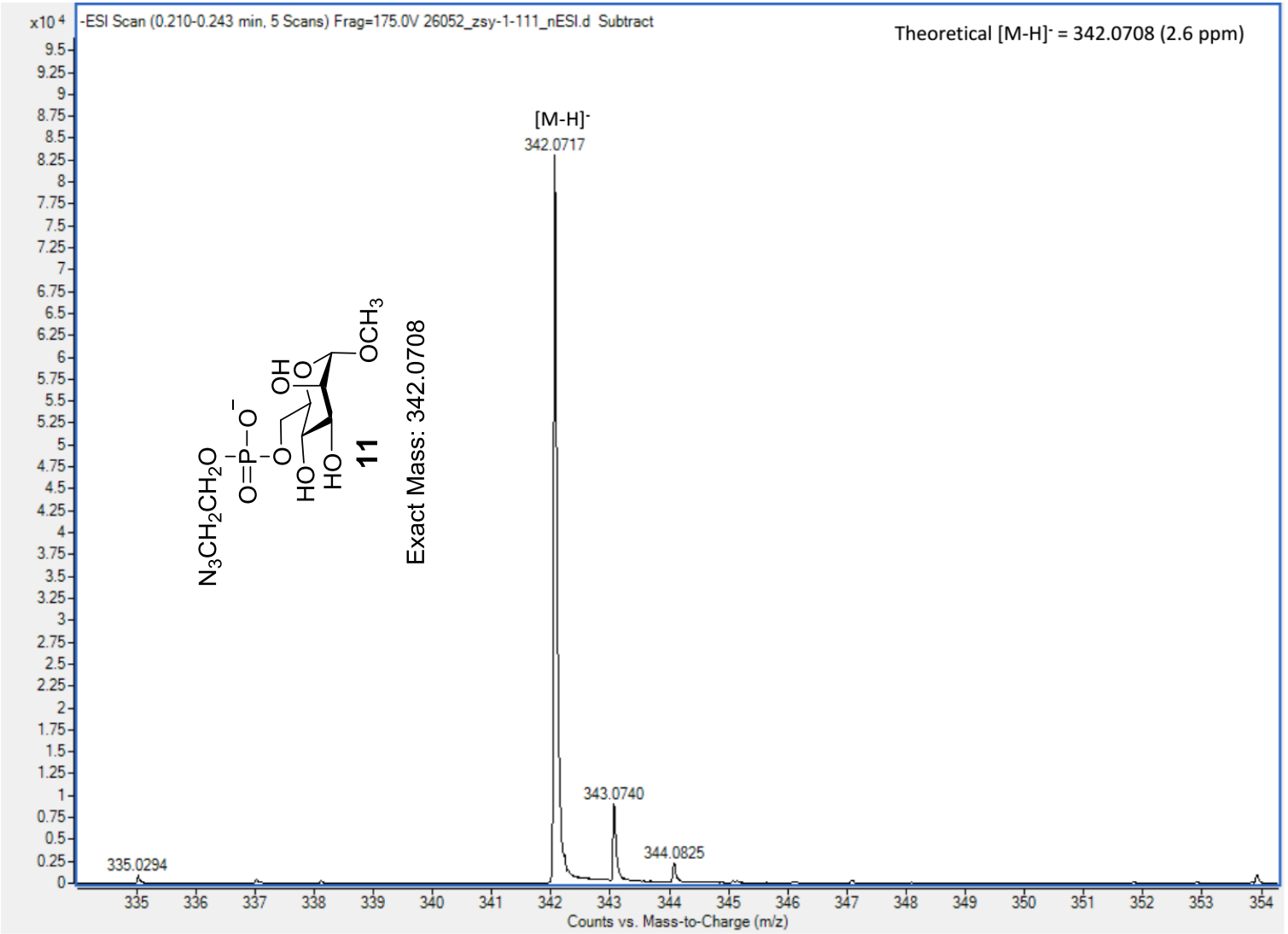
Minimum:				-1.5			
Maximum:		3.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
702.2446	702.2428	1.8	2.6	15.5	51.8	0.0	C33 H41 N3 O12 31P

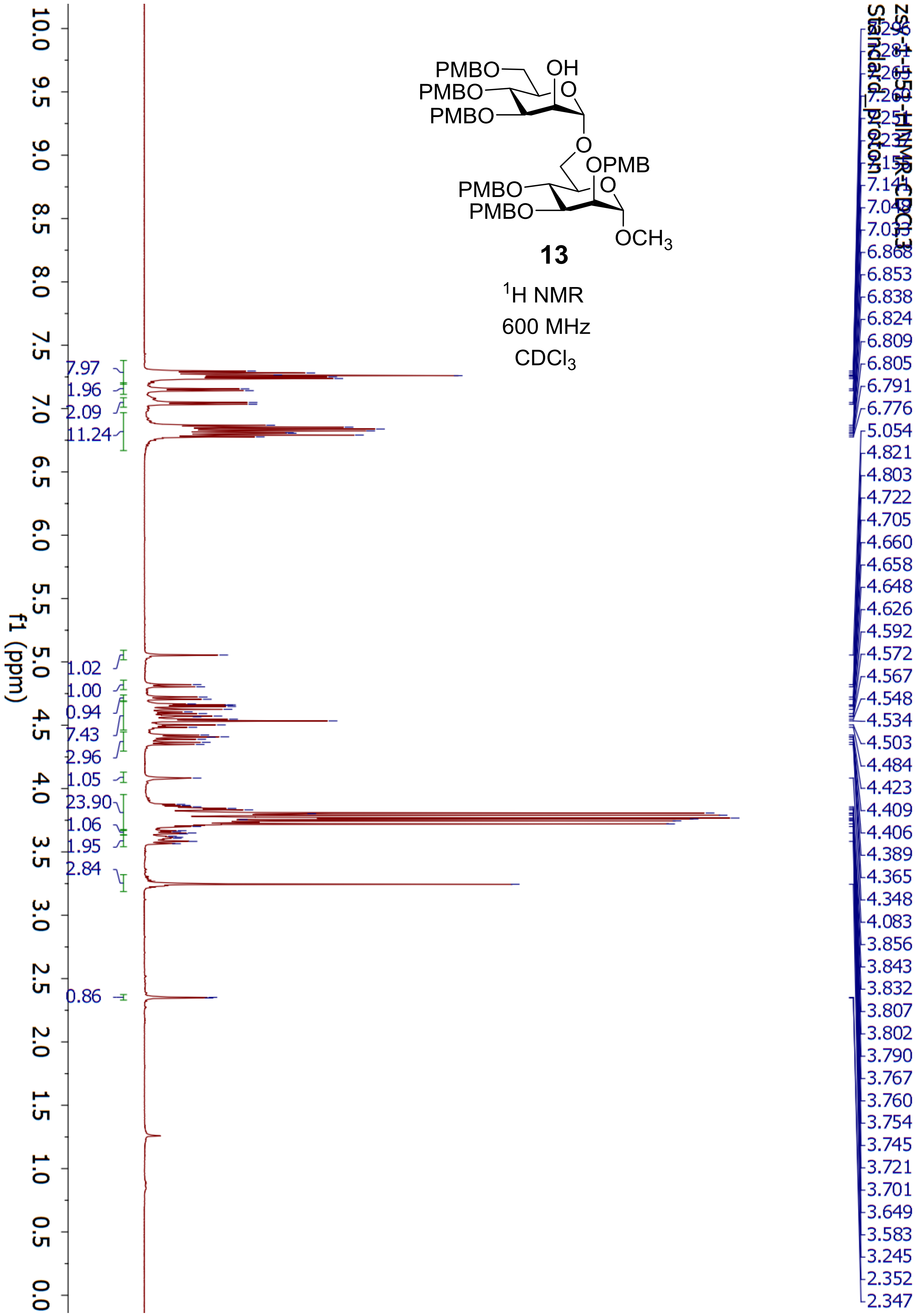

zsy-1-111-HNMR-CD3OD
Standard_proton
N3CH2CH2O
O=P–OH
O
OH
HO
O
HO
OCH3
11
1H NMR
600 MHz
CD3OD
f1 (ppm)
1.00
2.19
1.99
1.07
1.23
1.18
1.13
1.91
2.97
4.607
4.119
4.115
4.106
4.094
4.084
4.075
4.029
4.019
4.010
4.001
3.758
3.753
3.750
3.698
3.689
3.682
3.658
3.652
3.642
3.636
3.565
3.555
3.456
3.448
3.439


zsy-1-111-CNMR-CD3OD
Standard Carbon
$N_3CH_2CH_2O$
O=P–OH
O
OH
HO
HO
O
11
OCH_3
^{13}C NMR
150 MHz
CD_3OD
102.789
73.443
72.466
72.053
68.172
66.077
65.416
55.263
52.600
f1 (ppm)
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

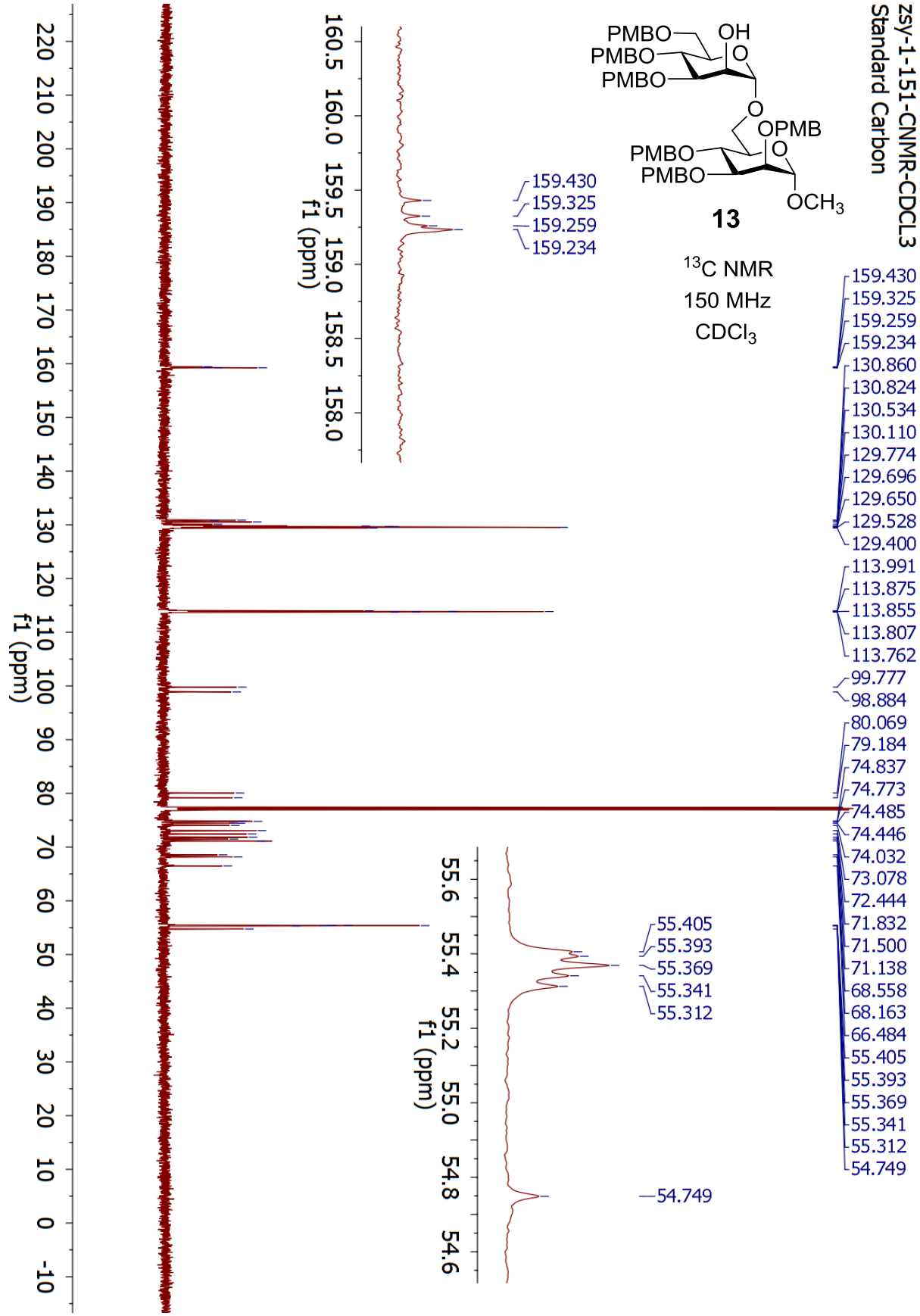

zsy-1-111-PNMR-CD3OD
P31 spectrum
N3CH2CH2O
O=P–OH
O
OH
HO
O
HO
11
OCH3
31P NMR
160 MHz
CD3OD
0.609
f1 (ppm)
60 55 50 45 40 35 30 25 20 15 10 5 0 -5 -10 -15 -20 -25 -30 -35 -40 -45 -50 -55 -60

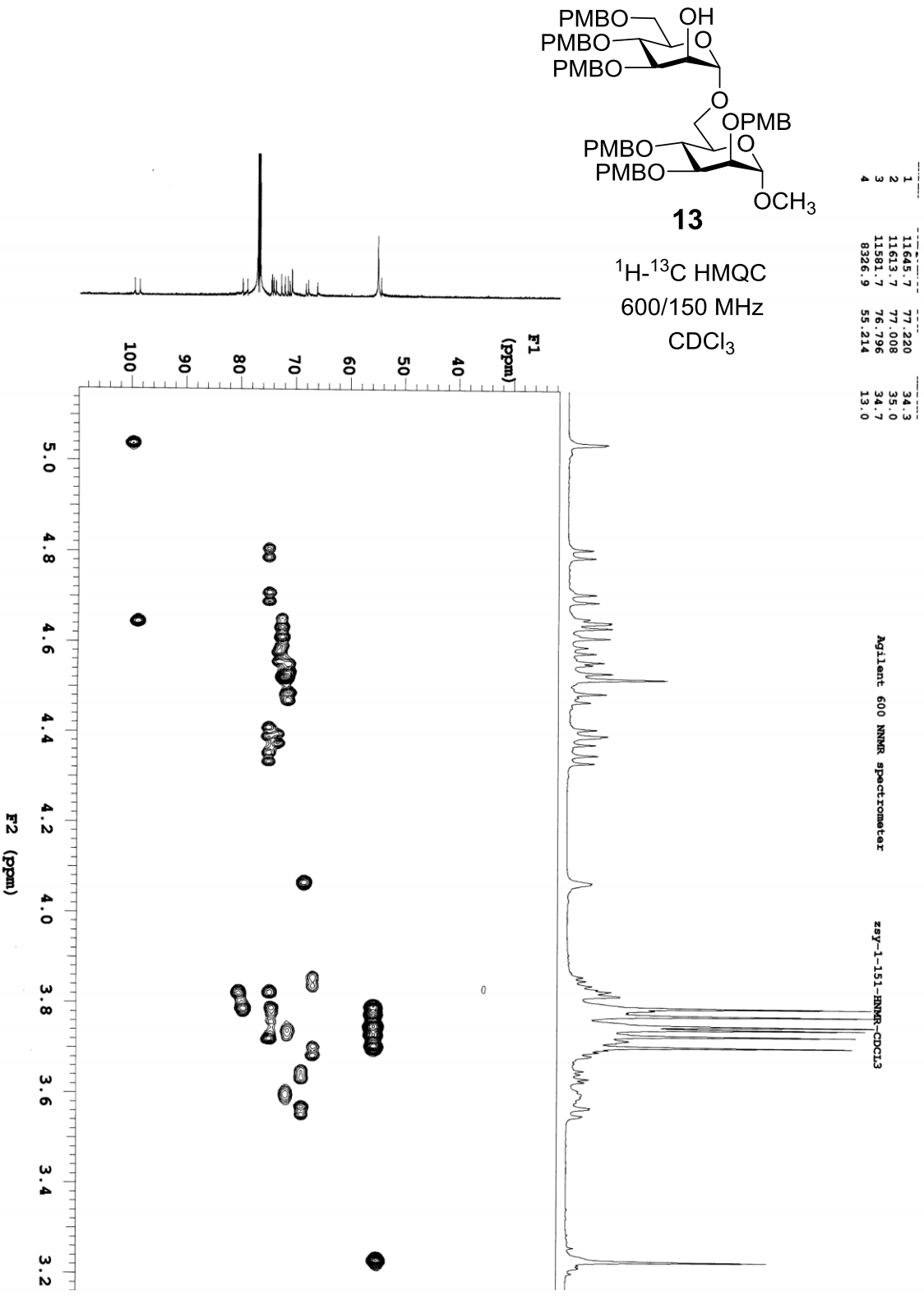

Agilent 600 NMR spectrometer
zsy-1-111-HMQC-CD3OD
F1 (ppm)
F2 (ppm)
N3CH2CH2O
O=P−OH
O
OH
HO
O
HO
OCH3
11
1H-13C HMQC
600/150 MHz
CD3OD

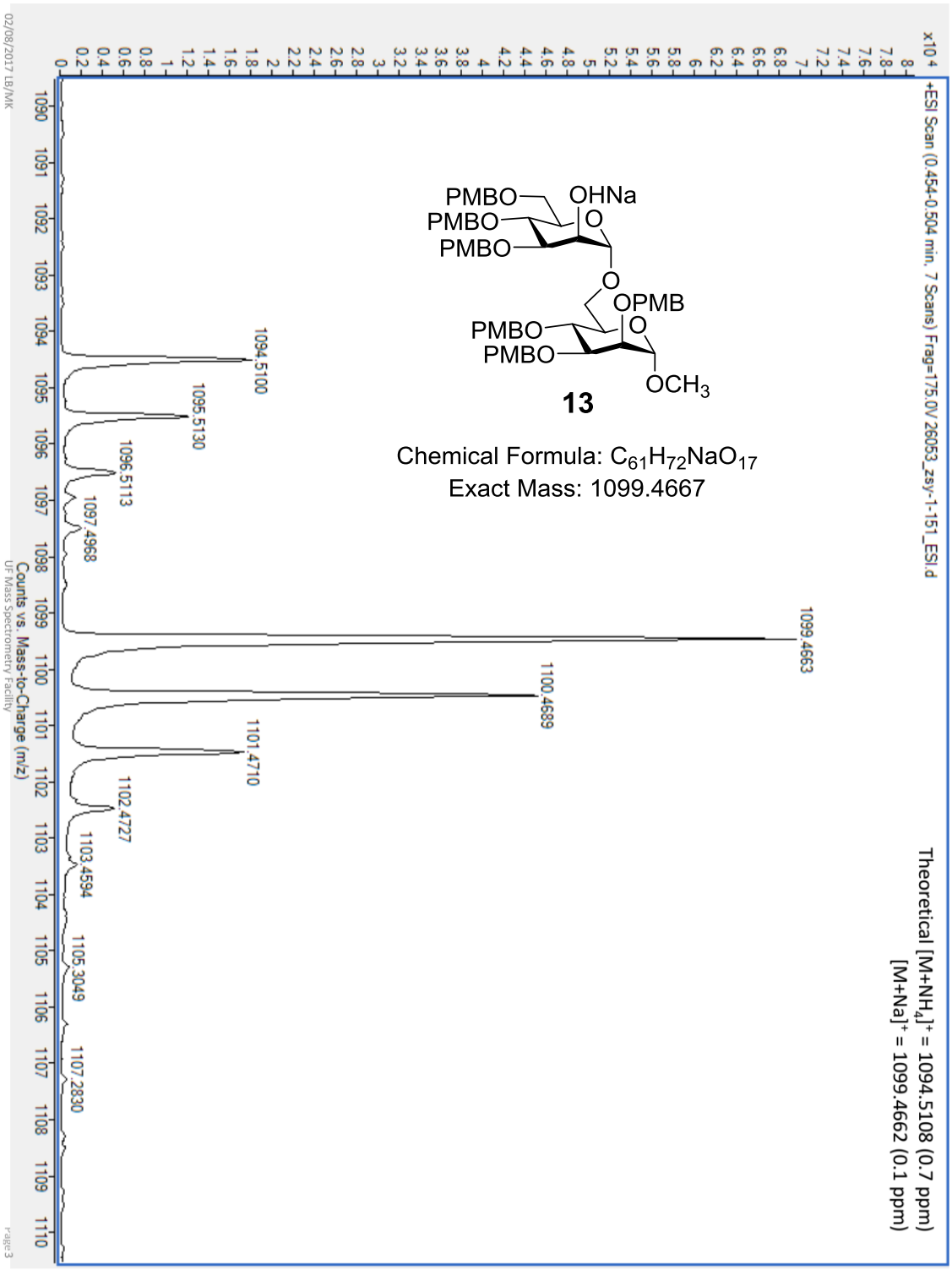

-ESI Scan (0.210-0.243 min, 5 Scans) Frag=175.0V 26052_zsy-1-111_nESI.d Subtract
x10 4
Theoretical [M-H]- = 342.0708 (2.6 ppm)
[M-H]-
342.0717
343.0740
344.0825
335.0294
Exact Mass: 342.0708
11
Counts vs. Mass-to-Charge (m/z)

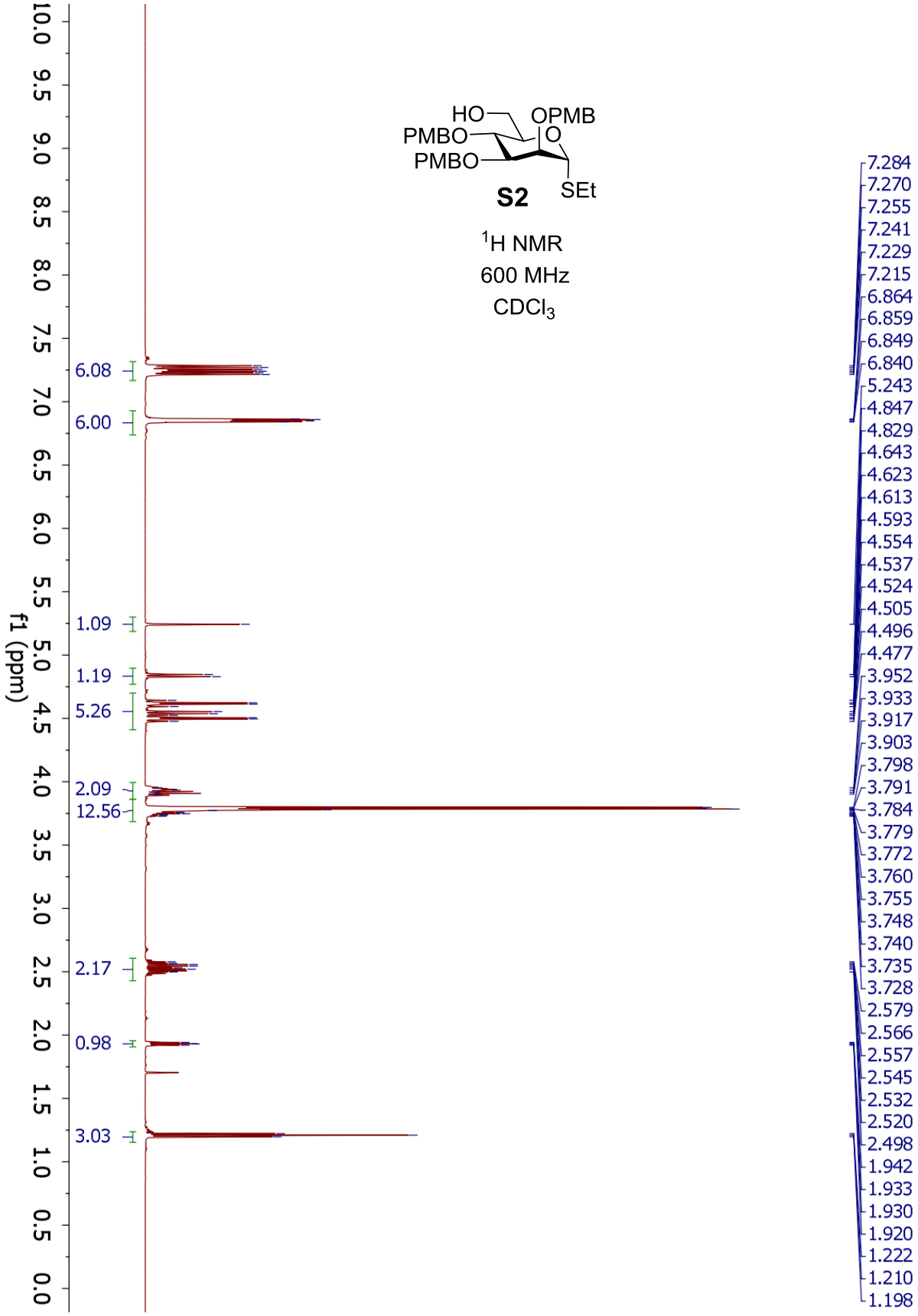


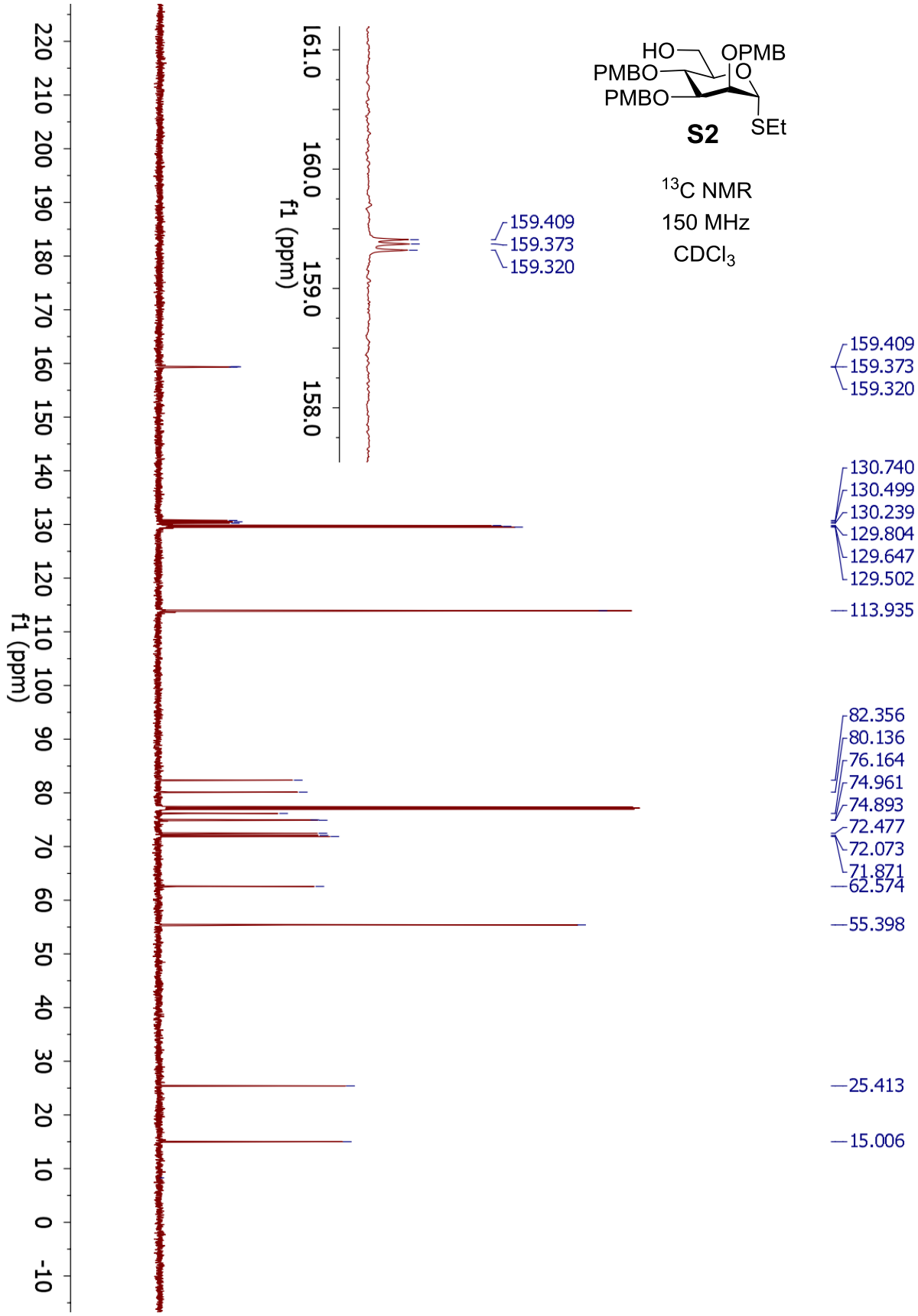
PMBO
PMBO
PMBO
OH
O
O
OPMB
PMBO
PMBO
O
OCH3
13
1H NMR
600 MHz
CDCl3
Standard proton
f1 (ppm)
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
7.97
1.96
2.09
11.24
1.02
1.00
0.94
7.43
2.96
1.05
23.90
1.06
1.95
2.84
0.86
6.868
6.853
6.838
6.824
6.809
6.805
6.791
6.776
5.054
4.821
4.803
4.722
4.705
4.660
4.658
4.648
4.626
4.592
4.572
4.567
4.548
4.534
4.503
4.484
4.423
4.409
4.406
4.389
4.365
4.348
4.083
3.856
3.843
3.832
3.807
3.802
3.790
3.767
3.760
3.754
3.745
3.721
3.701
3.649
3.583
3.245
2.352
2.347

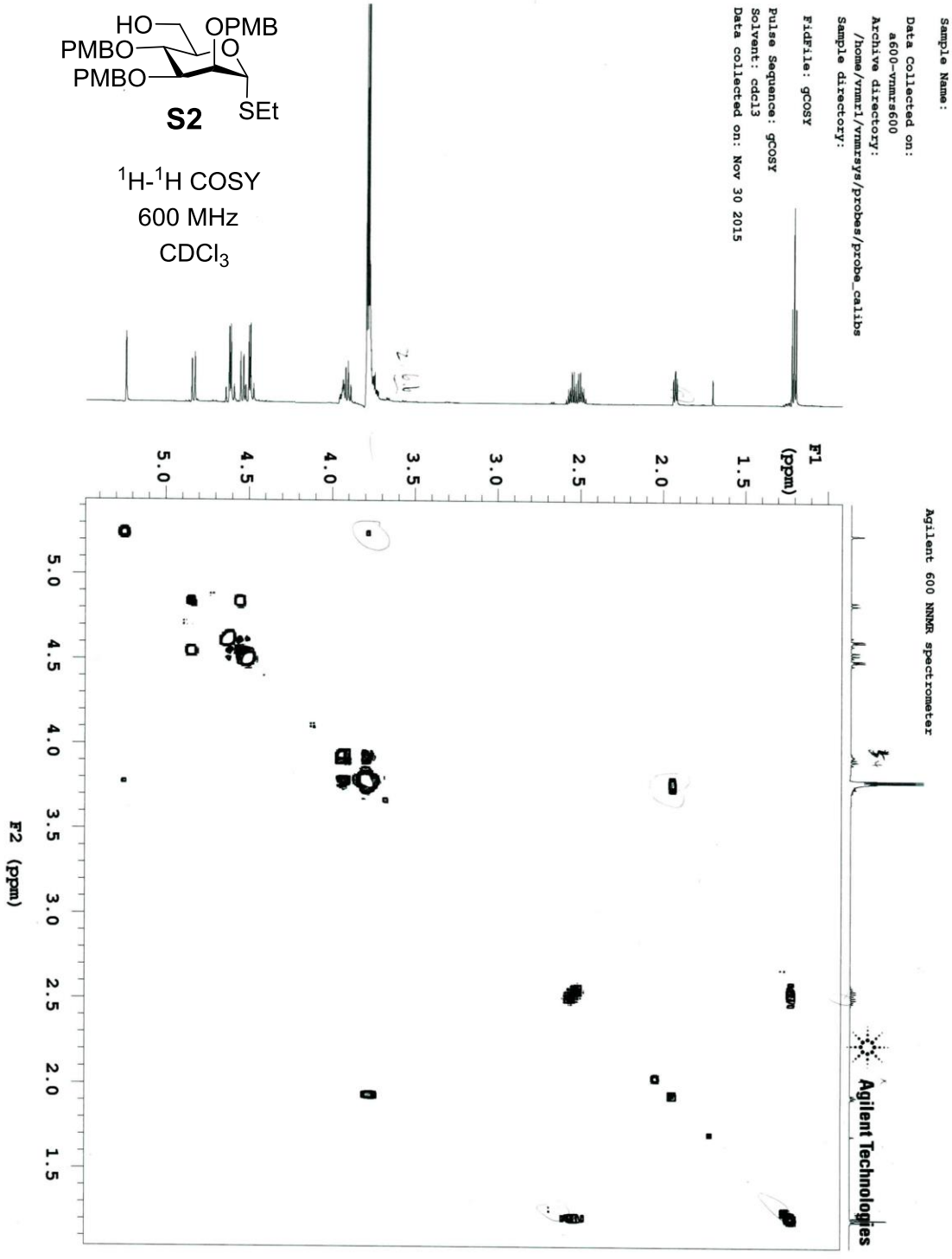

zsy-1-151-CNMR-CDCL3
Standard Carbon
PMBO
PMBO
PMBO
OH
O
O
OPMB
PMBO
PMBO
O
OCH3
13
13C NMR
150 MHz
CDCl3
159.430
159.325
159.259
159.234
130.860
130.824
130.534
130.110
129.774
129.696
129.650
129.528
129.400
113.991
113.875
113.855
113.807
113.762
99.777
98.884
80.069
79.184
74.837
74.773
74.485
74.446
74.032
73.078
72.444
71.832
71.500
71.138
68.558
68.163
66.484
55.405
55.393
55.369
55.341
55.312
54.749
f1 (ppm)

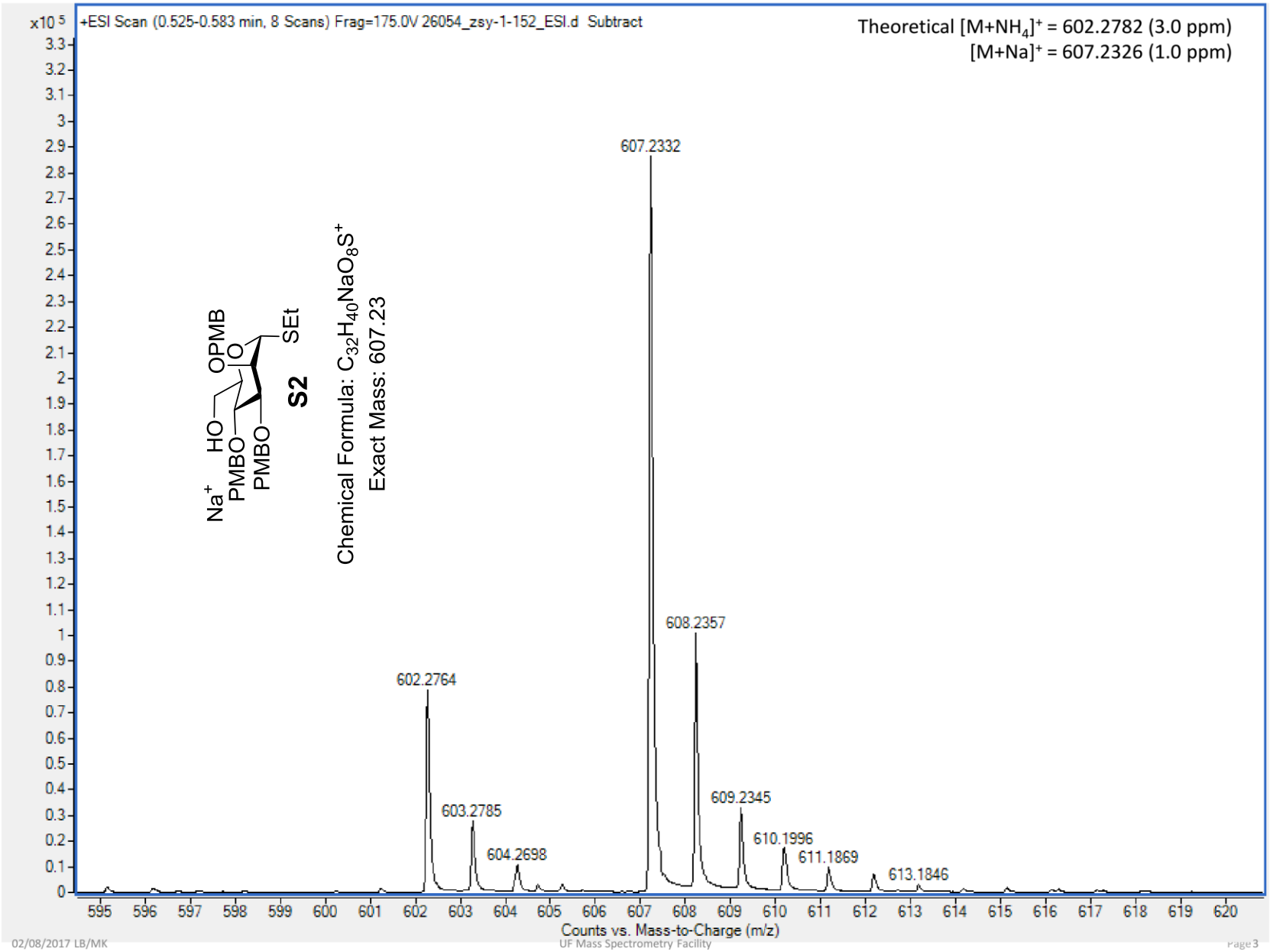

PMBO
PMBO
PMBO
OH
O
O
OPMB
PMBO
PMBO
O
OCH3
13
1H-13C HMQC
600/150 MHz
CDCl3
F1 (ppm)
F2 (ppm)
Agilent 600 NMNR spectrometer
zsy-1-151-HNMR-CDCL3

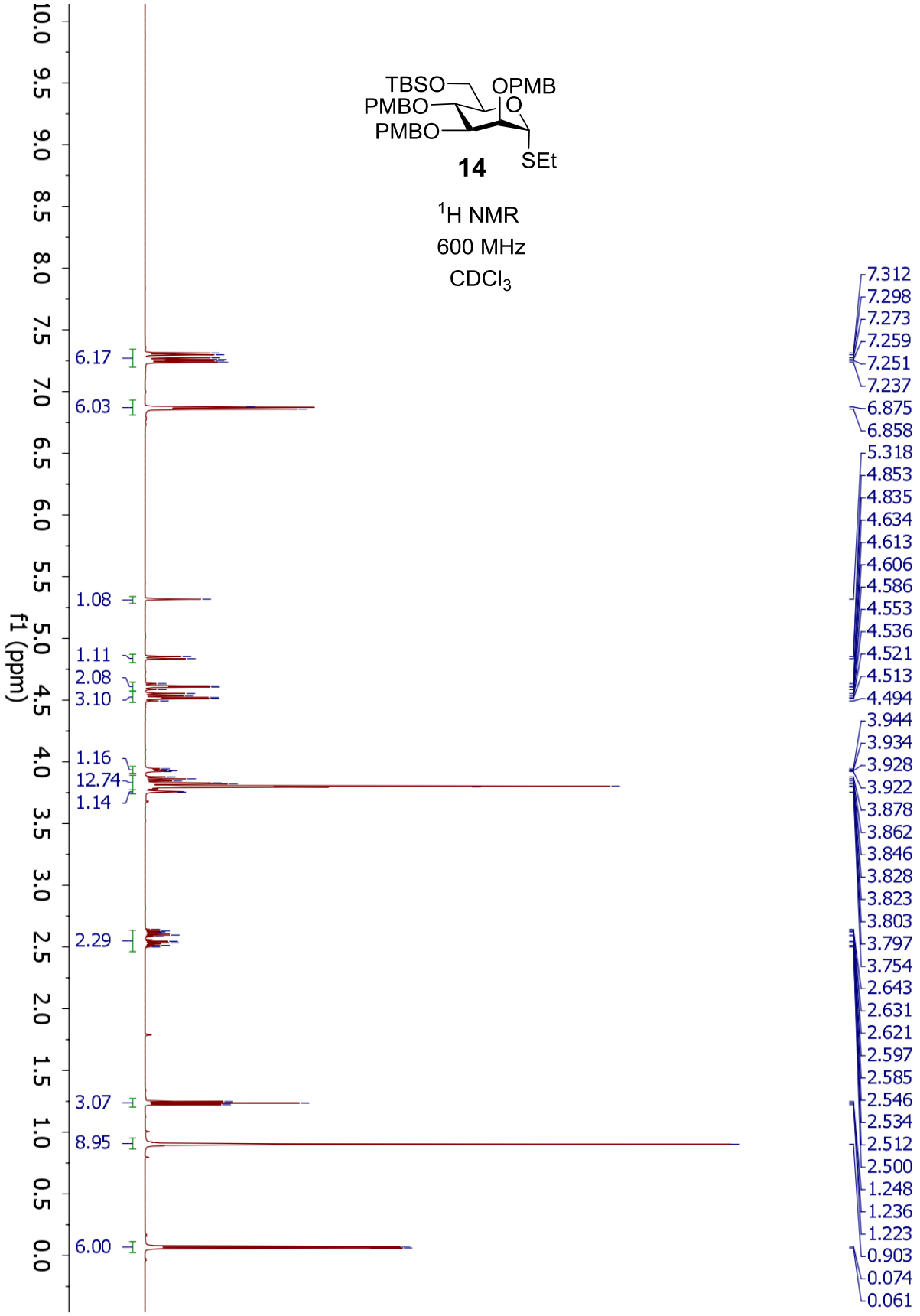

+ESI Scan (0.454-0.504 min, 7 Scans) Frag=175.0V 26053_zsy-1-151_ESI.d
Theoretical [M+NH4]+ = 1094.5108 (0.7 ppm)
[M+Na]+ = 1099.4662 (0.1 ppm)
PMBO
PMBO
PMBO
OHNa
O
O
OPMB
PMBO
PMBO
O
OCH3
13
Chemical Formula: C61H72NaO17
Exact Mass: 1099.4667
1094.5100
1095.5130
1096.5113
1097.4968
1099.4663
1100.4689
1101.4710
1102.4727
1103.4594
1105.3049
1107.2830
Counts vs. Mass-to-Charge (m/z)
UF Mass Spectrometry Facility
02/08/2017 LB/MK

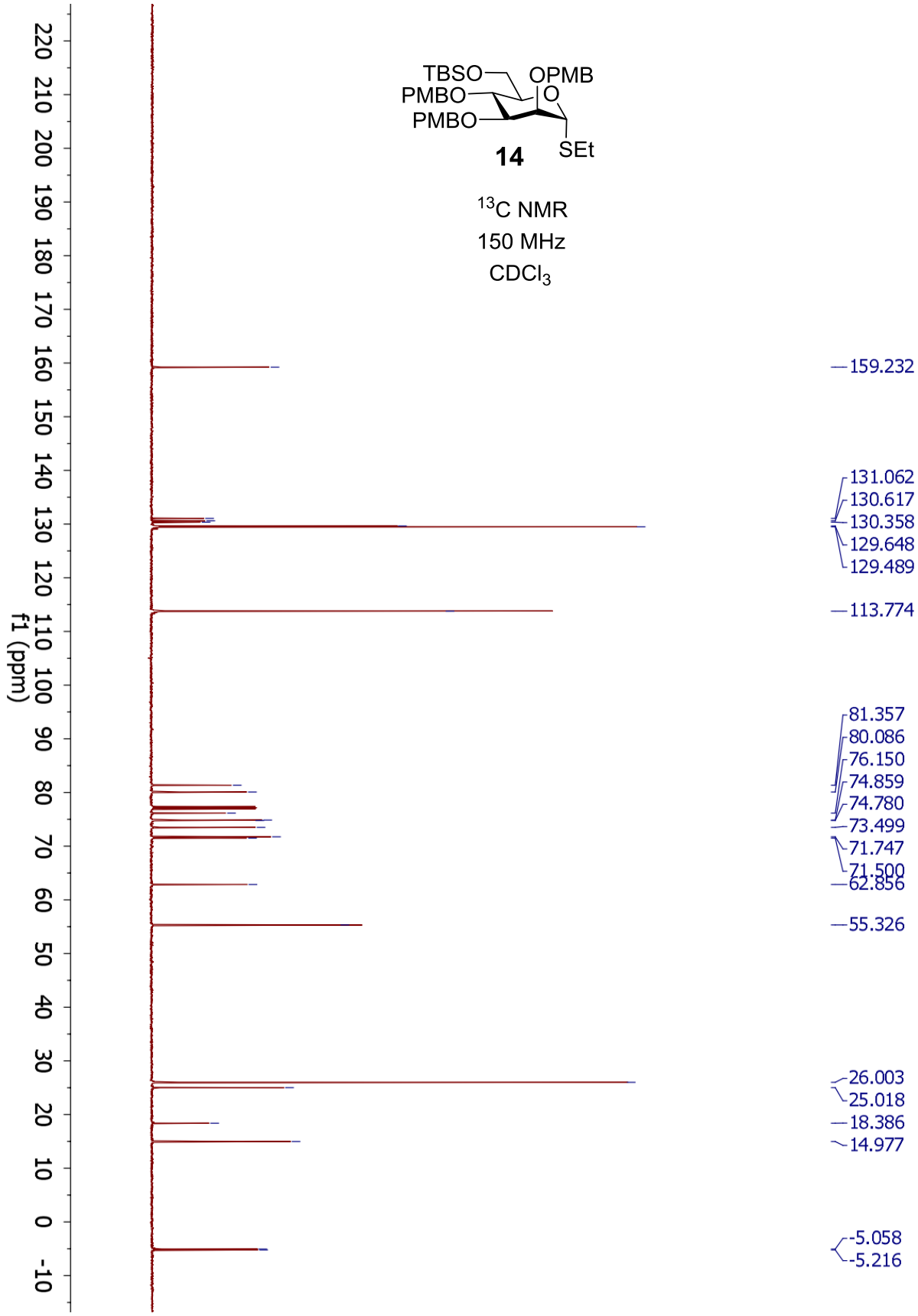

HO
OPMB
PMBO
O
PMBO
SEt
S2
1H NMR
600 MHz
CDCl3
f1 (ppm)
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
6.08
6.00
1.09
1.19
5.26
2.09
12.56
2.17
0.98
3.03
7.284
7.270
7.255
7.241
7.229
7.215
6.864
6.859
6.849
6.840
5.243
4.847
4.829
4.643
4.623
4.613
4.593
4.554
4.537
4.524
4.505
4.496
4.477
3.952
3.933
3.917
3.903
3.798
3.791
3.784
3.779
3.772
3.760
3.755
3.748
3.740
3.735
3.728
2.579
2.566
2.557
2.545
2.532
2.520
2.498
1.942
1.933
1.930
1.920
1.222
1.210
1.198

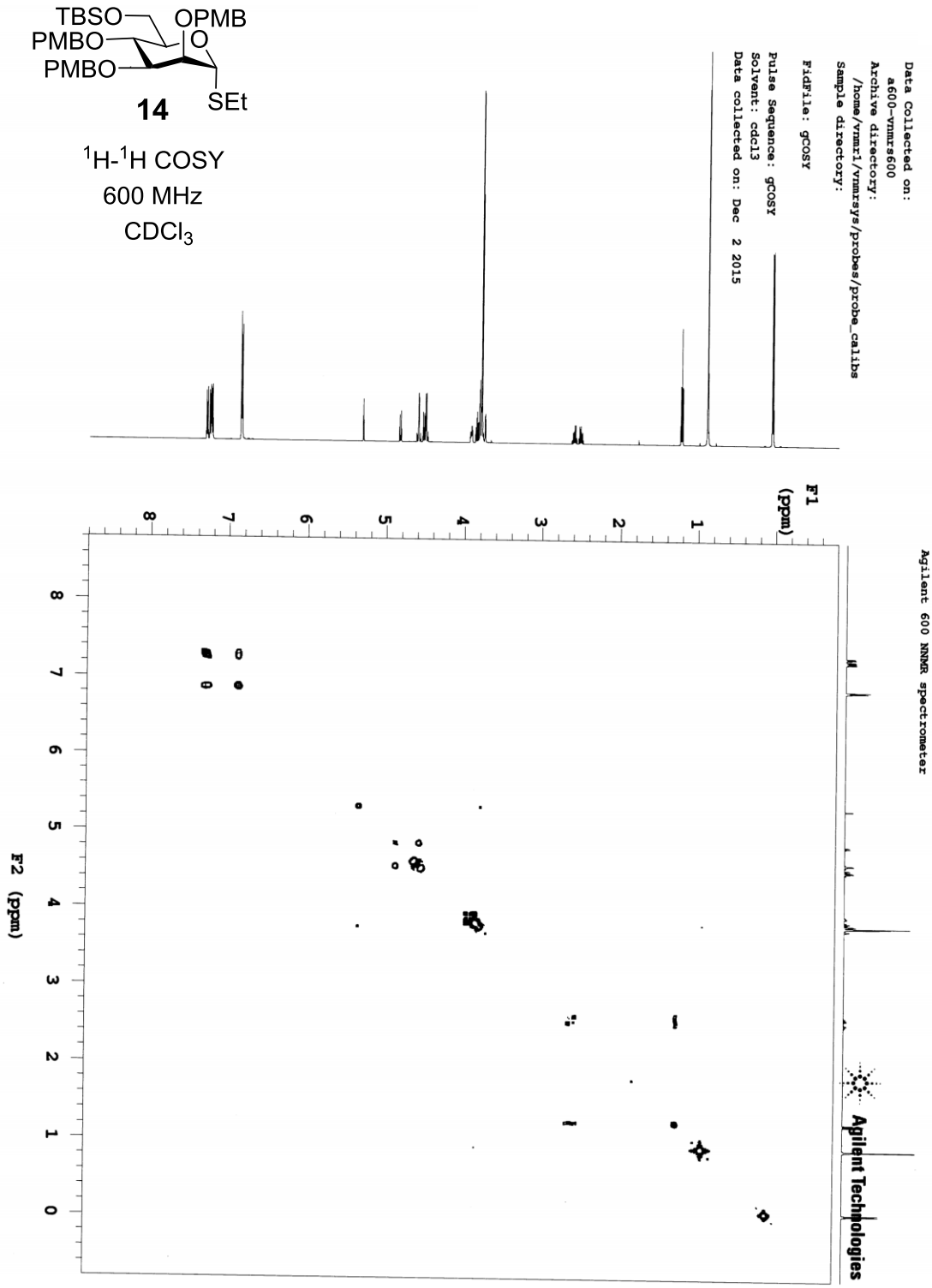

HO
OPMB
PMBO
O
PMBO
SEt
S2
^{13}C NMR
150 MHz
$CDCl_3$
159.409
159.373
159.320
130.740
130.499
130.239
129.804
129.647
129.502
113.935
82.356
80.136
76.164
74.961
74.893
72.477
72.073
71.871
62.574
55.398
25.413
15.006
f1 (ppm)

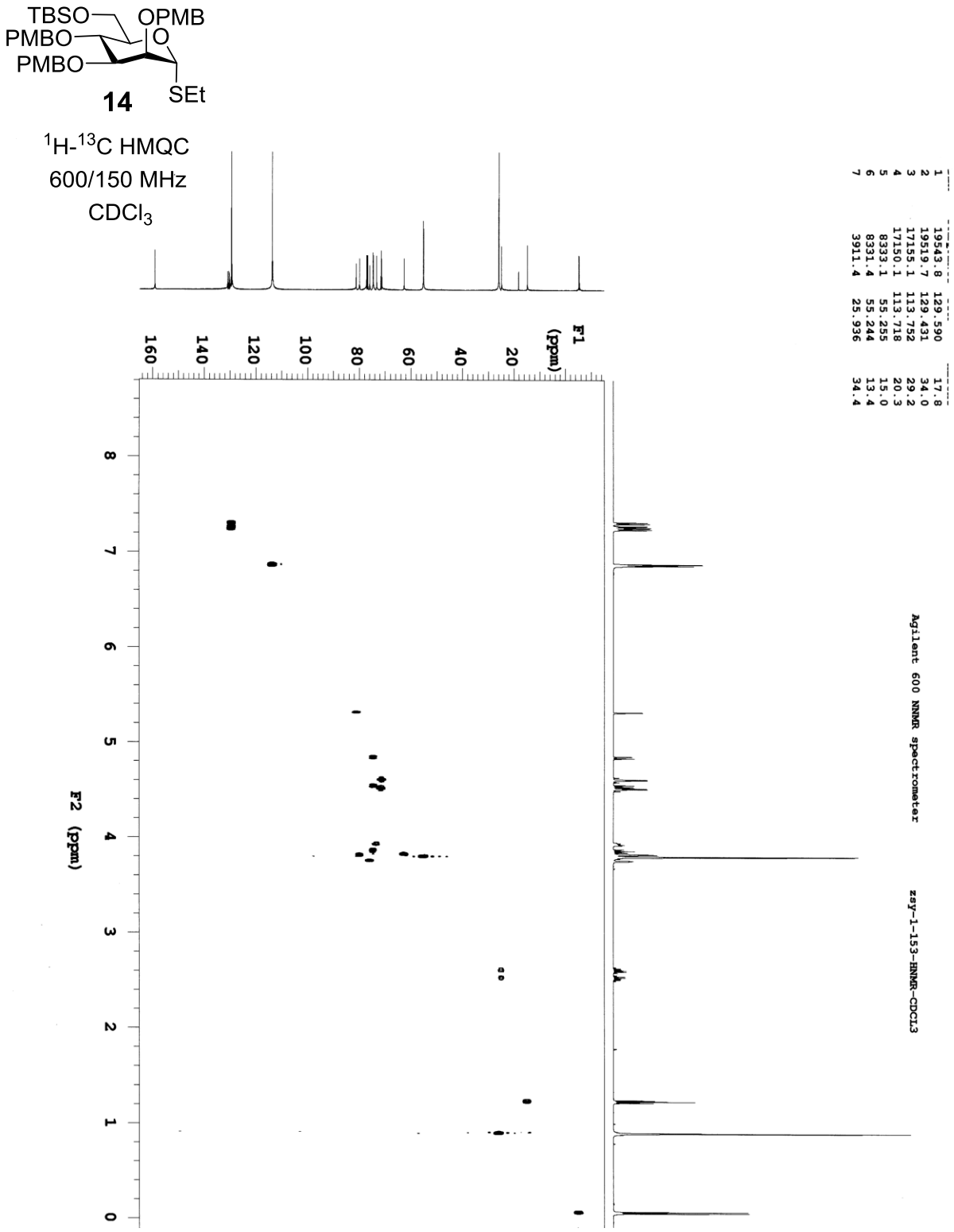

HO
OPMB
PMBO
O
PMBO
SEt
S2
^{1}H-^{1}H COSY
600 MHz
CDCl$_3$
Sample Name:
Data Collected on:
a600-vnmrs600
Archive directory:
/home/vnmrl/vnmrsys/probes/probe_calibs
Sample directory:
FidFile: gCOSY
Pulse Sequence: gCOSY
Solvent: cdcl3
Data collected on: Nov 30 2015
Agilent 600 NMR spectrometer
Agilent Technologies
F1 (ppm)
F2 (ppm)

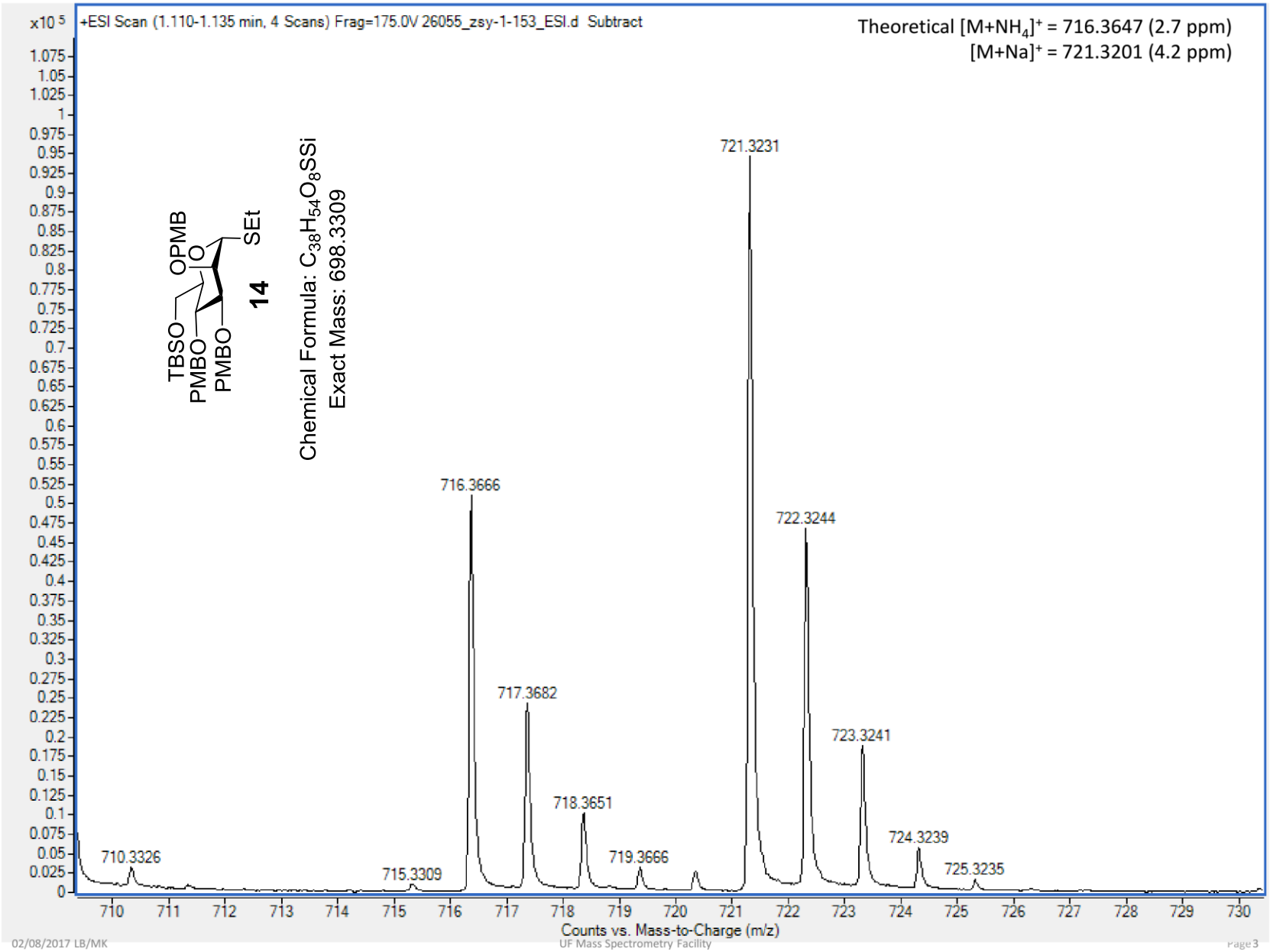

x10 5
+ESI Scan (0.525-0.583 min, 8 Scans) Frag=175.0V 26054_zsy-1-152_ESI.d Subtract
Theoretical [M+NH4]+ = 602.2782 (3.0 ppm)
[M+Na]+ = 607.2326 (1.0 ppm)
607.2332
608.2357
602.2764
609.2345
603.2785
610.1996
604.2698
611.1869
613.1846
Na+ PMBO HO PMBO OPMB O SEt
S2
Chemical Formula: C32H40NaO8S+
Exact Mass: 607.23
Counts vs. Mass-to-Charge (m/z)
02/08/2017 LB/MK
UF Mass Spectrometry Facility
Page 3

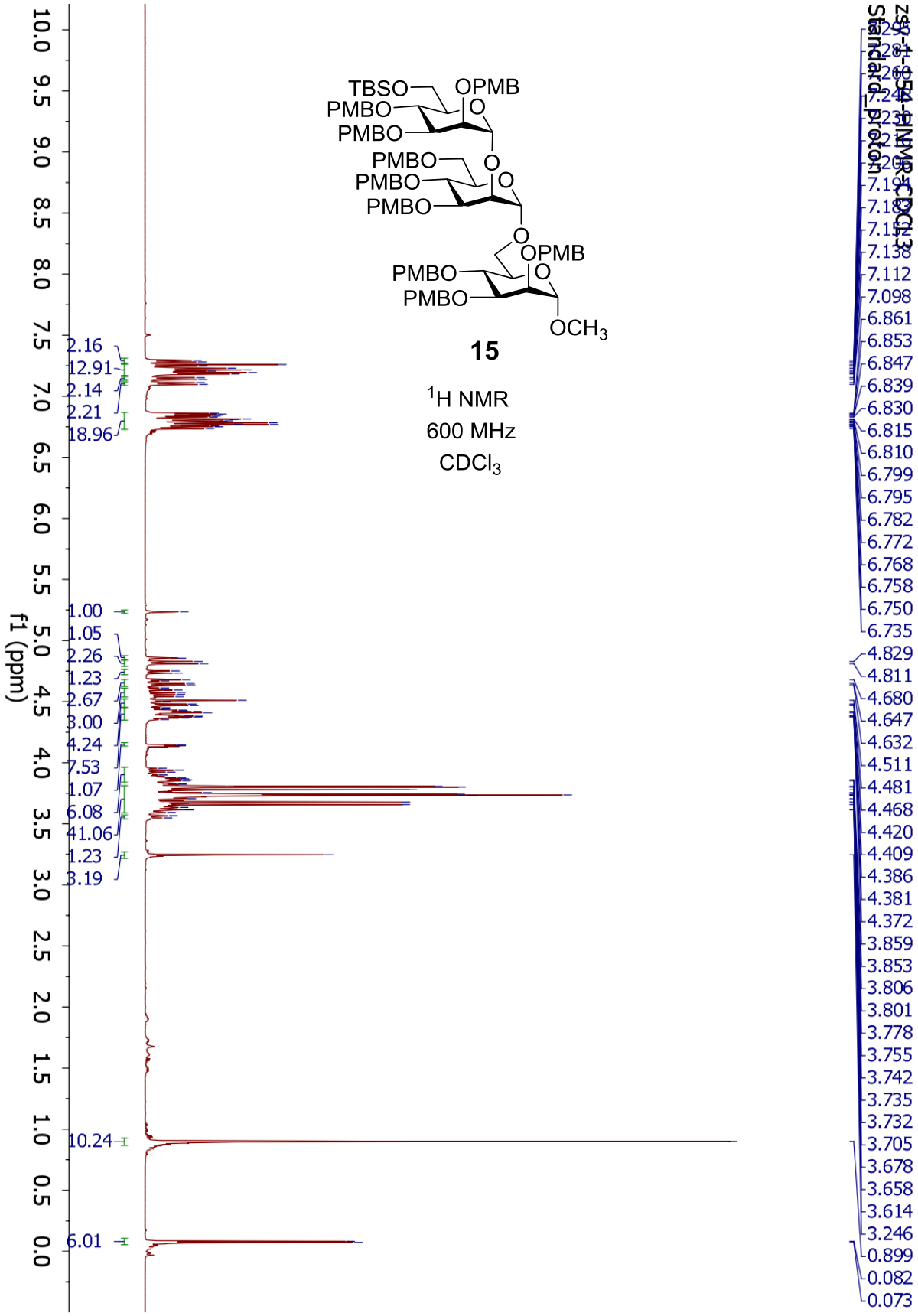

TBSO
OPMB
PMBO
O
PMBO
SEt
14
1H NMR
600 MHz
CDCl3
f1 (ppm)
6.17
6.03
1.08
1.11
2.08
3.10
1.16
12.74
1.14
2.29
3.07
8.95
6.00
7.312
7.298
7.273
7.259
7.251
7.237
6.875
6.858
5.318
4.853
4.835
4.634
4.613
4.606
4.586
4.553
4.536
4.521
4.513
4.494
3.944
3.934
3.928
3.922
3.878
3.862
3.846
3.828
3.823
3.803
3.797
3.754
2.643
2.631
2.621
2.597
2.585
2.546
2.534
2.512
2.500
1.248
1.236
1.223
0.903
0.074
0.061

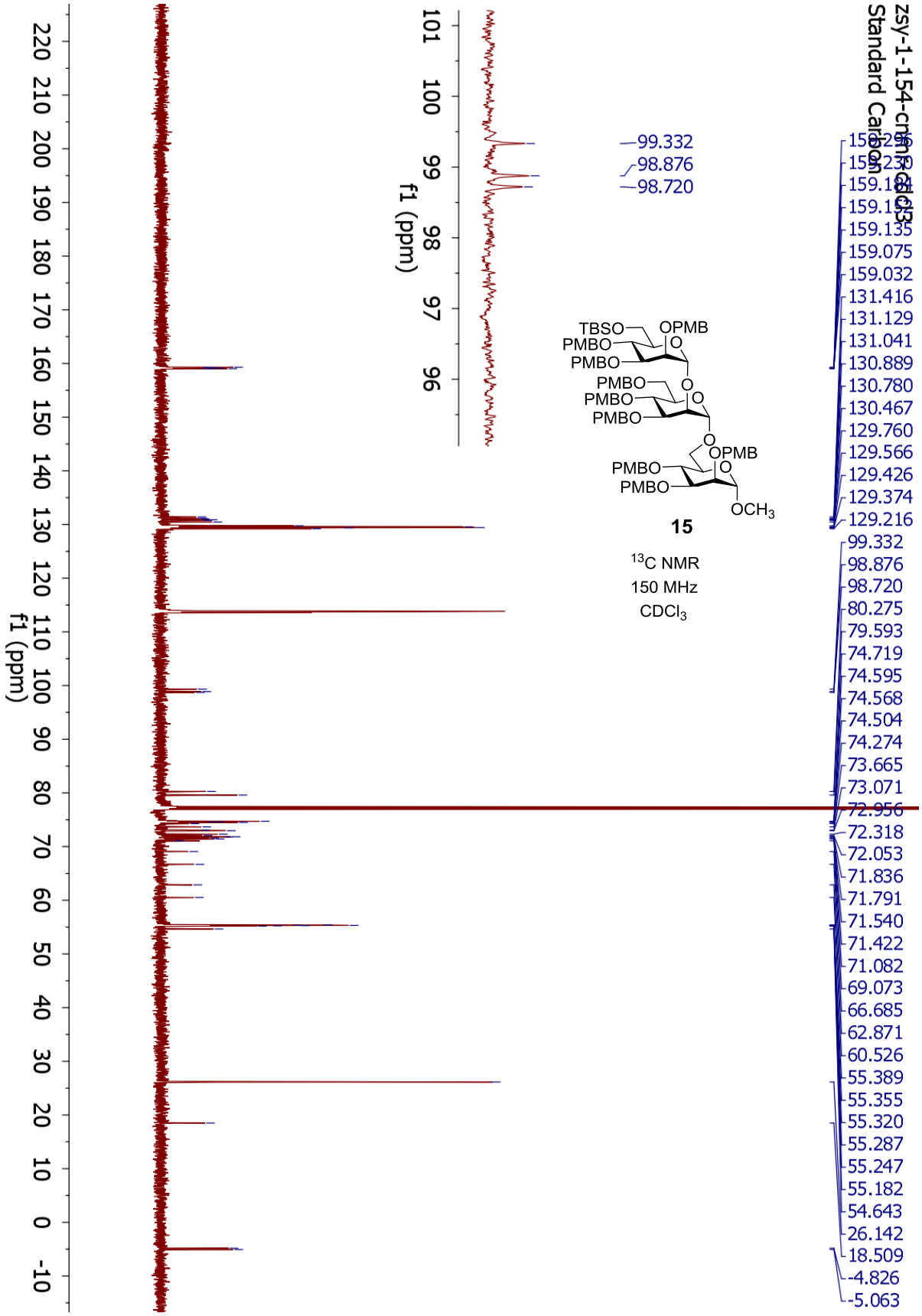

TBSO
OPMB
PMBO
O
PMBO
SEt
14
^{13}C NMR
150 MHz
$CDCl_3$
f1 (ppm)
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
159.232
131.062
130.617
130.358
129.648
129.489
113.774
81.357
80.086
76.150
74.859
74.780
73.499
71.747
71.500
62.856
55.326
26.003
25.018
18.386
14.977
-5.058
-5.216


TBSO
OPMB
PMBO
O
PMBO
SEt
14
^{1}H-^{1}H COSY
600 MHz
$CDCl_3$
Sample Name:
Data Collected on:
a600-vnmrs600
Archive directory:
/home/vnmrl/vnmrsys/probes/probe_calibs
Sample directory:
FidFile: gCOSY
Pulse Sequence: gCOSY
Solvent: cdcl3
Data collected on: Dec 2 2015
Agilent 600 NNMR spectrometer
F1 (ppm)
F2 (ppm)
Agilent Technologies

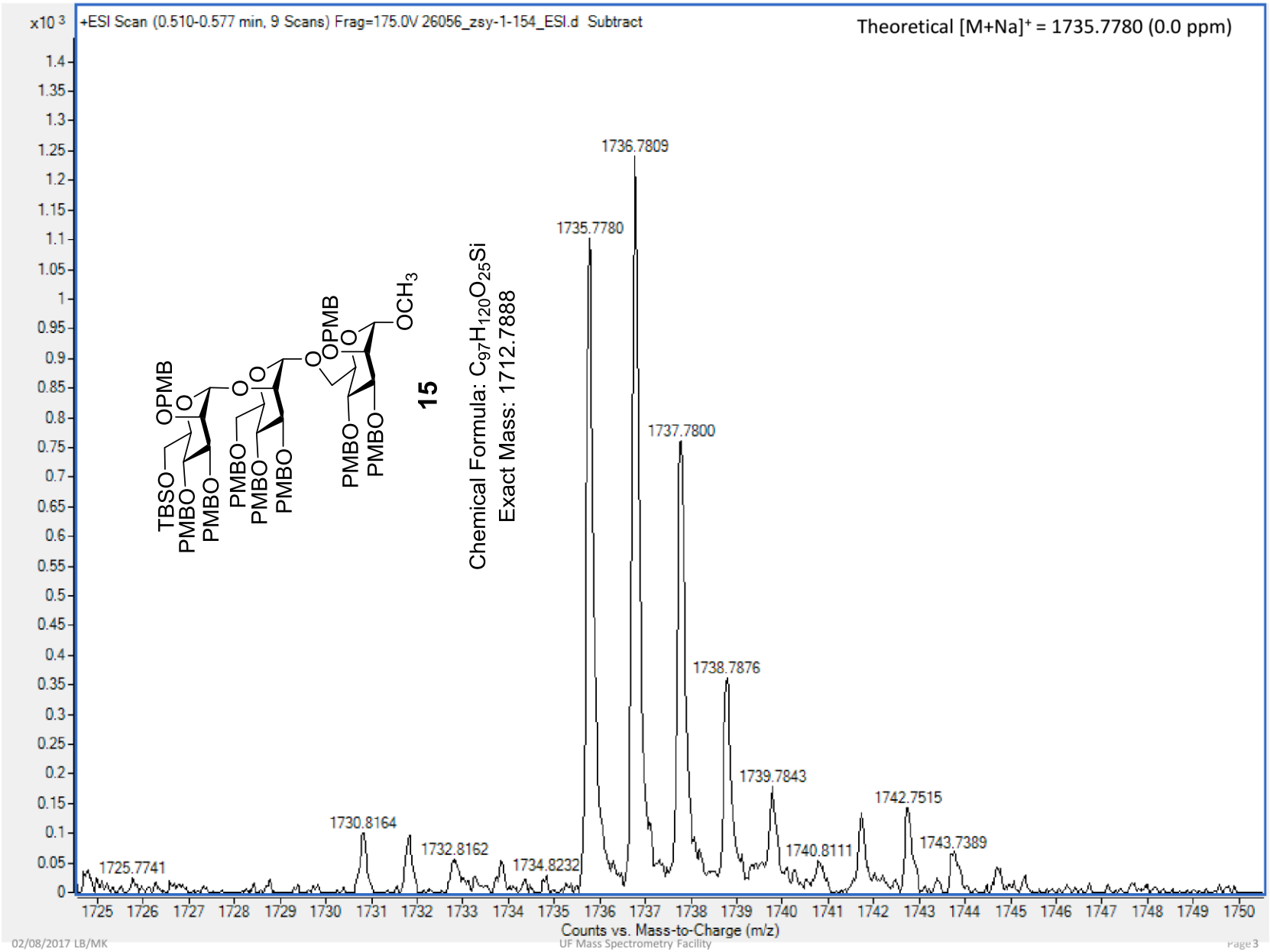

TBSO
OPMB
PMBO
O
PMBO
SEt
14
^{1}H-^{13}C HMQC
600/150 MHz
$CDCl_3$
F1 (ppm)
F2 (ppm)
Agilent 600 NMR spectrometer
zsy-1-153-HNMR-CDCl3

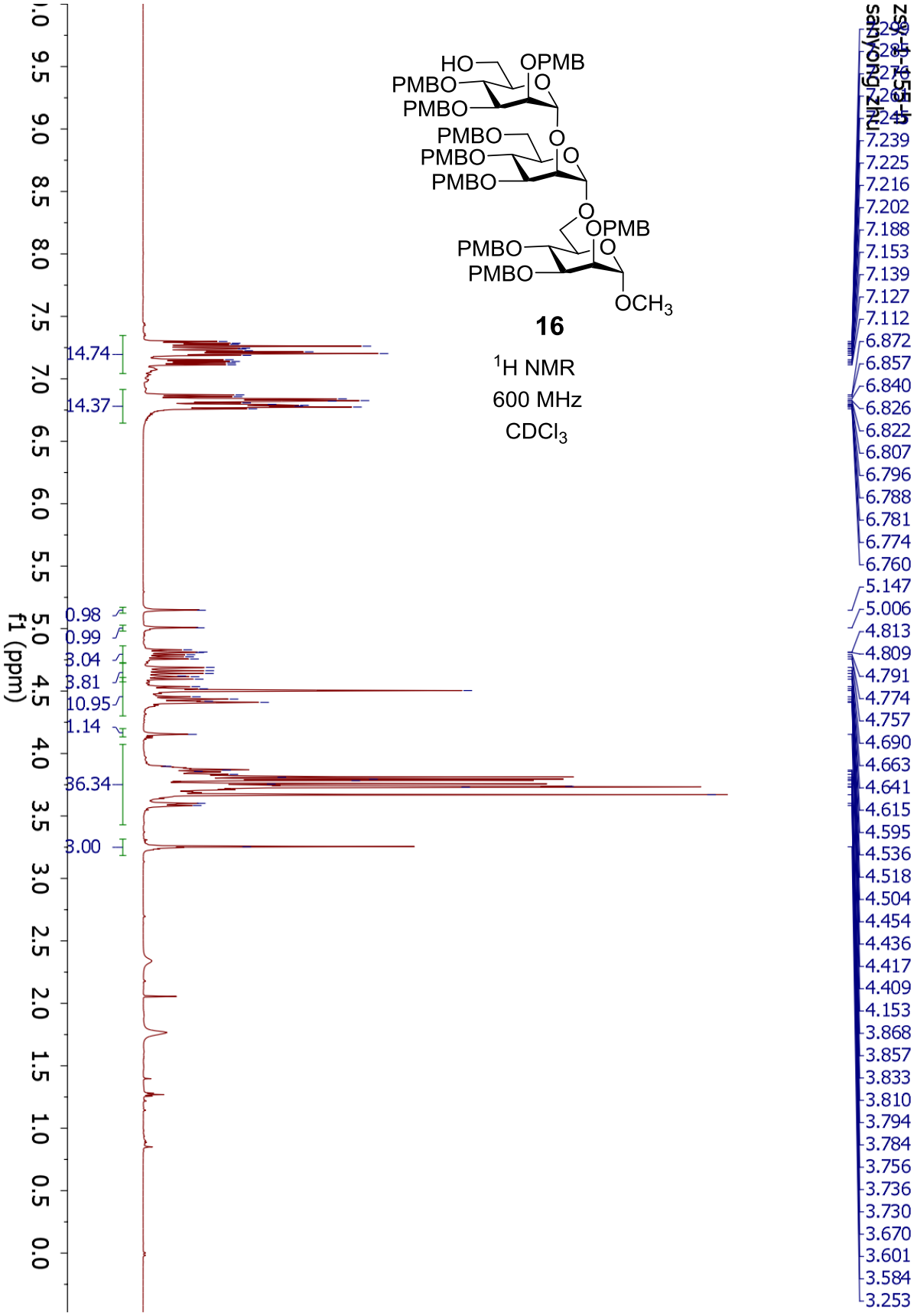

+ESI Scan (1.110-1.135 min, 4 Scans) Frag=175.0V 26055_zsy-1-153_ESI.d Subtract
x10 5
Theoretical [M+NH4]+ = 716.3647 (2.7 ppm)
[M+Na]+ = 721.3201 (4.2 ppm)
TBSO
PMBO
OPMB
O
SEt
PMBO
14
Chemical Formula: C38H54O8SSi
Exact Mass: 698.3309
721.3231
716.3666
722.3244
717.3682
723.3241
718.3651
724.3239
719.3666
725.3235
710.3326
715.3309
Counts vs. Mass-to-Charge (m/z)
02/08/2017 LB/MK
UF Mass Spectrometry Facility

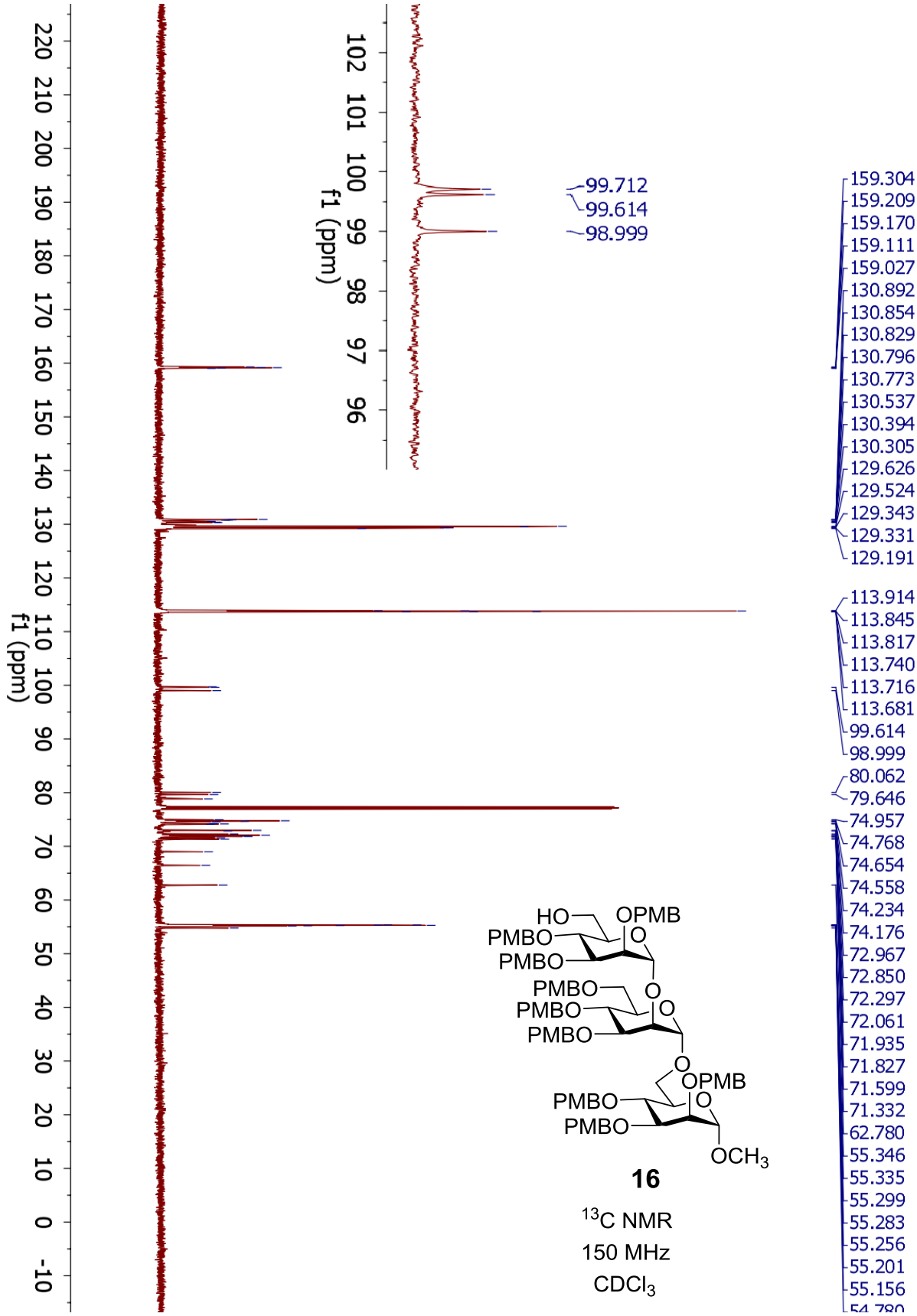

TBSO
OPMB
PMBO
PMBO
PMBO
PMBO
PMBO
OPMB
PMBO
PMBO
OCH3
15
1H NMR
600 MHz
CDCl3
f1 (ppm)
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
2.16
12.91
2.14
2.21
18.96
1.00
1.05
2.26
1.23
2.67
3.00
4.24
7.53
1.07
6.08
41.06
1.23
3.19
10.24
6.01
Standard_proton
7.295
7.281
7.266
7.240
7.225
7.211
7.206
7.192
7.183
7.157
7.138
7.112
7.098
6.861
6.853
6.847
6.839
6.830
6.815
6.810
6.799
6.795
6.782
6.772
6.768
6.758
6.750
6.735
4.829
4.811
4.680
4.647
4.632
4.511
4.481
4.468
4.420
4.409
4.386
4.381
4.372
3.859
3.853
3.806
3.801
3.778
3.755
3.742
3.735
3.732
3.705
3.678
3.658
3.614
3.246
0.899
0.082
0.073

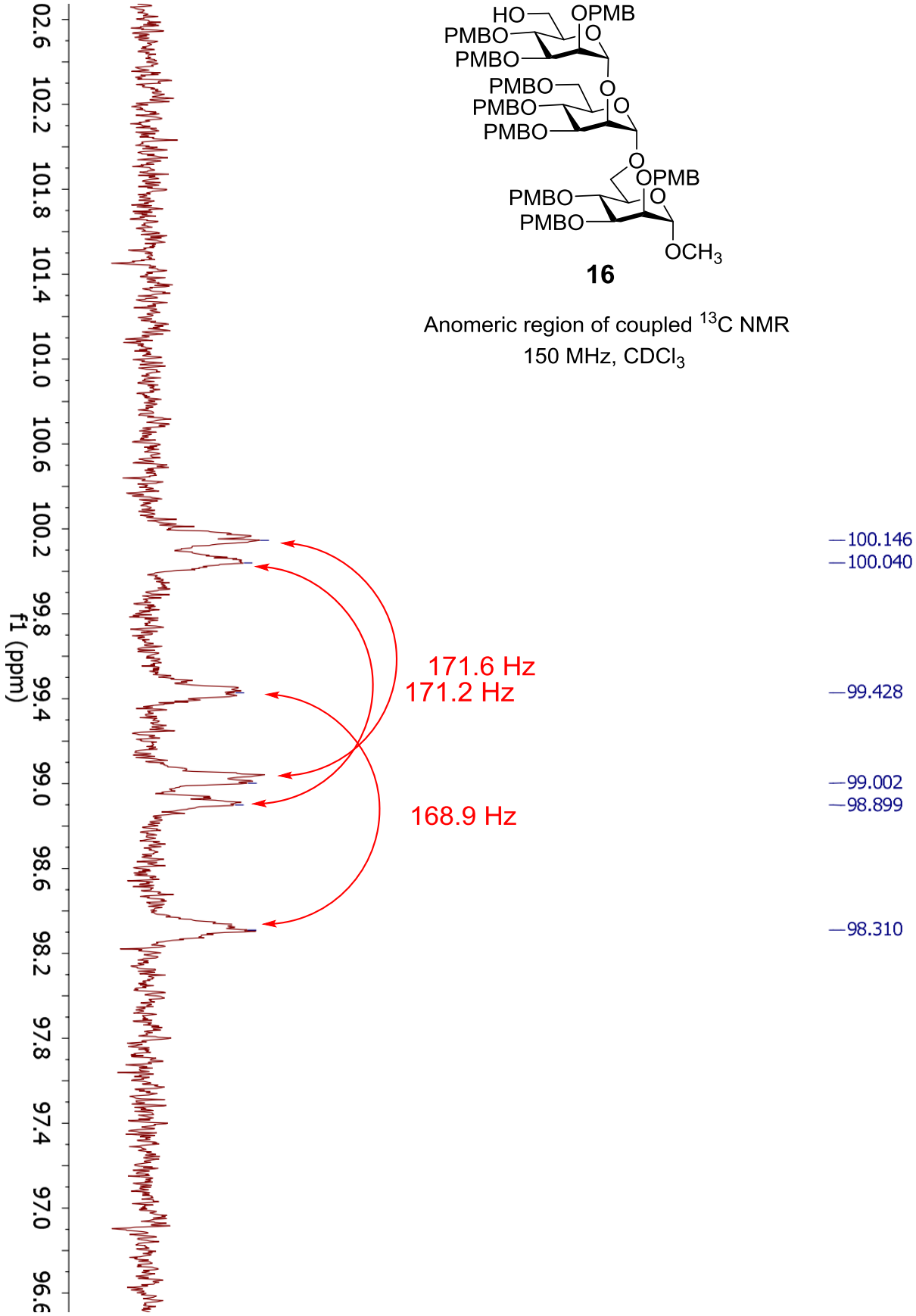

zsy-1-154-cmnr-600-CDCl3
Standard Carbon 2
99.332
98.876
98.720
TBSO
OPMB
PMBO
PMBO
PMBO
PMBO
PMBO
OPMB
PMBO
PMBO
OCH3
15
13C NMR
150 MHz
CDCl3
159.256
159.230
159.184
159.151
159.135
159.075
159.032
131.416
131.129
131.041
130.889
130.780
130.467
129.760
129.566
129.426
129.374
129.216
99.332
98.876
98.720
80.275
79.593
74.719
74.595
74.568
74.504
74.274
73.665
73.071
72.956
72.318
72.053
71.836
71.791
71.540
71.422
71.082
69.073
66.685
62.871
60.526
55.389
55.355
55.320
55.287
55.247
55.182
54.643
26.142
18.509
-4.826
-5.063
f1 (ppm)
f1 (ppm)

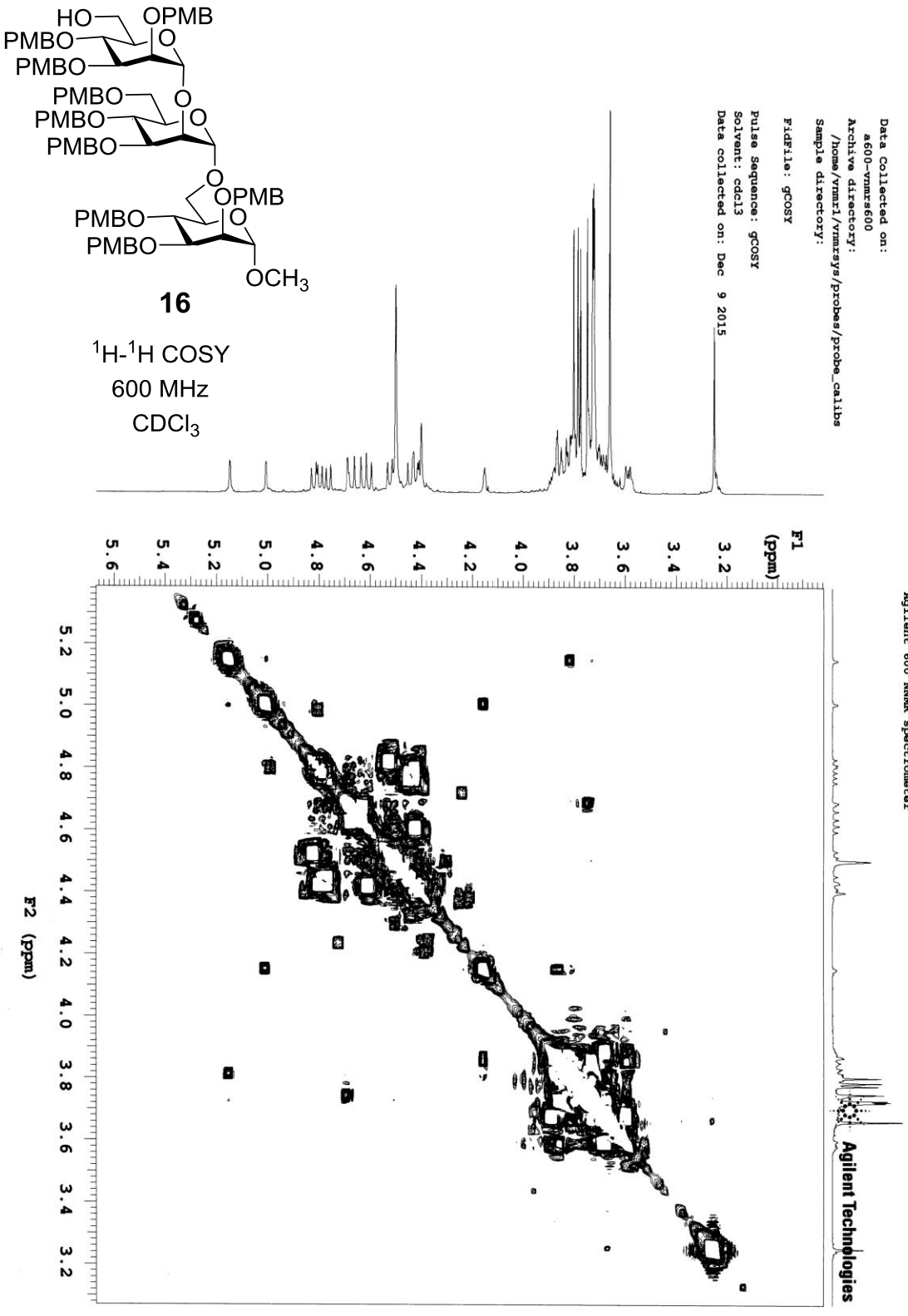
TBSO
OPMB
PMBO
PMBO
PMBO
PMBO
PMBO
PMBO
OPMB
PMBO
PMBO
OCH3
15
1H-13C HMQC
600/150 MHz
CDCl3
F1 (ppm)
F2 (ppm)
Agilent 600 NMNR spectrometer
zsy-1-154-HNMR-CDCL3

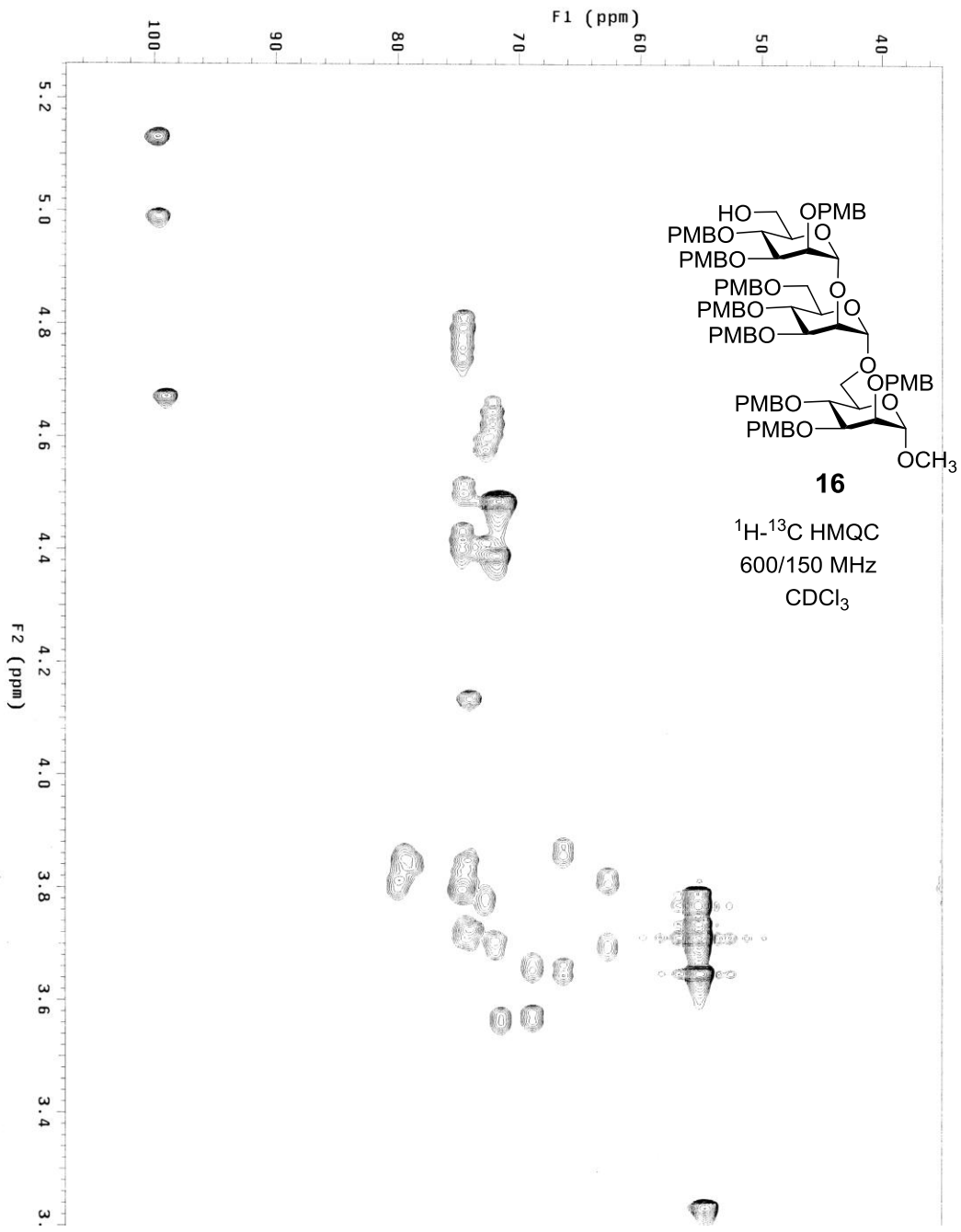

+ESI Scan (0.510-0.577 min, 9 Scans) Frag=175.0V 26056_zsy-1-154_ESI.d Subtract
Theoretical [M+Na]+ = 1735.7780 (0.0 ppm)
x10 3
1736.7809
1735.7780
1737.7800
1738.7876
1739.7843
1742.7515
1743.7389
1740.8111
1730.8164
1732.8162
1734.8232
1725.7741
Counts vs. Mass-to-Charge (m/z)
15
Chemical Formula: C97H120O25Si
Exact Mass: 1712.7888
TBSO
PMBO
PMBO
OPMB
PMBO
PMBO
PMBO
OPMB
PMBO
PMBO
OCH3

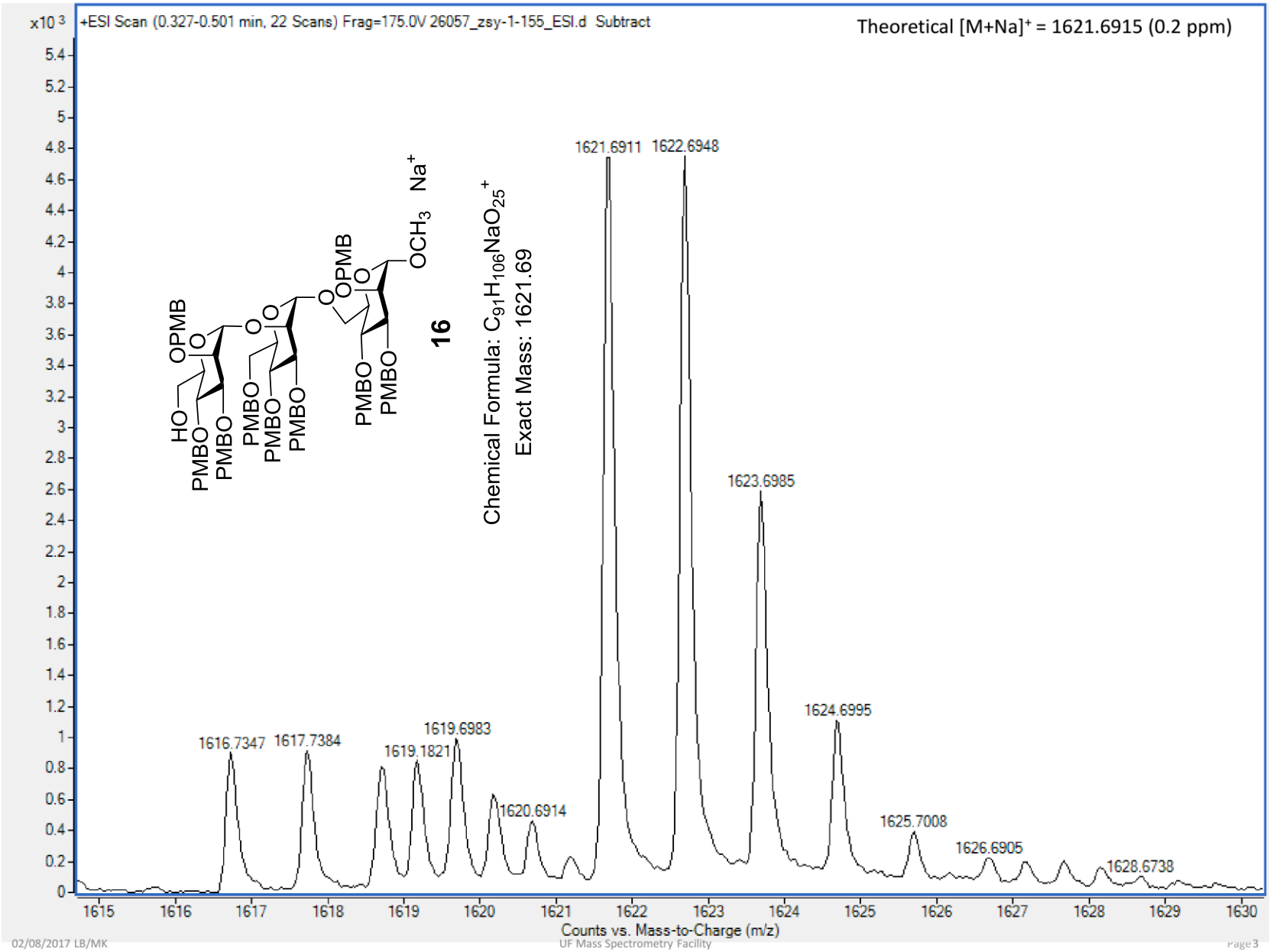

HO
OPMB
PMBO
PMBO
PMBO
PMBO
PMBO
OPMB
PMBO
PMBO
OCH3
16
1H NMR
600 MHz
CDCl3
f1 (ppm)
14.74
14.37
0.98
0.99
3.04
3.81
10.95
1.14
36.34
3.00

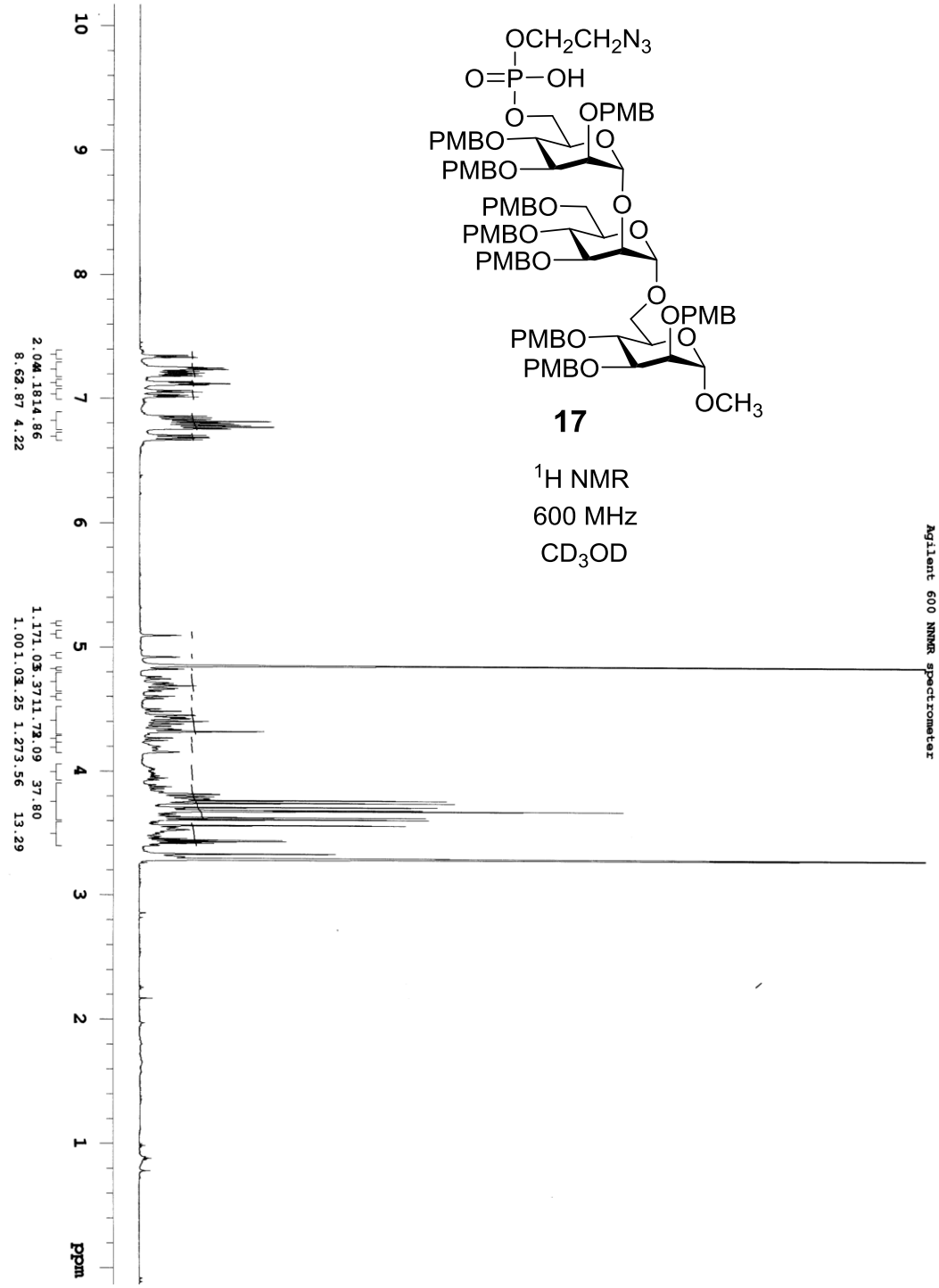

f1 (ppm)
99.712
99.614
98.999
159.304
159.209
159.170
159.111
159.027
130.892
130.854
130.829
130.796
130.773
130.537
130.394
130.305
129.626
129.524
129.343
129.331
129.191
113.914
113.845
113.817
113.740
113.716
113.681
99.614
98.999
80.062
79.646
74.957
74.768
74.654
74.558
74.234
74.176
72.967
72.850
72.297
72.061
71.935
71.827
71.599
71.332
62.780
55.346
55.335
55.299
55.283
55.256
55.201
55.156
HO
OPMB
PMBO
PMBO
PMBO
PMBO
PMBO
O
OPMB
PMBO
PMBO
OCH3
16
13C NMR
150 MHz
CDCl3

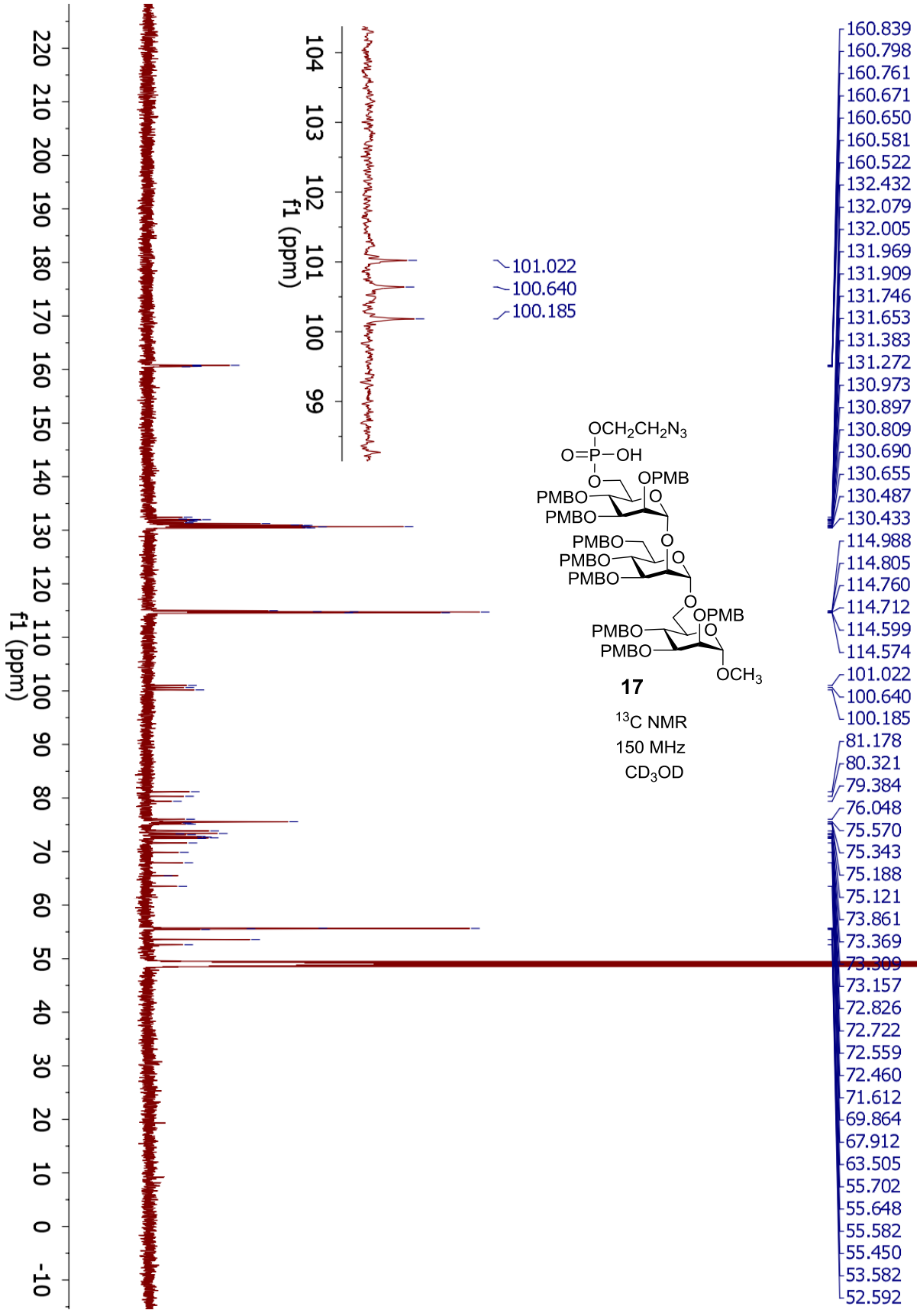

HO
OPMB
PMBO
PMBO
PMBO
PMBO
PMBO
O
OPMB
PMBO
PMBO
OCH3
16
Anomeric region of coupled 13C NMR
150 MHz, CDCl3
171.6 Hz
171.2 Hz
168.9 Hz
100.146
100.040
99.428
99.002
98.899
98.310
f1 (ppm)
102.6
102.2
101.8
101.4
101.0
100.6
100.2
99.8
99.4
99.0
98.6
98.2
97.8
97.4
97.0
96.6

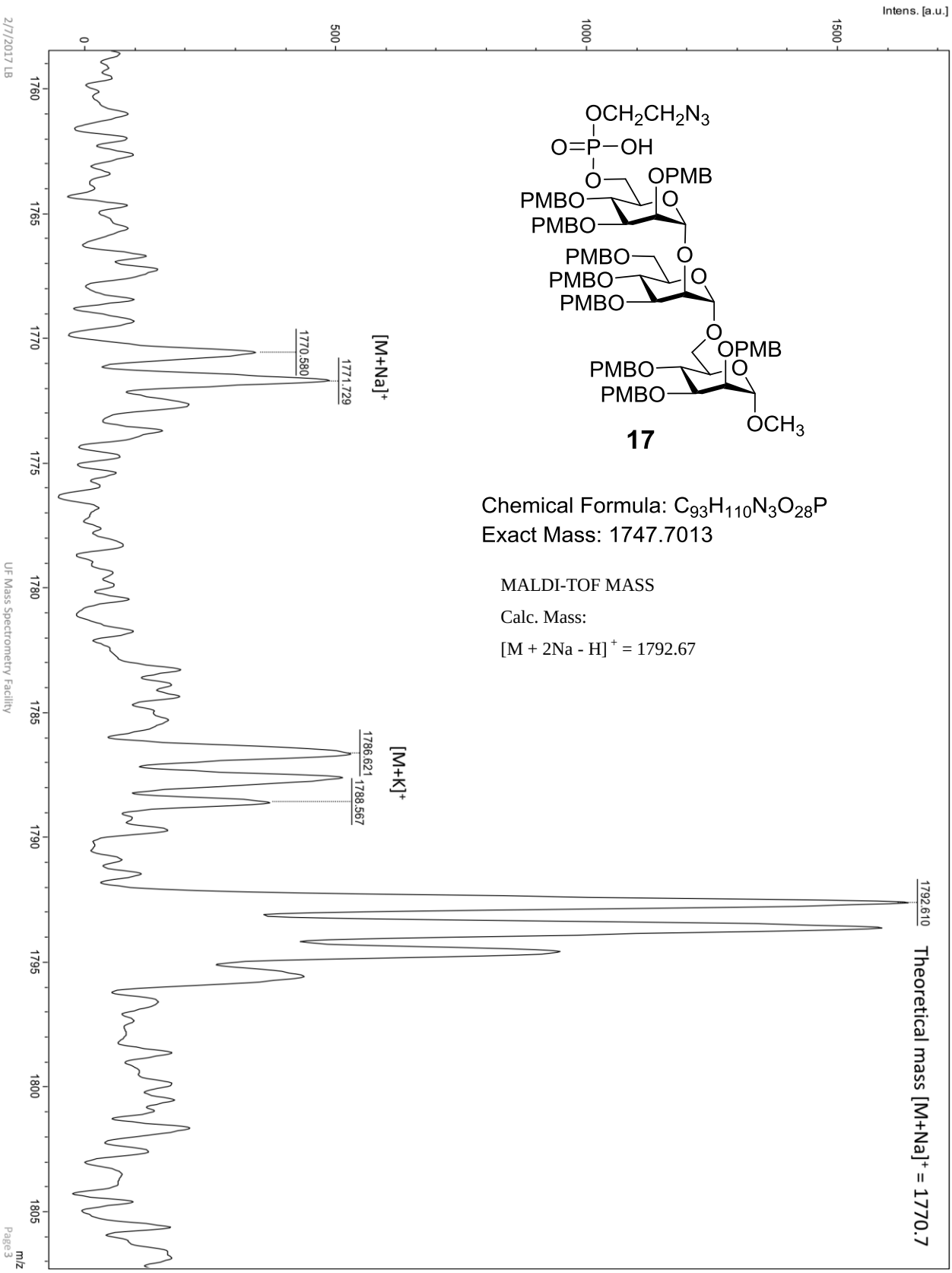

HO
OPMB
PMBO
PMBO
PMBO
O
PMBO
PMBO
PMBO
O
O
OPMB
PMBO
PMBO
O
OCH3
16
1H-1H COSY
600 MHz
CDCl3
Sample Name:
Data Collected on:
a600-vnmrs600
Archive directory:
/home/vnmrl/vnmrsys/probes/probe_calibs
Sample directory:
FidFile: gCOSY
Pulse Sequence: gCOSY
Solvent: cdcl3
Data collected on: Dec 9 2015
Agilent 600 NMR spectrometer
Agilent Technologies
F1 (ppm)
F2 (ppm)

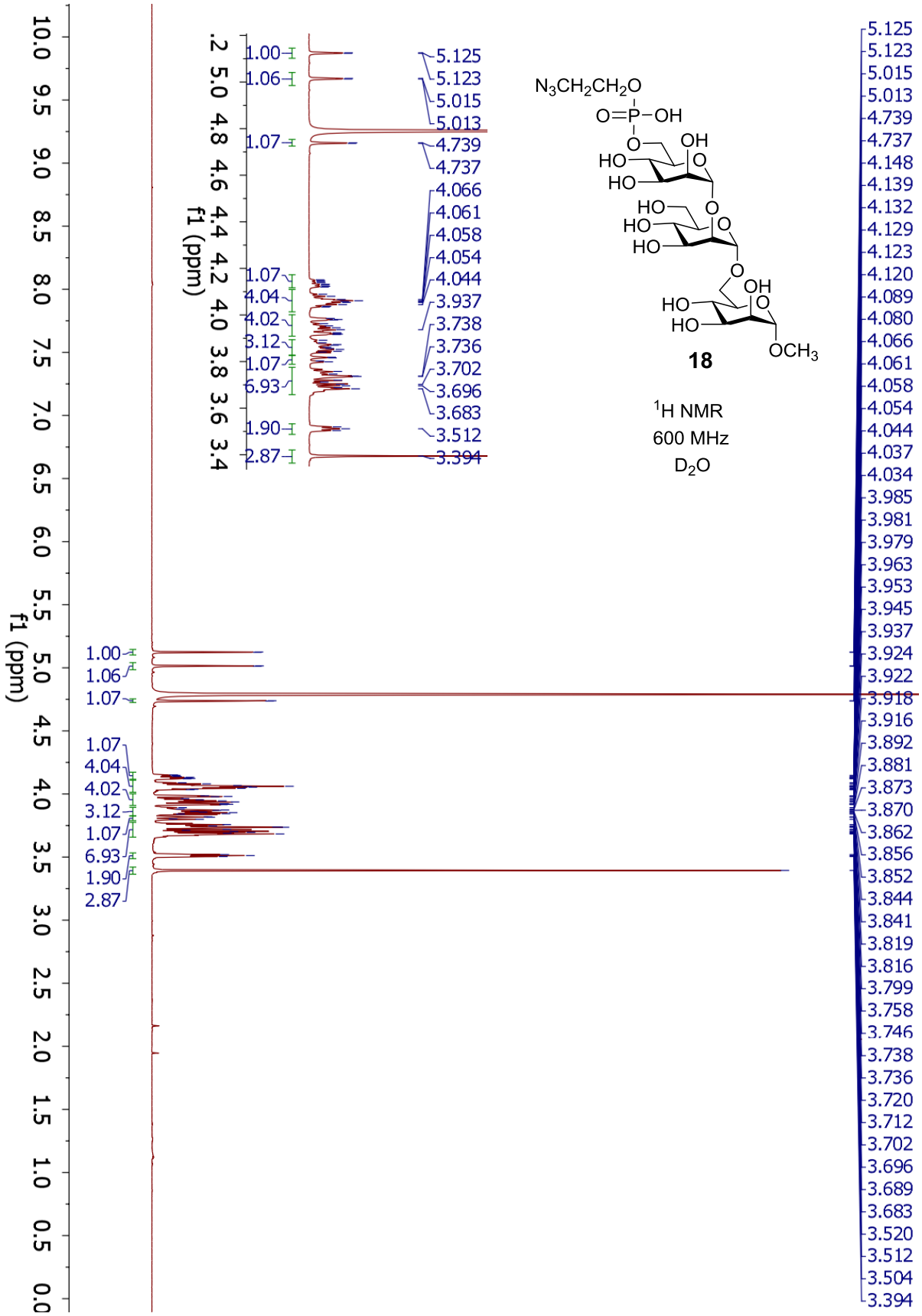

F1 (ppm)
F2 (ppm)
HO
OPMB
PMBO
PMBO
PMBO
PMBO
PMBO
PMBO
OPMB
PMBO
PMBO
OCH3
16
1H-13C HMQC
600/150 MHz
CDCl3

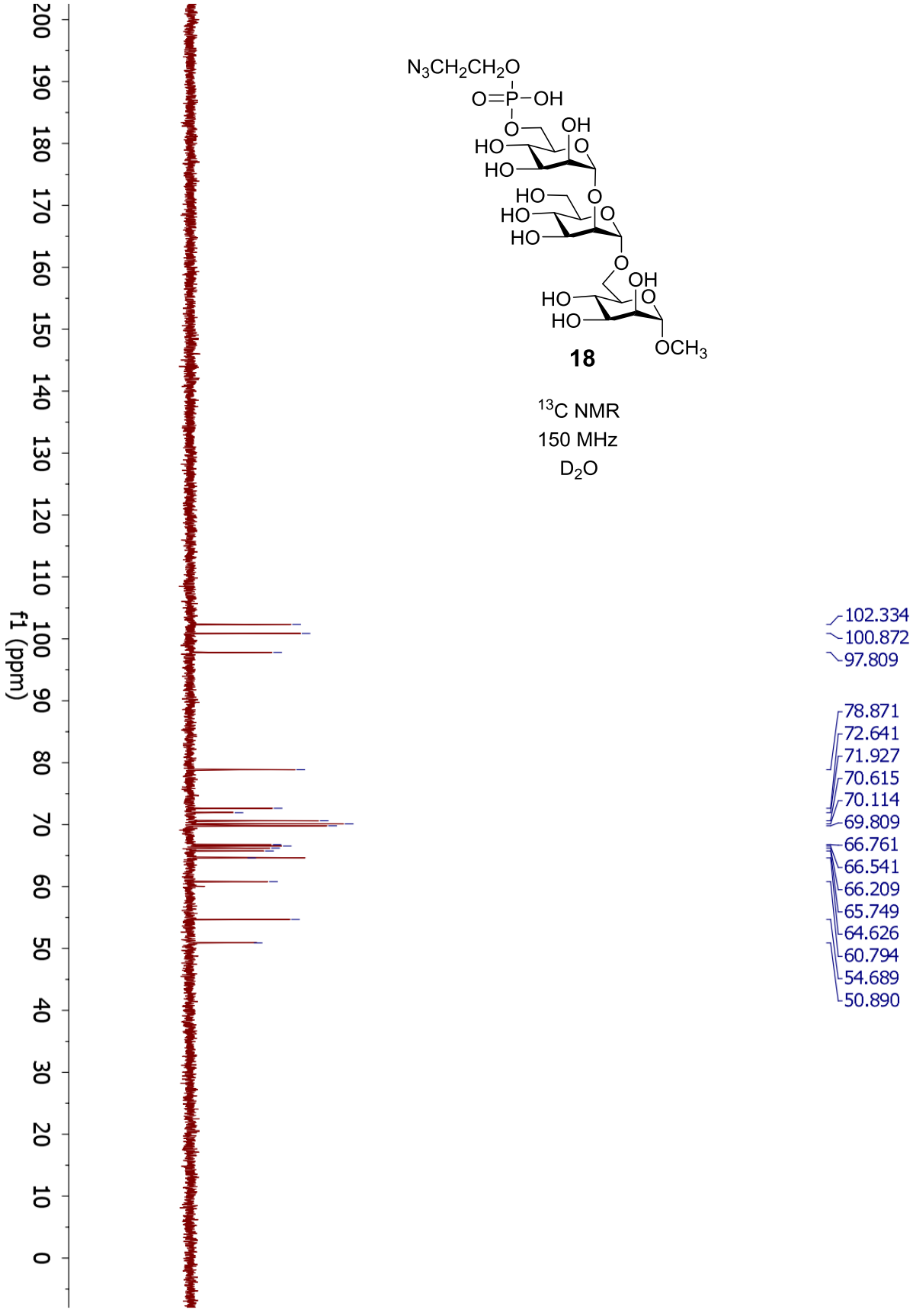

+ESI Scan (0.327-0.501 min, 22 Scans) Frag=175.0V 26057_zsy-1-155_ESI.d Subtract
Theoretical [M+Na]+ = 1621.6915 (0.2 ppm)
x10 3
Chemical Formula: C91H106NaO25+
Exact Mass: 1621.69
16
1621.6911
1622.6948
1623.6985
1624.6995
1625.7008
1626.6905
1628.6738
1616.7347
1617.7384
1619.1821
1619.6983
1620.6914
Counts vs. Mass-to-Charge (m/z)
02/08/2017 LB/MK
UF Mass Spectrometry Facility


OCH2CH2N3
O=P–OH
O
OPMB
PMBO
PMBO
PMBO
O
PMBO
PMBO
PMBO
O
O
OPMB
PMBO
PMBO
O
OCH3
17
1H NMR
600 MHz
CD3OD
Agilent 600 NMNR spectrometer
ppm


f1 (ppm)
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
104 103 102 101 100 99
f1 (ppm)
101.022
100.640
100.185
160.839
160.798
160.761
160.671
160.650
160.581
160.522
132.432
132.079
132.005
131.969
131.909
131.746
131.653
131.383
131.272
130.973
130.897
130.809
130.690
130.655
130.487
130.433
114.988
114.805
114.760
114.712
114.599
114.574
101.022
100.640
100.185
81.178
80.321
79.384
76.048
75.570
75.343
75.188
75.121
73.861
73.369
73.309
73.157
72.826
72.722
72.559
72.460
71.612
69.864
67.912
63.505
55.702
55.648
55.582
55.450
53.582
52.592
OCH2CH2N3
O=P–OH
O
OPMB
PMBO
PMBO
PMBO
PMBO
PMBO
O
O
O
OPMB
PMBO
PMBO
O
OCH3
17
13C NMR
150 MHz
CD3OD


Intens. [a.u.]
0
500
1000
1500
1760
1765
1770
1775
1780
1785
1790
1795
1800
1805
m/z
1770.580
1771.729
[M+Na]+
1786.621
1788.567
[M+K]+
1792.610
Theoretical mass [M+Na]+ = 1770.7
OCH2CH2N3
O=P−OH
O
OPMB
PMBO
PMBO
O
PMBO
PMBO
PMBO
O
O
OPMB
PMBO
PMBO
O
OCH3
17
Chemical Formula: C93H110N3O28P
Exact Mass: 1747.7013
MALDI-TOF MASS
Calc. Mass:
[M + 2Na - H]+ = 1792.67
2/7/2017 LB
UF Mass Spectrometry Facility
Page 3


18
1H NMR
600 MHz
D2O
f1 (ppm)

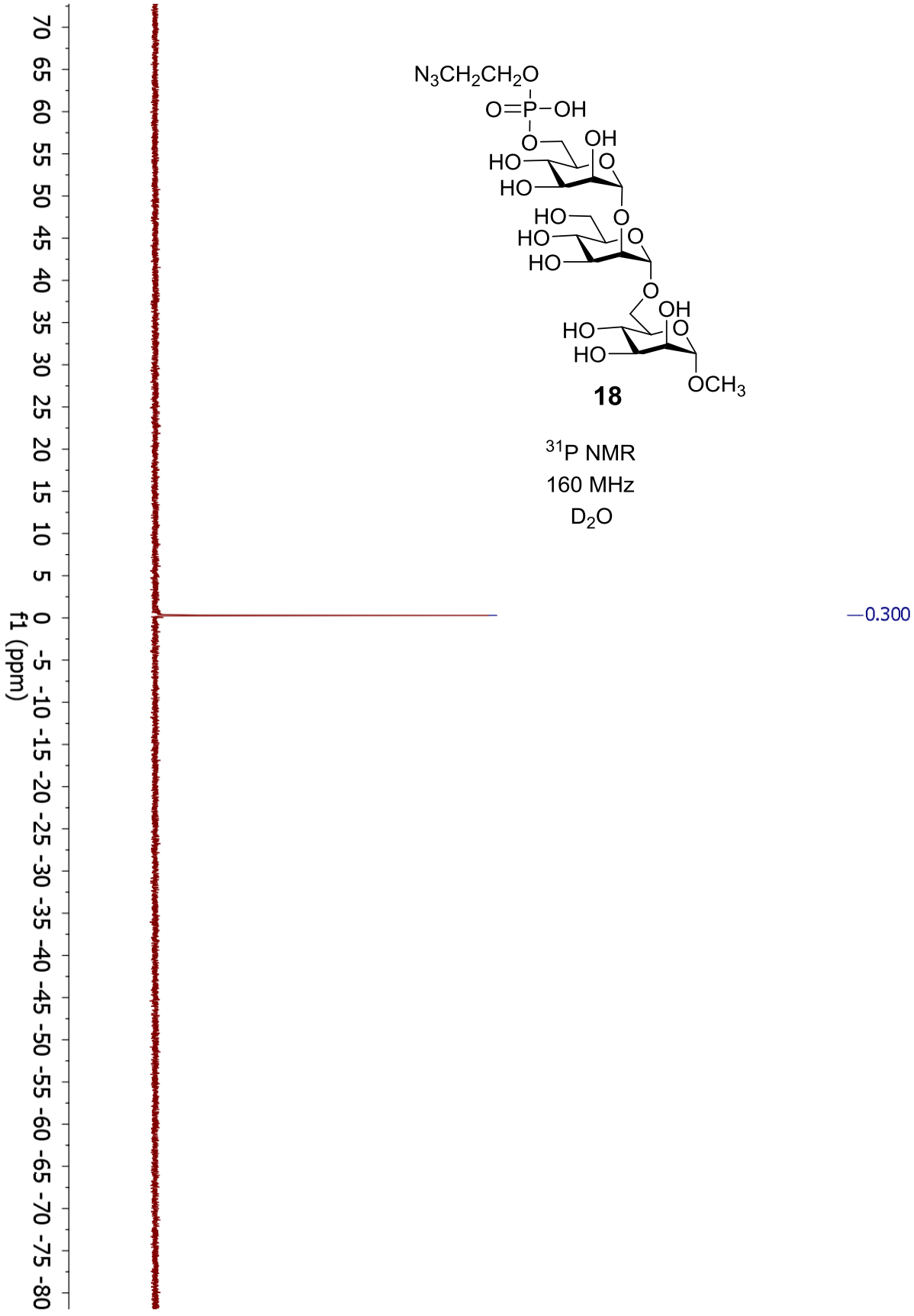


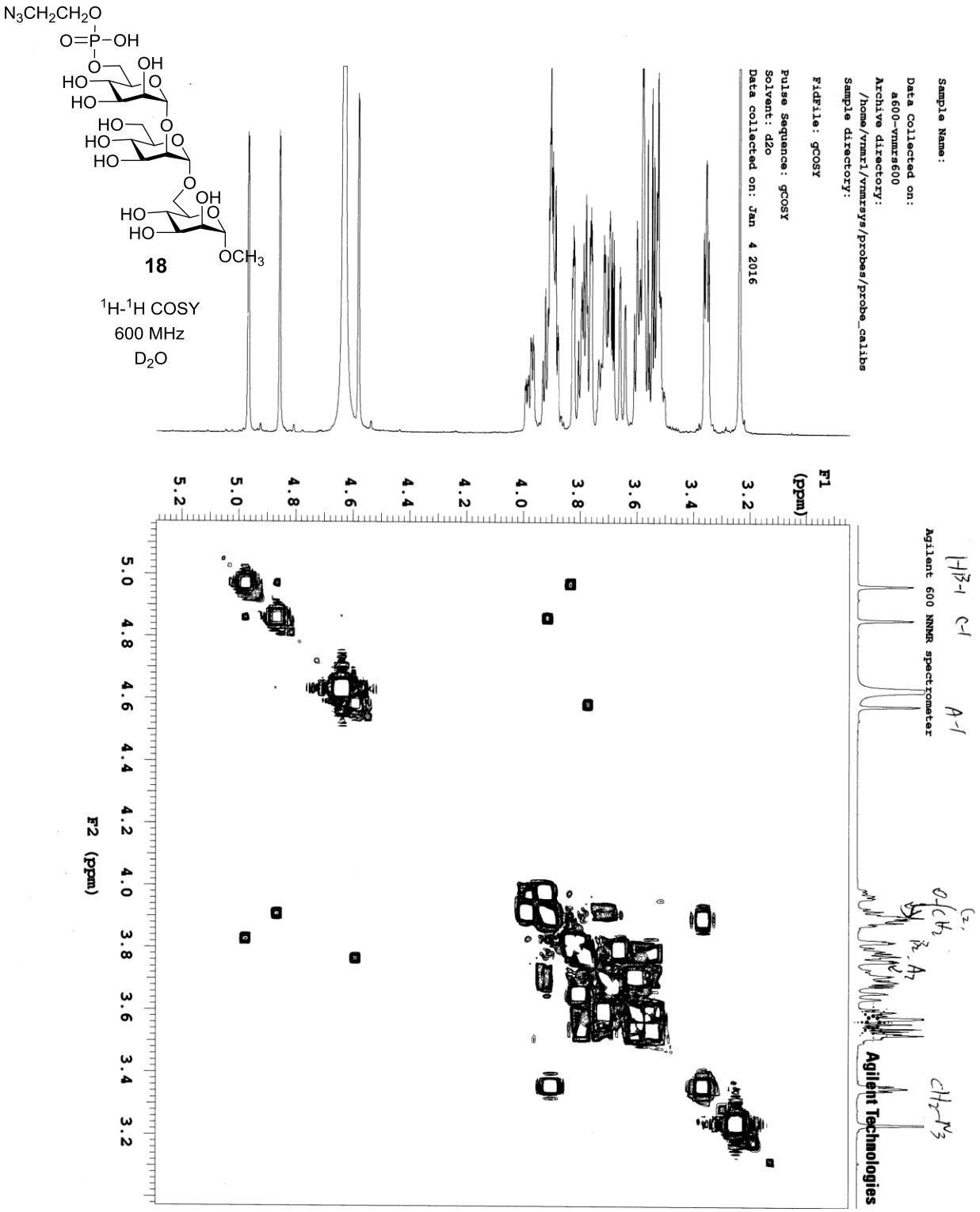
18

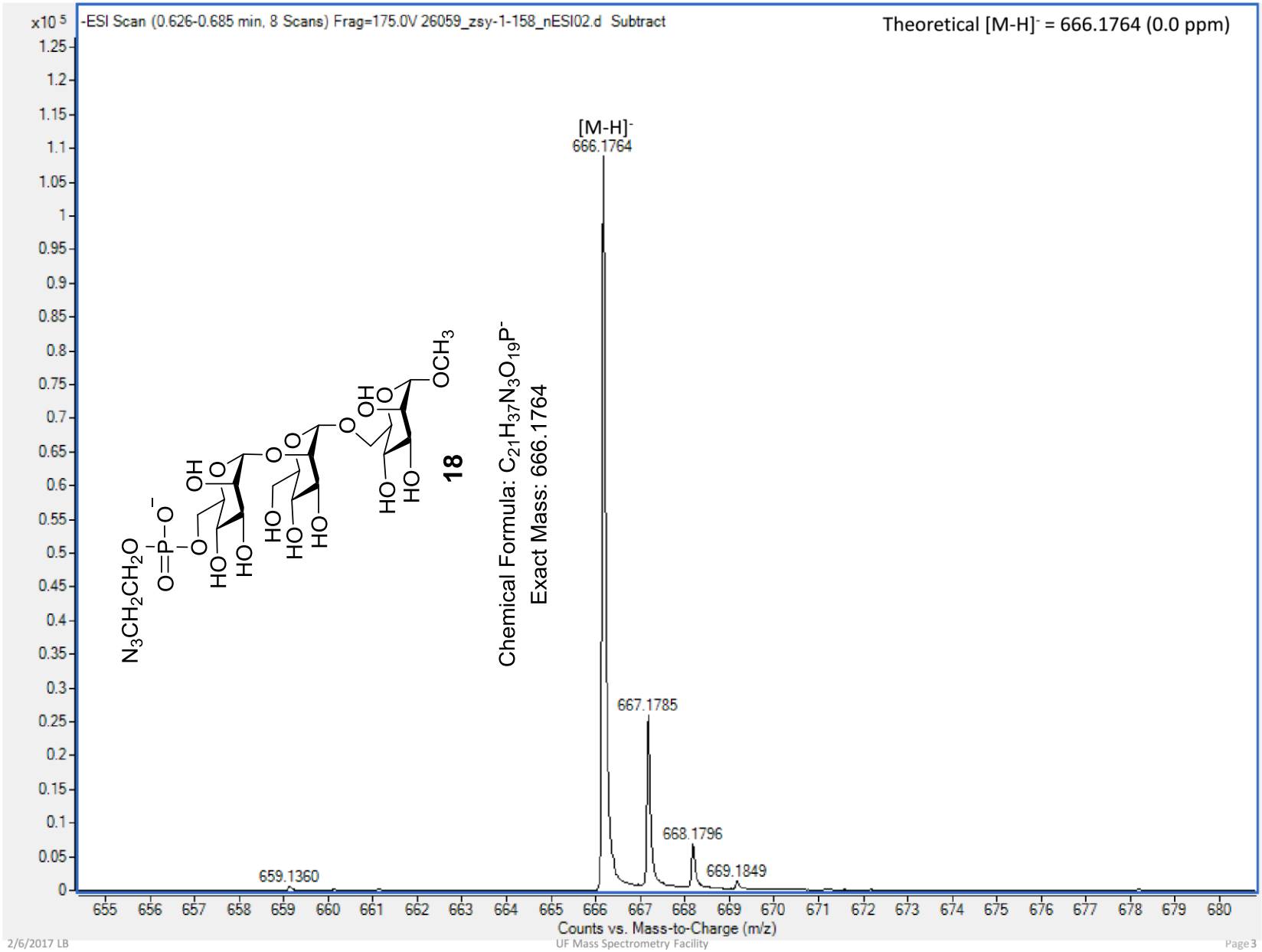
^{13}C NMR

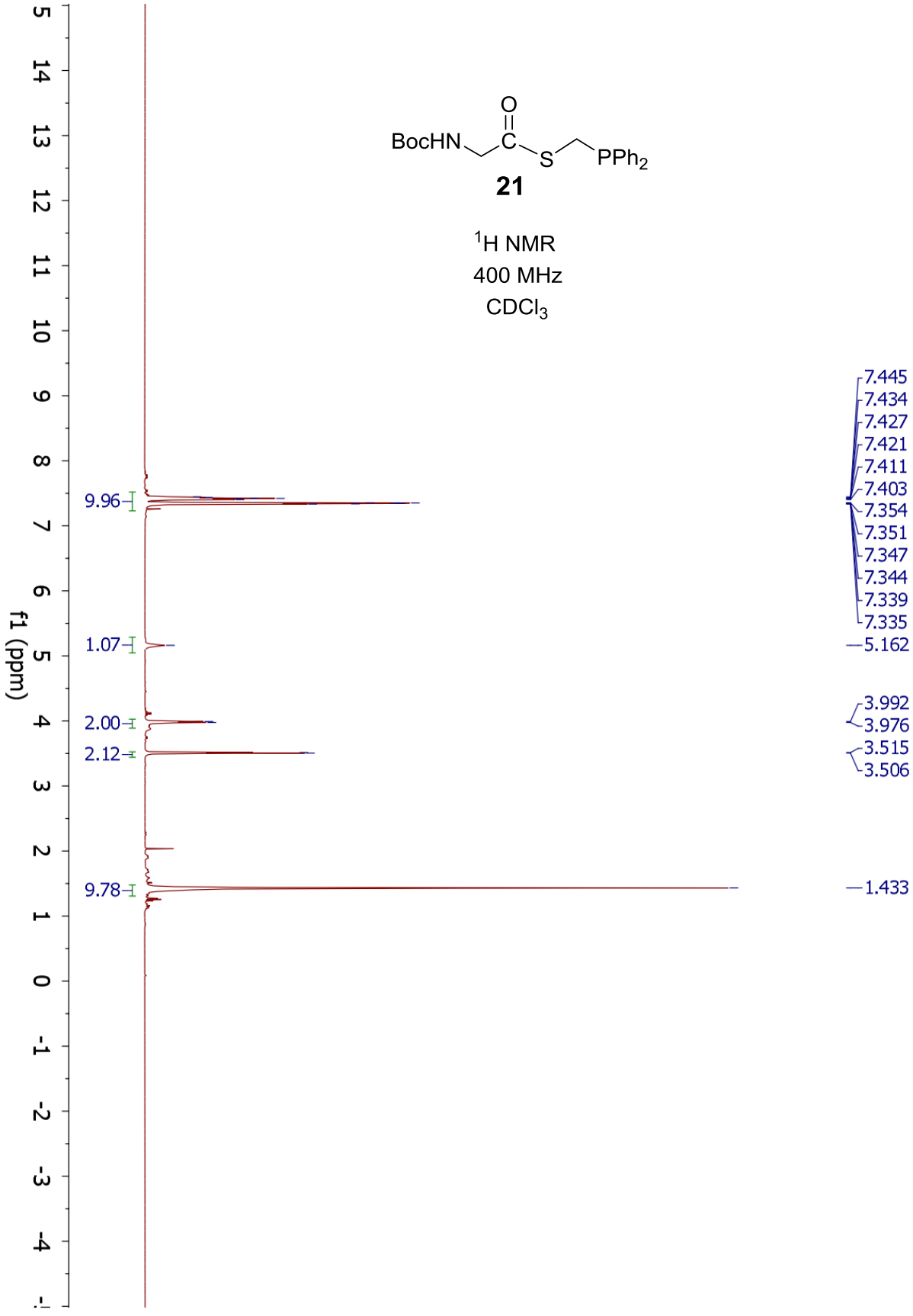
150 MHz

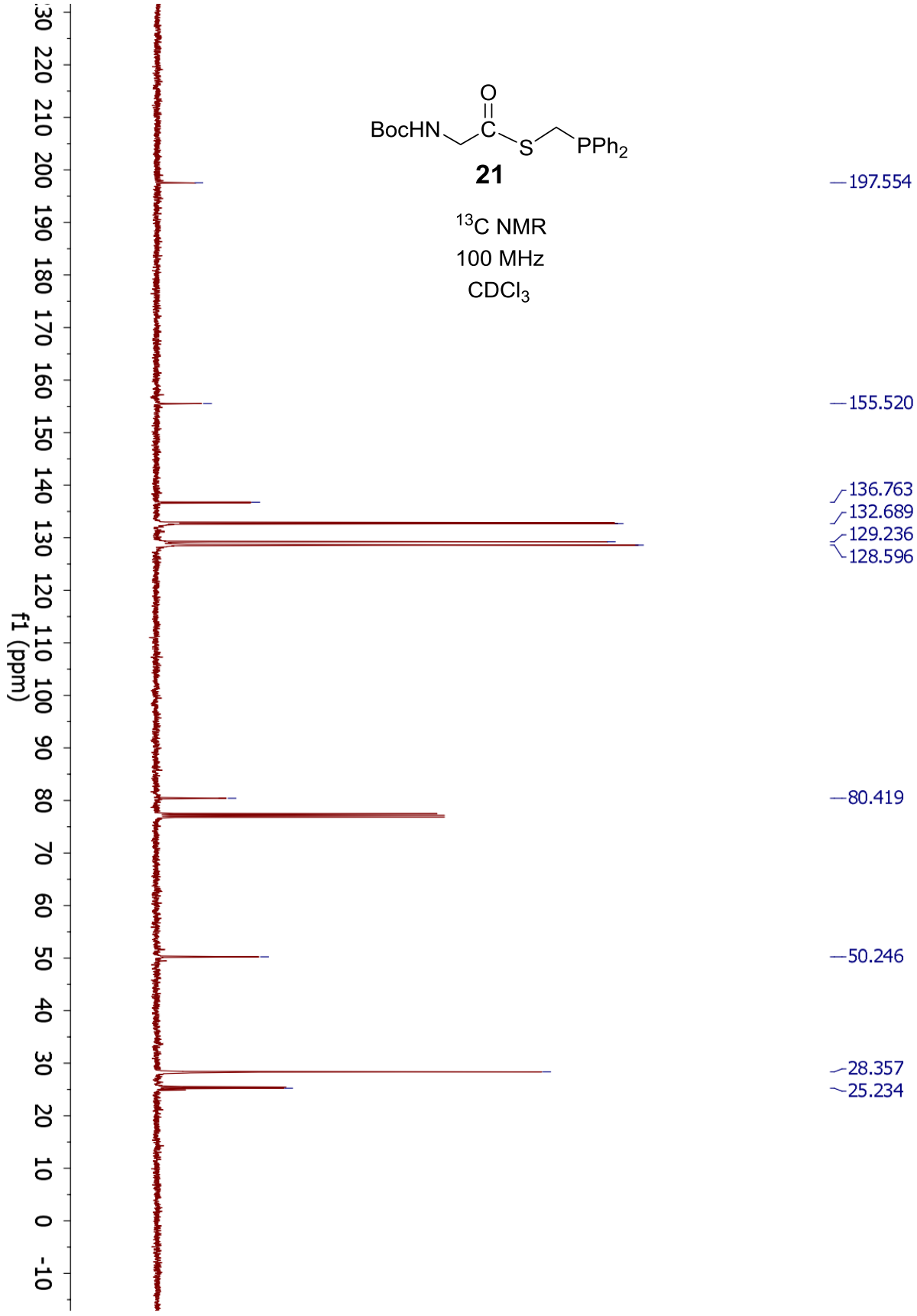
D_2O

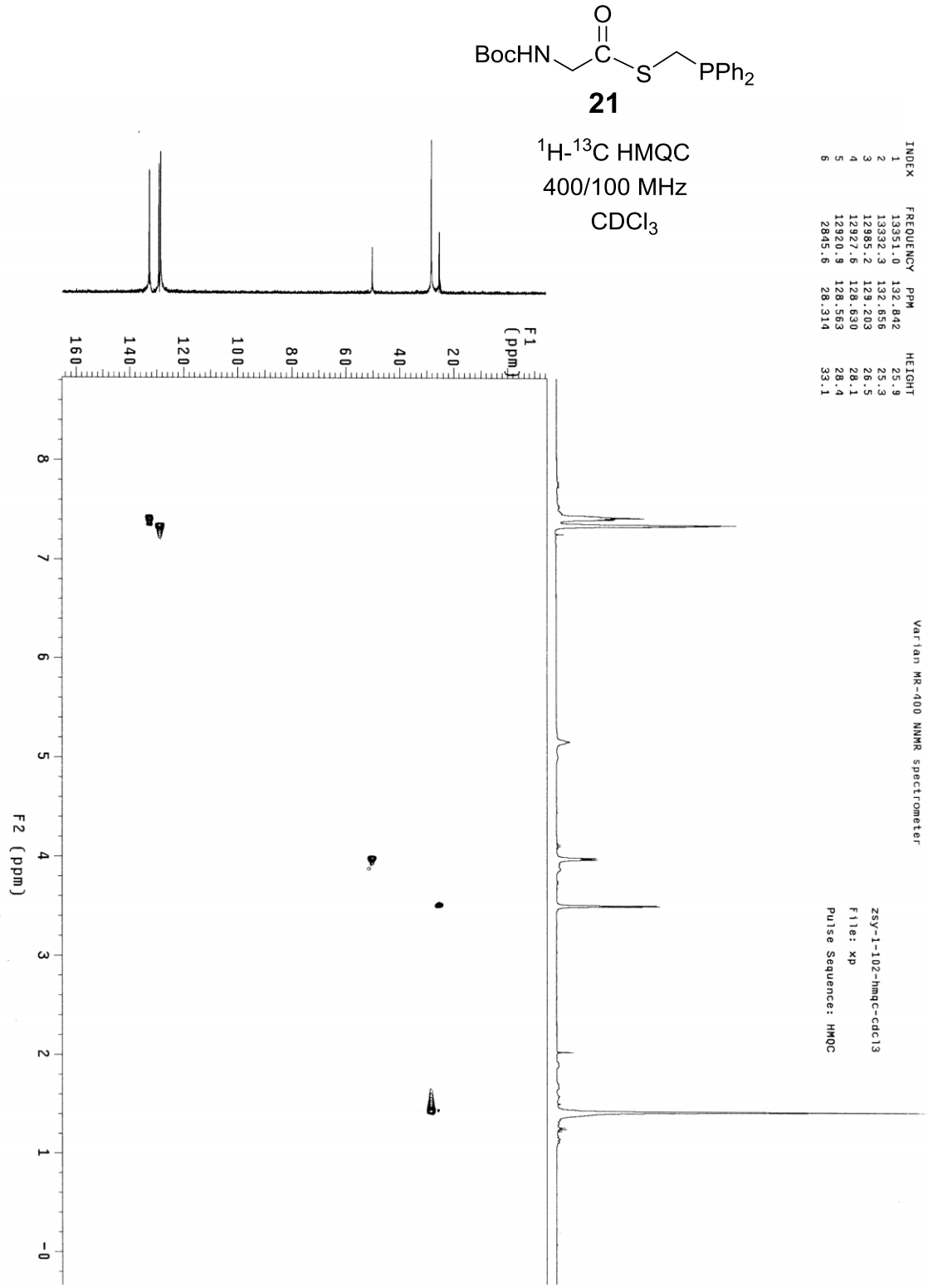

N3CH2CH2O
O=P-OH
O
OH
HO
HO
O
HO
HO
HO
O
O
OH
HO
HO
O
OCH3
18
31P NMR
160 MHz
D2O
—0.300
f1 (ppm)
70 65 60 55 50 45 40 35 30 25 20 15 10 5 0 -5 -10 -15 -20 -25 -30 -35 -40 -45 -50 -55 -60 -65 -70 -75 -80

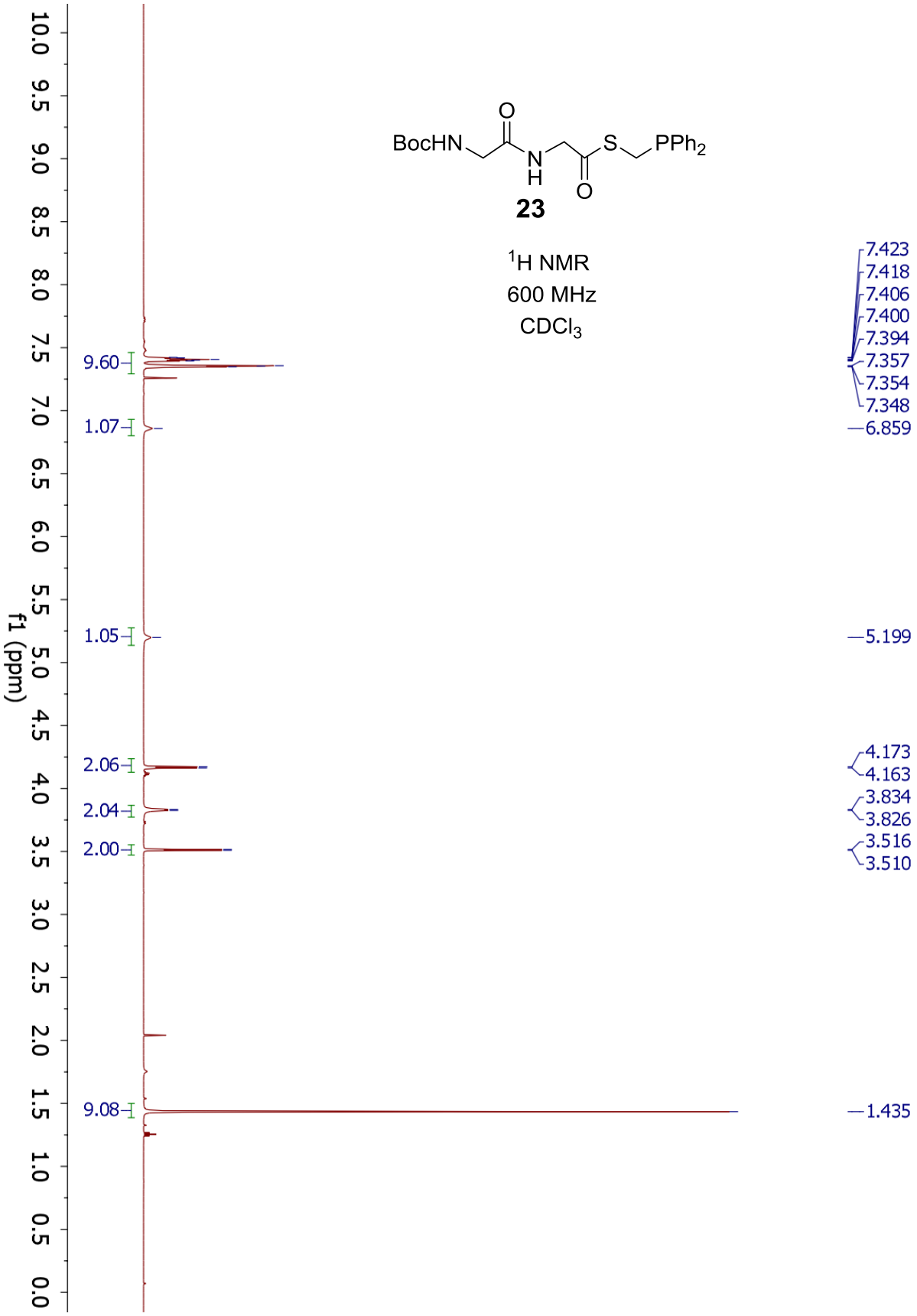

N3CH2CH2O
O=P–OH
OH
HO
HO
HO
HO
OH
HO
HO
OCH3
18
1H-1H COSY
600 MHz
D2O
Sample Name:
Data Collected on:
a600-vnmrs600
Archive directory:
/home/vnmrl/vnmrsys/probes/probe_calibs
Sample directory:
FidFile: gCOSY
Pulse Sequence: gCOSY
Solvent: d2o
Data collected on: Jan 4 2016
Agilent 600 NMR spectrometer
Agilent Technologies
F1 (ppm)
F2 (ppm)

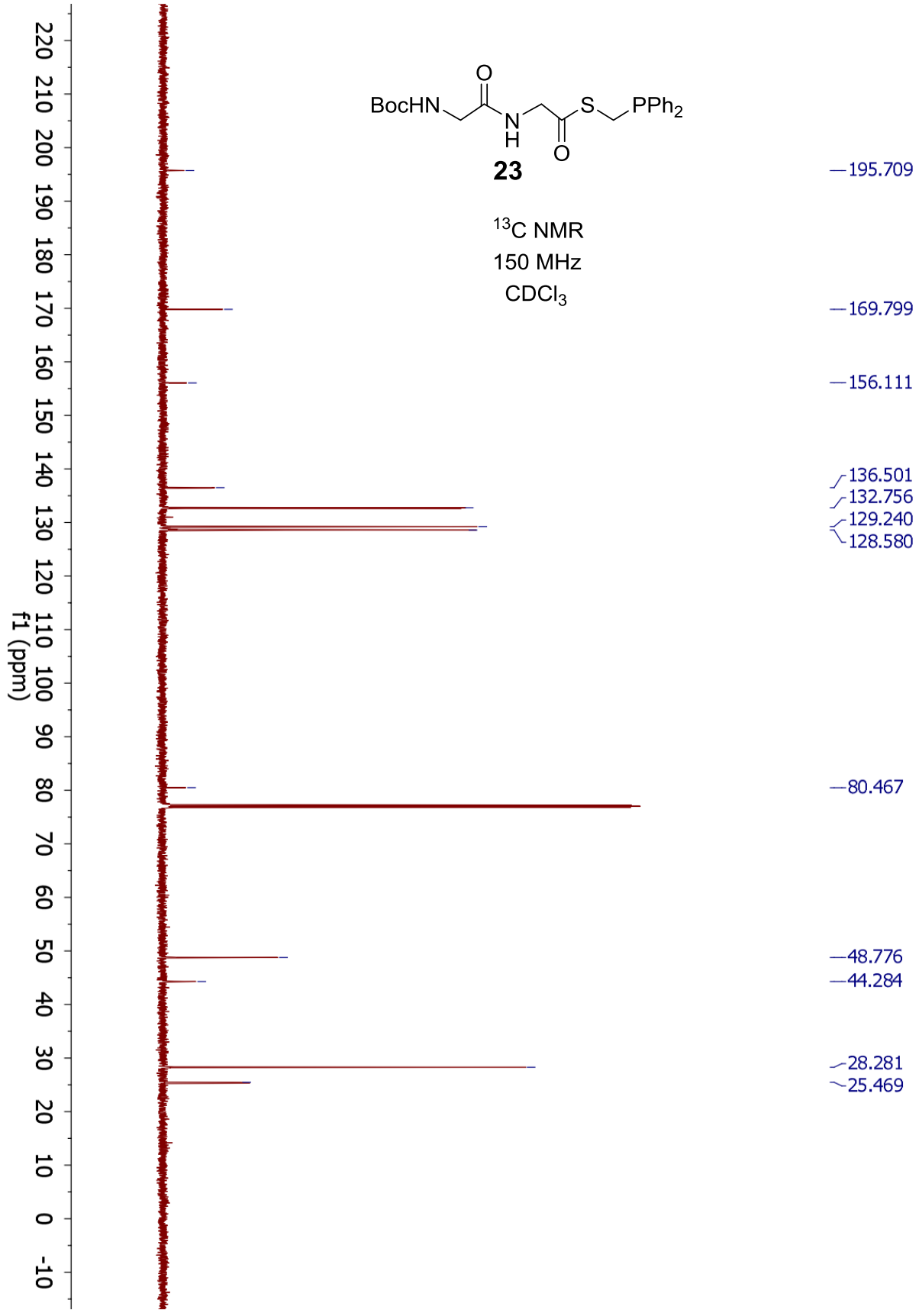

-ESI Scan (0.626-0.685 min, 8 Scans) Frag=175.0V 26059_zsy-1-158_nESI02.d Subtract
Theoretical [M-H]⁻ = 666.1764 (0.0 ppm)
x10 5
[M-H]⁻
666.1764
667.1785
668.1796
669.1849
659.1360
18
Chemical Formula: C21H37N3O19P⁻
Exact Mass: 666.1764
Counts vs. Mass-to-Charge (m/z)
2/6/2017 LB
UF Mass Spectrometry Facility
Page 3

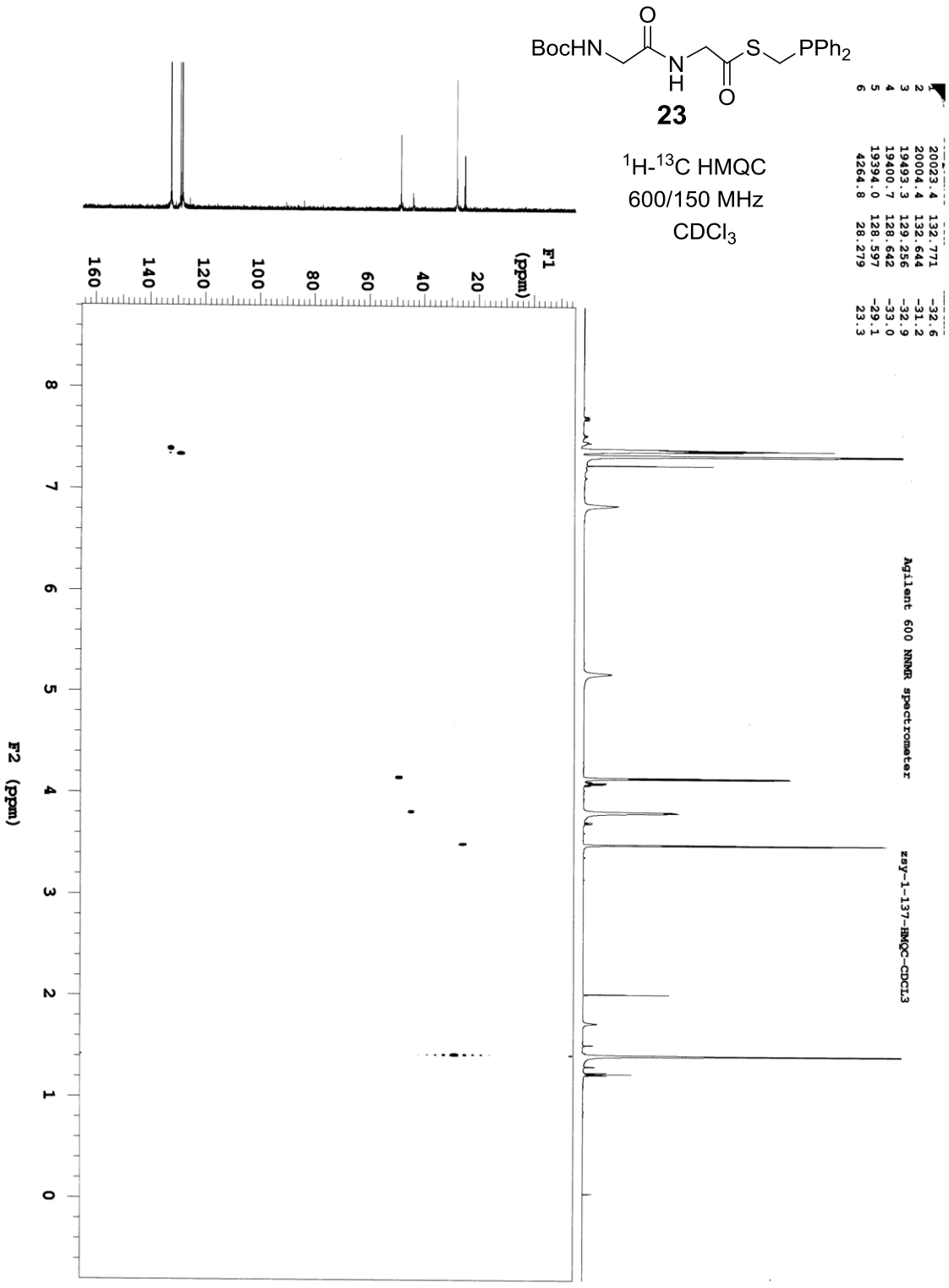

BocHN
O
C
S
PPh2
21
1H NMR
400 MHz
CDCl3
f1 (ppm)
9.96
1.07
2.00
2.12
9.78
7.445
7.434
7.427
7.421
7.411
7.403
7.354
7.351
7.347
7.344
7.339
7.335
5.162
3.992
3.976
3.515
3.506
1.433

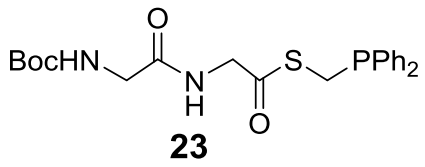

O
BocHN
C
S
PPh2
21
13C NMR
100 MHz
CDCl3
197.554
155.520
136.763
132.689
129.236
128.596
80.419
50.246
28.357
25.234
f1 (ppm)
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10


BocHN
O
C
S
PPh2
21
1H-13C HMQC
400/100 MHz
CDCl3
INDEX FREQUENCY PPM HEIGHT
1 13351.0 132.842 25.9
2 13332.3 132.656 25.3
3 12985.2 129.203 26.5
4 12927.6 128.630 28.1
5 12920.9 128.563 28.4
6 2845.6 28.314 33.1
Varian MR-400 NMR spectrometer
zsy-1-102-hmqc-cdcl3
File: xp
Pulse Sequence: HMQC
F1 (ppm)
F2 (ppm)


BocHN
O
N
H
S
PPh2
O
23
1H NMR
600 MHz
CDCl3
f1 (ppm)
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
0.5
0.0
9.60
1.07
1.05
2.06
2.04
2.00
9.08
7.423
7.418
7.406
7.400
7.394
7.357
7.354
7.348
6.859
5.199
4.173
4.163
3.834
3.826
3.516
3.510
1.435


BocHN
O
N
H
S
PPh2
O
23
13C NMR
150 MHz
CDCl3
195.709
169.799
156.111
136.501
132.756
129.240
128.580
80.467
48.776
44.284
28.281
25.469
f1 (ppm)
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10


BocHN
N
H
S
PPh2
O
O
O
23
1H-13C HMQC
600/150 MHz
CDCl3
F1 (ppm)
F2 (ppm)
Agilent 600 NMR spectrometer
zsy-1-137-HMQC-CDCl3



23

Chemical Formula: $C_{22}H_{27}N_2O_4PS$
Exact Mass: 446.1429

HR ESI-TOF MS

Calc. Mass:

$[M + H]^+ = 447.1507$

$[M + Na]^+ = 469.1327$

$[M + K]^+ = 485.1066$

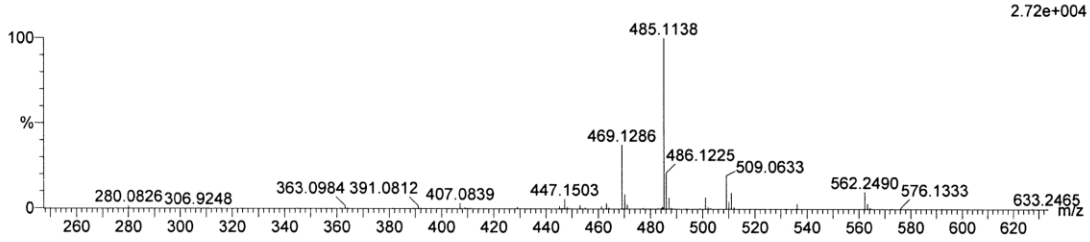
Elemental Composition Report

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 6

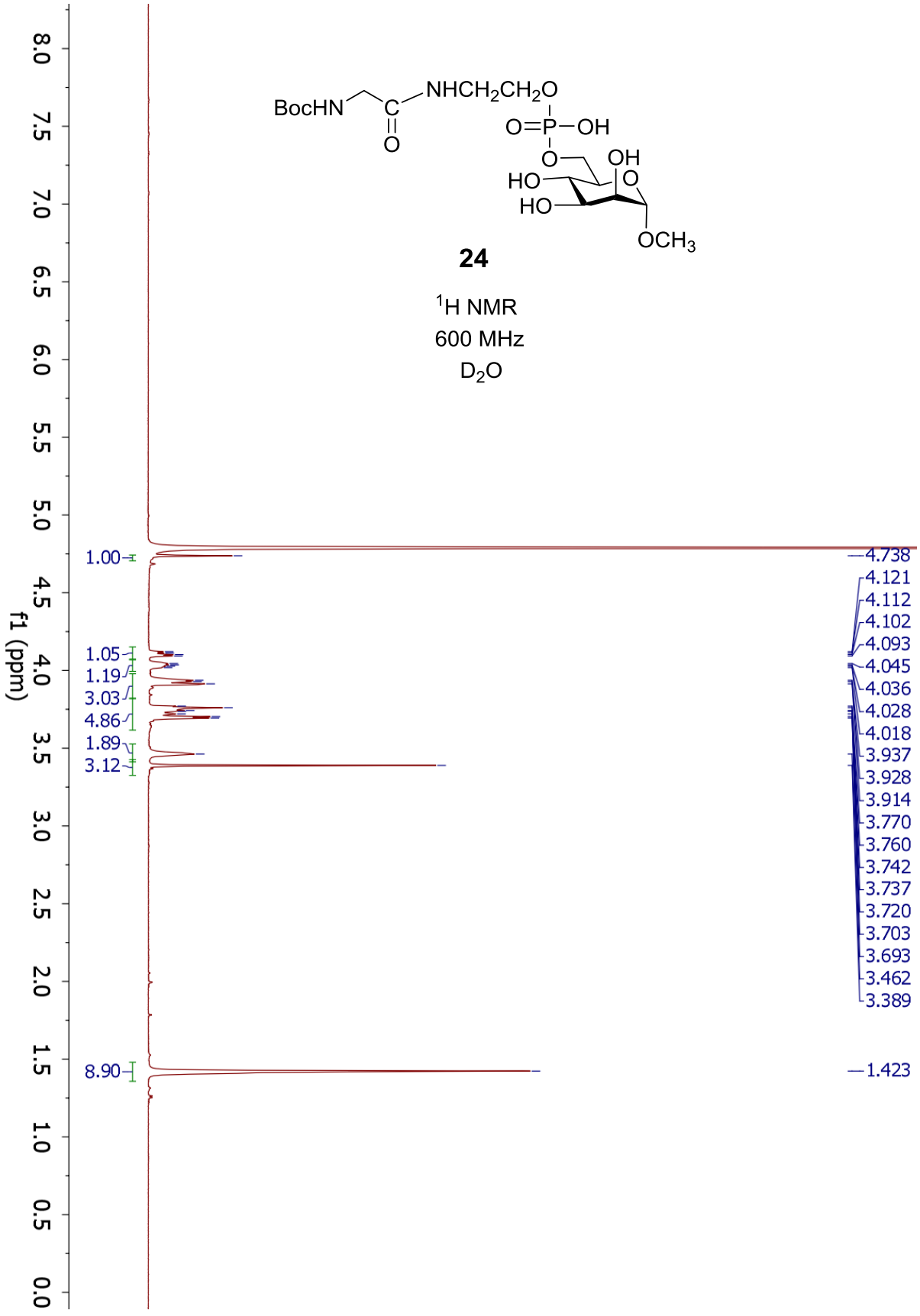
Monoisotopic Mass, Even Electron Ions
934 formula(e) evaluated with 4 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 22-22 H: 0-40 N: 0-5 O: 0-10 Na: 0-1 31P: 0-1 S: 0-1 39K: 0-1

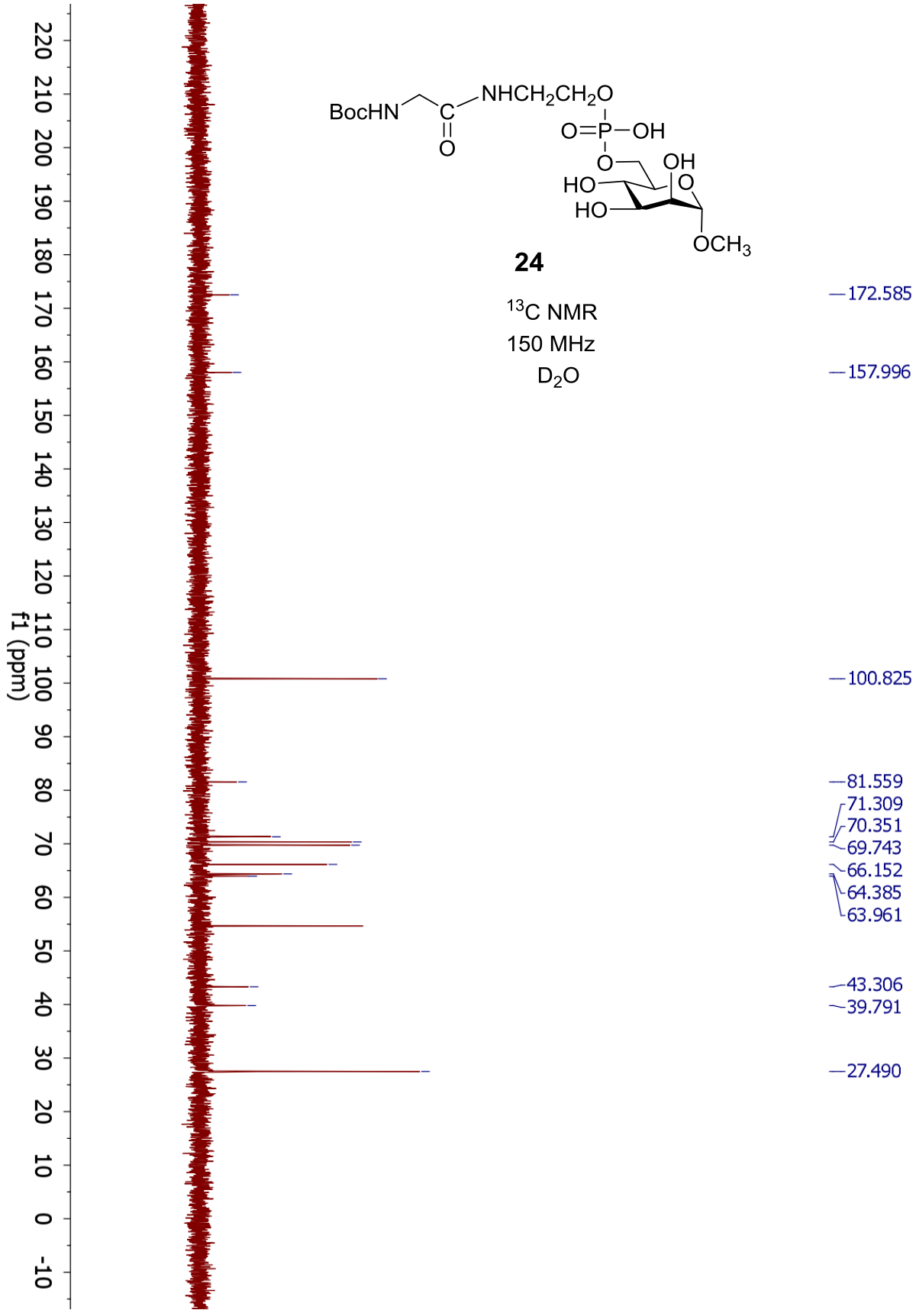
S.Zhu ZSY-1-137_in AcN Cone(V)50

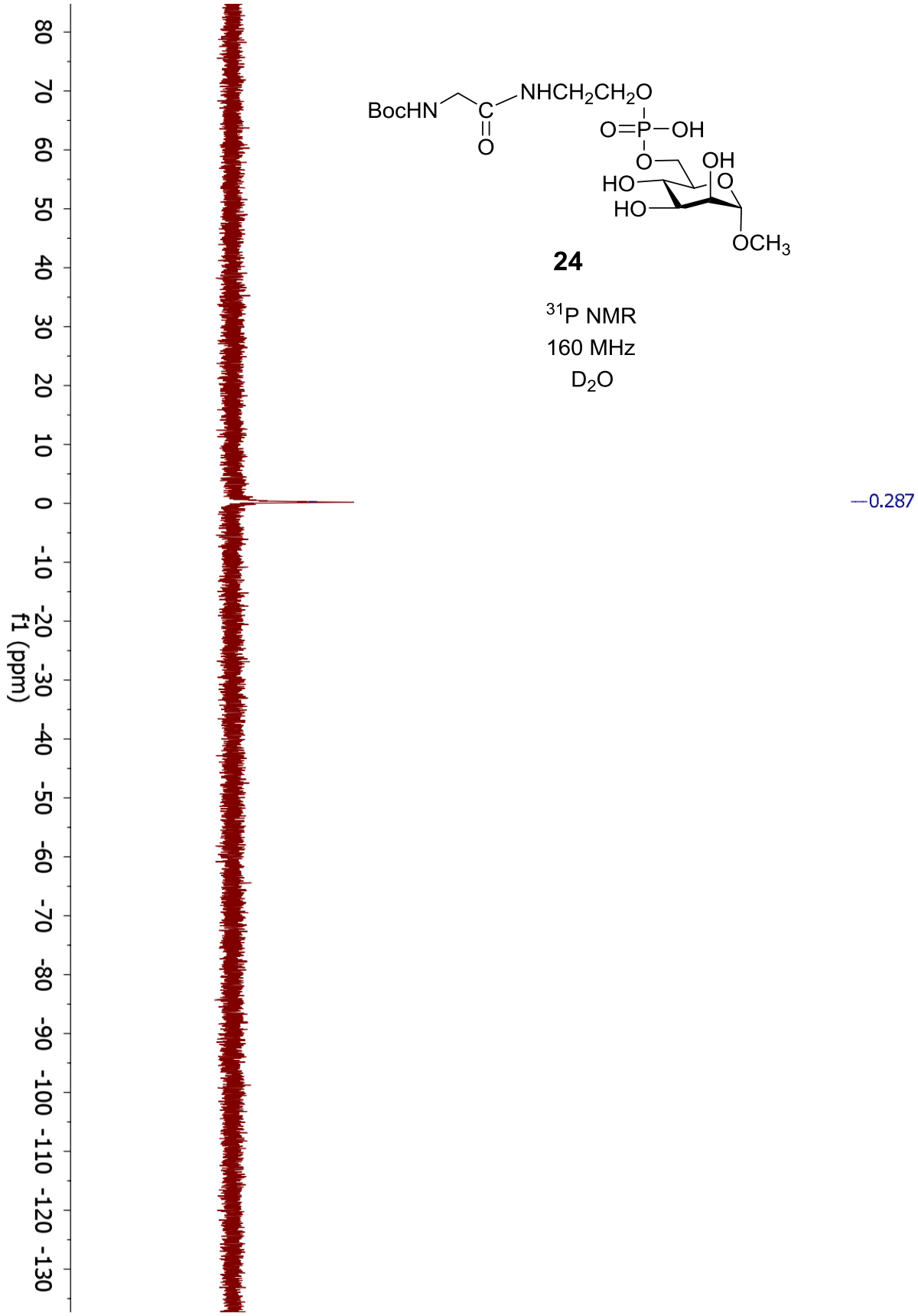
LCT Premier KD128
TOF MS ES+

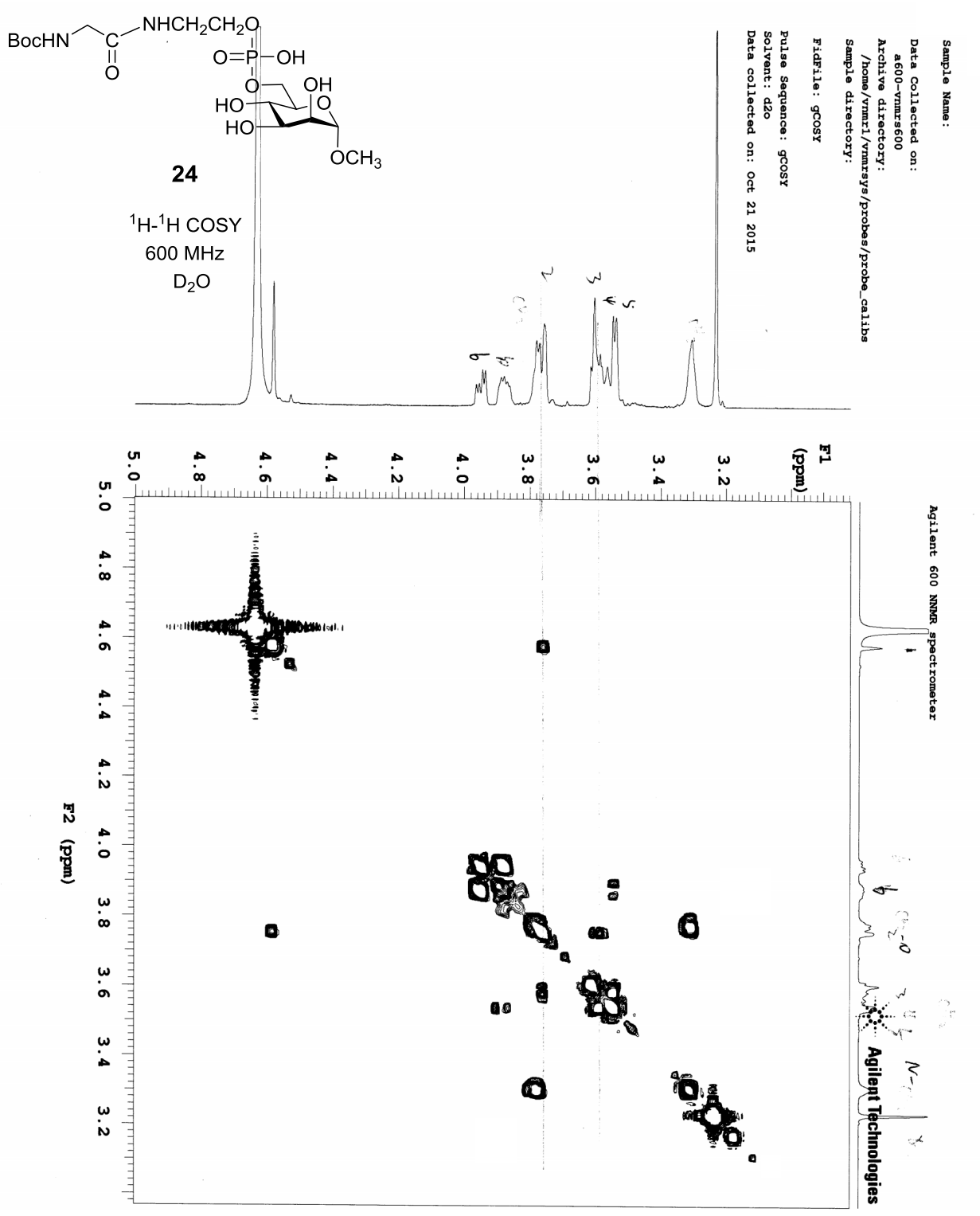


Minimum:				-1.5			
Maximum:		3.0	10.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
447.1503	447.1507	-0.4	-0.9	10.5	39.9	1.4	C22 H28 N2 O4 31P S
	447.1485	1.8	4.0	8.5	39.8	1.3	C22 H29 N2 O2 Na S 39K
	447.1525	-2.2	-4.9	6.5	40.0	1.5	C22 H33 O3 31P S 39K
	447.1467	3.6	8.1	12.5	39.8	1.3	C22 H24 N4 O3 Na S


BocHN
C
O
NHCH2CH2O
O=P–OH
O
OH
HO
O
HO
OCH3
24
1H NMR
600 MHz
D2O
f1 (ppm)
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
0.5
0.0
1.00
1.05
1.19
3.03
4.86
1.89
3.12
8.90
4.738
4.121
4.112
4.102
4.093
4.045
4.036
4.028
4.018
3.937
3.928
3.914
3.770
3.760
3.742
3.737
3.720
3.703
3.693
3.462
3.389
1.423


BocHN
C
O
NHCH2CH2O
O=P–OH
O
OH
HO
HO
O
OCH3
24
13C NMR
150 MHz
D2O
f1 (ppm)
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
172.585
157.996
100.825
81.559
71.309
70.351
69.743
66.152
64.385
63.961
43.306
39.791
27.490


BocHN
NHCH2CH2O
O=P–OH
HO
HO
OH
OCH3
24
31P NMR
160 MHz
D2O
–0.287
f1 (ppm)
80 70 60 50 40 30 20 10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130


BocHN
NHCH2CH2O
O=P-OH
HO
HO
OH
OCH3
24
1H-1H COSY
600 MHz
D2O
Sample Name:
Data Collected on:
a600-vnmrs600
Archive directory:
/home/vnmr1/vnmrsys/probes/probe_calibs
Sample directory:
FidFile: gCOSY
Pulse Sequence: gCOSY
Solvent: d2o
Data collected on: Oct 21 2015
Agilent 600 NMR spectrometer
F1 (ppm)
F2 (ppm)
Agilent Technologies

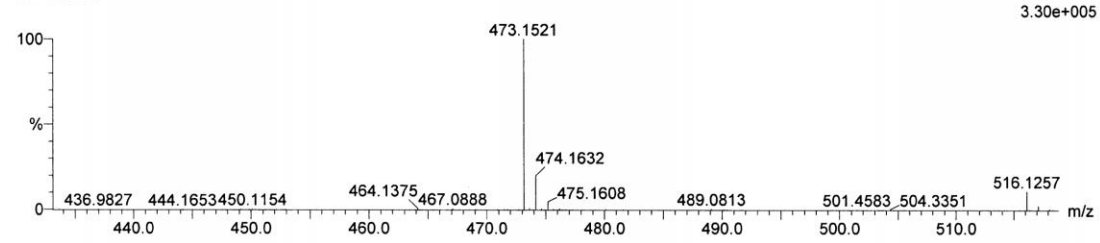
Elemental Composition Report

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 6

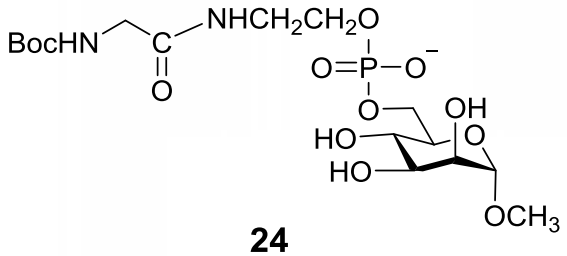
Monoisotopic Mass, Even Electron Ions
238 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 16-16 H: 0-35 N: 0-5 O: 0-20 31P: 0-1

S.Zhu ZSY-1-136 inMeOH Cone(V)50

LCT Premier KD128
TOF MS ES-

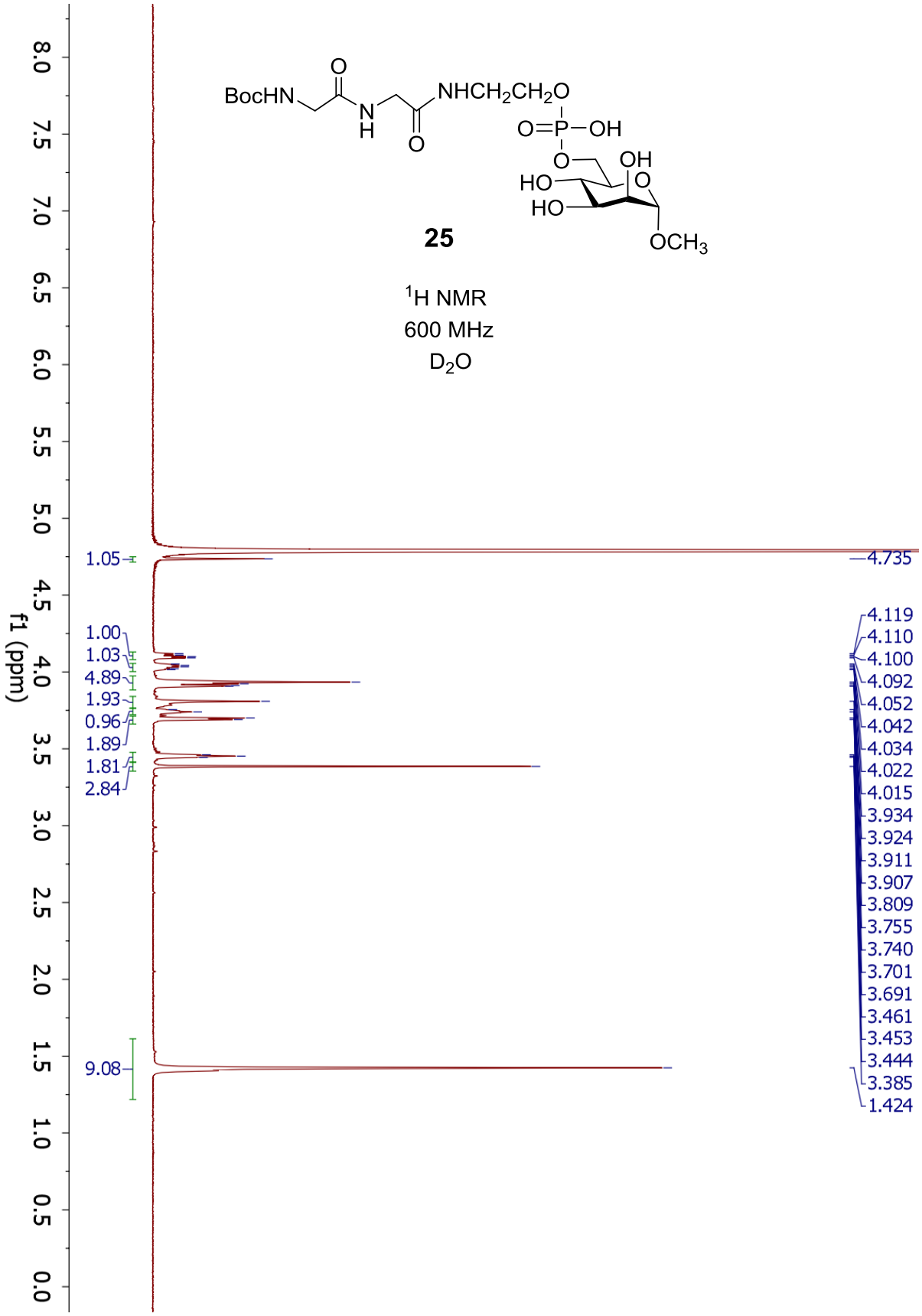


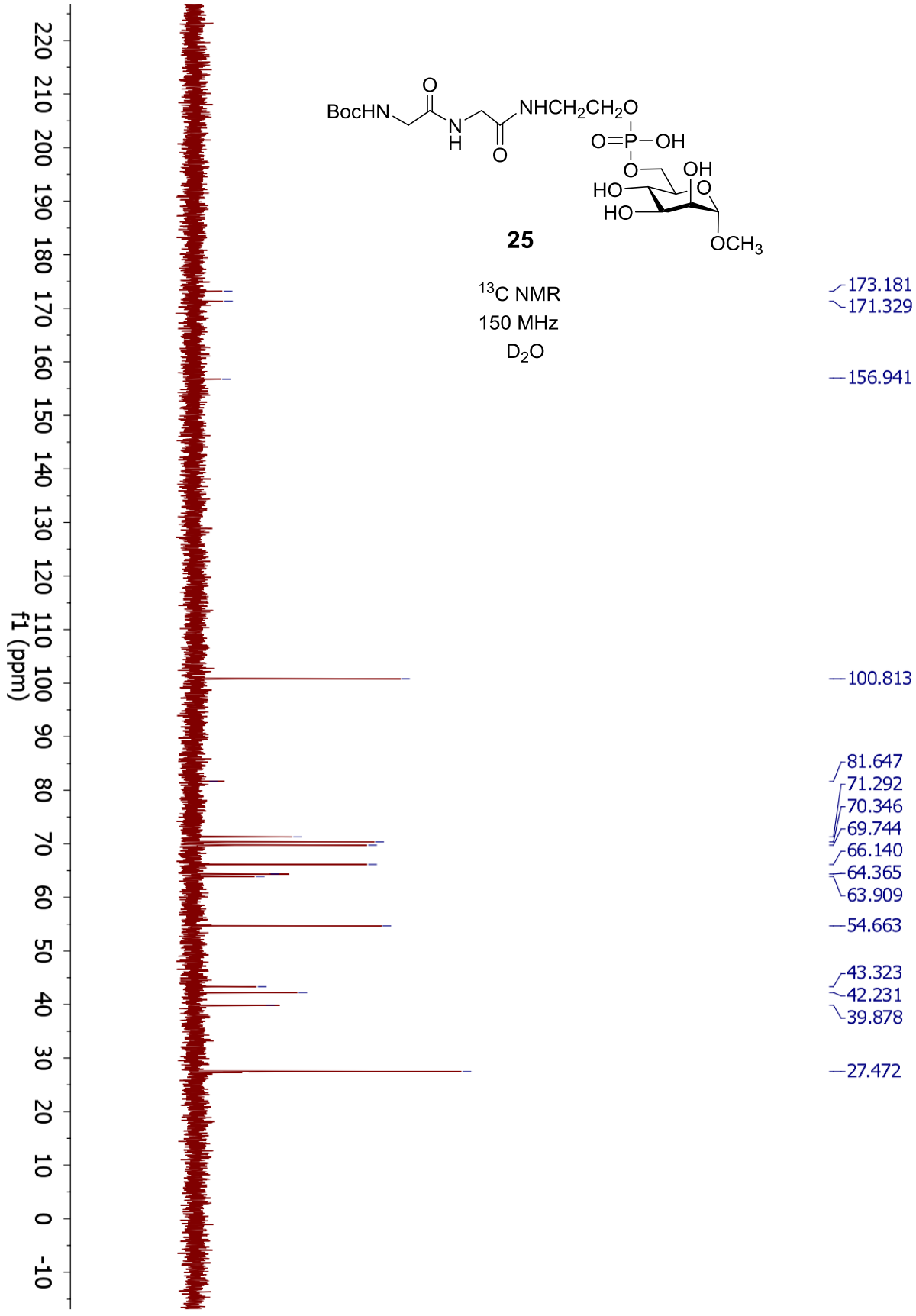
Minimum:				-1.5			
Maximum:		3.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
473.1521	473.1536	-1.5	-3.2	3.5	125.1	0.0	C16 H30 N2 O12 31P

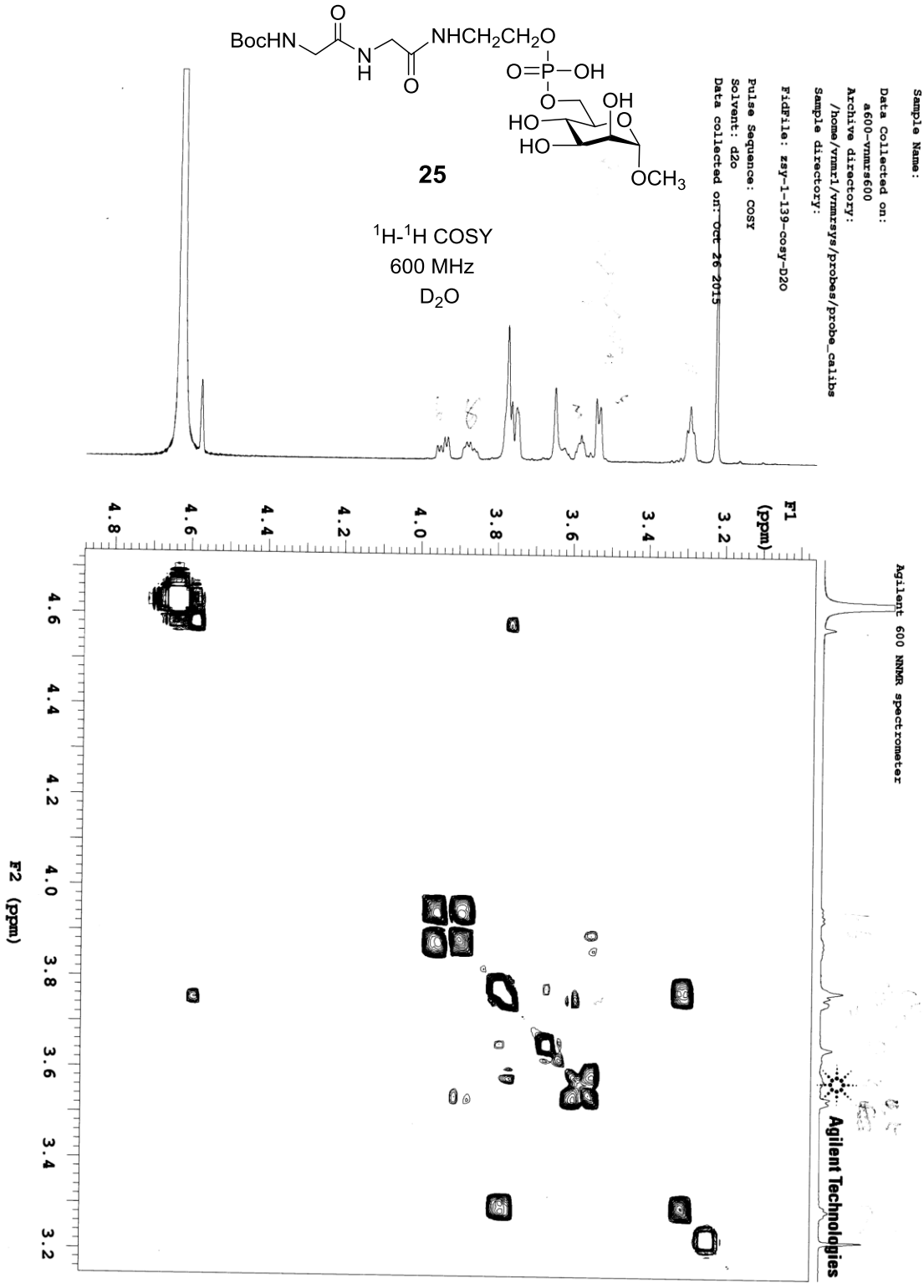


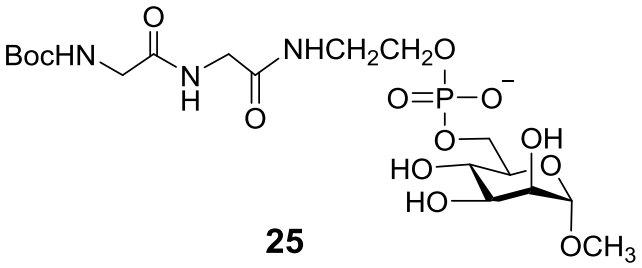
24

Chemical Formula: $C_{16}H_{30}N_2O_{12}P^-$
Exact Mass: 473.1542


BocHN
O
N
H
O
NHCH2CH2O
O=P–OH
O
OH
HO
O
HO
OCH3
25
1H NMR
600 MHz
D2O
f1 (ppm)
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
0.5
0.0
1.05
1.00
1.03
4.89
1.93
0.96
1.89
1.81
2.84
9.08
4.735
4.119
4.110
4.100
4.092
4.052
4.042
4.034
4.022
4.015
3.934
3.924
3.911
3.907
3.809
3.755
3.740
3.701
3.691
3.461
3.453
3.444
3.385
1.424


BocHN
O
N
H
O
NHCH2CH2O
O=P–OH
O
OH
HO
HO
O
OCH3
25
13C NMR
150 MHz
D2O
173.181
171.329
156.941
100.813
81.647
71.292
70.346
69.744
66.140
64.365
63.909
54.663
43.323
42.231
39.878
27.472
f1 (ppm)
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10


Sample Name:
Data Collected on:
a600-vnmrs600
Archive directory:
/home/vnmr1/vnmrsys/probes/probe_calibs
Sample directory:
FidFile: zsy-1-139-cosy-D2o
Pulse Sequence: COSY
Solvent: d2o
Data collected on: Oct 26 2015
BocHN
NHCH2CH2O
OH
HO
HO
OCH3
25
1H-1H COSY
600 MHz
D2O
F1 (ppm)
F2 (ppm)
Agilent 600 NNMR spectrometer
Agilent Technologies



25

Chemical Formula: $C_{18}H_{33}N_3O_{13}P^-$
Exact Mass: 530.1756

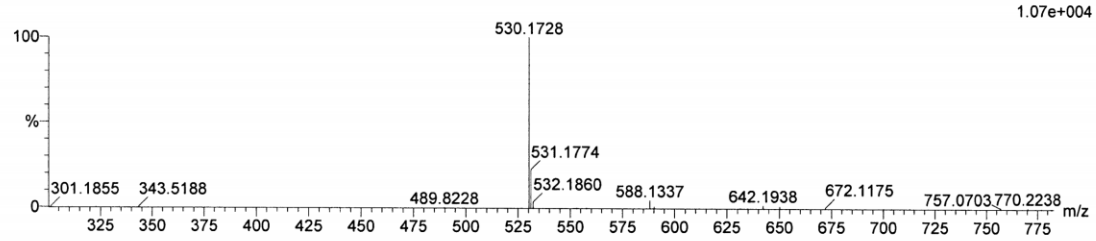
Elemental Composition Report

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 6

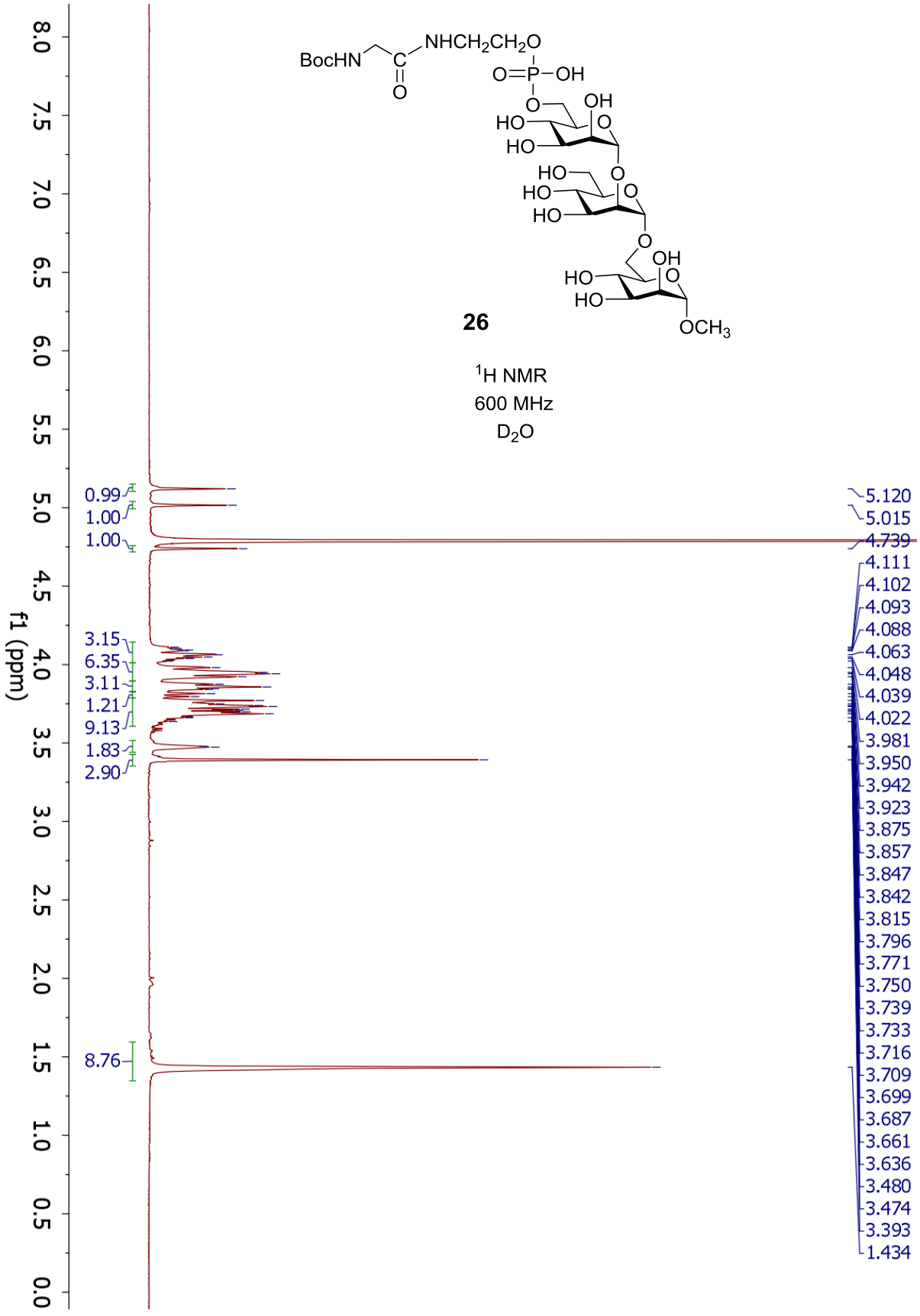
Monoisotopic Mass, Even Electron Ions
182 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 18-18 H: 0-40 N: 0-5 O: 0-15 31P: 0-1

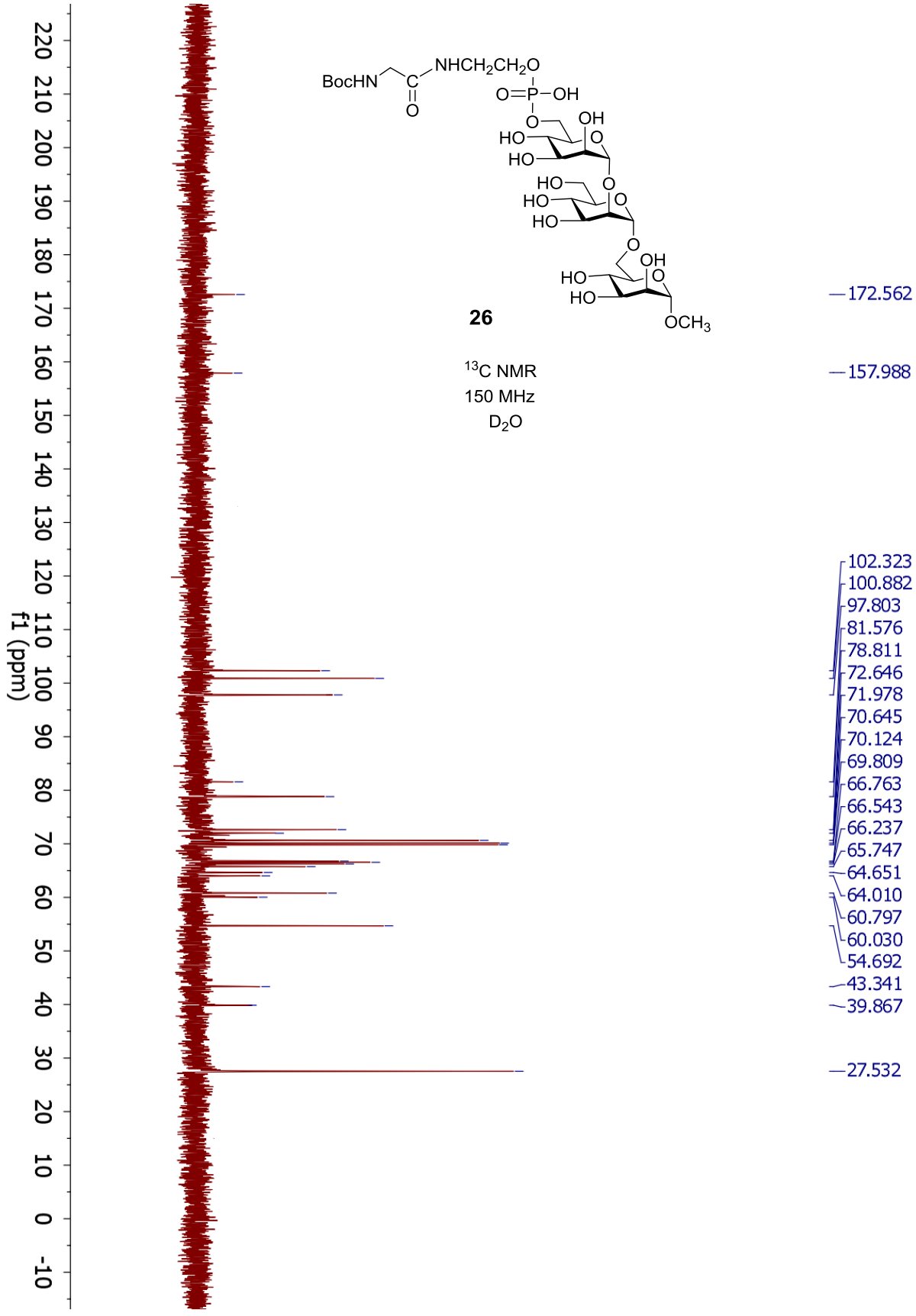
LCT Premier KD128
TOF MS ES-

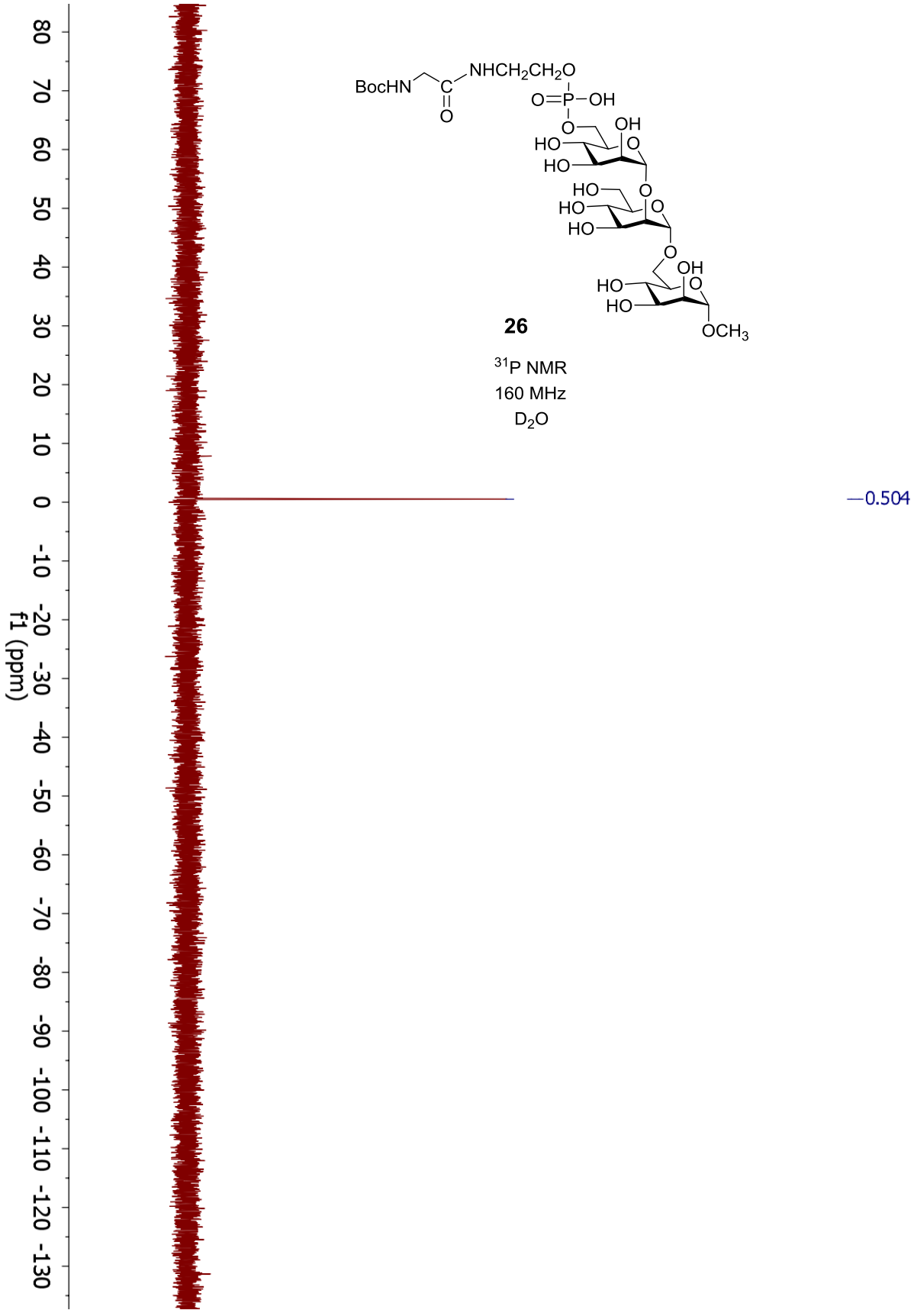
S.Zhu ZSY-1-139 in MeOH Cone(V)50

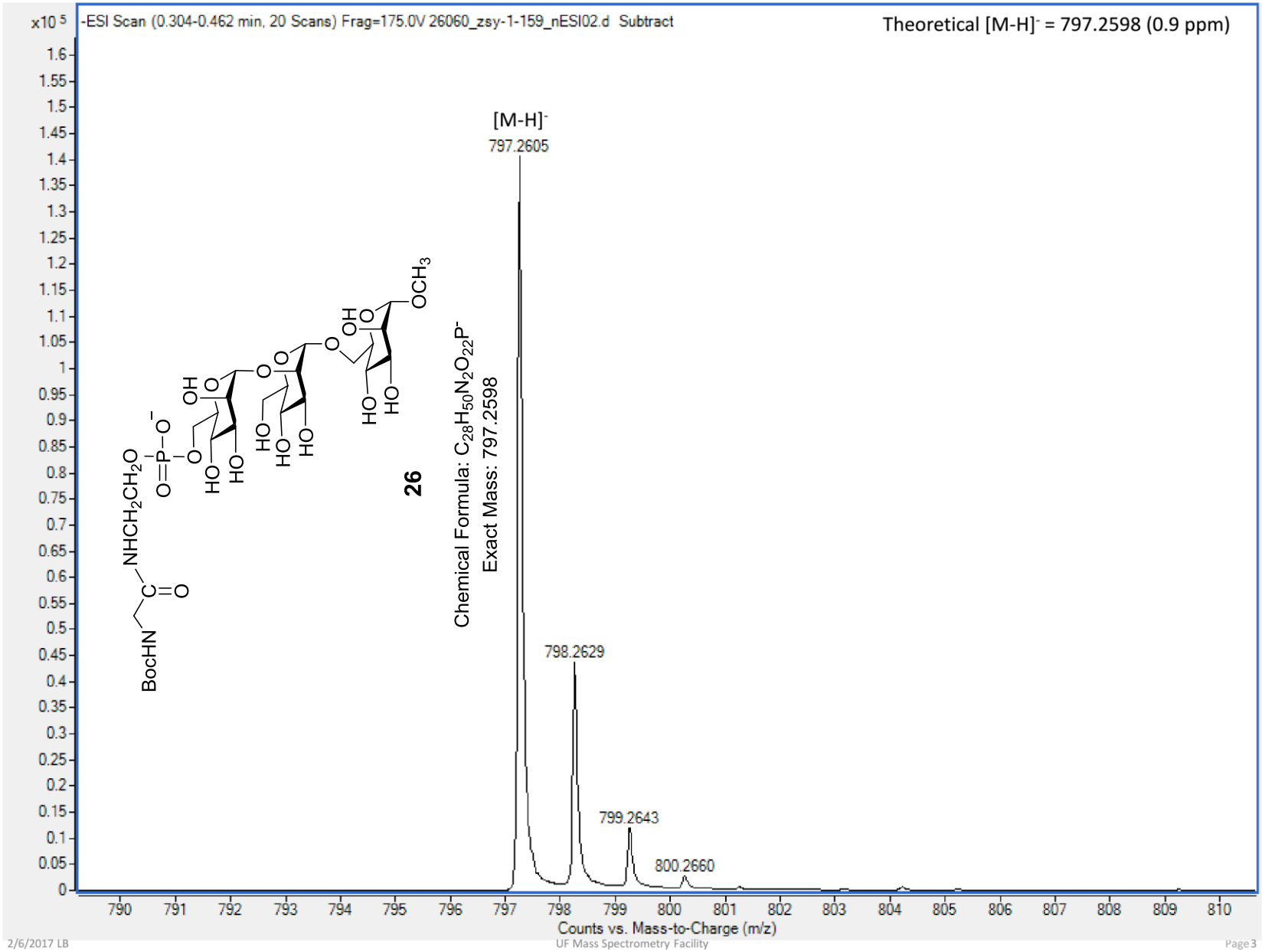


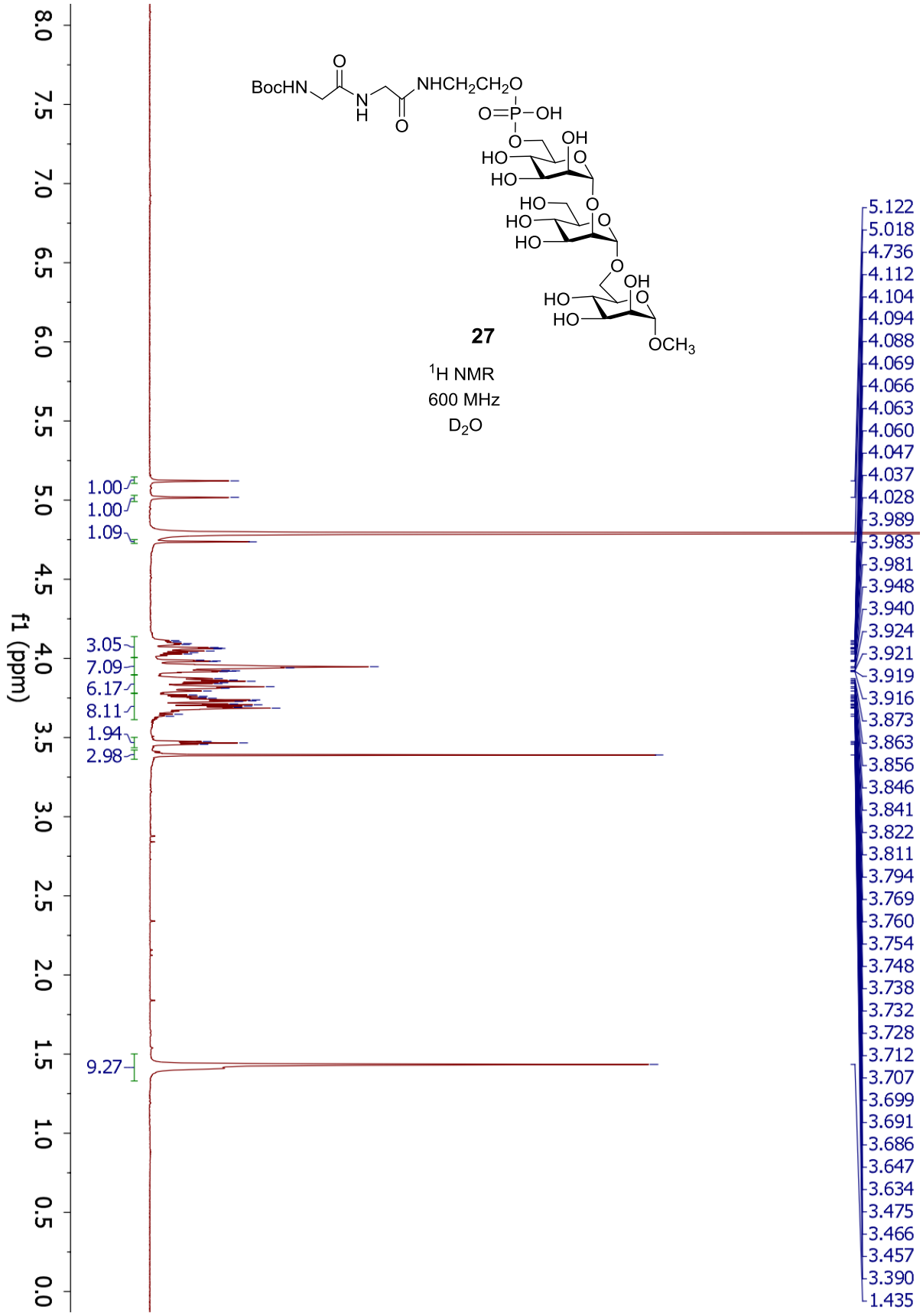
Minimum:				-1.5			
Maximum:		3.0	10.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
530.1728	530.1751	-2.3	-4.3	4.5	38.2	0.0	C18 H33 N3 O13 31P

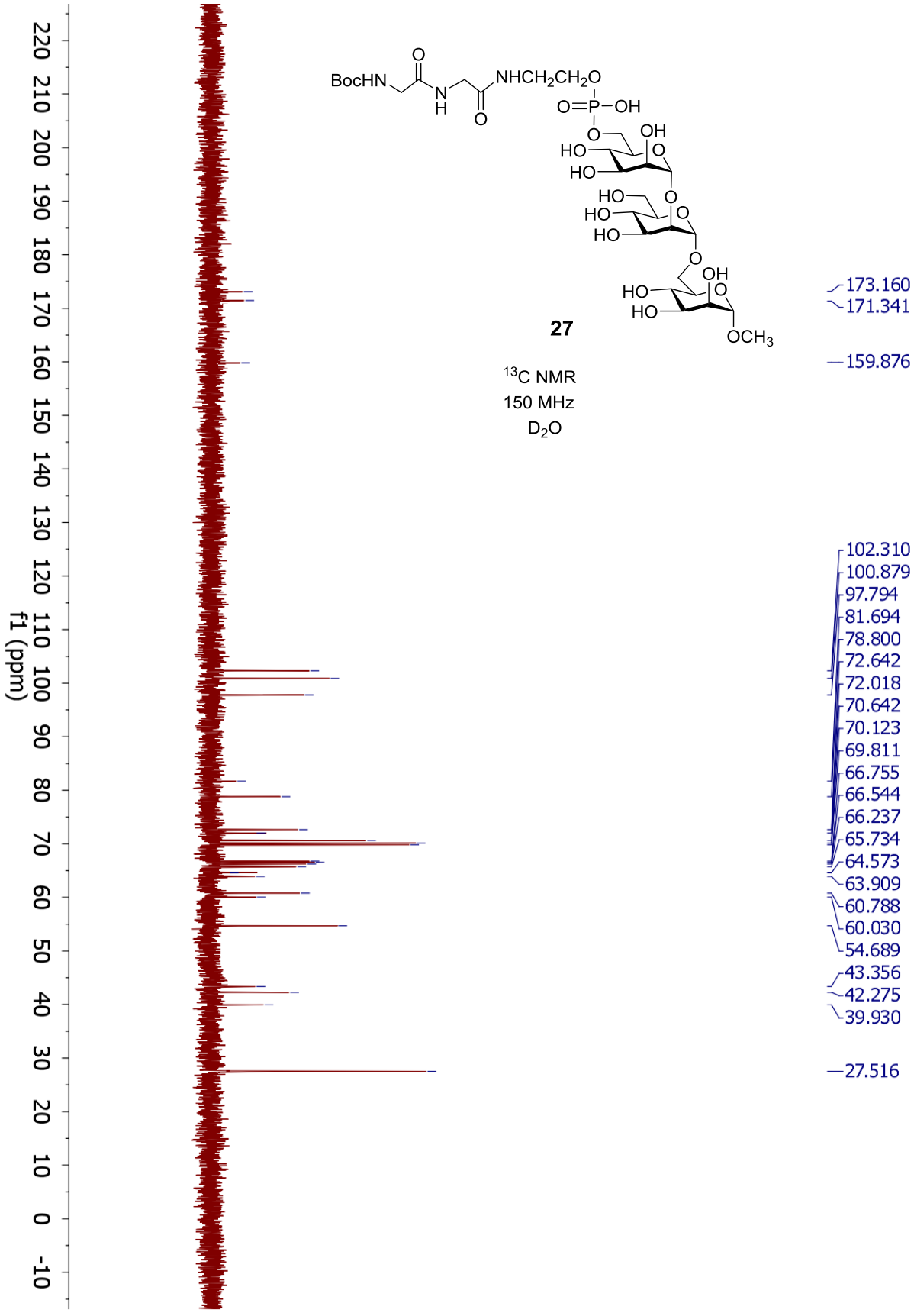

BocHN
C
O
NHCH2CH2O
O=P–OH
O
OH
HO
HO
O
HO
HO
HO
O
O
OH
HO
HO
O
OCH3
26
1H NMR
600 MHz
D2O
f1 (ppm)
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0.99
1.00
1.00
3.15
6.35
3.11
1.21
9.13
1.83
2.90
8.76
5.120
5.015
4.739
4.111
4.102
4.093
4.088
4.063
4.048
4.039
4.022
3.981
3.950
3.942
3.923
3.875
3.857
3.847
3.842
3.815
3.796
3.771
3.750
3.739
3.733
3.716
3.709
3.699
3.687
3.661
3.636
3.480
3.474
3.393
1.434

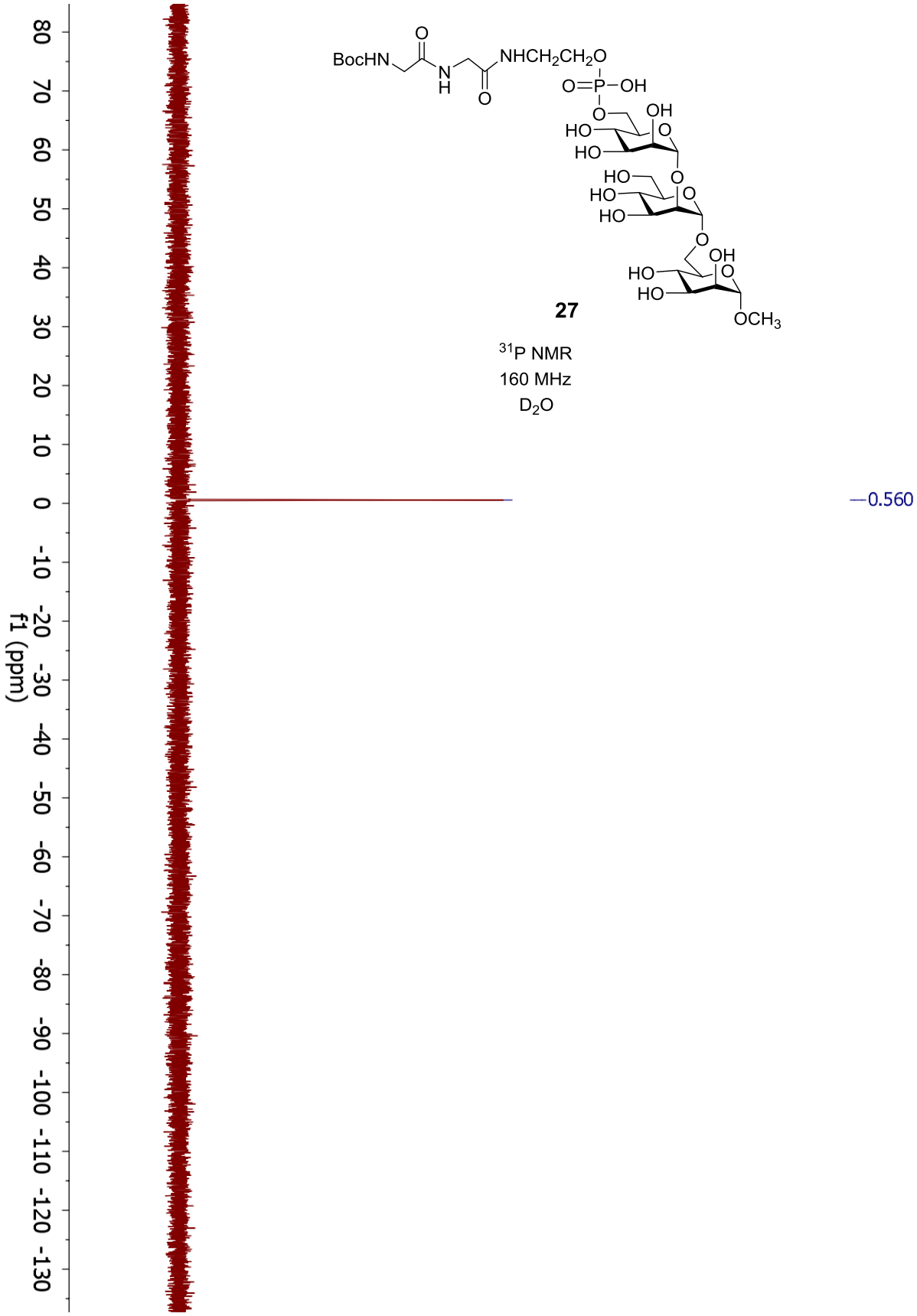

BocHN
NHCH2CH2O
O=P–OH
HO
OH
OCH3
26
13C NMR
150 MHz
D2O
172.562
157.988
102.323
100.882
97.803
81.576
78.811
72.646
71.978
70.645
70.124
69.809
66.763
66.543
66.237
65.747
64.651
64.010
60.797
60.030
54.692
43.341
39.867
27.532
f1 (ppm)

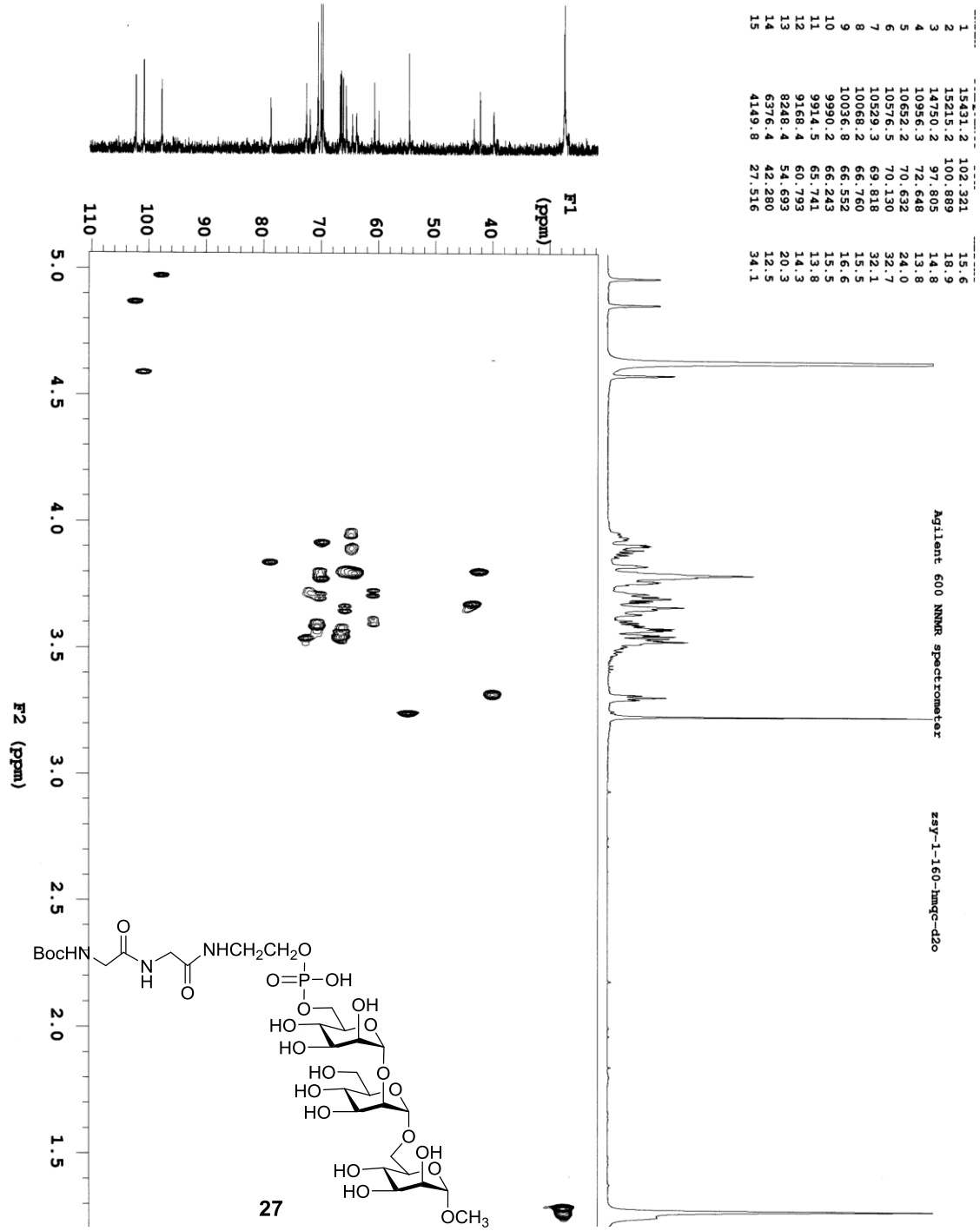

BocHN
C
O
NHCH2CH2O
O=P–OH
O
OH
HO
HO
O
HO
HO
HO
O
O
OH
HO
HO
O
OCH3
26
31P NMR
160 MHz
D2O
—0.504
80 70 60 50 40 30 20 10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130
f1 (ppm)


-ESI Scan (0.304-0.462 min, 20 Scans) Frag=175.0V 26060_zsy-1-159_nESI02.d Subtract
Theoretical [M-H]⁻ = 797.2598 (0.9 ppm)
[M-H]⁻
797.2605
798.2629
799.2643
800.2660
26
Chemical Formula: C28H50N2O22P⁻
Exact Mass: 797.2598
Counts vs. Mass-to-Charge (m/z)
2/6/2017 LB
UF Mass Spectrometry Facility
Page 3


BocHN
NHCH2CH2O
O=P–OH
OCH3
27
1H NMR
600 MHz
D2O
f1 (ppm)
1.00
1.00
1.09
3.05
7.09
6.17
8.11
1.94
2.98
9.27
5.122
5.018
4.736
4.112
4.104
4.094
4.088
4.069
4.066
4.063
4.060
4.047
4.037
4.028
3.989
3.983
3.981
3.948
3.940
3.924
3.921
3.919
3.916
3.873
3.863
3.856
3.846
3.841
3.822
3.811
3.794
3.769
3.760
3.754
3.748
3.738
3.732
3.728
3.712
3.707
3.699
3.691
3.686
3.647
3.634
3.475
3.466
3.457
3.390
1.435

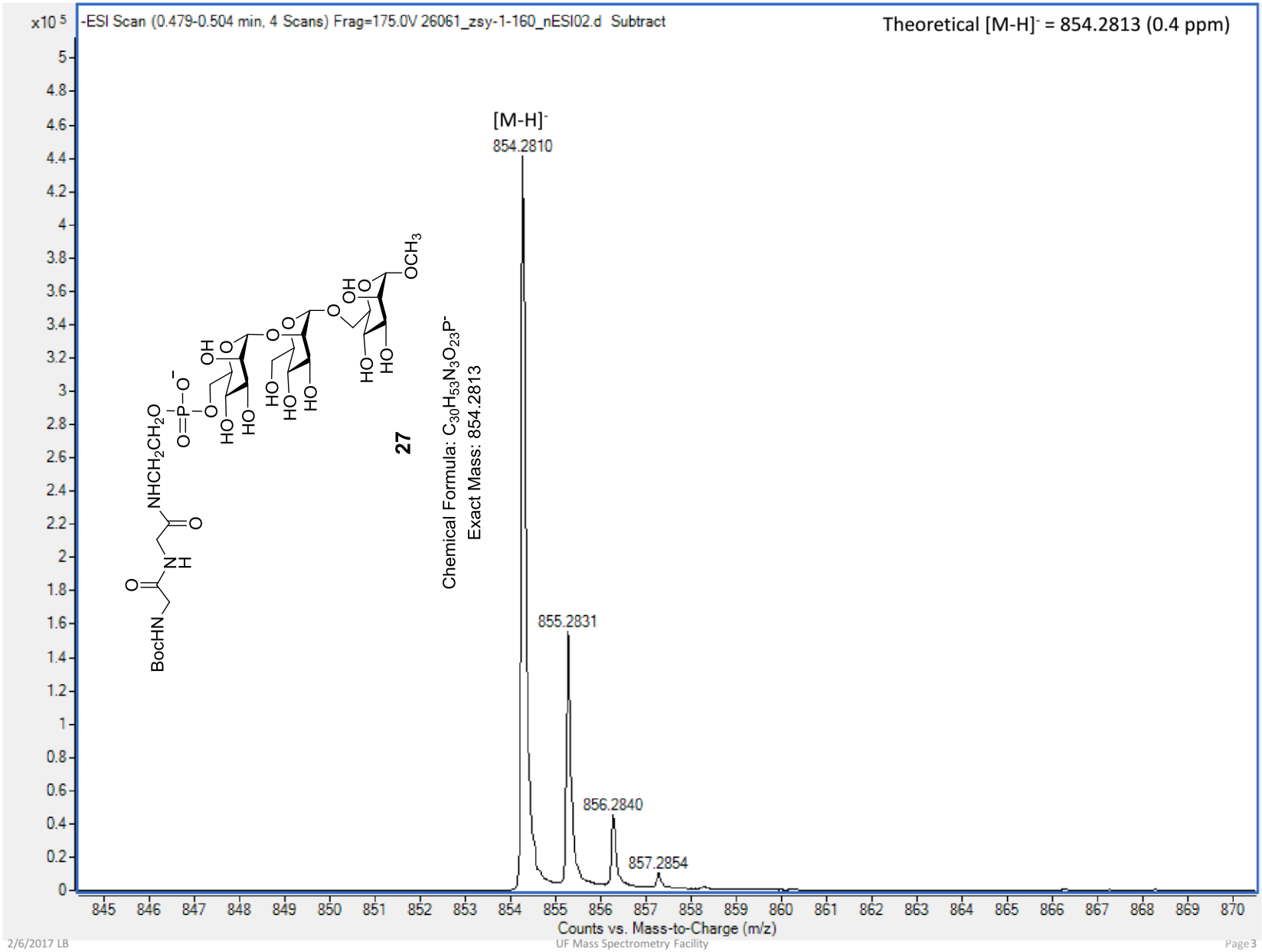

BocHN
NHCH2CH2O
O=P–OH
HO
OH
OCH3
27
13C NMR
150 MHz
D2O
173.160
171.341
159.876
102.310
100.879
97.794
81.694
78.800
72.642
72.018
70.642
70.123
69.811
66.755
66.544
66.237
65.734
64.573
63.909
60.788
60.030
54.689
43.356
42.275
39.930
27.516
f1 (ppm)


BocHN
O
N
H
O
NHCH2CH2O
O=P–OH
O
OH
HO
HO
O
HO
HO
HO
O
O
OH
HO
HO
O
OCH3
27
31P NMR
160 MHz
D2O
—0.560
80 70 60 50 40 30 20 10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130
f1 (ppm)



27

^{1}H-^{13}C HMQC
600/150 MHz
D_2O


-ESI Scan (0.479-0.504 min, 4 Scans) Frag=175.0V 26061_zsy-1-160_nESI02.d Subtract
Theoretical [M-H]⁻ = 854.2813 (0.4 ppm)
x10 5
[M-H]⁻
854.2810
855.2831
856.2840
857.2854
27
Chemical Formula: C30H53N3O23P⁻
Exact Mass: 854.2813
Counts vs. Mass-to-Charge (m/z)
2/6/2017 LB
UF Mass Spectrometry Facility
Page 3