

Supporting Information

Biosynthetic Glycan Labeling

Victoria M. Marando^{‡a}, Daria E. Kim^{‡a}, Phillip J. Calabretta^a, Matthew B. Kraft^b, Bryan D. Bryson^{c,d}, Laura L. Kiessling^{*a,b}

^aDepartment of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, United States

^bDepartment of Chemistry, University of Wisconsin Madison, Madison, Wisconsin 53706, United States

^cDepartment of Biological Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA

^dRagon Institute of MGH, MIT, and Harvard, Cambridge, Massachusetts 02139, USA

Table of Contents

Supplemental Procedures.....	S2
Supplemental Figures	S4
Chemical Synthesis and Characterization Data	S8
Supplemental References.....	S43

Supplemental Procedures

Strains and Growth Conditions

For bacterial assays, the strains employed include *Mycobacterium smegmatis mc²155*, *Corynebacterium glutamicum ATCC13032* and *Escherichia coli BL21*. *M. smegmatis* was grown in Middlebrook 7H9 broth (HiMedia, Mumbai, India) supplemented with 0.2% (w/v) dextrose, 0.2% (v/v) glycerol, 0.5% bovine serum albumin (United States Biological, Salem, MA), catalase (4 mg/liter) (Sigma-Aldrich), 15 mM sodium chloride and 0.05 % (v/v) Tween 80 in a shaking incubator at 37 °C. *C. glutamicum* was cultured in brain heart infusion (BHI) medium (BD, Franklin Lake, NJ) supplemented with 9% (w/v) sorbitol (BHIS) and *E. coli* was cultured in Luria broth (LB) liquid medium (Sigma-Aldrich) in a shaking incubator at 30 °C and 37 °C, respectively. Generally, starter cultures were incubated at the relevant temperature with shaking until saturation. Cells were then diluted into fresh media and grown to mid-logarithmic phase (determined by OD₆₀₀ measurement on a BioMate 3S Spectrophotometer).

Growth Inhibition Experiments

Experiments were performed following reported procedures.¹ In brief, a saturated culture was diluted down to the desired starting OD and plated in triplicate in a Corning black 96-well plate. Probes were added at the indicated concentration from DMSO stock solutions. *M. smegmatis* were grown with shaking at 37 °C for 24 h. *C. glutamicum* were grown with shaking at 30 °C for 16 h. Alamar Blue reagent (6 µL, Invitrogen) was added to each well and the plates were incubated again for 1 h at 37 °C or 30 °C. The fluorescence emission of each well was then measured on a Tecan Infinite M1000 Pro microplate reader. Monitoring of resorufin fluorescence was achieved by exciting at 570 nm ± 5 nm and detecting at 585 nm ± 5 nm. Z-position was set to 2 mm, and the fluorimeter gain was optimized and then kept constant between plates. Data are reported in relative fluorescence units (RFU) normalized to untreated controls (**Figure S1**).

Flow cytometry and Fluorescence Microscopy

Cells were plated from saturated starter cultures (OD₆₀₀ = 0.05) in a Corning black 96-well plate. *M. smegmatis* was cultured in Middlebrook 7H9 containing 0.2% (v/v) glycerol and 0.05 % Tween 80. *C. glutamicum* was cultured in BHIS medium containing 0.05 % Tween 80. *E. coli* was cultured in LB liquid medium containing 0.05 % Tween 80. AzFPA derivatives were added to the desired concentrations from 85 mM stocks in DMSO. Cultures were grown to mid-log (OD₆₀₀ 1.0-1.2) at 37 °C or 30 °C with shaking. Samples were immediately prepared for flow cytometry or microscopy.

Cells were pelleted for 5 min at 3000 x g. The pellets were washed with ice-cold phosphate-buffered saline (PBS) supplemented with 0.05% Tween 80 (100 µL) once. Cells were washed an additional time with PBS supplemented with 0.05% Tween 80 and 0.5% (w/v) bovine serum albumin (BSA) once then taken up in fresh 7H9 media supplemented with 0.5% Tween 80 (150 µL). AFDye™ 647 DBCO (Click Chemistry Tools #1302) was added from a 10 mM stock solution in DMSO to a final concentration of 500 µM. The samples were stained for 2 h rotating at 37 °C. When 1 hr and 45 mins minutes had passed, 0.1 µL (1500X dilution) of SytoBC™ Green Fluorescent Nucleic Acid Stain (ThermoFischer #S34855) was added for a 15-minute incubation

period. The stained cells were pelleted for 5 min at 3000 x g. The supernatant was removed, and the pellet was washed with PBS supplemented with 0.05% Tween 80 and 0.5% (w/v) BSA twice. For flow cytometry, stained cell pellets were taken up in 4% paraformaldehyde in PBS to be fixed at room temperature for 20 min. Following fixation, cells were pelleted then taken up in sterile PBS supplemented with 0.05% Tween 80 in flow tubes and analyzed using an Attune NxT Flow Cytometer (405 nm, 488 nm, 561 nm, and 640 nm lasers). 10,000 cells were counted at the low flow rate. Flow cytometry analysis was performed in triplicate, representative scatter plots are shown (**Figure S3**). The unstained controls were analyzed first to set gates. Data were analyzed using the FlowJo software package (FlowJo LLC). Mean fluorescence intensity was calculated using a geometric mean.

For analysis by microscopy, stained cell pellets were taken up in 7H9 supplemented with 0.5% Tween 80 (100 μ L). Each sample was spotted onto a glass-bottomed microwell dish (MatTek corporation # P35G-1.5-14-C) and covered with a pre-cooled and 0.6% (w/v) agarose pad. Images were collected RPI spinning-disk confocal microscope (100x oil immersion lens, 1.4 NA). Brightness and contrast were identically adjusted with the open-source Fiji distribution of ImageJ. Images were then converted to an RGB format to preserve normalization and then assembled into panels (**Figure S4**).

mAGP Isolation

Cell envelope material was extracted similarly to previously described protocols.¹⁻² *M. smegmatis* cultures (1 mL) were inoculated from a saturated starter culture ($OD_{600} = 0.05$) in Middlebrook 7H9 containing 0.05 % Tween 80. AzFPA derivatives were added from DMSO stock solutions to the desired concentrations (250 μ M), and the cultures were grown to saturation at 37 °C with shaking. *C. glutamicum* was cultured in BHIS medium (1 mL) in a shaking incubator at 30 °C. Cells were pelleted for 5 min at 3000 x g, normalizing across samples by OD. Cells were washed and reacted with AFDye™ 647 DBCO (Click Chemistry Tools #1302) as described above. After staining, cell pellets were resuspended in lysis buffer (2% Triton X-100 in PBS) and disrupted by sonication (6 x 20 s separated by 2 min off intervals on ice). The cell lysate was then pelleted by centrifugation at 15 000 g for 15 min. The supernatant was removed and the pellet was taken up in 2% SDS in PBS and heated to 95 °C for 1 hr before pelleting as above and discarding the supernatant. The pellet was then washed with water, 80% acetone/water and then acetone. After isolation, the mAGP complex was suspended in 2% SDS in PBS and the fluorescence was detected on a Tecan M1000 plate reader. Plates were shaken for 3 s (6 mm, orbital) immediately before the well fluorescence was read ($\lambda_{ex} = 648 \text{ nm} \pm 5$, $\lambda_{em} = 671 \pm 5 \text{ nm}$).

Uptake into THP-1 cells

The monocyte cell line THP-1 (ATCC TIB-202) was cultured in ATCC RPMI-1640 medium supplemented with 10% fetal bovine serum, 0.05 mM 2-mercaptoethanol and P/S in a 5% CO₂ humidified atmosphere. Monocyte cells were differentiated into macrophages by induction with phorbol-12-myristate-13-acetate (PMA, Sigma-Aldrich). Cells were seeded in six-well cell non-treated cell culture plates in complete RPMI with 100 ng/mL PMA. After 48 h of PMA stimulation, cells were adherent to the plate. They were washed with PBS and allowed to recover in complete RPMI media for another 24 h before using THP-1-derived macrophages for infection experiment. *M. smegmatis* cells were added at the indicated multiplicity of infection (MOI) and the co-cultures was incubated at 37 °C with 5% CO₂ for 1 h. Following infection,

samples were incubated with 1.5 $\mu\text{g}/\text{mL}$ fluorophore conjugated wheat germ agglutinin (CF[®]405S WGA, Biotium) on ice for 30 min. Macrophages were then washed PBS with 1% (w/v) BSA in PBS three times and spotted onto a glass-bottomed microwell dish. Images were collected RPI spinning-disk confocal microscope (100x oil immersion lens, 1.4 NA). Brightness and contrast were identically adjusted with the open-source Fiji distribution of ImageJ. Images were then converted to an RGB format to preserve normalization and then assembled into panels.

Supplemental Figures

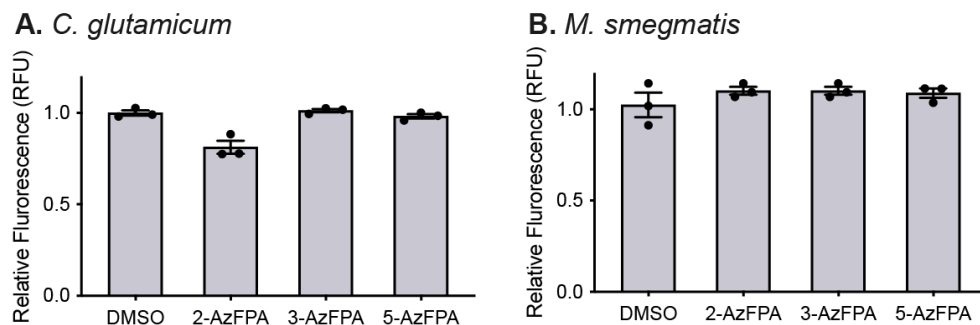


Figure S1. Probe Viability Determined by Alamar Blue Assay. Growth of *C. glutamicum* (A) and *M. smegmatis* (B) measured via the Alamar Blue assay in the presence of 250 μM AzFPA. The Y axis depicts the relative fluorescence compared to an untreated control sample. Error bars denote the standard error of the mean of three replicate experiments.

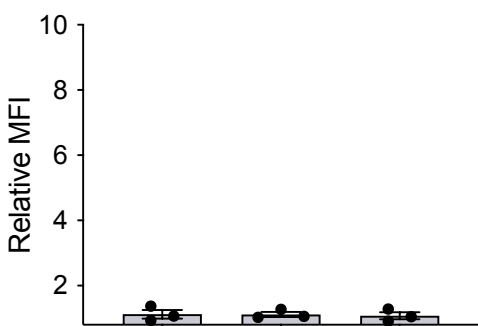


Figure S2. Cellular Labeling in *E. coli*. Flow cytometry of *E. coli* cultured in the presence of AzFPA (250 μM) following treatment with DBCO-AF647. MFI was calculated using the geometric mean and plotted relative to a dye only control. Error bars denote the standard error of the mean of three replicate experiments.

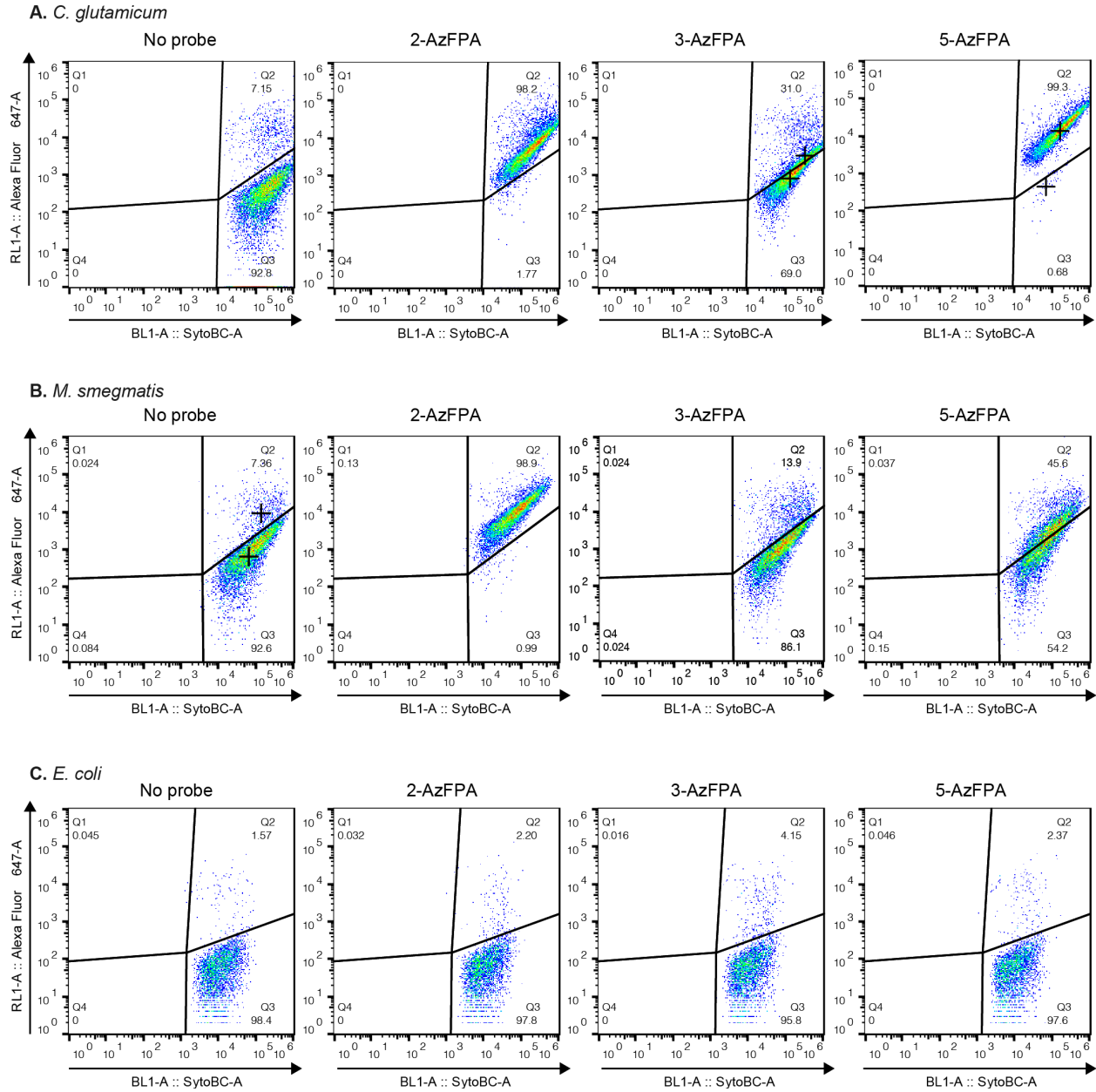


Figure S3. Flow Cytometry Scatter Plots. Representative scatter plots for *C. glutamicum* (A), *M. smegmatis* (B) or *E. coli* (C) treated with DBCO-AF647 in the absence or presence of AzFPA probe (250 μ M). Plots are representative of two independent experiments with three replicates for each strain and condition.

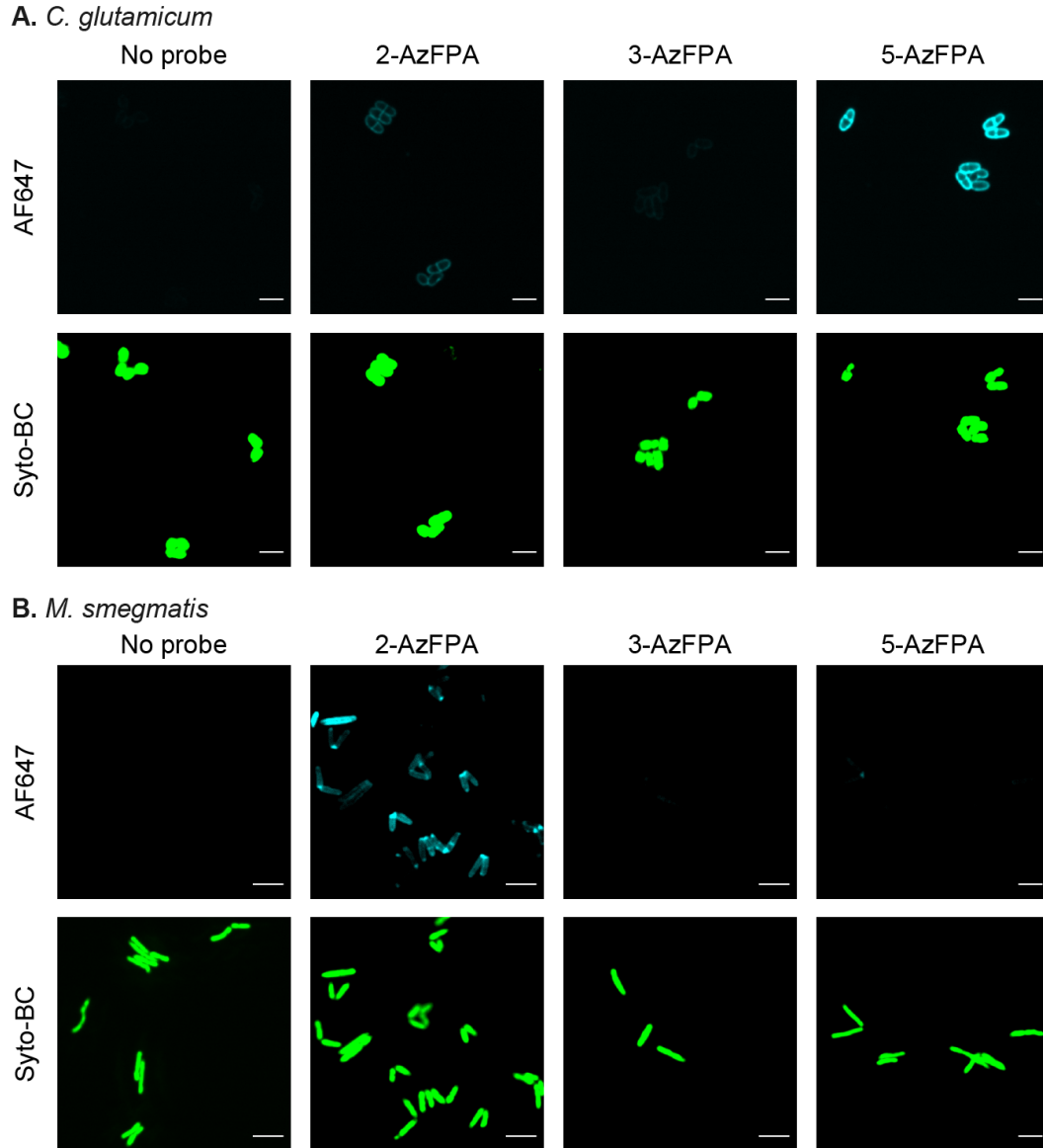


Figure S4. Confocal Microscopy. Fluorescence confocal microscopy of live AzFPA-labeled (250 μ M) *C. glutamicum* (A) and *M. smegmatis* (B) reacted with DBCO-AF647. Cells were identified using nuclear stain Syto-BC. (Scale bars: 3 μ m).

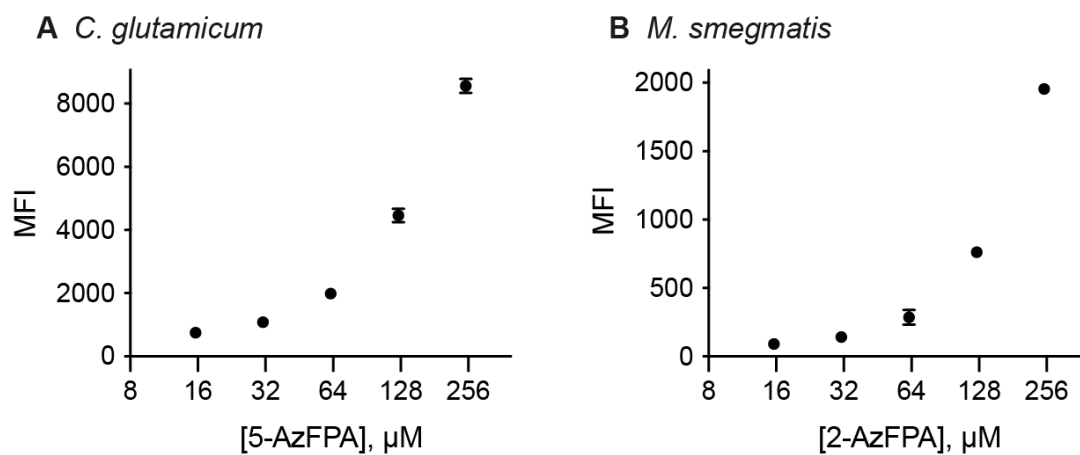


Figure S5. Dose-dependence of Staining. Flow cytometry quantification of staining for *C. glutamicum* (A), *M. smegmatis* (B) treated with DBCO-AF647 in the presence of increasing concentrations of AzFPA probe. MFI calculated using the geometric mean. Error bars denote the standard error of the mean of three replicate experiments.

Chemical Synthesis and Characterization Data

General information

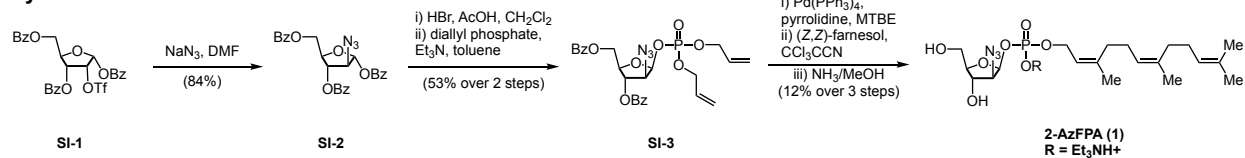
All chemicals were purchased from Sigma Aldrich unless otherwise stated. Dry solvents were obtained from a solvent purification system (Pure Process Technologies) under argon unless otherwise stated. DMF, MTBE and pyridine were used from sure seal bottles (Sigma Aldrich) without further purification. Triethylamine was distilled from CaH₂ just prior to use.

Analytical thin layer chromatography (TLC) was performed on EMD Millipore TM TLC silica gel 60 F254 (glass-backed). Plates were visualized under UV light and by staining with *p*-anisaldehyde stain with charring. Flash chromatography was performed on SiliCycle® SiliaFlash® P60 silica gel and Biotage® Selekt using Biotage Sfär silica cartridges.

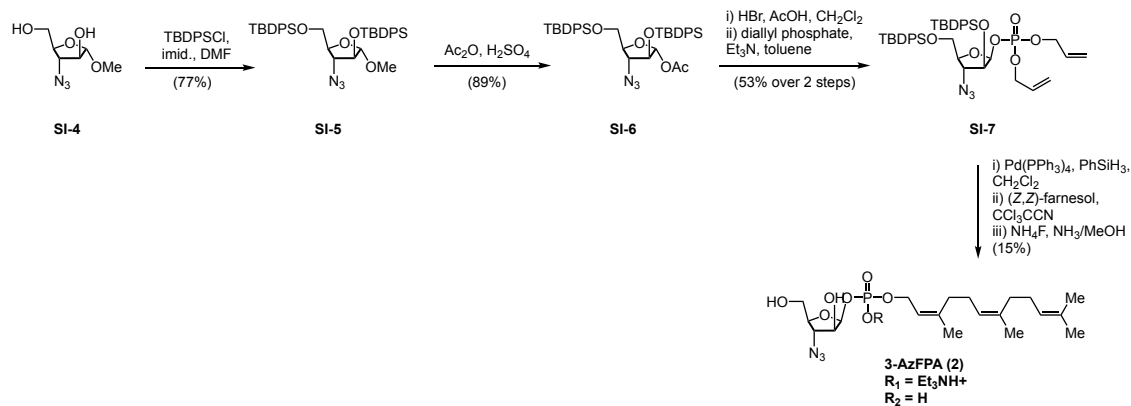
Nuclear magnetic resonance spectra were recorded on a 300 MHz spectrometer (acquired at 300 MHz for ¹H and 75 MHz for ¹³C), 400 MHz spectrometer (acquired at 400 MHz for ¹H and 100 MHz for ¹³C), a 500 MHz spectrometer (acquired at 500 MHz for ¹H and 125 MHz for ¹³C) or a 600 MHz spectrometer (acquired at 600 MHz for ¹H and 151 MHz for ¹³C). Chemical shifts are reported relative to residual solvent peaks in parts per million (CHCl₃: ¹H, 7.26, ¹³C, 77.16; MeOH: ¹H, 3.31, ¹³C, 49.00; C₆D₆: ¹H, 7.16, ¹³C, 128.06). High-resolution mass spectra (HRMS) were obtained on an electrospray ionization-time of flight (ESI-TOF) mass spectrometer. All IR spectra were taken on an FT-IR Bruker Alpha II.

Scheme S1. Synthesis of AzFPA regioisomers

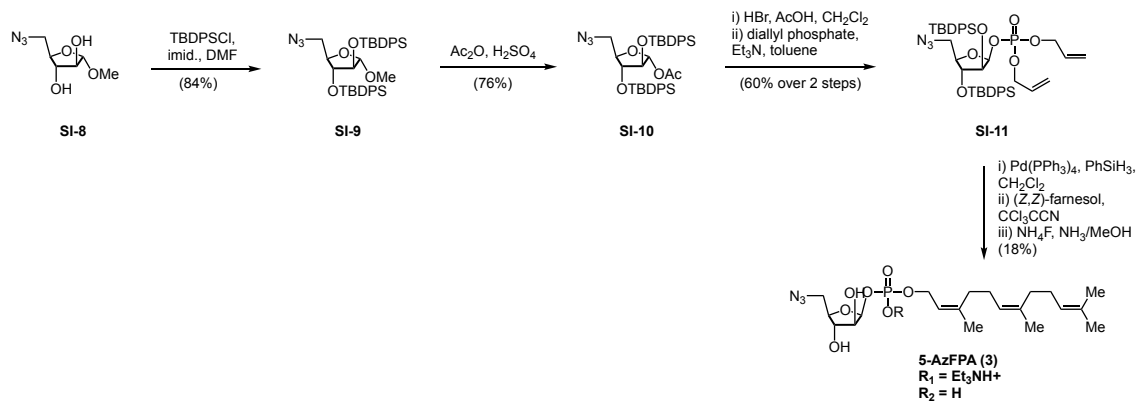
Synthesis of 2-AzFPA



Synthesis of 3-AzFPA



Synthesis of 5-AzFPA



2-azido-1,3,5-tri-O-benzoyl-2-deoxy- α -D-arabinofuranoside (SI-2).

A flame-dried, 50 mL round-bottomed flask equipped with a magnetic stir bar and a reflux condenser was charged with **SI-1** (5.00 g, 8.41 mmol, 1.0 equiv), sealed with a rubber septum, evacuated and backfilled with nitrogen three times, and placed under an argon atmosphere. **SI-1** was dissolved in anhydrous DMF (28 mL, 0.3 M) and NaN₃ (820.0 mg, 12.61 mmol, 1.5 equiv) was added to the resulting solution as a solid in a single portion. The reaction apparatus was transferred to an oil bath and heated to 70 °C.

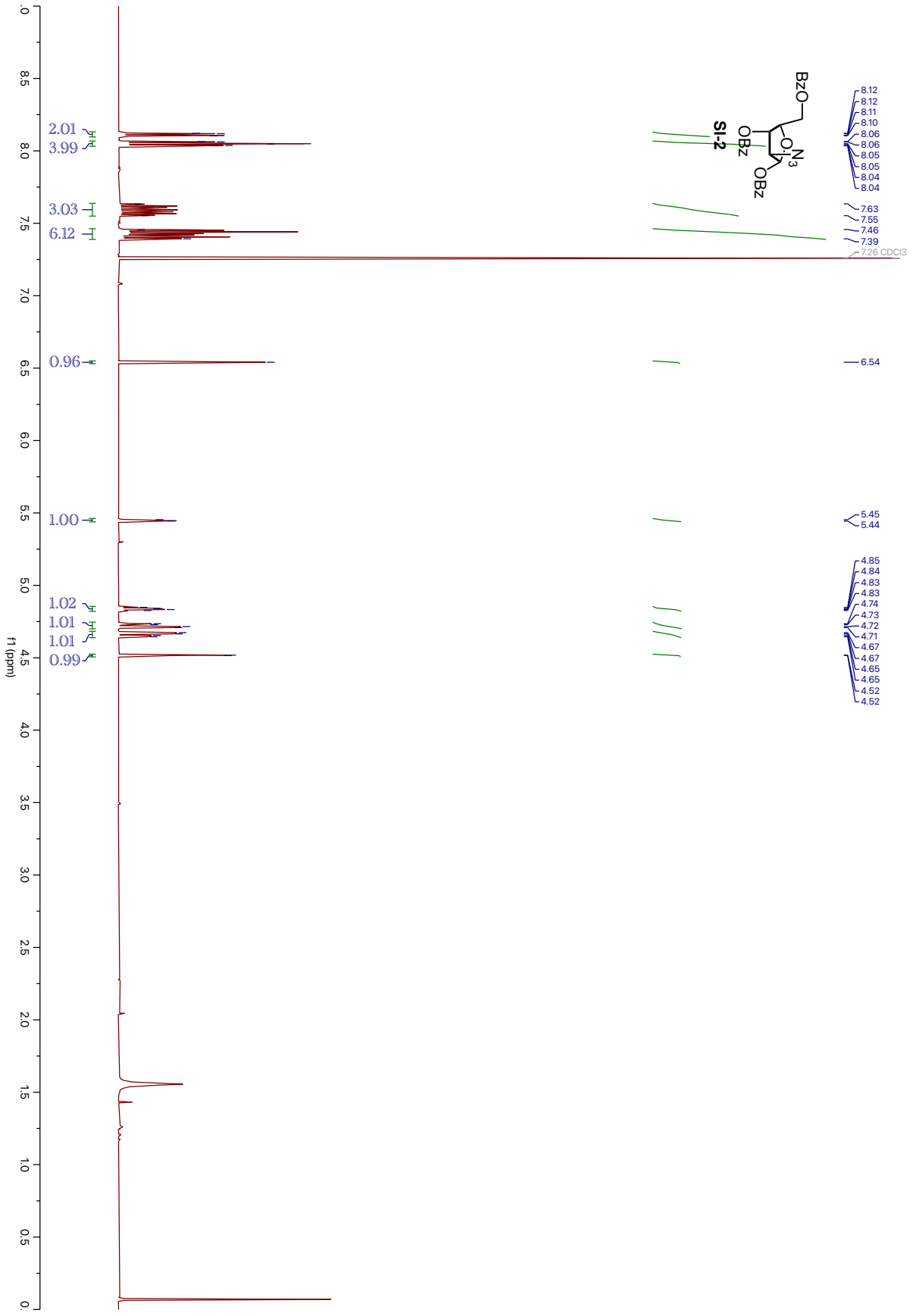
After stirring at 70 °C for 5 hours, the reaction apparatus was removed from the oil bath and allowed to cool to room temperature. The reaction was quenched by pouring into sat. aq. NaHCO₃ (300 mL). The layers were separated and the aqueous layer was extracted with Et₂O (3 × 300 mL). The combined organic layers were washed with brine (500 mL), dried over anhydrous Na₂SO₄, filtered, and concentrated under reduced pressure by rotary evaporation to provide a crude orange solid. Purification by flash column chromatography on silica gel (EtOAc/hexanes = 0/10 to 1/3) afforded **SI-2** (3.38 g, 84%) as a white solid.

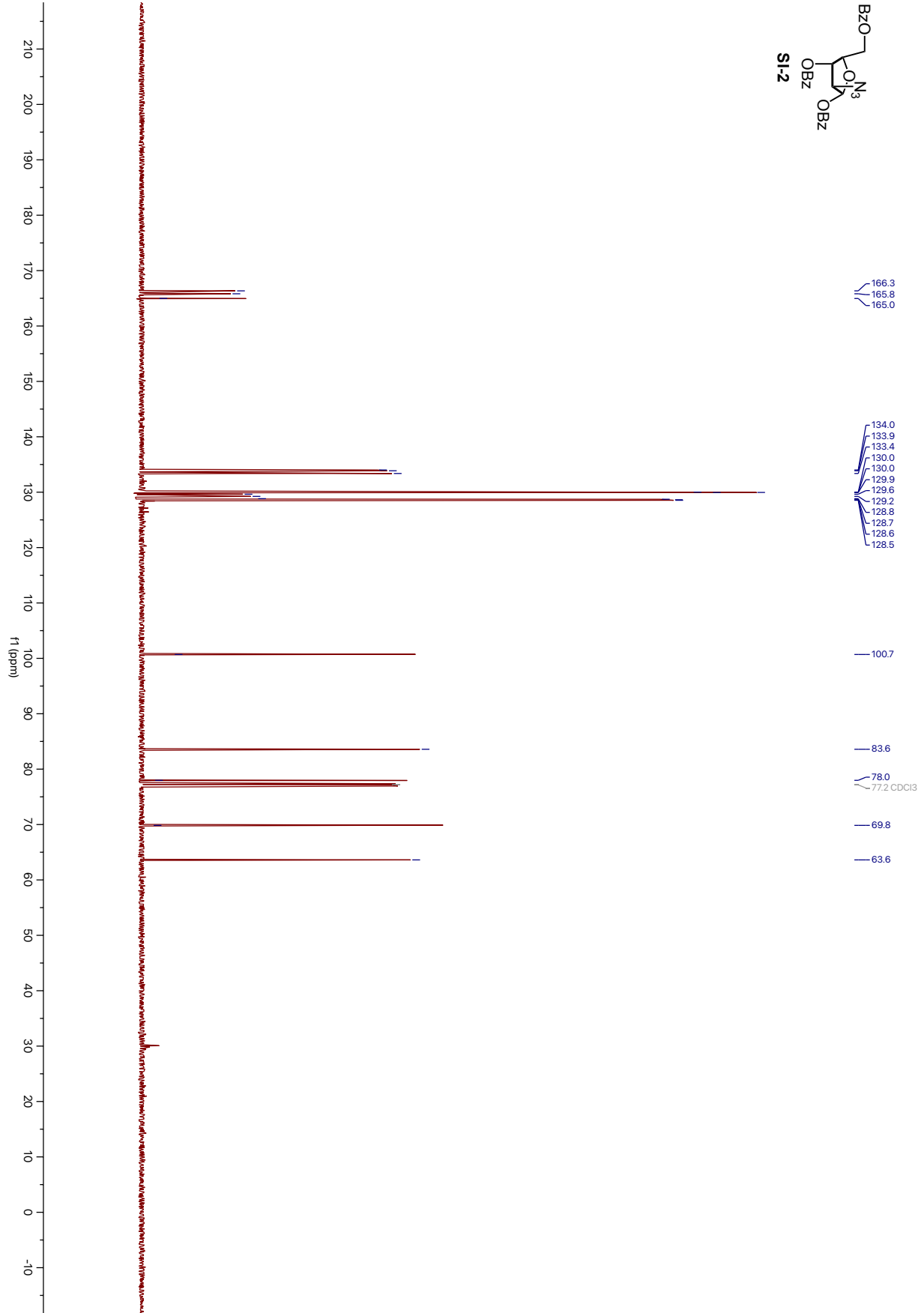
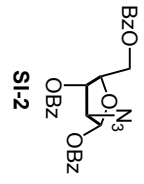
¹H NMR (600 MHz, CDCl₃) δ 8.11 (dd, J = 8.6, 1.1 Hz, 2H), 8.05 (dt, J = 8.1, 1.3 Hz, 4H), 7.63 – 7.55(m, 3H), 7.46 – 7.39 (m, 6H), 6.54 (s, 1H), 5.45 (d, J = 3.7 Hz, 1H), 4.84 (q, J = 4.4 Hz, 1H), 4.72 (dd, J = 12.1, 4.0 Hz, 1H), 4.66 (dd, J = 12.1, 5.3 Hz, 1H), 4.52 (d, J = 1.1 Hz, 1H)

¹³C NMR (151 MHz, CDCl₃) δ 166.3, 165.8, 165.0, 134.0, 133.9, 130.0, 130.0, 129.9, 129.6, 129.2, 128.8, 128.7, 128.6, 128.5, 100.7, 83.6, 78.0, 69.9, 63.6

IR (cm⁻¹): 3062, 2956, 2101, 1717, 1597, 1577, 1491, 1451, 1312, 1242, 1103, 1086, 1067, 1020, 938

HRMS (DART): calc. for C₂₆H₂₁N₃O₇⁺ (M + NH₄⁺): 505.1718, found 505.1720.





2-azido-3,5-di-O-benzoyl-1-O-diallylphosphoryl-2-deoxy-β-D-arabinofuranoside (SI-3).

A flame-dried, 10 mL round-bottomed flask equipped with a magnetic stir bar was charged with **SI-2** (1.00 g, 2.05 mmol, 1.0 equiv), sealed with a rubber septum, evacuated and backfilled with nitrogen three times, and placed under an argon atmosphere. **SI-2** was dissolved in anhydrous CH₂Cl₂ (4.1 mL, 0.5 M) and the resulting solution was cooled to 0 °C by transferring the reaction apparatus to an ice-water bath. After stirring at this temperature for 5 min, 33% HBr in AcOH (740 μL, 4.10 mmol, 2.0 equiv) was added to the reaction flask in a dropwise fashion. Following this addition, the reaction apparatus was immediately removed from the ice-water bath and allowed to equilibrate to room temperature.

After stirring for 1 hour, the stir bar was removed from the reaction flask and the reaction mixture was concentrated *in vacuo*. The resulting orange gum was dried on hi-vac for 1 hour. During this interim period, a second 25 mL round-bottomed flask equipped with a magnetic stir bar and 4 Å molecular sieves (1.00 g) was sealed with a rubber septum and evacuated. The flask was flame dried 3 times to activate the molecular sieves. Upon cooling, the reaction apparatus was evacuated and backfilled with nitrogen three times, and placed under an argon atmosphere. Diallylphosphate (730 mg, 4.10 mmol, 2.0 equiv) was transferred to the reaction flask in a single portion and dissolved in anhydrous toluene (4.1 mL, 0.5 M). Triethylamine (860 μL, 6.15 mmol, 3 equiv) was added to the reaction mixture in a dropwise fashion over the course of 5 minutes, after which the reaction was cooled to -10 °C by transferring the reaction apparatus to a NaCl-ice bath. After stirring at this temperature for 5 minutes, the glycosyl bromide intermediate was dissolved in anhydrous toluene (4.1 mL, 0.5 M). The resulting solution was transferred to the reaction flask in a dropwise fashion. Following this final addition, the reaction flask was allowed to slowly warm to room temperature.

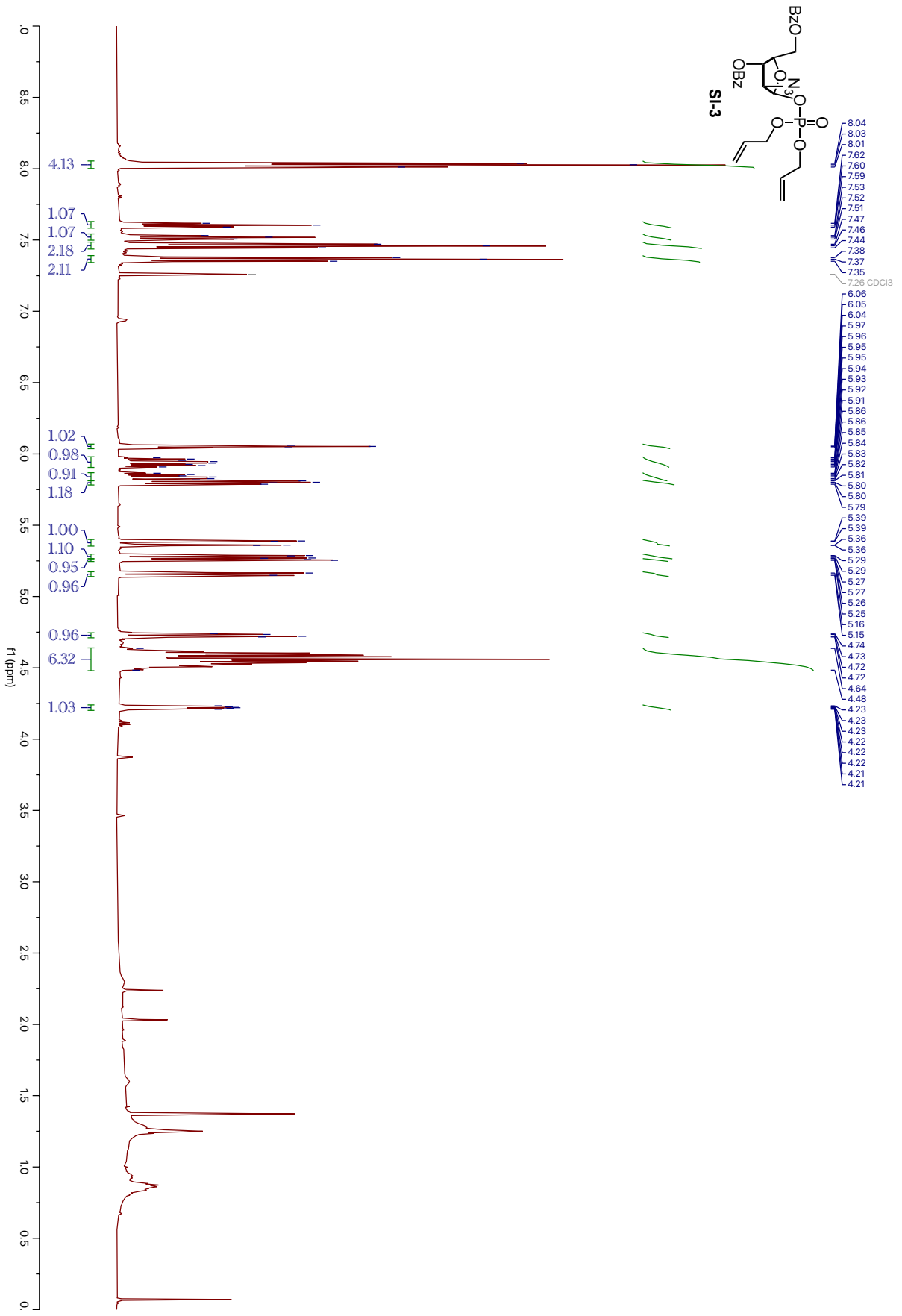
After stirring for 10 hours, the reaction was quenched by pouring into sat. aq. NH₄Cl (150 mL). The layers were separated and the aqueous layer was extracted with Et₂O (3 × 1500 mL). The combined organic layers were washed with brine (300 mL), dried over anhydrous Na₂SO₄, filtered, and concentrated under reduced pressure by rotary evaporation to provide a crude oil. Purification by flash column chromatography on silica gel (EtOAc/hexanes = 0/10 to 1/2) afforded **SI-3** (591 mg, 53% over 2 steps) as a colorless oil.

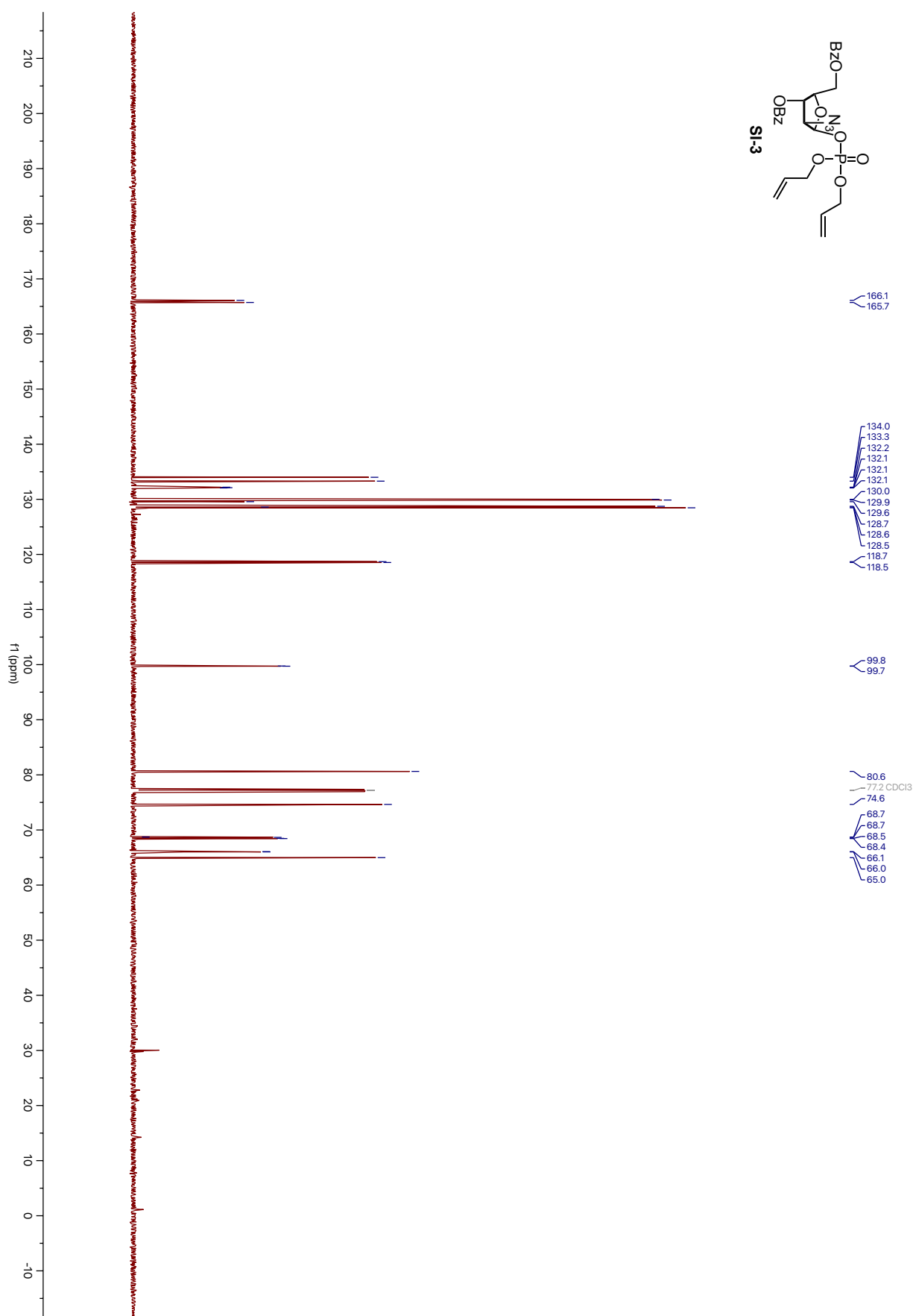
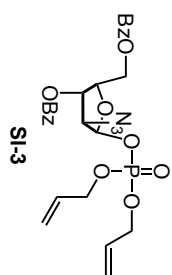
¹H NMR (600 MHz, CDCl₃) δ 8.03 (t, *J* = 6.9 Hz, 4H), 7.60 (t, *J* = 7.7 Hz, 1H), 7.52 (t, *J* = 6.9 Hz, 1H), 7.46 (t, *J* = 7.9 Hz, 2H), 7.37 (t, *J* = 7.7 Hz, 2H), 6.05 (t, *J* = 4.9 Hz, 1H), 5.94 (dq, *J* = 22.2, 10.3, 5.5 Hz, 1H), 5.83 (dq, *J* = 22.9, 10.7, 5.8 Hz, 1H), 5.80 (dd, *J* = 7.9, 5.8 Hz, 1H), 5.38 (dd, *J* = 17.3, 1.6 Hz, 1H), 5.30 (dd, *J* = 10.3, 1.6 Hz, 1H), 5.26 – 5.25 (m, 1H), 5.16 (d, *J* = 10.6 Hz, 1H), 4.73 (dd, *J* = 11.9, 4.0 Hz, 1H), 4.64 – 4.48 (m, 6H), 4.22 (ddd, *J* = 8.3, 4.4, 2.3 Hz, 1H)

¹³C NMR (151 MHz, CDCl₃) δ 166.1, 165.7, 134.0, 133.3, 132.2, 132.2, 132.1, 132.1, 123.0, 129.9, 129.6, 128.7, 128.6, 128.5, 118.7, 118.5, 99.8, 99.7, 80.6, 74.6, 68.7, 68.7, 68.5, 68.4, 66.1, 66.0, 65.0

IR (cm⁻¹): 3069, 2979, 2949, 2883, 2104, 1720, 1597, 1581, 1448, 1259, 1176, 1097, 1017

HRMS (ESI): calc. for C₂₅H₂₇N₃O P⁺ (M + H) 544.1479, found 544.1480.





1-*O*-(*Z,Z*)-farnesylphosphoryl-2-azido-2-deoxy- β -D-arabinofuranoside, 2-AzFPA (1**).**

A flame-dried, 5 mL microwave vial equipped with a magnetic stir bar was charged with **SI-3** (300 mg, 0.55 mmol, 1.0 equiv), sealed with a rubber septum, evacuated and backfilled with nitrogen three times, and placed under an argon atmosphere. **SI-3** was dissolved in anhydrous MTBE (1.4 mL, 0.4 M) and Pd(PPh₃)₄ (6 mg, 1 mol%) was added to the reaction vial in a single portion. Following this addition, the reaction vial was transferred to an ice-water bath to cool to 0 °C. After 5 min, pyrrolidine (180 μ L, 2.2 mmol, 4.0 equiv) was added to the reaction vial neat, in a dropwise fashion over the course of 2 minutes. After this addition, the reaction vial was removed from the ice-water bath and allowed to equilibrate to room temperature. Over the course of the reaction, the formation of a white precipitate was observed due to the insolubility of the de-allylated phosphate intermediate in MTBE.

After stirring for 2 h, stirring was ceased and the precipitate was allowed to settle for 5 min. After this time, the supernatant was decanted and the remaining white precipitate was washed with MTBE (3 \times 2 mL). The reaction vial was fitted with an aluminum–PTFE crimp cap and dried on hi-vac for 1 h to remove trace MTBE and pyrrolidine. The reaction vial was then evacuated and backfilled with nitrogen (3x) and placed under argon. The crude arabinofuranosyl phosphate was dissolved in anhydrous pyridine (1.1 mol, 0.5 M). (*Z,Z*)-farnesol (367 mg, 1.65 mmol, 3.0 equiv) and trichloroacetonitrile (553 μ L, 5.51 mmol, 10.0 equiv) were added to the reaction vial neat, in single portions. The resulting solution was heated to 40 °C in an oil bath.

After stirring at this temperature for 12 h, the reaction vial was removed from the oil bath and concentrated by rotary evaporation to yield a brown oil. The reaction vial was fitted with an aluminum–PTFE crimp cap and the crude farnesyl phosphate was further concentrated on hi-vac to remove traces of pyridine and trichloroacetonitrile. The resulting brown gum was dissolved in equal parts anhydrous methanol (550 μ L, 1.0 M) and 7 N ammonia solution in methanol (550 μ L, 1.0 M). The resulting solution was heated to 55 °C by transferring the reaction vial to an oil bath.

After stirring at this temperature for 12 h, the reaction vial was removed from the oil bath and concentrated by rotary evaporation to yield a brown gum. This crude product was purified directly by flash column chromatography on silica gel (CH₂Cl₂ w/ 0.5% Et₃N/MeOH = 0/1 to 1/5). The dissolved product was passed through a 0.2 micron PTFE syringe filter to afford **1** as a pale yellow oil. This oil was taken up in 1 mL of Milli-Q water, flash frozen and subjected to lyophilization to yield **1** (31 mg, 12% over 3 steps) as a pale-yellow solid.

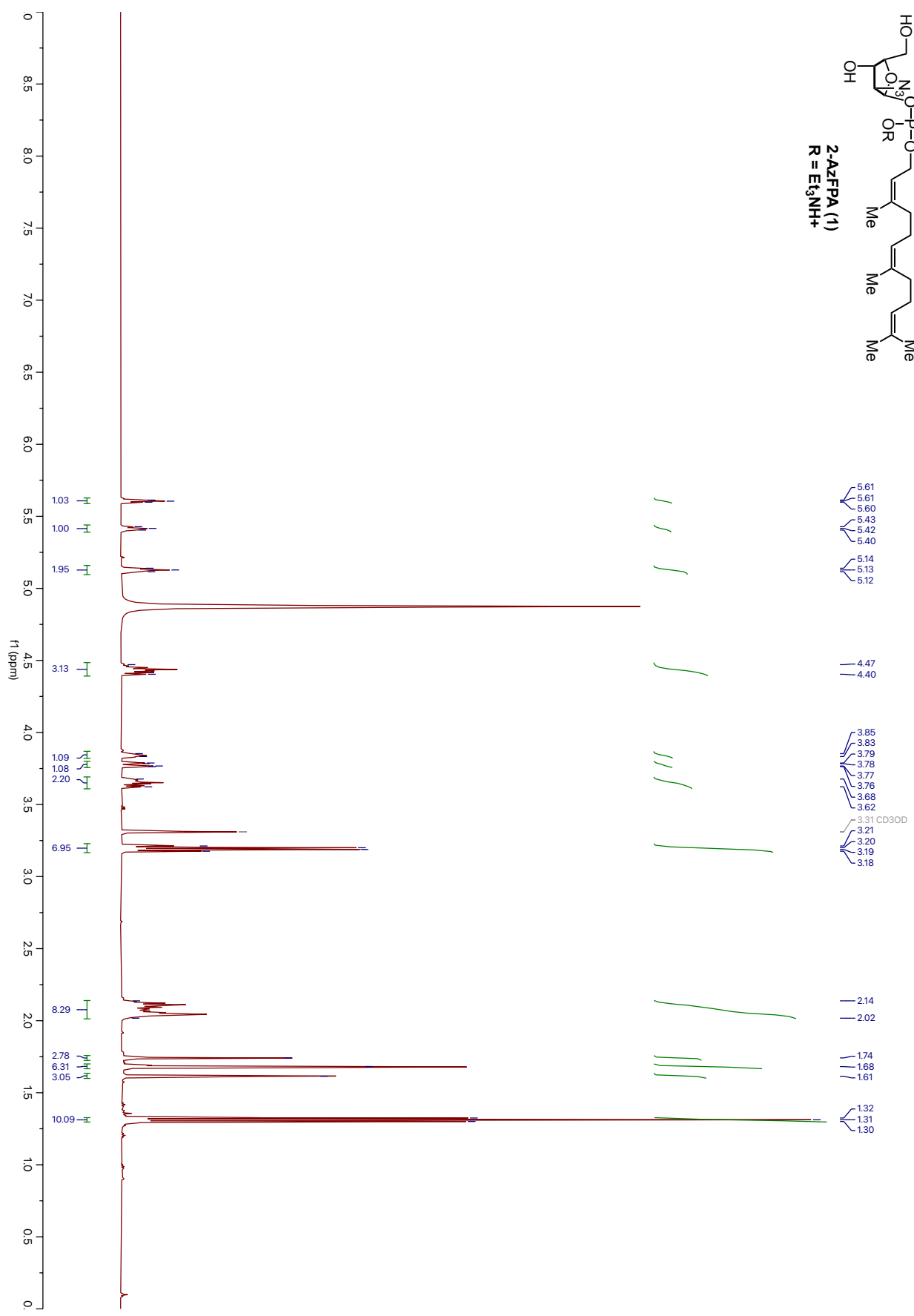
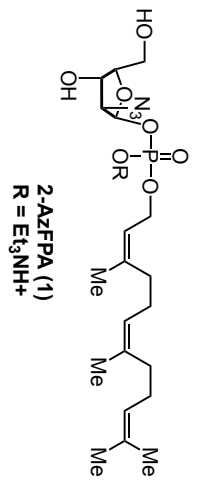
Isolated as triethylammonium adduct

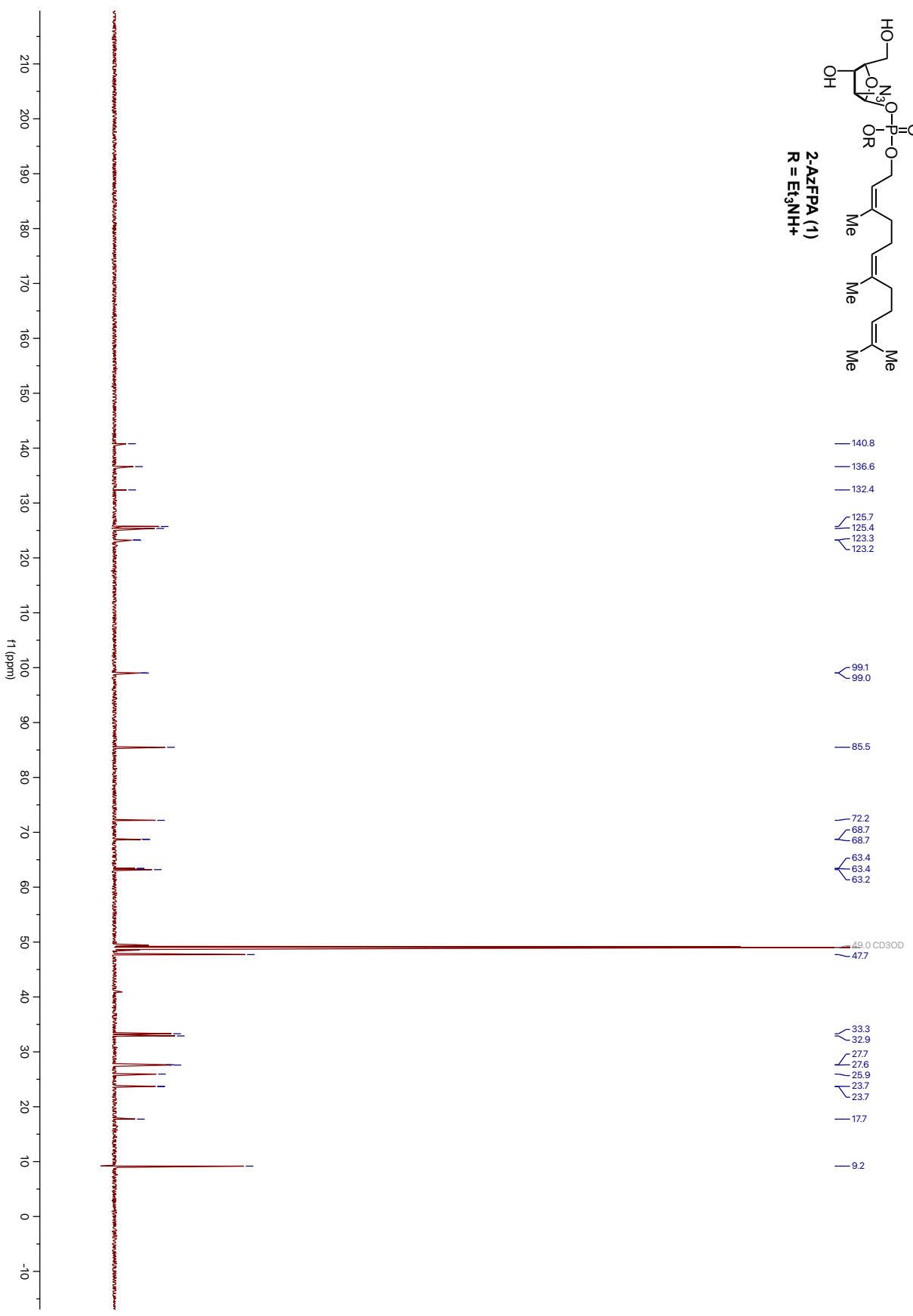
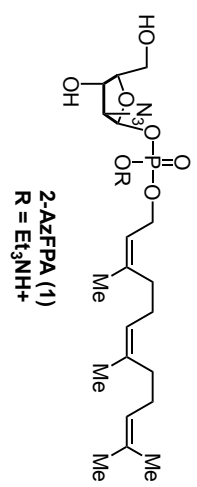
¹H NMR (600 MHz, CD₃OD) δ 5.61 (t, *J* = 4.4 Hz, 1H), 5.42 (t, *J* = 6.9 Hz, 1H), 5.13 (t, *J* = 6.4 Hz, 2H), 4.47 – 4.40 (m, 3H), 3.85 – 3.83 (m, 1H), 3.77 (dd, *J* = 12.5, 2.5 Hz, 1H), 3.68 – 3.62 (m, 2H), 2.14 – 2.02 (m, 8H), 1.74 (s, 3H), 1.68 (s, 6H), 1.61 (s, 3H)
triethyl ammonium adduct: 3.20 (q, *J* = 7.3 Hz, 6H), 1.31 (t, *J* = 7.3 Hz, 9H)

¹³C NMR (151 MHz, CD₃OD) δ 140.8, 136.6, 132.4, 125.7, 125.4, 123.3 (d), 123.2 (d), 99.1 (d), 99.0 (d), 85.5, 72.2, 68.7 (d), 68.7 (d), 63.4 (d), 63.4 (d), 63.2, 33.3, 32.9, 27.7, 27.6, 25.9, 23.7, 23.7, 17.7
triethyl ammonium adduct: 47.7, 9.2

IR (cm⁻¹): 3334, 2963, 2910, 2850, 2107, 1667, 1451, 1372, 1216, 1080, 1011

HRMS (ESI): calc. for C₂₀H₃₃N₃O₇P⁻ (M⁻) 458.2061, found 458.2060.





3-azido-3-deoxy-1-O-methyl-3,5-di-O-*t*-butyldiphenylsilyl-D-arabinofuranose (SI-5):

A flame-dried, 250 mL round-bottomed flask equipped with a magnetic stir bar was charged with **SI-4** (5.00 g, 26.43 mmol, 1.0 equiv), sealed with a rubber septum, evacuated and backfilled with nitrogen three times, and placed under an argon atmosphere.³ **SI-4** was dissolved in anhydrous DMF (88 mL, 0.3 M) and imidazole (7.19 g, 105.72 mmol, 4.0 equiv) was added to the resulting solution as a solid in a single portion. TBDPSCl (15.3 ml, 55.50 mmol, 2.1 equiv) was added to the reaction mixture neat, in a dropwise fashion over the course of 2 minutes. The resulting solution was stirred at room temperature for 6 hours.

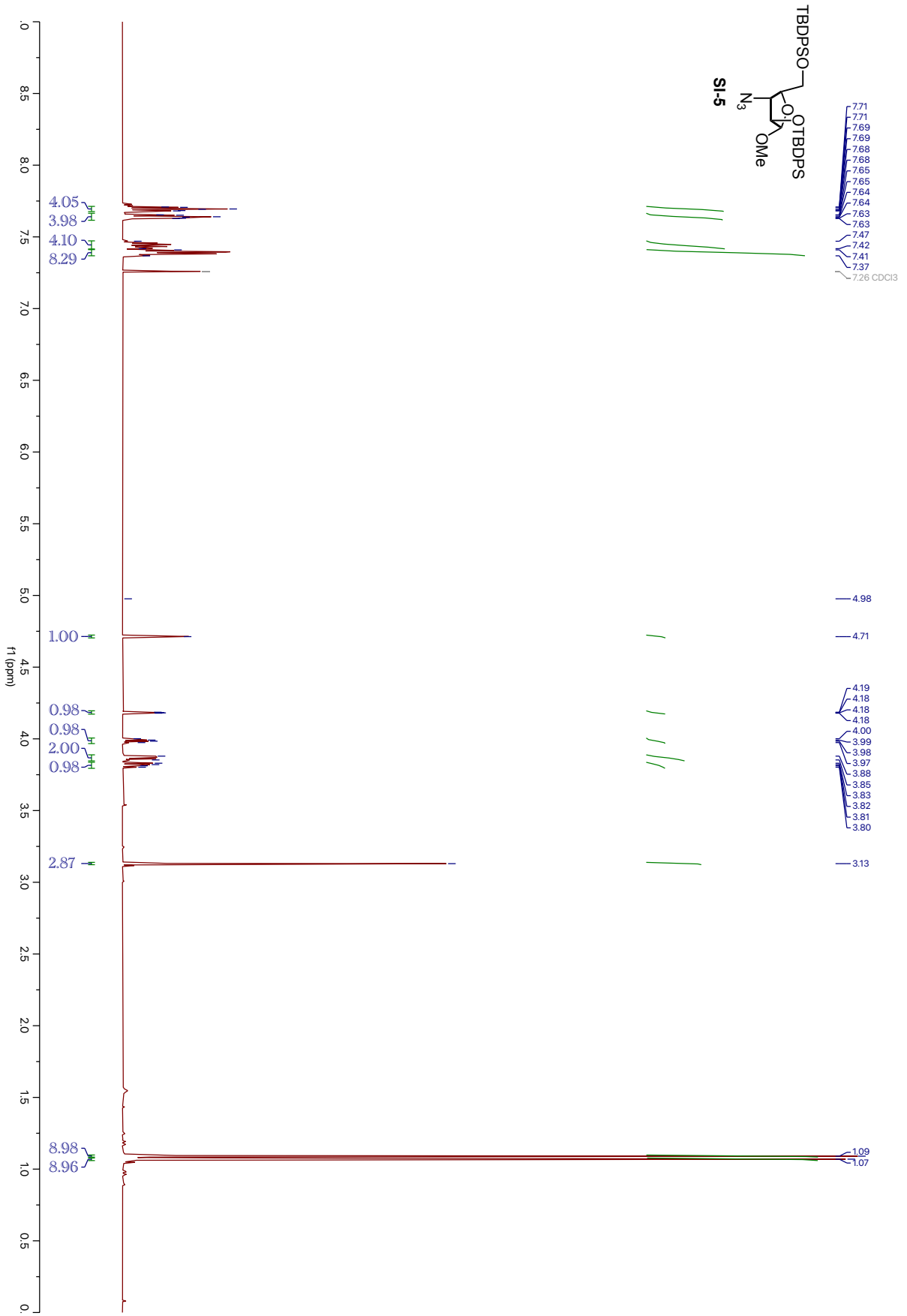
After this time, the reaction was quenched by pouring into sat. aq. NaHCO₃ (300 mL). The layers were separated and the aqueous layer was extracted with pentane (3 × 300 mL). The combined organic layers were washed with brine (500 mL), dried over anhydrous Na₂SO₄, filtered, and concentrated under reduced pressure by rotary evaporation to provide a crude yellow oil. Purification by flash column chromatography on silica gel (Et₂O/hexanes = 0/10 to 1/5) afforded **SI-5** (12.90 g, 77%) as a colorless oil.

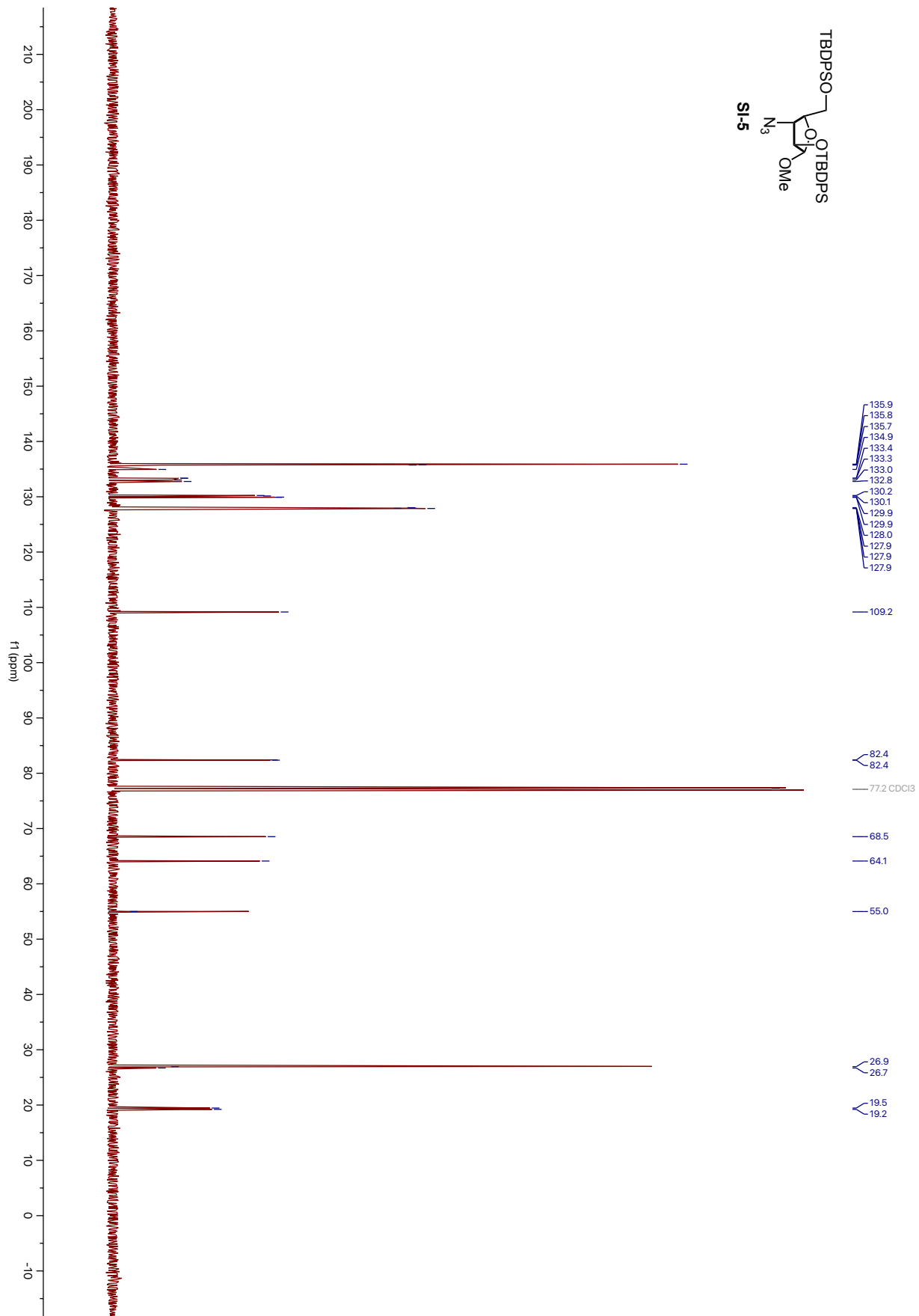
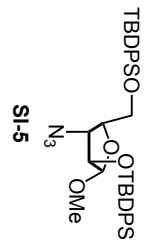
¹H NMR (600 MHz, CDCl₃) δ 7.69 (dt, *J* = 7.9, 1.3 Hz, 4H), 7.64 (dt, *J* = 7.86, 1.8 Hz, 4H), 7.47 – 7.42 (m, 4H), 7.41 – 7.37 (m, 8H), 4.71 (s, 1H), 4.18 (dd, *J* = 3.0, 1.3 Hz, 1H), 4.00 (q, *J* = 5.7 Hz, 1H), 3.88 – 3.85 (m, 2H), 3.82 (dd, *J* = 11.0, 5.7 Hz, 1H), 2.87 (s, 3H), 1.09 (s, 9H), 1.07 (s, 9H)

¹³C NMR (151 MHz, CDCl₃) δ 135.9, 135.8, 135.7, 134.9, 133.4, 133.3, 133.0, 132.8, 130.2, 130.2, 129.9, 129.9, 128.0, 127.9, 127.9, 127.9, 109.2, 82.4, 82.4, 68.5, 64.1, 55.0, 26.9, 26.7, 19.5, 19.2

IR (cm⁻¹): 3072, 3046, 2953, 2930, 2893, 2860, 2101, 1587, 1468, 1425, 1385, 1359, 1263, 1110

HRMS (ESI): calc. for C₃₈H₅₁N₄O₄Si₂⁺ (M + NH₄⁺): 683.3443, found 683.3441.





1-*O*-acetyl-3-azido-3-deoxy-3,5-di-*O*-*t*-butyldiphenylsilyl-D-arabinofuranose (SI-6).

A flame-dried, 250 mL round-bottomed flask equipped with a magnetic stir bar was charged with **SI-5** (5.00 g, 7.32 mmol, 1.0 equiv), sealed with a rubber septum, evacuated and backfilled with nitrogen three times, and placed under an argon atmosphere. **SI-5** was dissolved in Ac₂O (15 mL, 0.5 M). The resulting solution was cooled to 0 °C by transferring the reaction apparatus to an ice-water bath. After stirring for 5 minutes, a solution of H₂SO₄ in Ac₂O (200 μl in 3.0 ml) was added to the reaction mixture in a dropwise fashion over the course of 2 minutes. The reaction apparatus was removed from the ice-water bath and was allowed to warm to room temperature.

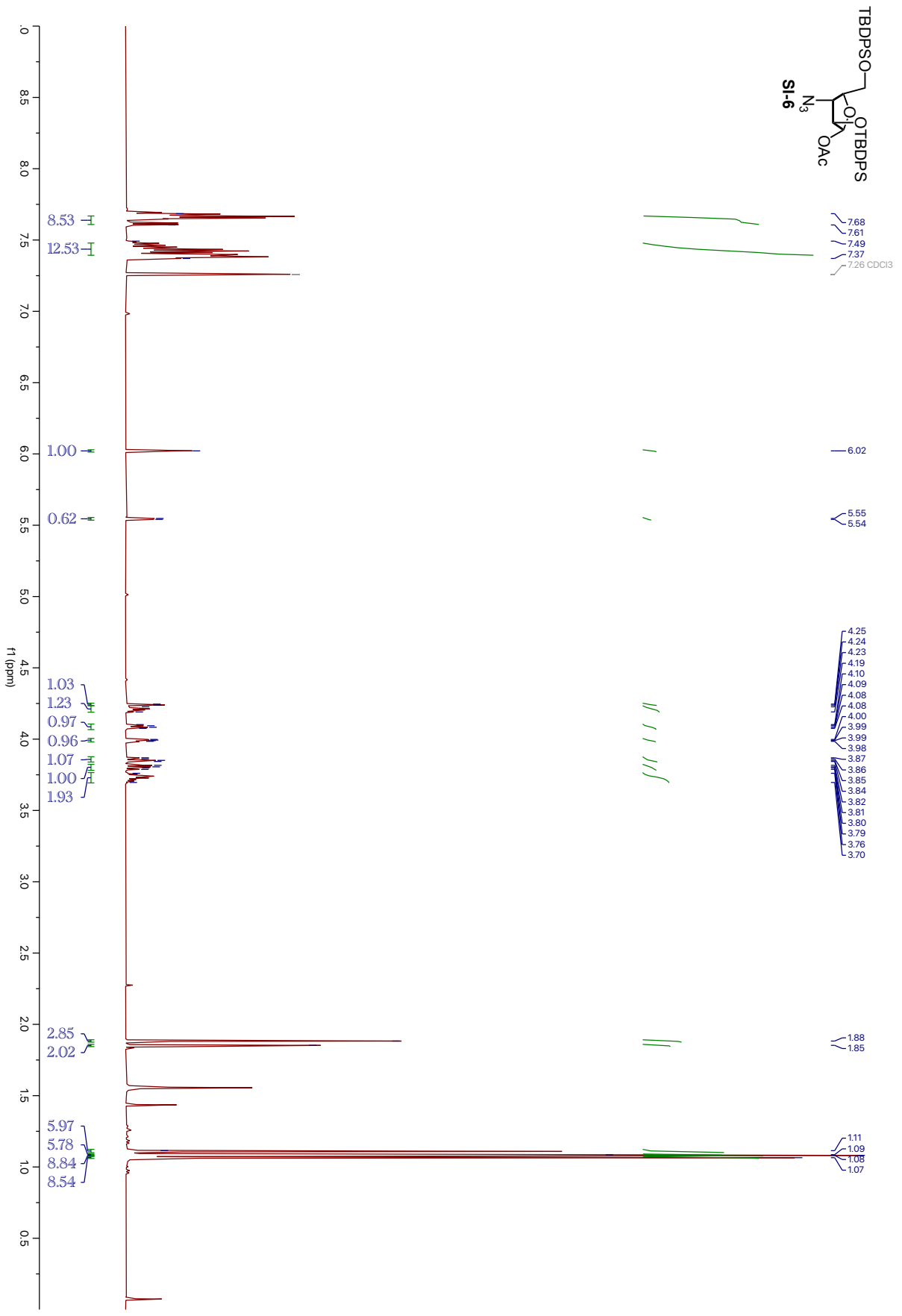
After stirring for 45 minutes, the reaction was quenched through the addition of solid Na₂CO₃. Once bubbling of the mixture ceased upon Na₂CO₃ addition, the reaction was poured into sat. aq. NaHCO₃ (300 mL). The layers were separated and the aqueous layer was extracted with Et₂O (3 × 300 mL). The combined organic layers were washed with brine (500 mL), dried over anhydrous Na₂SO₄, filtered, and concentrated under reduced pressure by rotary evaporation to provide a crude colorless oil. Purification by flash column chromatography on silica gel (Et₂O/hexanes = 0/10 to 1/5) afforded **SI-6** (4.51 g, 89%. α:β = 1.0:1.7) as a colorless oil.

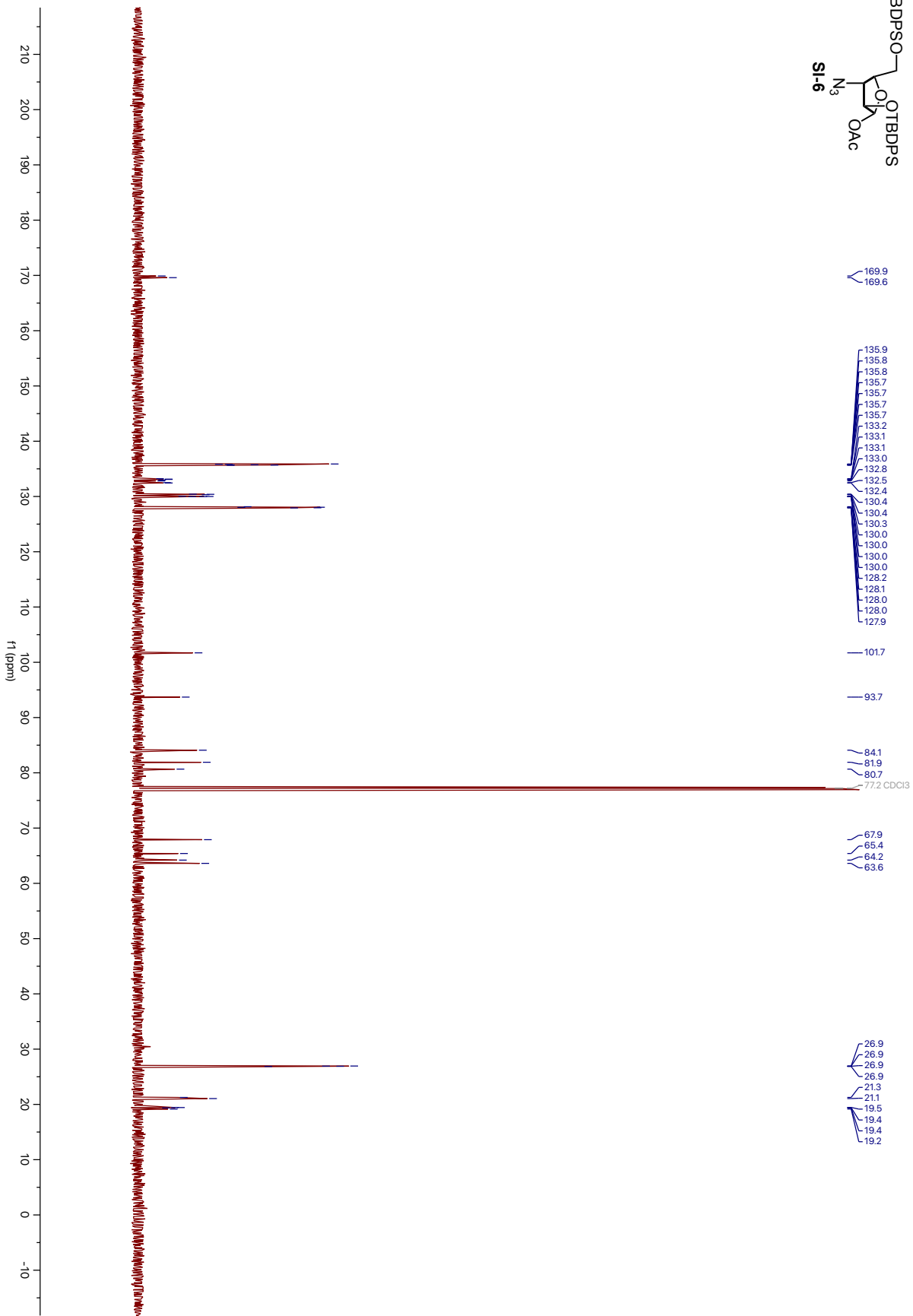
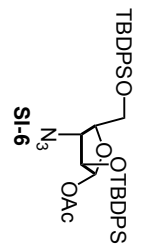
¹H NMR (600 MHz, CDCl₃) δ 7.68 – 7.61 (m, 8H), 7.49 – 7.37 (m, 12H), 6.02 (s, 1H), 5.55 (d, *J* = 4.2 Hz, 0.62H, H-1β), 4.24 (d, *J* = 2.05 Hz, 1H), 4.23 – 4.19 (m, 1cH), 4.09 (dd, *J* = 11.6, 5.0 Hz, 1H), 3.99 (dd, *J* = 5.3, 2.8 Hz, 1H), 3.85 (dd, *J* = 10.9, 4.5 Hz, 1H), 3.81 (dd, *J* = 10.9, 6.6 Hz, 1H), 3.78 – 3.72 (m, 2H), 1.88 (s, 3H), 1.85 (s, 2H, H-1β), 1.11 (s, 6H, H-1β), 1.09 (s, 6H, H-1β), 1.08 (s, 9H), 1.07 (s, 9H)

¹³C NMR (151 MHz, CDCl₃) δ 169.9, 169.6, 135.9, 135.8, 135.8, 135.7, 135.7, 135.7, 135.7, 133.2, 133.1, 133.1, 133.0, 132.8, 132.5, 132.5, 130.4, 130.4, 130.3, 130.0, 130.0, 130.0, 130.0, 128.2, 128.1, 128.0, 128.0, 128.0, 101.7, 93.7, 84.1, 81.9, 80.7, 67.9, 65.4, 64.2, 63.6, 27.0, 26.9, 26.9, 26.9, 21.3, 21.1, 19.5, 19.4, 19.4, 19.2

IR (cm⁻¹): 3072, 3042, 2956, 2926, 2890, 2857, 2104, 1750, 1587, 1471, 1428, 1355, 1223, 1134, 1007

HRMS (DART): calc. for C₃₉H₄₇N₃O₅Si₂⁺ (M – C₁₆H₁₉Si + NH₄⁺): 477.1690, found 477.1698.





1-*O*-diallylphosphoryl-3-azido-3-deoxy-3,5-di-*O*-*t*-butyldiphenylsilyl-D-arabinofuranose (SI-7).

A flame-dried, 10 mL round-bottomed flask equipped with a magnetic stir bar was charged with **SI-6** (1.00 g, 1.44 mmol, 1.0 equiv), sealed with a rubber septum, evacuated and backfilled with nitrogen three times, and placed under an argon atmosphere. **SI-6** was dissolved in anhydrous CH₂Cl₂ (2.9 ml, 0.5 M) and the resulting solution was cooled to 0 °C by transferring the reaction apparatus to an ice-water bath. After stirring at this temperature for 5 min, 33% HBr in AcOH (529 µl, 4.10 mmol, 2.0 equiv) was added to the reaction flask in a dropwise fashion. Following this addition, the reaction apparatus was immediately removed from the ice-water bath and allowed to equilibrate to room temperature.

After stirring for 1 hour, the stir bar was removed from the reaction flask and the reaction mixture was concentrated *in vacuo*. The resulting orange gum was dried on hi-vac for 1 hour. During this interim period, a second 25 mL round-bottomed flask equipped with a magnetic stir bar and 4 Å molecular sieves (1.00 g) was sealed with a rubber septum and evacuated. The flask was flame dried 3 times to activate the molecular sieves. Upon cooling, the reaction apparatus was evacuated and backfilled with nitrogen three times, and placed under an argon atmosphere. Diallylphosphate (513 mg, 4.10 mmol, 2.0 equiv) was transferred to the reaction flask in a single portion and dissolved in anhydrous toluene (2.9 ml, 0.5 M). Triethylamine (600 µl, 4.32 mmol, 3 equiv) was added to the reaction mixture in a dropwise fashion over the course of 5 minutes, after which the reaction was cooled to -10 °C by transferring the reaction apparatus to a NaCl-ice bath. After stirring at this temperature for 5 minutes, the glycosyl bromide intermediate was dissolved in anhydrous toluene (2.9 ml, 0.5 M). The resulting solution was transferred to the reaction flask in a dropwise fashion. Following this final addition, the reaction flask was allowed to slowly warm to room temperature.

After stirring for 10 hours, the reaction was quenched by pouring into sat. aq. NH₄Cl (150 mL). The layers were separated and the aqueous layer was extracted with Et₂O (3 × 1500 mL). The combined organic layers were washed with brine (300 mL), dried over anhydrous Na₂SO₄, filtered, and concentrated under reduced pressure by rotary evaporation to provide a crude oil. Purification by flash column chromatography on silica gel (EtOAc/hexanes = 0/10 to 1/2) afforded **SI-7** (701 mg, 60% over 2 steps) as a colorless oil.

¹H NMR (600 MHz, CDCl₃) δ 7.71 – 7.68 (m, 6H), 7.62 (dd, *J* = 8.1, 1.3 Hz, 2H), 7.47 – 7.42 (m, 4H), 7.40 – 7.33 (m, 8H), 5.87 – 5.75 (m, 2H), 5.27 (dq, *J* = 17.2, 1.5 Hz, 1H), 5.23 (dq, *J* = 17.0, 1.6 Hz, 1H), 5.18 – 5.15 (m, 3H), 4.48 – 4.43 (m, 1H), 4.41 – 4.27 (m, 5H), 3.82 (ddd, *J* = 9.5, 4.5, 3.3 Hz, 1H), 3.04 (dd, *J* = 12.9, 9.4 Hz, 1H), 2.08 (dd, *J* = 13.0, 3.3 Hz, 1H), 1.10 (s, 9H), 1.02 (s, 9H)

¹³C NMR (151 MHz, CDCl₃) δ 136.2, 136.0, 135.9, 135.8, 133.6, 132.9, 132.7, 132.5, 132.2, 130.5, 130.2, 130.2, 128.3, 128.1, 128.0, 128.0, 118.1, 99.8, 99.8, 83.6, 79.3, 79.3, 78.1, 68.2, 68.2, 68.2, 68.1, 52.9, 27.2, 27.1, 19.5, 19.2

IR (cm⁻¹): 3073, 3046, 2949, 2930, 2890, 2857, 2104, 1591, 1471, 1428, 1269, 1113, 1020, 931, 818

HRMS (DART): calc. for C₄₃H₅₄N₃O₇PSi₂⁺ (M/2 + H + NH₄⁺): 417.6601, found 417.6615.

1-O-(Z,Z)-farnesylphosphoryl-3-azido-3-deoxy-β-D-arabinofuranose, 3-AzFPA (2).

A flame-dried, 5 mL microwave vial equipped with a magnetic stir bar and was charged with **SI-7** (300 mg, 0.37 mmol, 1.0 equiv), sealed with a rubber septum, evacuated and backfilled with nitrogen three times, and placed under an argon atmosphere. **SI-7** was dissolved in anhydrous CH₂Cl₂ (1.4 mL, 0.4 M) and Pd(PPh₃)₄ (4 mg, 1 mol%) was added to the reaction vial in a single portion. Following this addition phenylsilane (90 μL, 0.74 mmol, 2.0 equiv) was added to the reaction vial neat, in a dropwise fashion over the course of 2 minutes.

After stirring for 3 hours, the contents of the reaction vial were concentrated *in vacuo*. The reaction vial was fitted with an aluminum–PTFE crimp cap and dried on hi-vac for 1 hour to remove trace solvent. Following this, the reaction vial was evacuated and backfilled with nitrogen three times, and placed under an argon atmosphere. The crude arabinofuranosyl phosphate was dissolved in anhydrous pyridine (740 μL, 0.5 M). (*Z,Z*)-farnesol (247 mg, 1.10 mmol, 3.0 equiv) and trichloroacetonitrile (370 μL, 3.70 mmol, 10.0 equiv) were added to the reaction vial neat, in single portions. The resulting solution was heated to 40 °C by transferring the reaction vial to an oil bath.

After stirring at this temperature for 12 hours, the reaction vial was removed from the oil bath and concentrated by rotary evaporation to yield a brown oil. The reaction vial was fitted with a rubber septum and the crude farnesyl phosphate was further concentrated on hi-vac to remove traces of pyridine and trichloroacetonitrile. The resulting brown gum was dissolved in equal parts anhydrous methanol (550 μL, 1.0 M) and 7 N ammonia solution in methanol (550 μL, 1.0 M). Ammonium fluoride (137 mg, 3.70 mmol, 10.0 equiv) was added to the reaction vial in a single portion and the vial was fitted with an aluminum–PTFE crimp cap. The resulting solution was heated to 55 °C by transferring the reaction vial to an oil bath.

After stirring at this temperature for 12 hours, the reaction vial was removed from the oil bath and concentrated by rotary evaporation to yield a brown gum. This crude product was purified directly by flash column chromatography on silica gel (CH₂Cl₂ w/ 0.5% Et₃N /MeOH = 0/1 to 1/5). Filtration of the dissolved product through a 0.2 micron PTFE syringe filter afforded **2** as a pale brown oil. This oil was taken up in 1 ml of Milli-Q water, flash frozen and subjected to lyophilization to yield **2** (25 mg, 15% over 3 steps) as a pale yellow solid.

Isolated as triethylammonium adduct with minor proteo- form

¹H NMR (600 MHz, CD₃OD) δ 5.47 (t, *J* = 4.5 Hz, 1H), 5.41 (t, *J* = 6.9 Hz, 1H), 5.14 – 5.11 (m, 2H), 4.45 – 4.42 (m, 2H), 4.08 – 4.01 (m, 2H), 3.77 – 3.70 (m, 2H), 3.66 – 3.60 (m, 1H), 2.14 – 2.03 (m, 8H), 1.74 (s, 3H), 1.68 (s, 6H), 1.61 (s, 3H).

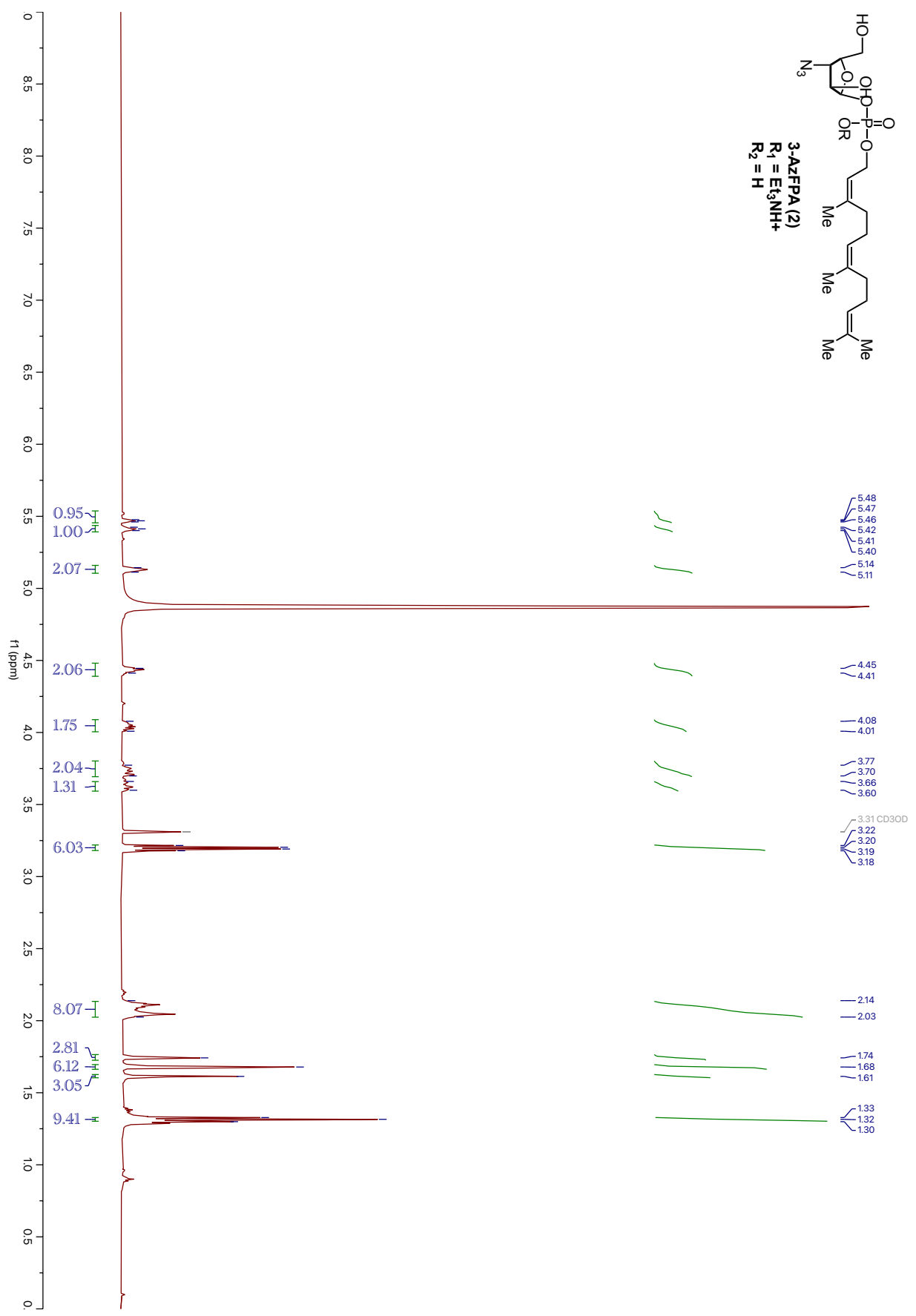
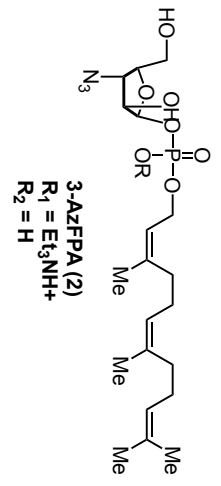
triethyl ammonium adduct: 3.20 (q, *J* = 7.7 Hz, 6H), 1.31 (t, *J* = 8.1 Hz, 9H)

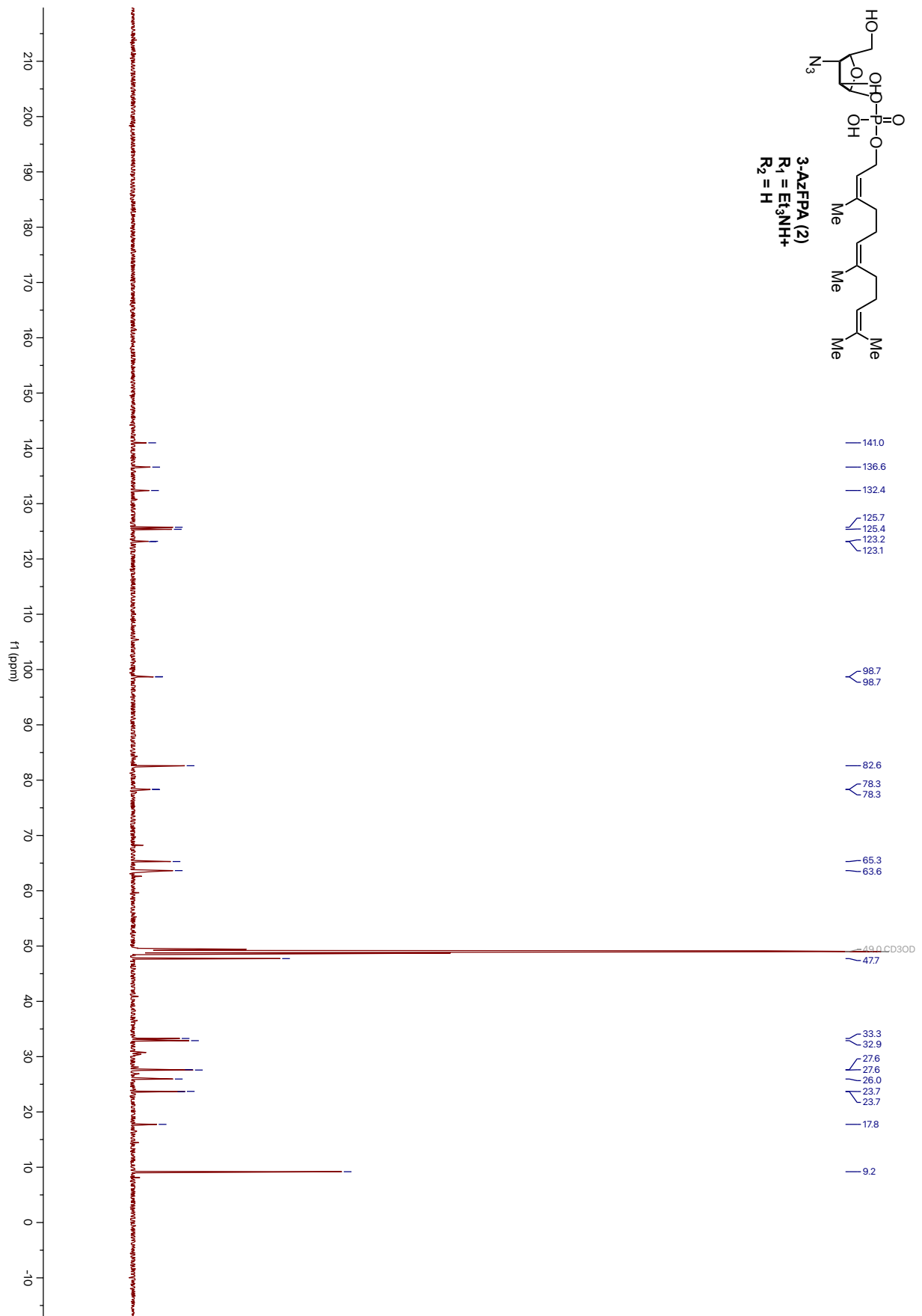
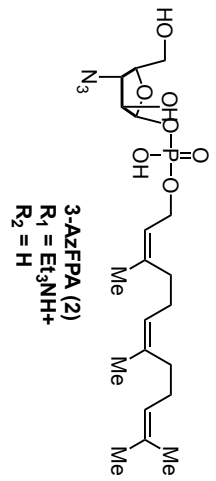
¹³C NMR (151 MHz, CD₃OD) δ 141.0, 136.6, 132.4, 125.7, 125.4, 123.2 (d), 123.1 (d), 98.7 (d), 98.7 (d), 82.6, 78.3, 78.3, 65.3, 63.6, 33.3, 32.9, 27.6, 27.6, 26.0, 23.7, 23.7, 17.8

triethyl ammonium adduct: 47.7, 9.2

IR (cm⁻¹): 3234, 2963, 2920, 2847, 2091, 1670, 1481, 1372, 1276, 1213, 1054, 1021, 937

HRMS (ESI): calc. for C₂₀H₃₃N₃O₇P⁻ (M⁻) 458.2061, found 458.2040.





5-azido-5-deoxy-1-O-methyl-2,3-di-O-*t*-butyldiphenylsilyl-D-arabinofuranose (SI-9):

A flame-dried, 250 mL round-bottomed flask equipped with a magnetic stir bar was charged with **SI-8** (5.00 g, 26.43 mmol, 1.0 equiv), sealed with a rubber septum, evacuated and backfilled with nitrogen three times, and placed under an argon atmosphere.⁴ **SI-8** was dissolved in anhydrous DMF (88 mL, 0.3 M) and imidazole (7.19 g, 105.72 mmol, 4.0 equiv) was added to the resulting solution as a solid in a single portion. TBDPSCl (15.3 ml, 55.50 mmol, 2.1 equiv) was added to the reaction mixture neat, in a dropwise fashion over the course of 2 minutes. The resulting solution was stirred at room temperature for 12 hours.

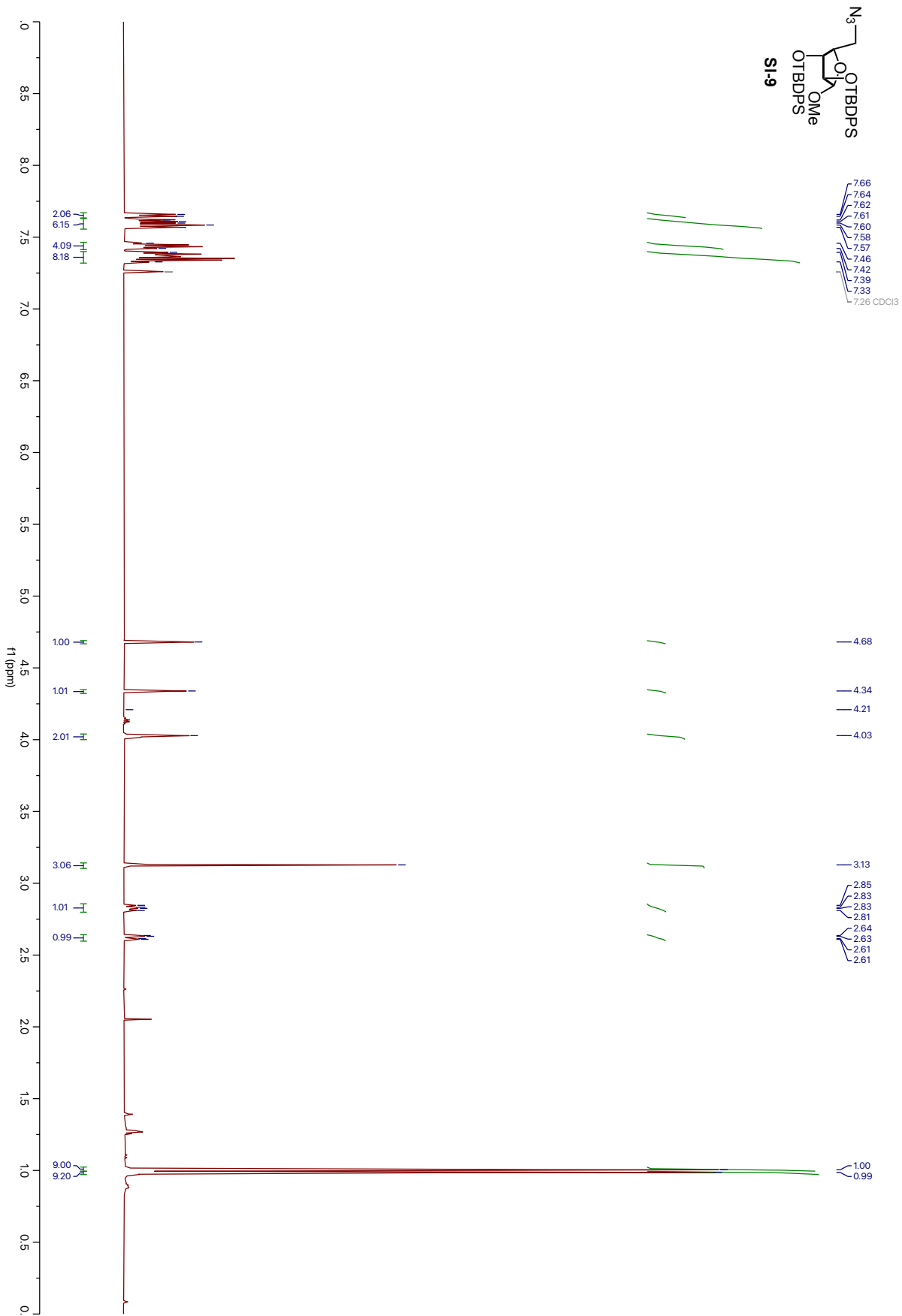
After this time, the reaction was quenched by pouring into sat. aq. NaHCO₃ (300 mL). The layers were separated and the aqueous layer was extracted with pentane (3 × 300 mL). The combined organic layers were washed with brine (500 mL), dried over anhydrous Na₂SO₄, filtered, and concentrated under reduced pressure by rotary evaporation to provide a crude yellow oil. Purification by flash column chromatography on silica gel (Et₂O/hexanes = 0/10 to 1/5) afforded **SI-9** (15.17 g, 84%) as a colorless oil.

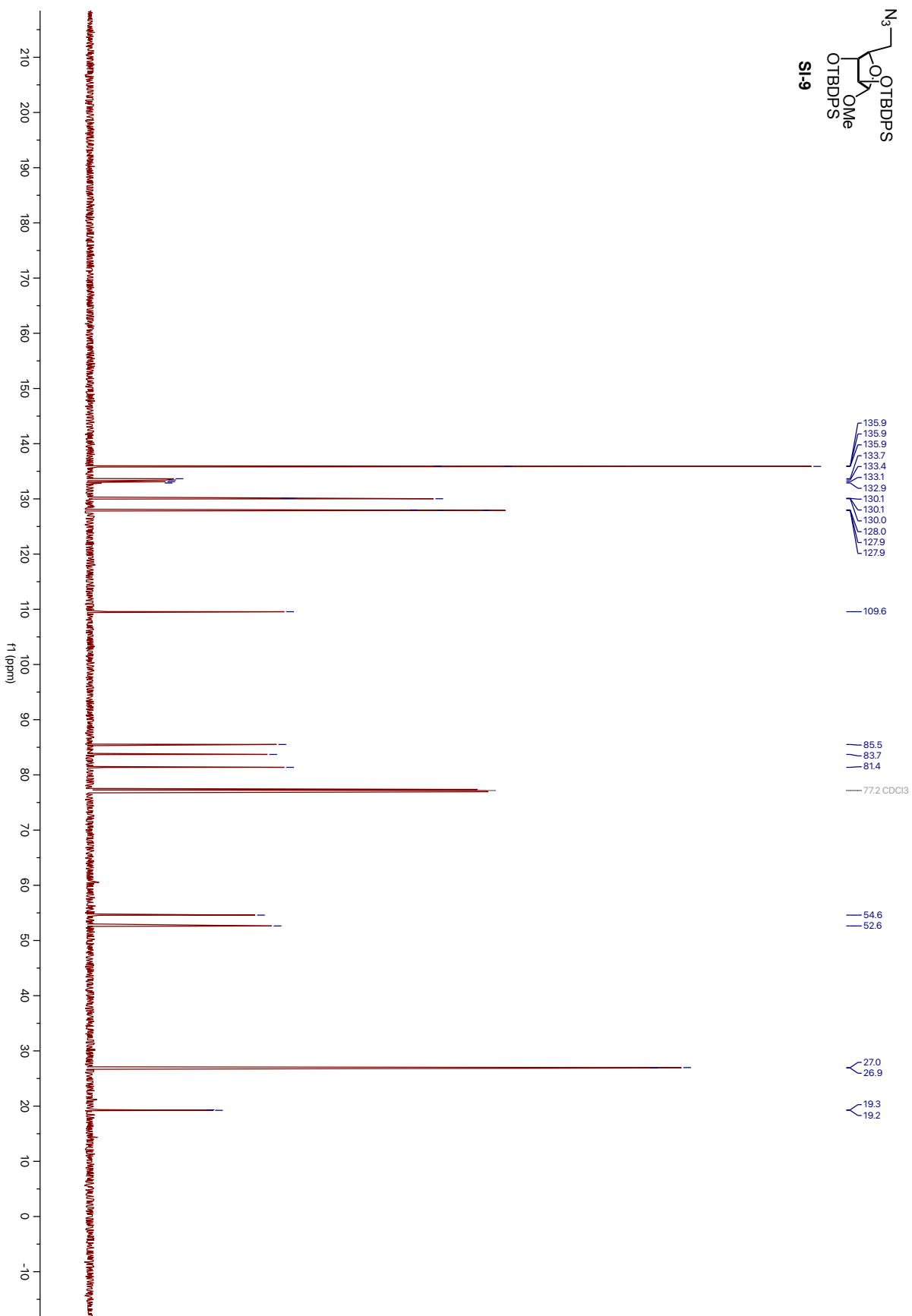
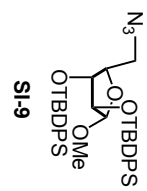
¹H NMR (600 MHz, CDCl₃) δ 7.65 (d, *J* = 7.3 Hz, 2H), 7.62 (d, *J* = 7.5 Hz, 2H), 7.58 (t, *J* = 8.3 Hz, 4H), 7.46 – 7.42 (m, 4H), 7.39 – 7.33 (m, 8H), 4.68 (s, 1H), 4.21 (s, 1H), 4.04 – 4.00 (m, 2H), 3.13 (s, 3H), 2.83 (dd, *J* = 12.0, 7.4 Hz, 1H), 2.62 (dd, *J* = 14.1, 3.3 Hz, 1H), 1.00 (s, 9H), 0.99 (s, 9H)

¹³C NMR (151 MHz, CDCl₃) δ 135.9, 135.9, 135.9, 133.7, 133.4, 133.1, 132.9, 130.1, 130.1, 130.0, 128.80, 127.9, 127.9, 109.6, 85.5, 83.7, 81.4, 54.46, 52.6, 27.0, 26.9, 19.3, 19.2

IR (cm⁻¹): 3049, 3069, 2959, 2926, 2853, 2098, 1750, 1471, 1428, 1355, 1226, 1007, 954

HRMS (ESI): calc. for C₃₈H₅₁N₄O₄Si₂⁺ (M + NH₄⁺): 683.3443, found 683.3446.





1-*O*-acetyl-5-azido-5-deoxy-2,3-di-*O*-*t*-butyldiphenylsilyl-D-arabinofuranose (SI-10).

A flame-dried, 250 mL round-bottomed flask equipped with a magnetic stir bar was charged with **SI-9** (5.00 g, 7.32 mmol, 1.0 equiv), sealed with a rubber septum, evacuated and backfilled with nitrogen three times, and placed under an argon atmosphere. **SI-9** was dissolved in Ac₂O (15 mL, 0.5 M). The resulting solution was cooled to 0 °C by transferring the reaction apparatus to an ice-water bath. After stirring for 5 minutes, a solution of H₂SO₄ in Ac₂O (200 μl in 3.0 ml) was added to the reaction mixture in a dropwise fashion over the course of 2 minutes. The reaction apparatus was removed from the ice-water bath and was allowed to warm to room temperature.

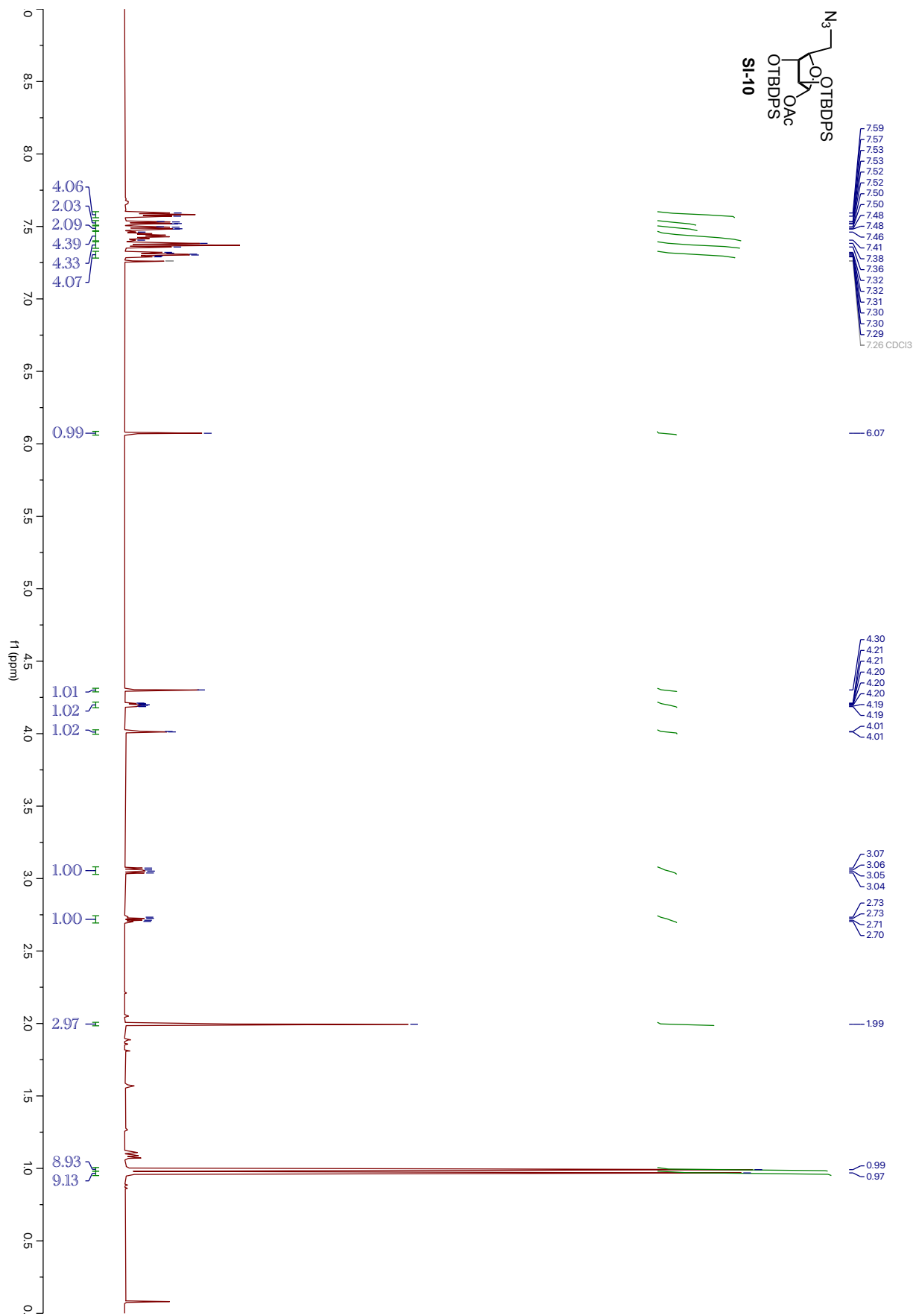
After stirring for 45 minutes, the reaction was quenched through the addition of solid Na₂CO₃. Once bubbling of the mixture ceased upon Na₂CO₃ addition, the reaction was poured into sat. aq. NaHCO₃ (300 mL). The layers were separated and the aqueous layer was extracted with Et₂O (3 × 300 mL). The combined organic layers were washed with brine (500 mL), dried over anhydrous Na₂SO₄, filtered, and concentrated under reduced pressure by rotary evaporation to provide a crude colorless oil. Purification by flash column chromatography on silica gel (Et₂O/hexanes = 0/10 to 1/5) afforded **SI-10** (3.85 g, 76%) as a colorless oil.

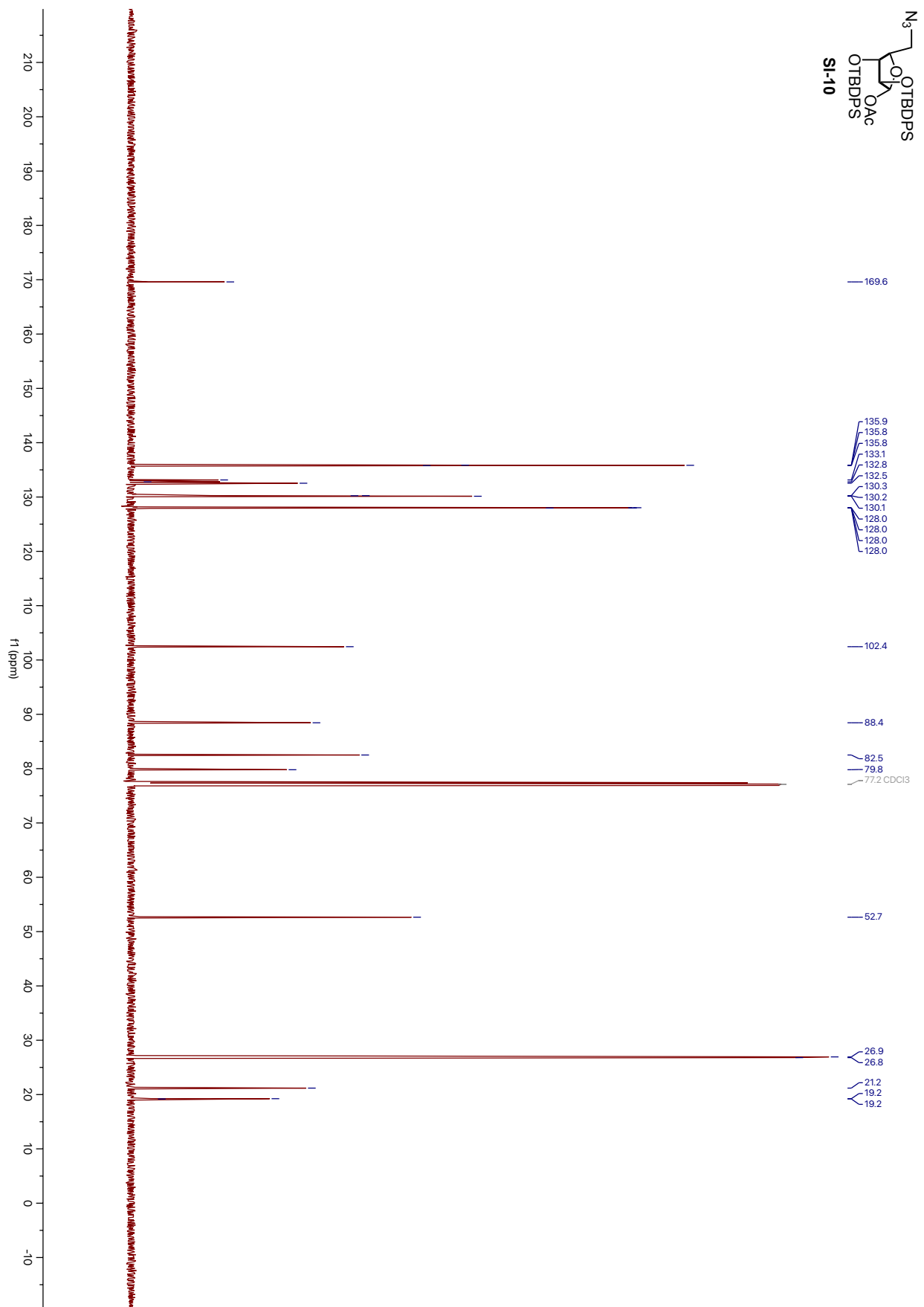
¹H NMR (600 MHz, CDCl₃) δ 7.59 – 7.57 (m, 4H), 7.53 (dd, *J* = 8.1, 1.5 Hz, 2H), 7.49 (dd, *J* = 8.0, 1.4 Hz, 2H), 7.46 – 7.41 (m, 4H), 7.38 – 7.36 (m, 4H), 7.31 (dt, *J* = 7.4, 3.4 Hz, 4H), 6.07 (s, 1H), 4.30 (s, 1H), 4.20 (ddd, *J* = 7.6, 5.0, 1.6 Hz, 1H), 4.01 (d, *J* = 1.9 Hz, 1H), 3.06 (dd, *J* = 12.4, 7.8 Hz, 1H), 2.72 (dd, *J* = 12.4, 5.0 Hz, 1H), 1.99 (s, 3H), 0.99 (s, 9H), 0.97 (s, 9H).

¹³C NMR (151 MHz, CDCl₃) δ 169.6, 135.9, 135.8, 135.8, 133.1, 132.8, 132.5, 130.3, 130.2, 130.1, 128.0, 128.0, 128.0, 128.0, 102.5, 88.4, 82.5, 79.8, 52.7, 26.9, 26.8, 21.2, 19.2, 19.2

IR (cm⁻¹): 2932, 2859, 2099, 1747, 1589, 1472, 1428, 1363, 1265, 1232, 1104, 1008, 959, 908, 850, 822, 779, 734, 699, 611, 554, 504, 485

HRMS (ESI): calc. for C₃₉H₄₈N₃O₅Si₂⁺ (M + H⁺): 694.3055, found 694.3011.





1-*O*-diallylphosphoryl-5-azido-5-deoxy-2,3-di-*O*-*t*-butyldiphenylsilyl-D-arabinofuranose (SI-11).

A flame-dried, 10 mL round-bottomed flask equipped with a magnetic stir bar was charged with **SI-10** (1.00 g, 1.44 mmol, 1.0 equiv), sealed with a rubber septum, evacuated and backfilled with nitrogen three times, and placed under an argon atmosphere. **SI-10** was dissolved in anhydrous CH₂Cl₂ (2.9 ml, 0.5 M) and the resulting solution was cooled to 0 °C by transferring the reaction apparatus to an ice-water bath. After stirring at this temperature for 5 min, 33% HBr in AcOH (529 μl, 4.10 mmol, 2.0 equiv) was added to the reaction flask in a dropwise fashion. Following this addition, the reaction apparatus was immediately removed from the ice-water bath and allowed to equilibrate to room temperature.

After stirring for 1 hour, the stir bar was removed from the reaction flask and the reaction mixture was concentrated *in vacuo*. The resulting orange gum was dried on hi-vac for 1 hour. During this interim period, a second 25 mL round-bottomed flask equipped with a magnetic stir bar and 4 Å molecular sieves (1.00 g) was sealed with a rubber septum and evacuated. The flask was flame dried 3 times to activate the molecular sieves. Upon cooling, the reaction apparatus was evacuated and backfilled with nitrogen three times, and placed under an argon atmosphere. Diallylphosphate (513 mg, 4.10 mmol, 2.0 equiv) was transferred to the reaction flask in a single portion and dissolved in anhydrous toluene (2.9 ml, 0.5 M). Triethylamine (600 μl, 4.32 mmol, 3 equiv) was added to the reaction mixture in a dropwise fashion over the course of 5 minutes, after which the reaction was cooled to -10 °C by transferring the reaction apparatus to a NaCl-ice bath. After stirring at this temperature for 5 minutes, the glycosyl bromide intermediate was dissolved in anhydrous toluene (2.9 ml, 0.5 M). The resulting solution was transferred to the reaction flask in a dropwise fashion. Following this final addition, the reaction flask was allowed to slowly warm to room temperature.

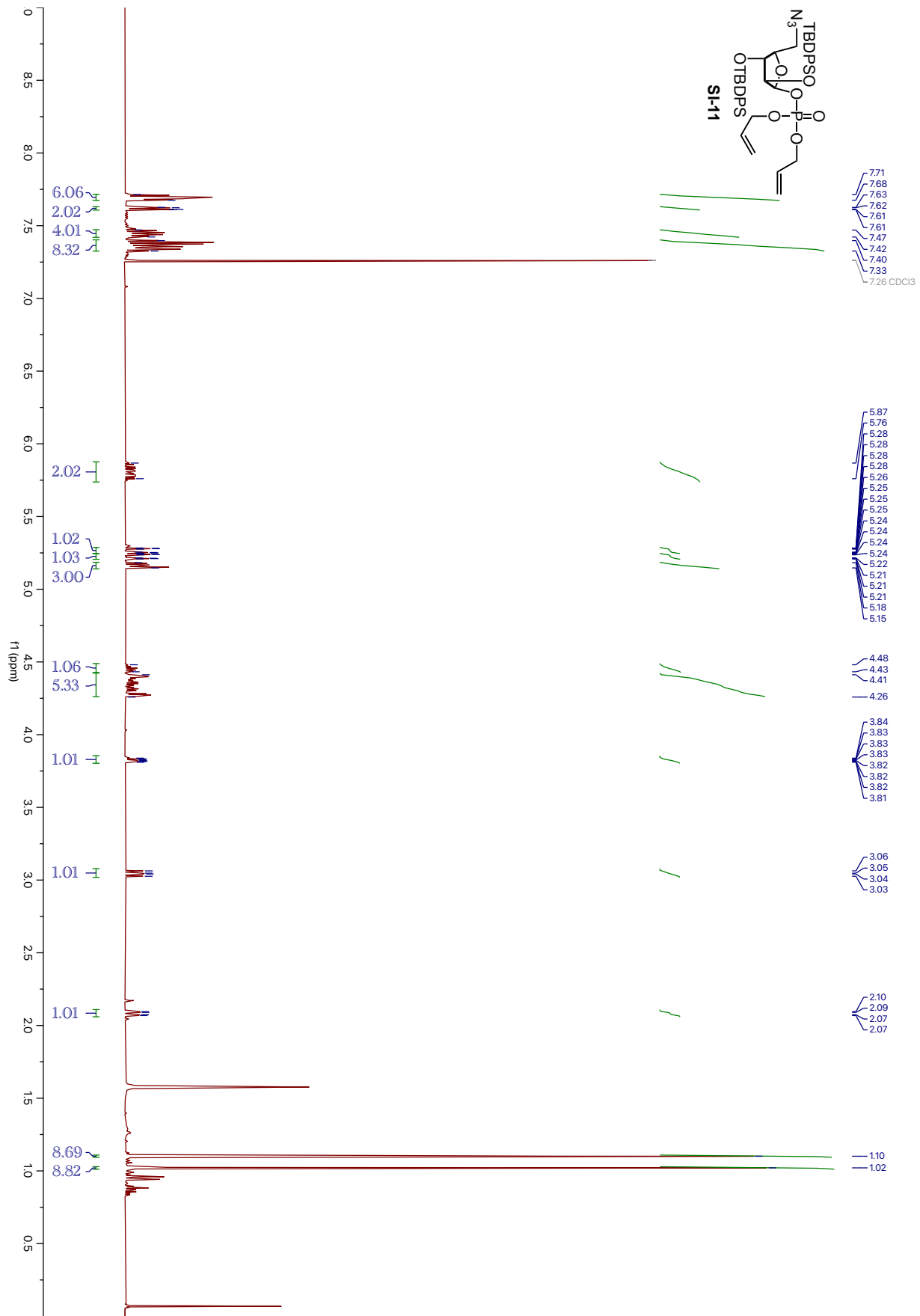
After stirring for 10 hours, the reaction was quenched by pouring into sat. aq. NH₄Cl (150 mL). The layers were separated and the aqueous layer was extracted with Et₂O (3 × 1500 mL). The combined organic layers were washed with brine (300 mL), dried over anhydrous Na₂SO₄, filtered, and concentrated under reduced pressure by rotary evaporation to provide a crude oil. Purification by flash column chromatography on silica gel (EtOAc/hexanes = 0/10 to 1/2) afforded **SI-11** (798 mg, 60% over 2 steps) as a colorless oil.

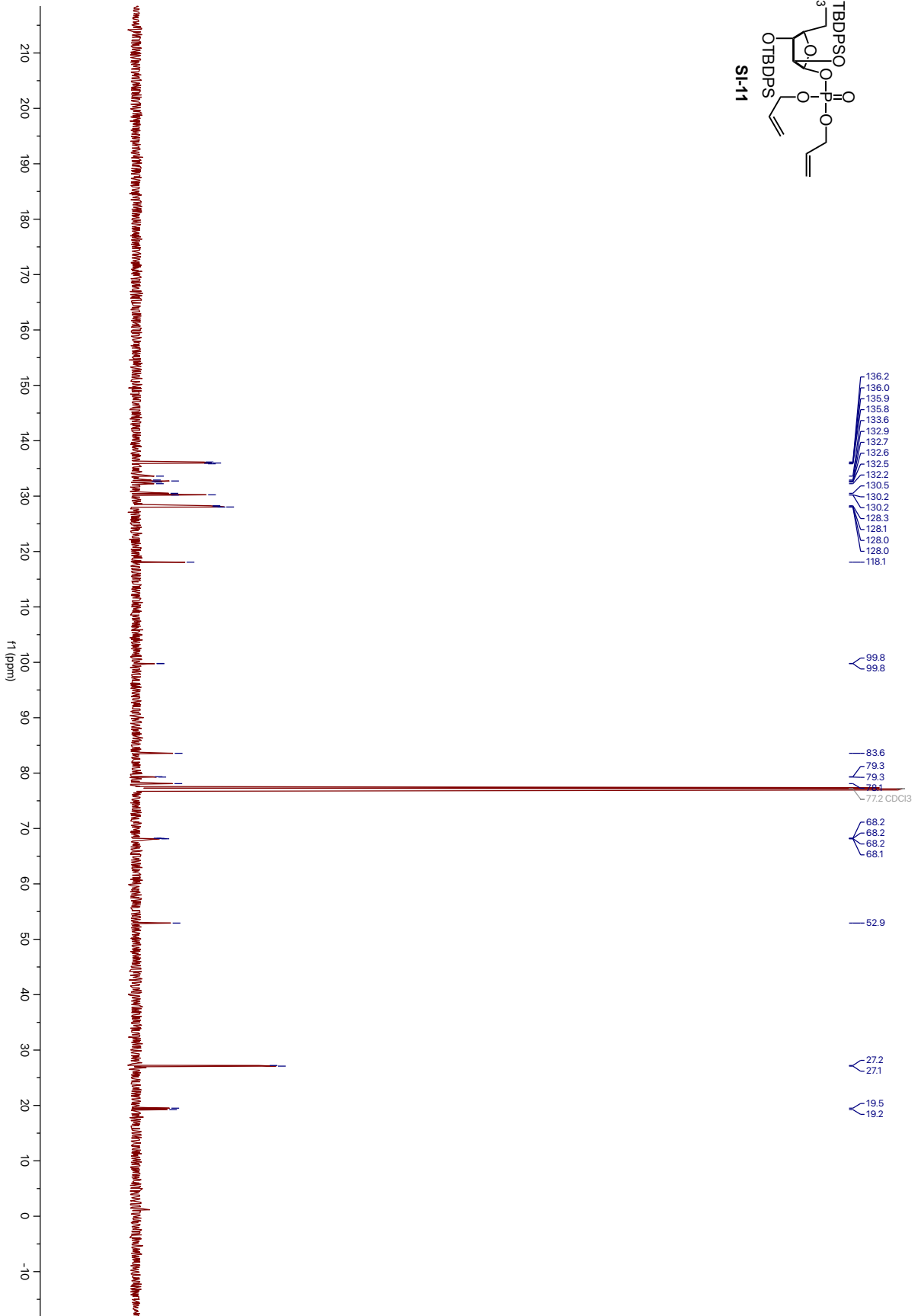
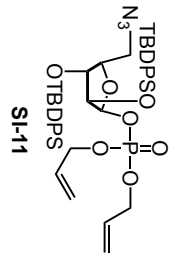
¹H NMR (600 MHz, CDCl₃) δ 7.71 – 7.68 (m, 6H), 7.61 (dd, *J* = 8.1, 1.3 Hz, 2H), 7.47 – 7.42 (m, 4H), 7.40 – 7.33 (m, 8H), 5.87 – 5.78 (m, 2H), 5.27 (dq, *J* = 17.4, 1.4 Hz, 1H), 5.23 (dq, *J* = 17.2, 1.7 Hz, 1H), 5.18 – 5.15 (m, 3H), 4.46 – 4.43 (m, 1H), 4.41 – 4.26 (m, 5H), 3.83 (qd, *J* = 4.5, 3.2 Hz, 1H), 3.04 (dd, *J* = 12.8, 9.5 Hz, 1H), 2.08 (dd, *J* = 12.7, 3.7 Hz, 1H), 1.10 (s, 9H), 1.01 (s, 9H).

¹³C NMR (151 MHz, CDCl₃) δ 136.2, 136.0, 135.9, 135.8, 133.6, 132.9, 132.7, 132.6, 132.5, 132.3, 130.5, 130.2, 130.2, 128.3, 128.1, 128.0, 128.0, 118.1, 99.8, 99.8, 83.6, 79.3, 79.2, 79.1, 68.2, 68.2, 68.2, 68.1, 52.9, 27.2, 27.1, 19.5, 19.3

IR (cm⁻¹): 3072, 3046, 2959, 2929, 2889, 2853, 2094, 1468, 1425, 1276, 1164, 1100, 1021

HRMS (DART): calc. for C₄₃H₅₄N₃O₇PSi₂⁺ (M – C₁₆H₁₉Si + CH₃OH + H): 605.2317, found 605.2350.





1-*O*-(*Z,Z*)-farnesylphosphoryl-5-azido-5-deoxy- β -D-arabinofuranose, 5-AzFPA (**3**).

A flame-dried, 5 mL microwave vial equipped with a magnetic stir bar and was charged with **SI-11** (300 mg, 0.37 mmol, 1.0 equiv), sealed with a rubber septum, evacuated and backfilled with nitrogen three times, and placed under an argon atmosphere. **SI-11** was dissolved in anhydrous CH₂Cl₂ (1.4 mL, 0.4 M) and Pd(PPh₃)₄ (4 mg, 1 mol%) was added to the reaction vial in a single portion. Following this addition phenylsilane (90 μ l, 0.74 mmol, 2.0 equiv) was added to the reaction vial neat, in a dropwise fashion over the course of 2 minutes.

After stirring for 3 hours, the contents of the reaction vial were concentrated *in vacuo*. The reaction vial was fitted with an aluminum–PTFE crimp cap and dried on hi-vac for 1 hour to remove trace solvent. Following this, the reaction vial was evacuated and backfilled with nitrogen three times, and placed under an argon atmosphere. The crude arabinofuranosyl phosphate was dissolved in anhydrous pyridine (740 μ l, 0.5 M). (*Z,Z*)-farnesol (247 mg, 1.10 mmol, 3.0 equiv) and trichloroacetonitrile (370 μ l, 3.70 mmol, 10.0 equiv) were added to the reaction vial neat, in single portions. The resulting solution was heated to 40 °C by transferring the reaction vial to an oil bath.

After stirring at this temperature for 12 hours, the reaction vial was removed from the oil bath and concentrated by rotary evaporation to yield a brown oil. The reaction vial was fitted with a rubber septum and the crude farnesyl phosphate was further concentrated on hi-vac to remove traces of pyridine and trichloroacetonitrile. The resulting brown gum was dissolved in equal parts anhydrous methanol (550 μ l, 1.0 M) and 7 N ammonia solution in methanol (550 μ l, 1.0 M). Ammonium fluoride (137 mg, 3.70 mmol, 10.0 equiv) was added to the reaction vial in a single portion and the vial was fitted with an aluminum–PTFE crimp cap. The resulting solution was heated to 55 °C by transferring the reaction vial to an oil bath.

After stirring at this temperature for 12 hours, the reaction vial was removed from the oil bath and concentrated by rotary evaporation to yield a brown gum. This crude product was purified directly by flash column chromatography on silica gel (CH₂Cl₂ w/ 0.5% Et₃N/MeOH = 0/1 to 1/5). Filtration of the dissolved product through a 0.2 micron PTFE syringe filter afforded **3** as a pale brown oil. This oil was taken up in 1 ml of Milli-Q water, flash frozen and subjected to lyophilization to yield **3** (31 mg, 18% over 3 steps) as a pale yellow solid.

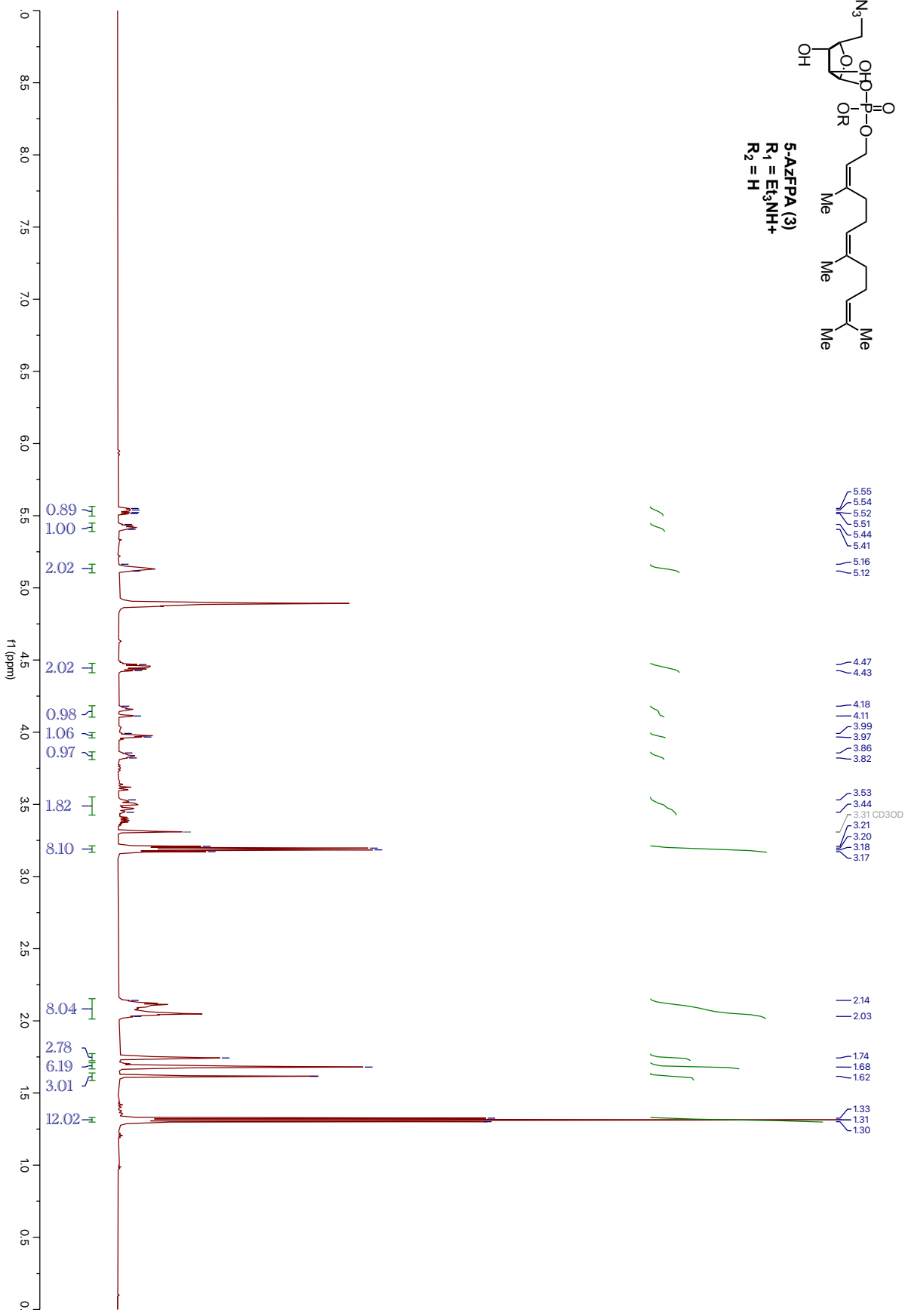
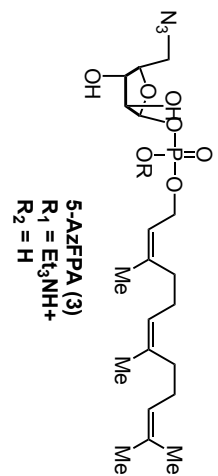
Isolated as triethylammonium adduct with minor proteo- form

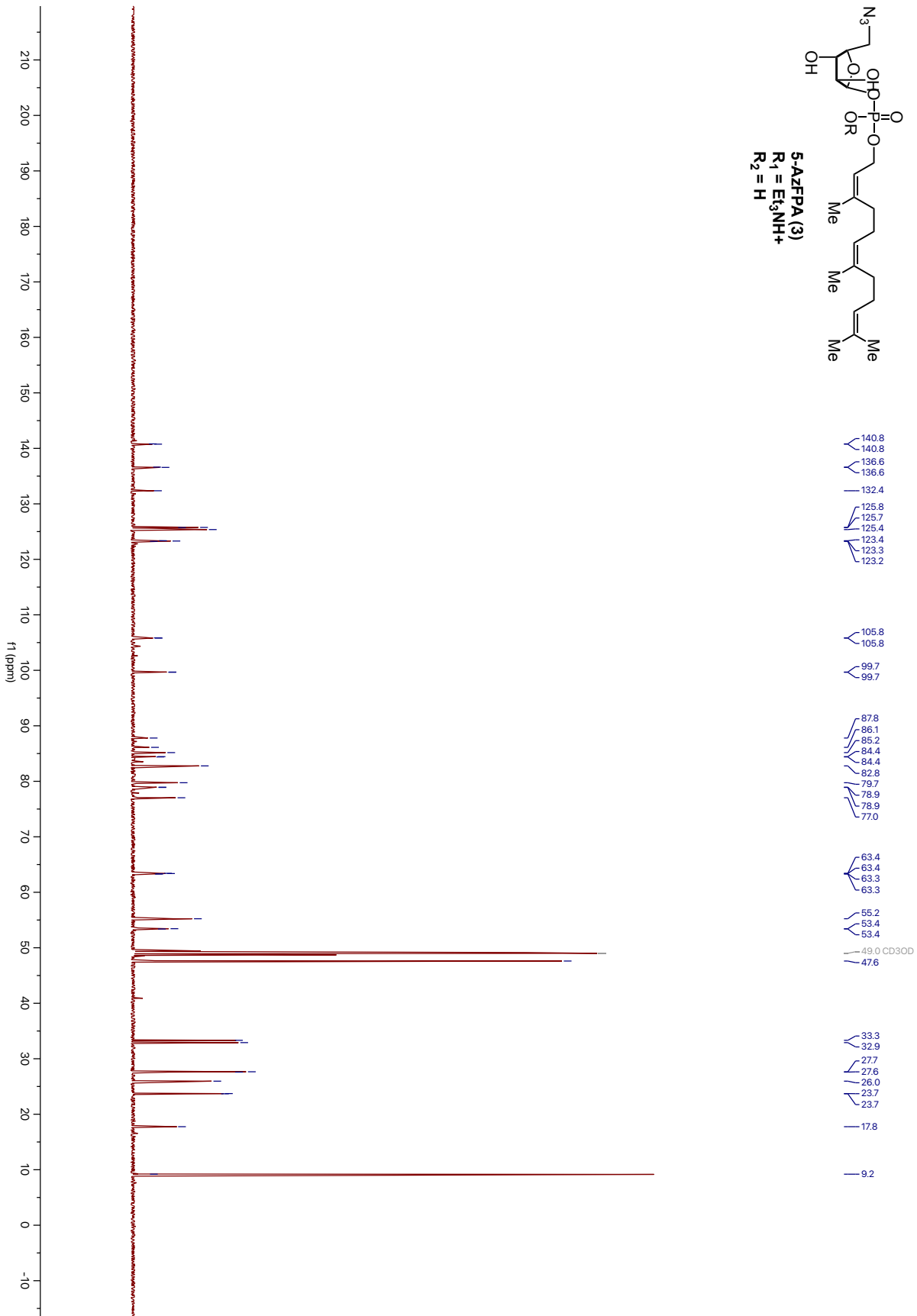
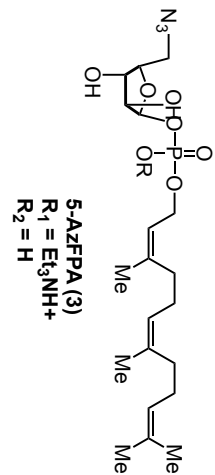
¹H NMR (600 MHz, CD₃OD) δ 5.53 (dd, *J* = 15.9, 5.2 Hz, 1H), 5.44 – 5.41 (m, 1H), 5.16 – 5.12 (m, 2H), 4.47 – 4.43 (m, 2H), 4.18 – 4.11 (m, 1H), 3.99 – 3.97 (m, 1H), 3.86 – 3.82 (m, 1H), 3.53 – 3.44 (m, 2H), 2.14 – 2.03 (m, 8H), 1.74 (s, 3H), 1.68 (s, 6H), 1.62 (s, 3H)
triethyl ammonium adduct: 3.19 (q, *J* = 7.4 Hz, 8H), 1.31 (t, *J* = 7.4 Hz, 12H)

¹³C NMR (151 MHz, CD₃OD) δ 140.1 (d), 140.8 (d), 132.4, 125.8 (d), 125.7 (d), 125.4, 123.4 (t), 123.3 (t), 123.2 (t), 105.8 (d), 105.8 (d), 99.7 (d), 99.7 (d), 87.8, 86.1, 85.2, 84.4 (d), 84.4 (d), 82.8, 79.7, 78.9 (d), 78.9 (d), 77.0, 63.4 (q), 63.4 (q), 63.3 (q), 63.3 (q), 55.2, 53.4 (d), 53.4 (d), 33.3, 32.9, 27.7 (d), 27.6 (d), 26.0, 23.7 (d), 23.7 (d), 17.8
triethyl ammonium adduct: 47.6, 9.2

IR (cm⁻¹): 3234, 2963, 2920, 2847, 2091, 1670, 1451, 1372, 1276, 1213, 1137, 1154, 1054, 1021

HRMS (ESI): calc. for C₂₀H₃₃N₃O₇P⁻ (M⁻) 458.2061, found 458.2037.





Supplemental References

1. Calabretta, P. J.; Hodges, H. L.; Kraft, M. B.; Marando, V. M.; Kiessling, L. L., Bacterial Cell Wall Modification with a Glycolipid Substrate. *J Am Chem Soc* **2019**, *141* (23), 9262-9272.
2. Besra, G. S.; Khoo, K.; McNeil, M. R.; Dell, A.; Morris, H. R.; Brennan, P. J., A New Interpretation of the Structure of the Mycolyl-Arabinogalactan Complex of *Mycobacterium tuberculosis* As Revealed through Characterization of Oligoglycosylalditol Fragments by Fast-Atom Bombardment Mass Spectrometry and ¹H Nuclear Magnetic Resonance Spectroscopy. *Biochemistry* **1995**, *34*, 4257-4266.
3. Cociorva, O. M.; Gurcha, S. S.; Besra, G. S.; Lowary, T. L., Oligosaccharides as inhibitors of mycobacterial arabinosyltransferases. Di- and trisaccharides containing C-3 modified arabinofuranosyl residues. *Bioorg Med Chem* **2005**, *13* (4), 1369-79.
4. Hanessian, S., Neighboring-Group Participation across a Furanose Ring. Synthesis of 5-Acetamido-5-deoxy-D-lyxopyranose from D-Arabinose Precursors. *J. Org. Chem.* **1963**, *28*, 442.