Israel Journal of Chemistry

Supporting Information

A Fluorescent Unnatural Mannosamine Derivative with Enhanced Emission Upon Complexation with Cucurbit[7]uril

Anna Kataki-Anastasakou, Shang Jia, Jonathan C. Axtell, and Ellen M. Sletten*

Supporting Information

for

A fluorescent unnatural mannosamine derivative with enhanced emission upon complexation with cucurbit[7]uril

Anna Kataki-Anastasakou[1], Shang Jia[1], Jonathan C. Axtell[1], Ellen M. Sletten[1,*]

[1] Department of Chemistry and Biochemistry, University of California Los Angeles, 607 Charles E. Young Drive East, Los Angeles, California 90095, USA.

[*]Corresponding author: sletten@chem.ucla.edu

Table of Contents

Page #

1. Supporting Figures……..……. S3

2. General Experimental Procedures…….…….. S21

3. Figure Experimental Procedures…….…… S23

4. Synthetic Procedures……...…. S24

5. ^{1}H NMR Spectra ……..…… S27

6. ^{13}C NMR Spectra …….……. S34

7. References……..…….……S38

Supporting Figures

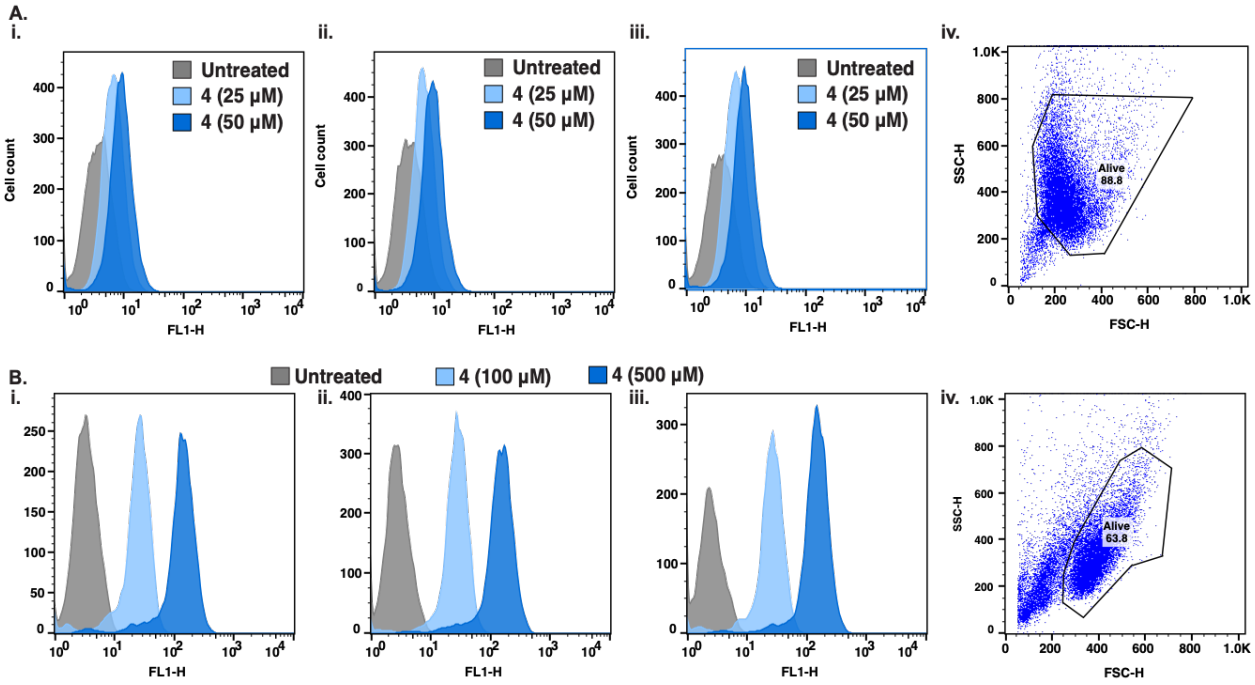


Figure S1: (**A**) Jurkat cells grown in the presence or absence of **4** at 37 ºC, 5% CO_2 for three days. (**i-iii**) Histograms of triplicate experiments exhibiting **4** (25-50 µM) fluorescence and (**iv**) Jurkat cell population used for analysis. (**B**) (**i-iii**) Histograms of triplicate experiments exhibiting **4** (100-500 µM) fluorescence and (**iv**) Jurkat cell population used for analysis.

Jurkat cells were grown in RPMI media containing **4** (0, 25, 50 µM) (**A**) or **4** (0, 100, 500 µM) (**B**) for 3 days at 37 ºC, 5% CO_2. Cells from culture were washed with PBS (10 mL) by centrifugation (526 x *g*, 3 min, 4 ˚C) thrice and plated on a 96-well plate (400,000 cells/well) in 1% FACS. Cells were incubated in 1% FACS for 1 h and washed with 1% FACS (200 µL/well) by centrifugation (526 x *g*, 5 min, 4 ˚C) thrice and transferred to FACS tubes with a final volume of 400 µL 1% FACS. **4** fluorescence was measured on the FL1, FL2, FL3 channels of a FACSCalibur flow cytometer with 15,000 cells collected per sample. Error bars represent the standard deviation of three replicate experiments. Flow cytometry data were processed using FlowJo™ v10 software.

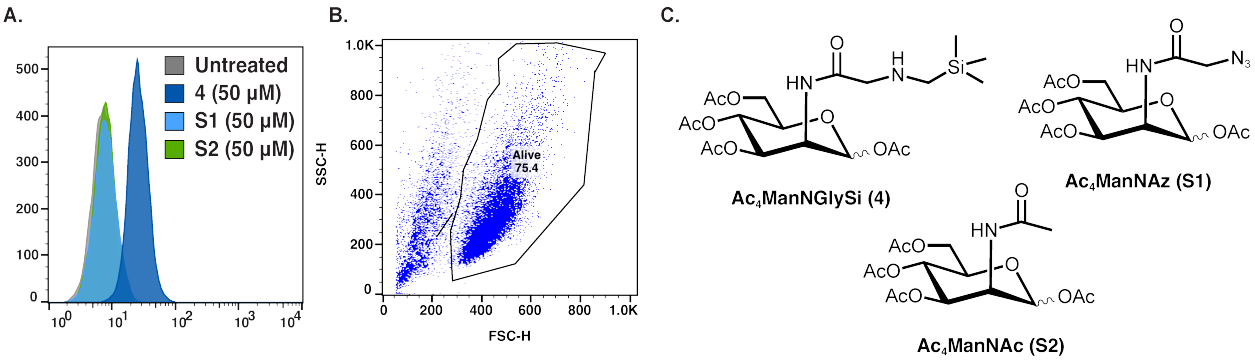


Figure S2: (**A**) Single replicate of Jurkat cells incubated with peracetylated mannosamine compounds (**4, S1, S2**) used in a comparison experiment of intrinsic fluorescent properties after incubation at 37 ºC, 5% CO_2 for three days. (**B**) Jurkat cell population used for analysis and (**C**) Peracetylated mannosamine compounds (**4, S1, S2**) used in the comparison experiment.

Jurkat cells were grown in RPMI media containing **4** (50, 100 or 250 µM), **S1** (50, 100 or 250 µM), or **S2** (50, 100 or 250 µM) for 3 days at 37 ºC, 5% CO_2. Cells from culture were washed with PBS (10 mL) by centrifugation (526 x *g*, 3 min, 4 ˚C) thrice and transferred to FACS tubes (400,000 cells/tube) with a final volume of 400 µL in 1% FACS. **4** fluorescence was measured on the FL1, FL2, FL3 channels of a FACSCalibur flow cytometer with 15,000 cells collected per sample. Flow cytometry data were processed using FlowJo™ v10 software. Note: Cells incubated with ≥100 µM of **S1** and **S2** did not survive the 3-day incubation and analysis was not possible.

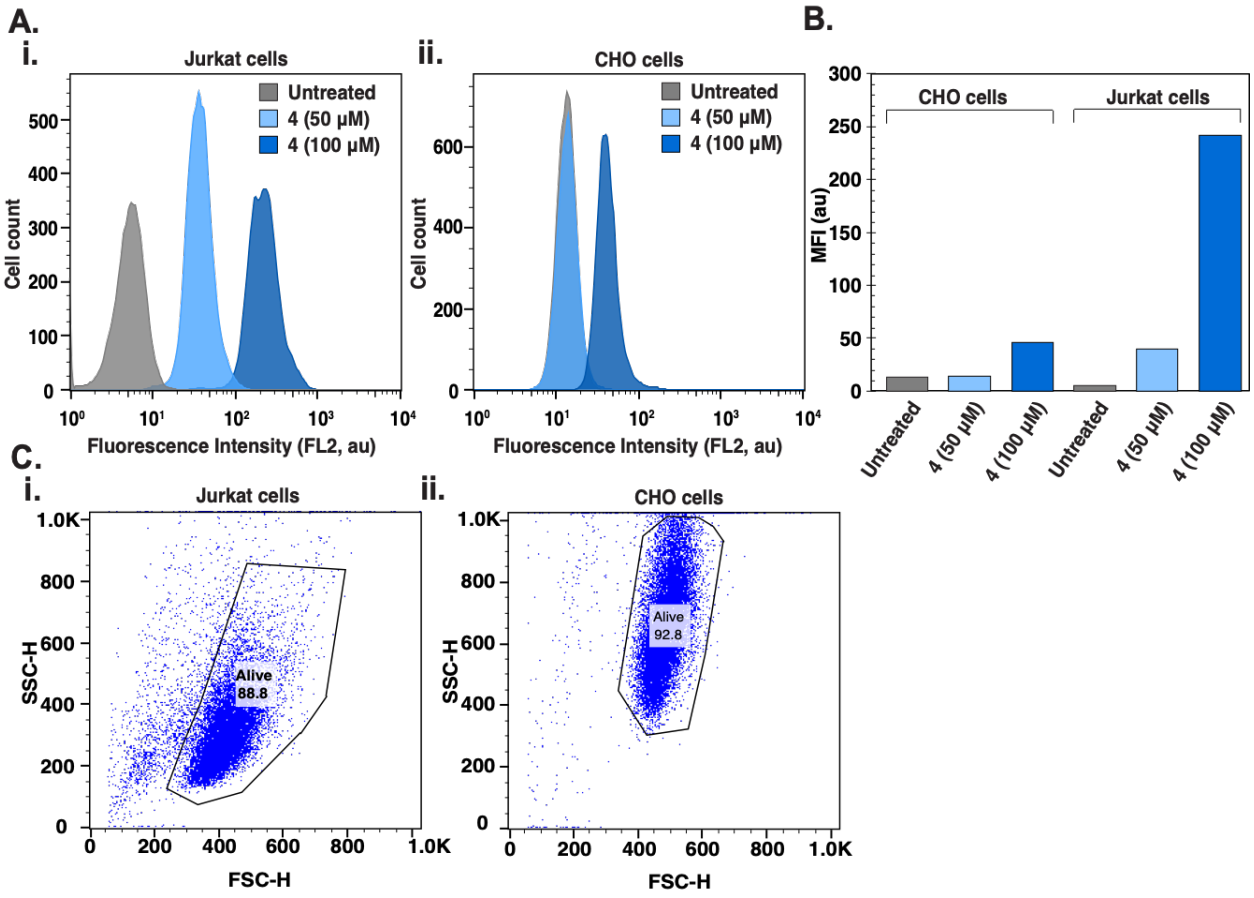


Figure S3: (**A**) Histograms and (**B**) bar graph representing fluorescence from a single replicate of Jurkat (**i**) or CHO (**ii**) cells treated with **4** (0-50 µM) at 37 ºC, 5% CO_2 for three days. (**C**) Analysis of fluorescence intensity from Jurkat cells grown in the presence of **4** (50 or 100 µM) at 37 ºC, 5% CO_2 for 1-48 h. **(C)** Gate of Jurkat (**i**) and CHO (**ii**) cell population chosen for analysis based on FSC/SSC.

Jurkat or CHO cells were grown in RPMI or FK-12 media, respectively, containing **4** (0, 50, 100 µM), respectively, for 3 days at 37 ºC, 5% CO_2. Cells from culture were washed with PBS (10 mL) by centrifugation (526 x *g*, 3 min, 4 ˚C) thrice and transferred to FACS tubes (400,000 cells/tube) with a final volume of 400 µL in 1% FACS. **4** fluorescence was measured on the FL1, FL2, FL3 channels of a FACSCalibur flow cytometer with 15,000 cells collected per sample. Flow cytometry data were processed using FlowJo™ v10 software.

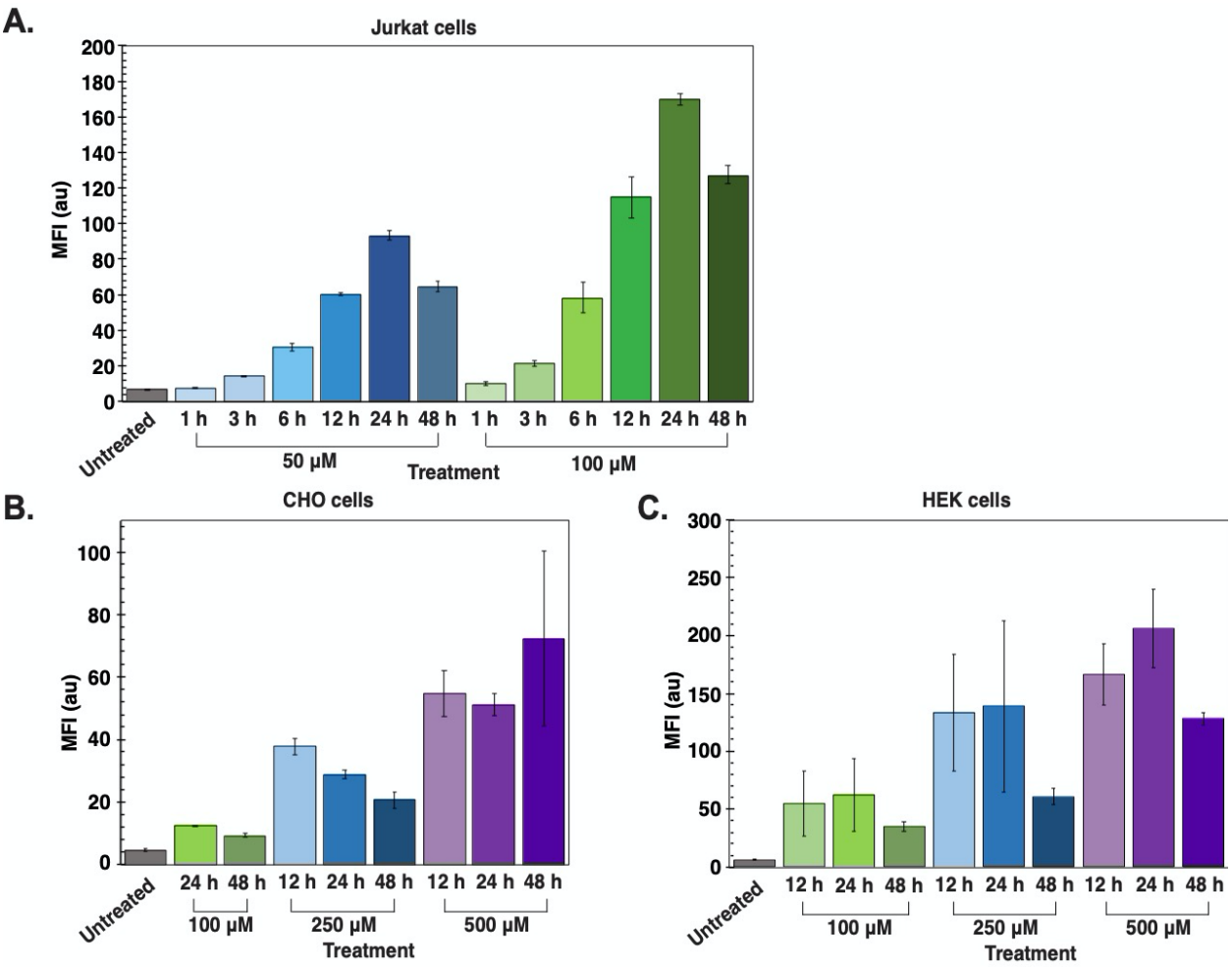


Figure S4: (**A**) Analysis of fluorescence intensity from Jurkat cells grown in the presence of **4** (50 or 100 µM) at 37 ºC, 5% CO_2 for 1-48 h. (**B**) Analysis of fluorescence intensity from CHO cells grown in the presence of **4** (50 or 100 µM) at 37 ºC, 5% CO_2 for 1-48 h. (**C**) Analysis of fluorescence intensity from HEK cells grown in the presence of **4** (50 or 100 µM) at 37 ºC, 5% CO_2 for 1-48 h. Error bars represent the standard deviation of triplicate experiments.

Jurkat cells from culture were washed with PBS (10 mL) by centrifugation (526 x *g*, 3 min, 4 ˚C) thrice and transferred to a flat-bottom 96-well plate (100,000 cells per well). Cells were resuspended in RPMI media and **4** (50 or 100 µM) was added to respective wells at 1-48 h timepoints. Cells were transferred to a V-bottom 96-well plate and washed with 1% FACS (200 µL/well) by centrifugation (526 x *g*, 5 min, 4 ˚C) thrice and transferred to FACS tubes with a final volume of 400 µL 1% FACS. **4** fluorescence was measured on the FL1, FL2, FL3 channels of a FACSCalibur flow cytometer with 15,000 cells collected per sample. Error bars represent the standard deviation of three replicate experiments. Flow cytometry data were processed using FlowJo™ v10 software.

HEK or CHO cells from culture were washed with PBS (10 mL) by centrifugation (526 x *g*, 3 min, 4 ˚C) thrice and transferred to a flat-bottom 96-well plate (100,000 cells per well). Cells were resuspended in RPMI media and **4** (100, 250 or 500 µM) was added to respective wells at 12-48 h

timepoints. Cells were transferred to a V-bottom 96-well plate and washed with 1% FACS (200 μL/well) by centrifugation (526 x *g*, 5 min, 4 ˚C) thrice and transferred to FACS tubes with a final volume of 400 μL 1% FACS. **4** fluorescence was measured on the FL1, FL2, FL3 channels of a FACSCalibur flow cytometer with 15,000 cells collected per sample. Error bars represent the standard deviation of three replicate experiments. Flow cytometry data were processed using FlowJo™ v10 software.

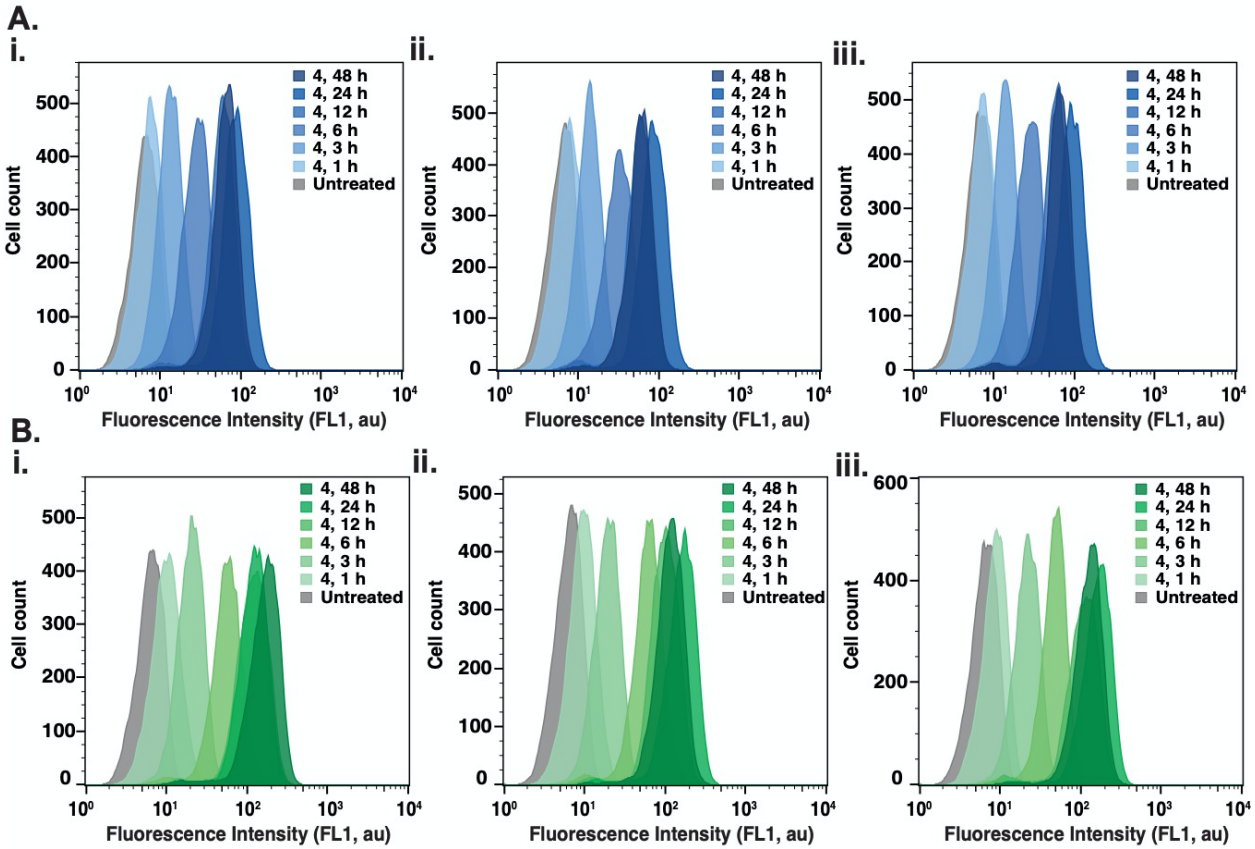


Figure S5: (A) Histograms of triplicate experiments (**i-iii**) of Jurkat cells treated with **4** (50 μM) from 1-48 h. (**B**) Histograms of triplicate experiments (**i-iii**) of Jurkat cells treated with **4** (100 μM) from 1-48 h. See Figure S4 for details on experimental procedure.

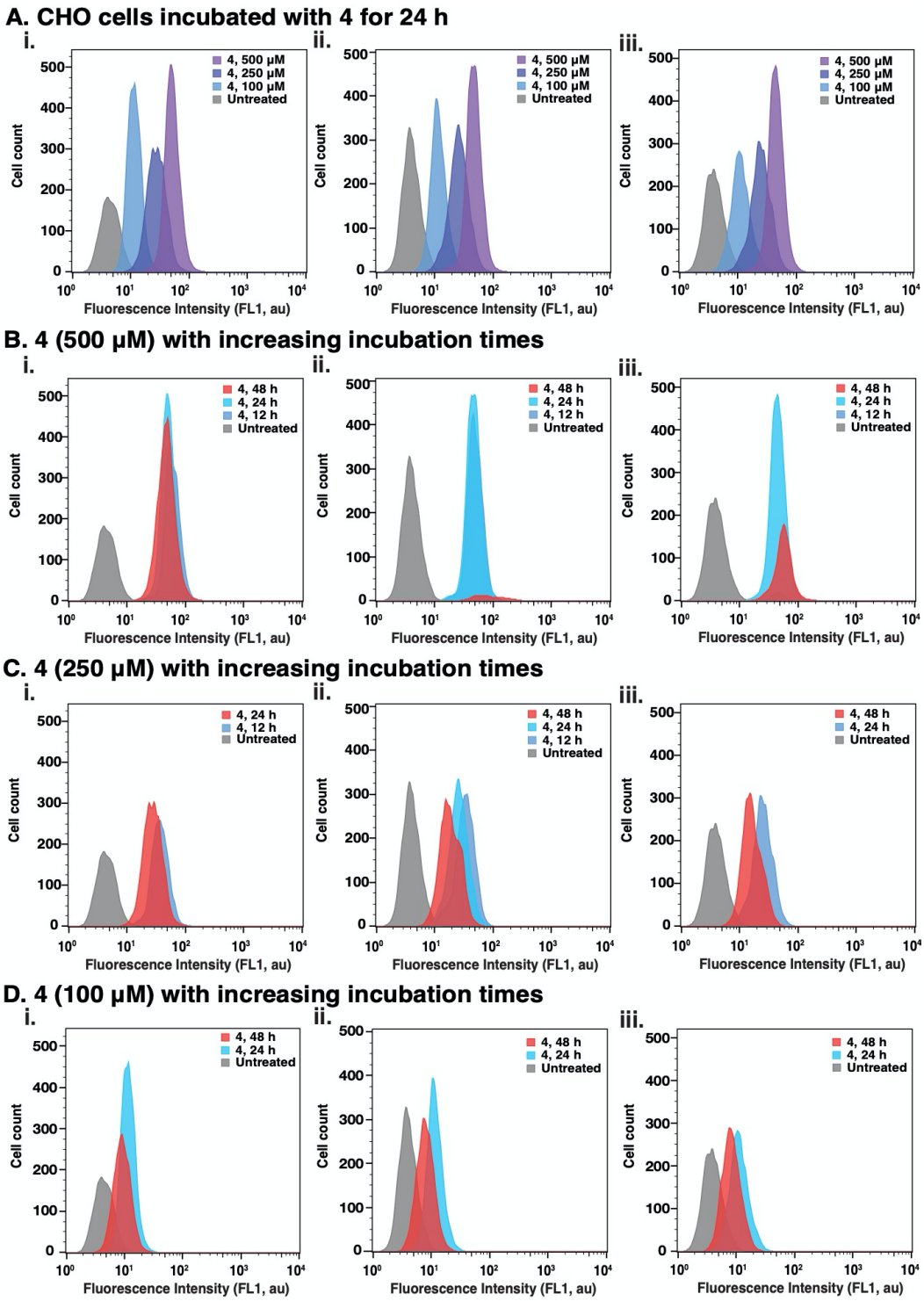


Figure S6: (**A**) Histograms of triplicate (**i-iii**) experiments showing **4** (100-500 µM) fluorescence after 24 h incubation. (**B**) Histograms of triplicate (**i-iii**) experiments showing **4** (500 µM) fluorescence after 12-48 h incubation. (**C**) Histograms of triplicate (**i-iii**) experiments showing **4** (250 µM) fluorescence after 12-48 h incubation. (**D**) Histograms of triplicate (**i-iii**) experiments showing **4** (100 µM) fluorescence after 12-48 h incubation. See Figure S4 for experimental procedures.

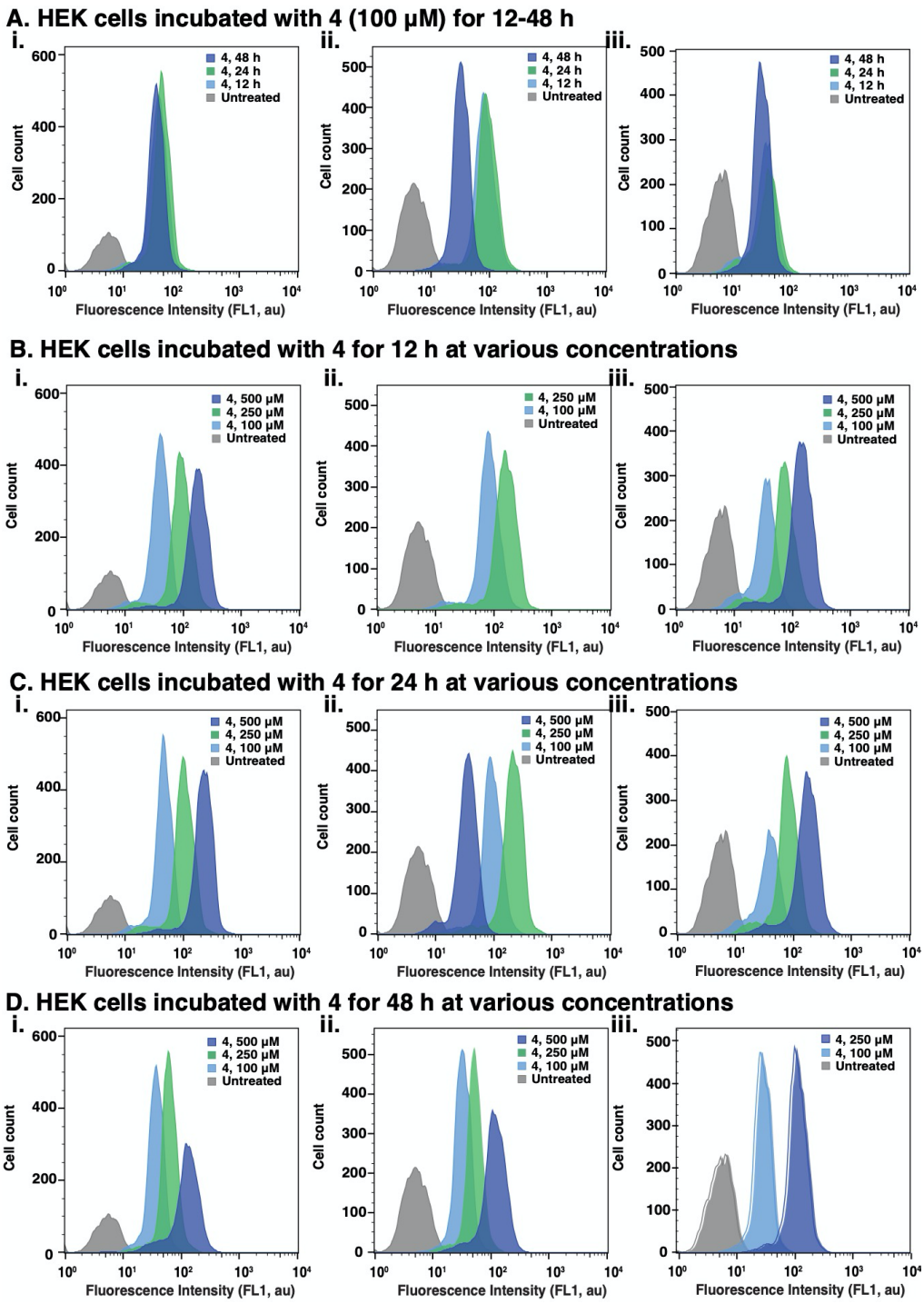


Figure S7: (**A**) Histograms of triplicate (**i-iii**) experiments showing **4** (100 µM) fluorescence after 12-48 h incubation. (**B**) Histograms of triplicate (**i-iii**) experiments showing **4** (100-500 µM) fluorescence after 12 h incubation. (**C**) Histograms of triplicate (**i-iii**) experiments showing **4** (100-500 µM) fluorescence after 24 h incubation. (**D**) Histograms of triplicate (**i-iii**) experiments showing **4** (100-500 µM) fluorescence after 48 h incubation. See Figure S4 for experimental procedures.

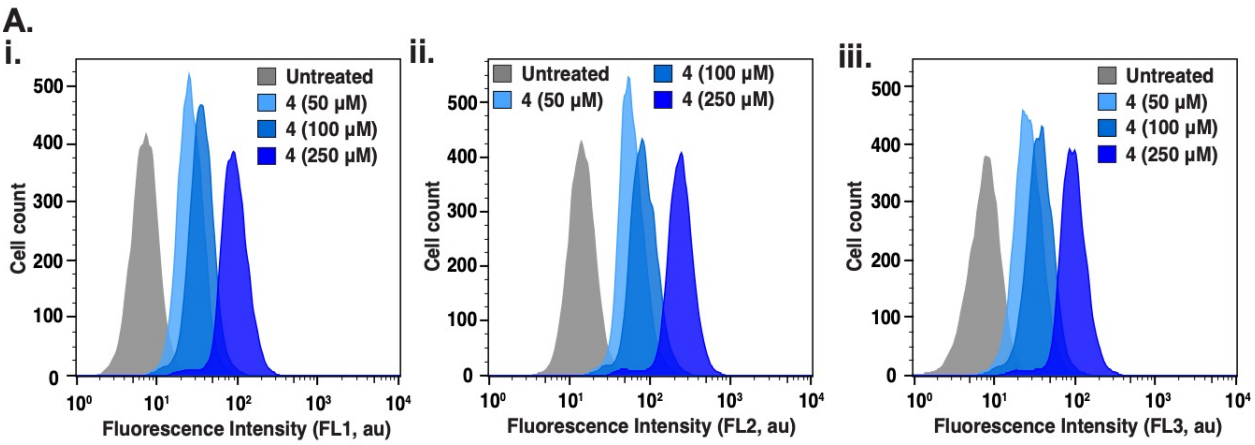


Figure S8: (A) Histograms exhibiting **4** (50-250 µM) fluorescence in FL1 (**i**), FL2 (**ii**) and FL3 (**iii**) channels after incubation at 37 ºC, 5% CO_2 for three days. Error bars represent the standard deviation of triplicate experiments.

Jurkat cells were grown in RPMI media containing **4** (0, 50, 100, 250 µM) for 3 days at 37 ºC, 5% CO_2. Cells from culture were washed with PBS (10 mL) by centrifugation (526 x *g*, 3 min, 4 ˚C) thrice and transferred to FACS tubes (400,000 cells/tube) with a final volume of 400 µL in 1% FACS. **4** fluorescence was measured on the FL1, FL2, FL3 channels of a FACSCalibur flow cytometer with 15,000 cells collected per sample. Flow cytometry data were processed using FlowJo™ v10 software.

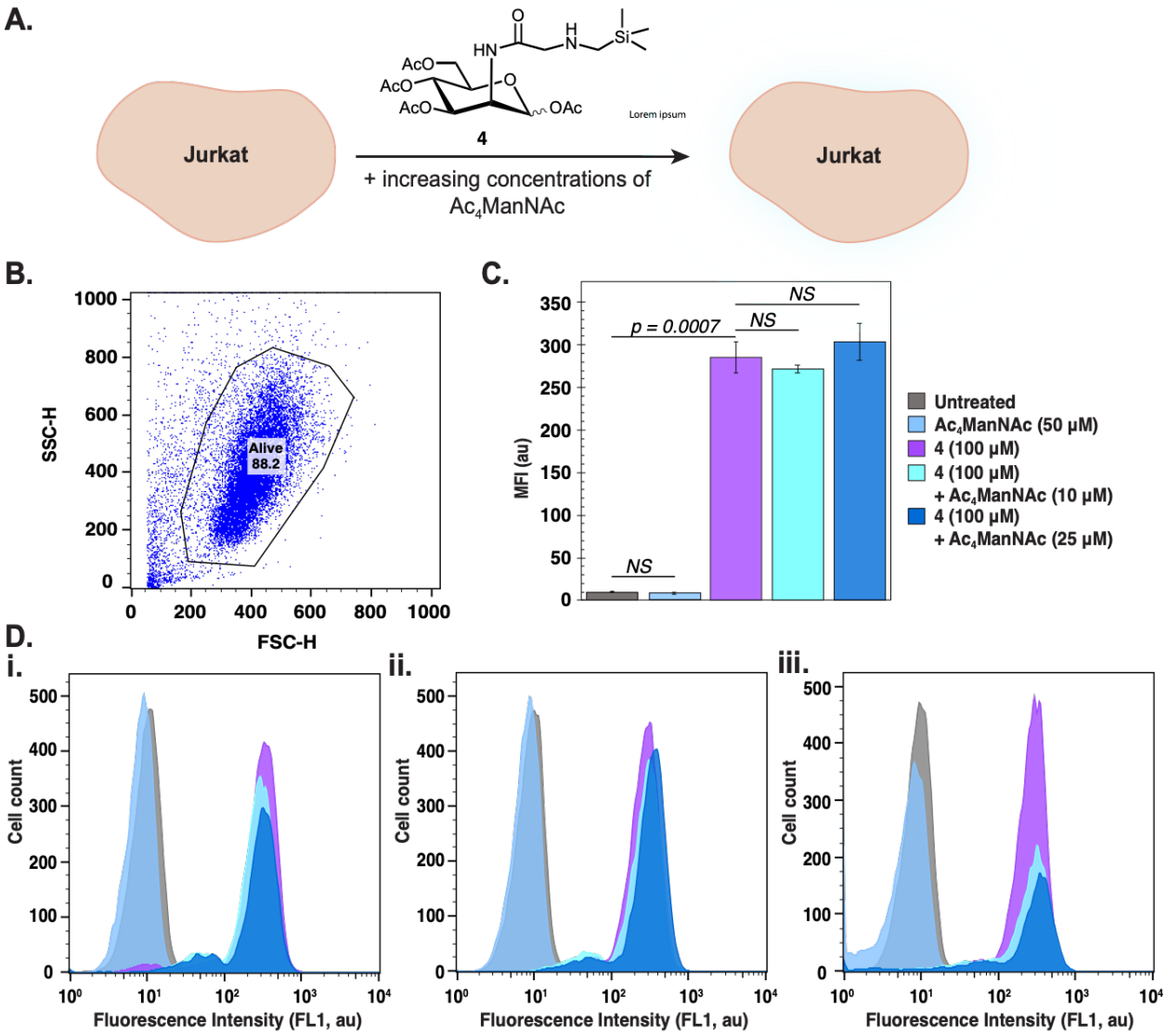


Figure S9: (**A**) Scheme representing the competition experiment of Jurkat cells incubated with **4** (100 µM) in the presence of increasing concentrations of **Ac$_4$ManNAc** (0-25 µM) at 37 ºC, 5% CO_2 for three days. (**B**) Dot plot representing the gated cell population used for analysis. (**C**) Analysis of **4** fluorescence in the presence or absence of **Ac$_4$ManNAc**. (**D**) (**i-iii**) Histograms for the triplicate experiments. Error bars represent the standard deviation of triplicate experiments.

Jurkat cells were grown in RPMI media containing **4** (0, 100, 250 µM) and/or Ac$_4$ManNAc (10, 25, 50 µM) for 3 days at 37 ºC, 5% CO_2. Cells from culture were washed with PBS (10 mL) by centrifugation (526 x *g*, 3 min, 4 ˚C) thrice and transferred to FACS tubes (400,000 cells/tube) with a final volume of 400 µL in 1% FACS. **4** fluorescence was measured on the FL1, FL2, FL3 channels of a FACSCalibur flow cytometer with 15,000 cells collected per sample. Flow cytometry data were processed using FlowJo™ v10 software. Cells grown in media containing 250 µM **4** and 50 µM Ac$_4$ManNAc did not survive the incubation and were not used in the analysis.

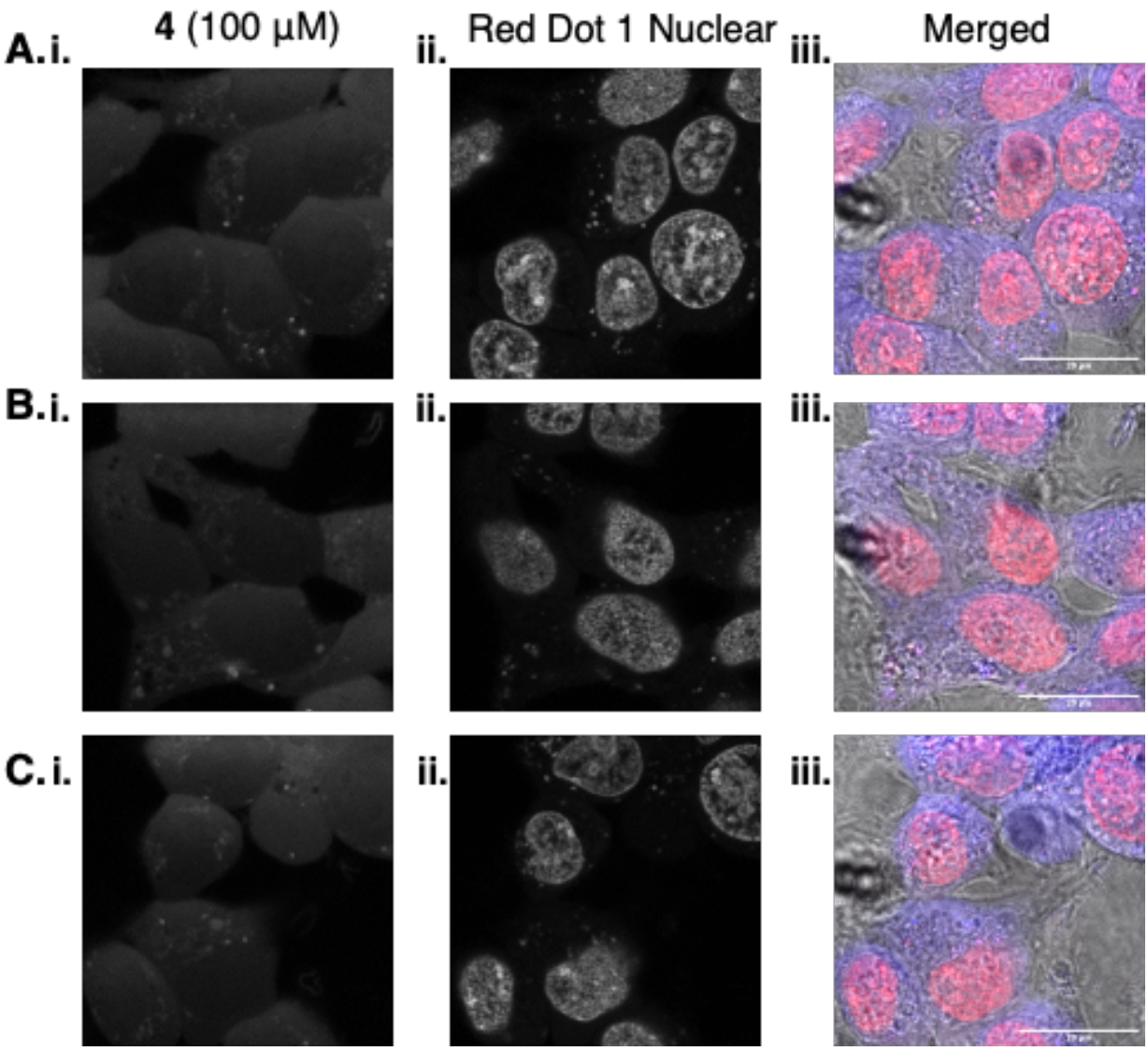


Figure S10: Confocal microscopy of live HEK cells. (**A-C**) Triplicate images of cells incubated with (**i**) **4** (100 µM) for 24 h and imaged using 405 nm laser, and (**ii**) Red Dot 1 Nuclear stain (100µM) using a white laser set at 662 nm. (**iii**) Merge of blue and far red channels to determine colocalization.

Two days prior to imaging, HEK293 cells were passed to an 8-well chamber slide (80826, ibidi) for 40-50% confluency on the day of imaging. The second day, a 100x solution of **4** were added to each well to a final concentration of 100 µM for 24 h incubation. Red Dot 1 Nuclear stain (200X) was added 15 min prior to imaging to a final concentration of 100 µM (1X). Media were then removed and cells were washed with PBS containing 10% FBS. Imaging was performed using a SP8 Leica confocal microscope (100x oil immersion lens); **4** excited with 405 nm diode laser; other dyes excited with a white laser set at the absorption maximum.

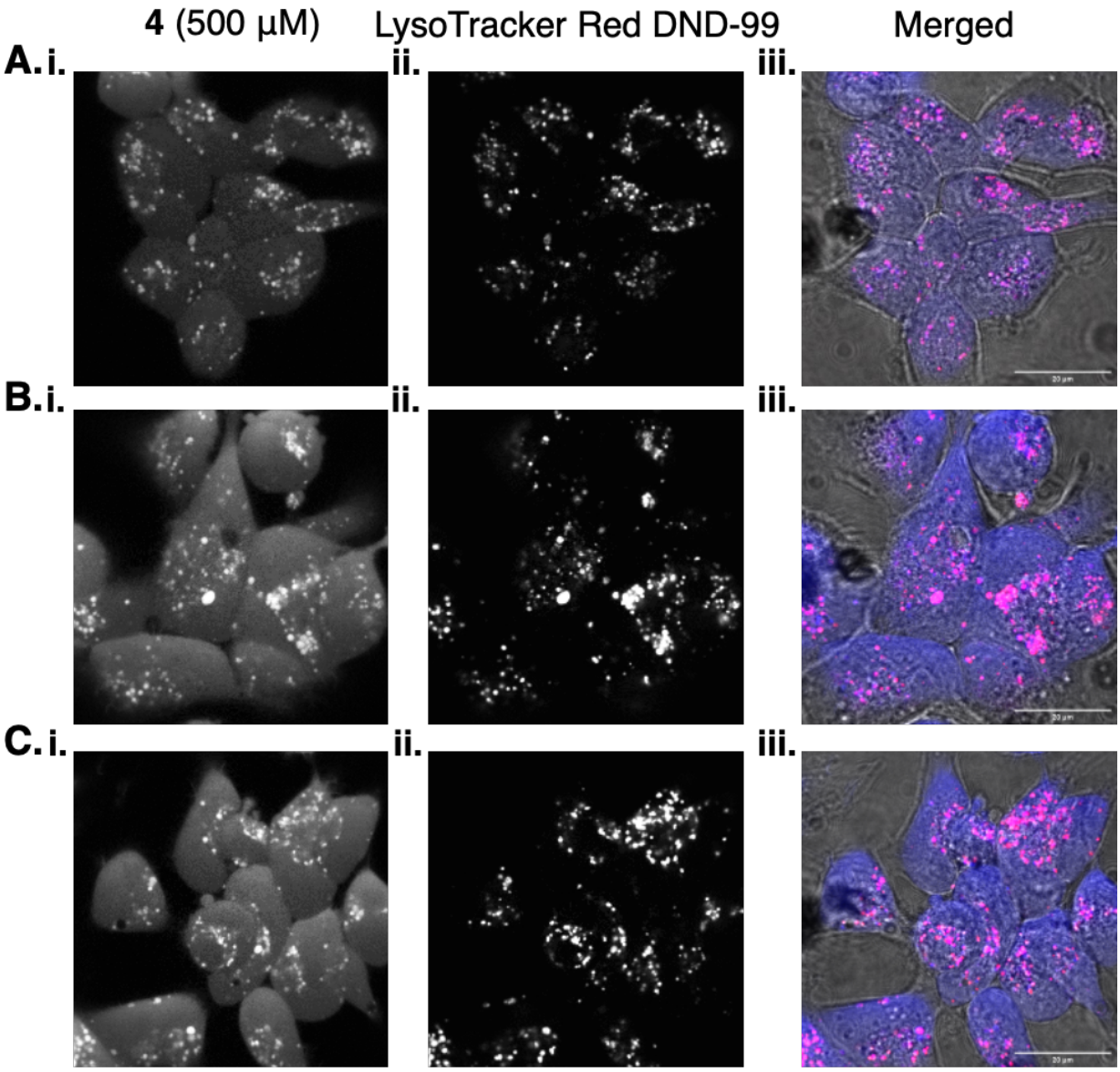


Figure S11: Confocal microscopy of live HEK cells. (**A-C**) Triplicate images of cells incubated with (**i**) **4** (500 µM) for 24 h and imaged using 405 nm laser, and (**ii**) LysoTracker™ Red DND-99 lysosome stain (0.1 µM) using the 577 nm laser. (**iii**) Merge of blue and far red channels to determine colocalization.

Two days prior to imaging, HEK293 cells were passed to an 8-well chamber slide (80826, ibidi) for 40-50% confluency on the day of imaging. The second day, a 100x solution of **4** were added to each well to a final concentration of 500 µM for 24 h incubation. Lysotracker Red ™ DND-99was added 15 min prior to imaging to a final concentration of 0.1 µM. Media were then removed and cells were washed with PBS containing 10% FBS. Imaging was performed using a SP8 Leica confocal microscope (100x oil immersion lens); **4** excited with 405 nm diode laser; other dyes excited with a white laser set at the absorption maximum.

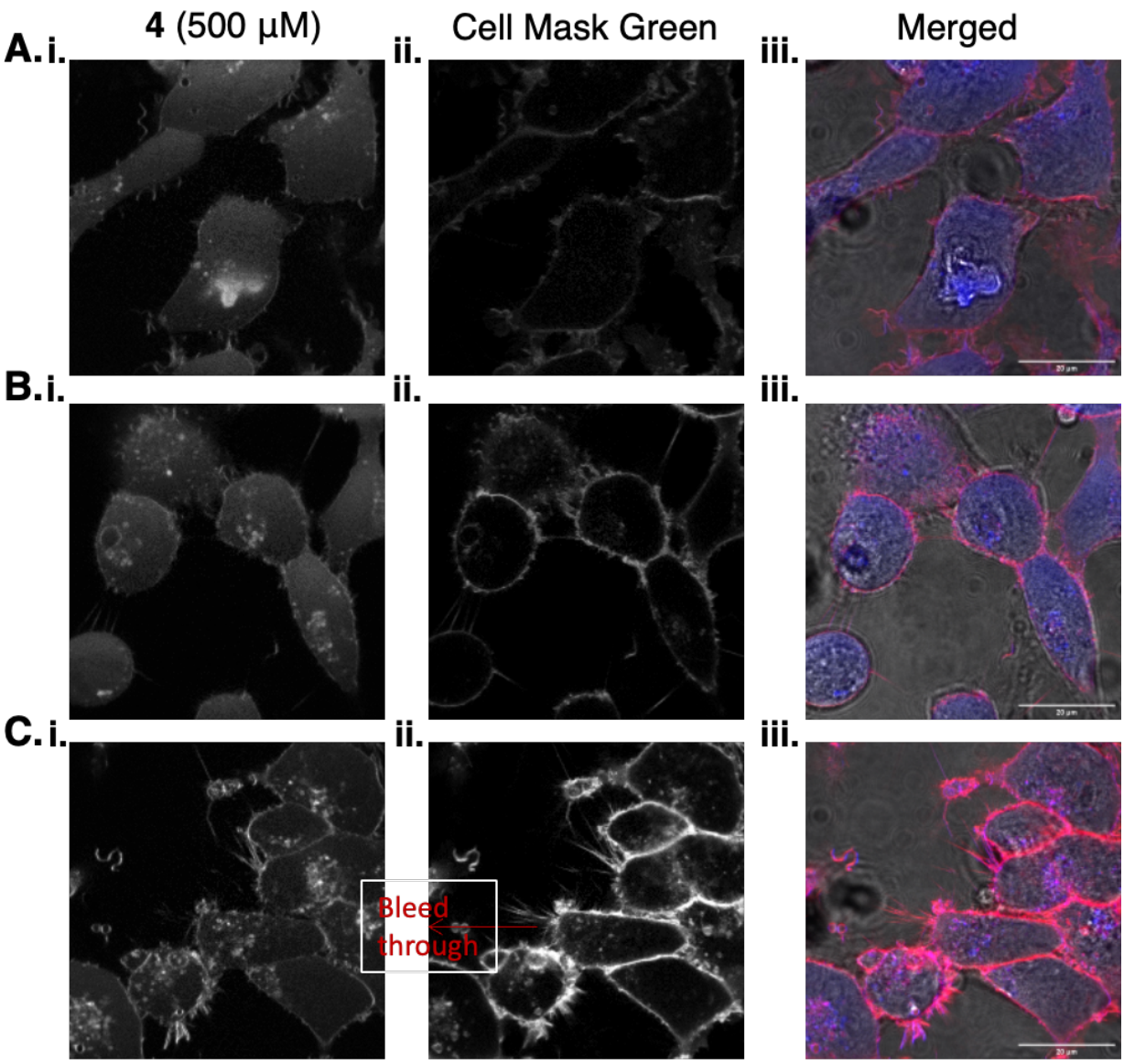


Figure S12: Confocal microscopy of live HEK cells. (**A-C**) Triplicate images of cells incubated with (**i**) **4** (500 µM) for 24 h and imaged using 405 nm laser, and (**ii**) Cell Mask Green stain (1:100 stock dilution) using the 522 nm laser. (**iii**) Merge of 405/535 channels to determine colocalization.

Two days prior to imaging, HEK293 cells were passed to an 8-well chamber slide (80826, ibidi) for 40-50% confluency on the day of imaging. The second day, a 100x solution of **4** were added to each well to a final concentration of 500 µM for 24 h incubation. Cell Mask Green was added 15 min prior to imaging (1:100 dilution). Media were then removed and cells were washed with PBS containing 10% FBS. Imaging was performed using a SP8 Leica confocal microscope (100x oil immersion lens); **4** excited with 405 nm diode laser; other dyes excited with a white laser set at the absorption maximum.

Given the broad but weaker emission profile of **4,** bleed-through from the green channel was unavoidable and can be observed in single channel images, as well as in colocalization plots.

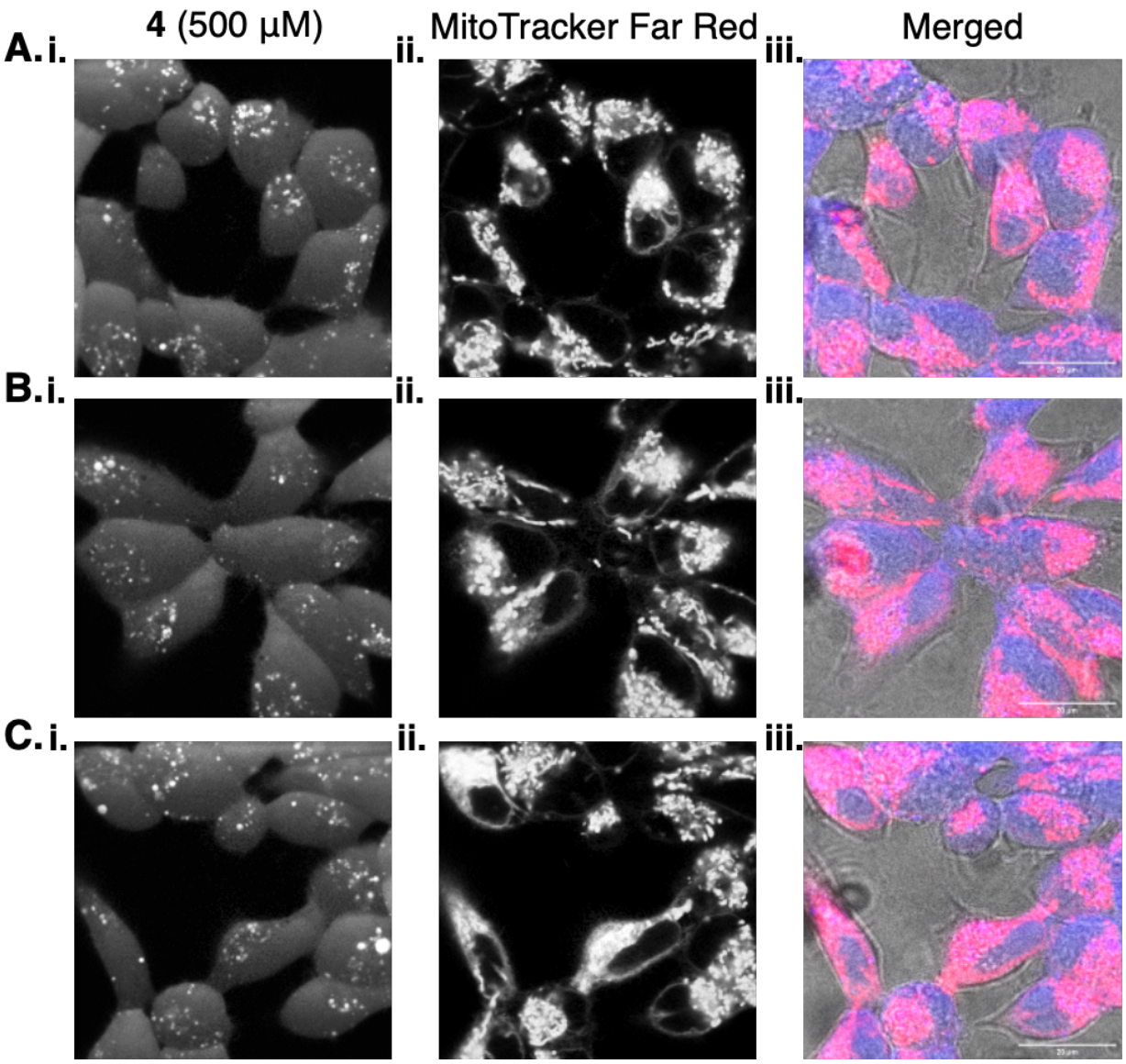


Figure S13: Confocal microscopy of live HEK cells. (**A-C**) Triplicate images of cells incubated with (**i**) **4** (500 μM) for 24 h and imaged using 405 nm laser, and (**ii**) MitoTracker™ Deep Red FM stain (0.1 μM) using a white laser set at 644 nm. (**iii**) Merge of Blue and Far Red channels to determine colocalization.

Two days prior to imaging, HEK293 cells were passed to an 8-well chamber slide (80826, ibidi) for 40-50% confluency on the day of imaging. The second day, a 100x solution of **4** were added to each well to a final concentration of 500 μM for 24 h incubation. MitoTracker™ Deep Red FM was added 15 min prior to imaging to a final concentration of 0.1 μM. Media were then removed and cells were washed with PBS containing 10% FBS. Imaging was performed using a SP8 Leica confocal microscope (100x oil immersion lens); **4** excited with 405 nm diode laser; other dyes excited with a white laser set at the absorption maximum.

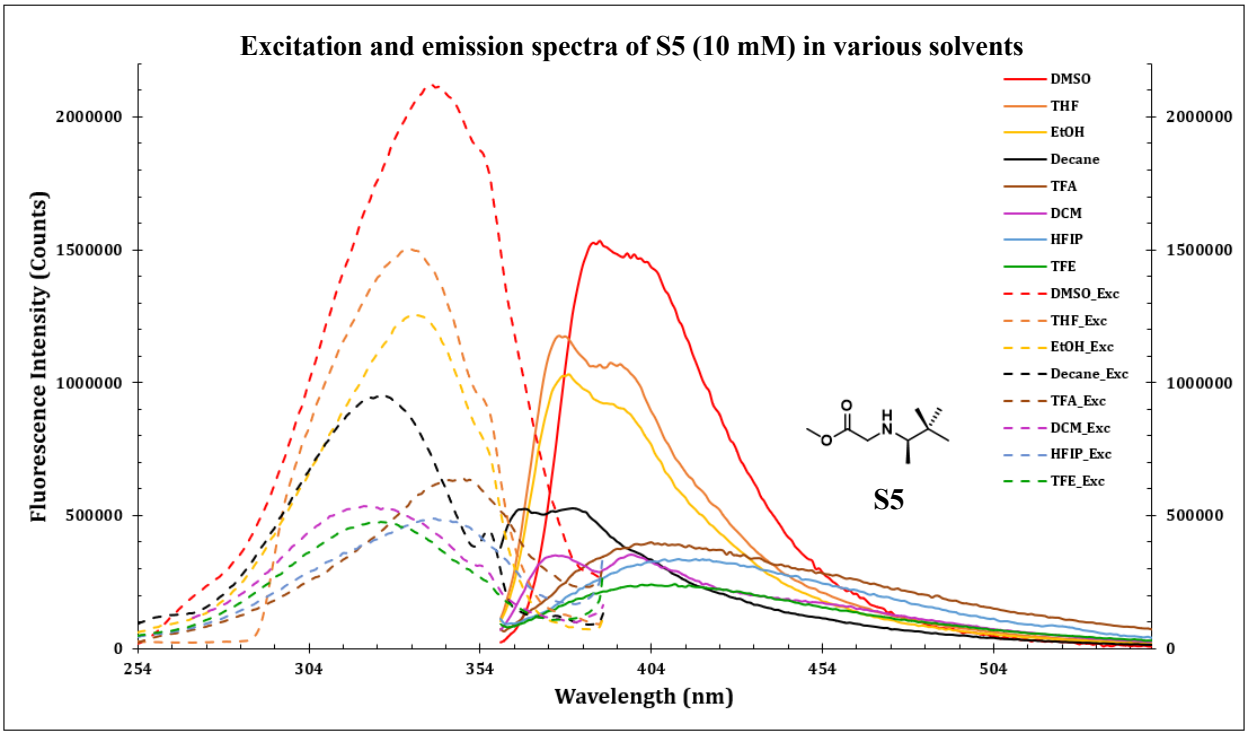


Figure S14: Excitation (dotted, monitored at 400nm) and emission spectra (solid, λ_{exc} = 350 nm) of **S5** (10 mM) in solvents with varied proton donor ability.
DMSO: Dimethyl sulfoxide**, THF:** Tetrahydrofuran**, EtOH:** Ethanol**, TFA:** Trifluoroacetic acid**, DCM:** Dichloromethane**, HFIP:** Hexafluoroisopropanol**, TFE:** Trifluoroethanol**.**

Note:
A model study was conducted with alkyl (aminomethyl)ester **S5** to further interrogate the nature of the emission of **4**. Subtle shifts in emission are observed as solvent polarity is modified, which do not fully support a pure charge-transfer mechanism. However, when analogous emission experiments are performed in more Brønsted acidic environments (TFE, TFA, HFIP), dramatically reduced emission under otherwise identical conditions are observed. These data imply the importance of Lewis basic sites, the secondary amine and possibly that of the carbonyl oxygen as well. The excitation spectra also show pronounced reduction in intensity, suggesting the acidic environment largely plays a role in the ground state manifold of **S5**. In acidic environments, Lewis basic electron lone pairs interact with Bronsted acidic donors, decreasing 1) the efficiency of charge transfer from amine to carbonyl under UV irradiation and 2) ability for carbonyl to accept proton in the excited state. While in the vast majority of excited-state proton transfer studies a large Stokes shift is observed in the emission spectra due to emissive tautomeric states[1], **S5** is incapable of forming tautomers, so no large Stokes shift or pronounced dependence of emission on solvent polarity is observed, explaining the similarity in spectra shown in Figure S14. In compound **S5**, proton transfer in the excited state could proceed through a 5-membered transition state, minimizing charge separation. This would result in only subtle changes of emission depending on solvent polarity, which is consistent with our observations. Taken to together, the data suggest that excitation is most likely charge-transfer in nature, but coupled with a redistribution of proton location equilibrium, resulting in diminished charge separation explaining the lack of more dramatic solvatochromism but clear dependence of emission on medium acidity.

Furthermore, the broad, tailing emission profiles in all solvents is characteristic of charge transfer emission bands.

An analogous study performed by Nishimura and co-workers[2] showed a comparable system in which ground state properties of amine electron donors were modulated using electron-withdrawing and -accepting groups on amine substituents in a charge-transfer-coupled excited state proton transfer system. In their case, electron-withdrawing groups, while increasing the acidity of the urea amine proton, reduced efficiency of charge transfer, resulting in decreased emission; conversely, electron-donating groups enhanced emission efficiency.

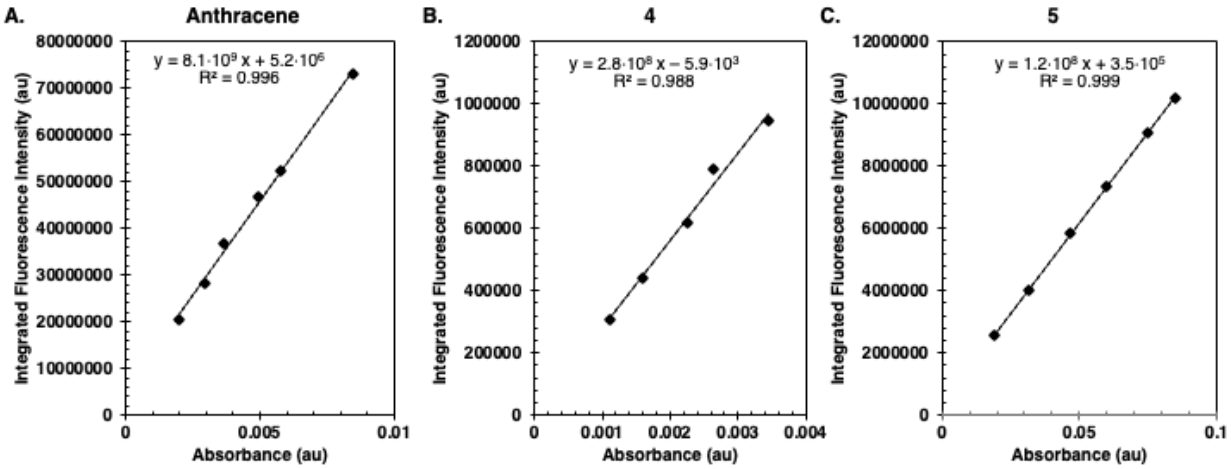


Figure S15: Quantum yield determination for compounds **4** and **5** using anthracene as a reference **(A)** Integrated fluorescence vs absorbance plot for anthracene in EtOH. (**B**) Integrated fluorescence vs absorbance plot of **4** (250 – 750 μM) in EtOH. (**C**) Integrated fluorescence vs absorbance plot of **5** (25 – 150 μM) in EtOH.

Quantum yield determination procedure: The photoluminescence quantum yield (Φ_F) of a molecule or material is defined as follows, $\mathbf{\Phi_F = P_E / P_A}$, where PE and PA are the number of photons absorbed and emitted, respectively. To determine the quantum yield, we either use a relative method with a known standard in the same region of the electromagnetic spectrum, or an absolute method, in which the number of photons absorbed and emitted are measured independently. Here, due to the excitation-dependent emission exhibited by compounds of interest, we use a relative method, with anthracene as the known standard.

To compare an unknown to a reference with a known quantum yield, the following relationship was used: $\boldsymbol{\Phi_{F,x} = \Phi_{F,r}\,(m_x/m_f)(\eta_x^2/\eta_r^2)}$, where m represents the slope of the line (y = mx + b) obtained from graphing integrated fluorescence intensity versus optical density across a series of samples, C is the refractive index of the solvent, and the subscripts x and r represent values of the unknown and reference, respectively.

The ($\Phi_{F,r}$) of anthracene was taken to be a constant, 27%, as reported by Dawson and Windsor[3]. Here, we measured relative fluorescence quantum yields of **4** and **5** in EtOH: To obtain a plot of integrated fluorescence intensity versus absorbance for the reference and unknown, five solutions and a solvent blank were prepared and their absorbance and emission spectra (with an excitation wavelength of 365 nm) were acquired. Anthracene and **4** and **5** were diluted in ethanol to concentrations with optical densities less than 0.1 to minimize effects of reabsorption. The background was subtracted (375 – 600 nm), fluorescence traces were integrated, and the raw integrals were corrected by subtracting the integral over an identical range from fluorescence traces of the blank solvent. The integrated fluorescence intensities were then plotted against the baseline corrected absorbance values at the relevant wavelength (365 nm), and the slope and error in slope were obtained ($R^2 > 0.99$ for all traces)

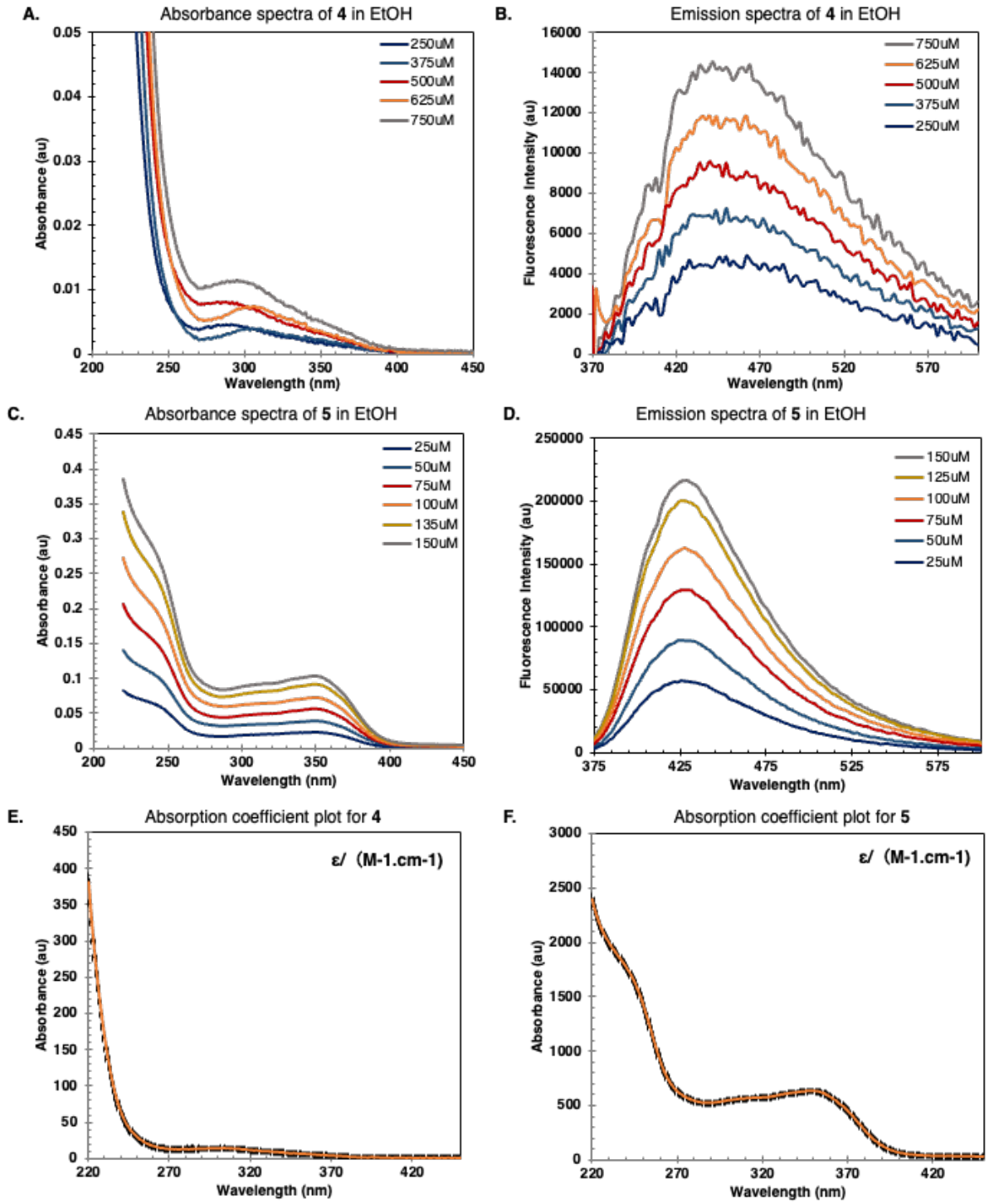


Figure S16: (**A**) Absorbance spectra of **4** in EtOH at increasing concentrations (250 – 750 µM) (**B**) Emission spectra of **4** in EtOH at increasing concentrations (250 – 750 µM). (**C**) Absorbance spectra of **5** in EtOH at increasing concentrations (25 – 150 µM) (**D**) Emission spectra of **5** in EtOH at increasing concentrations (25 – 150 µM). (**E**) Absorbance vs. wavelength plot used to determine absorption coefficient of **4** in EtOH. (**F**) Absorbance vs. wavelength plot used to determine absorption coefficient of **4** in EtOH.

General experimental procedures:

Materials: Chemical reagents were purchased from Sigma-Aldrich, Fisher Scientific, TCI America or Acros Organics and used without purification unless noted otherwise. Anhydrous dimethylsulfoxide (DMSO) was obtained from a Sure-Seal^{TM} bottle (Aldrich) that after opening was stored in a Schlenk-bomb flask over 4Å molecular sieves. Anhydrous and deoxygenated solvents dimethylformamide (DMF), methanol (MeOH), tetrahydrofuran (THF) and dichloromethane (DCM) were dispensed from a Grubb's-type Phoenix Solvent Drying System constructed by JC Meyer. $CDCl_3$ and D_2O were purchased from Cambridge Isotope Laboratories and used as received. Thin layer chromatography was performed using Silica Gel 60 F254 (EMD Millipore) plates. Flash chromatography was executed with technical grade silica gel with 60 Å pores and 40 – 63 μm mesh particle size (Sorbtech Technologies).

Instruments: Masses for analytical measurements were taken on a Sartorius MSE6.6S-000-DM or MSA6.6S-000-DM Cubis Micro Balance. Centrifugation was performed on a Thermo Scientific Sorvall ST 16 Centrifuge. All sonication was done in a Branson M-Series Model 3800 120V bath sonicator. Solvent was removed under reduced pressure with a Büchi Rotovapor with a Welch self-cleaning dry vacuum pump and further dried with a Welch DuoSeal pump. Bath sonication was performed using a Branson 3800 ultrasonic cleaner. ^{1}H and $^{13}C\{^{1}H\}$ NMR spectra were acquired on a Bruker AV 500, DRX 500 or DRX 400 spectrometer. ^{1}H and $^{13}C\{^{1}H\}$ NMR spectra were referenced to residual solvent resonances in deuterated solvents ($CDCl_3$: ^{1}H, 7.26 ppm; ^{13}C, 77.16 ppm, Note: due to high humidity H_2O resonances are often present; D_2O: ^{1}H, 4.79 ppm) and are reported relative to tetramethylsilane ($\delta = 0$ ppm). Spectra were processed with MestReNova or TopSpin software. All deuterated solvents were referenced according to Nudelman and coworkers.[4] Low resolution mass spectra were taken using an Agilent 1260 series HPLC-tandem MS and high resolution mass spectra on a Thermo Q Exactive plus Orbitrap instrument. Reverse-phase chromatography for analysis and purification was conducted on an Agilent 1260 II series HPLC. Compounds were purified by HPLC a semi-prep Zorbax SB C18 column (5 μm, 250 mm x 9.4 mm) was used. High purity LCMS grade water, MeCN, FA and TFA solvents were used for HPLC runs. Absorbance spectra were collected on a JASCO V-770 UV-Visible/NIR spectrophotometer with a 2000 nm/min scan rate after blanking with the appropriate solvent. Photoluminescence spectra were obtained on a Horiba Instruments PTI QuantaMaster Series fluorometer. Quartz cuvettes (1 cm) were used for absorbance and photoluminescence measurements.

Abbreviations:
CB[7] : Cucurbit[7]uril, DCM: Dichloromethane, MeOH: Methanol, MeCN: Acetonitrile, THF: Tetrahydrofuran, DMF: Dimethylformamide, TFA: Trifluoroacetic acid, TFE: trifluoroethanol, FA: Formic acid, HFIP: hexafluoroisopropanol, LCMS: Liquid-chromatography tandem mass-spectroscopy, NMR: Nuclear Magnetic Resonance, DIPEA: Diisopropylethylamine, TEA: Triethylamine, PFP: Pentafluorophenyl, RPMI 1640: Roswell Park Memorial Institute 1640, FBS: Fetal bovine serum, PS: Penicillin-streptomycin.

General cell culture procedures

Jurkat-97 cells were purchased from ATCC© (Cat # TIB-152) and were cultured in RPMI 1640 media (Genessee #25-506H) supplemented with 10% fetal bovine serum (FBS) (Corning, lot# 35016109) and 1% penicillin-streptomycin (PS) (Life Technologies, cat# 15070063). HEK-293 cells were purchased from ATCC© (Cat # CRL-1573) and were in Minimum Essential Media (Gibco, cat# 11095080) supplemented with 10% fetal bovine serum (Corning, lot# 35016109) and 1% penicillin/streptomycin (Life Technologies, cat# 15070063). CHO-K1 cells were purchased from ATCC© (Cat # CCL-61) and were cultured in FK-12 media (Gibco # 211277030) supplemented with 10% fetal bovine serum (Corning, lot# 35016109) and 1% penicillin/streptomycin (Life Technologies, cat# 15070063). Cells were washed with phosphate buffered saline (PBS), or PBS supplemented with 1% fetal bovine serum (FACS buffer). Cells were incubated at 37 ˚C, 5% CO_2, throughout culturing, in HERACell 150i CO_2 incubators. Cells were pelleted through use of Sorvall ST 40R centrifuge. All cell work was performed in 1300 Series A2 biosafety cabinets. Confocal microscopy was performed on a SP8 Leica NucRed Dot 1 (Biotium, cat # 40060) was used as a far-red nuclear stain. CellMask Green (Thermofisher cat # C37608) was used as a green cell membrane stain. MitoTracker™ Deep Red FM (Thermofisher cat # M22426) was used as a far red mitochondrial stain. LysoTracker™ Red DND-99 (Thermofisher cat # L7528) was used as a far red lysosomal stain. Flow cytometry was performed on a BDBiosciences FACSCalibur equipped with 488 nm and 635 nm lasers. Fluorescence was measured primarily on the FL1 channel although compounds were visible on FL2 and FL3 channels, too.

Figure Procedures

Figure 2: Experimental procedures can be found in supplemental Figures S1-S9.

Figure 3 Experimental procedures can be found in supplemental Figures S10-S13

Colocalization plot determination
Initially, using Coloc2 plug-in on Fiji / ImageJ and by selecting both channels for each experiment (blue channel for **4** and far red / green channels for organelles), a 2D histogram and Pearson's R coefficient was determined. However, due to the widespread staining of **4**, accurate measurements were not possible, even using thresholds to remove cytosolic staining.

Instead, 2D cross section plots were used in Fiji / Image J. An ROI on the organelle staining image was used to plot pixel intensity vs distance for each image. The data were exported and Pearson's R coefficient was calculated for each pair. The cross-section line drawn can be seen in Figure 3 in the merged (**iii**) images.

Figure 4: Photophysical characterization of 4
(**A**) A solution of **4** in EtOH (10 mM) was prepared. Absorption spectra were collected at 2000 scans/min with 200–800 nm range. Fluorescence spectra were taken with slits 5 nm, step size 1 nm, integration time 0.1 s, λ_{exc} = 345 nm and a 360-750 nm spectral range.

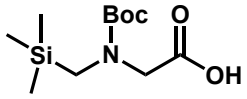
(B) A solution of **4** in EtOH (10 mM) was prepared. Fluorescence spectra were taken with slits 5 nm, step size 1 nm, integration time 0.1 s. Emission spectra with λ_{exc} = 300-450 nm (stepwise every 10 nm) and a 310–650 nm spectral range were collected to observe the excitation-dependent emission.

Figure 5: Photophysical characterization of 5 and interaction with CB[7]
(**B**) A solution of **5** in EtOH (10 mM) was prepared. Absorption spectra were collected at 2000 scans/min with 220–450 nm range. Fluorescence spectra were taken with slits 5 nm, step size 1 nm, integration time 0.1 s, λ_{exc} = 365 nm and a 370–620 nm spectral range.

(**E-G**) Equimolar solutions of **4** (50 μM) and **5** (5 μM) with **CB[7]** (1:1) in H_2O were prepared. Solutions of **4** (50 μM) and **5** (5 μM) alone were prepared for comparison. Absorption spectra were collected at 2000 scans/min with 220–450 nm range. Fluorescence spectra were taken with slits 5 nm, step size 1 nm, integration time 0.1 s, λ_{exc} = 345 nm and a 360–650 nm spectral range.

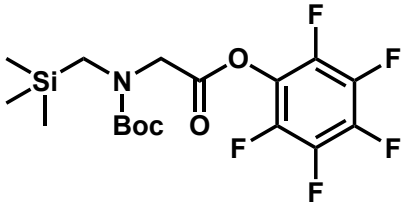
Synthetic procedures:

_N_-(tert-butoxycarbonyl)-_N_-((trimethylsilyl)methyl)glycine (2):



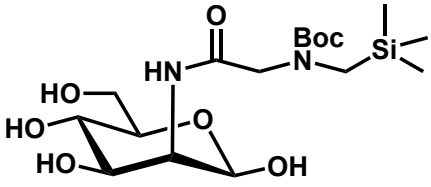
Trimethylsilylglycine methyl ester (**1**)[5] (0.709 g, 4.04 mmol, 1 equiv.) was dissolved in DCM (8 mL, anhydrous) under N_2 and cooled down to 0 °C. TEA (0.562 mL, 4.04, 1 equiv.) was added followed by dropwise addition of Boc anhydride (1.06 g, 4.85 mmol, 1.2 equiv.) in DCM (4 mL, anhydrous). The resulting reaction was stirred at 0 °C for 2 h and was allowed to warm up to room temp. over 3 h. Excess solvent was evaporated to give the crude product as a yellow oil. The crude product was passed through a silica plug with 10% EtOAc/Hexanes to give *N*-Boc-trimethylsilylglycine methyl ester (1.97 g, 2.79 mmol, 69%) as a yellow oil. The oil was dissolved in THF (8 mL) and added to a solution of LiOH (0.174 g 7.27 mmol, 5 equiv.) in H_2O (2 mL). The resulting suspension was vigorously stirred at room temp. for 5 h. The THF was evaporated and the aqueous mixture was acidified to pH 2-3 (1 M HCl) and extracted with DCM (5 x 10 mL). The organic layers were combined, dried (Na_2SO_4) and DCM evaporated to give the desired product (0.309 g, 1.18 mmol, 81%). ^{1}H NMR (400 MHz, MeOD): δ 3.87 (d, *J* = 6.3 Hz, 2H), 2.84 (d, *J* = 16.8 Hz, 2H), 1.45 (d, *J* = 20.2 Hz, 9H), 0.08 (d, *J* = 5.1 Hz, 9H). ^{13}C NMR (101 MHz, MeOD): δ 173.4, 157.8, 157.2, 81.3, 52.0, 40.8, 28.6, -1.8. HRMS (ESI–): calculated for $C_{11}H_{23}NO_4Si^-$ $[M-H]^-$: 260.1319; found: 260.1319.

Pentafluorophenyl _N_-(_tert_-butoxycarbonyl)-_N_-((trimethylsilyl)methyl)glycinate (S3):



In a flame-dried 50 mL round-bottom flask under N_2 was added a solution of **2** (0.65 g, 2.5 mmol, 1 equiv.) in DCM (anhydrous, 10 mL) and the reaction was cooled down to 0 °C. TEA (0.35 mL, 2.5 mmol, 1 equiv.) was added followed by slow, dropwise addition of pentafluorophenyltrifluoro acetate (0.52 mL, 3.0 mmol, 1.2 equiv.). After the addition was complete, the reaction was allowed to warm up to room temp and was stirred for 3 h. The reaction was concentrated to about 2 mL and purified using column chromatography (0-10% EtOAc/Hex) to give the product as a yellow oil (0.985 g, 2.31 mmol, 92%). The product was used directly for conjugation to Mannosamine without further purification/characterization.

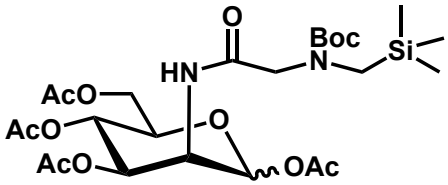
_N_-tert-butyl(2-oxo-2-(((2R,3S,4R,5S,6R)-2,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-3-yl)amino)ethyl)((trimethylsilyl)methyl)carbamate (S4):



Mannosamine.HCl (84 mg, 0.39 mmol, 1 equiv.) was dissolved in MeOH (anhydrous, 3 mL) after 15 min of stirring at room temp under N_2. To the colorless solution was added TEA (0.11 mL, 0.78 mmol, 2 equiv.) followed by a solution of **S3** (0.20 g, 0.47 mmol, 1.2 equiv.) in DCM (anhydrous, 0.5 mL). The resulting reaction was stirred at room temp for 16 h. Excess solvents were evaporated and the crude product was purified by column chromatography (0-10% MeOH/DCM) to give a mixture of product with TEA salt (0.187 g) that was used without further purification.

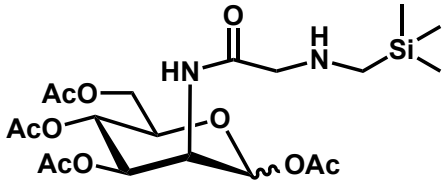
A small amount of the crude (≈ 5 mg) was purified by semi-prep HPLC (5-95% MeCN/H_2O + 0.01% TFA) to give the product, eluting at 19 min, for characterization. ^{1}H NMR (500 MHz, MeOD): δ 5.03 (s, 1H), 4.48 – 4.31 (m, 1H), 4.28 (s, 1H), 4.05 – 3.91 (m, 2H), 3.87 – 3.82 (m, 1H), 3.79 (s, 1H), 3.78 – 3.75 (m, 1H), 3.61 (d, *J* = 7.0 Hz, 1H), 3.53 (t, *J* = 9.2 Hz, 1H), 3.43 (t, *J* = 9.6 Hz, 1H), 2.91 (d, *J* = 14.3 Hz, 1H), 2.88 – 2.74 (m, 1H), 2.58 – 2.51 (m, 1H), 2.16 (s, 2H), 2.04 (s, 1H), 1.45 (d, *J* = 19.6 Hz, 9H), 1.30 (s, 3H), 0.08 (d, *J* = 4.1 Hz, 9H). R_f = 0.42 (in 10 % MeOH/DCM). LRMS (ESI+): calculated for $C_{17}H_{34}N_2O_8SiNa^+$ $[M+Na]^+$: 445.2; found: 445.2.

(3S,4R,5S,6R)-6-(acetoxymethyl)-3-(2-((*tert*-butoxycarbonyl)((trimethylsilyl)methyl)amino) acetamido)tetrahydro-2*H*-pyran-2,4,5-triyl triacetate (3):



Crude **S4** (0.187 g) was dissolved in pyridine (anhydrous, 3 mL) under N_2. To the colorless solution was added acetic anhydride (3 mL) and the reaction was stirred at room temp for 8 h. Excess solvents were evaporated *in vacuo* and the crude product purified by column chromatography (20-40% EtOAc/Hexanes) to give the desired product as a white solid (0.20 g, 0.34 mmol, 86% over 2 steps). ^{1}H NMR (500 MHz, MeOD): δ 5.97 (s, 1H), 5.28 (d, *J* = 8.4 Hz, 2H), 5.16 (d, *J* = 9.0 Hz, 1H), 4.75 (s, 1H), 4.58 (d, *J* = 12.2 Hz, 1H), 4.30 (dd, *J* = 12.2, 5.5 Hz, 1H), 4.12 (s, 1H), 4.09 – 4.04 (m, 1H), 4.03 – 3.85 (m, 3H), 2.93 – 2.76 (m, 3H), 2.17 (s, 3H), 2.06 (s, 9H), 1.98 (s, 3H), 1.45 (d, *J* = 19.6 Hz, 9H), 0.10 (d, *J* = 6.9 Hz, 9H). R_f = 0.47 (2:1 EtOAc/Hexanes). LRMS (ESI+): calculated for $C_{25}H_{42}N_2O_{12}SiNa^+$ $[M+Na]^+$: 613.2; found: 613.0.

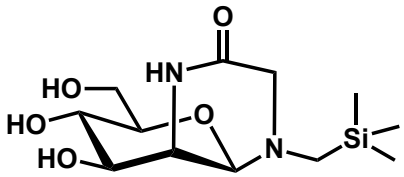
(3S,4R,5S,6R)-6-(acetoxymethyl)-3-(2-(((trimethylsilyl)methyl)amino)acetamido)tetrahydro -2*H*-pyran-2,4,5-triyl triacetate (4):



Compound **3** (0.20 g, 0.34 mmol) was dissolved in DCM (2 mL) and to the resulting pale yellow solution, TFA (0.5 mL) was slowly added over 2 minutes. The reaction was stirred at room temp for 2 h. Excess solvents and reagents were evaporated by diluting and evaporating with toluene (2 x 1 mL) and DCM (3 x 5 mL) to give the desired product as a pink solid. A silica plug (100:3:1 EtOAc:MeOH:H_2O) was used to remove colored contaminants and give the desired product as a mixture of anomers (α/β 2:1) (0.17 g, 0.27 mmol, 70% over 3 steps). Separation of anomers was achieved by purification by semi-prep HPLC (5-95% MeCN/H_2O + 0.01%TFA) with the β-anomer eluting at 17.5 min and the α-anomer at 19 min. R_f = 0.33 (in 2:1 EtOAc/Hexanes). α-anomer: ^{1}H NMR (500 MHz, MeOD): δ 5.97 (s, 1H), 5.28 (d, *J* = 8.4 Hz, 2H), 5.16 (d, *J* = 9.0 Hz, 1H), 4.75 (s, 1H), 4.58 (d, *J* = 12.2 Hz, 1H), 4.30 (dd, *J* = 12.2, 5.5 Hz, 1H), 4.12 (s, 1H), 4.09 – 4.04 (m, 1H), 4.03 – 3.85 (m, 3H), 2.93 – 2.76 (m, 3H), 2.17 (s, 3H), 2.06 (s, 9H), 1.98 (s, 3H), 1.45 (d, *J* = 19.6 Hz, 9H), 0.10 (d, *J* = 6.9 Hz, 9H). β-anomer: ^{1}H NMR (400 MHz, MeOD): δ 5.97 (d, *J* = 2.0 Hz, 1H), 5.23 (dd, *J* = 9.9, 4.2 Hz, 1H), 5.13 (t, *J* = 9.8 Hz, 1H), 4.81 – 4.76 (m, 2H), 4.32 (dd, *J* = 12.2, 6.4 Hz, 1H), 4.12 (dd, *J* = 12.2, 2.8 Hz, 1H), 3.97 (td, *J* = 6.5, 3.2 Hz, 1H), 3.93 (d, *J* = 1.2 Hz, 2H), 2.54 (s, 9H), 2.10 – 2.02 (m, 9H), 1.99 (s, 3H), 0.23 (s, 9H). ^{13}C NMR (126 MHz, Methanol-*d*$_4$): δ 172.3, 171.5, 171.5, 171.4,

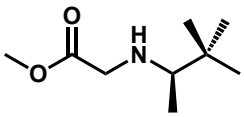
171.4, 170.0, 169.9, 167.4, 167.1, 93.0, 92.0, 74.5, 72.55, 71.8, 70.5, 67.1, 67.0, 63.7, 52.5, 51.2, 51.1, 39.3, 39.2, 28.8, 20.7, 20.6, 20.6, 20.6, 14.5, -2.6. HRMS (ESI+): calculated for $C_{20}H_{35}N_2O_{10}Si^+$ $[M+H]^+$: 491.2061; found: 491.2051.

(4aR,6R,7S,8R,8aS)-7,8-dihydroxy-6-(hydroxymethyl)-4-((trimethylsilyl)methyl)hexahydro-1*H*-pyrano[2,3-b]pyrazin-2(3H)-one (5):



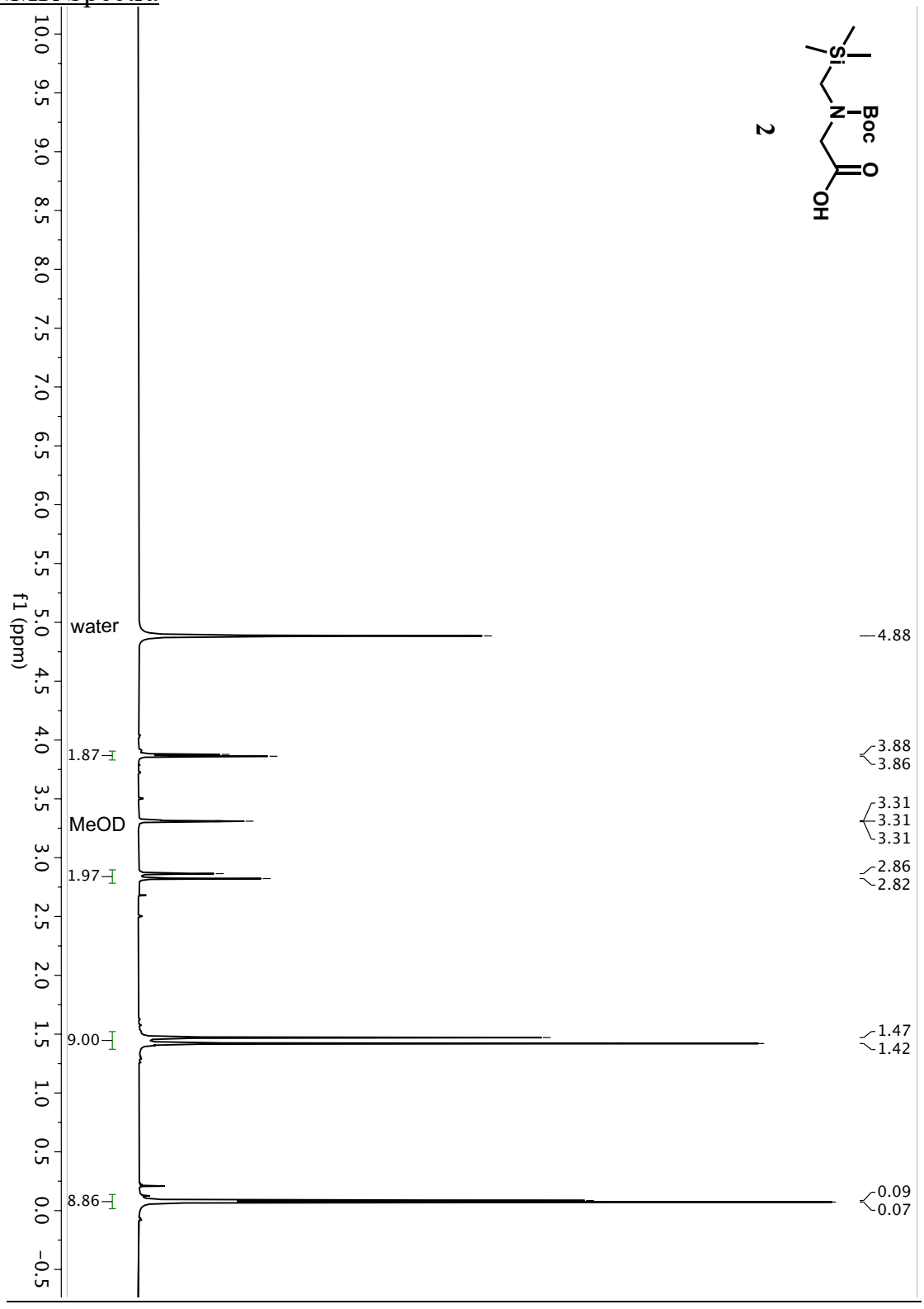
Compound **4** (15 mg, 0.027 mmol, 1 equiv.) was dissolved in MeOH (1 mL) and to the colorless solution was added 0.5 M aq. NaOMe (0.1 mL). The reaction was stirred at room temp overnight. Excess solvents were evaporated to give the product (some acetate remaining) as a white solid (16 mg, 0.031 mmol). The product was used without further purification. ^{1}H NMR (400 MHz, MeOD): δ 4.39 (d, *J* = 2.1 Hz, 1H), 3.85 (dd, *J* = 12.0, 2.4 Hz, 1H), 3.80 (dd, *J* = 4.2, 1.5 Hz, 1H), 3.71 – 3.64 (m, 2H), 3.38 (s, 1H), 3.35 (d, *J* = 3.0 Hz, 2H), 3.27 (s, 1H), 3.23 (s, 1H), 3.19 (ddd, *J* = 9.7, 5.7, 2.4 Hz, 1H), 2.42 (d, *J* = 14.5 Hz, 1H), 2.24 (d, *J* = 14.5 Hz, 1H), 0.09 (s, 9H). ^{13}C NMR (126 MHz, D_2O): δ 172.3, 86.8, 78.7, 76.0, 73.7, 72.4, 68.2, 64.4, 62.7, 57.1, 54.5, 46.3, 9.2, 2.0, 1.1, -1.55. HRMS (ESI+): calculated for $C_{12}H_{25}N_2O_5Si^+$ $[M+H]^+$: 305.1533; found: 305.1537.

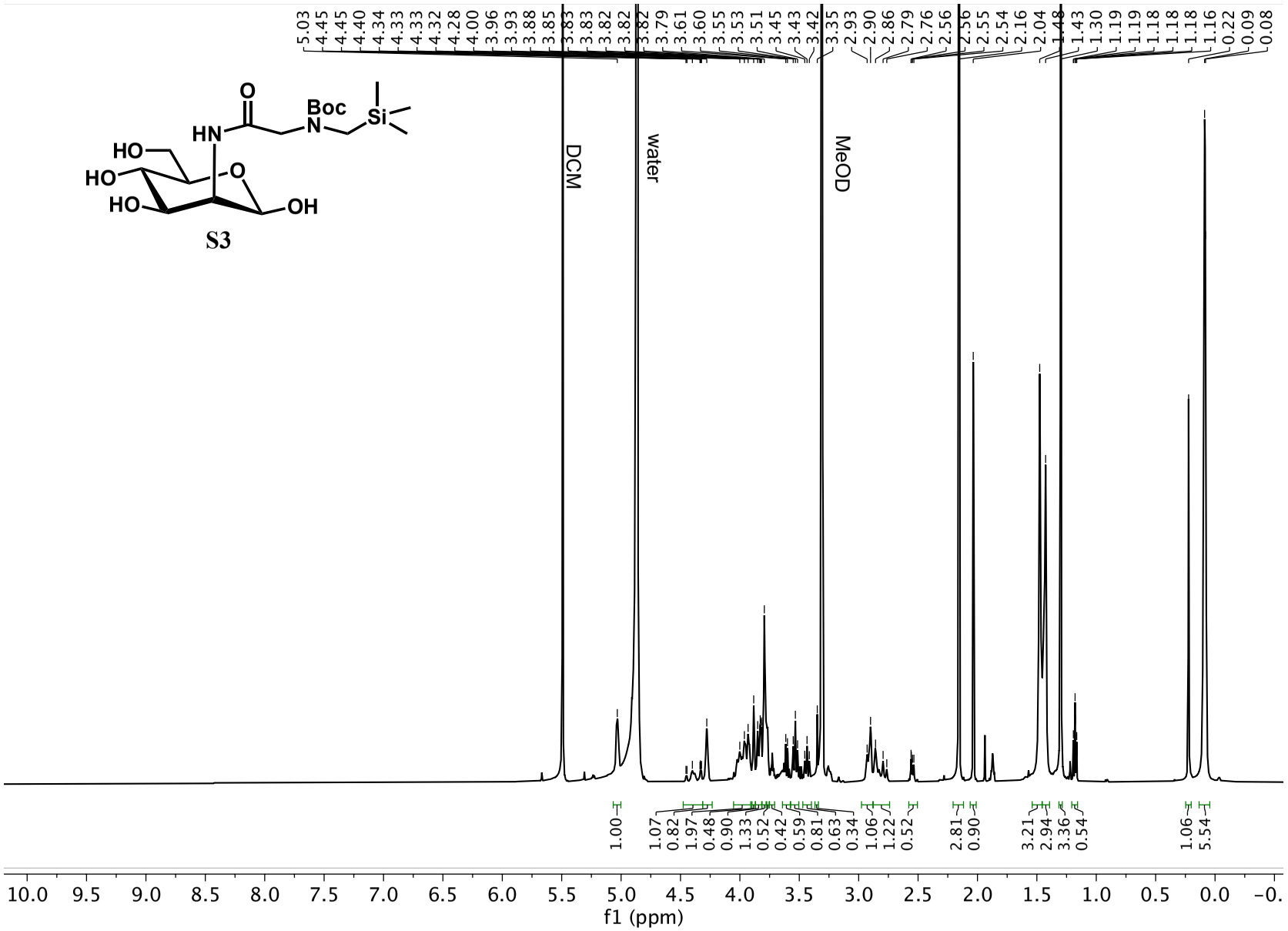
***N*-*sec*-butylglycine methyl ester (S5):**



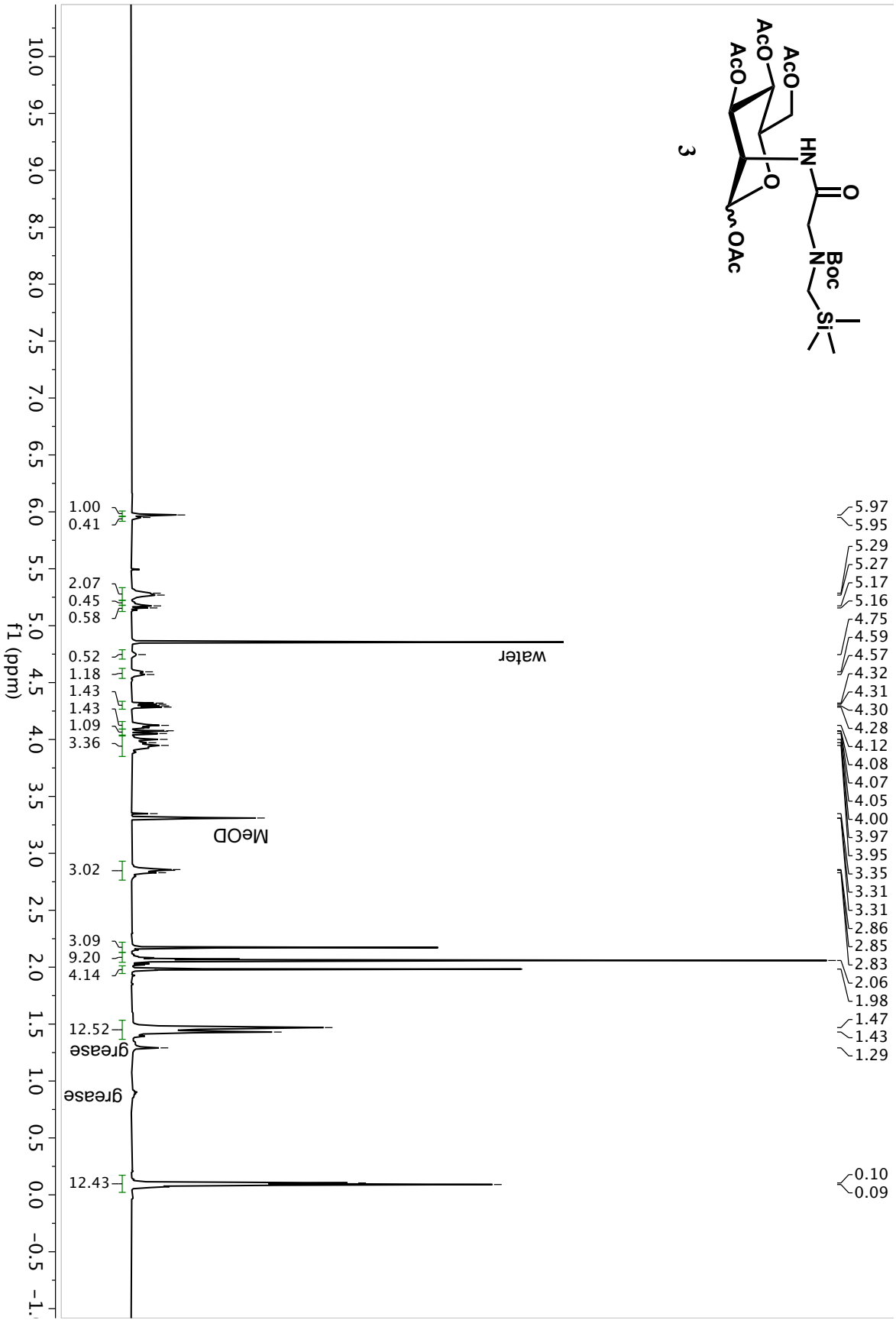
Sec-butylamine (863 μL, 6.34 mmol, 2 equiv.) and potassium carbonate (876 mg, 6.34 mmol, 2 equiv.) were suspended in MeCN (3 mL). A solution of 2-Methylbromoacetate (0.300 mL, 3.17 mmol, 1 equiv.) in MeCN (1 mL) was added to the suspension and the resulting reaction was heated to 80 ºC overnight. The reaction mixture was diluted with Et_2O (10 mL) and filtered through Celite. The supernatant was concentrated to give the desired product as a yellow oil (460 mg, 2.64 mmol, 78%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.73 – 3.66 (m, 3H), 3.52 – 3.29 (m, 2H), 2.27 – 2.18 (m, 1H), 0.94 (d, J = 6.5 Hz, 3H), 0.90 – 0.86 (m, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.54, 62.10, 51.78, 49.94, 34.46, 26.47, 26.30, 26.20, 14.77. HRMS (ESI+): calculated for $C_9H_{19}NO_2^+$ $[M+H]^+$: 174.1494; found: 174.1485.

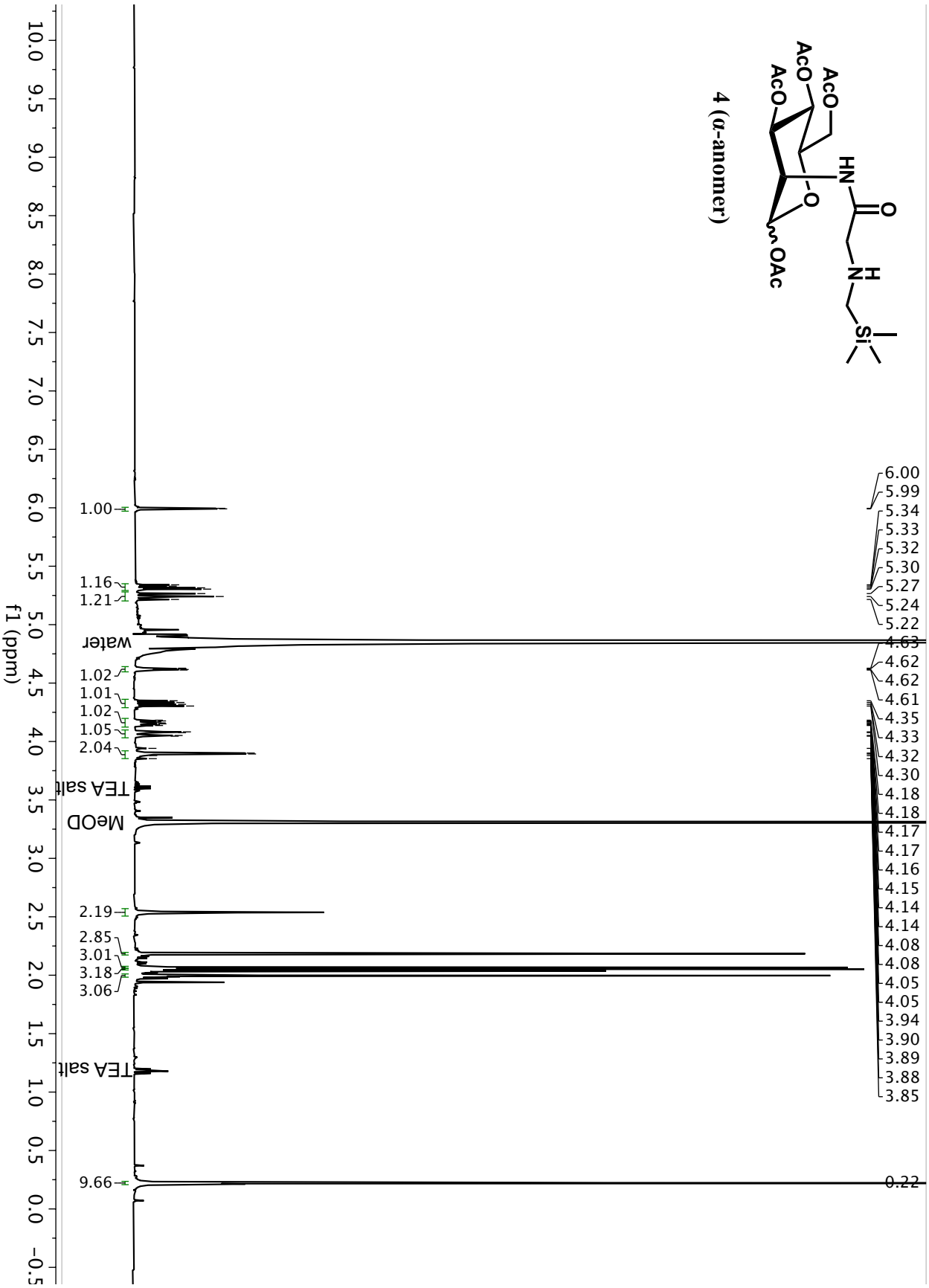
[1]H NMR Spectra

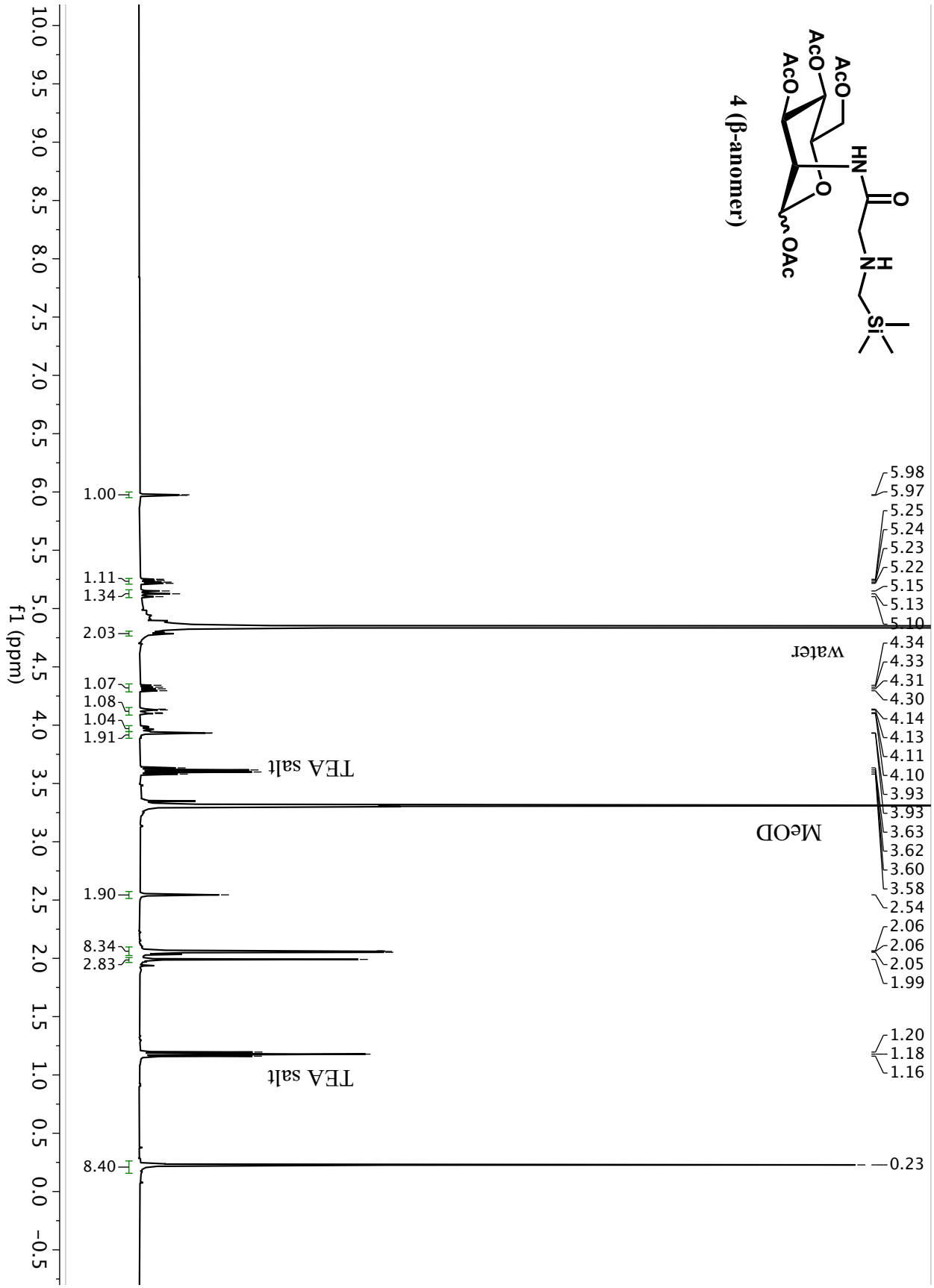


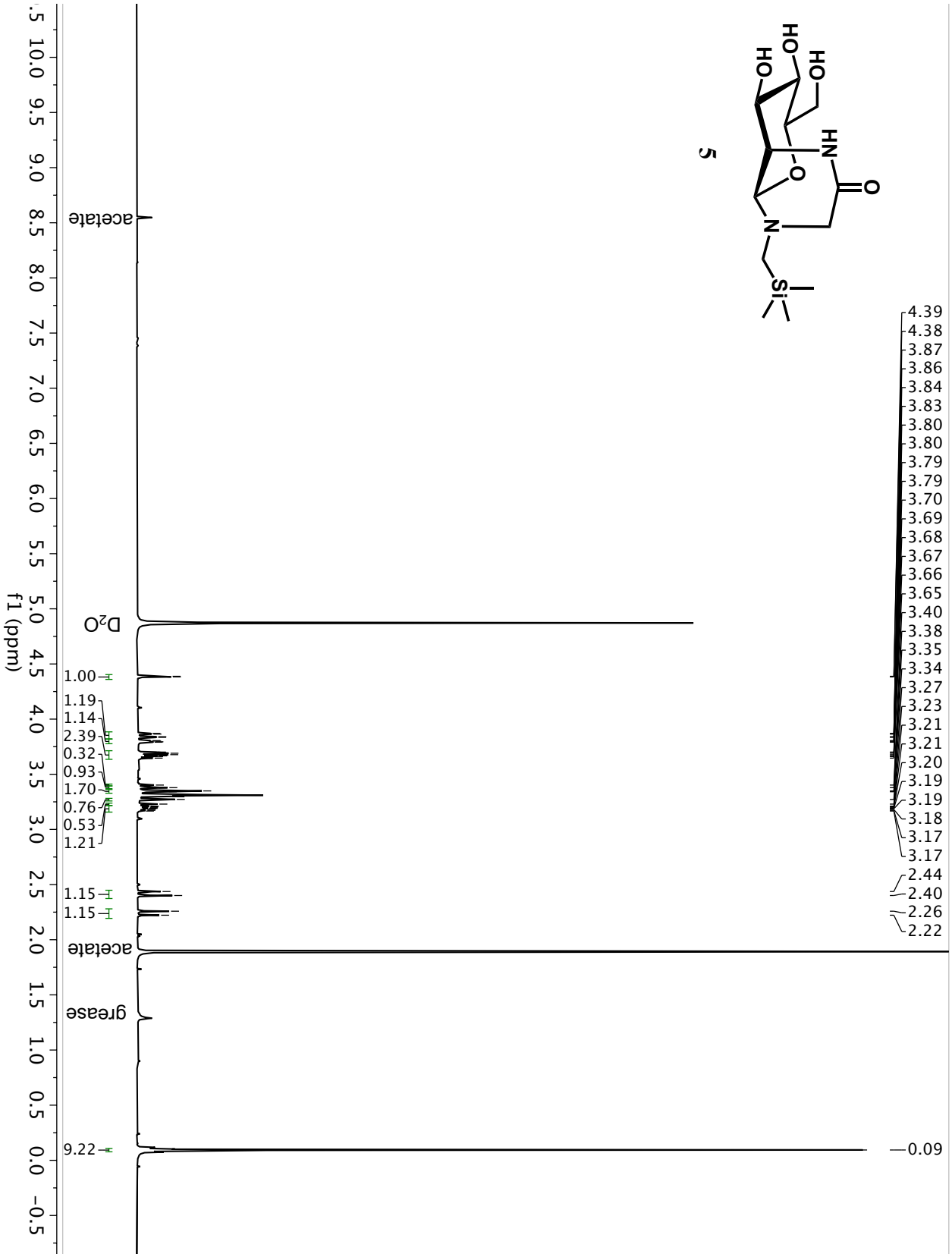


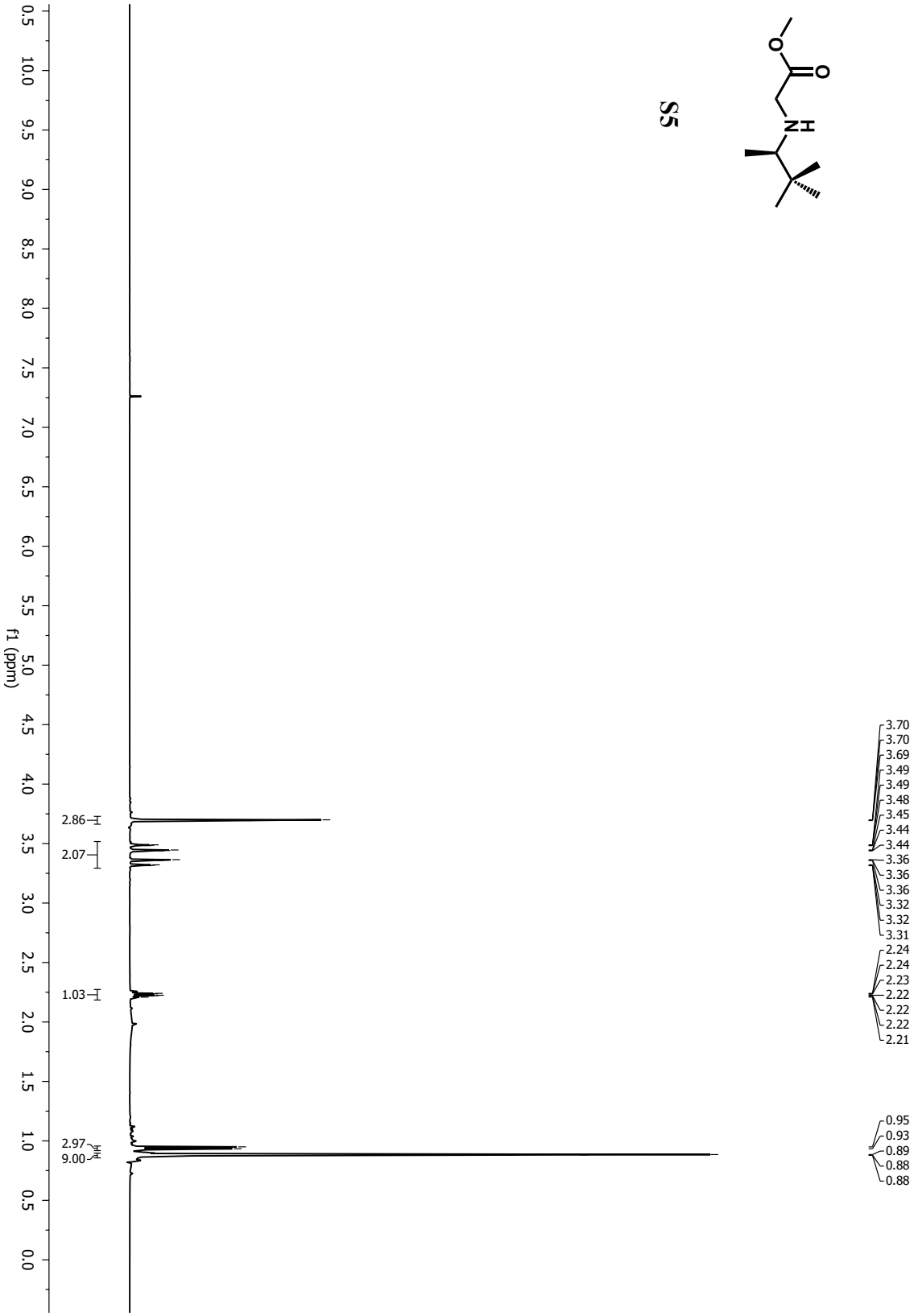
5.3


3
AcO
AcO
AcO
OAc
HN
O
O
Boc
N
Si
f1 (ppm)
water
MeOD
grease
grease
1.00
0.41
2.07
0.45
0.58
0.52
1.18
1.43
1.43
1.09
3.36
3.02
3.09
9.20
4.14
12.52
12.43
5.97
5.95
5.29
5.27
5.17
5.16
4.75
4.59
4.57
4.32
4.31
4.30
4.28
4.12
4.08
4.07
4.05
4.00
3.97
3.95
3.35
3.31
3.31
2.86
2.85
2.83
2.06
1.98
1.47
1.43
1.29
0.10
0.09

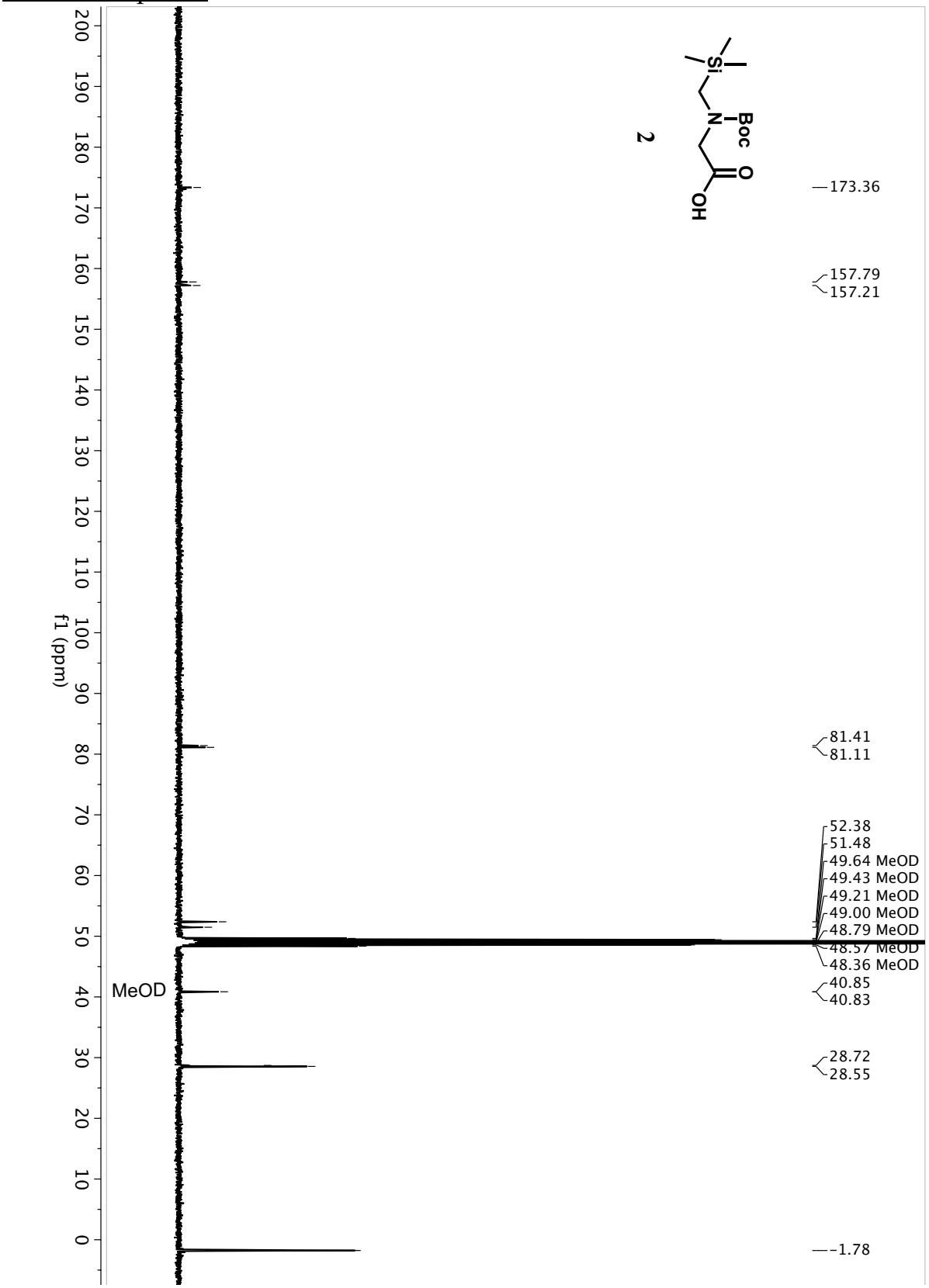

4 (α-anomer)
AcO
AcO
AcO
OAc
HN
O
H
N
Si
f1 (ppm)
water
MeOD
TEA salt
TEA salt
1.00
1.16
1.21
1.02
1.01
1.02
1.05
2.04
2.19
2.85
3.01
3.18
3.06
9.66
6.00
5.99
5.34
5.33
5.32
5.30
5.27
5.24
5.22
4.63
4.62
4.62
4.61
4.35
4.33
4.32
4.30
4.18
4.18
4.17
4.17
4.16
4.15
4.14
4.14
4.08
4.08
4.05
4.05
3.94
3.90
3.89
3.88
3.85
0.22

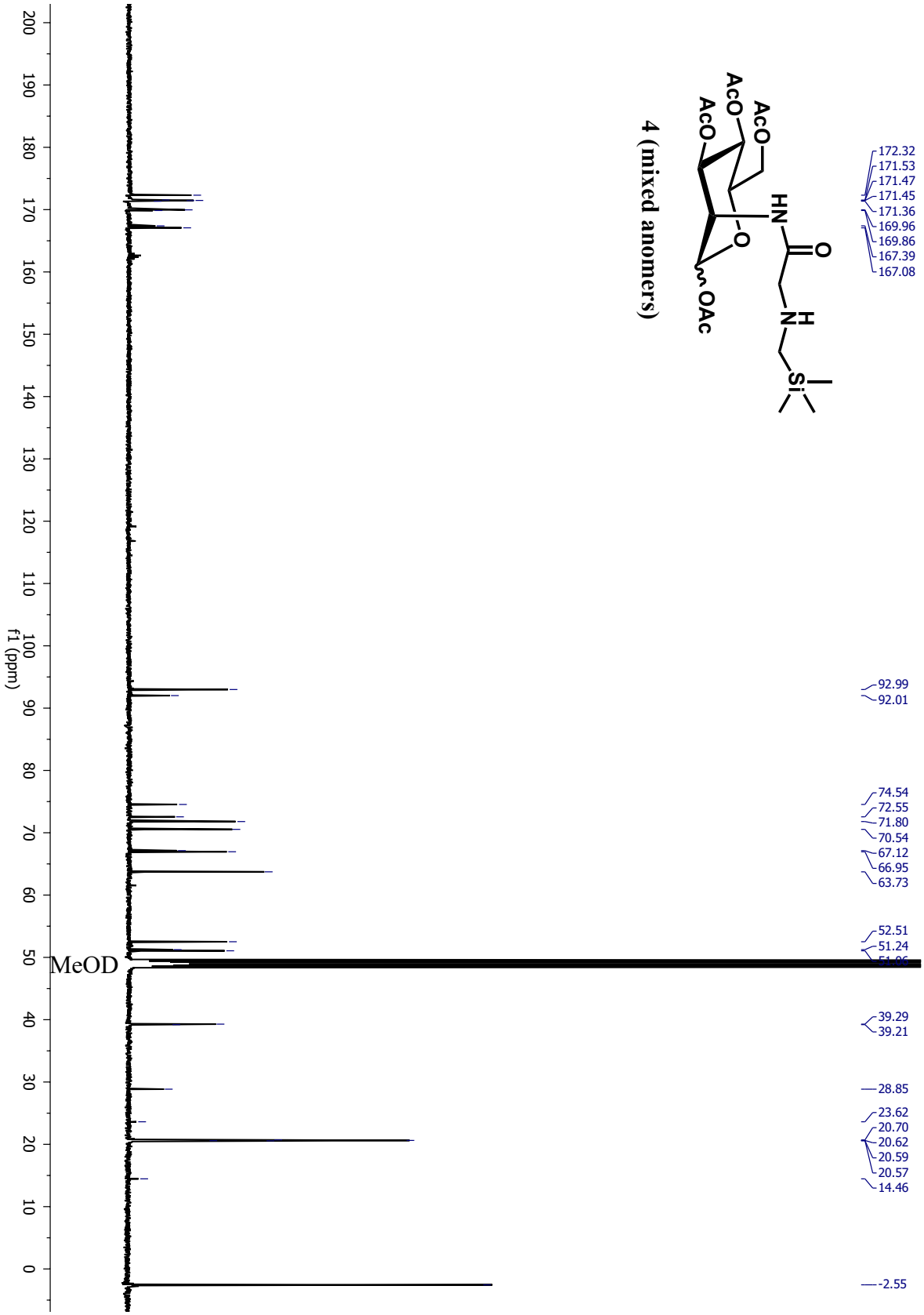

4 (β-anomer)
AcO
AcO
AcO
OAc
HN
O
H
N
Si
f1 (ppm)
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 −0.5
1.00
1.11
1.34
2.03
1.07
1.08
1.04
1.91
1.90
8.34
2.83
8.40
TEA salt
TEA salt
water
MeOD
5.98
5.97
5.25
5.24
5.23
5.22
5.15
5.13
5.10
4.34
4.33
4.31
4.30
4.14
4.13
4.11
4.10
3.93
3.93
3.63
3.62
3.60
3.58
2.54
2.06
2.06
2.05
1.99
1.20
1.18
1.16
0.23

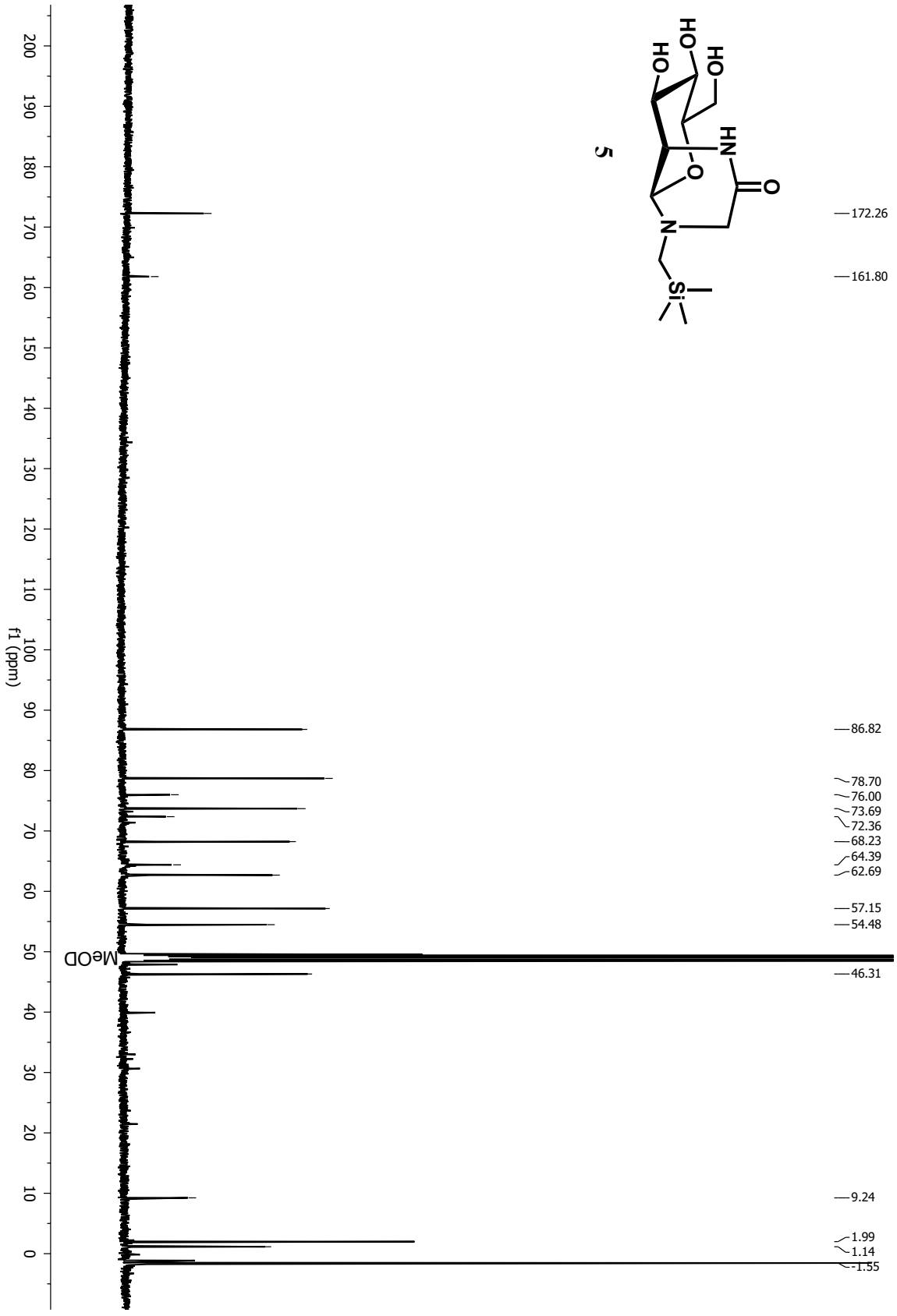

5
HO
HO
HO
HN
O
O
N
Si
D2O
acetate
grease
acetate
f1 (ppm)
1.00
1.19
1.14
2.39
0.32
0.93
1.70
0.76
0.53
1.21
1.15
1.15
9.22
4.39
4.38
3.87
3.86
3.84
3.83
3.80
3.80
3.79
3.79
3.70
3.69
3.68
3.67
3.66
3.65
3.40
3.38
3.35
3.34
3.27
3.23
3.21
3.21
3.20
3.19
3.19
3.18
3.17
3.17
2.44
2.40
2.26
2.22
0.09

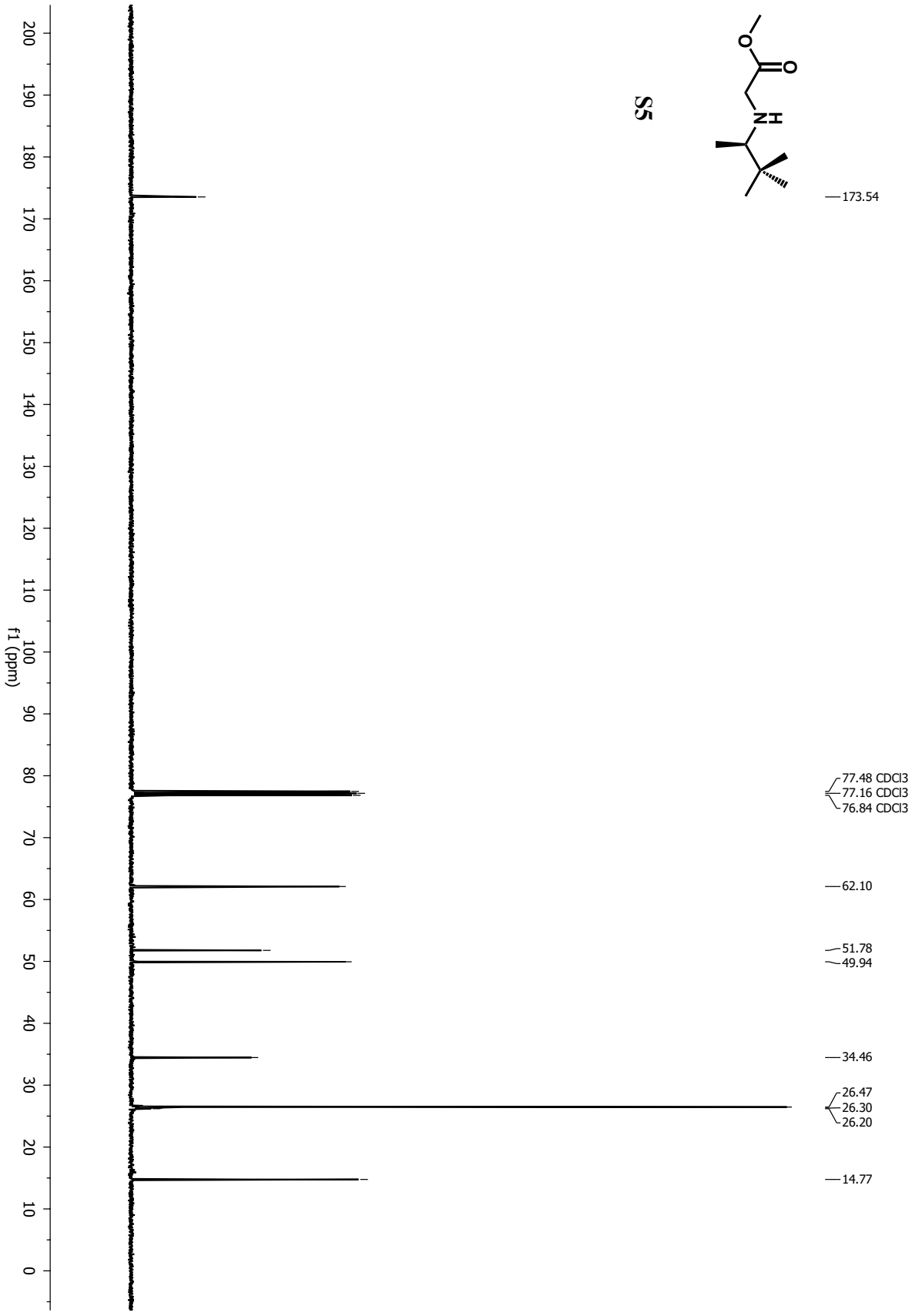

S5
f1 (ppm)
2.86
2.07
1.03
2.97
9.00
3.70
3.70
3.69
3.49
3.49
3.48
3.45
3.44
3.44
3.36
3.36
3.36
3.32
3.32
3.31
2.24
2.24
2.23
2.22
2.22
2.22
2.21
0.95
0.93
0.89
0.88
0.88

^{13}C NMR Spectra




4 (mixed anomers)
AcO
AcO
AcO
AcO
O
HN
O
H
N
Si
OAc
172.32
171.53
171.47
171.45
171.36
169.96
169.86
167.39
167.08
92.99
92.01
74.54
72.55
71.80
70.54
67.12
66.95
63.73
52.51
51.24
MeOD
39.29
39.21
28.85
23.62
20.70
20.62
20.59
20.57
14.46
-2.55
f1 (ppm)


5
HO
HO
HO
HN
O
O
N
Si
172.26
161.80
86.82
78.70
76.00
73.69
72.36
68.23
64.39
62.69
57.15
54.48
MeOD
46.31
9.24
1.99
1.14
1.55
f1 (ppm)


S5
173.54
77.48 CDCl3
77.16 CDCl3
76.84 CDCl3
62.10
51.78
49.94
34.46
26.47
26.30
26.20
14.77
f1 (ppm)

References:

1. Demchenko, A. P., Tang, K. C. & Chou, P. T. Excited-state proton coupled charge transfer modulated by molecular structure and media polarization. *Chem Soc Rev* **2013**, *42*, 1379–1408.
2. Togasaki, K., Arai, T. & Nishimura, Y. Opposite substituent effects in the ground and excited states on the acidity of N-H fragments involved in proton transfer reaction in aromatic urea compounds. *Photochem Photobiol Sci* **2021**, *20*, 523–532.
3. Dawson, W. R. & Windsor, M. W. Fluorescence Yields of Aromatic Compounds 3251 Fluorescence Yields of Aromatic Compounds. *J. Phys. Chem.* **1968,** *72*, 3251-3260.
4. Gottlieb, H. E.; Kotlyar, V. A.; Nudelman, A. NMR Chemical Shifts of Common Laboratory Solvents as Trace Impurities. *Journal of Organic Chemistry* **1997**, *62*, 7512–7515.
5. Badir, S. O.; Sim, J.; Billings, K.; Csakai, A.; Zhang, X.; Dong, W.; Molander, G. A. Multifunctional Building Blocks Compatible with Photoredox-Mediated Alkylation for DNA-Encoded Library Synthesis. *Organic Letters* **2020**, *22*, 1046–1051.