Development of rare bacterial monosaccharide analogs for metabolic glycan labeling in pathogenic bacteria

Emily L. Clark,[1] Madhu Emmadi,[2] Katharine L. Krupp,[1] Ananda R. Podilapu,[2] Jennifer D. Helble,[1] Suvarn S. Kulkarni,[2,*] and Danielle H. Dube[1,*]

[1]Department of Chemistry & Biochemistry, Bowdoin College, 6600 College Station, Brunswick, Maine 04011; [2]Department of Chemistry, Indian Institute of Technology Bombay, Powai, Mumbai 400076, India

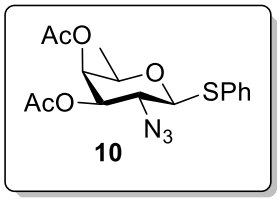
Supporting Information

Table of Contents

Synthesis

General. All reactions were conducted under a dry nitrogen atmosphere. Solvents (CH_2Cl_2 >99%, THF 99.5%, Acetonitrile 99.8%, DMF 99.5%) were purchased in capped bottles and dried under sodium or CaH_2. All other solvents and reagents were used without further purification. All glasswares used were oven dried before use. TLC was performed on pre-coated Aluminium plates of Silica Gel 60 F254 (0.25 mm, E. Merck). Developed TLC plates were visualized under a short-wave UV lamp and by heating plates that were dipped in ammonium molybdate/cerium (IV) sulfate solution. Silica gel column chromatography was performed using Silica Gel (100-200 mesh) and employed a solvent polarity correlated with TLC mobility. NMR experiments were conducted on 400 MHz and 500 MHz instruments using $CDCl_3$ (D, 99.8%) or $(CD_3)_2CO$ (D, 99.9%) as solvents. Chemical shifts are relative to the deuterated solvent peaks and are in parts per million (ppm). ^1H-^1H COSY was used to confirm proton assignments. Mass spectra were acquired in the ESI mode. Melting points were determined by capillary apparatus. Specific rotation experiments were measured at 589 nm (Na) and 25 °C. IR spectra were recorded on an FT-IR spectrometer using CsCl plates. Ac_4GlcNAc (**1**), Ac_4GlcNAz (**2**), Ac_4GalNAz, $Ac_3$6Az-GlcNAc (**3**), and Phos-FLAG were synthesized as previously described.[1-3]

Phenyl 3,4-*O*-diacetyl-2-azido-2,6-dideoxy-1-thio-β-D-galactopyranoside (10)

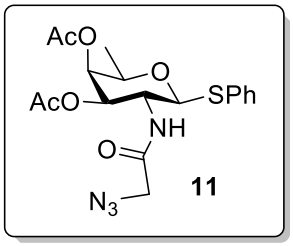


10

To a clear solution of **9** (0.12 g, 0.38 mmol) in CH_2Cl_2 (2 mL), was added AcCl (0.08 mL, 1.13 mmol) and pyridine (0.09 mL, 1.13 mmol). After 1 h, reaction mixture was concentrated in *vacuo* and chromatographed on silica gel (5% ethyl acetate: petroleum ether) to obtain compound **10**

as a pale yellowish liquid (0.13 g, 95%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65-7.62 (m, 2H, ArH), 7.38-7.33 (m, 3H, ArH), 5.19 (d, *J* = 2.8 Hz, 1H, H-4), 4.87 (dd, *J* = 9.4, 2.8 Hz, 1H, H-3), 4.99 (d, *J* = 9.4 Hz, 1H, H-1), 3.28 (q, *J* = 6.2 Hz, 1H, H-5), 3.62 (t, *J* = 9.4 Hz, 1H, H-2), 2.11 (s, 3H, CH_3), 2.01 (s, 3H, CH_3), 1.23 (d, *J* = 6.2 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.5, 169.9, 133.4, 131.6, 129.1, 128.5, 86.5, 73.5, 73.2, 69.8, 59.4, 20.8, 20.7, 16.7; HR-ESI-MS (*m/z*): $[M + Na]^+$ calcd for $C_{16}H_{19}NaN_3O_5S$, 388.0938, found 388.0938.

Phenyl 3,4-*O*-diacetyl-2-(azidoacetyl amino)-2,6-dideoxy-1-thio-*β*-D-galactopyranoside (11)

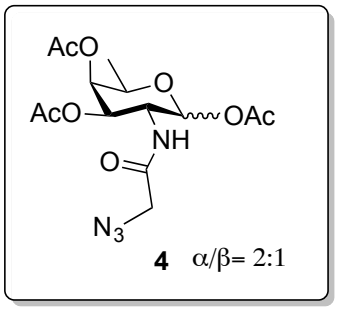


Palladium on carbon (0.1 g) was added to a clear solution of **10** (0.13 g, 0.35 mmol) in EtOH (8 mL) and the solution was stirred at room temperature under hydrogen (1 atm) for 3 h. After complete consumption of starting material the reaction mixture was filtered through celite pad and washed with EtOH. The filtrate was concentrated and the residue was used for the next step without any purification.

The residue obtained after solvents removal was dissolved in CH_3CN (2 mL), to this $NaHCO_3$ (84 mg, 1.0 mmol) and azido acetic acid (53 mg, 0.53 mmol) were added. After 5 min, EDC (0.1 g, 0.53 mmol) and HOBT (15 mg, 0.09 mmol) were added and the reaction was continued with stirring at rt for 2 h. After complete consumption of starting materials solvent was removed and the crude residue was purified on silica gel (25% ethyl acetate: petroleum ether) to obtain desired product **11** as a foam (0.13 g, 88%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.54-7.51 (m, 2H, ArH), 7.35-7.30 (m, 3H, ArH), 6.41 (d, *J* = 9.2 Hz, 1H, NH), 5.28-5.24 (m, 2H, H-3 & H-4),

4.97 (d, *J* = 10.0 Hz, 1H, H-1), 4.19 (q, *J* = 10.0 Hz, 1H, H-2), 3.97 (ABq, *J* = 16.6, 5.0 Hz, 2H, CH_2), 3.86 (q, *J* = 6.2 Hz, 1H, H-5), 2.17 (s, 3H, CH_3), 2.01 (s, 3H, CH_3), 1.26 (d, *J* = 6.2 Hz, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.8, 170.7, 167.2, 133.1, 132.3, 129.1, 128.0, 86.8, 73.4, 71.4, 70.1, 52.8, 49.9, 20.9, 16.8; HR-ESI-MS (*m/z*): $[M + Na]^+$ calcd for $C_{18}H_{22}NaN_4O_6S$, 445.1152, found 445.1154.

Acetyl 3,4-*O*-diacetyl-2-(azidoacetyl amino)-2,6-dideoxy-α/β-D-galactopyranoside (4)

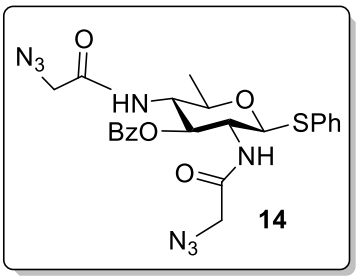


4 α/β= 2:1

NBS (0.1 g, 0.5 mmol) was added at 0 °C to a cooled solution of **11** (0.07 g, 0.17 mmol) in THF: H_2O (2.5 mL, 4:1). After 10 min, reaction mixture was brought to rt and stirred for 30 min. After complete consumption of starting material reaction mixture was diluted with EtOAc and washed with aq. $NaHCO_3$. Separated organic layer dried over Na_2SO_4 and concentrated *in vacuo*. The crude product obtained in above step was dissolved in CH_2Cl_2 (1.5 mL) and to this clear solution Ac_2O (30 µL, 0.3 mmol) and Et_3N (0.13 mL, 0.9 mmol) were added. After stirring at rt for 4 h, solvents were removed in vacuo and the crude product was purified by column chromatography on silica gel (50% ethyl acetate: pet ether) to afford the desired product **4** as a viscous liquid (50 mg, 76%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.48 (d, *J* = 9.4 Hz, 1H, NHα), 6.33 (d, *J* = 9.4 Hz, 1H, NHβ), 6.18 (d, *J* = 3.7 Hz, 1H, H-1α), 5.74 (d, *J* = 10.0 Hz, 1H, H-1β), 5.27-5.14 (m, 2H, H-3 & H-4), 4.68-4.63 (m, 1H, H-2α), 4.39 (q, *J* = 10.0 Hz, 1H, H-2β), 4.16 (q, *J* = 6.2 Hz, 1H, H-5α), 3.93-3.89 (m, 3H, H-5β & CH_2), 2.18 (s, 3H, CH_3), 2.16, 2.10 (s, 3H, CH_3), 2.01, 1.99 (s, 3H, CH_3), 1.21 (d, *J* = 6.2 Hz, 3H, CH_3β), 1.14 (d, *J* = 6.2 Hz, 3H, CH_3α); ^{13}C NMR (125 MHz,

$CDCl_3$) δ 171.3, 170.8, 169.8, 169.4, 169.3, 167.2, 92.8, 91.4, 70.6, 69.9, 69.5, 68.2, 67.4, 52.7, 52.6, 49.9, 47.1, 29.7, 21.5, 21.0, 20.8, 16.2; HR-ESI-MS (*m/z*): $[M + Na]^+$ calcd for $C_{14}H_{20}NaN_4O_8$, 395.1173, found 395.1105.

Phenyl 2,4-(diazidoacetyl amino)-3-*O*-benzoyl-2,4,6-trideoxy-1-thio-*β*-D-glucopyranoside (14)



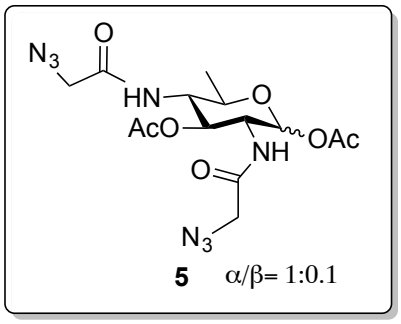
14

Palladium on carbon (0.2 g) was added to a clear solution of **13** (0.15 g, 0.36 mmol) in EtOH (10 mL) and the solution was stirred at room temperature under hydrogen (1 atm) for 10 h. After complete consumption of starting material the reaction mixture was filtered through celite pad and washed with EtOH. The filtrate was concentrated and the residue was used for the next step without any purification.

The residue obtained after solvents removal was dissolved in CH_3CN (2 mL), to this $NaHCO_3$ (0.18 g, 2.1 mmol) and azido acetic acid (0.11 g, 1.1 mmol) were added. After 5 min, EDC (0.21 g, 1.1 mmol) and HOBT (30 mg, 0.18 mmol) were added and the reaction was continued with stirring at rt for 2 h. After complete consumption of starting materials solvent was removed and the crude residue was purified on silica gel (30% ethyl acetate: petroleum ether) to obtain desired product **14** as a foam (0.12 g, 72%). ^1H NMR (500 MHz, $CDCl_3$) δ 7.94 (d, *J* = 8.2 Hz, 1H, ArH), 7.61-7.54 (m, 3H, ArH), 7.45-7.41 (m, 2H, ArH), 7.37-7.31 (m, 3H, ArH), 7.12 (d, *J* = 8.8 Hz, 1H, NH), 7.08 (d, *J* = 8.8 Hz, 1H, NH), 6.00 (t, *J* = 10.0 Hz, 1H, H-3), 5.26 (d, *J* =

10.0 Hz, 1H, H-1), 4.14-3.91 (m, 3H, H-2, H-4 & H-5), 3.89-3.70 (m, 4H, $2CH_2$), 1.41 (d, *J* = 6.2 Hz, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.9, 167.5, 167.2, 134.2, 133.0, 132.6, 130.0, 129.2, 128.9, 128.7, 128.3, 85.7, 75.1, 73.8, 55.9, 54.4, 52.7, 52.6, 18.4; HR-ESI-MS (*m/z*): [M + Na]$^+$ calcd for $C_{23}H_{24}NaN_8O_5S$, 547.1483, found 547.1475.

Acetyl 3-*O*-acetyl-2,4-(diazidoacetyl amino)-2,4,6-trideoxy-α/β-D-glucopyranoside (5)

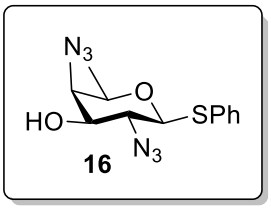


5 α/β= 1:0.1

NaOMe (30 mg) was added to clear solution of **14** (0.3 g. 0.56 mmol) in MeOH (3 mL) and stirred at rt for 30 min. After complete consumption of starting material, reaction mixture was neutralized with Amberlite (H^+, 0.3 g) and the reaction mixture was filtered and concentrated. NBS (0.3 g, 1.7 mmol) was added at 0 °C to a cooled solution of above obtained residue in THF: H_2O (10.0 mL, 4:1). After 10 min, reaction mixture was brought to rt and stirred for 30 min. After complete consumption of starting material, reaction mixture was diluted with EtOAc and washed with aq. $NaHCO_3$. Separated organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The crude product obtained in above step was dissolved in CH_2Cl_2 (1.5 mL) and to this clear solution Ac_2O and Et_3N were added. After stirring at rt for 6 h, solvents were removed *in vacuo* and the crude product was purified by column chromatography on silica gel (70% ethyl acetate: pet ether) to afford the desired product **5** as a white solid (0.16 g, 68%). ^1H NMR (400 MHz, $CDCl_3$) δ 6.33 (d, *J* = 9.2 Hz, 1H, NH), 6.29 (d, *J* = 9.2 Hz, 1H, NH), 6.16 (d, *J* = 3.7 Hz, 1H, H-1), 5.21 (t, *J* = 10.0 Hz, 1H, H-3), 4.47-4.41 (m, 1H, H-2), 3.99-3.90 (m, 6H, H-4, H-5 & $2CH_2$), 2.08 (s, 3H, CH_3), 2.07 (s, 3H, CH_3), 1.23 (d, *J* = 6.2 Hz, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ

172.2, 169.2, 166.9, 90.7, 70.1, 69.1, 55.0, 52.7, 52.6, 51.4, 21.1, 20.9, 18.0; HR-ESI-MS (*m/z*): $[M + Na]^+$ calcd for $C_{14}H_{20}NaN_8O_7$, 435.1347, found 435.1362.

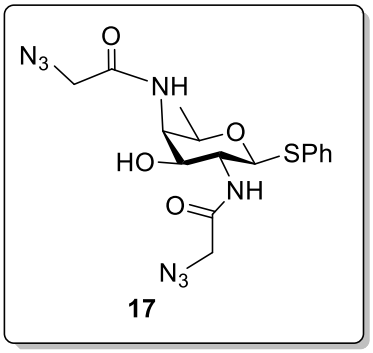
Phenyl 2,4-diazido-2,4,6-trideoxy-1-thio-β-D-galactopyranoside (16)



16

NaOMe (50 mg) was added to clear solution of **15** (0.5 g. 1.22 mmol) in MeOH (5 mL) and stirred at rt for 30 min. After complete consumption of starting material reaction mixture was neutralized with Amberlite (H^+, 0.5 g) and the reaction mixture was filtered, concentrated and the crude product was purified by column chromatography on silica gel (15% ethyl acetate: pet ether) to afford the desired product **16** as a yellowish liquid (0.36 g, 96%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.62-7.61 (m, 2H, ArH), 7.36-7.28 (m, 3H, ArH), 4.40 (d, *J* = 10.0 Hz, 1H, H-1), 3.72-3.66 (m, 3H, H-3, H-4 & H-5), 3.54 (t, *J* = 9.4 Hz, 1H, H-2), 1.41 (d, *J* = 6.2 Hz, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 133.2, 131.7, 129.2, 128.5, 86.9, 74.5, 74.1, 65.1, 62.8, 18.0; HR-ESI-MS (*m/z*): $[M + Na]^+$ calcd for $C_{12}H_{14}NaN_6O_2S$, 329.0791, found 329.0796.

Phenyl 2,4-(diazidoacetyl amino)-2,4,6-trideoxy-1-thio-β-D-galactopyranoside (17)



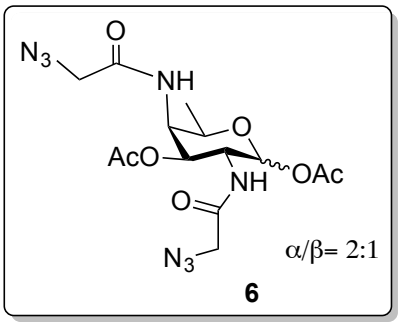
17

Palladium on carbon (0.35 g) was added to a clear solution of **16** (0.2 g, 0.65 mmol) in EtOH (14

mL) and the solution was kept for stirring at room temperature under hydrogen (1 atm) 10 h. After complete consumption of starting material, the reaction mixture was filtered through celite pad and washed with EtOH. The filtrate was concentrated and the residue was used for the next step without any purification.

The residue obtained after solvents removal was dissolved in CH_3CN (3 mL), to this $NaHCO_3$ (0.47 g, 5.6 mmol) and azido acetic acid (0.3 g, 2.9 mmol) were added. After 5 min, EDC (0.56 g, 2.93 mmol) and HOBT (75 mg, 0.49 mmol) were added and the stirring was continued at rt for 2 h. After complete consumption of starting material, solvent was removed under reduced pressure. The crude residue was dissolved in MeOH (2 mL) and to this NaOMe (20 mg) was added. After 20 min, reaction mixture was neutralized with Amberlite (H^+, 0.2 g) and filtered. Filtrate was concentrated and the crude product was purified by column chromatography on silica gel (35% ethyl acetate: pet ether) to afford the desired product **17** as a yellowish liquid (0.20 g, 74%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.54-7.51 (m, 2H, ArH), 7.36-7.29 (m, 3H, ArH), 6.72 (d, *J* = 8.4 Hz, 1H, NH), 6.56 (d, *J* = 8.4 Hz, 1H, NH), 4.80 (d, *J* = 10.0 Hz, 1H, H-1), 4.31 (dd, *J* = 10.0, 3.2 Hz, 1H, H-4), 4.10-3.98 (m, 5H, H-3 & $2CH_2$), 3.84 (q, *J* = 6.4 Hz, 1H, H-5), 3.62 (q, *J* = 10.0 Hz, 1H, H-2), 1.23 (d, *J* = 6.4 Hz, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.4, 168.7, 132.9, 132.3, 129.4, 128.2, 86.5, 73.9, 72.2, 53.6, 52.6, 52.5, 52.3, 17.2; HR-ESI-MS (*m/z*): $[M + Na]^+$ calcd for $C_{16}H_{20}NaN_8O_4S$, 443.1220, found 443.1220.

Acetyl 3-O-acetyl-2,4-(diazidoacetyl amino)-2,4,6-trideoxy-α/β-D-galactopyranoside (6)



6

NBS (0.11 g, 0.6 mmol) was added at 0 °C to a cooled solution of **17** (87 mg, 0.2 mmol) in THF: H_2O (2.5 mL, 4:1). After 10 min, reaction mixture was brought to rt and stirred for 30 min. After complete consumption of starting material, reaction mixture was diluted with EtOAc and washed with aq. $NaHCO_3$. Separated organic layer dried over Na_2SO_4 and concentrated *in vacuo*. The crude product obtained in above step was dissolved in CH_2Cl_2 (1.5 mL) and to this clear solution Ac_2O (60 µL, 0.6 mmol) and Et_3N (0.26 mL, 1.8 mmol) were added. After stirring at rt for 4 h, solvents were removed in vacuo and the crude product was purified by column chromatography on silica gel (70% ethyl acetate: pet ether) to afford the desired product **6** as a viscous liquid (62 mg, 72%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.66 (d, *J* = 9.4 Hz, 1H, NH), 6.62(d, *J* = 9.4 Hz, 1H, NH), 6.38 (d, *J* = 9.4 Hz, 1H, NH), 6.32(d, *J* = 9.4 Hz, 1H, NH), 6.18 (d, *J* = 3.8 Hz, 1H, H-1α), 5.67 (d, *J* = 10.0 Hz, 1H, H-1β), 5.21 (d, *J* = 2.8, 10.0 Hz, 1H, H-3α), 5.09 (d, *J* = 2.8, 10.0 Hz, 1H, H-3β), 4.54-4.13 (m, 3H, H-2, H-4 & H-5), 4.09 (s, 2H, CH_2), 3.94 (s, 1H, CH_2), 3.92 (s, 1H, CH_2), 1.24 (d, *J* = 6.2 Hz, 3H, CH_3), 1.16 (d, *J* = 6.2 Hz, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.1, 167.8, 167.5, 167.3, 93.3, 90.8, 71.4, 70.8, 68.9, 67.1, 52.7, 52.66, 52.62, 50.7, 50.5, 50.0, 47.2, 29.8, 21.1, 20.9, 16.73, 16.70; HR-ESI-MS (*m/z*): $[M + Na]^+$ calcd for $C_{14}H_{20}NaN_8O_7$, 435.1347, found 435.1364.

Biology

General. All biological reagents were obtained from commercial suppliers and used without further purification. *Helicobacter pylori* strain G27[4] was a gift from Manuel Amieva (Stanford University). *Mycobacterium smegmatis* strain Mc^{2}155::*otsA treS treY*[5] was a gift from Peter Woodruff (University of Southern Maine). *Campylobacter jejuni* (ATCC 33560). *Burkholderia thailandensis* (ATCC 700388), *Ralstonia solanacearum* ATCC 33291* (*incorrectly cataloged as *C. jejuni*; species confirmed as *Ralstonia solanacearum* by 16S ribosomal RNA sequencing),

Bacteroides fragilis ATCC 23745, *Pseudomonas aeruginosa* ATCC 39324, and MDCK cells (ATCC CCL-34) were purchased from ATCC and grown as described below.

Bacterial growth conditions. Six bacterial species were used to examine incorporation of unnatural azide-containing sugars. *H. pylori* was grown on horse blood agar plates (4% Columbia agar, 5% horse blood, 10 μg/mL vancomycin, 5 μg/mL cefsulodin, 0.3 μg/mL polymixin B, 5 μg/mL trimethoprim, and 8 μg/mL amphotericin B) or in Brucella Broth (with 10% Fetal Bovine Serum (FBS) and 6 μg/mL vancomycin) for 3-5 days in 14% CO_2 at 37 °C. *M. smegmatis* was grown on Middlebrook 7H9 agar plates or in Middlebrook 7H9 broth (with 0.25% Tween 80) at 37 °C in an aerobic environment for 3-5 days. *R. solanacearum* and *C. jejuni* were grown for 3-5 days on 4% Müller-Hinton agar plates or in Müller-Hinton broth in 14% CO_2 at 37 °C. *B. thailandensis* was grown on 2.3% nutrient agar plates or in 2.5% nutrient broth at 30 °C in an aerobic environment for 3-5 days. *B. fragilis* was grown overnight on brain-heart infusion plates (1.5% Bacto agar, 3.7% brain-heart infusion broth, 0.5% yeast extract, and 15 μg/mL hematin porcine) or in brain-heart infusion broth (3.7% brain-heart infusion broth, 0.5% yeast extract, and 15 μg/mL hematin porcine). *B. fragilis* cultures were incubated at 37 °C under anaerobic conditions generated using a GasPak™ EZ Anaerobe Pouch System (Becton Dickinson).[6]

Mammalian cell growth and metabolic labeling. MDCK cells were grown in Dulbecco's Modified Eagle Medium (DMEM) (Life Technologies) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (P/S) in a 5% CO_2, water saturated incubator at 37°C. Cells were passaged every 3 days by lifting using trypsin-EDTA (Gibco) and plating at a density of 2 x 10^5 cells/mL in supplemented DMEM. For metabolic labeling, cells were seeded into DMEM with 10% fetal bovine serum (FBS), penicillin/streptomycin, and 250 μM of

Ac_4GlcNAc (**1**), Ac_4GlcNAz (**2**), $Ac_3$6-AzGlcNAc (**3**), Ac_3FucNAz (**4**), Ac_2Bac-diNAz (**5**), Ac_2DATDH-diNAz (**6**), or Ac_4GalNAz, then grown for 3 days in a 5% CO_2, water saturated incubator at 37°C. After three days of metabolic labeling, cells were lifted using trypsin-EDTA, rinsed three times with PBS, then analyzed for azides via Staudinger ligation (see below).

Metabolic labeling of bacterial strains. Bacterial strains from a frozen stock were streaked onto agar plates using a sterile tip applicator and then incubated under appropriate conditions for the bacterial strain (see *Bacterial growth conditions*). The bacteria from plates were transferred with a sterile tip applicator to 3 mL of liquid growth containing 1 mM of Ac_4GlcNAc (**1**), Ac_4GlcNAz (**2**), $Ac_3$6-AzGlcNAc (**3**), Ac_3FucNAz (**4**), Ac_2Bac-diNAz (**5**), Ac_2DATDH-diNAz (**6**), or Ac_4GalNAz. Once cells reached stationary phase, they were centrifuged at 3500 rpm using a Sorvall Legend RT^+ centrifuge (Thermo Scientific, Waltham, MA) and washed three times with PBS.

Probing for azides on cells and in lysates via Staudinger ligation. To probe for total azide-labeled glycoproteins in cells, metabolically labeled and rinsed cells were lysed in lysis buffer (20 mM Tris-HCl, pH 7.4, 1% Igepal, 150 mM NaCl, 1 mM EDTA) containing protease inhibitor cocktail (Sigma Aldrich, St. Louis, MO). Lysates were subsequently standardized to ~3 mg/mL, reacted 1:1 with 500 μM Phos-FLAG at 37°C for 7-12 hours, and analyzed by Western blot, as described below. Alternatively, the presence of cell surface azides on metabolically labeled bacteria was determined by performing Staudinger ligation with cell-impermeable Phos-FLAG (500 μM for 5-6 hours) on intact cells.[7] Phos-FLAG reacted cells were then rinsed three times with PBS and lysed to facilitate analysis by Western blot.

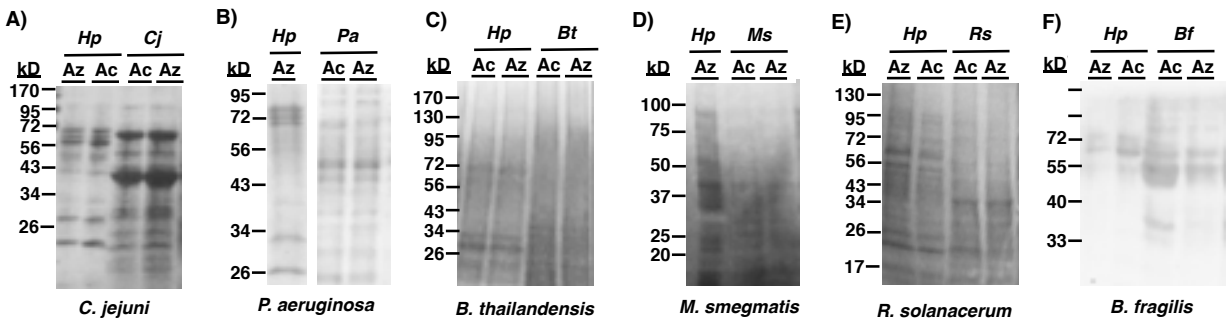
Western blot analysis of azide-labeled proteins. Protein concentrations of samples were

measured using the DC Protein Assay (Bio-Rad, Hercules, CA) and standardized to equal concentrations (~3 mg/mL). All samples were then combined in a 1:1 ratio with 2X SDS reducing loading buffer and boiled at 95°C for 5-10 minutes. Samples (20 μg for bacterial samples; 14 μg for MDCK samples), alongside a molecular weight ladder (EZ-Run Prestained Rec Protein Ladder, Fisher Scientific), were loaded onto a 4-15% Tris-HCl gradient gel (Bio-Rad) or a 12% Tris-HCl SDS-PAGE gel with a 4% stacking layer. After electrophoresis at 200 V for 40 minutes on ice, proteins were transferred to a nitrocellulose membrane (Bio-Rad) at 100 V for 1 hour or stained with Coomassie (Stain: 45% deionized water, 45% Methanol, 10% acetic acid, 0.25% Coomassie brilliant blue/Destain: 50% deionized water, 40% methanol, 10% acetic acid) or Zinc Stain (BioRad) to visualize equal protein loading. Immunoblots were blocked for 1 hour with 5% non-fat dried milk in 0.05% TBS-T buffer (5 mM Tris-HCl, 0.05% Tween-20 (BioRad), pH 7.4. Anti-FLAG-HRP (1:1000 dilution; Sigma Aldrich) was employed to visualize FLAG-tagged proteins via chemiluminescence with film (Thermo Scientific) or G:BOX Chemi XRQ gel doc system (Syngene).

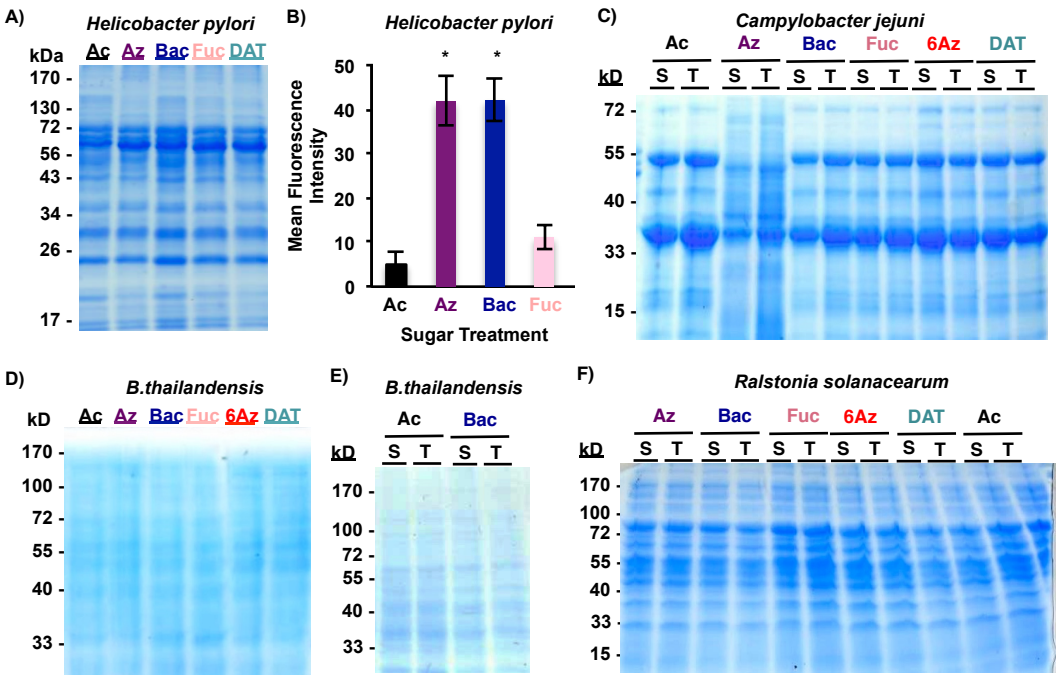
Flow cytometry experiments. To complement Western blot analyses, the presence of azides on cell surfaces was also probed via flow cytometry. For these experiments, metabolically labeled cells were reacted with Phos-FLAG (500 μM in PBS) for 1 hour. After washing with FACS buffer (1X PBS containing 1% fetal bovine serum (FBS)), cells were immunostained with α-FLAG-FITC (1:100 dilution in FACS buffer; Sigma-Aldrich; ex: 488/em: 519) for 1 hour, rinsed with FACS buffer, and analyzed using a BD FACSCalibur flow cytometer. Alternatively, azide-containing sugars were detected using Click-IT Alexa Fluor 488 DIBO Alkyne for copper free click chemistry detection of azide (Thermo Fisher Scientific). In this case, whole cells metabolically labeled as described above (see “Metabolic Labeling”) were reacted with 20 μM Click-IT Alexa Fluor 488 DIBO Alkyne for copper free click chemistry detection of azide (ex:

488/em: 519)[8] under standard growth conditions for 5 hours in the dark. Cells were then rinsed with PBS supplemented with 3% BSA, and analyzed using a BD FACSCalibur flow cytometer. For this single color analysis, 20,000 live cells were gated on for each replicate. Labeling was performed in triplicate and is reported as number of cells versus fluorescence intensity. Alternatively, flow data are reported as the mean fluorescence intensity (MFI) of a population of cells from replicate experiments, as calculated using FlowJo software (Ashland, OR).

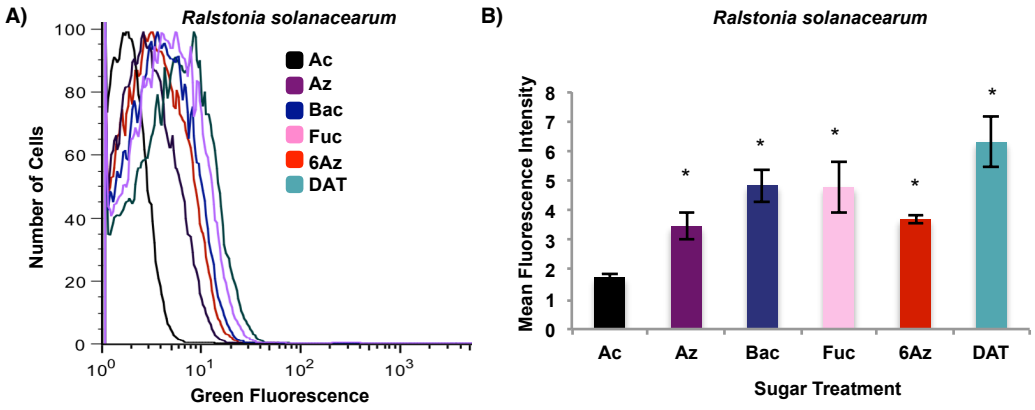
Supplemental Figures



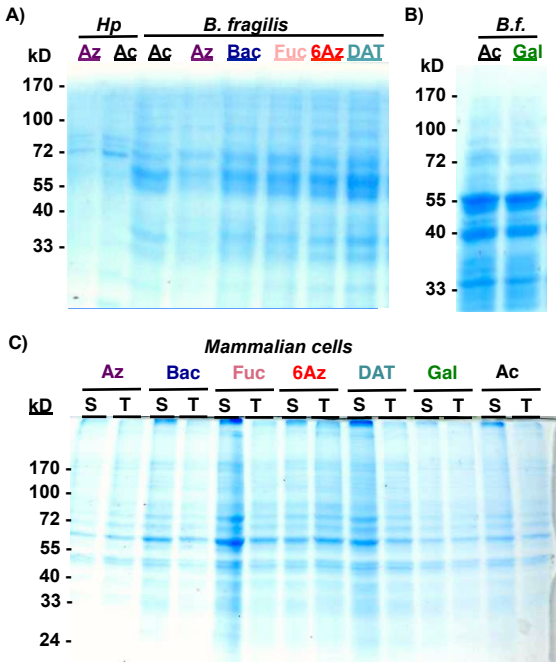
Supplementary Figure 1. Samples probed by Western blot in Figure 3 were electrophoresed and stained with Coomassie (A, B, F) or zinc stain (C, D, E) to reveal that samples contain protein in each experiment. For these experiments, lysates from bacterial cells treated with 1 mM Ac_4GlcNAz (**2**, Az) or the azide-free control sugar Ac_4GlcNAc (**1**, Ac) were reacted with Phos-FLAG (250 μM), then analyzed by SDS-PAGE with Coomassie or zinc stain (BioRad) to detect protein levels.



Supplementary Figure 2. Complementary data to those presented in Figure 4. (A, C, D, E, F) Coomassie staining of electrophoresed samples from Figure 4 reveal that all Western samples contain roughly equivalent protein levels. (B) Flow cytometry indicate that *H. pylori* robustly express surface-accessible azides after treatment with Ac_4GlcNAz (**2**, Az) and Ac_2Bac-diNAz (**5**, Bac). For this experiment, *H. pylori* were metabolically labeled in media containing 1 mM of the indicated sugars, probed for the presence of azides using Phos-FLAG and anti-FLAG-FITC, and analyzed by flow cytometry. Flow cytometry data are presented as mean fluorescence intensity from triplicate samples. Error bars represent standard deviations of triplicate samples. Asterisks indicate significantly elevated MFI relative to the azide-free control Ac_4GlcNAc (**1**, Ac) ($p < 0.05$ by Student's t-test).



Supplementary Figure 3. Flow cytometry data reveal that *R. solanacearum* robustly express surface-accessible azides after treatment with Ac_4GlcNAc (**1**, Ac), Ac_4GlcNAz (**2**, Az), Ac_2Bac-diNAz (**5**, Bac), Ac_3FucNAz (**4**, Fuc), $Ac_3$6Az-GlcNAc (**3**, 6Az), and Ac_2DATDG-diNAz (**6**, DAT). For this experiment, *R. solanacearum* were metabolically labeled in liquid media containing 1 mM of the indicated sugars, probed for the presence of azides using Phos-FLAG and anti-FLAG-FITC, and analyzed by flow cytometry. Flow cytometry data are presented as single parameter histograms (A) and mean fluorescence intensity from triplicate samples (B). Error bars represent standard deviations of triplicate samples. Asterisks indicate significantly elevated MFI relative to the azide-free control Ac_4GlcNAc (**1**, Ac) ($p < 0.05$ by Student's t-test).



Supplementary Figure 4. Samples probed by Western blot in Figure 5 were electrophoresed and stained with Coomassie to reveal that all samples contain roughly equivalent protein levels. (A, B) SDS-PAGE analysis of samples from *H. pylori* and *B. fragilis* treated with 1 mM Ac_4GlcNAc (**1**, Ac), Ac_4GlcNAz (**2**, Az), Ac_2Bac-diNAz (**5**, Bac), Ac_3FucNAz (**4**, Fuc), $Ac_3$6Az-GlcNAc (**3**, 6Az), and Ac_2DATDG-diNAz (**6**, DAT), then reacted with Phos-FLAG (250 μM). Proteins were visualized via Coomassie stain. (C) SDS-PAGE analysis of MDCK samples from cells cultured in media supplemented with unnatural sugars (0.25 mM), reacted with Phos-FLAG (250 μM), then electrophoresed and stained with Coomassie.

Supplemental References

[1] Luchansky, S. J., Hang, H. C., Saxon, E., Grunwell, J. R., Yu, C., Dube, D. H., and Bertozzi, C. R. (2003) Constructing azide-labeled cell surfaces using polysaccharide biosynthetic pathways, *Methods in Enzymology 362*, 249-272.

[2] Laughlin, S. T., and Bertozzi, C. R. (2007) Metabolic labeling of glycans with azido sugars and subsequent glycan-profiling and visualization via Staudinger ligation, *Nature Protocols 2*, 2930 - 2944.

[3] Chuh, K. N., Zaro, B. W., Piller, F., Piller, V., and Pratt, M. R. (2014) Changes in metabolic chemical reporter structure yield a selective probe of O-GlcNAc modification, *Journal of the American Chemical Society 136*, 12283-12295.

[4] Baltrus, D. A., Amieva, M. R., Covacci, A., Lowe, T. M., Merrell, D. S., Ottemann, K. M., Stein, M., Salama, N. R., and Guillemin, K. (2009) *The complete genome sequence of Helicobacter pylori* strain G27, *J. Bacteriol. 191*, 447-448.

[5] Woodruff, P. J., Carlson, B. L., Siridechadilok, B., Pratt, M. R., Senaratne, R. H., Mougous, J. D., Riley, L. W., Williams, S. J., and Bertozzi, C. R. (2004) Trehalose is required for growth of *Mycobacterium smegmatis*, *Journal of Biological Chemistry 279*, 28835-28843.

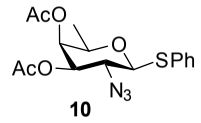
[6] Brigham, C., Caughlan, R., Gallegos, R., Dallas, M. B., Godoy, V. G., and Malamy, M. H. (2009) Sialic acid (N-acetyl neuraminic acid) utilization by *Bacteroides fragilis* requires a novel N-acetyl mannosamine epimerase, *Journal of Bacteriology 191*, 3629-3638.

[7] Kaewsapsak, P., Esonu, O., and Dube, D. H. (2013) Recruiting the host's immune system to target *Helicobacter pylori's* surface glycans, *ChemBioChem 14*, 721-726.

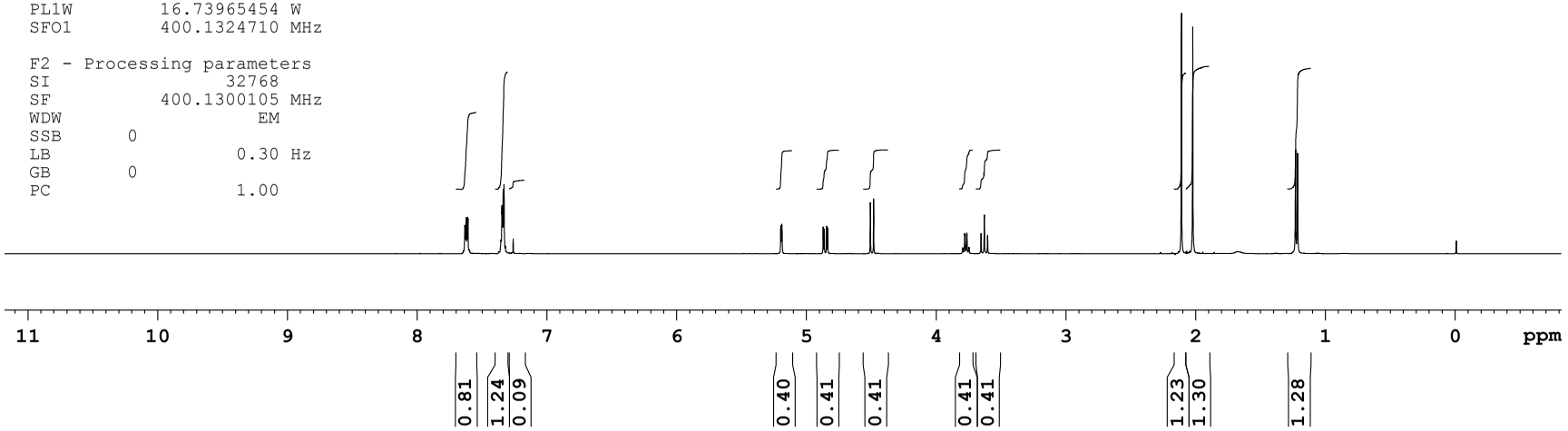
[8] Geva-Zatorsky, N., Alvarez, D., Hudak, J. E., Reading, N. C., Erturk-Hasdemir, D., Dasgupta, S., von Andrian, U. H., and Kasper, D. L. (2015) *In vivo* imaging and tracking of host-microbiota interactions via metabolic labeling of gut anaerobic bacteria, *Nature Medicine 21*, 1091-1100.

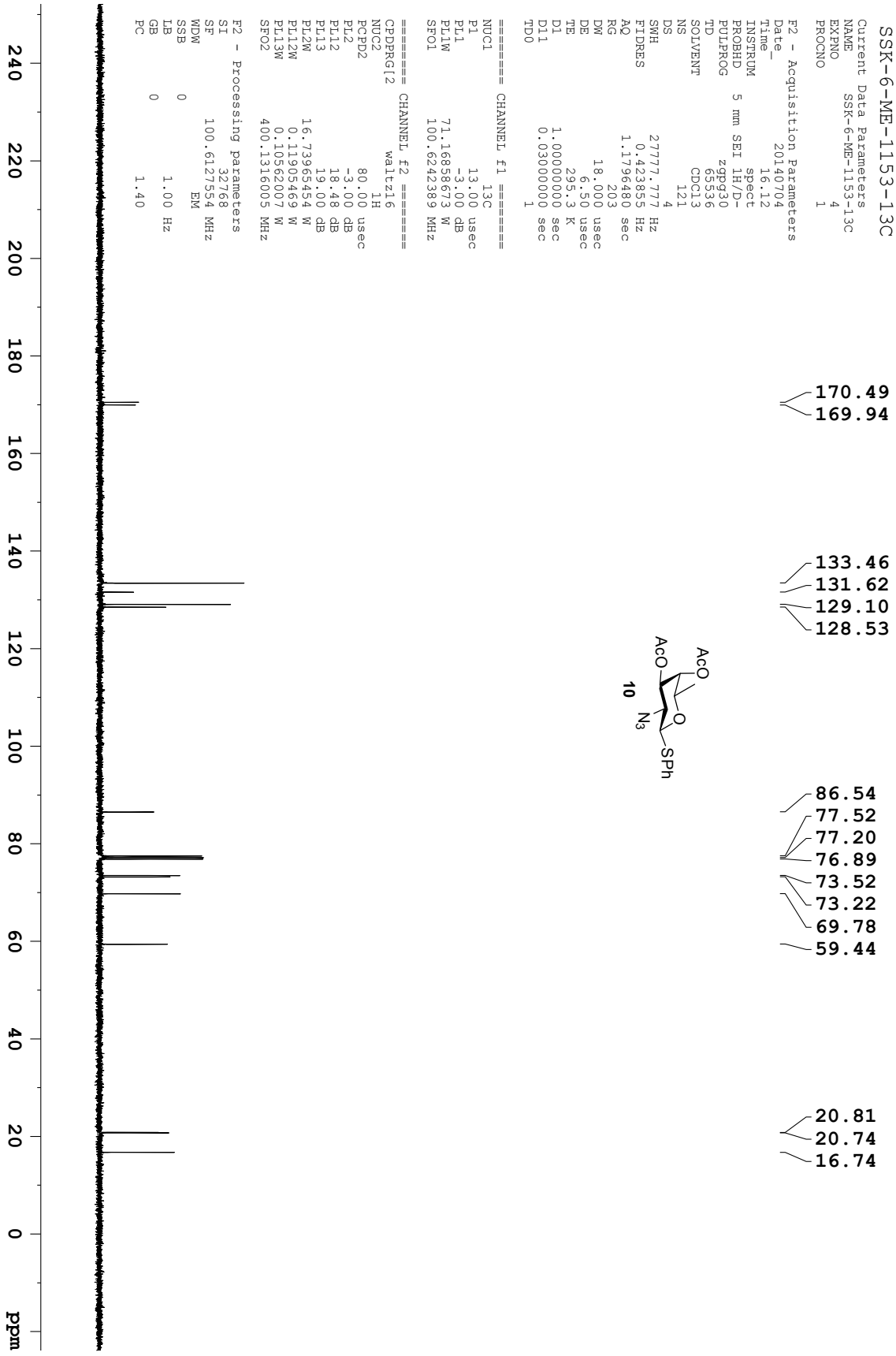
SSK-6-ME-1153-1H

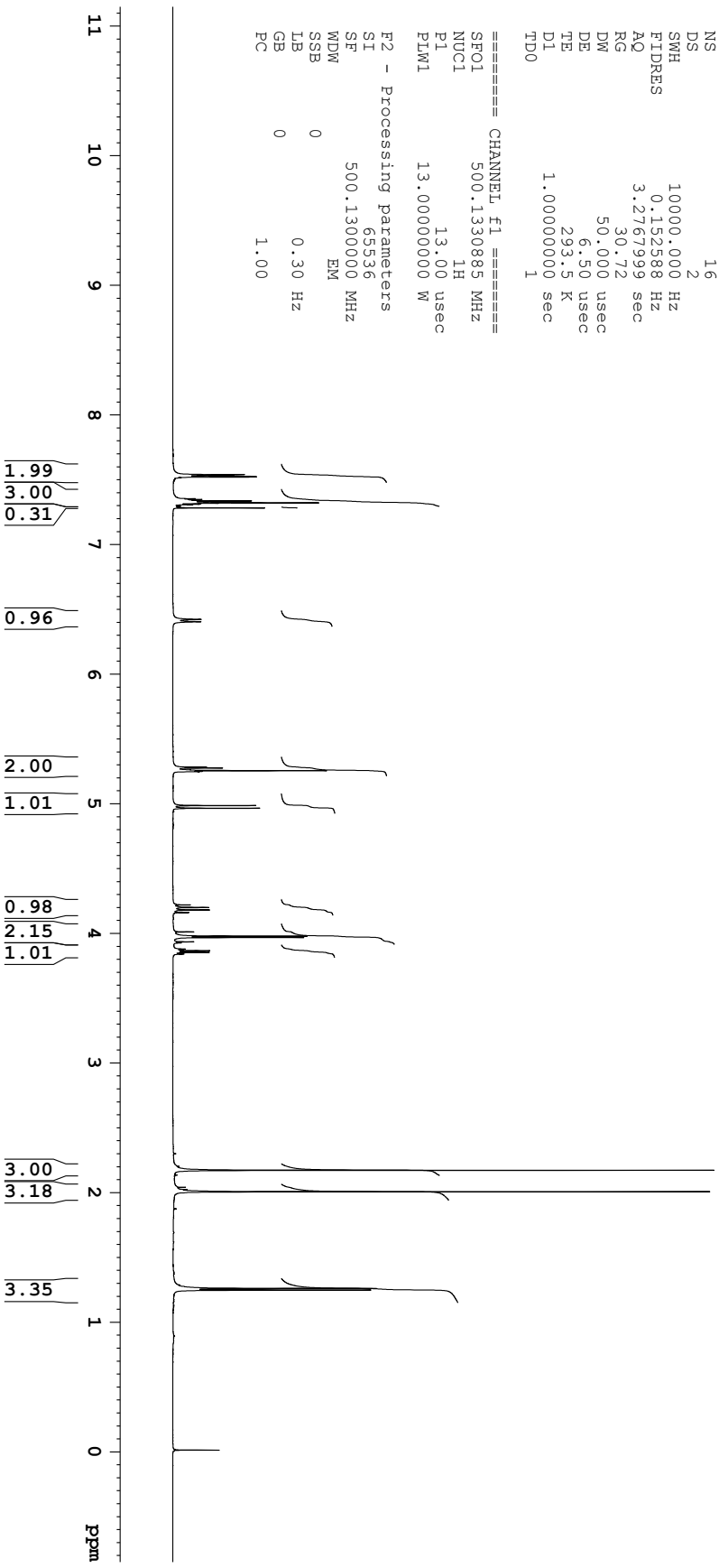
```
Current Data Parameters
NAME      SSK-6-ME-1153-1H
EXPNO                    3
PROCNO                   1

F2 - Acquisition Parameters
Date_           20140704
Time               16.09
INSTRUM            spect
PROBHD   5 mm SEI 1H/D-
PULPROG              zg30
TD                  49340
SOLVENT             CDCl3
NS                     31
DS                      0
SWH              8223.685 Hz
FIDRES           0.166674 Hz
AQ              2.9998720 sec
RG                     57
DW                 60.800 usec
DE                  6.50 usec
TE                 295.2 K
D1             1.00000000 sec
TD0                     1

======== CHANNEL f1 ========
NUC1                   1H
P1                   6.75 usec
PL1                 -3.00 dB
PL1W         16.73965454 W
SFO1         400.1324710 MHz

F2 - Processing parameters
SI                  32768
SF           400.1300105 MHz
WDW                    EM
SSB       0
LB                  0.30 Hz
GB        0
PC                  1.00
```

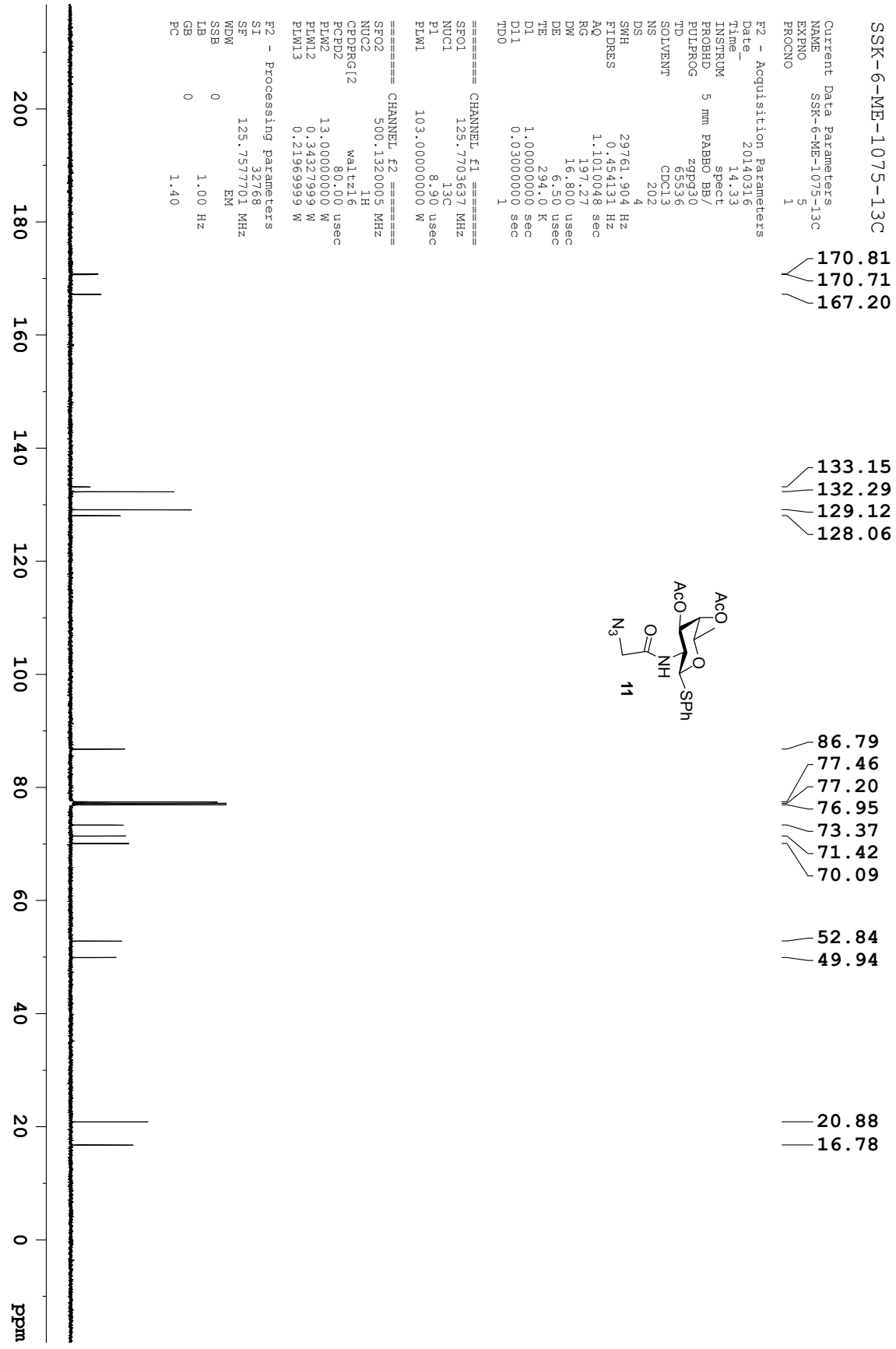


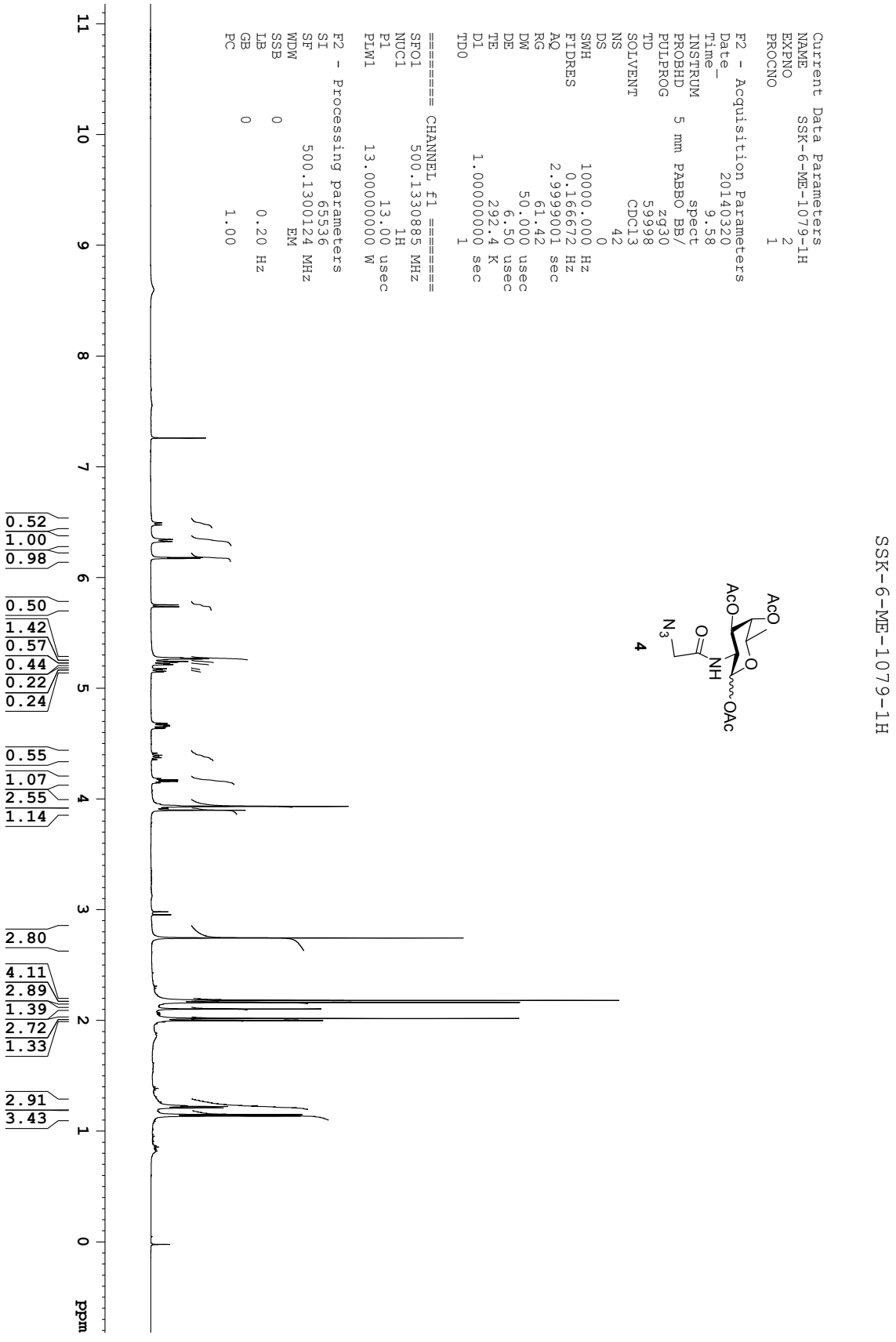
10

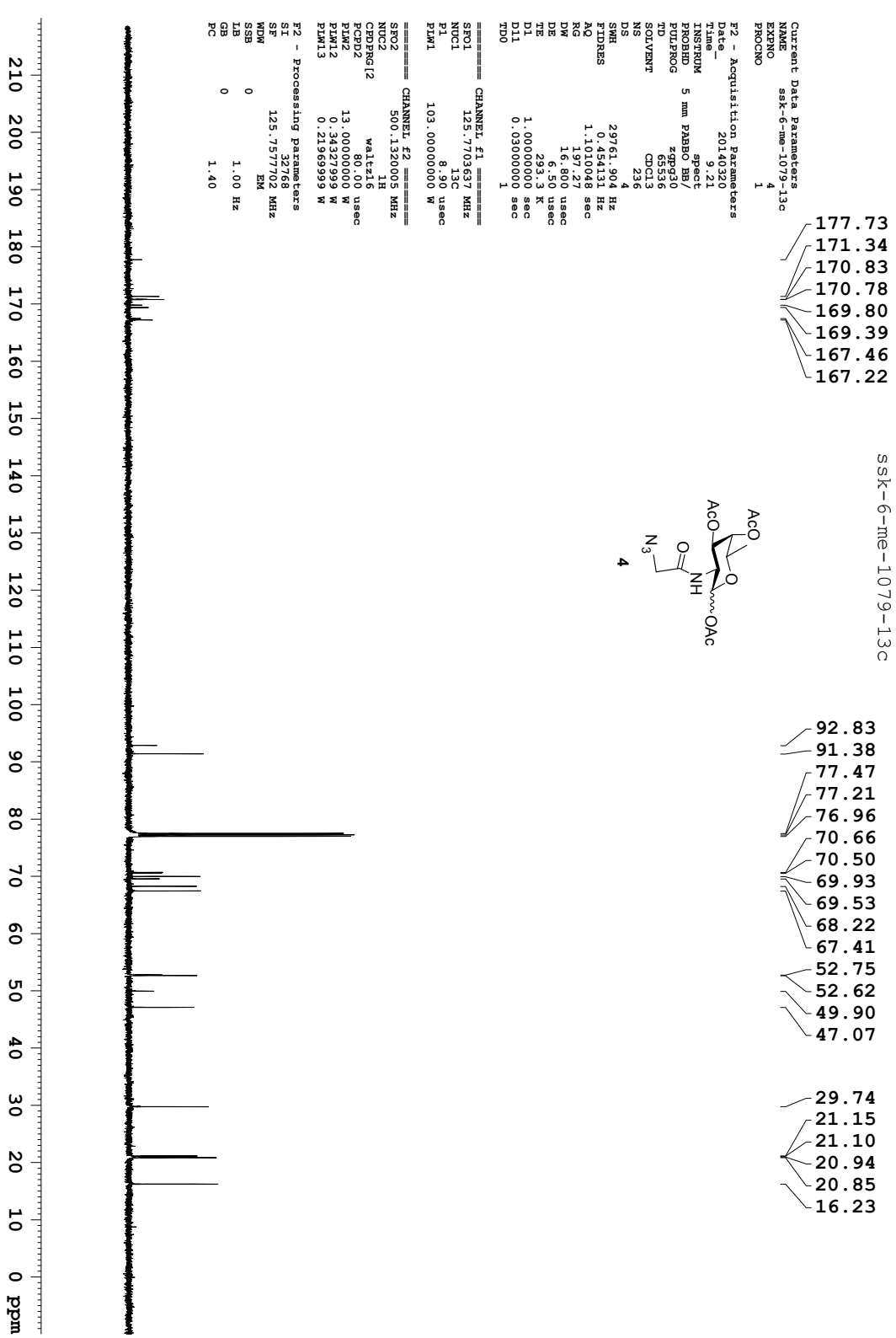


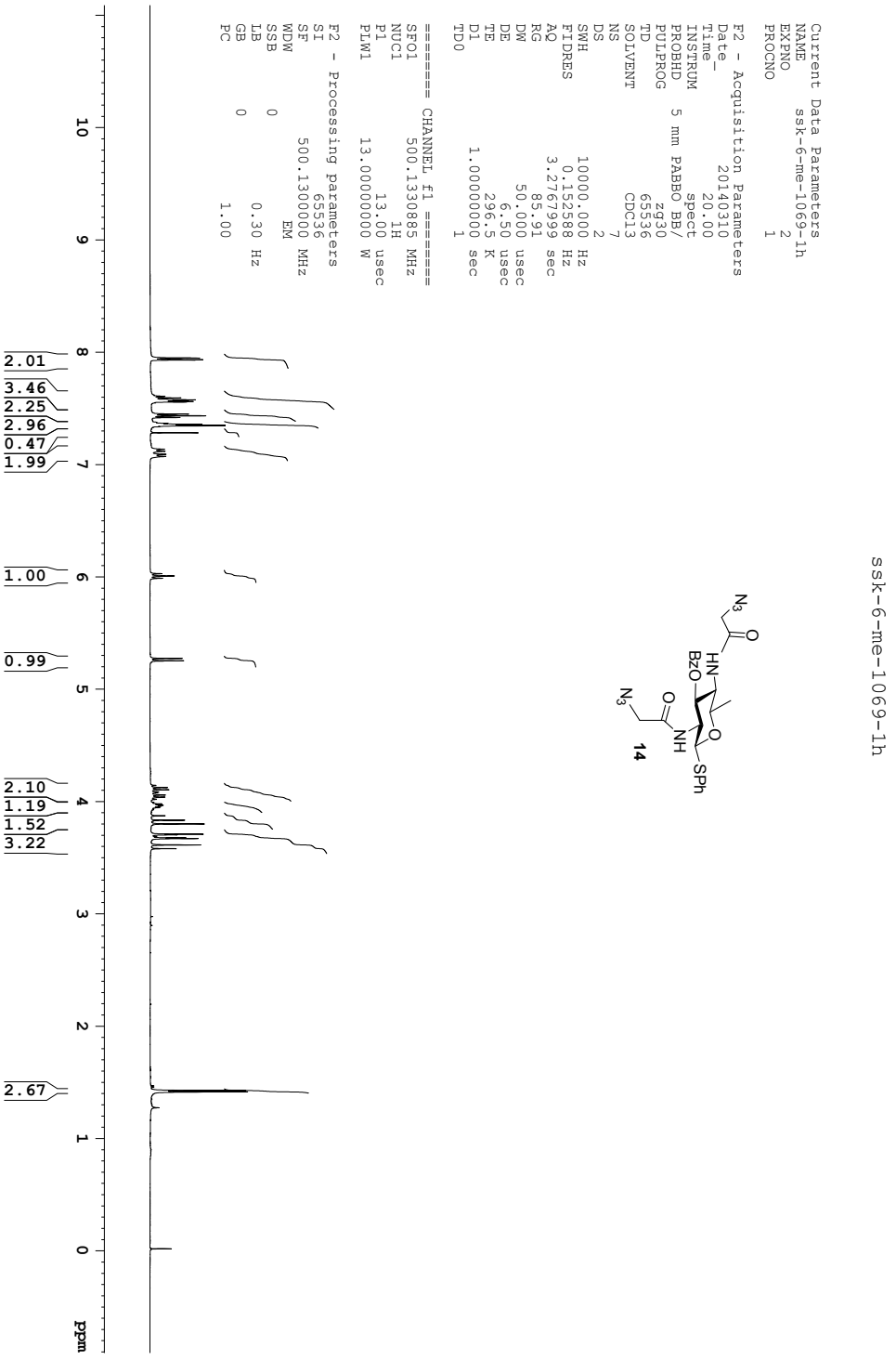

SSK-6-ME-1153-13C
Current Data Parameters
NAME SSK-6-ME-1153-13C
EXPNO 4
PROCNO 1
F2 - Acquisition Parameters
Date_ 20140704
Time_ 16.12
INSTRUM spect
PROBHD 5 mm SEI 1H/D-
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 121
DS 4
SWH 27777.777 Hz
FIDRES 0.423855 Hz
AQ 1.1796480 sec
RG 203
DW 18.000 usec
DE 6.50 usec
TE 295.3 K
D1 1.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
NUC1 13C
P1 13.00 usec
PL1 -3.00 dB
PL1W 71.16858673 W
SFO1 100.6242389 MHz
======== CHANNEL f2 ========
CPDPRG[2 waltz16
NUC2 1H
PCPD2 80.00 usec
PL2 -3.00 dB
PL12 18.48 dB
PL13 19.00 dB
PL2W 16.73965454 W
PL12W 0.11905469 W
PL13W 0.10562007 W
SFO2 400.1316005 MHz
F2 - Processing parameters
SI 32768
SF 100.6127554 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
170.49
169.94
133.46
131.62
129.10
128.53
86.54
77.52
77.20
76.89
73.52
73.22
69.78
59.44
20.81
20.74
16.74
AcO
AcO
O
10
N3
SPh
240
220
200
180
160
140
120
100
80
60
40
20
0
ppm

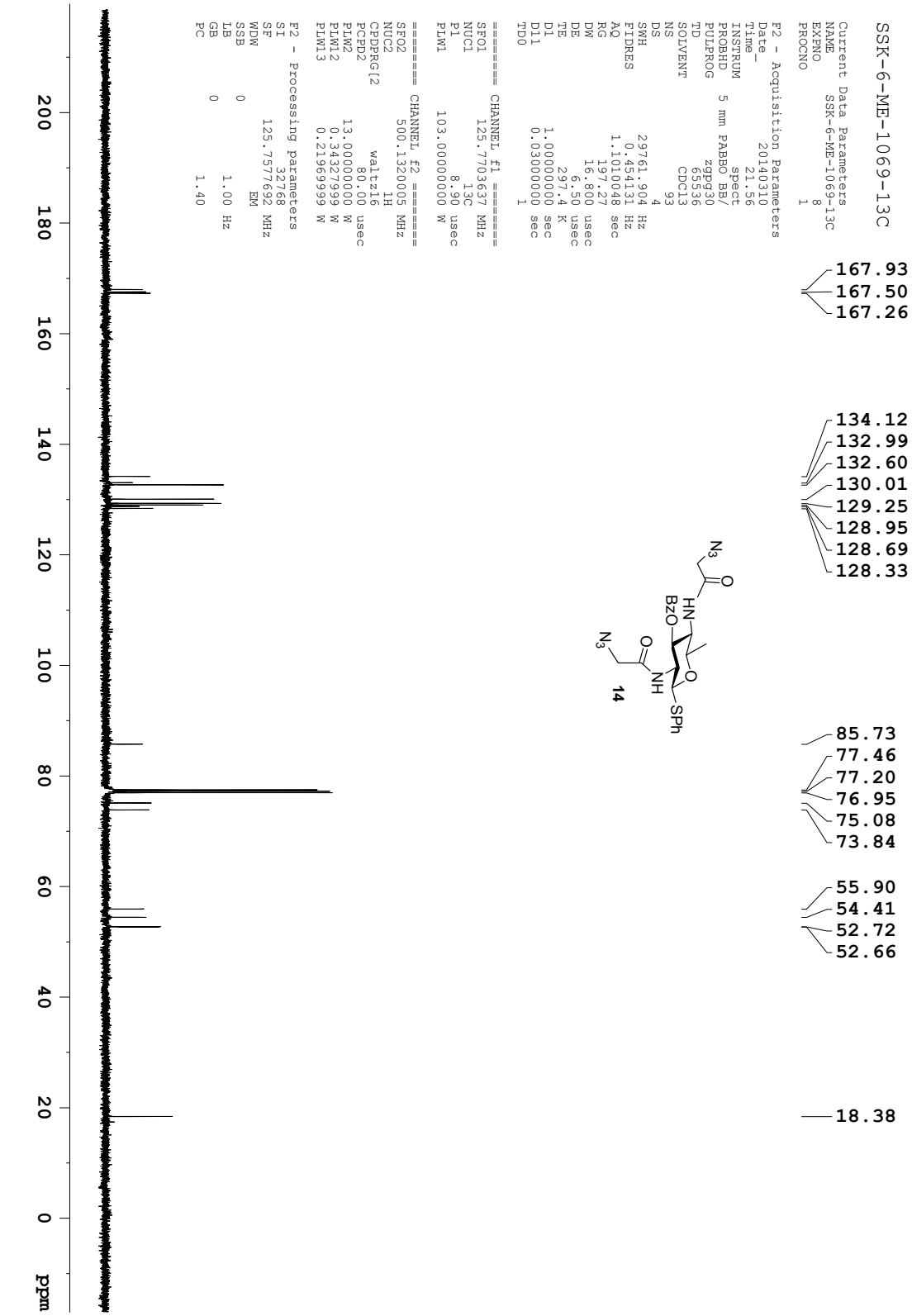

SSK-6-ME-1075-1H
Current Data Parameters
NAME SSK-6-ME-1075-1H
EXPNO 4
PROCNO 1
F2 - Acquisition Parameters
Date_ 20140316
Time_ 14.30
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 2
SWH 10000.000 Hz
FIDRES 0.152588 Hz
AQ 3.2767999 sec
RG 30.72
DW 50.000 usec
DE 6.50 usec
TE 293.5 K
D1 1.00000000 sec
TD0 1
========== CHANNEL f1 ========
SFO1 500.1330885 MHz
NUC1 1H
P1 13.00 usec
PLW1 13.00000000 W
F2 - Processing parameters
SI 65536
SF 500.1300000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
AcO
AcO
O
NH
O
SPh
N3
11
1.99
3.00
0.31
0.96
2.00
1.01
0.98
2.15
1.01
3.00
3.18
3.35
11
10
9
8
7
6
5
4
3
2
1
0
ppm

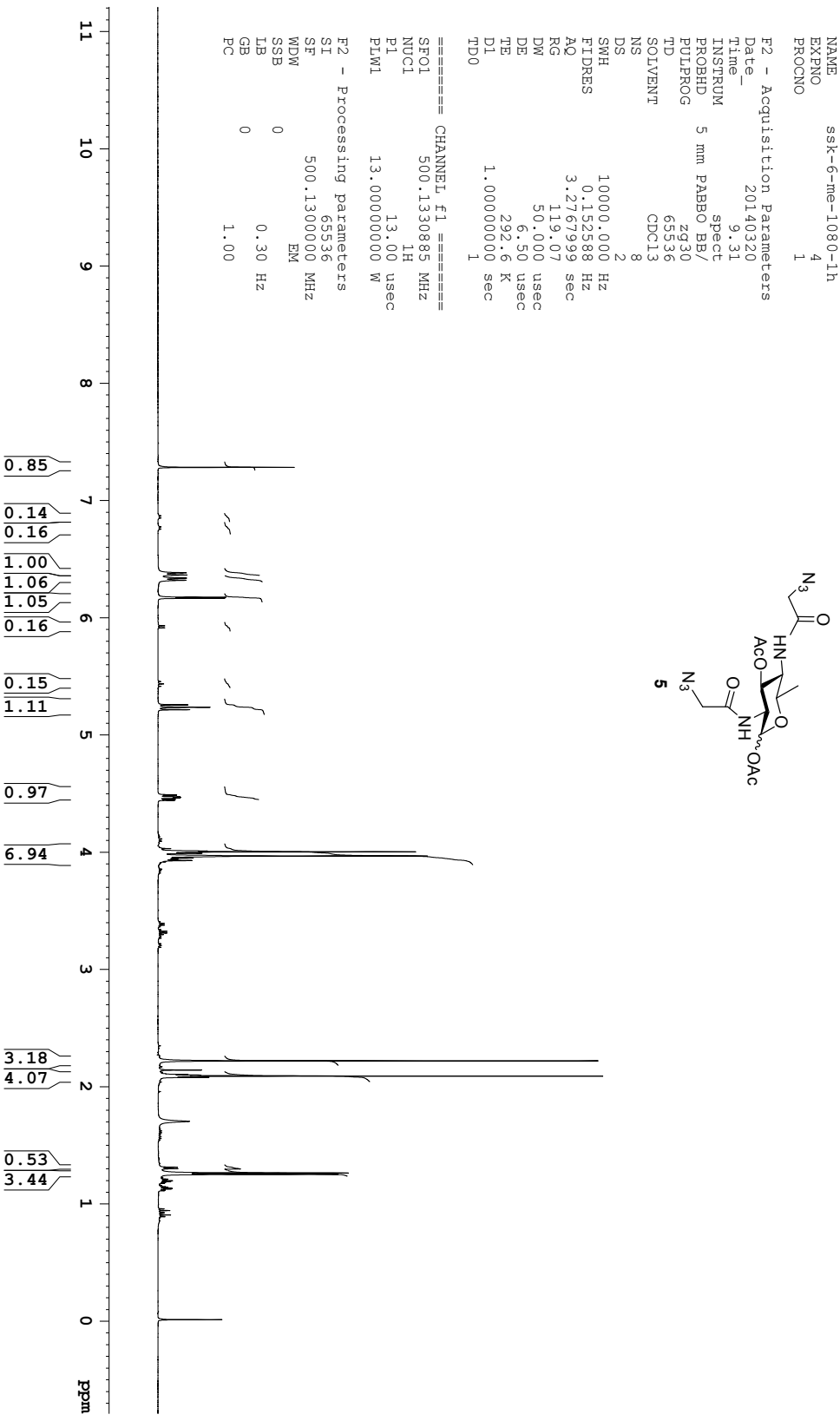

SSK-6-ME-1075-13C
Current Data Parameters
NAME SSK-6-ME-1075-13C
EXPNO 5
PROCNO 1
F2 - Acquisition Parameters
Date_ 20140316
Time_ 14.33
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 202
DS 4
SWH 29761.904 Hz
FIDRES 0.454131 Hz
AQ 1.1010048 sec
RG 197.27
DW 16.800 usec
DE 6.50 usec
TE 294.0 K
D1 1.00000000 sec
D11 0.03000000 sec
TD0 1
========== CHANNEL f1 ==========
SFO1 125.7703637 MHz
NUC1 13C
P1 8.90 usec
PLW1 103.00000000 W
========== CHANNEL f2 ==========
SFO2 500.1320005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 13.00000000 W
PLW12 0.34327999 W
PLW13 0.21969999 W
F2 - Processing parameters
SI 32768
SF 125.7577701 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
170.81
170.71
167.20
133.15
132.29
129.12
128.06
AcO
AcO
O
NH
O
N3
SPh
11
86.79
77.46
77.20
76.95
73.37
71.42
70.09
52.84
49.94
20.88
16.78
200
180
160
140
120
100
80
60
40
20
0
ppm

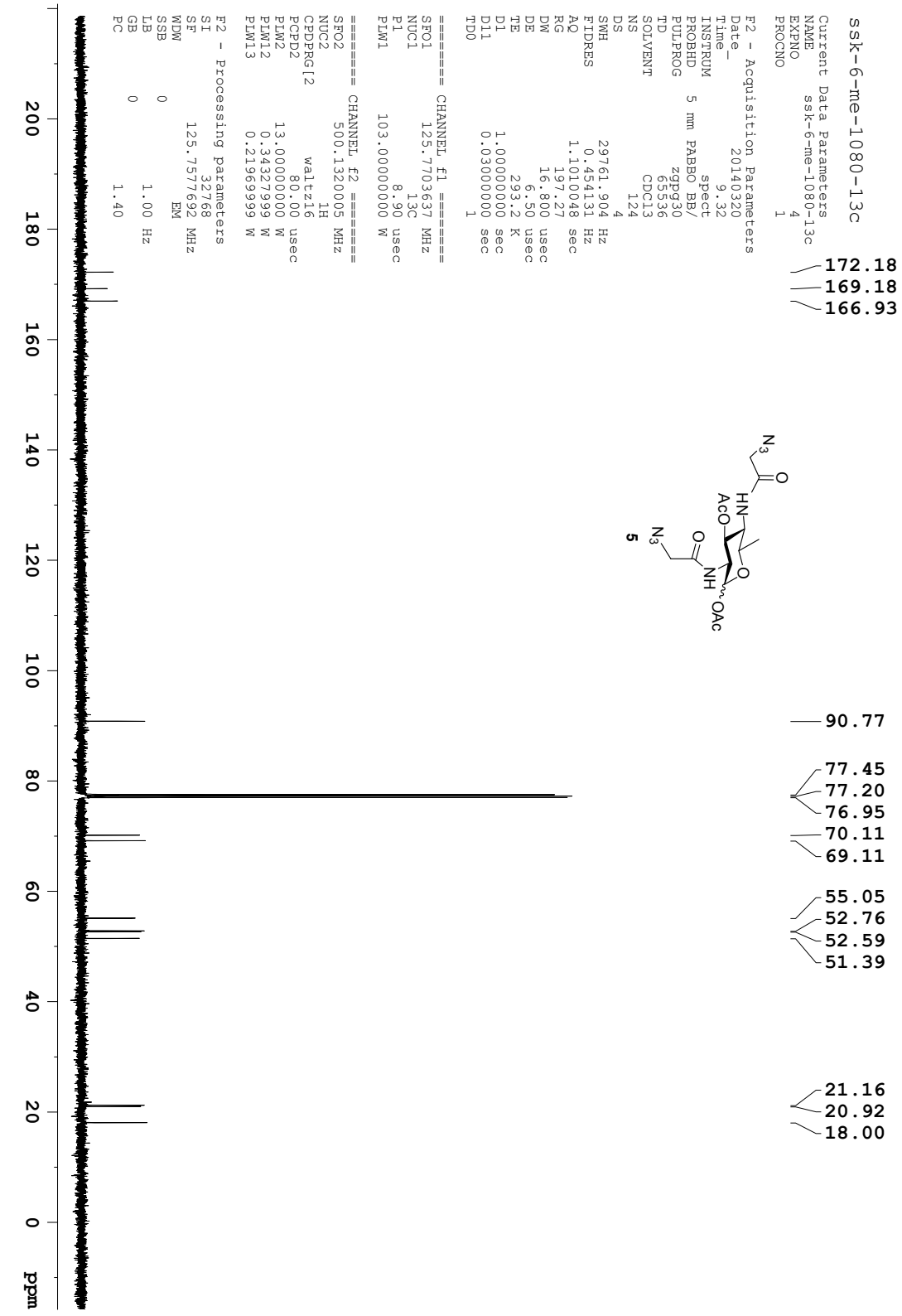

Current Data Parameters
NAME SSK-6-ME-1079-1H
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20140320
Time_ 9.58
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zg30
TD 59998
SOLVENT CDCl3
NS 42
DS 0
SWH 10000.000 Hz
FIDRES 0.166672 Hz
AQ 2.9999001 sec
RG 61.42
DW 50.000 usec
DE 6.50 usec
TE 292.4 K
D1 1.00000000 sec
TD0 1
========== CHANNEL f1 ==========
SFO1 500.1330885 MHz
NUC1 1H
P1 13.00 usec
PLW1 13.00000000 W
F2 - Processing parameters
SI 65536
SF 500.1300124 MHz
WDW EM
SSB 0
LB 0.20 Hz
GB 0
PC 1.00
AcO
AcO
O
NH
OAc
O
N3
4
SSK-6-ME-1079-1H
0.52
1.00
0.98
0.50
1.42
0.57
0.44
0.22
0.24
0.55
1.07
2.55
1.14
2.80
4.11
2.89
1.39
2.72
1.33
2.91
3.43
11
10
9
8
7
6
5
4
3
2
1
0
ppm


ssk-6-me-1079-13c
177.73
171.34
170.83
170.78
169.80
169.39
167.46
167.22
92.83
91.38
77.47
77.21
76.96
70.66
70.50
69.93
69.53
68.22
67.41
52.75
52.62
49.90
47.07
29.74
21.15
21.10
20.94
20.85
16.23
AcO
AcO
O
NH
O
N3
OAc
4
Current Data Parameters
NAME ssk-6-me-1079-13c
EXPNO 4
PROCNO 1
F2 - Acquisition Parameters
Date_ 20140320
Time_ 9.21
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 236
DS 4
SWH 29761.904 Hz
FIDRES 0.454131 Hz
AQ 1.1010048 sec
RG 197.27
DW 16.800 usec
DE 6.50 usec
TE 293.3 K
D1 1.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 125.7703637 MHz
NUC1 13C
P1 8.90 usec
PLW1 103.00000000 W
======== CHANNEL f2 ========
SFO2 500.1320005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 13.00000000 W
PLW12 0.34327999 W
PLW13 0.21969999 W
F2 - Processing parameters
SI 32768
SF 125.7577702 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

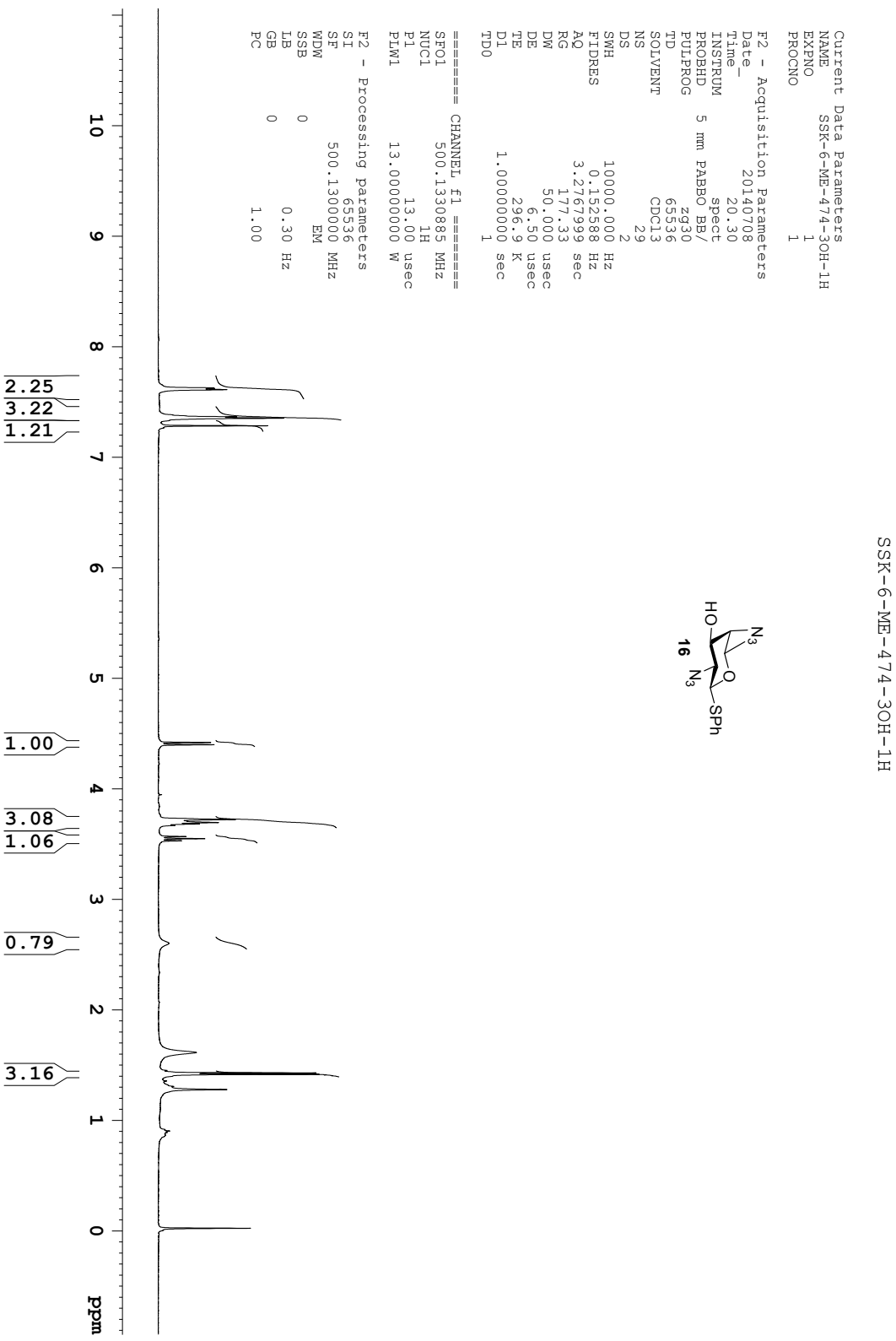

ssk-6-me-1069-1h
Current Data Parameters
NAME ssk-6-me-1069-1h
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20140310
Time_ 20.00
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 7
DS 2
SWH 10000.000 Hz
FIDRES 0.152588 Hz
AQ 3.2767999 sec
RG 85.91
DW 50.000 usec
DE 6.50 usec
TE 296.5 K
D1 1.00000000 sec
TD0 1
========== CHANNEL f1 ==========
SFO1 500.1330885 MHz
NUC1 1H
P1 13.00 usec
PLW1 13.00000000 W
F2 - Processing parameters
SI 65536
SF 500.1300000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
14
2.01
3.46
2.25
2.96
0.47
1.99
1.00
0.99
2.10
1.19
1.52
3.22
2.67
10
9
8
7
6
5
4
3
2
1
0
ppm

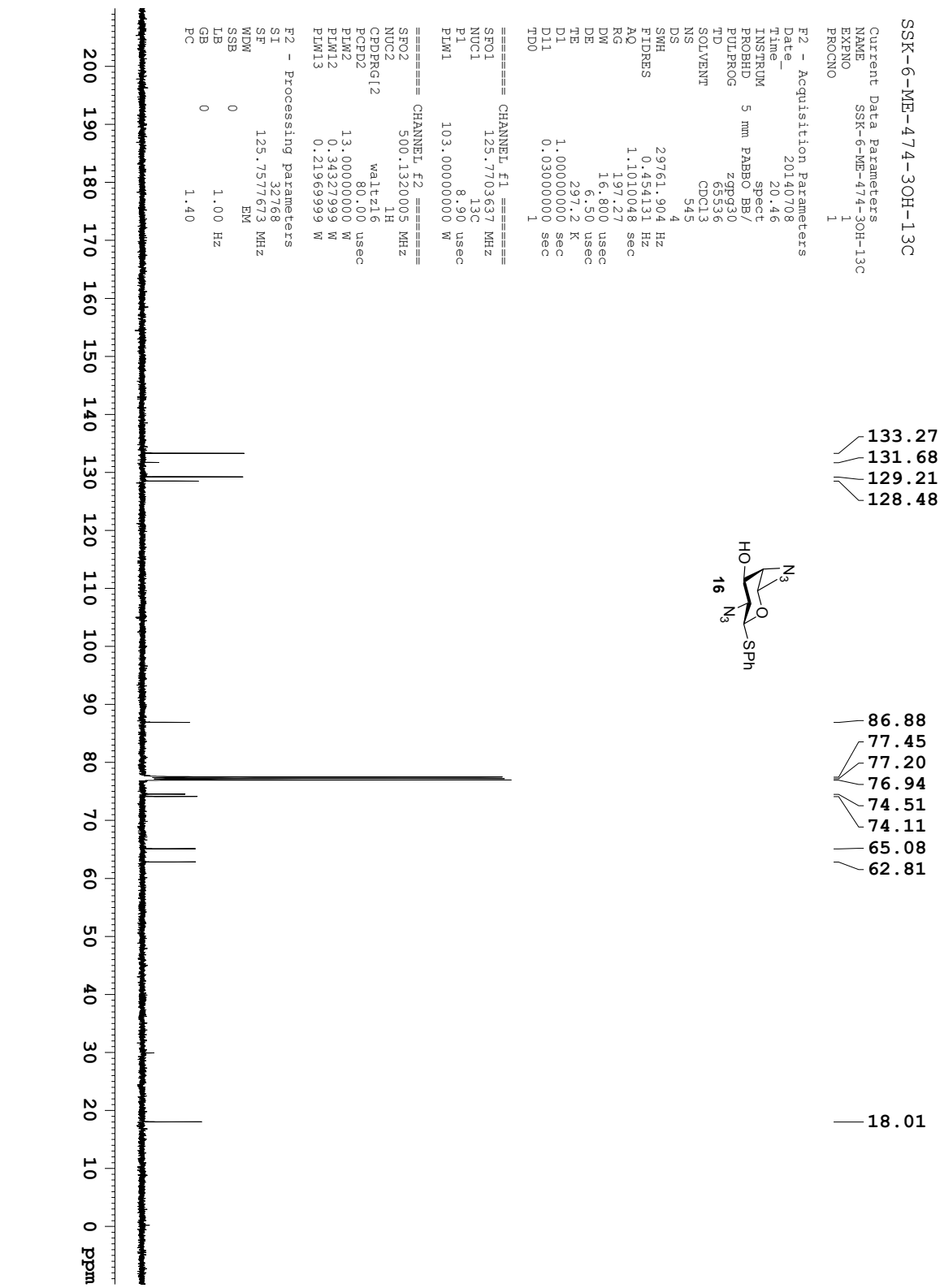

SSK-6-ME-1069-13C
Current Data Parameters
NAME SSK-6-ME-1069-13C
EXPNO 8
PROCNO 1
F2 - Acquisition Parameters
Date_ 20140310
Time_ 21.56
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 93
DS 4
SWH 29761.904 Hz
FIDRES 0.454131 Hz
AQ 1.1010048 sec
RG 197.27
DW 16.800 usec
DE 6.50 usec
TE 297.4 K
D1 1.00000000 sec
D11 0.03000000 sec
TD0 1
========== CHANNEL f1 ==========
SFO1 125.7703637 MHz
NUC1 13C
P1 8.90 usec
PLW1 103.00000000 W
========== CHANNEL f2 ==========
SFO2 500.1320005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 13.00000000 W
PLW12 0.34327999 W
PLW13 0.21969999 W
F2 - Processing parameters
SI 32768
SF 125.7577692 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
167.93
167.50
167.26
134.12
132.99
132.60
130.01
129.25
128.95
128.69
128.33
85.73
77.46
77.20
76.95
75.08
73.84
55.90
54.41
52.72
52.66
18.38
200
180
160
140
120
100
80
60
40
20
0
ppm
N3
O
HN
BzO
O
O
NH
N3
SPh
14

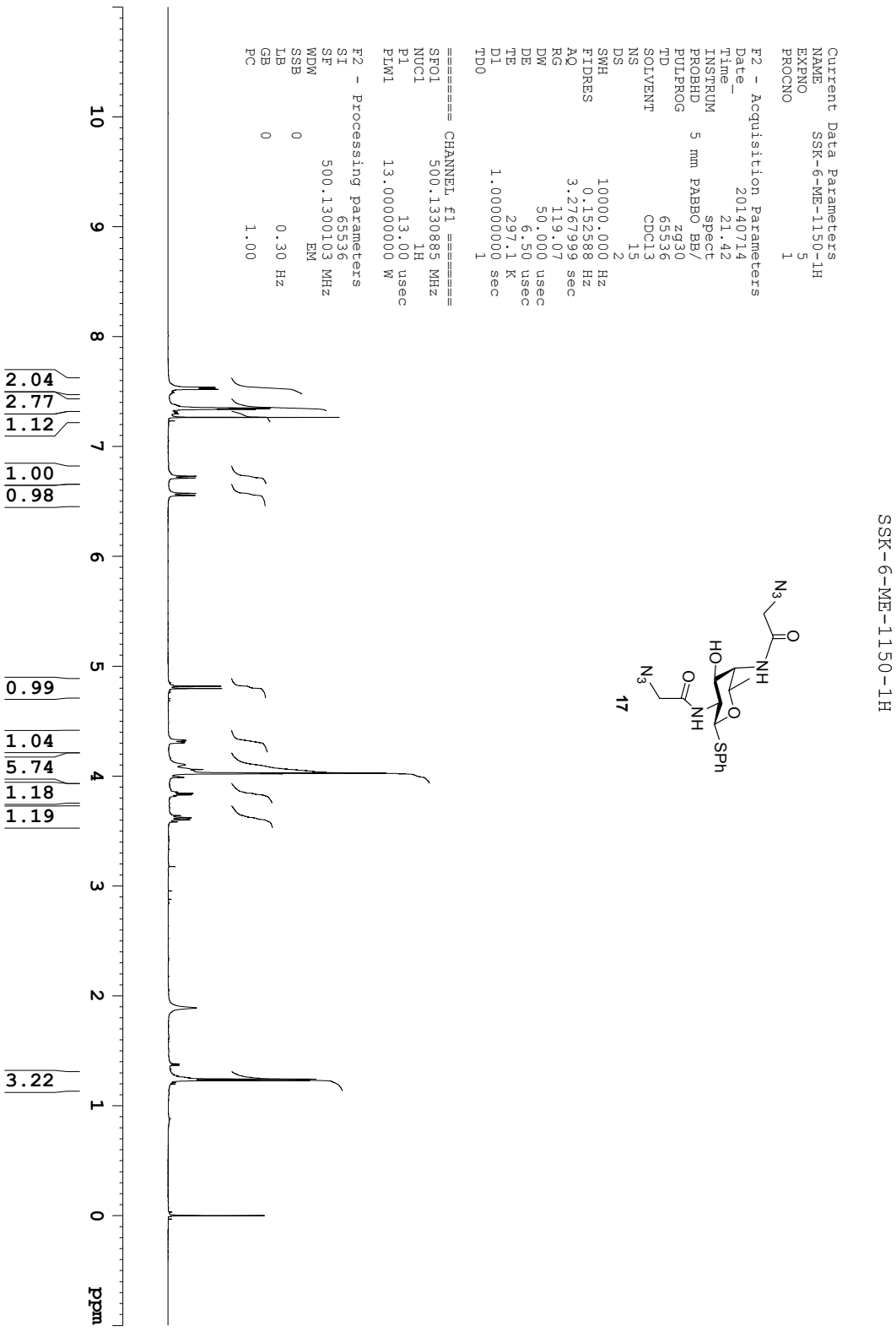

ssk-6-me-1080-1h
Current Data Parameters
NAME ssk-6-me-1080-1h
EXPNO 4
PROCNO 1
F2 - Acquisition Parameters
Date_ 20140320
Time_ 9.31
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 8
DS 2
SWH 10000.000 Hz
FIDRES 0.152588 Hz
AQ 3.2767999 sec
RG 119.07
DW 50.000 usec
DE 6.50 usec
TE 292.6 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 500.1330885 MHz
NUC1 1H
P1 13.00 usec
PLW1 13.00000000 W
F2 - Processing parameters
SI 65536
SF 500.1300000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
0.85
0.14
0.16
1.00
1.06
1.05
0.16
0.15
1.11
0.97
6.94
3.18
4.07
0.53
3.44
11
10
9
8
7
6
5
4
3
2
1
0
ppm
N3
O
HN
AcO
O
O
NH
OAc
N3
5

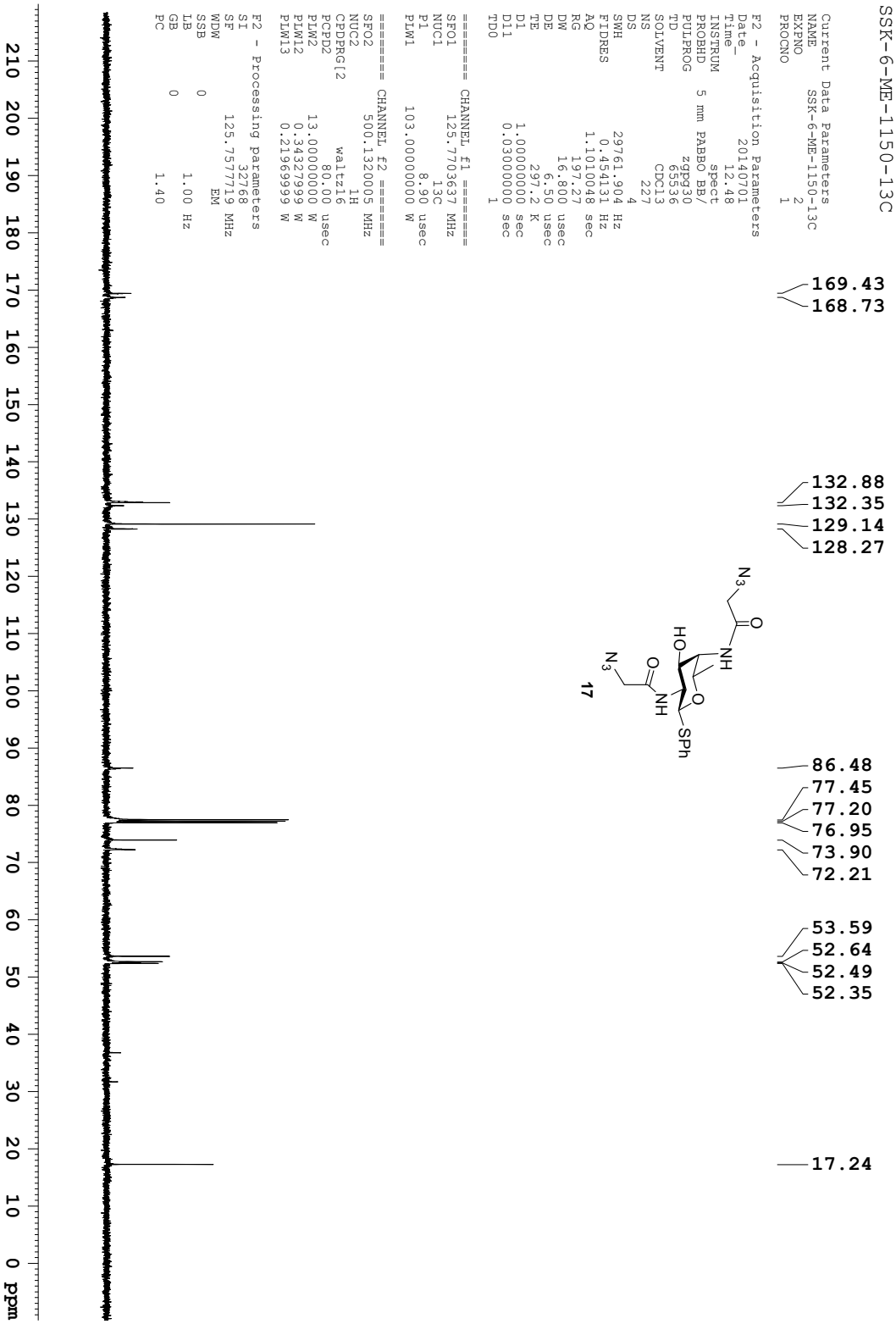

ssk-6-me-1080-13c
Current Data Parameters
NAME ssk-6-me-1080-13c
EXPNO 4
PROCNO 1
F2 - Acquisition Parameters
Date_ 20140320
Time_ 9.32
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 124
DS 4
SWH 29761.904 Hz
FIDRES 0.454131 Hz
AQ 1.1010048 sec
RG 197.27
DW 16.800 usec
DE 6.50 usec
TE 293.2 K
D1 1.00000000 sec
D11 0.03000000 sec
TD0 1
========== CHANNEL f1 ==========
SFO1 125.7703637 MHz
NUC1 13C
P1 8.90 usec
PLW1 103.00000000 W
========== CHANNEL f2 ==========
SFO2 500.1320005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 13.00000000 W
PLW12 0.34327999 W
PLW13 0.21969999 W
F2 - Processing parameters
SI 32768
SF 125.7577692 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
172.18
169.18
166.93
90.77
77.45
77.20
76.95
70.11
69.11
55.05
52.76
52.59
51.39
21.16
20.92
18.00
200
180
160
140
120
100
80
60
40
20
0
ppm

5

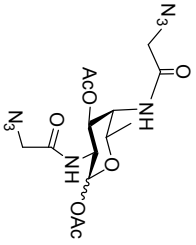

SSK-6-ME-474-3OH-1H
16
HO
N3
O
N3
SPh
Current Data Parameters
NAME SSK-6-ME-474-3OH-1H
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20140708
Time_ 20.30
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 29
DS 2
SWH 10000.000 Hz
FIDRES 0.152588 Hz
AQ 3.2767999 sec
RG 177.33
DW 50.000 usec
DE 6.50 usec
TE 296.9 K
D1 1.00000000 sec
TD0 1
========== CHANNEL f1 ========
SFO1 500.1330885 MHz
NUC1 1H
P1 13.00 usec
PLW1 13.00000000 W
F2 - Processing parameters
SI 65536
SF 500.1300000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
2.25
3.22
1.21
1.00
3.08
1.06
0.79
3.16
10
9
8
7
6
5
4
3
2
1
0
ppm


SSK-6-ME-474-3OH-13C
Current Data Parameters
NAME SSK-6-ME-474-3OH-13C
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20140708
Time_ 20.46
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 545
DS 4
SWH 29761.904 Hz
FIDRES 0.454131 Hz
AQ 1.1010048 sec
RG 197.27
DW 16.800 usec
DE 6.50 usec
TE 297.2 K
D1 1.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 125.7703637 MHz
NUC1 13C
P1 8.90 usec
PLW1 103.00000000 W
======== CHANNEL f2 ========
SFO2 500.1320005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 13.00000000 W
PLW12 0.34327999 W
PLW13 0.21969999 W
F2 - Processing parameters
SI 32768
SF 125.7577673 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
133.27
131.68
129.21
128.48
HO
N3
O
SPh
16
N3
86.88
77.45
77.20
76.94
74.51
74.11
65.08
62.81
18.01
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm


SSK-6-ME-1150-1H
Current Data Parameters
NAME SSK-6-ME-1150-1H
EXPNO 5
PROCNO 1
F2 - Acquisition Parameters
Date_ 20140714
Time_ 21.42
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 15
DS 2
SWH 10000.000 Hz
FIDRES 0.152588 Hz
AQ 3.2767999 sec
RG 119.07
DW 50.000 usec
DE 6.50 usec
TE 297.1 K
D1 1.00000000 sec
TD0 1
========== CHANNEL f1 ========
SFO1 500.1330885 MHz
NUC1 1H
P1 13.00 usec
PLW1 13.00000000 W
F2 - Processing parameters
SI 65536
SF 500.1300103 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
17
2.04
2.77
1.12
1.00
0.98
0.99
1.04
5.74
1.18
1.19
3.22
10
9
8
7
6
5
4
3
2
1
0
ppm

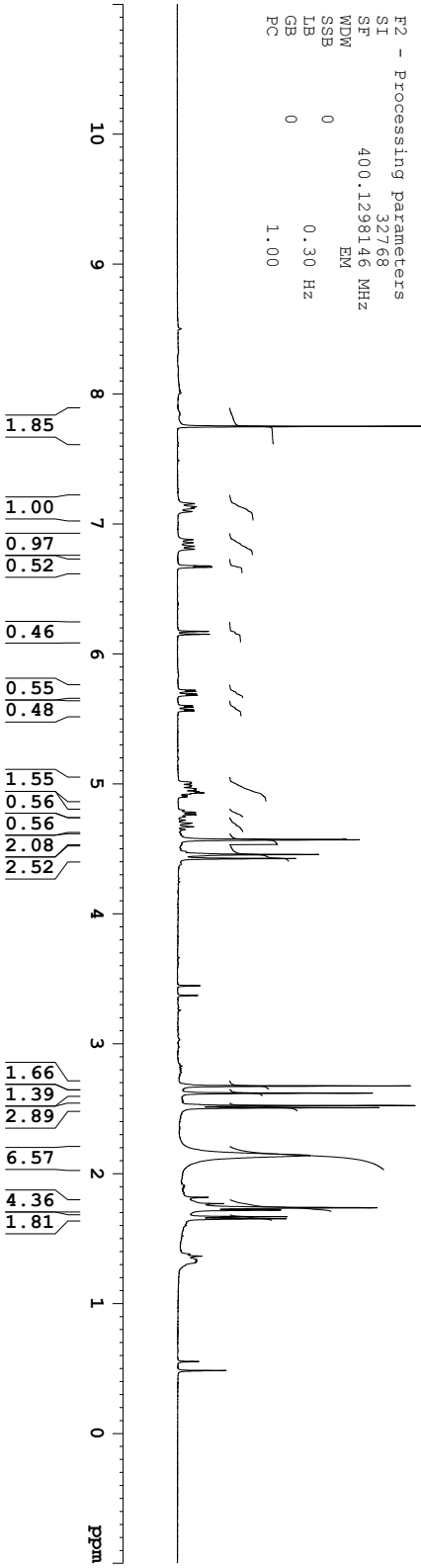

SSK-6-ME-1150-13C
Current Data Parameters
NAME SSK-6-ME-1150-13C
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20140701
Time_ 12.48
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 227
DS 4
SWH 29761.904 Hz
FIDRES 0.454131 Hz
AQ 1.1010048 sec
RG 197.27
DW 16.800 usec
DE 6.50 usec
TE 297.2 K
D1 1.00000000 sec
D11 0.03000000 sec
TD0 1
========== CHANNEL f1 ==========
SFO1 125.7703637 MHz
NUC1 13C
P1 8.90 usec
PLW1 103.00000000 W
========== CHANNEL f2 ==========
SFO2 500.1320005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 13.00000000 W
PLW12 0.34327999 W
PLW13 0.21969999 W
F2 - Processing parameters
SI 32768
SF 125.7577719 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
169.43
168.73
132.88
132.35
129.14
128.27
86.48
77.45
77.20
76.95
73.90
72.21
53.59
52.64
52.49
52.35
17.24
17
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

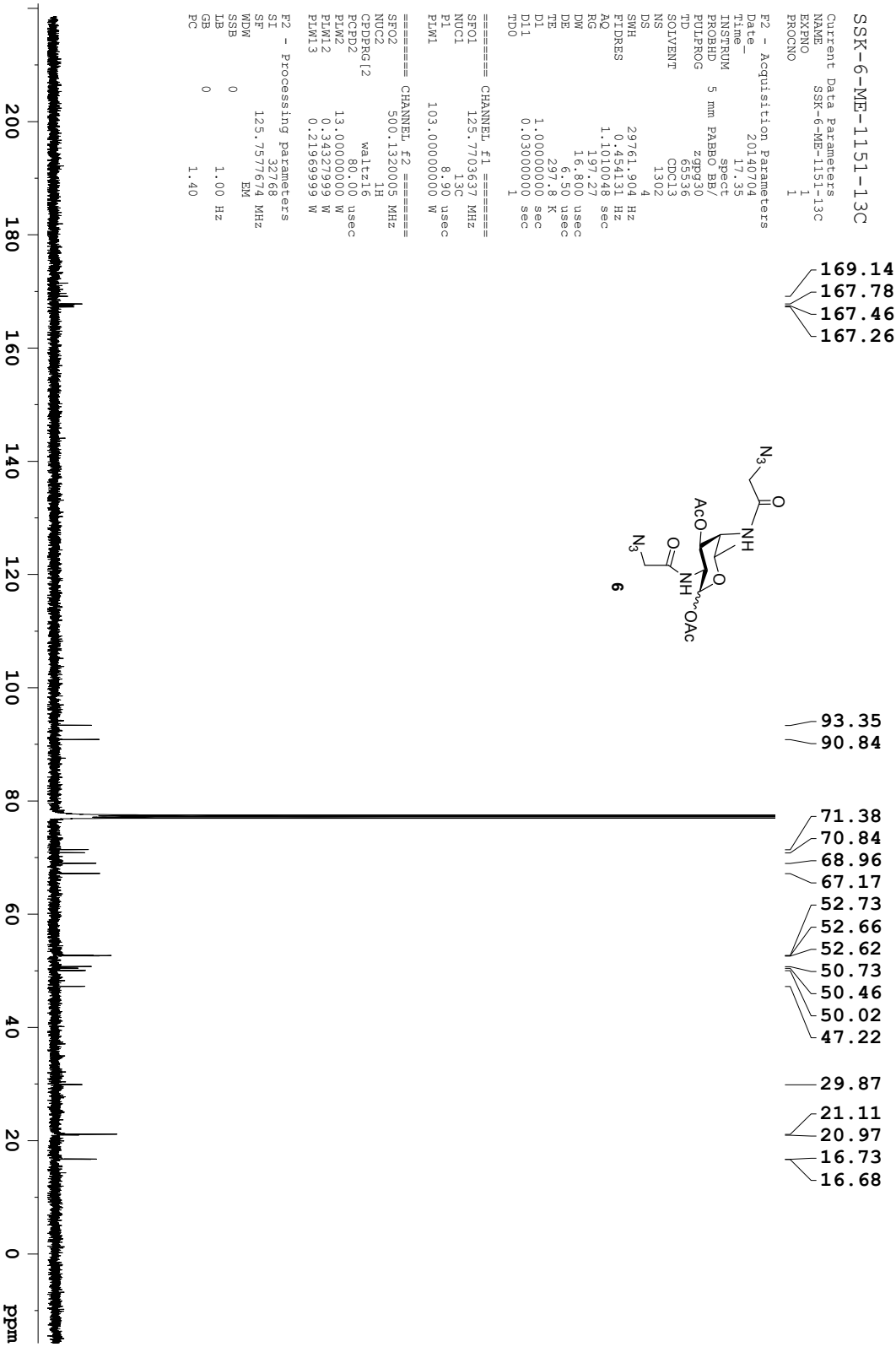
SSK-6-ME-1151-1H



6

```
Current Data Parameters
NAME                  SSK-6-ME-1151-1H
EXPNO                                3
PROCNO                               1

F2 - Acquisition Parameters
Date_                         20140704
Time_                             2.30
INSTRUM                           spect
PROBHD     5 mm SEI 1H/D-
PULPROG                           zg30
TD                               65536
SOLVENT                         CDC13
NS                                  16
DS                                   0
SWH                           8223.685 Hz
FIDRES                        0.125483 Hz
AQ                            3.9845889 sec
RG                                 114
DW                            60.800 usec
DE                             6.50 usec
TE                             295.5 K
D1                            1.00000000 sec
TD0                                  1

========== CHANNEL f1 ========
NUC1                               1H
P1                            13.50 usec
PL1                           -1.00 dB
PL1W                     10.56200695 W
SFO1                    400.1324710 MHz

F2 - Processing parameters
SI                               32768
SF                      400.1298146 MHz
WDW                                EM
SSB                   0
LB                             0.30 Hz
GB                    0
PC                             1.00
```




SSK-6-ME-1151-13C
Current Data Parameters
NAME SSK-6-ME-1151-13C
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20140704
Time_ 17.35
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 1302
DS 4
SWH 29761.904 Hz
FIDRES 0.454131 Hz
AQ 1.1010048 sec
RG 197.27
DW 16.800 usec
DE 6.50 usec
TE 297.8 K
D1 1.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 125.7703637 MHz
NUC1 13C
P1 8.90 usec
PLW1 103.00000000 W
======== CHANNEL f2 ========
SFO2 500.1320005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 13.00000000 W
PLW12 0.34327999 W
PLW13 0.21969999 W
F2 - Processing parameters
SI 32768
SF 125.7577674 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
169.14
167.78
167.46
167.26
93.35
90.84
71.38
70.84
68.96
67.17
52.73
52.66
52.62
50.73
50.46
50.02
47.22
29.87
21.11
20.97
16.73
16.68
6
200
180
160
140
120
100
80
60
40
20
0
ppm