Supporting Information

Control of RNA with quinone methide reversible acylating reagents

Hyun Shin Park, Biswarup Jash, Lu Xiao, Yong Woong Jun and Eric T. Kool*
Department of Chemistry, Stanford University, Stanford, CA 94305
*Author to whom correspondence should be addressed: kool@stanford.edu

Table of Contents

Instrumentation and Reagents

Instrumentation

^{1}H- and ^{13}C-NMR spectra were recorded on Varian Mercury 400 MHz NMR or Varian Inova 300 MHz spectrometer. ^{1}H- and ^{13}C-NMR spectra were internally referenced to the residual solvent signal. Matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectra (MS) were acquired using a Bruker MALDI Microflex LRF instrument using an AnchorChip™ target. The 3-hydroxypicolinic acid matrix was prepared with the addition of diammonium hydrogen citrate and trifluoroacetic acid (TFA) following the manufacturer's manual. Reverse-phase HPLC was performed on a SunFire preparative C18 column (250 x 10 mm). The flow rate was maintained throughout at 1.5 mL/min, with detection at λ =260 nm. The obtained mass spectra were analyzed with MestReNova software (v. 14). Liquid chromatography MS and high-resolution MS analysis were performed by Vincent Coates Foundation Mass Spectrometry Laboratory at Stanford University. Fluorescence studies were performed on a Fluorolog 3-11 instrument (Jobin Yvon-SPEX). EGFP mRNA expression in HeLa cells was measured with Tecan Infinite M1000 plate reader at the High-throughput Biosciences Center at Stanford University.

Chemical Reagents

Chemical reagents were purchased from Sigma-Aldrich, Ambeed, Oakwood Chemicals, and Combi-blocks and were used without further purification. Vinyl chloroformate was purchased from Fluorochem. (4-chloropyridin-3-yl)methanol was purchased from Enamine. TCO-OH equatorial and axial were purchased from Axis Pharm. 2DPBM[1], 2DPBM-S[1], and HBC[2] were synthesized following published procedures. The precursor carboxylic acids and DMSO stocks of 1 or 2M NAI-N_3[3] and AEGAI[4] were prepared according to the published procedures. Phosphine solutions in DMSO were freshly prepared or routinely checked with ^{31}P NMR for oxidation.

Nucleic Acids

Cy5-tRF-3005 (5'-Cy5-AUC CUG CCG ACU ACGCCA-3', RNA) was ordered from Stanford Protein and Nucleic Acid Facility (PAN). RNA oligomers were purified by high performance liquid chromatography (HPLC) or polyacrylamide gel electrophoresis (PAGE). eGFP mRNA (CleanCap® EGFP mRNA (5moU), L-7201) was ordered from Trilink Biotechnologies. RNA concentrations were measured using a NanoDrop One microvolume UV-Vis spectrophotometer. When available, molar concentrations were calculated based on Cy5 absorbance.

NMR Experiments

Measurement of hydrolysis half-life of acylation agents

In an NMR tube, the imidazole carbamate compounds (final concentration 10 mM) were dissolved in a 1:1 mixture of DMSO-d_6 and D_2O at room temperature, and hydrolysis was over monitored by NMR. The half-lives were approximated by fitting the obtained data to an exponential decay curve with the following equation, $f(x)=Ae^{-bx}$ and $t_{1/2} = \ln(2)/b$.

Chemical deacylation of RNA 2'-ester or carbonate mimics

RNA 2'-ester or carbonate mimic compounds and TCO-OH (equatorial) were prepared as 62.8 mM DMSO-d_6 stocks. THPP and TPPMS were prepared as 628 mM and 126 mM DMSO-d_6 stocks, respectively. In an NMR tube, 50 µL of the acyl compound stock was added to 650 µL D_2O, and an NMR spectrum was collected for t = 0. To initiate de-acylation, 50 µL of the phosphine (2 or 10 equiv.) or TCO-OH (1 equiv.) stock was added. The resulting solution was mixed, incubated at room temperature for phosphines or 37°C for TCO-OH, and monitored with NMR.

Hydrolytic stability of RNA 2'-ester or carbonate mimics

In an NMR tube, 50 µL of the 62.8 mM of RNA 2'-ester or carbonate mimic solution (DMSO-d_6) was added to 700 µL D_2O. The resulting mixture was mixed, incubated at 37°C, and monitored with NMR.

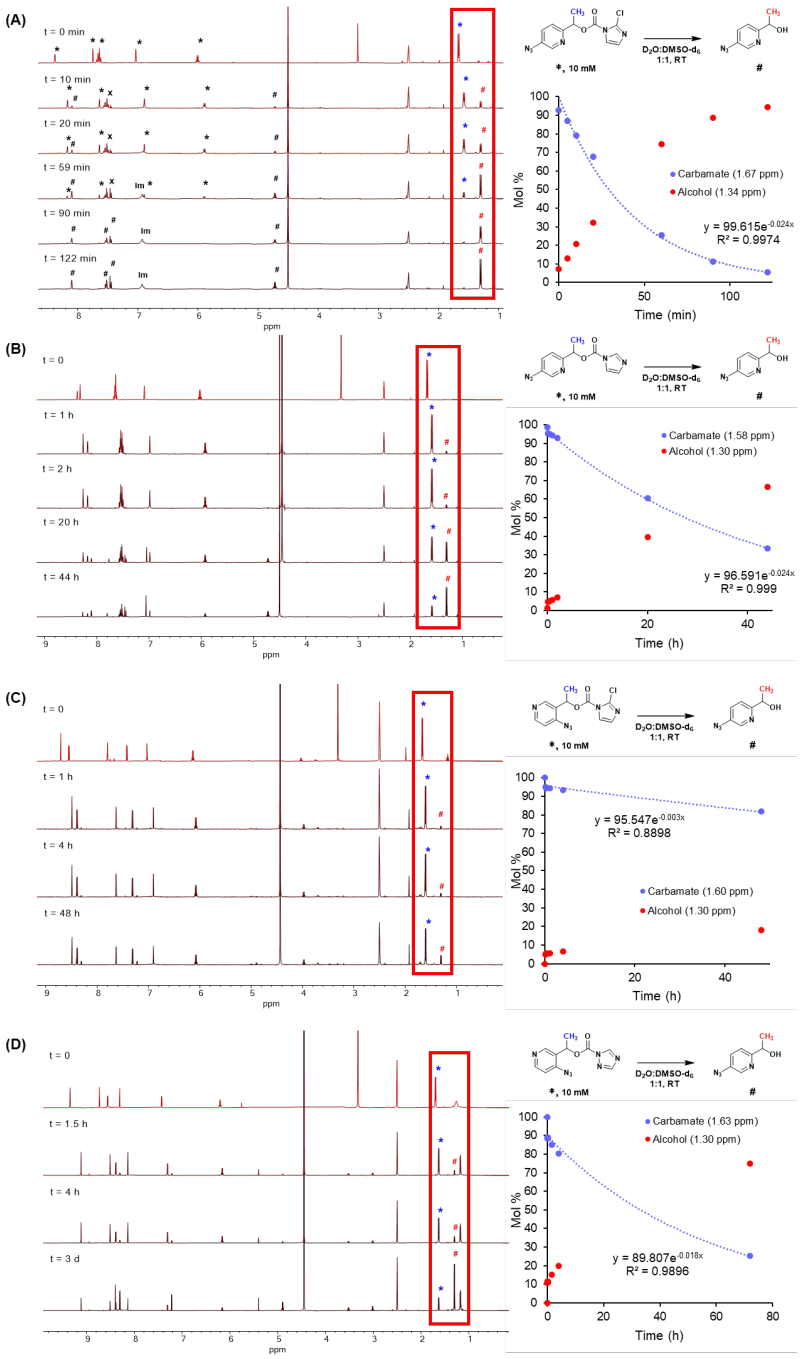


Figure S1. NMR analysis of hydrolysis rate of imidazole carbamate acylating agents. (A) Stacked NMR plots of **A-3** in 1:1 DMSO-d_6:D_2O. Hydrolysis is monitored with the color-coded methyl peaks (blue: carbamate, 1.67 ppm; red: alcohol, 1.34 ppm) in the indicated red box. Hydrolysis half-life was approximately 30 minutes. (B) Stacked NMR plots of hydrolysis of **A-5** in 1:1 DMSO-d_6:D_2O. Hydrolysis is monitored with the color-coded methyl peaks (blue: carbamate, 1.58 ppm; red: alcohol, 1.30 ppm) in the indicated red box. Hydrolysis half-life was approximately 30 hours. (C) Stacked NMR plots of hydrolysis of **A-6** in 1:1 DMSO-d_6:D_2O. Hydrolysis is monitored with the color-coded methyl peaks (blue: carbamate, 1.60 ppm; red: alcohol, 1.30 ppm) in the indicated red box. Hydrolysis half-life well exceeded 48 hours. (D) Stacked NMR plots of hydrolysis of **A-7** in 1:1 DMSO-d_6:D_2O. Hydrolysis is monitored with the color-coded methyl peaks (blue: carbamate, 1.63 ppm; red: alcohol, 1.30 ppm) in the indicated red box. Hydrolysis half-life was approximately 40 hours. *: imidazole carbamates, #: hydrolyzed alcohol product, **X**: mixture of * and #

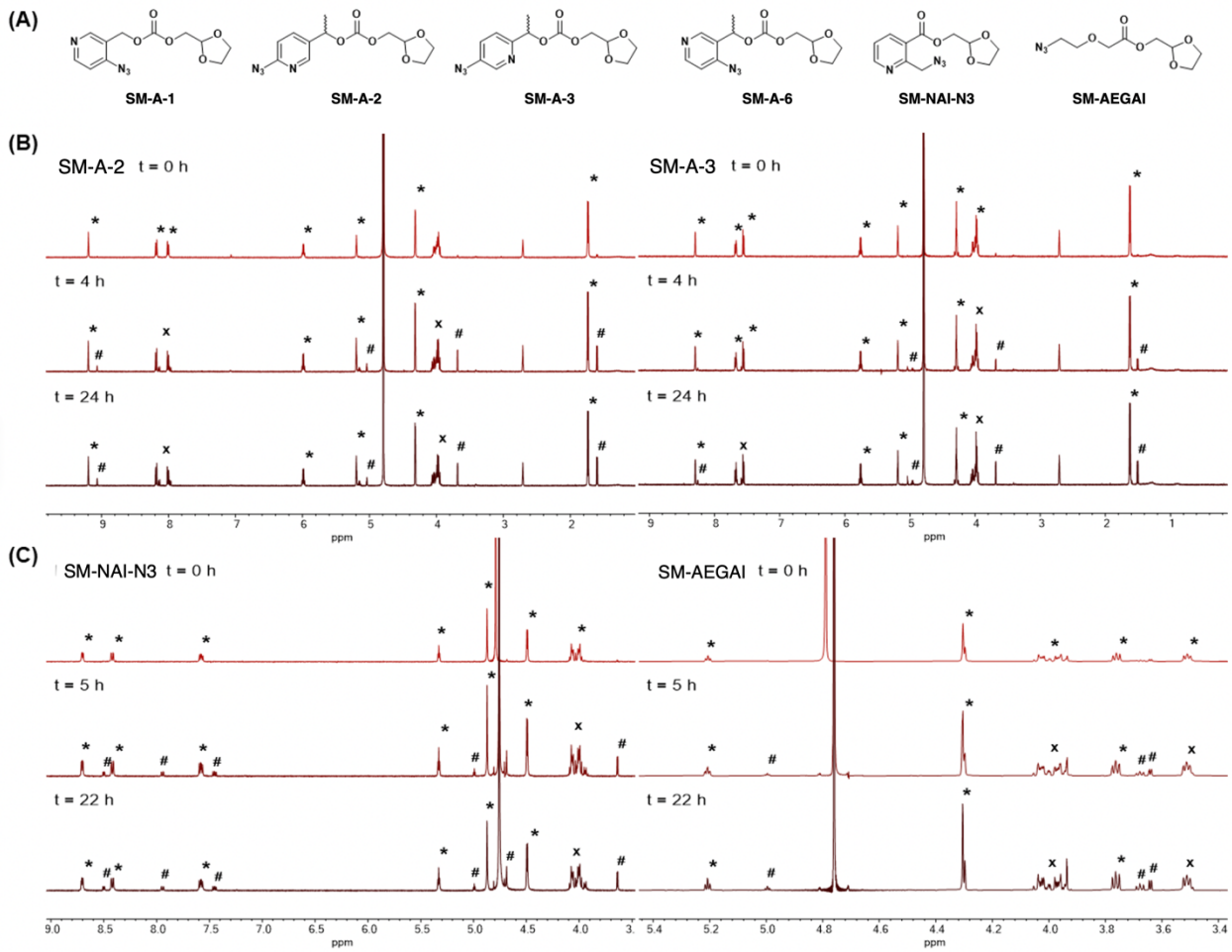


Figure S2. NMR analysis of hydrolytic stability of adducts: RNA 2'-esters or carbonate mimics. (A) Structures of RNA 2'-ester or carbonate mimics. (B) Hydrolytic stability of carbonate mimics. 4.2 mM of RNA 2'-carbonate mimics were incubated in D_2O at 37°C and analyzed with NMR at the indicated times. (C) Hydrolytic stability of ester mimics. 4.2 mM of RNA 2'-ester mimics were incubated in D_2O at 37°C and analyzed with NMR at the indicated times. *: carbonate or ester mimic, #: hydrolysis products, **X**: mixture of * and #.

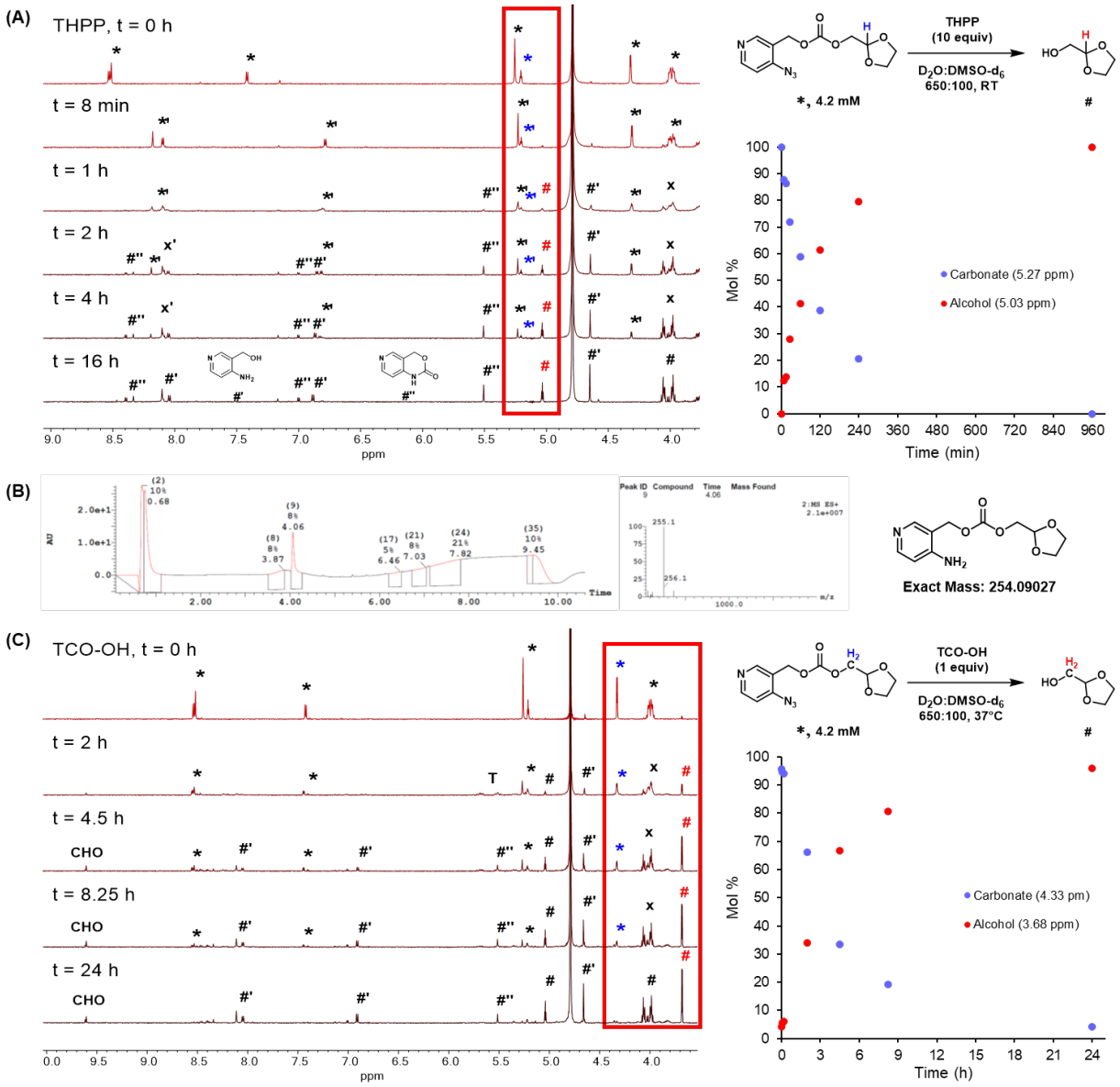


Figure S3. NMR analysis of deacylation of RNA 2' **A-1** carbonate model adducts. (A) THPP induced deacylation. Reversal is monitored with the color-coded methine peaks (blue: carbonate, 5.27 ppm; red: alcohol, 5.03 ppm) in the indicated red box. Upon phosphine addition, the azido group is reduced rapidly by 8 minutes as shown by shift in the aromatic peaks (7-9 ppm). (B) LC/MS confirmation of the **SM-A-1** amino intermediate during phosphine induced deacylation. 5.8 mM of NMR-3 was treated with 38.3 mM of THPP in water for 5 minutes at room temperature, then diluted with water, and analyzed with LC/MS. (C) TCO-OH eq induced deacylation. Reversal is monitored with the color-coded methylene peaks (blue: carbonate, 4.33 ppm; red: alcohol, 3.68 ppm) in the indicated red box. *: **SM-A-1**, *': **SM-A-1** amino intermediate, #: released alcohol, #': released product 1 (structure above), #'': released product 2 (structure above), **T**: TCO-OH, **CHO**: cycloheptane carboxaldehyde, **X**: mixture of */*' and #.

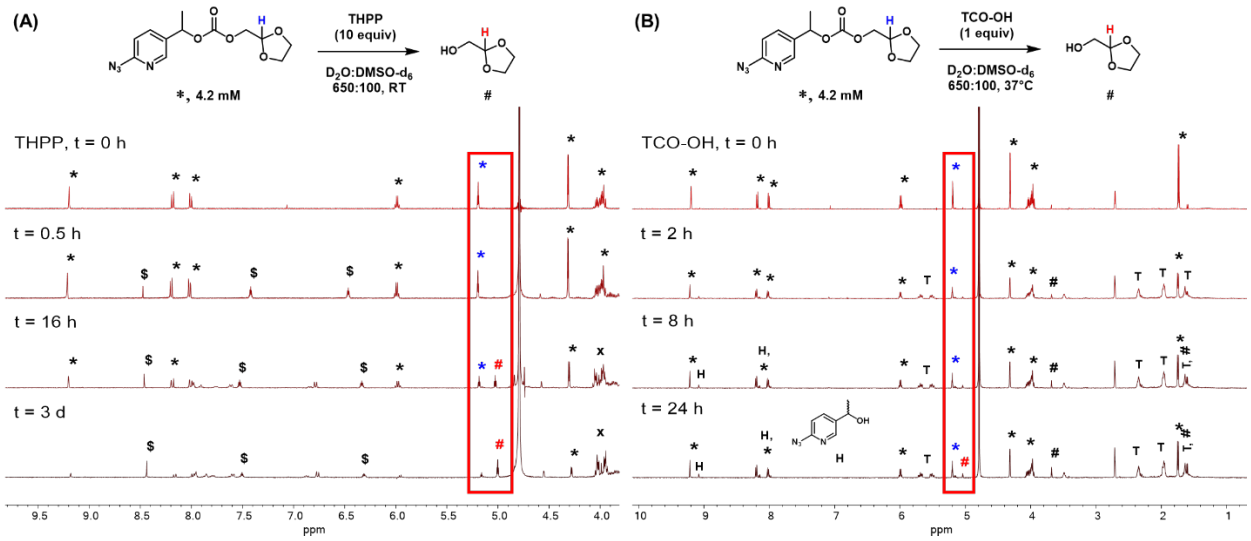


Figure S4. NMR analysis of deacylation of RNA 2' **A-2** carbonate model adducts. (A) THPP-induced deacylation. Reversal is monitored with the color-coded methine peaks (blue: carbonate, 5.19 ppm; red: alcohol, 5.03 ppm) in the indicated red box. (B) TCO-OH equatorial induced deacylation. Reversal is monitored with the color-coded methine peaks (blue: carbonate, 5.19 ppm; red: alcohol, 5.03 ppm) in the indicated red box. No reaction with TCO-OH is observed, and the released alcohol # is likely from background carbonate hydrolysis. *: **SM-A-2**, #: released alcohol, **T**: TCO-OH, **H**: alcohol **A-2-OH**, **$**: impurities from THPP stock.

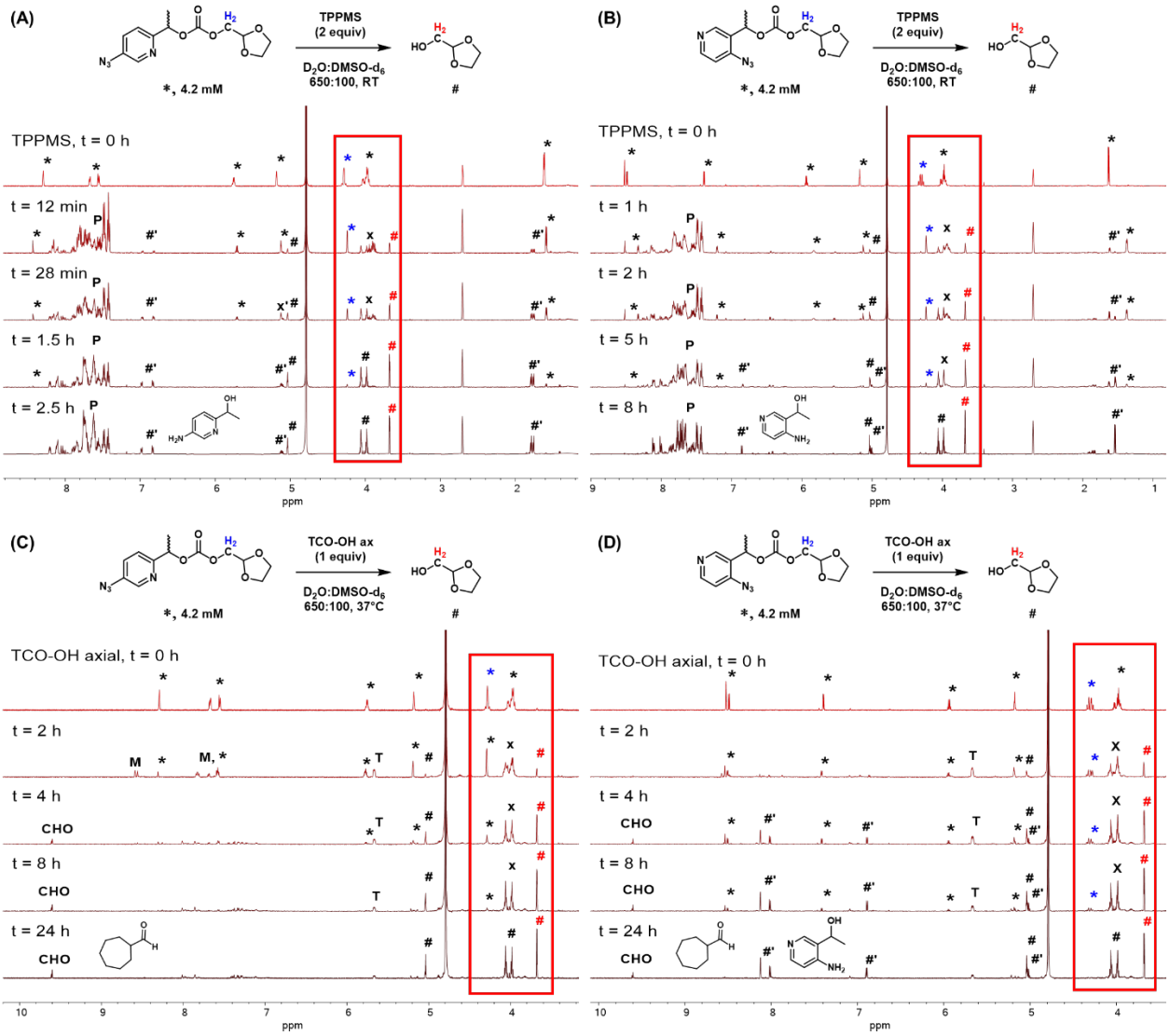


Figure S5. NMR analysis of deacylation of RNA 2' **A-3** and **6** carbonate model adducts. (A) TPPMS induced deacylation of **SM-A-3**. (B) TPPMS induced deacylation of **SM-A-6**. (C) TCO-OH ax induced deacylation of **SM-A-3**. (D) TCO-OH ax induced deacylation of **SM-A-6**. Reversal is monitored with the color-coded methylene peaks in the indicated red box. *: **SM-A-3** or **6**, #: released alcohol, X: mixture of * and #, #': released pyridinone methide, T: TCO-OH axial, M: unidentified intermediate, CHO: cycloheptane carboxaldehyde, P: TPPMS and phosphine oxide.

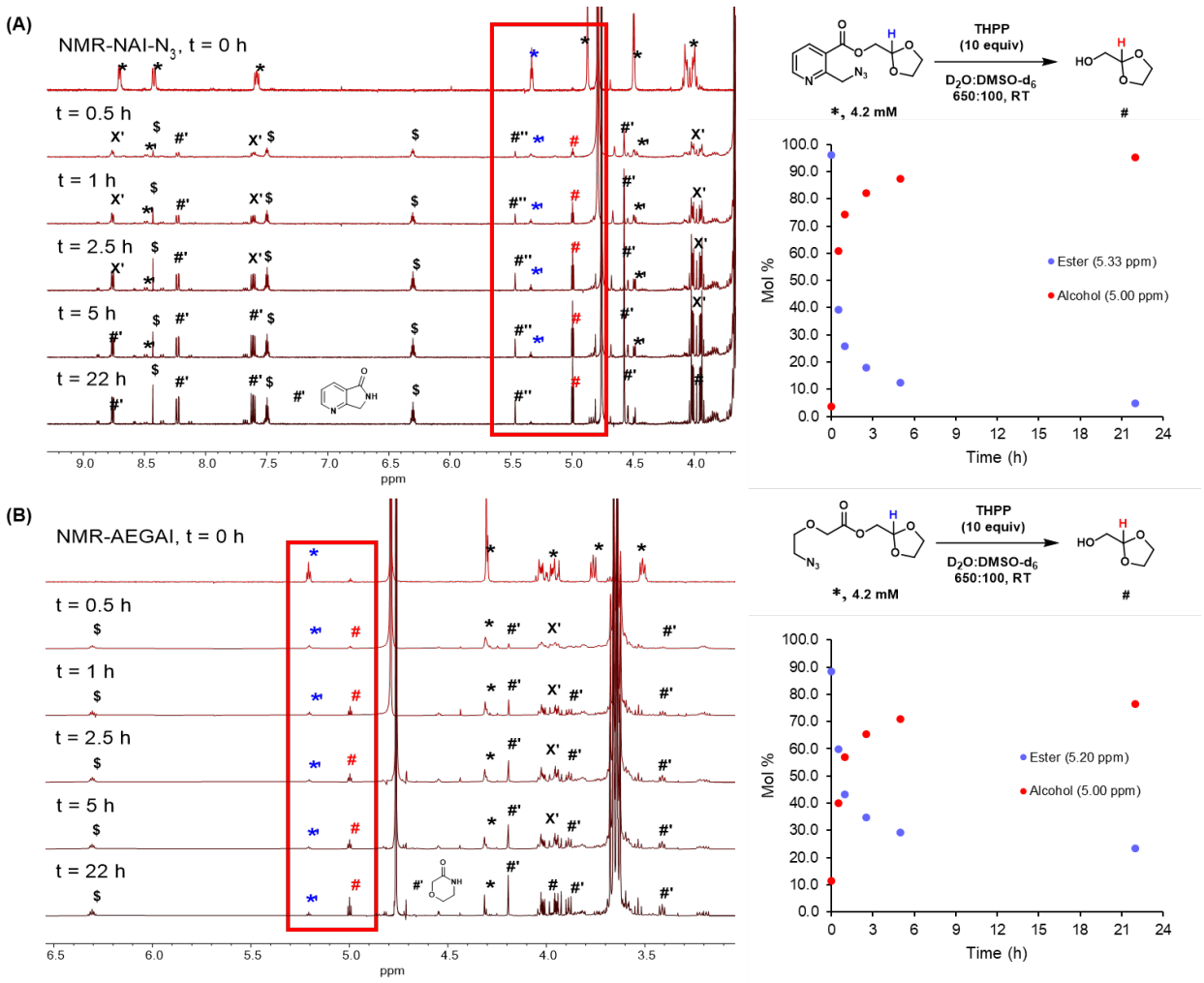


Figure S6. NMR analysis of deacylation of RNA 2'-ester model adducts. (A) Deacylation of **SM-NAI-N$_3$** with THPP. Reversal is monitored with the color-coded methine peaks (blue: ester, 5.33 ppm; red: alcohol, 5.00 ppm) in the indicated red box. Upon phosphine addition, the azido group is reduced rapidly by 0.5 h as shown by the disappearance of the azidomethylene peak at 4.88 ppm (amino intermediate not observed due to overlap with D_2O). (B) Deacylation of **SM-AEGAI** with THPP. Reversal is monitored with the color-coded methine peaks (blue: ester, 5.20 ppm; red: alcohol, 5.00 ppm) in the indicated red box. Upon phosphine addition, the azido group is reduced rapidly by 0.5 h as shown by the disappearance of the methylenes near the azide at 3.76 and 3.51 ppm (amino intermediate not observed due to overlap with THPP). *: RNA 2'-ester mimics, #: released alcohol, #': released lactam products, #'': unidentified minor product, **X**: mixture of *' and #, **$**: impurities from THPP stock.

Cy5-tRF-3005 Oligonucleotide Cloaking & Uncloaking

General RNA polyacylation (cloaking) procedure

6 μM RNA (36 ng/μL) was treated with 0.1 M acylating probe (1 M stock in DMSO) in 70% DMSO RNase-free water solution for 4 h at room temperature in a total volume of 50 μL. The polyacylated RNA was then purified via ethanol precipitation via the following procedure: the reaction was quenched with addition of 200 ul RNase-free water to dilute away DMSO. To the diluted mixture, 25 μL of 3 M NaOAc pH 5.2, 0.5 μL of 20 mg/mL glycogen, and 1031 μL of absolute EtOH were added. The mixture was preciptiated at -80 °C overnight. The RNA pellet was obtained by centrifuging (21000 RCF) for 60 min at 4 °C and removing the supernatant. The pellet was washed with 75% EtOH and centrifuged (21000 RCF) for 5 min at room temperature. The supernatant was removed, and the RNA was air dried for 20 minutes, dissolved in RNase-free water, and stored at -20 °C until analysis by MALDI-TOF MS.

General RNA deacylation (uncloaking) procedure

1 μM polyacylated RNA (ca. 6 ng/μL) was treated with 0.5-5 mM uncloaking agents (phosphine, tetrazine, and TCO-OH 10X stocks in DMSO) in 300 mM Tris-HCl buffer pH 7.5 (10% DMSO) in a total volume of 100 μL. The reaction was incubated for 1 to 4 h at 37 °C. The uncloaked RNA was purified via ethanol precipitation as described above. To quench the reaction mixture, 10 μL of 3 M NaOAc pH 5.2, 0.5 μL of 20 mg/ml glycogen, and 414 μL of absolute EtOH were added. The remaining steps are identical to the RNA cloaking method above.

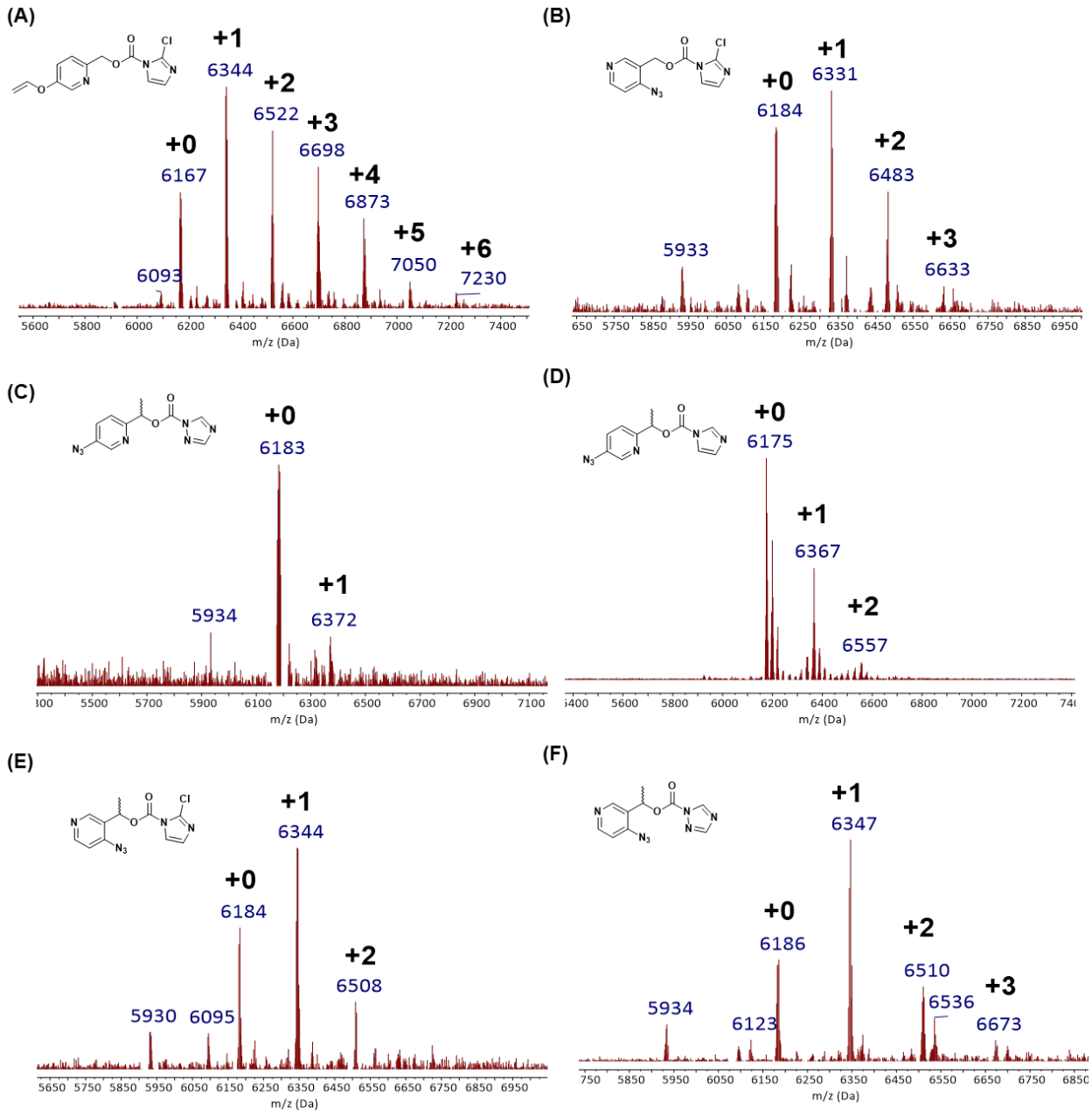


Figure S7. Polyacylation efficiency of the novel acylating agents with model RNA oligonucleotide (Cy5-tRF-3005). (A) MALDI-TOF spectrum of **T-1** polyacylated RNA. 6 µM RNA was treated with 0.2 M of acylating agent in a 2:8 water:DMSO for 4 h at 37 °C. (B) MALDI-TOF spectrum of **A-1** polyacylated RNA. 6 µM RNA was treated with 0.1 M of cloaking agent in a 2:8 water:DMSO for 4 h at 37 °C. The azido groups are observed as amino groups due to reduction during MALDI-TOF analysis.[5–7] (C) MALDI-TOF spectrum of **A-4** polyacylated RNA. 6 µM RNA was treated with 0.1 M of cloaking agent in a 4:6 water:DMSO for 4 h at 37 °C. (D) MALDI-TOF spectrum of **A-5** polyacylated RNA. 6 µM RNA was treated with 0.1 M of cloaking agent in a 3:7 water:DMSO for 4 h at 37 °C. (E) MALDI-TOF spectrum of **A-6** polyacylated RNA. 6 µM RNA was treated with 0.1 M of cloaking agent in a 3:7 water:DMSO for 4 h at 37 °C. (F) MALDI-TOF spectrum of **A-7** polyacylated RNA. 6 µM RNA was treated with 0.1 M of cloaking agent in a 3:7 water:DMSO for 4 h at 37 °C. Black numbers indicate the number of adducts per RNA.

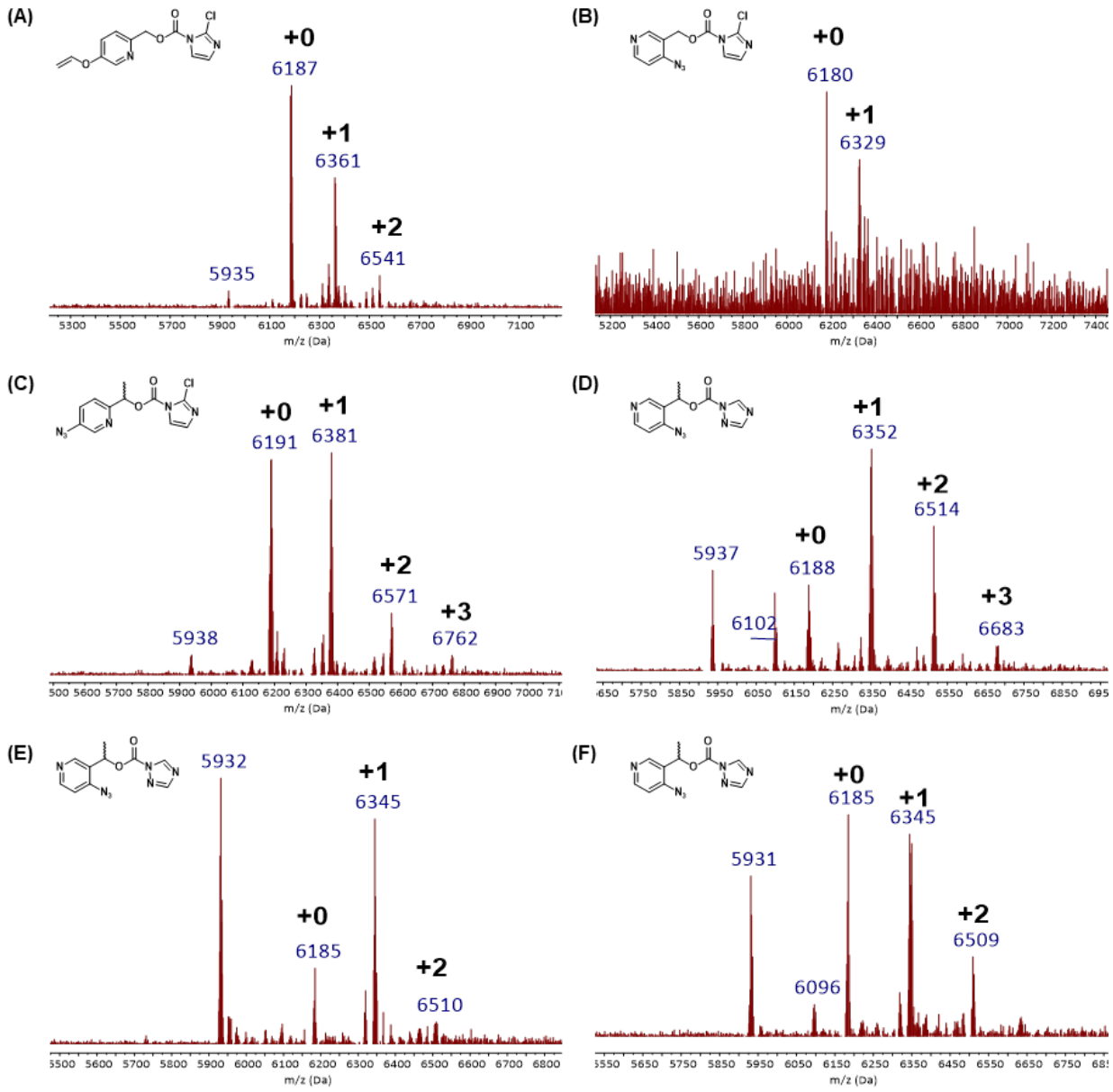


Figure S8. Deacylation efficiency of the novel acylating agents with model RNA oligonucleotide, Cy5-tRF-3005. Refer to Fig. S7 for starting points. (A) MALDI-TOF spectrum of **T-1** polyacylated RNA reversed with THPP. 1 µM of cloaked RNA was treated with 1 mM Py_2-Tz in 300 mM Tris-HCl buffer pH 7.5 (20% DMSO) for 16 h at 37 °C. (B) MALDI-TOF spectrum of **A-1** polacylated RNA reversed with THPP. 1 µM of cloaked RNA was treated with 5 mM THPP in 300 mM Tris-HCl buffer pH 7.5 (10% DMSO) for 4 h at 37 °C. (C) MALDI-TOF spectrum of **A-3** polyacylated RNA incubated in buffer without uncloaking agents. 1 µM of cloaked RNA was incubated in 50 mM Tris-HCl buffer pH 7.5 (10% DMSO) for 4 h at 37 °C. (D) & (E) MALDI-TOF spectra of **A-7** polyacylated RNA reversed with TPPMS. 1 µM RNA was treated with 0.5 mM TPPMS in 50 mM Tris-HCl buffer pH 7.5 (10% DMSO) for 1h (D) or 5 mM TPPMS in 300 mM Tris-HCl buffer pH 7.5 (10% DMSO) for 4 h (E) at 37 °C. (F) MALDI-TOF spectra of **A-7** polyacylated RNA reversed with TCO-OH eq. 1 µM RNA was treated with 5 mM TCO-OH eq in 50 mM Tris-HCl buffer pH 7.5 (10% DMSO) for 4 h at 37 °C. Black numbers indicate the number of adducts.

mRNA Polyacylation Experiments

General mRNA polyacylation (cloaking) procedure

35 ng/μL EGFP mRNA was treated with 50 mM **A-3** (0.25 M stock in DMSO) in 20% DMSO MOPS SHAPE buffer (100 mM MOPS pH 7.5, 6 mM $MgCl_2$, 100 mM NaCl) in a total volume of 20 μL. The fine cloudy suspension was incubated for 3 h at room temperature, which clears over time. The cloaked RNA was purified by ethanol precipitation. The reaction was quenched by the addition of 9 volumes (180 μL) of precipitation solution (0.33 M NaOAc, pH 5.2, glycogen 0.2 mg/mL) and 30 volumes (600 μL) of absolute EtOH. The resulting mixture was incubated at -80 °C overnight. The remaining steps are identical to RNA oligonucleotide cloaking above. For mRNA cloaked for *in vitro* translation, the cloaked RNA was purified using RNA Clean & Concentrator™-5 (Zymo Research) following the manufacturer's protocol due to glycogen inhibiting translation.

In vitro deacylation (uncloaking) procedure

23.3 ng/μL EGFP mRNA was treated with 10 mM uncloaking agent (phosphines and TCO-OH 10X stocks in DMSO) in 20% DMSO 2X PBS pH 7.4 for 4 h at 37 °C in total volume of 17.5 μL. The cloaked RNA was purified with ethanol precipitation or RNA Clean & Concentrator™-5.

mRNA *in vitro* translation

mRNA was expressed *in vitro* with wheat germ extract (WGE, Promega L4380) with optimization of KOAc concentration following the manufacturer's protocol. On a 384 well plate, 200 ng of EGFP mRNA was expressed in reaction volume of 25 μL including additional 2 μL of 1 M KOAc. The reaction was incubated at 25 ℃ for 3 hours. The resulting fluorescence signals were recorded on a Fluoroskan Ascent™ Microplate Fluorometer (λ_{ex} = 485 nm, λ_{em} = 538 nm) at 25 °C.

For directly uncloaking in WGE, 200 ng of cloaked EGFP mRNA was added to WGE translation mixture at the volume of 21 μL and then 4 μL of 6.25X uncloaking agent in water was added. The reaction was incubated at 25 ℃ for 3 hours, and the fluorescence was measured as above.

mRNA transfection and cellular uncloaking

In a 96-well plate, HeLa cells were seeded overnight at a concentration of $1.5x10^4$ cells per well. The cells were incubated in supplemented DMEM culture medium (10% FBS, Gibco, 100 U/ml Penicillin/Streptomycin, Thermo Fisher Scientific) at 37 °C and 5% CO_2 with 95% humidity. Old medium was aspirated away and replenished with 100 μL of fresh culture medium. mRNA lipofectamine complex (100 ng mRNA in 2 μL water, 10 μL of OptiMEM medium (Gibco), 0.3 μL of Lipofectamine™ messengerMAX™ (Thermo Fisher Scientific) was prepared following the manufacturer's protocol. The transfection mixture was added dropwise to each well containing culture media and incubated for 16 h. EGFP fluorescence was measured with a microplate reader Tecan Infinite M1000 (λ_{ex} 488 ± 5 nm, λ_{em} 507 ± 5 nm). Fluorescence from cells treated with only lipofectamine was subtracted as the background, and the resulting values were normalized to untreated mRNA or DMSO mock treated conditions. All experiments were conducted with at least 3 replications.

For *in cellulo* uncloaking, the seeded cells were washed with DMEM media (no FBS or antibiotics) and then transfected for 4 h in DMEM media. The transfection medium was aspirated away and replenished with 100 μL of culture medium (1% DMSO) with 1 or 2 mM phosphine or TCO-OH. The cells were incubated for an additional 4 h until measurement.

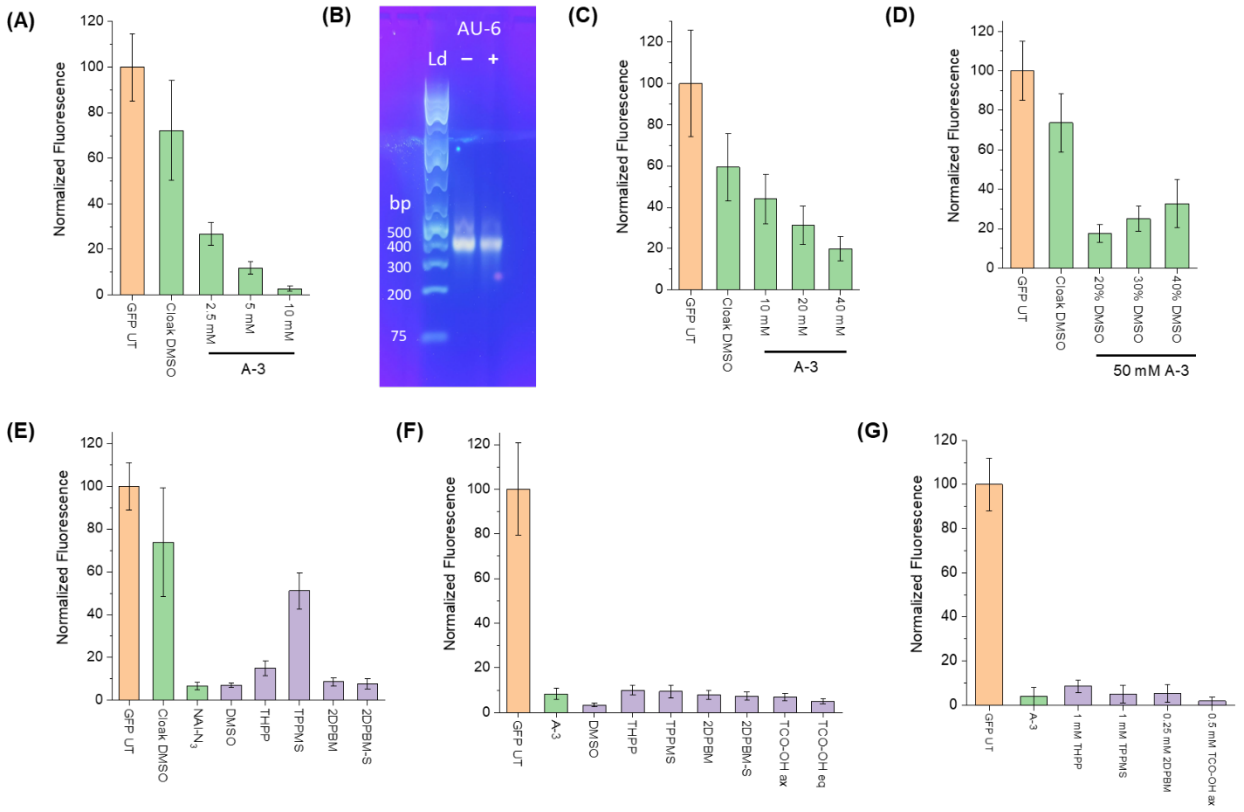


Figure S9. Modulation of EGFP mRNA expression in HeLa cells with reversible polyacylation. (A) Optimization of **A-3** concentration for EGFP mRNA acylation in water. 35 ng/μL EGFP mRNA was treated with 2.5-10 mM **A-3** in 8:2 water:DMSO for 1 h at room temperature. The cloaked mRNA was transfected into HeLa cells via lipofection for 16 h, and the resulting fluorescence was measured with a microplate reader. (B) Agarose (7.5%) gel electrophoresis of **A-3** cloaked EGFP mRNA. Nucleic acids were visualized with SBYR gold. Ld indicates DNA ladder. EGFP mRNA is 996 nt long. (C) Optimization of **A-3** concentration for EGFP mRNA acylation in MOPS SHAPE buffer. 35 ng/μL EGFP mRNA was treated with 10-40 mM **A-3** in 8:2 buffer:DMSO for 2 h at room temperature. (D) Optimization of DMSO% for **A-3** acylation of EGFP mRNA in MOPS SHAPE buffer. 35 ng/μL EGFP mRNA was treated with 50 mM **A-3** in buffer with 20-40% DMSO for 3 h at room temperature. (E) *In vitro* phosphine screening with NAI-N_3 cloaked EGFP mRNA. 35 ng/μL EGFP mRNA was treated with 0.1 M NAI-N_3 in 9:1 MOPS SHAPE buffer:DMSO for 10 minutes. For *in vitro* deacylation, 23.3 ng/μL of cloaked EGFP mRNA was treated with 10 mM uncloaking agent in 8:2 2X PBS pH 7.4:DMSO for 4 h at 37 °C. (F) *In vitro* uncloaking of EGFP mRNA cloaked with **A-3** in water. 35 ng/μL EGFP mRNA was treated with 10 mM **A-3** in 8:2 water:DMSO for 1 h at room temperature. 23.3 ng/μL of cloaked EGFP mRNA was treated with 10 mM uncloaking agent in 1:1 PBS pH 7.4:DMSO for 4 h at 37 °C. (G) Cellular uncloaking of EGFP mRNA cloaked with **A-3** in water. 35 ng/μL EGFP mRNA was treated with 10 mM **A-3** in 8:2 water:DMSO for 1 h at room temperature. HeLa cells were transfected with the cloaked EGFP mRNA for 12 h and subsequently treated with uncloaking agents for 8 h. Error bars represent standard deviation. Orange: untreated RNA, green: cloaked RNA, purple: uncloaked RNA.

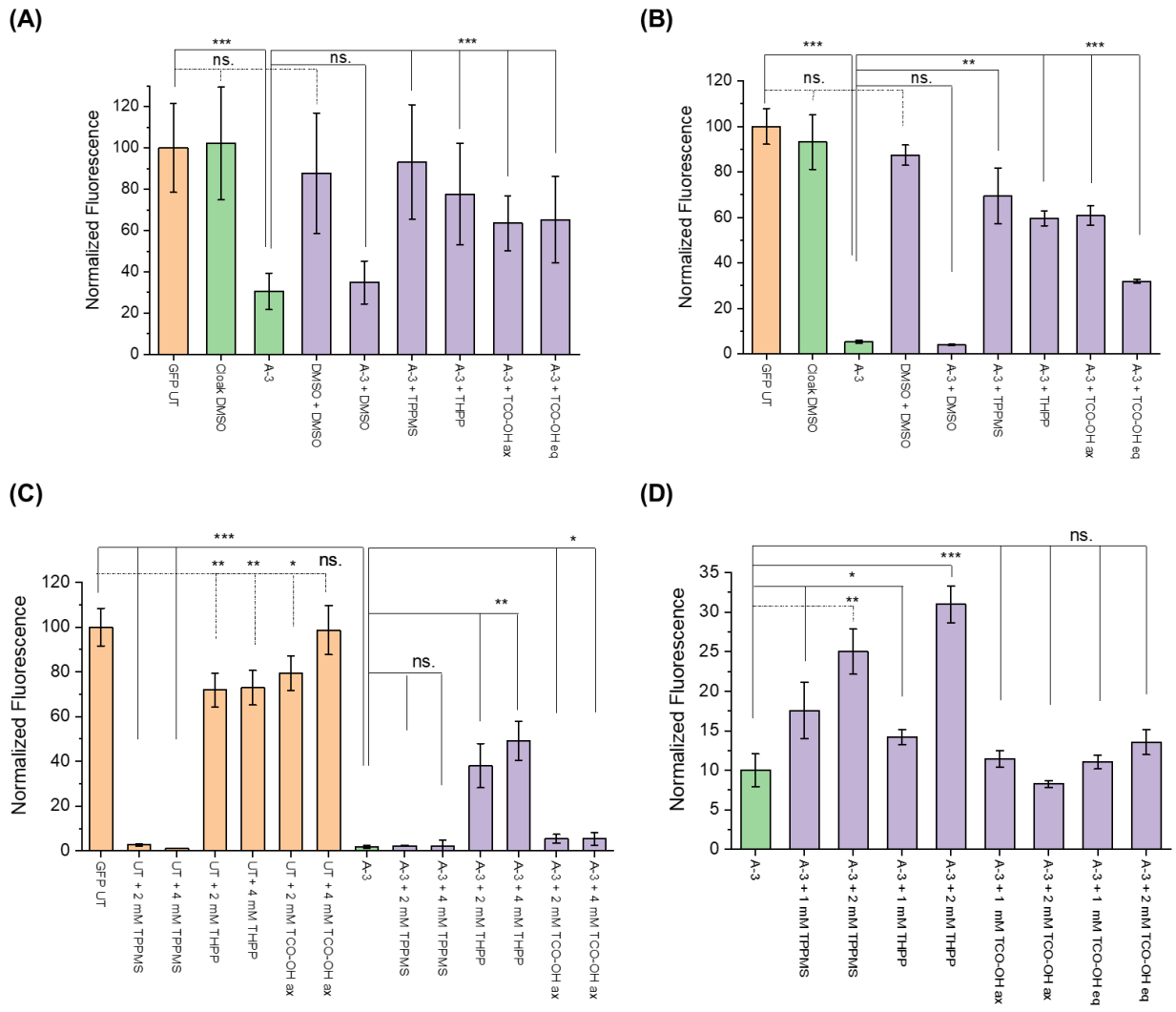


Figure S10. Control of EGFP mRNA translation with **A-3**. (A) Cellular expression of *in vitro* uncloaked EGFP mRNA. 23.3 ng/μL of **A-3** cloaked EGFP mRNA was treated with 10 mM uncloaking agent in 2x PBS pH 7.4 (20% DMSO) for 4 h at 37 °C. (B) *In vitro* translation of uncloaked EGFP mRNA. **A-3** cloaked and subsequently uncloaked EGFP mRNA was expressed with WGE for 3 hours at 25 °C. (C) Direct activation of **A-3** cloaked EGFP mRNA in WGE. **A-3** cloaked EGFP mRNA in WGE was treated with various reversal agents for 3 hours at 25 °C. (D) Cellular control of EGFP mRNA expression. **A-3** cloaked mRNA was transfected into HeLa cells for 4 hours and subsequently treated with various reversal agents for 4 hours. Error bars represent standard deviation and p-values: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, ns. – not significant. Orange: untreated RNA, green: cloaked RNA, purple: uncloaked RNA.

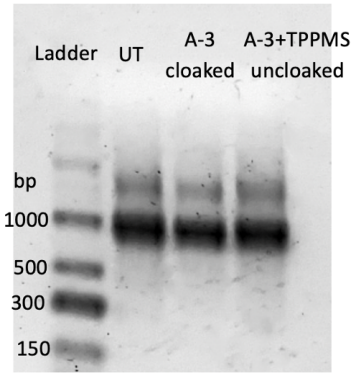


Figure S11. Agarose (1%) gel electrophoresis of **A-3** cloaked and uncloaked EGFP mRNA. Nucleic acids were visualized with SBYR gold. EGFP mRNA is 996nt long. UT: untreated mRNA, A-3: cloaked mRNA, A-3+TPPMS: uncloaked mRNA by treating with 2mM TPPMS.

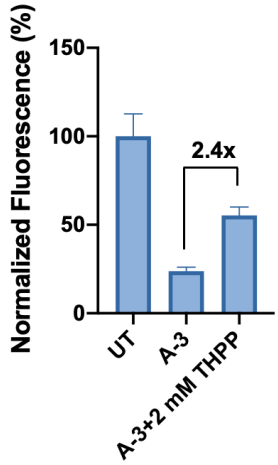


Figure S12. Cellular control of EGFP mRNA expression. A-3 cloaked mRNA was transfected into HeLa cells for 4 hours; cells were subsequently treated with 2mM THPP for 4 hours and then replaced with normal growth media for additional 17 h incubation before testing with plate reader.

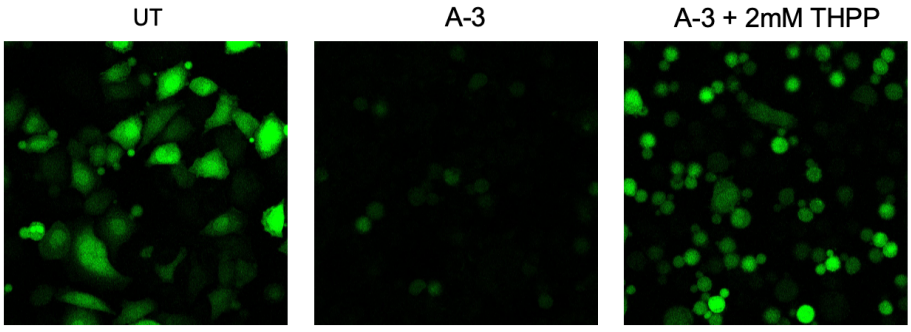


Figure S13. Microscopy images of HeLa cells following transfection of cloaked mRNA and phosphine treatment to activate EGFP expression. Cells were transfected with 10ng mRNA for 4 h and treated with 2mM THPP for 3 h before fixation and imaging. UT: untreated mRNA, A-3: cloaked mRNA, A-3+THPP: uncloaked mRNA.

Model studies

Acylation of DNA with A-3

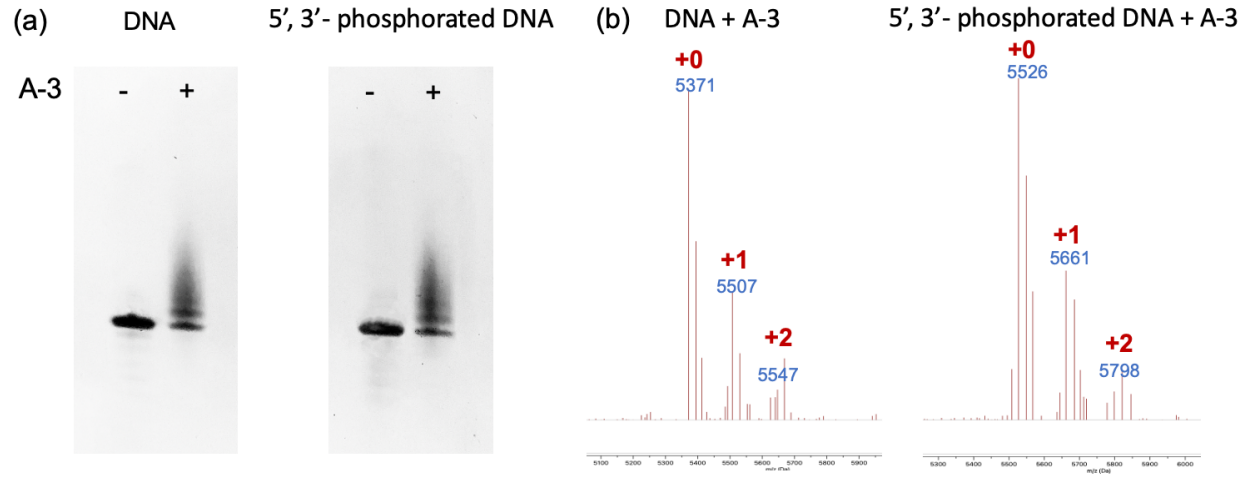
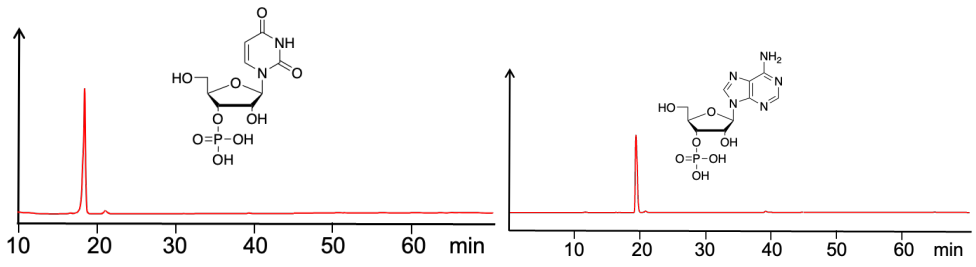


Figure S14. (a) PAGE (20%) gel electrophoresis of DNA and 5', 3'-ends phosphorated DNA reacting with 0.1M A-3 in 70% DMSO/H_2O at room temperature for 4 h. The appearing shifted bands indicate that some acylation occur at DNA. (b) MALDI-TOF spectrum of A-3 reacted DNA and 5', 3'-ends phosphorated DNA. Red number indicates the number of adducts.

HPLC trace of nucleotides



Determination of the extinction coefficient of A-3

An aqueous solution of known concentration of **A-3** was prepared. The corresponding UV absorbance at 260 nm was recorded. The solution was diluted into various concentration and the corresponding absorbance was recorded. Finally, the absorbance was plotted against the concentration by following the Beer-Lambert's law. The slope of the fitted straight line resulted in the extinction coefficient of the compound at 260 nm.

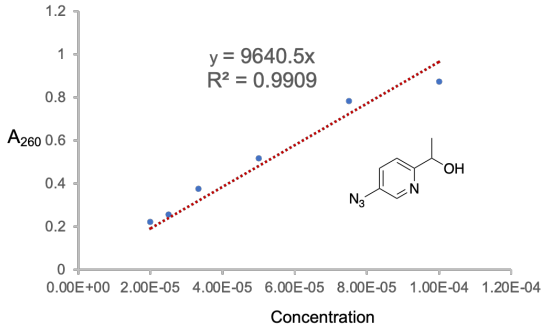


Figure S15: Straight line fitting for the determination of the molar extinction coefficient of **A-3**.

Reaction of 3'-UMP with A-3

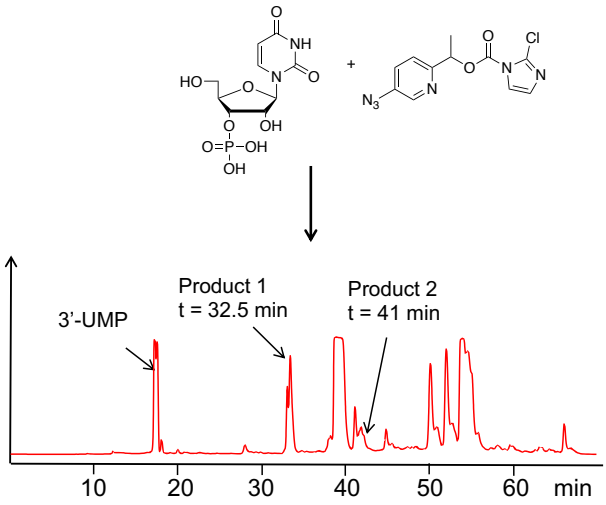


Figure S16. HPLC trace of the assay with 3'-UMP and A-3 after 14 h reaction time. Conditions: 21 mM nucleotide, 250 mM acylating reagent, r.t., Total reaction vol = 30 µL; 50 % DMSO content. The assay solution was diluted with water and methanol before injection to HPLC.

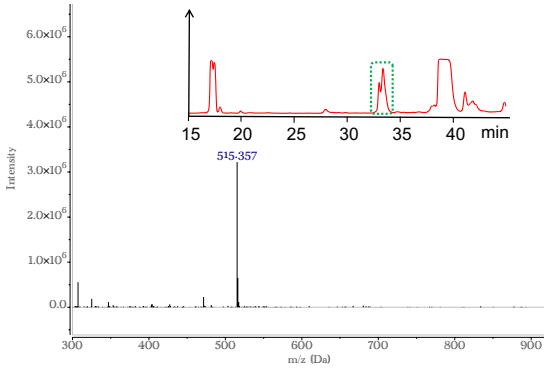


Figure S17. ESI-MS of the fraction eluted at t =32.5 min, from the assay with 3'-UMP and A-3. *m/z*, calculated for $C_{17}H_{20}N_6O_{11}P$, $[M+H]^+$: 515.35, found: 515.36.

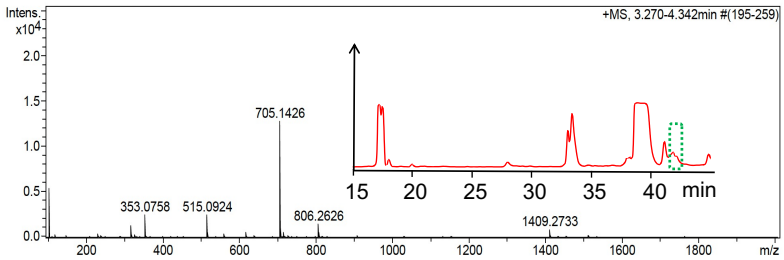


Figure S18. HRMS of the fraction eluted at t =41 min, from the assay with 3'-UMP and A-3. *m/z*, calculated for $C_{25}H_{26}N_{19}O_{13}P$, $[M+H]^+$: 705.1418, found: 705.1426.

Reaction of 3'-AMP with A-3

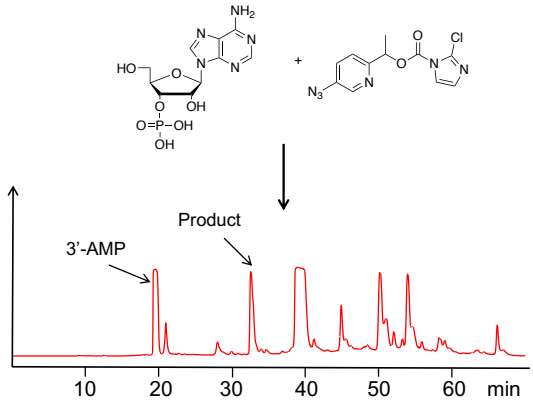


Figure S19. HPLC trace of the assay with 3'-AMP and A-3 after 14 h reaction time. Conditions: 21 mM nucleotide, 250 mM acylating reagent, r.t., Total reaction vol = 30 µL; 50 % DMSO content. The assay solution was diluted with water and methanol before injection to HPLC.

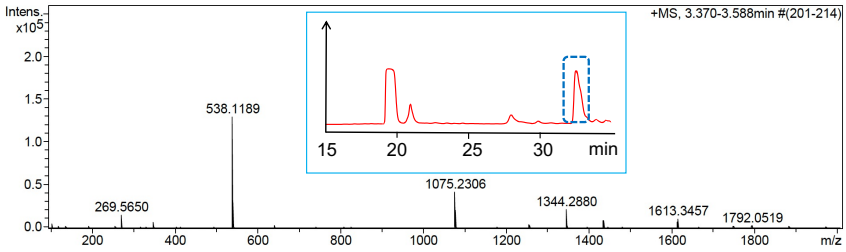


Figure S20. HRMS of the fraction eluted at t =32 min, from the assay with 3'-AMP and A-3. *m/z*, calculated for $C_{18}H_{21}N_9O_9P$, $[M+H]^+$: 538.1200, found: 538.1189.

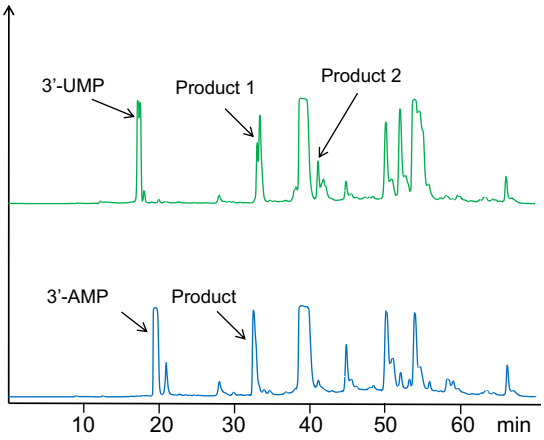


Figure S21. HPLC traces from acylation assays involving A-3 and mononucleotide after 14 h reaction time

Table S1. Results from assays with mononucleotide and A-3, as expressed in conversion to product based on HPLC chromatogram (%).[a,b]

Entry	substrate	monoacylated product (%)	bisacylated product (%)
1	3'-UMP	21	3
2	3'-AMP	30	n.d.*

[a] As detected by the absorbance from HPLC trace at 260 nm. The extinction coefficient of A-3 (ε_{260} = 9640 $M^{-1}cm^{-1}$) was taken into consideration during the yield calculation.
[b] Conditions: 21 mM nucleotide, 250 mM acylating reagent, r.t., Total reaction vol = 30 µL; 50 % DMSO content. The assay solution was diluted with water and methanol before injection to HPLC.

*n.d. = not detected

Reaction of 9-methyl adenine with A-3

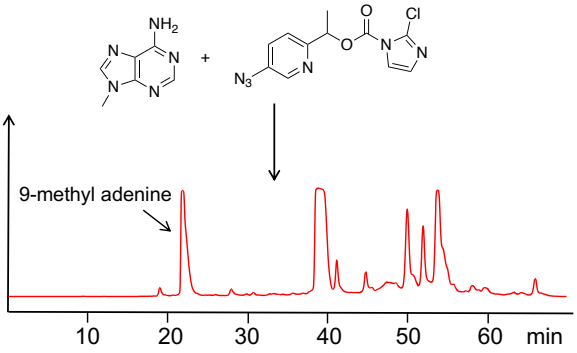


Figure S22. HPLC trace of the assay with 9-methyl adenine and A-3 after 14 h reaction time. Conditions: 21 mM nucleotide, 250 mM acylating reagent, r.t., Total reaction vol = 30 µL; 50 % DMSO content. The assay solution was diluted with water and methanol before injection to HPLC.

Reaction of 1-methyl uracil with A-3

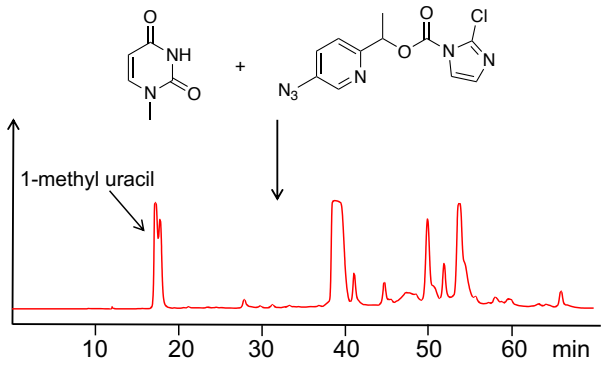
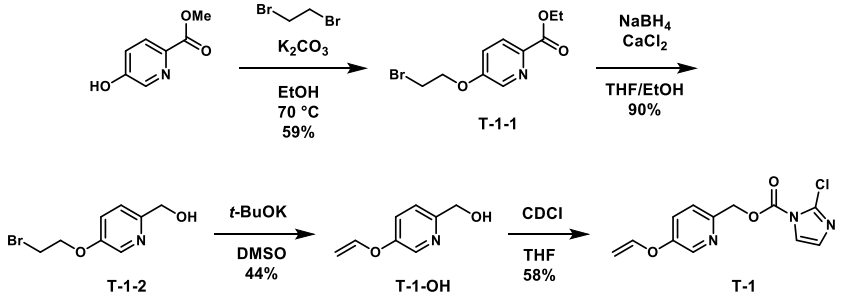


Figure S23. HPLC trace of the assay with 1-methyl uracil and A-3 after 14 h reaction time. Conditions: 21 mM nucleotide, 250 mM acylating reagent, r.t., Total reaction vol = 30 µL; 50 % DMSO content. The assay solution was diluted with water and methanol before injection to HPLC.

Synthetic Procedures

Synthesis of T-1



Ethyl 5-(2-bromoethoxy)picolinate (**T-1-1**)

In a 100 mL round-bottom flask, 20 mL of abs. EtOH was added to methyl 5-hydroxypicolinate (2.50 g, 16.3 mmol) and potassium carbonate (4.51 g, 32.7 mmol). To the reaction vessel, 14.1 mL of 1,2-dibromoethane (30.1 g, 163 mmol) was added. The reaction was stirred 70 °C for 2 d. The reaction was cooled to room temperature and filtered through a celite pad. EtOH was evaporated under vacuum, and the remaining residues were diluted with 60 mL DCM. The organic layer was washed with 2 x 30 mL of water and 10 mL of brine, dried with $MgSO_4$, and concentrated under vacuum. The crude product was purified via flash chromatography (DCM to 2% MeOH in DCM).

Off-white powder, 2.62 g, 59% yield

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.38 (d, *J* = 2.8 Hz, 1H), 8.07 (d, *J* = 8.7 Hz, 1H), 7.24 (dd, *J* = 8.7, 2.9 Hz, 1H), 4.41 (q, *J* = 7.2 Hz, 2H), 4.36 (t, *J* = 6.1 Hz, 2H), 3.64 (t, *J* = 6.1 Hz, 2H), 1.39 (t, *J* = 7.1 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 164.79, 156.73, 141.21, 138.37, 126.51, 120.74, 68.30, 61.76, 28.27, 14.43.

ESI-MS: [M+H] calculated: 274.0; observed mass: 274.0

(5-(2-bromoethoxy)pyridin-2-yl)methanol (**T-1-2**)

In 25 mL round-bottom flask, **T-1-1** (632 mg, 2.31 mmol) and anhydrous calcium chloride (1.02 g, 9.22 mmol) was suspended in 15 mL of 1:1 THF:EtOH. The heterogenous solution was stirred at room temperature for 30 minutes and then chilled on an ice bath. Sodium borohydride (218 mg, 5.76 mmol) was added in portions, and the reaction was warmed up to room temparture and stirred overnight. To quench the reaction, 50 mL of saturated aqueous ammonium chloride solution was added. The solution was then extracted with 3 x 50 mL DCM. The organics were combined, dried with Na_2SO_4, concentrated under vacuum. The obtained product was used without further purification.

Yellow oil, 325 mg, 90% yield

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.27 (d, *J* = 2.8 Hz, 1H), 7.25 (dd, *J* = 8.6, 2.8 Hz, 1H), 7.20 (d, *J* = 8.5 Hz, 1H), 4.71 (s, 2H), 4.35 (t, *J* = 6.1 Hz, 2H), 3.66 (t, *J* = 6.1 Hz, 2H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 153.81, 152.10, 135.94, 123.52, 121.51, 68.58, 63.89, 28.80.

ESI-MS: [M+H] calculated: 232.0; observed mass: 231.9

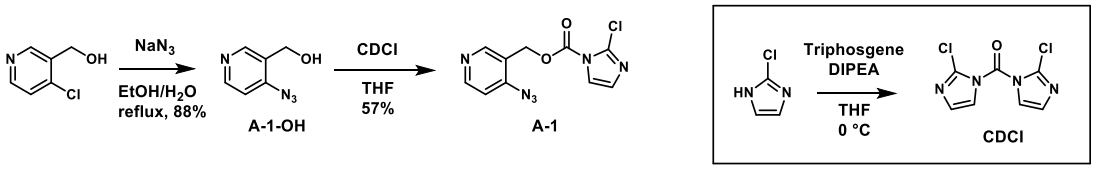
(5-(vinyloxy)pyridin-2-yl)methanol (**T-1-OH**)

In 25 mL round-bottom flask, **T-1-2** (503 mg, 2.17 mmol) was dissolved in 11 mL of anhydrous DMSO. Potassium *tert*-butoxide (486 mg, 4.34 mmol) was added in one portion, and the reaction was stirred at room temperature for 2 h. To quench the reaction, 55 mL of DCM was added. The solution was washed with 3 x 25 mL of water and 25 mL of brine. The organic layer was dried with $MgSO_4$ and concentrated under vacuum. The crude product was purified via flash chromatography (3:1 to 1:1 hexanes:ethyl acetate).

Yellow oil, 145 mg, 44% yield
^{1}H NMR (500 MHz, $CDCl_3$) δ 8.33 (dd, *J* = 2.8, 0.7 Hz, 1H), 7.35 (dd, *J* = 8.5, 2.8 Hz, 1H), 7.24 (dd, *J* = 8.5, 0.7 Hz, 1H), 6.63 (dd, *J* = 13.6, 6.0 Hz, 1H), 4.81 (dd, *J* = 13.7, 2.1 Hz, 1H), 4.74 (s, 3H), 4.53 (dd, *J* = 6.0, 2.0 Hz, 1H), 3.42 (s, 1H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 154.01, 152.29, 147.73, 138.53, 125.56, 121.34, 96.65, 64.08.
ESI-MS: [M+H] calculated: 152.1; observed mass: 152.1

See below for 2-chloroimidazole carbamate activation (**T-1**)

Synthesis of A-1

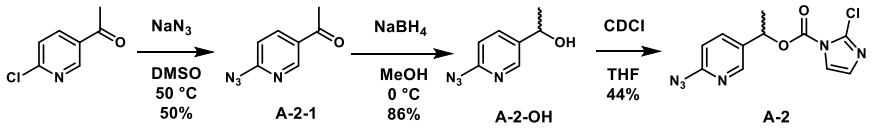


(4-azidopyridin-3-yl)methanol (A-1-OH)
In a 25 mL round-bottom flask, (4-chloropyridin-3-yl)methanol (461 mg, 3.21 mmol) and sodium azide (417 mg, 6.41 mmol) was dissolved in 7 mL of 1:1 water:EtOH. The reaction was refluxed for 3 d. The reaction mixture was cooled to room temperature and saturated with sodium chloride. The aqueous solution was extracted with 4 x 10 mL ethyl acetate. The organic layer was dried with $MgSO_4$ and concentrated under vacuum. The crude product was purified via flash chromatography (ethyl acetate).

Off-white powder, 420 mg, 88% yield
^{1}H NMR (500 MHz, $CDCl_3$) δ 8.48 (s, 1H), 8.43 (d, *J* = 5.4 Hz, 1H), 7.04 (d, *J* = 5.4 Hz, 1H), 4.63 (s, 2H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 150.11, 149.94, 147.01, 127.54, 112.90, 58.67.
NMR values are in agreement with published data.[8]

See below for 2-chloroimidazole carbamate activation (**A-1**)

Synthesis of A-2



1-(6-azidopyridin-3-yl)ethan-1-one (**A-2-1**)

In a 100 mL round-bottom flask, 1-(6-chloropyridin-3-yl)ethan-1-one (1250 mg, 8.03 mmol) and sodium azide (627 mg, 9.64 mmol) were dissolved in 5.3 mL anhydrous DMSO. The reaction was stirred at 50 °C for 1 d. To quench the reaction, the reaction mixture was cooled to room temperature, and 50 mL of water was added. The resulting solution was extracted with 3 x 50 mL of ethyl acetate. The organics were combined, washed with 3 x 50 mL of water and 50 mL of brine, dried with $MgSO_4$, and concentrated under vacuum. The crude product was purified via flash chromatography (3:1 to 1:1 hexanes:ethyl acetate).

White powder, 656 mg, 50% yield

^{1}H NMR (500 MHz, $CDCl_3$) δ 9.43 (dd, *J* = 1.6, 1.0 Hz, 1H), 8.22 (dd, *J* = 9.3, 1.5 Hz, 1H), 8.08 (dd, *J* = 9.3, 1.0 Hz, 1H), 2.74 (s, 3H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 193.09, 149.34, 130.81, 128.12, 127.30, 115.99, 26.73.

ESI-MS: [M+H] calculated: 163.1; observed mass: 163.1

1-(6-azidopyridin-3-yl)ethan-1-ol (**A-2-OH**)

In a 50 mL round-bottom flask, **A-2-1** (300 mg, 1.85 mmol) was dissolved in 9.5 mL of MeOH and chilled on an ice bath. Sodium borohydride (35 mg, 0.925 mmol) was added in one portion, and the reaction mixture was stirred at 0 °C for 30 minutes. To quench the reaction, 20 mL of water was added. MeOH was evaporated under vacuum, and the resulting aqueous solution was extracted with 5 x 20 mL of ethyl acetate. The organics were combined and washed with 10 mL of brine. The brine layer was back extracted with 2 x 10 mL of ethyl acetate. All organics were combined, dried with $MgSO_4$, and concentrated under vacuum. The crude product was purified via flash chromatography (3:1 to 1:1 hexanes:ethyl acetate).

White powder, 261 mg, 86% yield

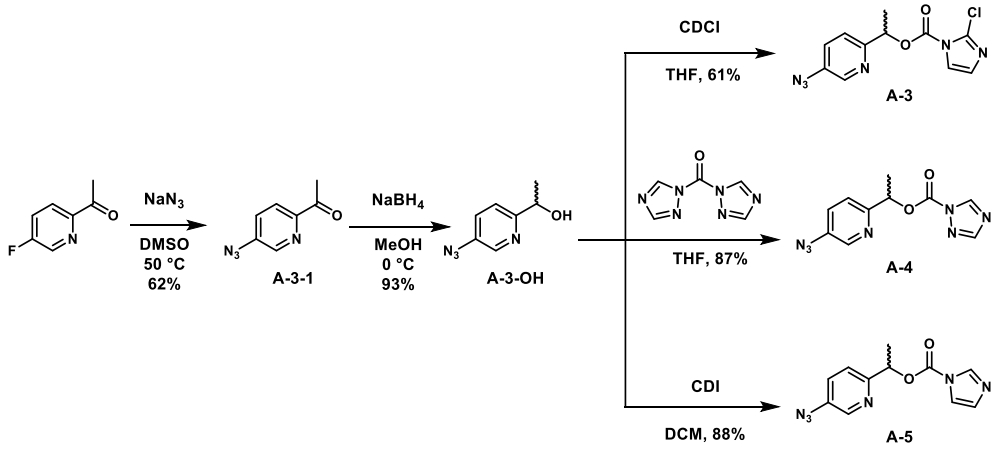
^{1}H NMR (500 MHz, $CDCl_3$) δ 8.86 (q, *J* = 1.1 Hz, 1H), 7.99 (dd, *J* = 9.2, 1.1 Hz, 1H), 7.67 (dd, *J* = 9.3, 1.6 Hz, 1H), 5.16 – 5.07 (m, 1H), 2.46 (d, *J* = 4.0 Hz, 1H), 1.62 (d, *J* = 6.5 Hz, 3H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 148.33, 135.75, 131.29, 121.75, 115.63, 67.23, 25.19.

ESI-MS: [M+H] calculated: 165.1; observed mass: 165.1

See below for 2-chloroimidazole carbamate activation (**A-2**)

Synthesis of A-3, 4, and 5



1-(5-azidopyridin-2-yl)ethan-1-one (**A-3-1**)

In a 25 mL round-bottom flask, 1-(5-fluoropyridin-2-yl)ethan-1-one (200 mg, 1.44 mmol) and sodium azide (280 mg, 4.31 mmol) was dissolved in 2 mL of anhydrous DMSO. The reaction was stirred at 50 °C overnight. To quench the reaction, the reaction mixture was cooled to room temperature, and 15 mL of water was added. The resulting solution was extracted with 3 x 15 mL of ethyl acetate. The organics were combined, washed with 3 x 15 mL of water and 15 mL of brine, dried with $MgSO_4$, and concentrated under vacuum. The crude product was purified via flash chromatography (20:1 hexanes:ethyl acetate).

Off-white powder, 145 mg, 62% yield

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.37 (dd, *J* = 2.7, 0.7 Hz, 1H), 8.06 (dd, *J* = 8.5, 0.7 Hz, 1H), 7.45 (dd, *J* = 8.5, 2.6 Hz, 1H), 2.69 (s, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 198.91, 150.33, 140.72, 140.33, 126.31, 122.99, 25.84.

ESI-MS: [M+H] calculated: 163.1; observed mass: 163.1

1-(5-azidopyridin-2-yl)ethan-1-ol (**A-3-OH**)

In a 25 mL round-bottom flask, **A-3-1** (135 mg, 0.833 mmol) was dissolved in 4.5 mL of MeOH and chilled on an ice bath. Sodium borohydride (15.75 mg, 0.416 mmol) was added in one portion, and the reaction mixture was stirred at 0 °C for 30 minutes. To quench the reaction, 10 mL of water was added. MeOH was evaporated under vacuum, and the resulting aqueous solution was extracted with 3 x 10 mL of ethyl acetate. The organics were combined, washed with 10 mL of brine, dried with $MgSO_4$, and concentrated under vacuum. The obtained product was used without further purification.

Yellow oil, 126 mg, 93 % yield

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.28 (dd, *J* = 2.6, 0.8 Hz, 1H), 7.36 (dd, *J* = 8.4, 2.6 Hz, 1H), 7.30 (dt, *J* = 8.4, 0.7 Hz, 1H), 4.89 (s, 1H), 3.79 (d, *J* = 4.6 Hz, 1H), 1.49 (d, *J* = 6.6 Hz, 3H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 159.95, 139.71, 135.98, 127.03, 120.55, 69.00, 24.37.

ESI-MS: [M+H] calculated: 165.1; observed mass: 165.1

See below for 2-chloroimidazole carbamate activation (**A-3**)

1-(5-azidopyridin-2-yl)ethyl 1H-1,2,4-triazole-1-carboxylate (A-4)

In a 4 mL vial, **A-3-OH** (50 mg, 0.305 mmol) and 1,1′-carbonyl-di-(1,2,4-triazole) (100 mg, 0.610 mmol) were dissolved in 1 mL of anhydrous THF. The reaction was stirred at room temperature for 2 h. The reaction mixture was diluted with 15 mL of DCM, washed with 3 x 10 mL of water, dried with $MgSO_4$, and concentrated under vacuum. The obtained product was used without further purification and prepared as 0.5 or 1 M stocks in anhydrous DMSO, aliquoted, and stored at -80 °C.

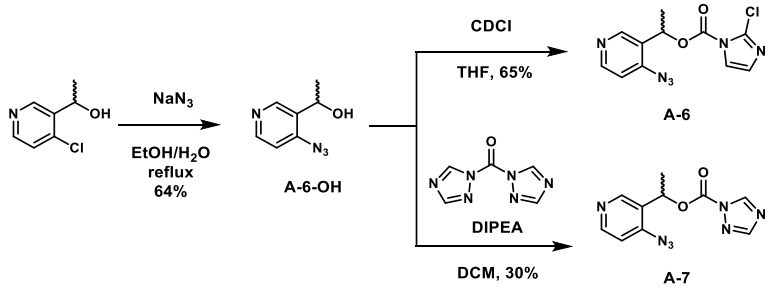
Brown oil, 70 mg, 87% yield
^{1}H NMR (500 MHz, $CDCl_3$) δ 8.85 (s, 1H), 8.34 (dd, *J* = 2.7, 0.8 Hz, 1H), 8.08 (s, 1H), 7.49 – 7.44 (m, 1H), 7.39 (dd, *J* = 8.4, 2.7 Hz, 1H), 6.17 (q, *J* = 6.7 Hz, 1H), 1.82 (d, *J* = 6.7 Hz, 3H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 154.16, 153.89, 147.06, 145.85, 141.14, 137.20, 126.91, 121.80, 78.15, 20.37.
ESI-MS: [M+H] calculated: 260.1; observed mass: 260.1
HRMS [+ Scan]; calculated m/z for $C_{10}H_9N_7O_2$ [M+H] 260.0896; observed mass 260.0889

1-(5-azidopyridin-2-yl)ethyl 1H-imidazole-1-carboxylate (A-5)

In a 10 mL round-bottom flask, **A-3-OH** (60 mg, 0.365 mmol) and 1,1′-carbonyldiimidazole (119 mg, 0.731 mmol) were dissolved in 2.5 mL of anhydrous DCM. The reaction was stirred at room temperature for 2 h under argon. The reaction mixture was diluted with 15 mL of DCM, washed with 3 x 10 mL of water and 1 x 10 mL brine. The organic layer was dried with $MgSO_4$ and concentrated under vacuum. The crude product was purified via flash chromatography (5:1 to 1:1 hexanes:ethyl acetate). The product was prepared as 0.5 or 1 M stocks in anhydrous DMSO, aliquoted, and stored at -80 °C.

Off-white solid, 83 mg, 88% yield
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.35 (dd, *J* = 2.2, 1.2 Hz, 1H), 8.17 (t, *J* = 1.1 Hz, 1H), 7.45 (dd, *J* = 1.7, 1.3 Hz, 1H), 7.42 – 7.36 (m, 2H), 7.07 (dd, *J* = 1.7, 0.9 Hz, 1H), 6.09 (q, *J* = 6.6 Hz, 1H), 1.77 (d, *J* = 6.7 Hz, 3H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 154.64, 148.22, 141.19, 137.29, 137.13, 130.90, 126.86, 121.76, 117.30, 76.99, 20.34.
ESI-MS: [M+H] calculated: 259.1; observed mass: 259.2
HRMS was attempted, but only the parent alcohol (**A-3-OH**) was detected likely due to fragmentation after ionization.

Synthesis of A-6 and 7



1-(4-azidopyridin-3-yl)ethan-1-ol (A-6-OH)

1-(4-chloropyridin-3-yl)ethan-1-ol was prepared following a published procedure.[9] In a 25 mL round-bottom flask, (4-chloropyridin-3-yl) ethan-1-ol (520 mg, 3.30 mmol) and sodium azide (643 mg, 9.90 mmol) was dissolved in 11 mL of 1:1 water:EtOH. The reaction was refluxed for 3 d. The reaction mixture was cooled to room temperature and saturated with sodium chloride. The aqueous solution was extracted with 4 x 15 mL ethyl acetate. The organic layer was dried with $MgSO_4$ and concentrated under vacuum. The crude product was purified via flash chromatography (2:1 hexanes:ethyl acetate to ethyl acetate).

White powder, 347 mg, 64% yield
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.63 (s, 1H), 8.46 (d, *J* = 5.4 Hz, 1H), 7.04 (d, *J* = 5.4 Hz, 1H), 5.06 (q, *J* = 6.5 Hz, 1H), 1.51 (d, *J* = 6.6 Hz, 3H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 149.80, 148.70, 145.56, 131.66, 112.76, 64.80, 23.77.
ESI-MS: [M+H] calculated: 165.1; observed mass: 165.1

See below for 2-chloroimidazole carbamate activation (**A-6**)

1-(4-azidopyridin-3-yl)ethyl 1H-1,2,4-triazole-1-carboxylate (**A-7**)

In a 10 mL vial, **A-6-OH** (50 mg, 0.305 mmol) and 1,1′-carbonyl-di-(1,2,4-triazole) (100 mg, 0.610 mmol) were dissolved in 2 mL of anhydrous DCM. 59 µL of DIPEA was added, and the reaction was stirred at room temperature overnight. The reaction mixture was diluted with 15 mL of DCM, washed 3 x 10 mL of water and 1 x 10 mL brine. The organic layer was dried with $MgSO_4$ and concentrated under vacuum. The crude product was purified on a short silica column with DCM wash to elute the desired product. The product was prepared as 0.5 or 1 M stocks in anhydrous DMSO, aliquoted, and stored at -80 °C.

Yellow oil, 24 mg, 30% yield
^{1}H NMR (500 MHz, $CDCl_3$) δ 8.82 (s, 1H), 8.69 (s, 1H), 8.57 (d, *J* = 5.5 Hz, 1H), 8.07 (s, 1H), 7.11 (d, *J* = 5.5 Hz, 1H), 6.34 (q, *J* = 6.7 Hz, 1H), 1.79 (d, *J* = 6.7 Hz, 3H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 153.92, 151.17, 148.84, 146.87, 146.48, 145.82, 125.88, 113.07, 72.16, 20.64.
ESI-MS: [M+H] calculated: 260.1; observed mass: 260.1
HRMS [+ Scan]; calculated m/z for $C_{10}H_9N_7O_2$ [M+H] 260.0896; observed mass 260.0889

General procedure for 2-chloroimidazole carbamate activation

In 25 mL round-bottom flask, 2-chloroimidazole (300 mg, 2.92 mmol) and DIPEA (1.13 g, 8.77 mmol) were dissolved in 6 mL of anhydrous THF and chilled on ice. Triphosgene (434 mg, 1.46 mmol) was added in portions. The ice bath was removed, and the reaction was stirred under Ar at room temperature for 30 minutes. The benzyl alcohol derivative (0.731 mmol) dissolved in 1.5 mL of THF was added dropwise. The reaction was stirred under Ar at room temperature for an additional 60 minutes. HCl salt precipitates were filtered away, and the volatiles were evaporated under vacuum. The remaining residue was dissolved in 20 mL of DCM and washed with 3 x 10 mL of water and 10 mL of brine. The organic layer was dried with $MgSO_4$ and concentrated under vacuum. The crude product was purified via flash chromatography using a hexanes ethyl acetate gradient. The acylating agents were prepared as 0.5 or 1M stocks in anhydrous DMSO, aliquoted, and stored at -80 °C.

(5-(vinyloxy)pyridin-2-yl)methyl 2-chloro-1*H*-imidazole-1-carboxylate (T-1)

Chromatography gradient: 3:1 to 1:1 hexanes:ethyl acetate

Brown oil, 58% yield

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.38 (d, *J* = 2.7 Hz, 1H), 7.47 (d, *J* = 1.6 Hz, 1H), 7.44 (d, *J* = 8.5 Hz, 1H), 7.36 (dd, *J* = 8.5, 2.7 Hz, 1H), 6.91 (d, *J* = 1.6 Hz, 1H), 6.63 (dd, *J* = 13.6, 6.0 Hz, 1H), 5.47 (s, 2H), 4.87 (dd, *J* = 13.6, 1.8 Hz, 1H), 4.59 (dd, *J* = 6.0, 1.8 Hz, 1H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 153.16, 147.86, 147.73, 146.98, 139.91, 139.22, 133.21, 128.81, 124.65, 120.45, 97.70, 70.05.

ESI-MS: [M+H] calculated: 280.0; observed mass: 280.1

HRMS [+ Scan]; calculated m/z for $C_{12}H_{10}ClN_3O_3$ [M+H] 280.0489; observed mass 280.0485

(4-azidopyridin-3-yl)methyl 2-chloro-1*H*-imidazole-1-carboxylate (A-1)

Chromatography gradient: 1:1 to 1:3 hexanes:ethyl acetate

Off-white powder, 57% yield

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.64 (s, 1H), 8.62 (d, *J* = 5.5 Hz, 1H), 7.42 (d, *J* = 1.9 Hz, 1H), 7.17 (d, *J* = 5.5 Hz, 1H), 6.92 (d, *J* = 1.9 Hz, 1H), 5.40 (s, 2H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 151.99, 151.89, 148.56, 147.58, 133.23, 128.97, 120.47, 120.33, 113.27, 63.35.

ESI-MS: [M+H] calculated: 279.0; observed mass: 279.0

HRMS [+ Scan]; calculated m/z for $C_{10}H_8ClN_6O_2$ [M+H] 279.0397; observed mass 279.0391

1-(6-azidopyridin-3-yl)ethyl 2-chloro-1*H*-imidazole-1-carboxylate (A-2)

Chromatography gradient: 8:3 to 2:3 hexanes:ethyl acetate

White powder, 44% yield

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.95 (dt, *J* = 1.6, 0.8 Hz, 1H), 8.11 (dd, *J* = 9.3, 1.0 Hz, 1H), 7.76 (dd, *J* = 9.4, 1.7 Hz, 1H), 7.46 (d, *J* = 1.9 Hz, 1H), 6.96 (d, *J* = 1.9 Hz, 1H), 6.21 (q, *J* = 6.7 Hz, 1H), 1.88 (d, *J* = 6.7 Hz, 3H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 148.51, 147.06, 133.03, 130.63, 129.38, 129.33, 123.77, 120.28, 116.88, 74.08, 21.40.

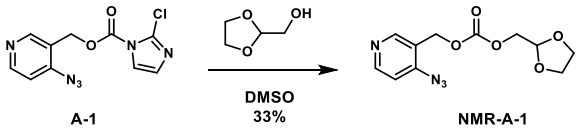
ESI-MS: [M+H] calculated: 293.1; observed mass: 293.2

HRMS [+ Scan]; calculated m/z for $C_{11}H_9ClN_6O_2$ [M+H] 293.0554; observed mass 293.0547

1-(5-azidopyridin-2-yl)ethyl 2-chloro-1*H*-imidazole-1-carboxylate (**A-3**)
Chromatography gradient: 10:1 to 1:3 hexanes:ethyl acetate
Yellow oil, 61% yield
^{1}H NMR (500 MHz, $CDCl_3$) δ 8.35 (dd, *J* = 2.6, 0.9 Hz, 1H), 7.49 (d, *J* = 1.9 Hz, 1H), 7.43 (dd, *J* = 8.4, 0.9 Hz, 1H), 7.39 (dd, *J* = 8.4, 2.6 Hz, 1H), 6.93 (d, *J* = 1.9 Hz, 1H), 6.08 (q, *J* = 6.6 Hz, 1H), 1.77 (d, *J* = 6.7 Hz, 3H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 154.46, 147.32, 141.18, 137.18, 133.11, 128.87, 126.90, 121.86, 120.48, 77.62, 20.37.
ESI-MS: [M+H] calculated: 293.1; observed mass: 293.2
HRMS was attempted, but ~~but~~ only the parent alcohol (**A-3-OH**) was detected likely due to fragmentation after ionization.

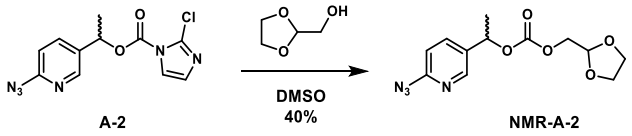
1-(4-azidopyridin-3-yl)ethyl 2-chloro-1H-imidazole-1-carboxylate (**A-6)**
Chromatography gradient: 2:1 to 1:3 hexanes:ethyl acetate
Yellow oil, 65% yield
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.66 (s, 1H), 8.57 (d, *J* = 5.5 Hz, 1H), 7.46 (d, *J* = 1.8 Hz, 1H), 7.11 (d, *J* = 5.5 Hz, 1H), 6.94 (d, *J* = 1.8 Hz, 1H), 6.27 (q, *J* = 6.6 Hz, 1H), 1.73 (d, *J* = 6.7 Hz, 3H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 151.12, 148.87, 147.05, 146.20, 133.09, 128.99, 126.24, 120.35, 113.04, 71.52, 20.96.
ESI-MS: [M+H] calculated: 293.1; observed mass: 293.1
HRMS [+ Scan]; calculated m/z for $C_{11}H_9ClN_6O_2$ [M+H] 293.0554; observed mass 293.0547

Synthesis of NMR probes



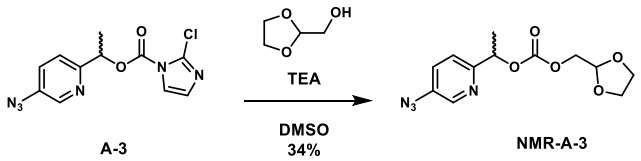
(1,3-dioxolan-2-yl)methyl ((4-azidopyridin-3-yl)methyl) carbonate (**SM-A-1**)
In a 2 mL vial, 192 µL of 0.5 M **A-1** solution in DMSO (0.096 mmol) and 17.2 µL of (1,3-dioxolan-2-yl)methanol (20 mg, 0.192 mmol) were added with a pipette. The reaction was stirred at room temperature overnight. The reaction mixture was diluted with 10 mL of DCM. The organic layer was washed with 3 x 10 mL of water and then 10 mL of brine, dried with $MgSO_4$, and concentrated under vacuum. The crude product was purified via flash chromatography (1:1 hexanes:ethyl acetate to ethyl acetate).

Yellow oil, 8.8 mg, 33% yield
^{1}H NMR (500 MHz, $CDCl_3$) δ 8.58 – 8.54 (m, 2H), 7.09 (d, *J* = 5.5 Hz, 1H), 5.19 – 5.10 (m, 2H), 4.20 (d, *J* = 3.8 Hz, 2H), 4.04 – 3.97 (m, 2H), 3.95 – 3.88 (m, 2H).
ESI-MS: [M+H] calculated: 281.1; observed mass: 281.1
HRMS [+ Scan]; calculated m/z for $C_{11}H_{12}N_4O_5$ [M+H] 281.0886; observed mass 281.0879



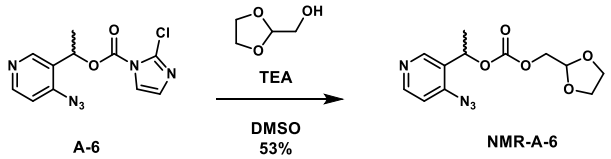
(1,3-dioxolan-2-yl)methyl (1-(6-azidopyridin-3-yl)ethyl) carbonate (**SM-A-2**)
In a 2 mL vial, 115 µL of 0.5 M **A-2** solution in DMSO (0.058 mmol) and 25.8 µL of (1,3-dioxolan-2-yl)methanol (30 mg, 0.288 mmol) were added with a pipette. The reaction was stirred at 37 °C overnight. The reaction mixture was diluted with 10 mL of water. The resulting solution was extracted with 3 x 10 mL ethyl acetate. The organics were combined, washed with 3 x 10 mL of water and then 10 mL of brine, dried with $MgSO_4$, and concentrated under vacuum. The crude product was purified via flash chromatography (4:1 to 1:1 hexanes:ethyl acetate).

Clear oil, 6.8 mg, 40% yield
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.87 (dt, *J* = 1.7, 0.9 Hz, 1H), 8.04 (dd, *J* = 9.3, 1.0 Hz, 1H), 7.68 (dd, *J* = 9.3, 1.6 Hz, 1H), 5.86 (q, *J* = 6.6 Hz, 1H), 5.14 (t, *J* = 3.8 Hz, 1H), 4.24 – 4.13 (m, 2H), 4.06 – 3.86 (m, 5H), 1.71 (d, *J* = 6.6 Hz, 3H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 154.11, 148.42, 130.93, 123.07, 116.26, 100.79, 73.08, 67.55, 65.47, 65.45, 21.76.
ESI-MS: [M+H] calculated: 295.1; observed mass: 295.2
HRMS [+ Scan]; calculated m/z for $C_{12}H_{14}N_4O_5$ [M+H] 295.1042; observed mass 295.1035



(1,3-dioxolan-2-yl)methyl (1-(5-azidopyridin-2-yl)ethyl) carbonate (**SM-A-3**)
In a 2 mL vial, 115 µL of 0.5 M **A-3** solution in DMSO (0.058 mmol) and 25.8 µL of (1,3-dioxolan-2-yl)methanol (30 mg, 0.288 mmol) were added with a pipette. The reaction was stirred at 37 °C overnight. The reaction mixture was diluted with 10 mL of water. The resulting solution was extracted with 3 x 10 mL ethyl acetate. The organics were combined, washed with 3 x 10 mL of water and then 10 mL of brine, dried with $MgSO_4$, and concentrated under vacuum. The crude product was purified via flash chromatography (4:1 to 1:1 hexanes:ethyl acetate).

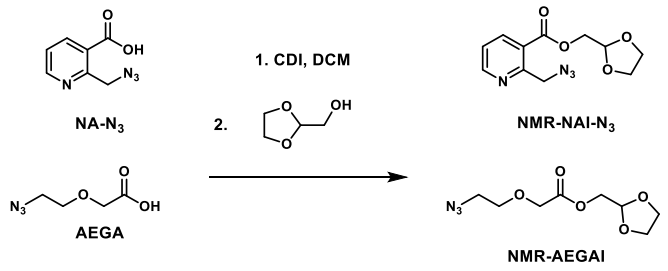
Yellow oil, 5.8 mg, 34% yield
^{1}H NMR (500 MHz, $CDCl_3$) δ 8.31 (dd, *J* = 2.7, 0.9 Hz, 1H), 7.40 (d, *J* = 8.4 Hz, 1H), 7.35 (dd, *J* = 8.4, 2.6 Hz, 1H), 5.78 (q, *J* = 6.7 Hz, 1H), 5.16 (t, *J* = 3.9 Hz, 1H), 4.22 – 4.13 (m, 2H), 4.04 – 3.88 (m, 4H), 1.63 (d, *J* = 6.7 Hz, 3H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 156.40, 154.45, 140.75, 136.48, 126.81, 121.05, 101.02, 67.36, 65.42, 65.41, 20.86.
ESI-MS: [M+H] calculated: 295.1; observed mass: 295.2
HRMS [+ Scan]; calculated m/z for $C_{12}H_{14}N_4O_5$ [M+H] 295.1042; observed mass 295.1036



(1,3-dioxolan-2-yl)methyl (1-(4-azidopyridin-3-yl)ethyl) carbonate (**SM-A-6**)
In a 2 mL vial, 120 µL of 0.5 M **A-6** solution in DMSO (0.060 mmol) and 26.6 µL of (1,3-dioxolan-2-yl)methanol (31 mg, 0.298 mmol) were added with a pipette. The reaction was stirred at room temperature overnight. The reaction mixture was diluted with 10 mL of water. The resulting solution was extracted with 3 x 10 mL ethyl acetate. The organics were combined, washed with 3 x 10 mL of water and then 10 mL of brine, dried with $MgSO_4$, and concentrated under vacuum. The crude product was purified via flash chromatography (4:1 to 1:1 hexanes:ethyl acetate).

Yellow oil, 9.2 mg, 53% yield
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.61 (s, 1H), 8.51 (d, *J* = 5.5 Hz, 1H), 7.06 (dd, *J* = 5.5, 0.6 Hz, 1H), 5.96 – 5.89 (m, 1H), 5.14 (t, *J* = 3.8 Hz, 1H), 4.16 (dd, *J* = 3.8, 2.9 Hz, 2H), 4.03 – 3.95 (m, 2H), 3.95 – 3.88 (m, 2H), 1.58 (d, *J* = 6.7 Hz, 3H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 154.24, 150.51, 148.76, 145.76, 127.72, 117.47, 112.80, 100.94, 70.52, 67.34, 65.43, 21.24.
ESI-MS: [M+H] calculated: 295.1; observed mass: 295.2
HRMS [+ Scan]; calculated m/z for $C_{12}H_{14}N_4O_5$ [M+H] 295.1042; observed mass 295.1035

Synthesis of NMR-NAI-N_3 and NMR-AEGAI

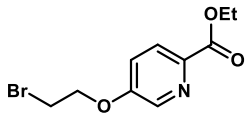


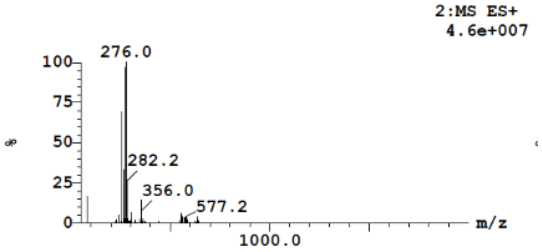
In a 2 mL vial, 1,1′-carbonyldiimidazole (50.1 mg, 0.309 mmol) was added. Carboxylic acid (0.281 mmol) dissolved in 0.6 mL of THF was added dropwise. The reaction was stirred at room temperature for 1 h. 25.1 µL of (1,3-dioxolan-2-yl)methanol (29 mg, 0.281 mmol) were added with a pipette. The reaction was stirred at room temperature overnight. The reaction mixture was diluted with 10 mL of ethyl acetate. The resulting solution was washed with 3 x 5 mL of water and then 5 mL of brine, dried with $MgSO_4$, and concentrated under vacuum. The crude product was purified via flash chromatography using a hexanes ethyl acetate gradient.

(1,3-dioxolan-2-yl)methyl 2-(azidomethyl)nicotinate (SM-NAI-N_3)
Chromatography gradient: 2:1 hexanes:ethyl acetate
Clear oil, 56% yield
^{1}H NMR (500 MHz, $CDCl_3$) δ 8.77 (dd, *J* = 4.8, 1.8 Hz, 1H), 8.32 (dd, *J* = 7.9, 1.8 Hz, 1H), 7.37 (dd, *J* = 7.9, 4.8 Hz, 1H), 5.27 (t, *J* = 4.0 Hz, 1H), 4.89 (s, 2H), 4.40 (d, *J* = 3.9 Hz, 2H), 4.14 – 3.89 (m, 4H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 165.23, 156.90, 152.64, 139.26, 124.83, 123.00, 101.10, 65.47, 64.93, 54.39.
ESI-MS: [M+H] calculated: 265.1; observed mass: 265.2
HRMS [+ Scan]; calculated m/z for $C_{11}H_{12}N_4O_4$ [M+H] 265.0937; observed mass 265.0929

(1,3-dioxolan-2-yl)methyl 2-(2-azidoethoxy)acetate (SM-AEGAI)
Chromatography gradient: 4:1 hexanes:ethyl acetate
Clear oil, 24% yield
^{1}H NMR (500 MHz, cdcl$_3$) δ 5.16 (t, *J* = 4.0 Hz, 1H), 4.21 (d, *J* = 4.2 Hz, 4H), 4.05 – 3.88 (m, 4H), 3.75 (dd, *J* = 5.5, 4.6 Hz, 2H), 3.45 (t, *J* = 5.0 Hz, 2H).
^{13}C NMR (126 MHz, cdcl$_3$) δ 169.85, 101.04, 70.52, 68.42, 65.41, 64.32, 50.88.
ESI-MS: [M+H] calculated: 232.1; observed mass: 232.2
HRMS was attempted, but no result was obtained.

NMR and MS spectra





B (d)
8.07
J(8.69)

E (t)
4.36
J(6.10)

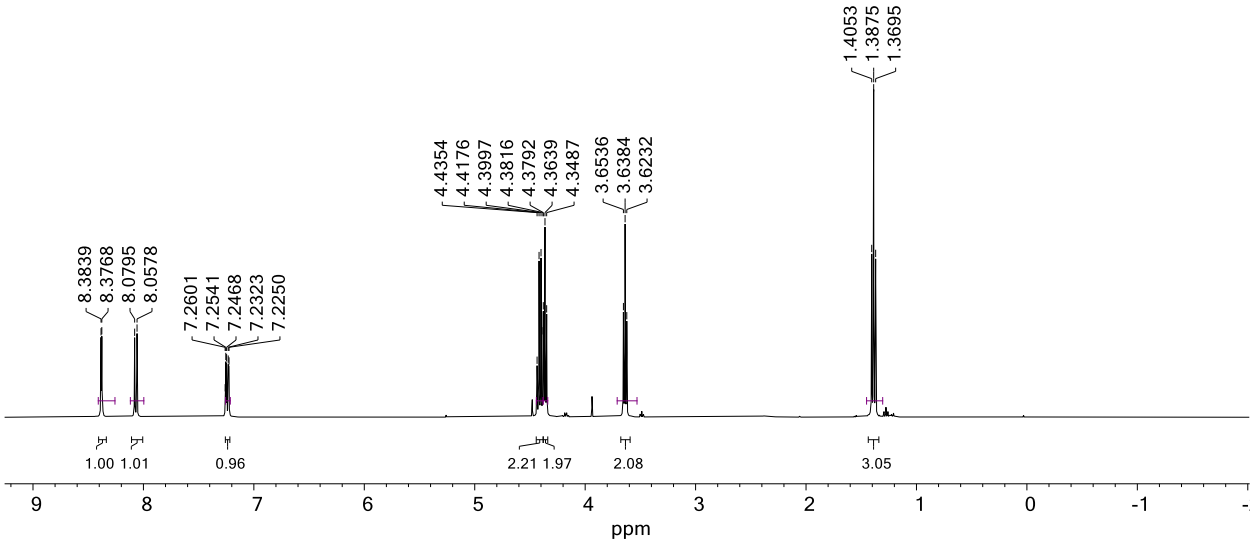
A (d)
8.38
J(2.85)

C (dd)
7.24
J(8.72, 2.92)

D (q)
4.41
J(7.20)

F (t)
3.64
J(6.07)

G (t)
1.39
J(7.14)



B (s)
156.73

D (s)
138.37

F (s)
120.74

H (s)
61.76

A (s)
164.79

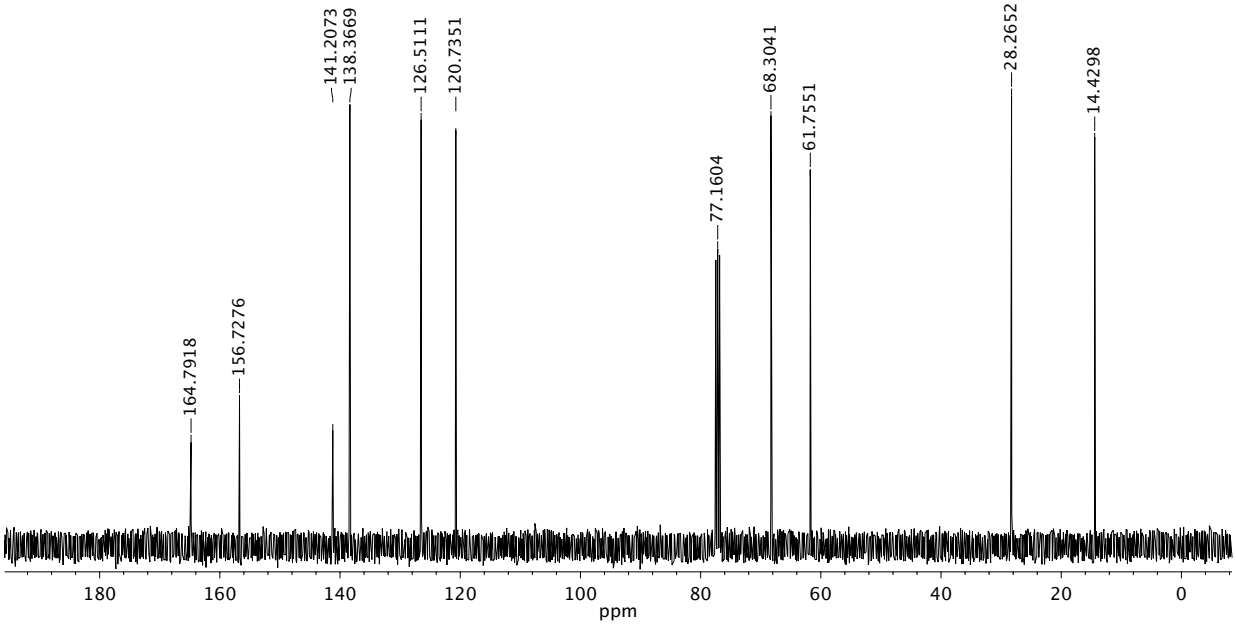
C (s)
141.21

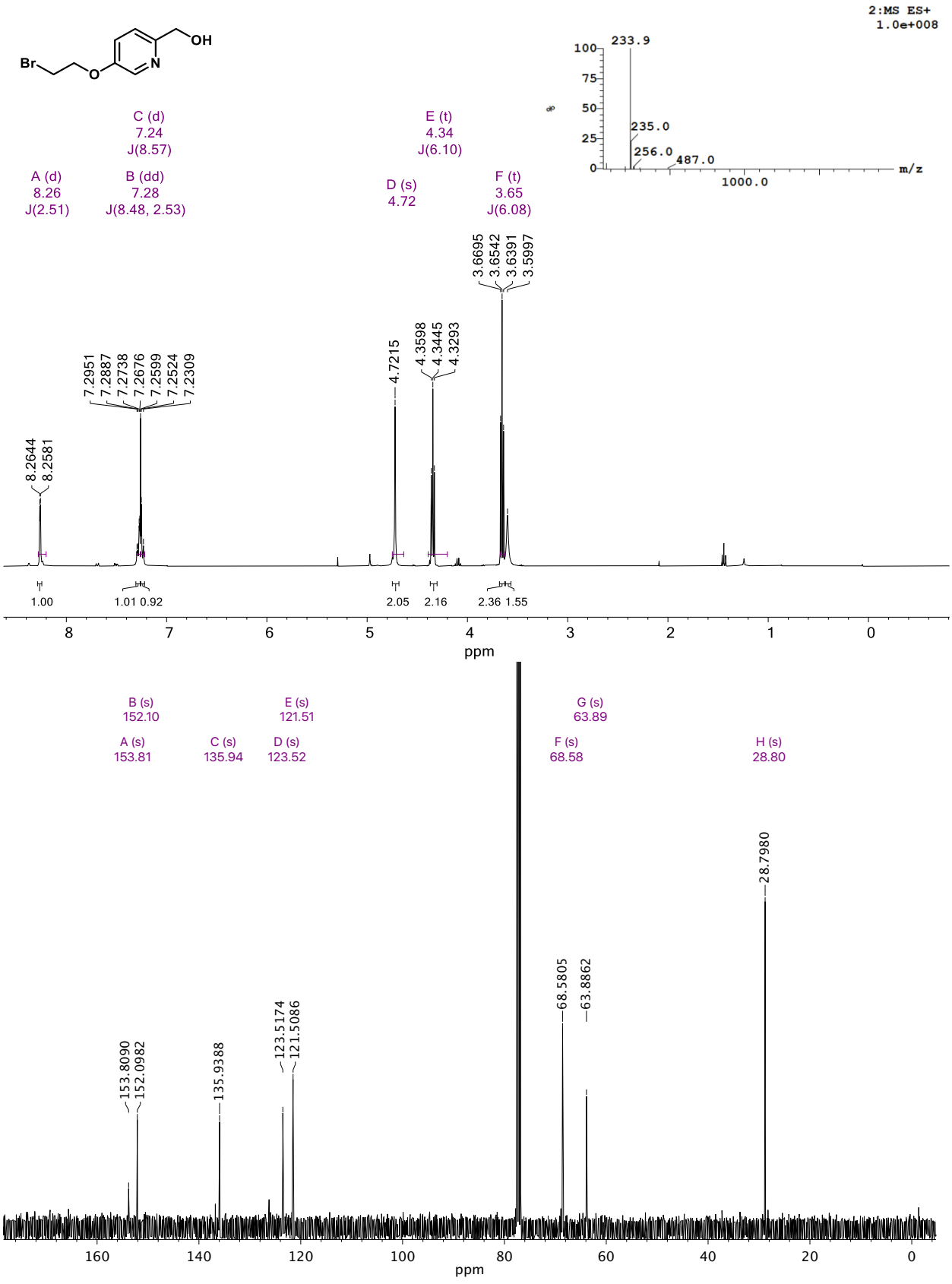
E (s)
126.51

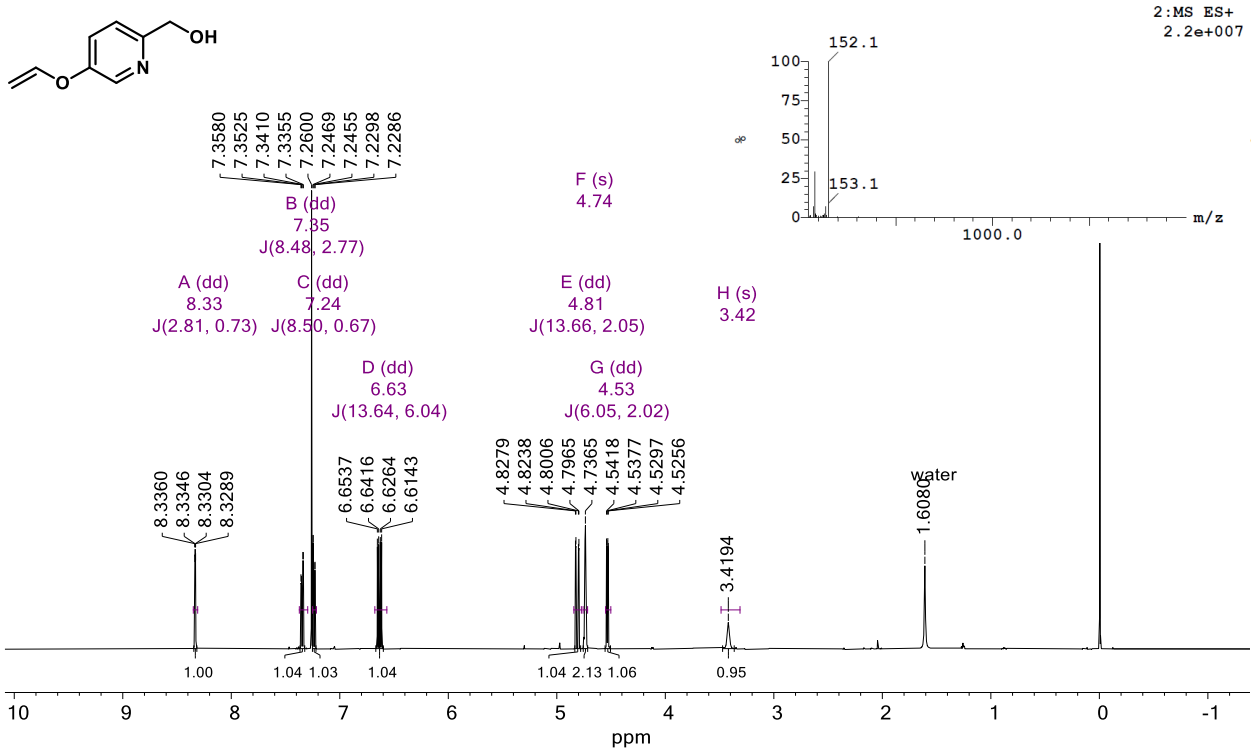
G (s)
68.30

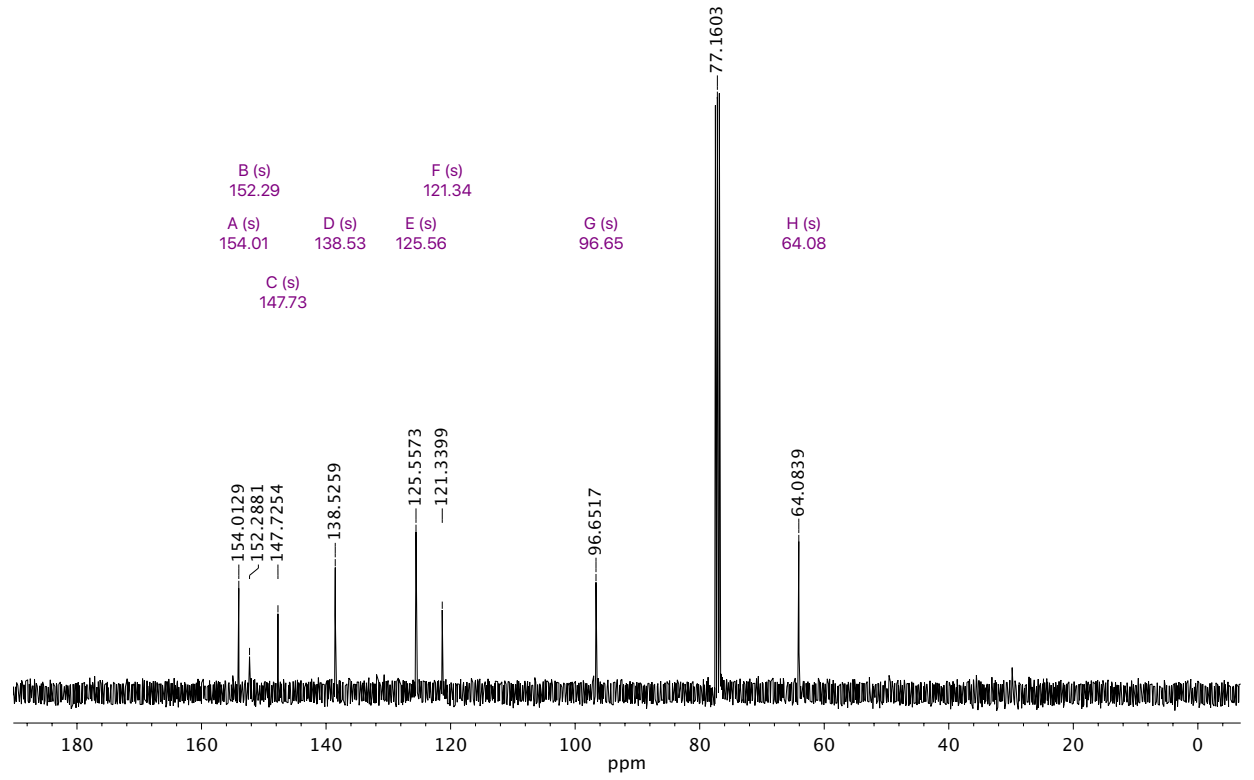
I (s)
28.27

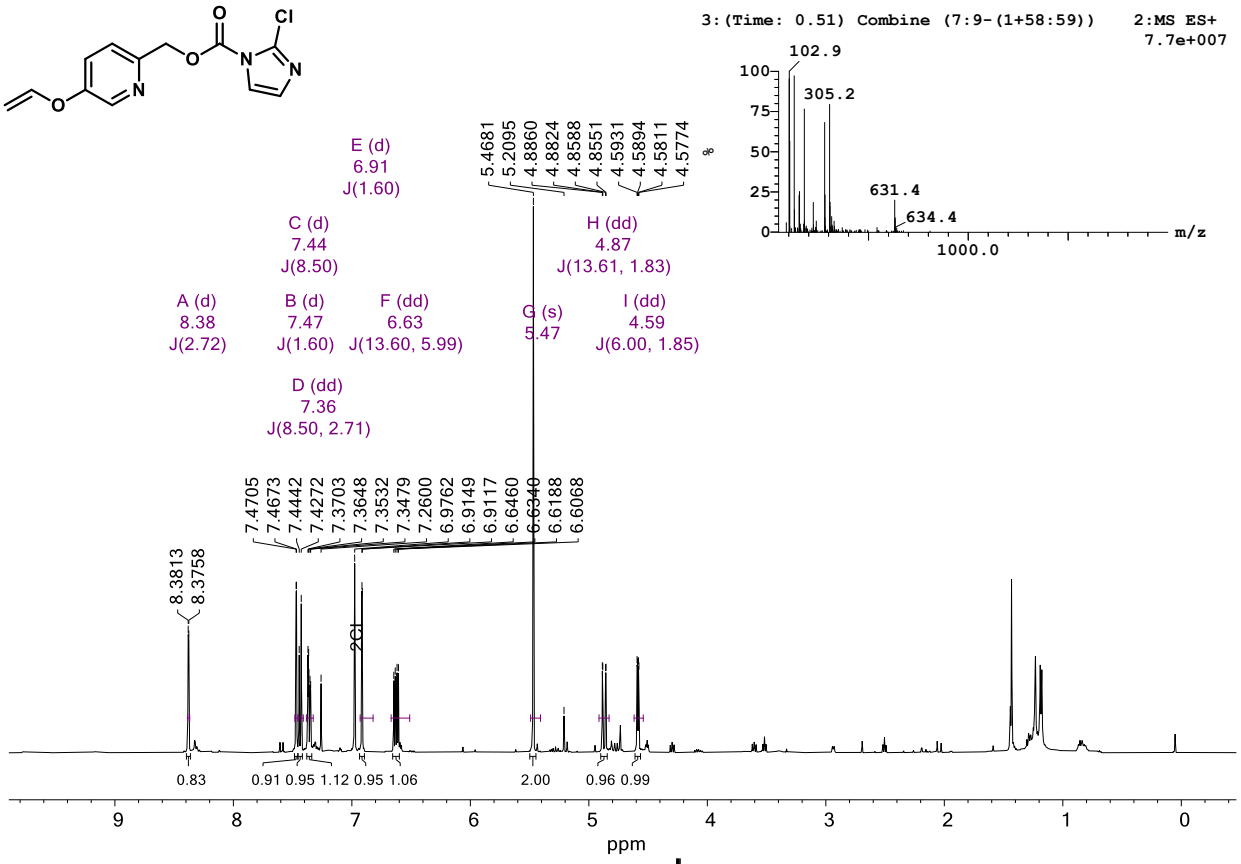
J (s)
14.43

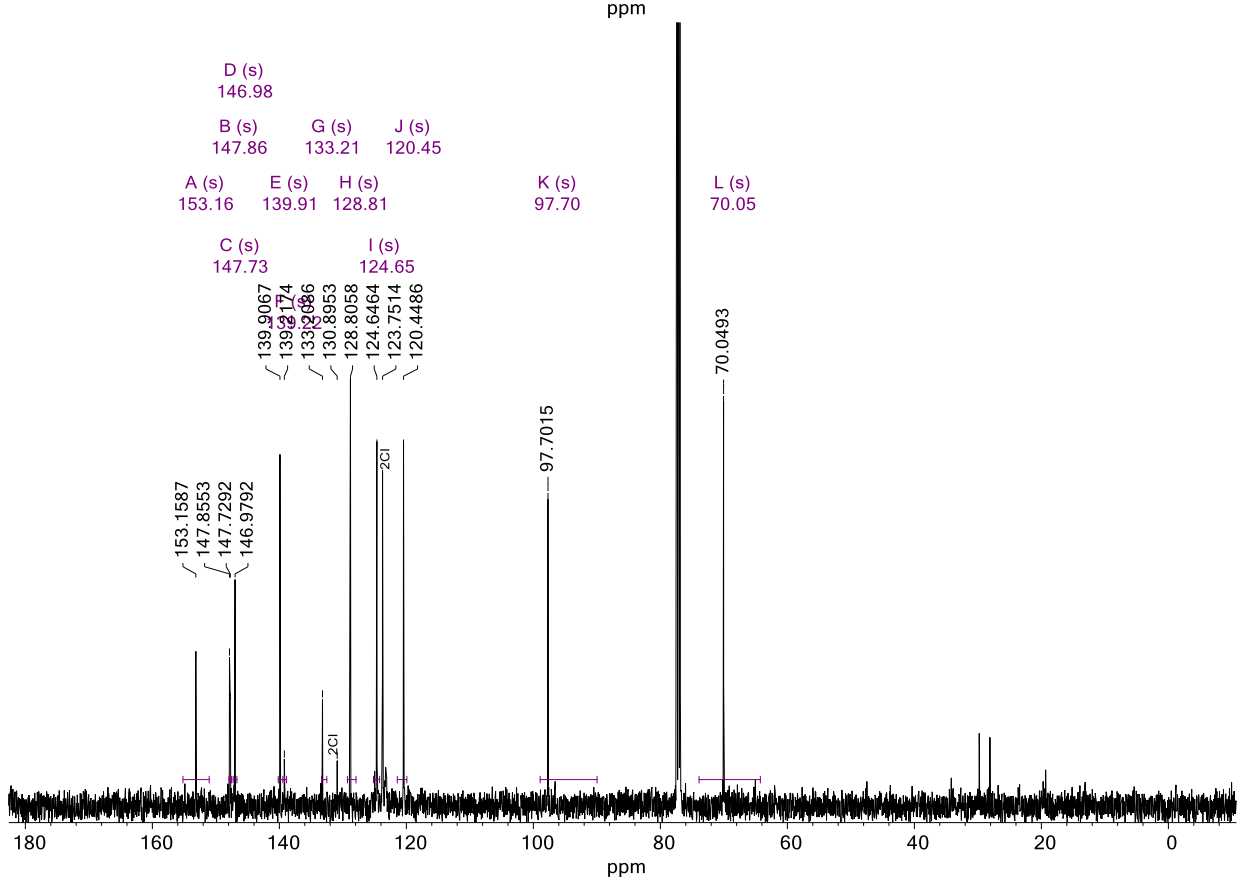


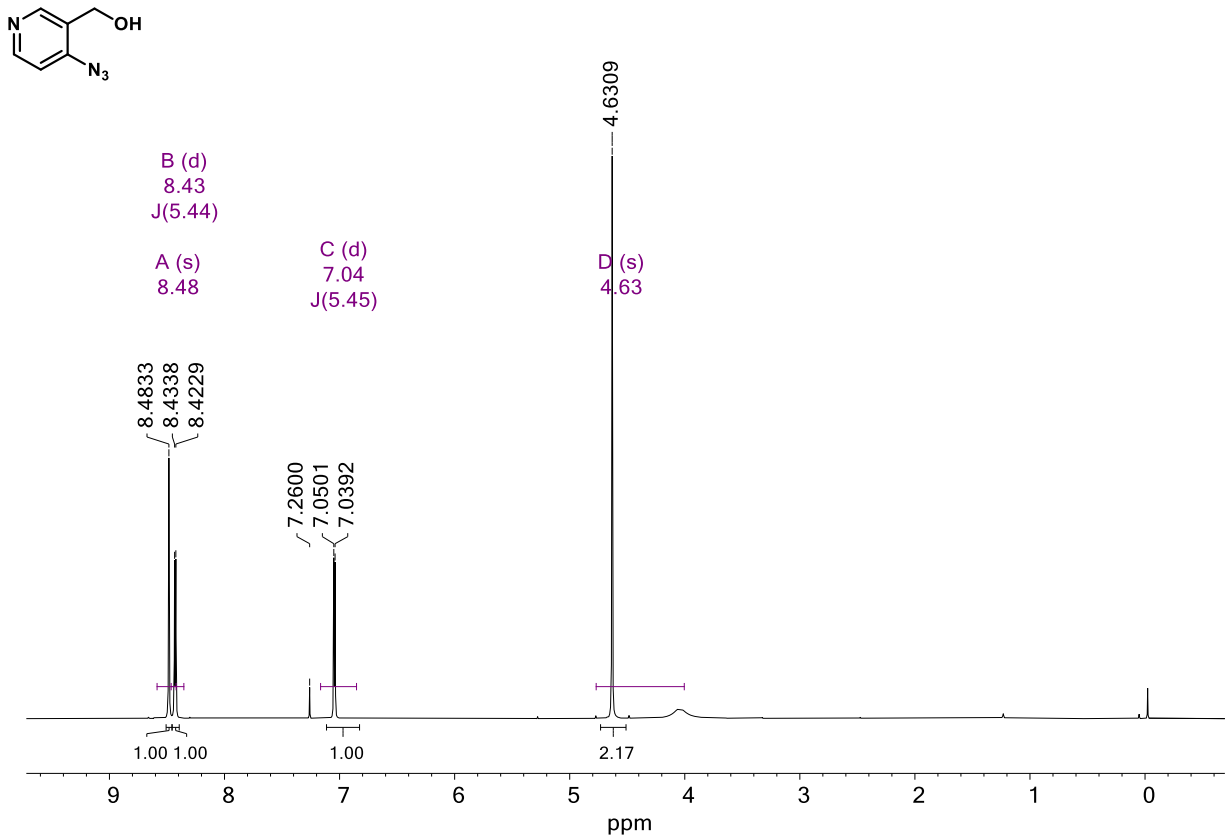

Br
O
N
OH
2:MS ES+
1.0e+008
233.9
235.0
256.0
487.0
1000.0
m/z
A (d)
8.26
J(2.51)
B (dd)
7.28
J(8.48, 2.53)
C (d)
7.24
J(8.57)
D (s)
4.72
E (t)
4.34
J(6.10)
F (t)
3.65
J(6.08)
8.2644
8.2581
7.2951
7.2887
7.2738
7.2676
7.2599
7.2524
7.2309
4.7215
4.3598
4.3445
4.3293
3.6695
3.6542
3.6391
3.5997
1.00
1.01 0.92
2.05
2.16
2.36 1.55
ppm
A (s)
153.81
B (s)
152.10
C (s)
135.94
D (s)
123.52
E (s)
121.51
F (s)
68.58
G (s)
63.89
H (s)
28.80
153.8090
152.0982
135.9388
123.5174
121.5086
68.5805
63.8862
28.7980
ppm

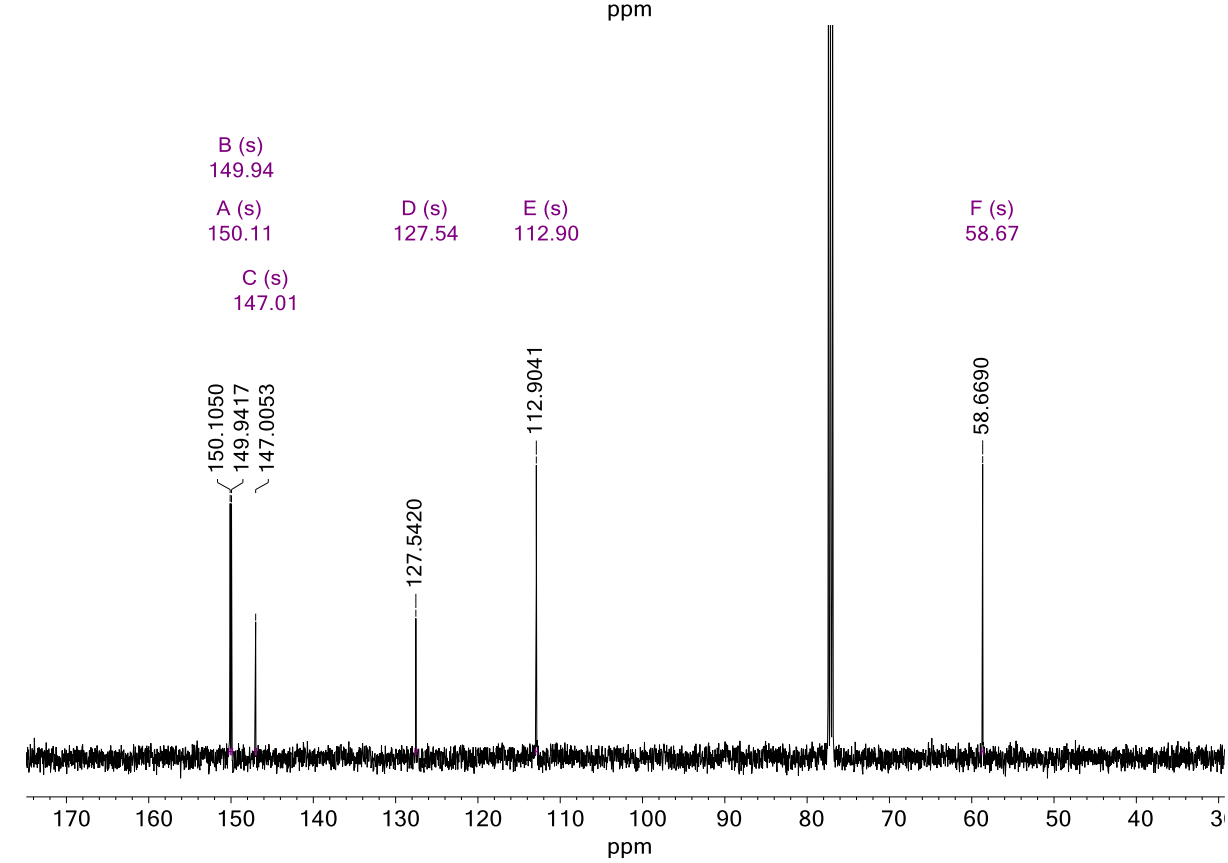

OH
N
O
2:MS ES+
2.2e+007
152.1
153.1
1000.0
m/z
7.3580
7.3525
7.3410
7.3355
7.2600
7.2469
7.2455
7.2298
7.2286
B (dd)
7.35
J(8.48, 2.77)
F (s)
4.74
A (dd)
8.33
J(2.81, 0.73)
C (dd)
7.24
J(8.50, 0.67)
E (dd)
4.81
J(13.66, 2.05)
H (s)
3.42
D (dd)
6.63
J(13.64, 6.04)
G (dd)
4.53
J(6.05, 2.02)
8.3360
8.3346
8.3304
8.3289
6.6537
6.6416
6.6264
6.6143
4.8279
4.8238
4.8006
4.7965
4.7365
4.5418
4.5377
4.5297
4.5256
3.4194
water
1.6080
1.00
1.04
1.03
1.04
1.04
2.13
1.06
0.95
ppm

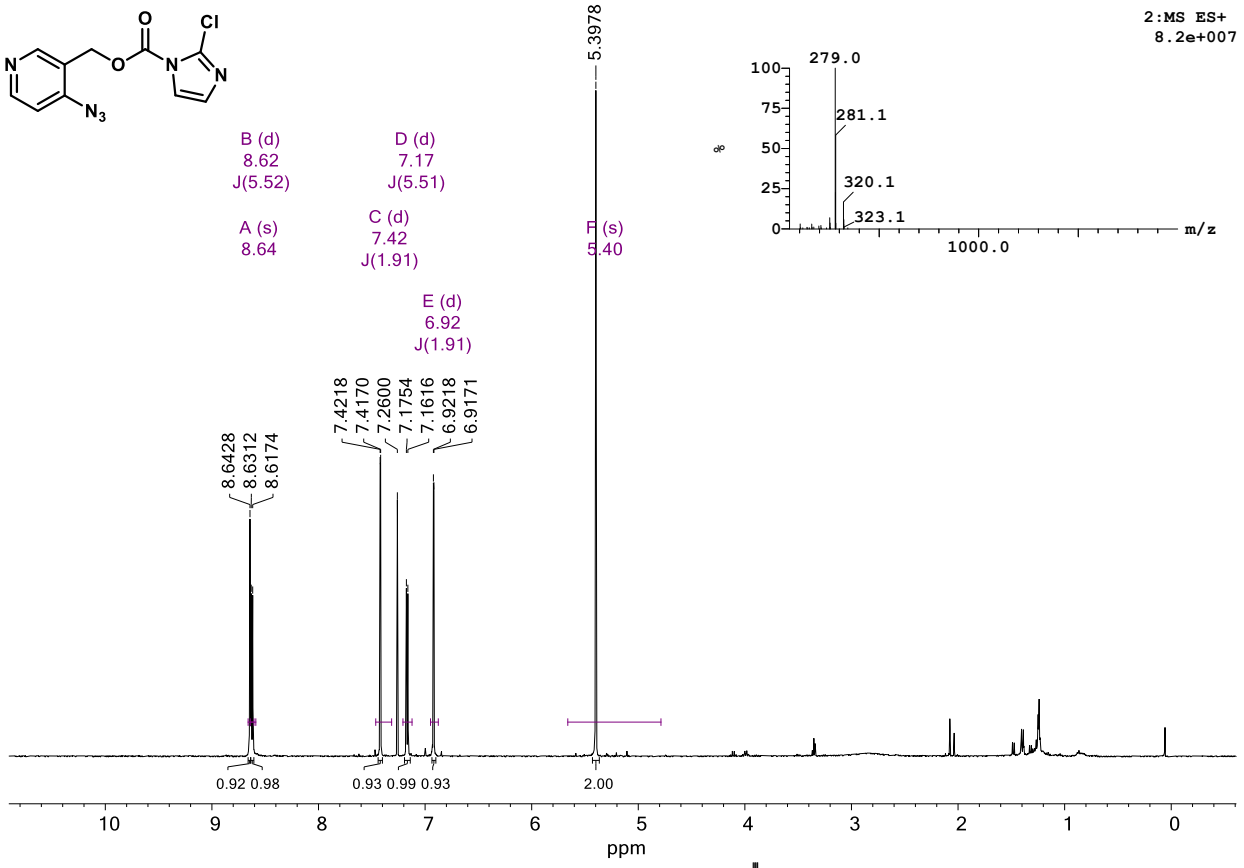

B (s)
152.29
F (s)
121.34
A (s)
154.01
D (s)
138.53
E (s)
125.56
G (s)
96.65
H (s)
64.08
C (s)
147.73
77.1603
154.0129
152.2881
147.7254
138.5259
125.5573
121.3399
96.6517
64.0839
ppm


3:(Time: 0.51) Combine (7:9-(1+58:59))
2:MS ES+
7.7e+007
102.9
305.2
631.4
634.4
1000.0
m/z
E (d)
6.91
J(1.60)
C (d)
7.44
J(8.50)
H (dd)
4.87
J(13.61, 1.83)
A (d)
8.38
J(2.72)
B (d)
7.47
J(1.60)
F (dd)
6.63
J(13.60, 5.99)
G (s)
5.47
I (dd)
4.59
J(6.00, 1.85)
D (dd)
7.36
J(8.50, 2.71)
2Cl
0.83
0.91 0.95 1.12 0.95 1.06
2.00
0.96 0.99
ppm

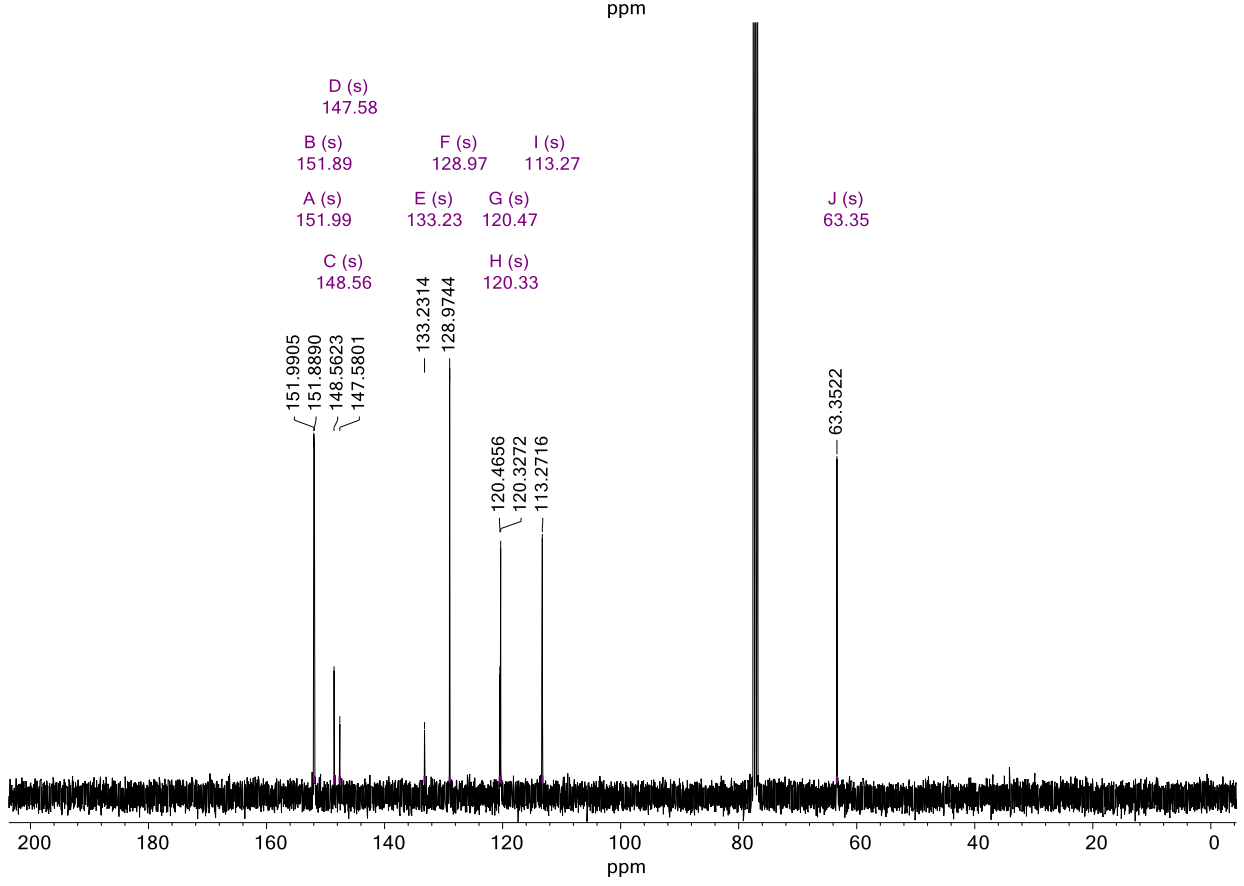

D (s)
146.98
B (s)
147.86
G (s)
133.21
J (s)
120.45
A (s)
153.16
E (s)
139.91
H (s)
128.81
K (s)
97.70
L (s)
70.05
C (s)
147.73
I (s)
124.65
2Cl
ppm

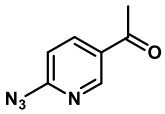

N
OH
N3
B (d)
8.43
J(5.44)
A (s)
8.48
C (d)
7.04
J(5.45)
D (s)
4.63
4.6309
8.4833
8.4338
8.4229
7.2600
7.0501
7.0392
1.00 1.00
1.00
2.17
9
8
7
6
5
4
3
2
1
0
ppm

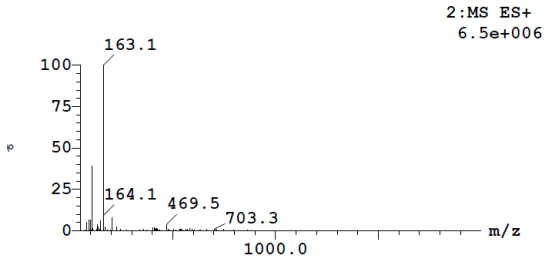

B (s)
149.94
A (s)
150.11
C (s)
147.01
D (s)
127.54
E (s)
112.90
F (s)
58.67
150.1050
149.9417
147.0053
127.5420
112.9041
58.6690
170
160
150
140
130
120
110
100
90
80
70
60
50
40
ppm

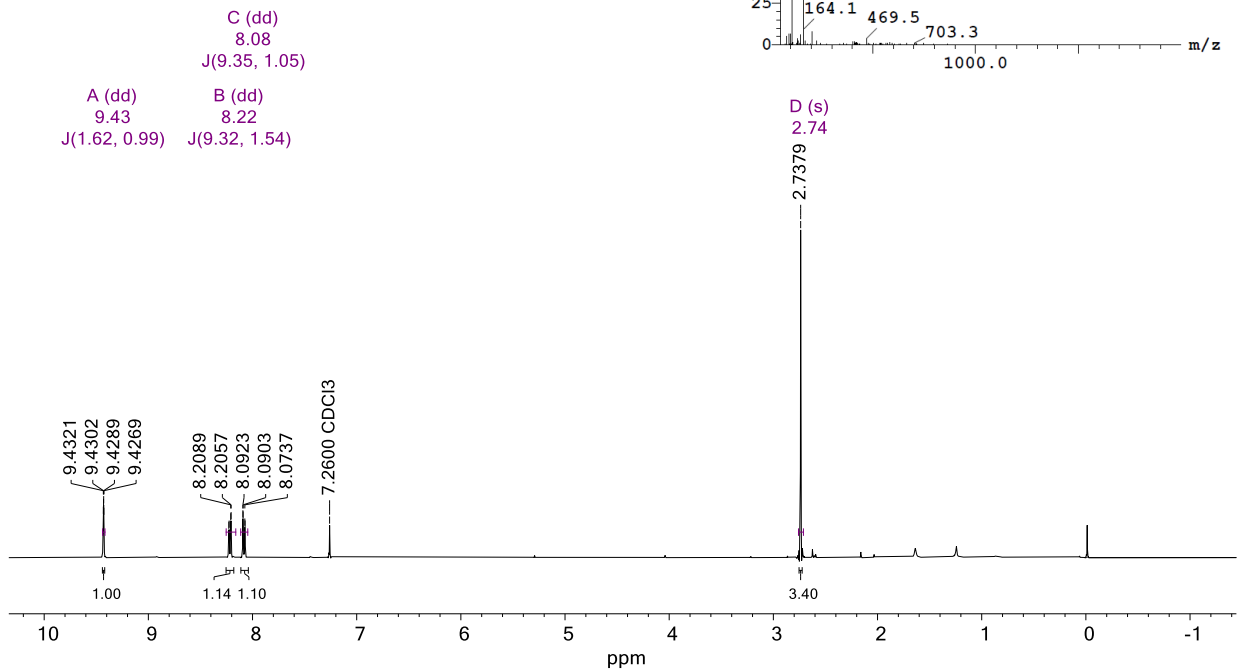

B (d)
8.62
J(5.52)
A (s)
8.64
D (d)
7.17
J(5.51)
C (d)
7.42
J(1.91)
E (d)
6.92
J(1.91)
F (s)
5.40
8.6428
8.6312
8.6174
7.4218
7.4170
7.2600
7.1754
7.1616
6.9218
6.9171
5.3978
0.92 0.98
0.93 0.99 0.93
2.00
ppm

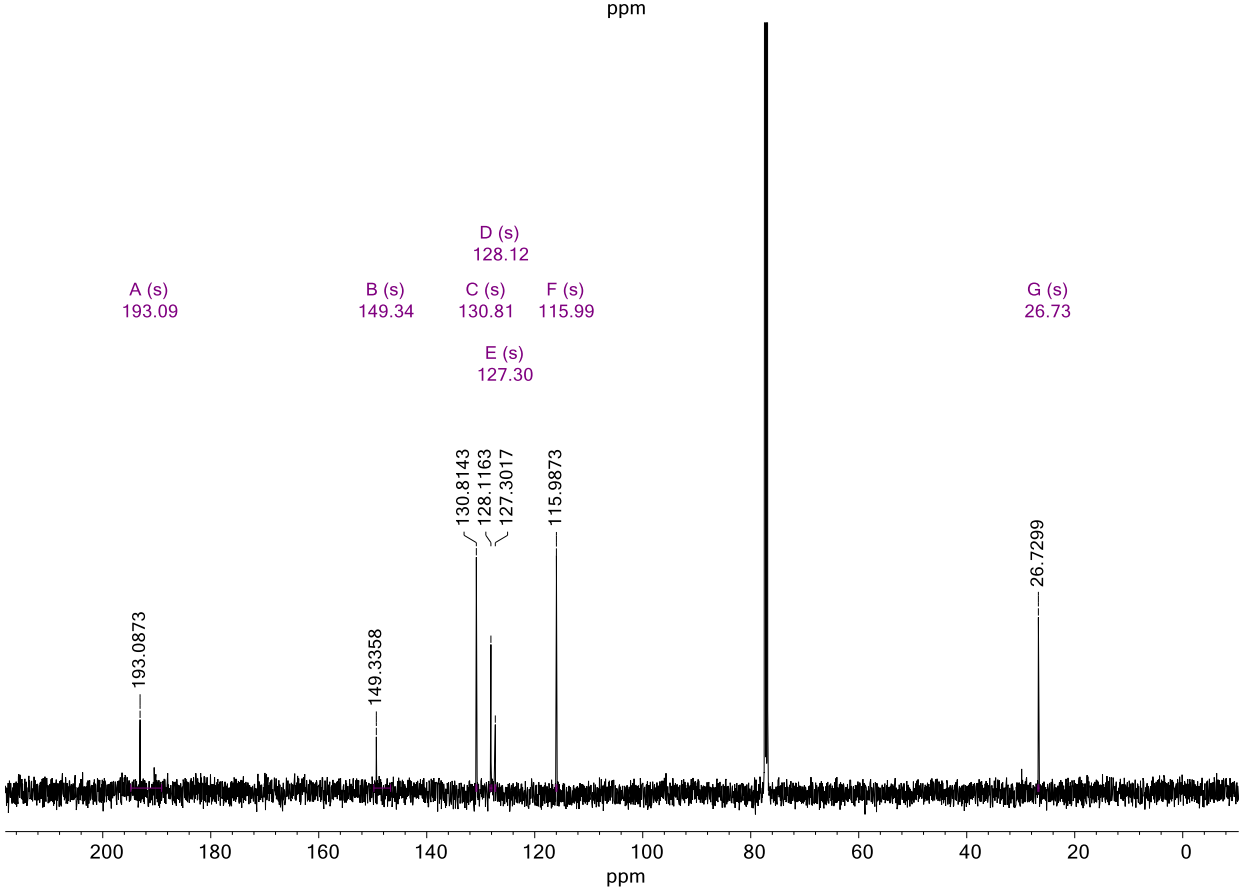
2:MS ES+
8.2e+007
279.0
281.1
320.1
323.1
1000.0
m/z

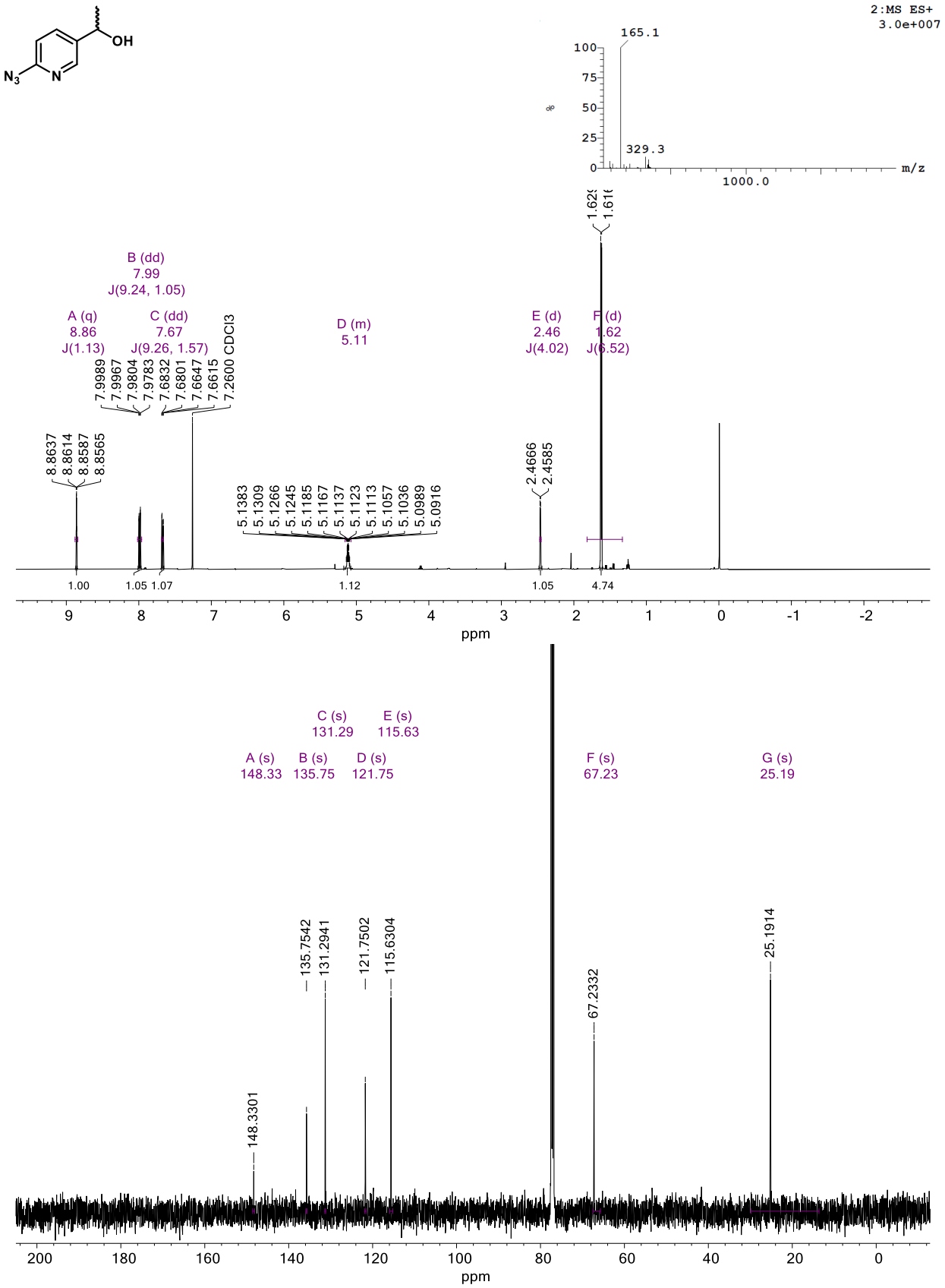

D (s)
147.58
B (s)
151.89
A (s)
151.99
C (s)
148.56
F (s)
128.97
E (s)
133.23
G (s)
120.47
H (s)
120.33
I (s)
113.27
J (s)
63.35
151.9905
151.8890
148.5623
147.5801
133.2314
128.9744
120.4656
120.3272
113.2716
63.3522
ppm

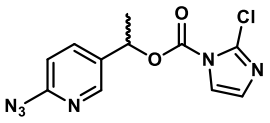

O
N3
N

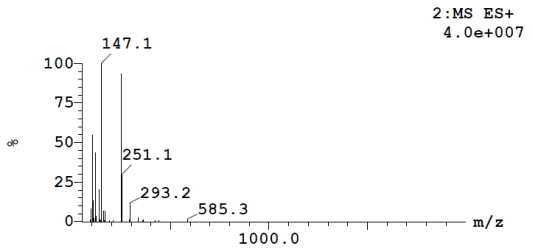

2:MS ES+
6.5e+006
163.1
100
75
50
25
0
164.1
469.5
703.3
1000.0
m/z

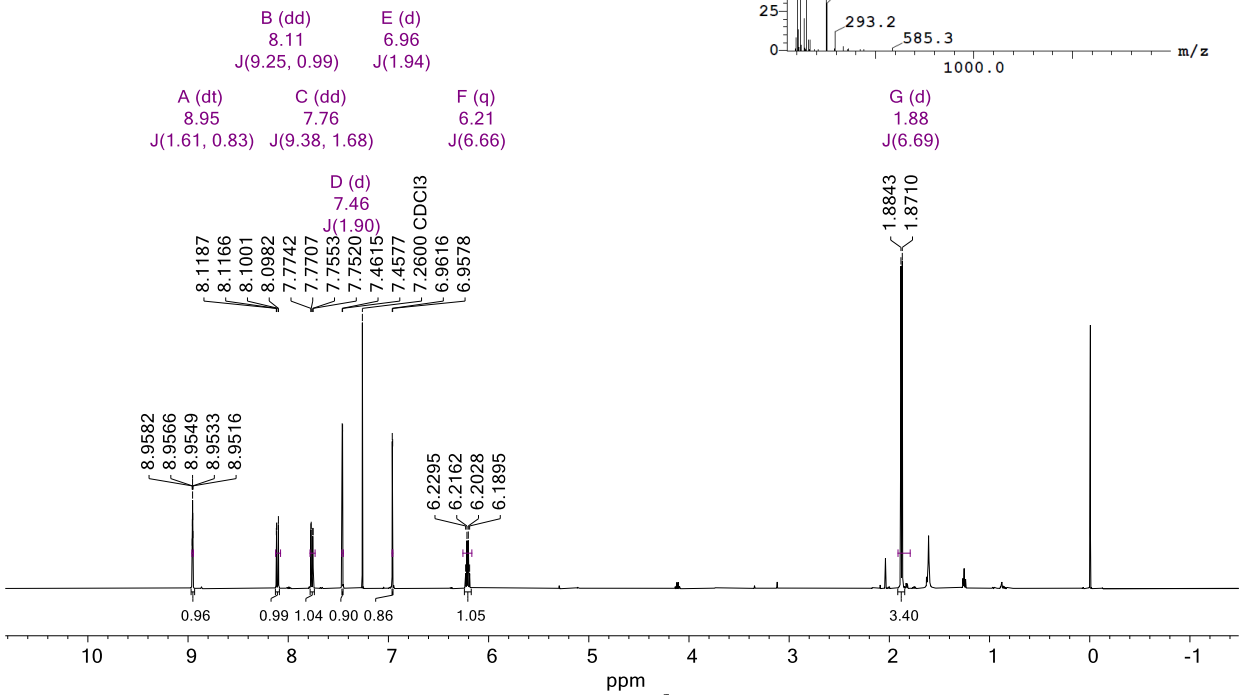

C (dd)
8.08
J(9.35, 1.05)
A (dd)
9.43
J(1.62, 0.99)
B (dd)
8.22
J(9.32, 1.54)
D (s)
2.74
2.7379
9.4321
9.4302
9.4289
9.4269
8.2089
8.2057
8.0923
8.0903
8.0737
7.2600 CDCl3
1.00
1.14
1.10
3.40
10
9
8
7
6
5
4
3
2
1
0
-1
ppm

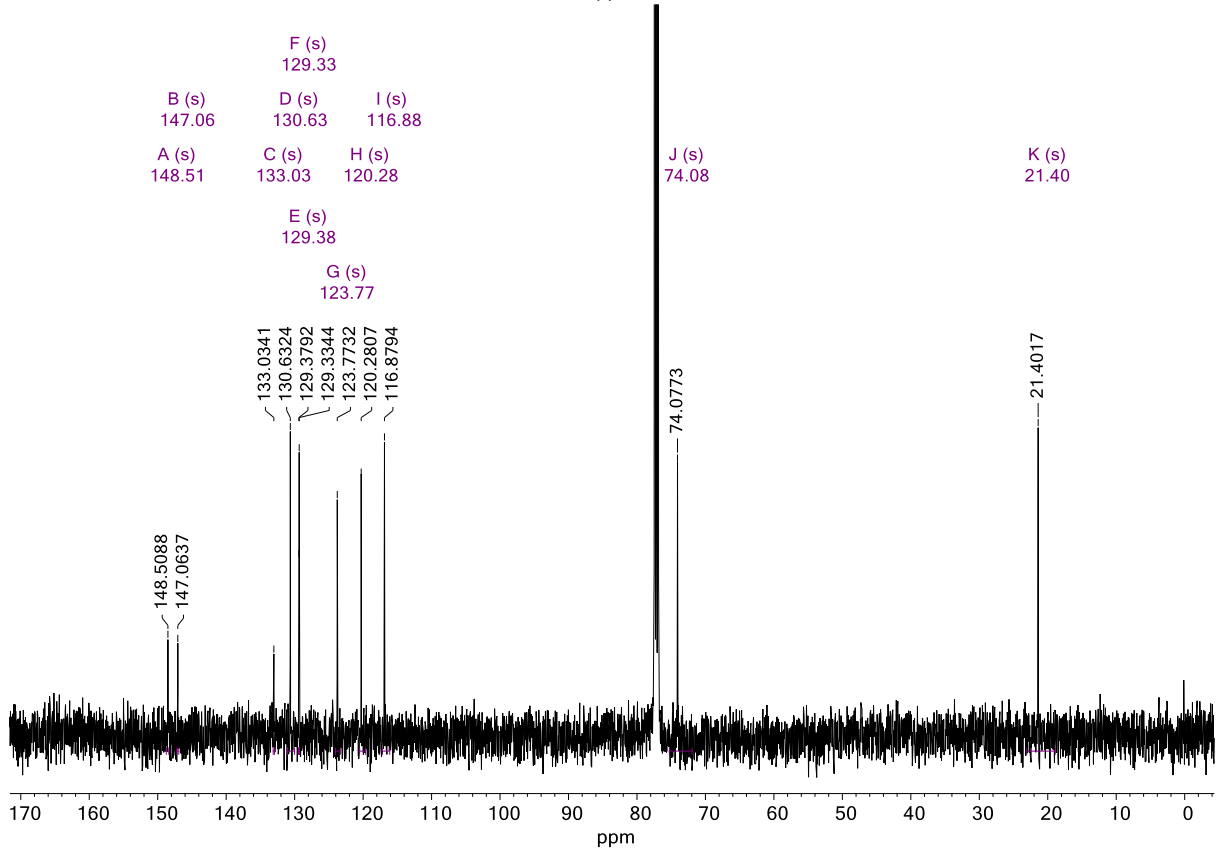

D (s)
128.12
A (s)
193.09
B (s)
149.34
C (s)
130.81
F (s)
115.99
E (s)
127.30
G (s)
26.73
130.8143
128.1163
127.3017
115.9873
193.0873
149.3358
26.7299
200
180
160
140
120
100
80
60
40
20
0
ppm

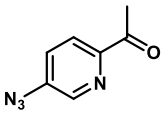

OH
N3
N
2:MS ES+
3.0e+007
165.1
329.3
m/z
1000.0
B (dd)
7.99
J(9.24, 1.05)
A (q)
8.86
J(1.13)
C (dd)
7.67
J(9.26, 1.57)
D (m)
5.11
E (d)
2.46
J(4.02)
F (d)
1.62
J(6.52)
CDCl3
ppm
C (s)
131.29
E (s)
115.63
A (s)
148.33
B (s)
135.75
D (s)
121.75
F (s)
67.23
G (s)
25.19
ppm

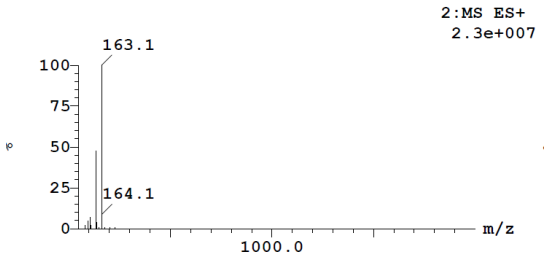

O
Cl
O
N
N
N_3
N

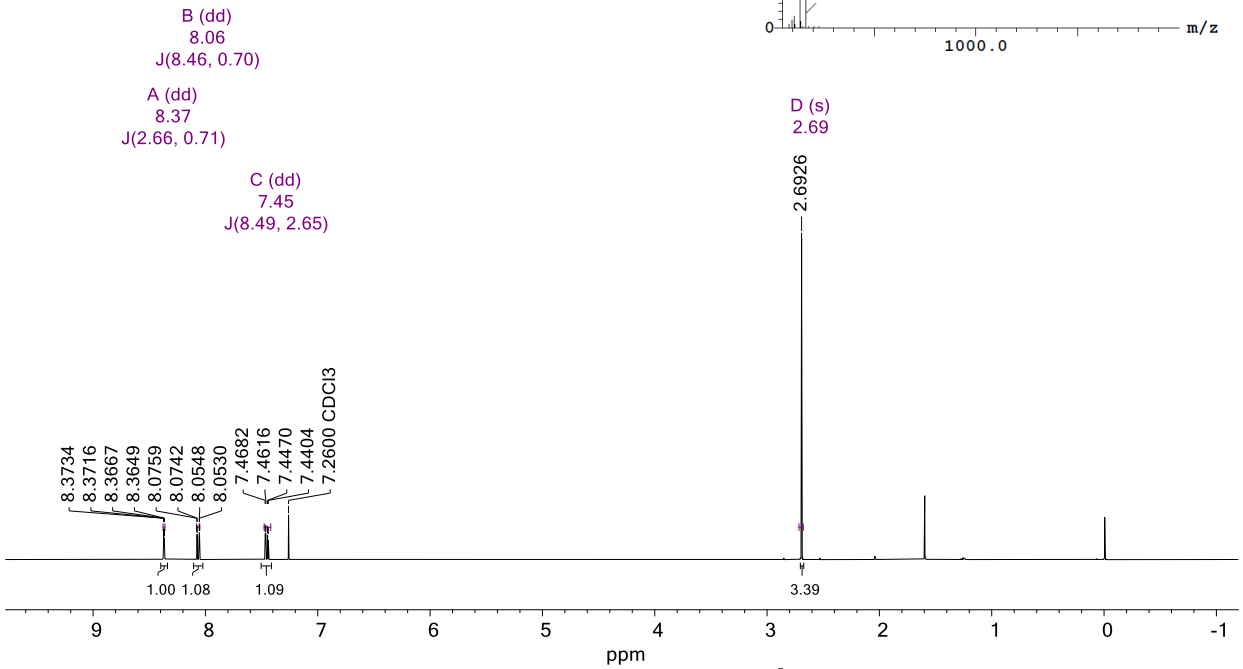

2:MS ES+
4.0e+007
147.1
100
75
50
25
0
%
251.1
293.2
585.3
1000.0
m/z

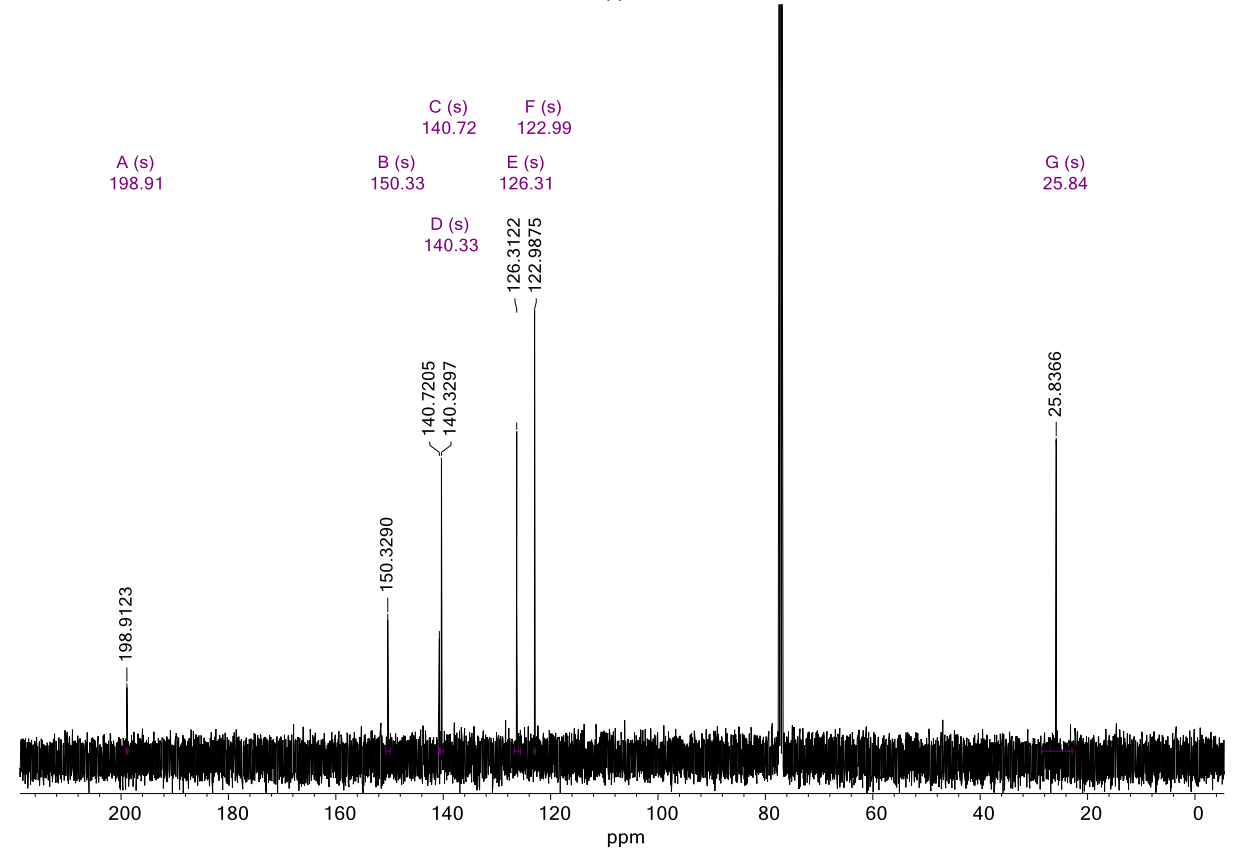

B (dd)
8.11
J(9.25, 0.99)
E (d)
6.96
J(1.94)
A (dt)
8.95
J(1.61, 0.83)
C (dd)
7.76
J(9.38, 1.68)
F (q)
6.21
J(6.66)
G (d)
1.88
J(6.69)
D (d)
7.46
J(1.90)
CDCl3
8.1187
8.1166
8.1001
8.0982
7.7742
7.7707
7.7553
7.7520
7.4615
7.4577
7.2600
6.9616
6.9578
1.8843
1.8710
8.9582
8.9566
8.9549
8.9533
8.9516
6.2295
6.2162
6.2028
6.1895
0.96
0.99
1.04
0.90
0.86
1.05
3.40
10
9
8
7
6
5
4
3
2
1
0
-1
ppm

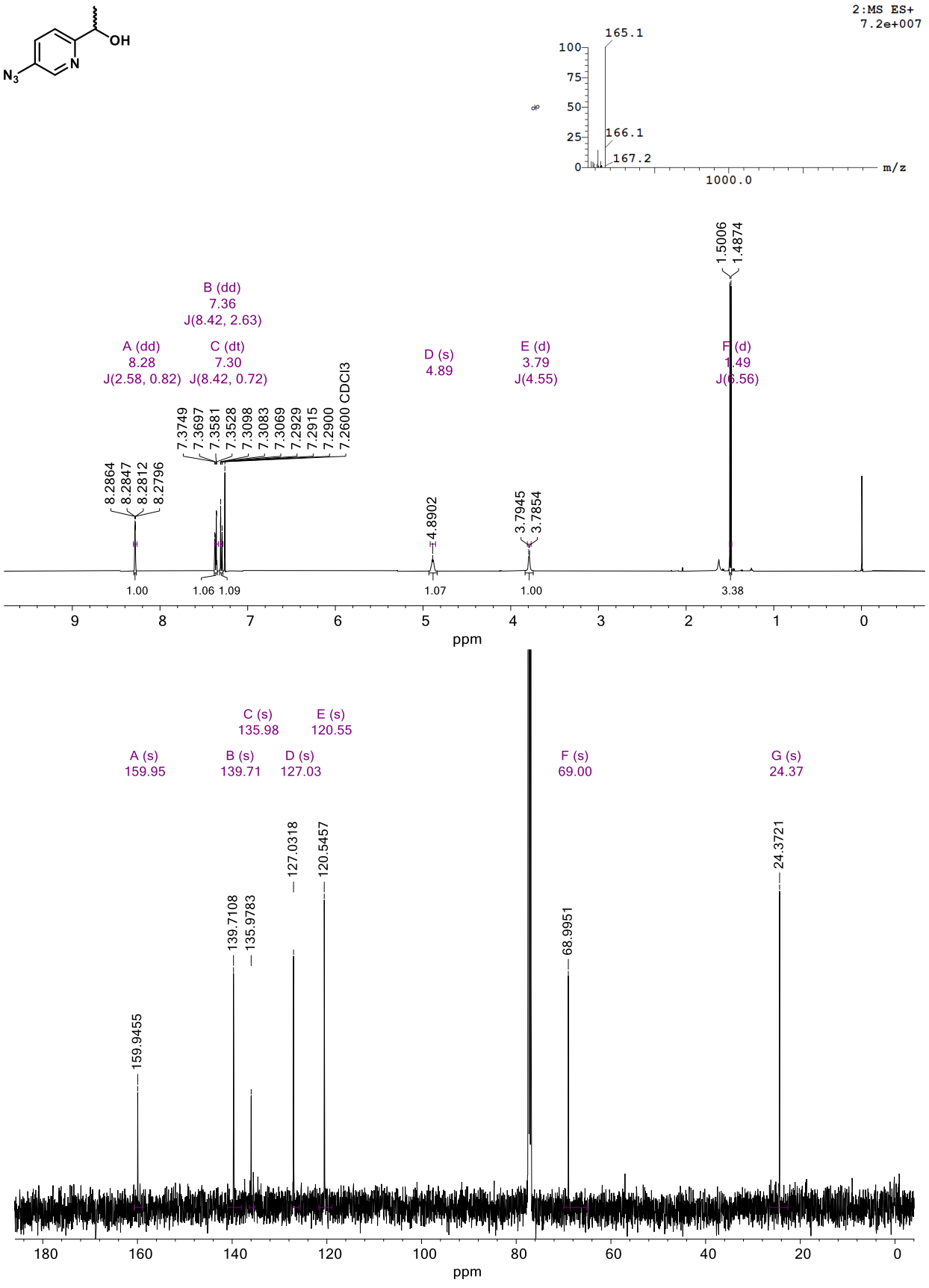

F (s)
129.33
B (s)
147.06
D (s)
130.63
I (s)
116.88
A (s)
148.51
C (s)
133.03
H (s)
120.28
J (s)
74.08
K (s)
21.40
E (s)
129.38
G (s)
123.77
133.0341
130.6324
129.3792
129.3344
123.7732
120.2807
116.8794
74.0773
21.4017
148.5088
147.0637
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm

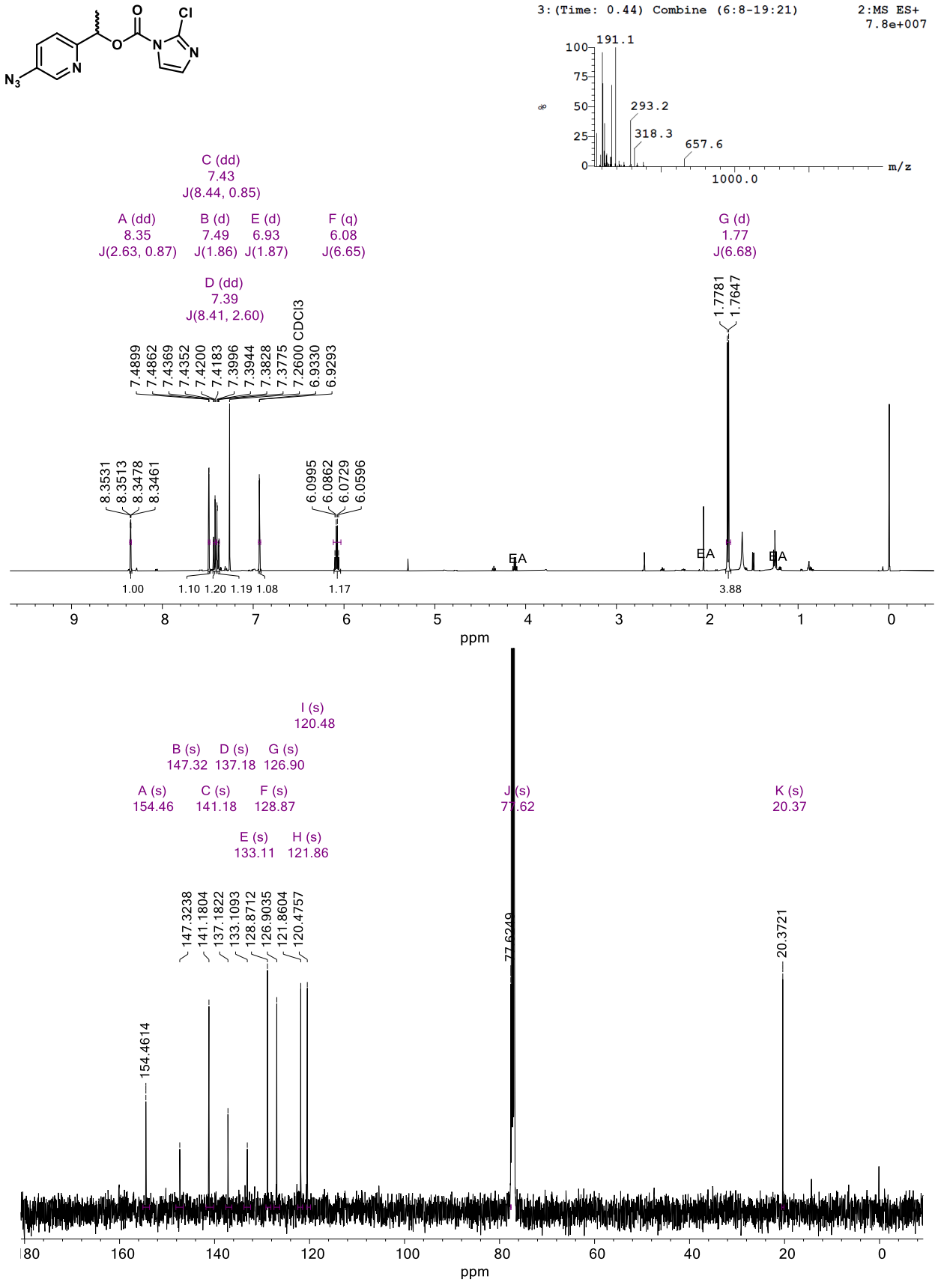

O
N
N_3

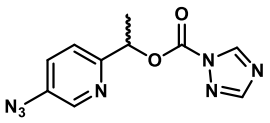

2:MS ES+
2.3e+007
163.1
164.1
100
75
50
25
0
m/z
1000.0

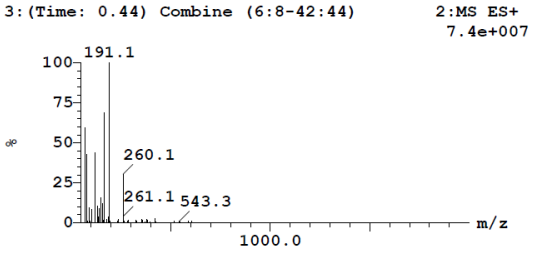

B (dd)
8.06
J(8.46, 0.70)
A (dd)
8.37
J(2.66, 0.71)
C (dd)
7.45
J(8.49, 2.65)
D (s)
2.69
2.6926
8.3734
8.3716
8.3667
8.3649
8.0759
8.0742
8.0548
8.0530
7.4682
7.4616
7.4470
7.4404
7.2600 CDCl3
1.00 1.08 1.09 3.39
9 8 7 6 5 4 3 2 1 0 -1
ppm

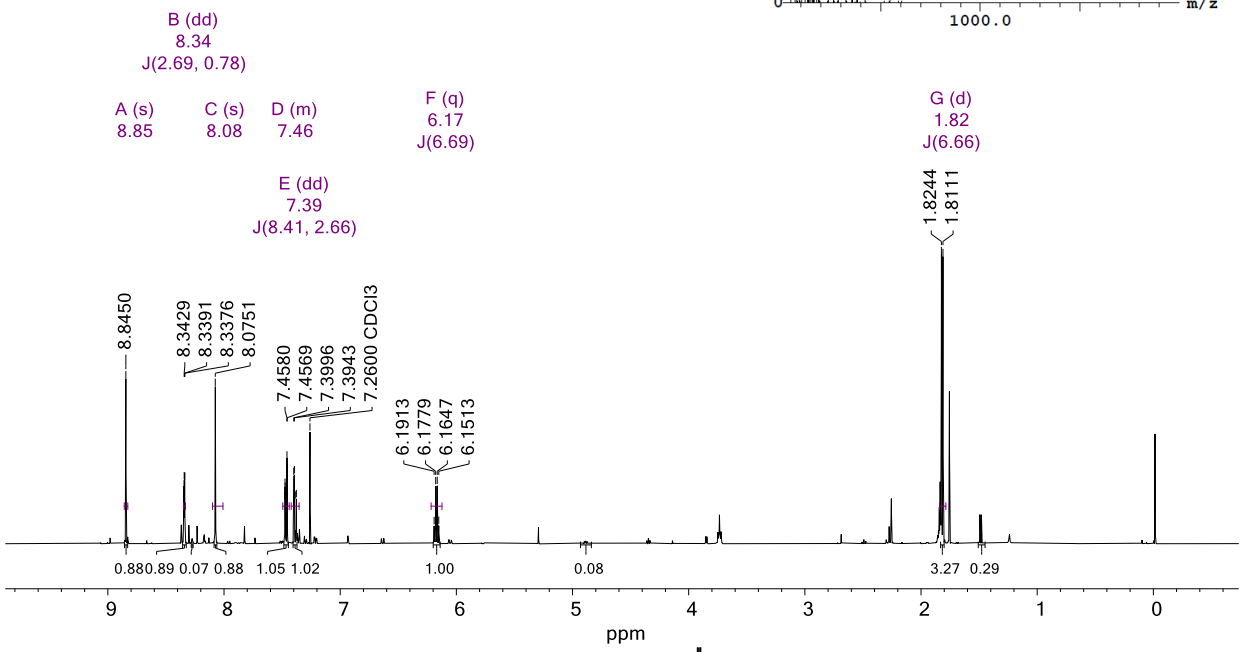

A (s)
198.91
B (s)
150.33
C (s)
140.72
D (s)
140.33
E (s)
126.31
F (s)
122.99
G (s)
25.84
198.9123
150.3290
140.7205
140.3297
126.3122
122.9875
25.8366
200 180 160 140 120 100 80 60 40 20 0
ppm

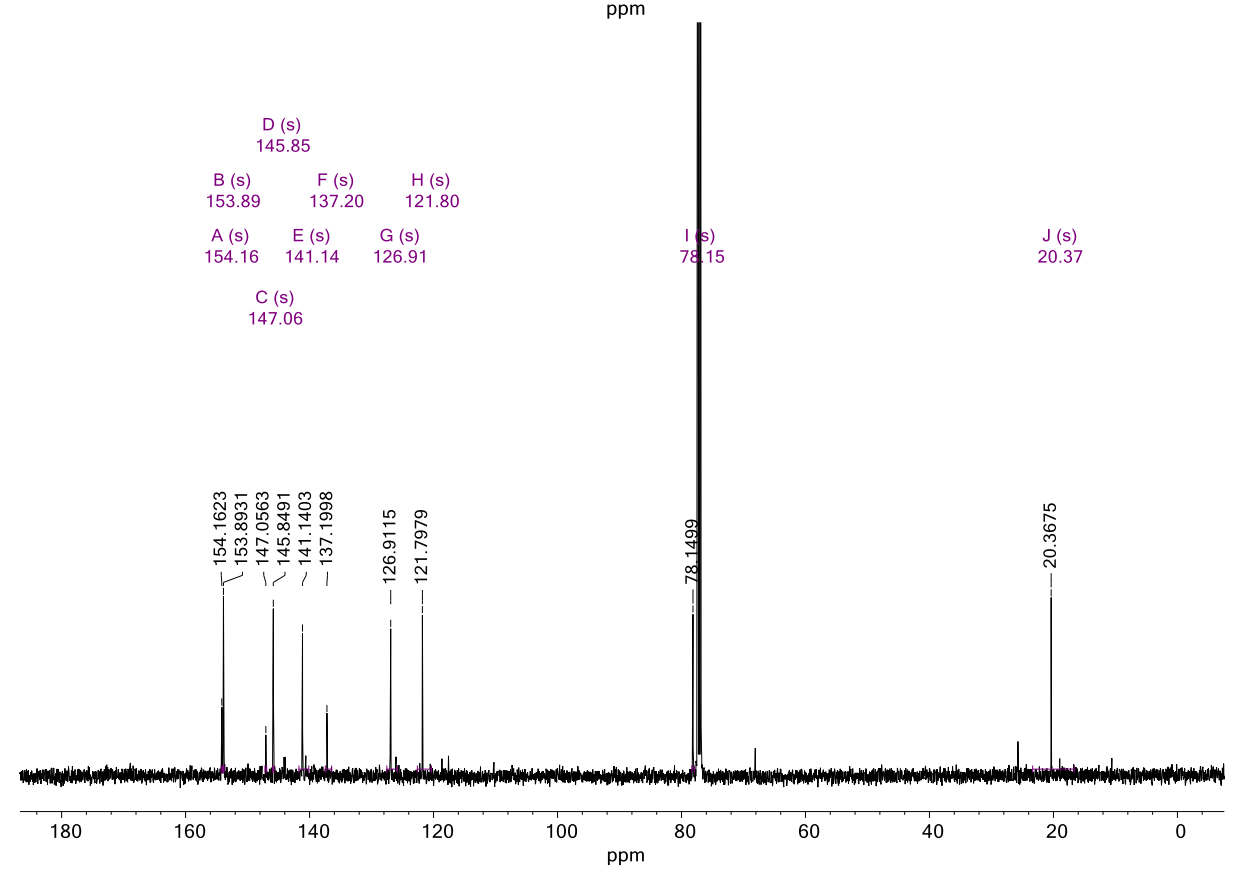

OH
N
N3
2:MS ES+
7.2e+007
165.1
166.1
167.2
m/z
1000.0
B (dd)
7.36
J(8.42, 2.63)
A (dd)
8.28
J(2.58, 0.82)
C (dt)
7.30
J(8.42, 0.72)
D (s)
4.89
E (d)
3.79
J(4.55)
F (d)
1.49
J(6.56)
7.2600 CDCl3
ppm
C (s)
135.98
E (s)
120.55
A (s)
159.95
B (s)
139.71
D (s)
127.03
F (s)
69.00
G (s)
24.37
159.9455
139.7108
135.9783
127.0318
120.5457
68.9951
24.3721
ppm

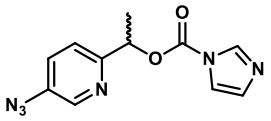

3:(Time: 0.44) Combine (6:8-19:21)
2:MS ES+
7.8e+007
191.1
293.2
318.3
657.6
1000.0
m/z
C (dd)
7.43
J(8.44, 0.85)
A (dd)
8.35
J(2.63, 0.87)
B (d)
7.49
J(1.86)
E (d)
6.93
J(1.87)
F (q)
6.08
J(6.65)
G (d)
1.77
J(6.68)
D (dd)
7.39
J(8.41, 2.60)
CDCl3
EA
ppm
I (s)
120.48
B (s)
147.32
D (s)
137.18
G (s)
126.90
A (s)
154.46
C (s)
141.18
F (s)
128.87
J (s)
77.62
K (s)
20.37
E (s)
133.11
H (s)
121.86
ppm

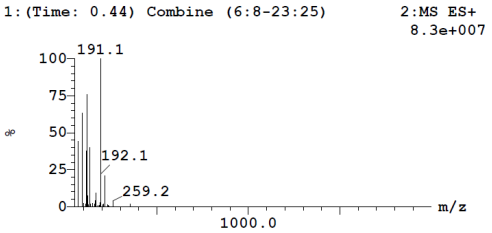


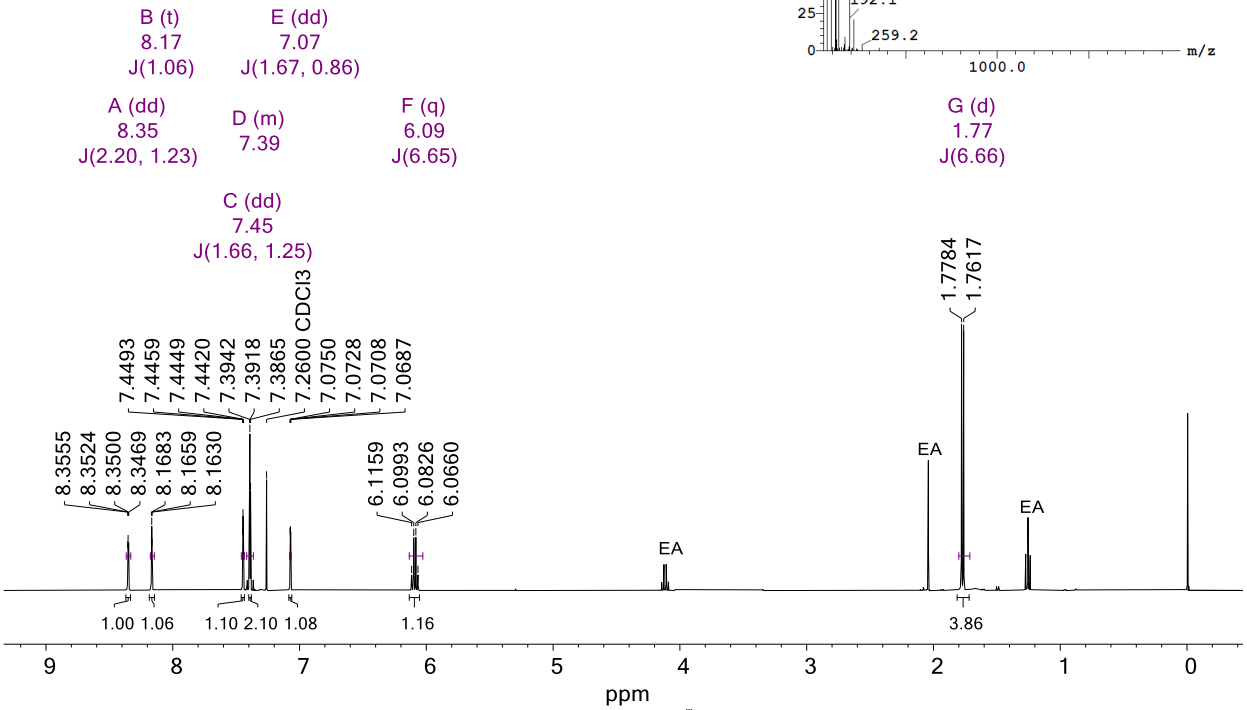

3:(Time: 0.44) Combine (6:8-42:44)
2:MS ES+
7.4e+007
191.1
260.1
261.1
543.3
1000.0
m/z

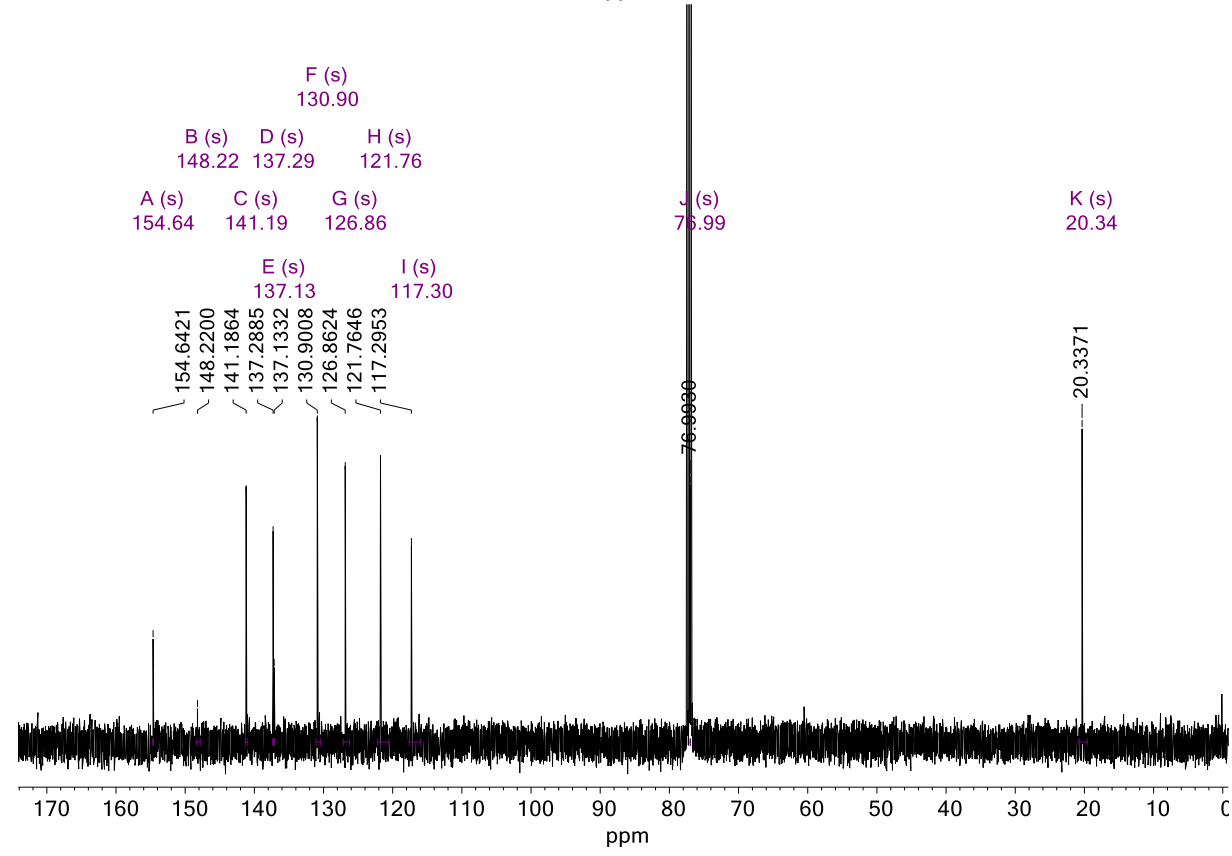

B (dd)
8.34
J(2.69, 0.78)
A (s)
8.85
C (s)
8.08
D (m)
7.46
F (q)
6.17
J(6.69)
G (d)
1.82
J(6.66)
E (dd)
7.39
J(8.41, 2.66)
8.8450
8.3429
8.3391
8.3376
8.0751
7.4580
7.4569
7.3996
7.3943
7.2600 CDCl3
6.1913
6.1779
6.1647
6.1513
1.8244
1.8111
0.88 0.89 0.07 0.88 1.05 1.02 1.00 0.08 3.27 0.29
ppm

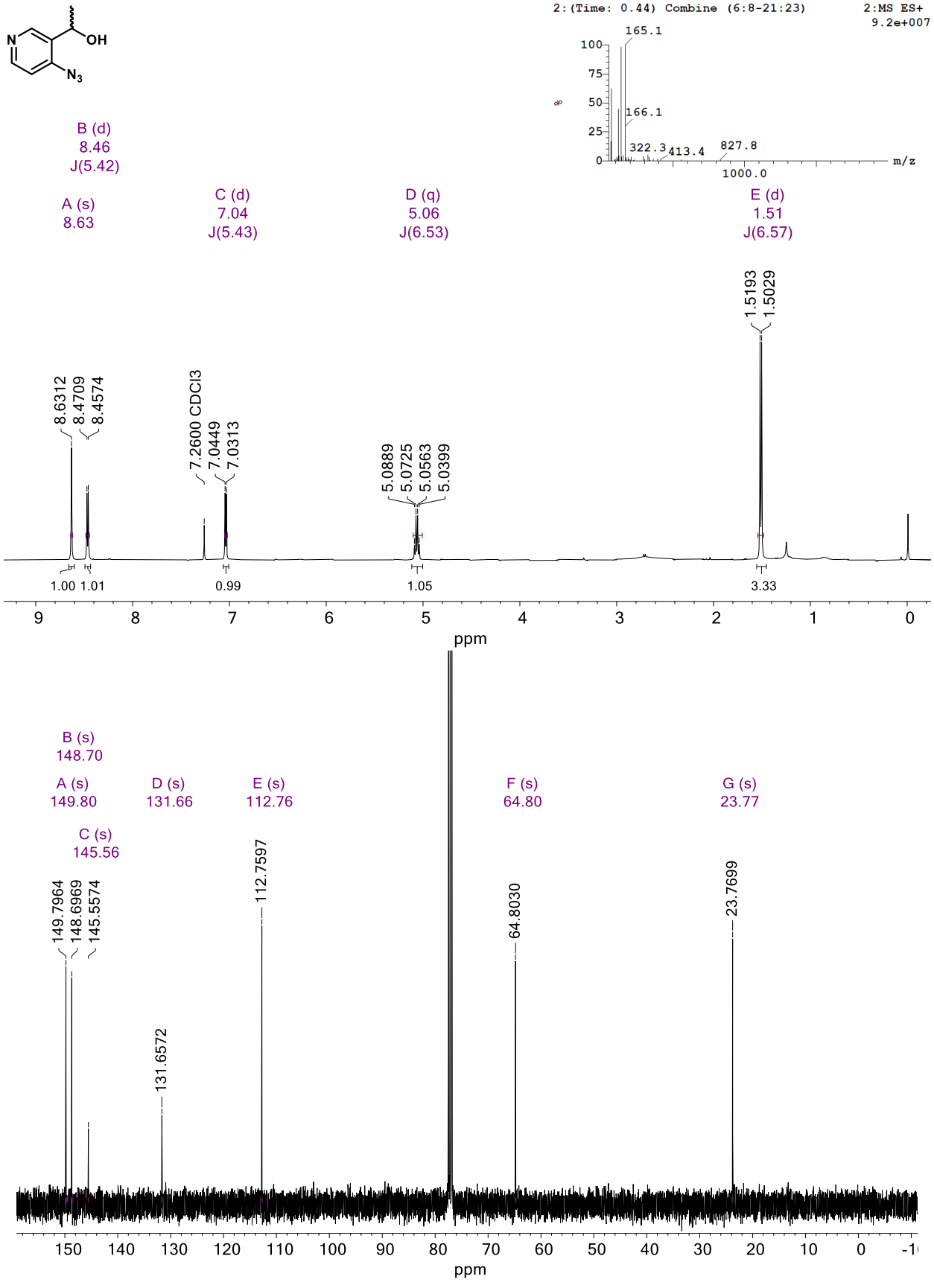

D (s)
145.85
B (s)
153.89
F (s)
137.20
H (s)
121.80
A (s)
154.16
E (s)
141.14
G (s)
126.91
C (s)
147.06
I (s)
78.15
J (s)
20.37
154.1623
153.8931
147.0563
145.8491
141.1403
137.1998
126.9115
121.7979
78.1499
20.3675
ppm

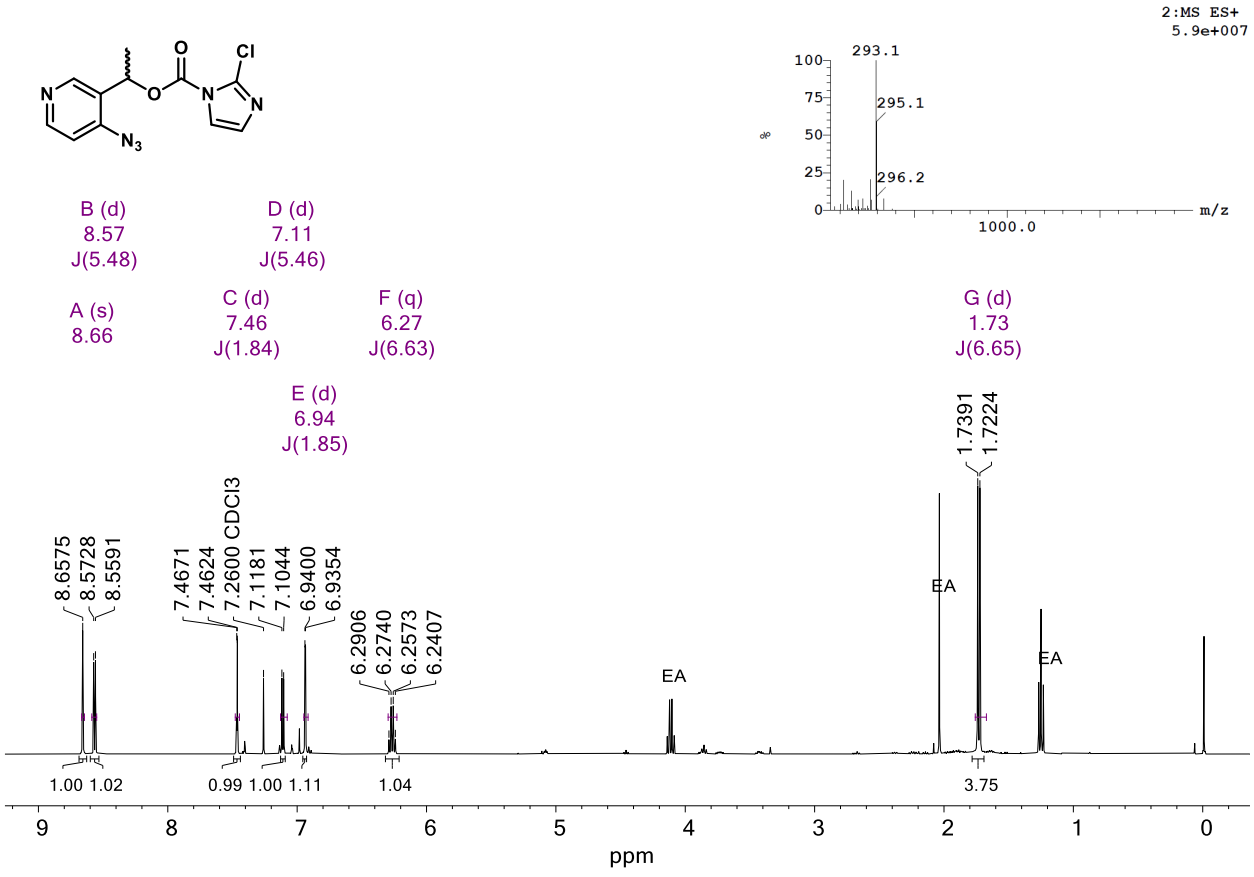


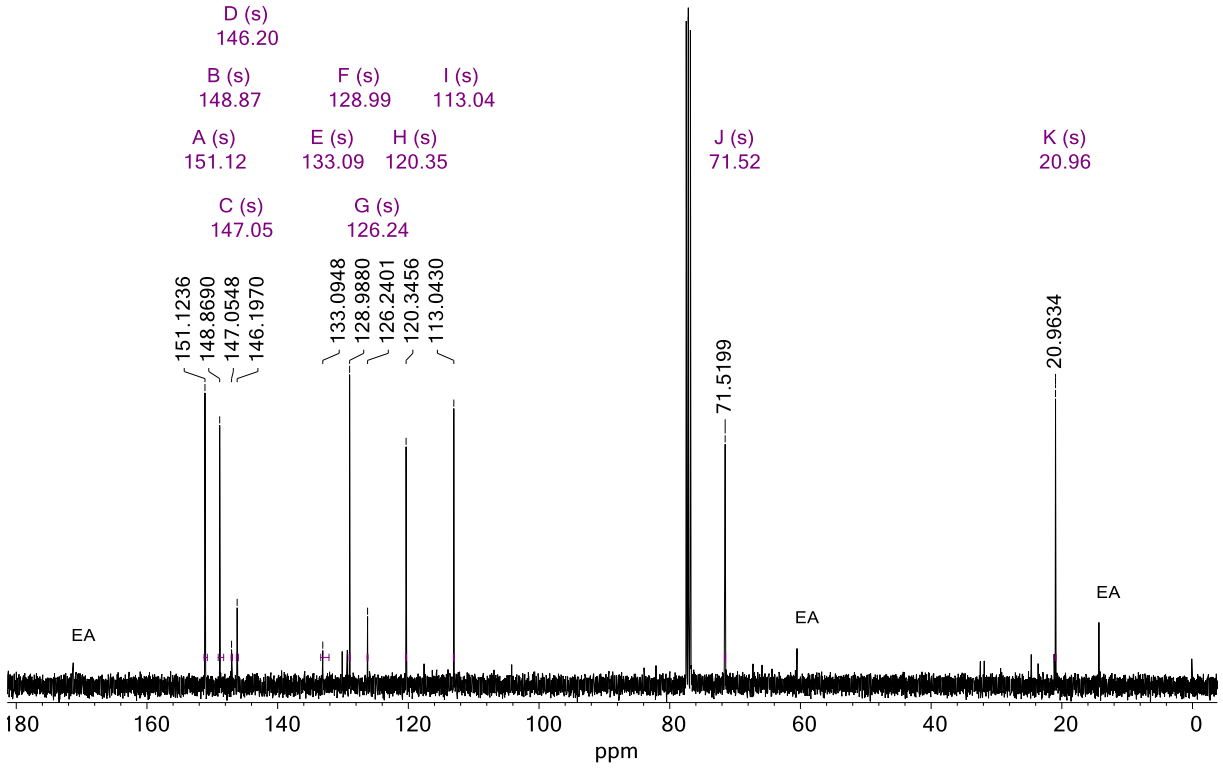

1:(Time: 0.44) Combine (6:8-23:25)
2:MS ES+
8.3e+007
191.1
192.1
259.2
1000.0
m/z

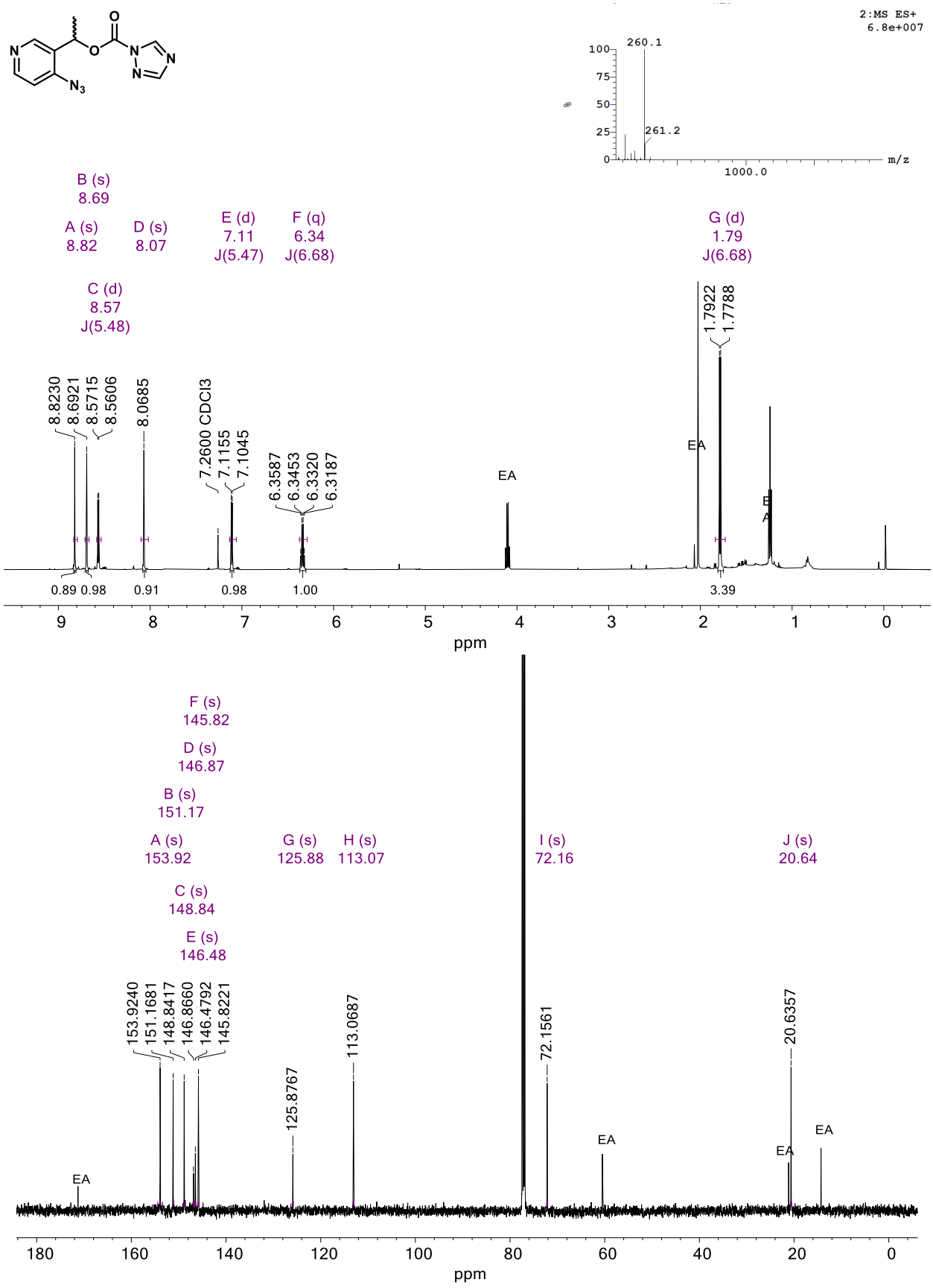

B (t)
8.17
J(1.06)
E (dd)
7.07
J(1.67, 0.86)
A (dd)
8.35
J(2.20, 1.23)
D (m)
7.39
F (q)
6.09
J(6.65)
G (d)
1.77
J(6.66)
C (dd)
7.45
J(1.66, 1.25)
CDCl3
EA
1.00 1.06
1.10 2.10 1.08
1.16
3.86
ppm

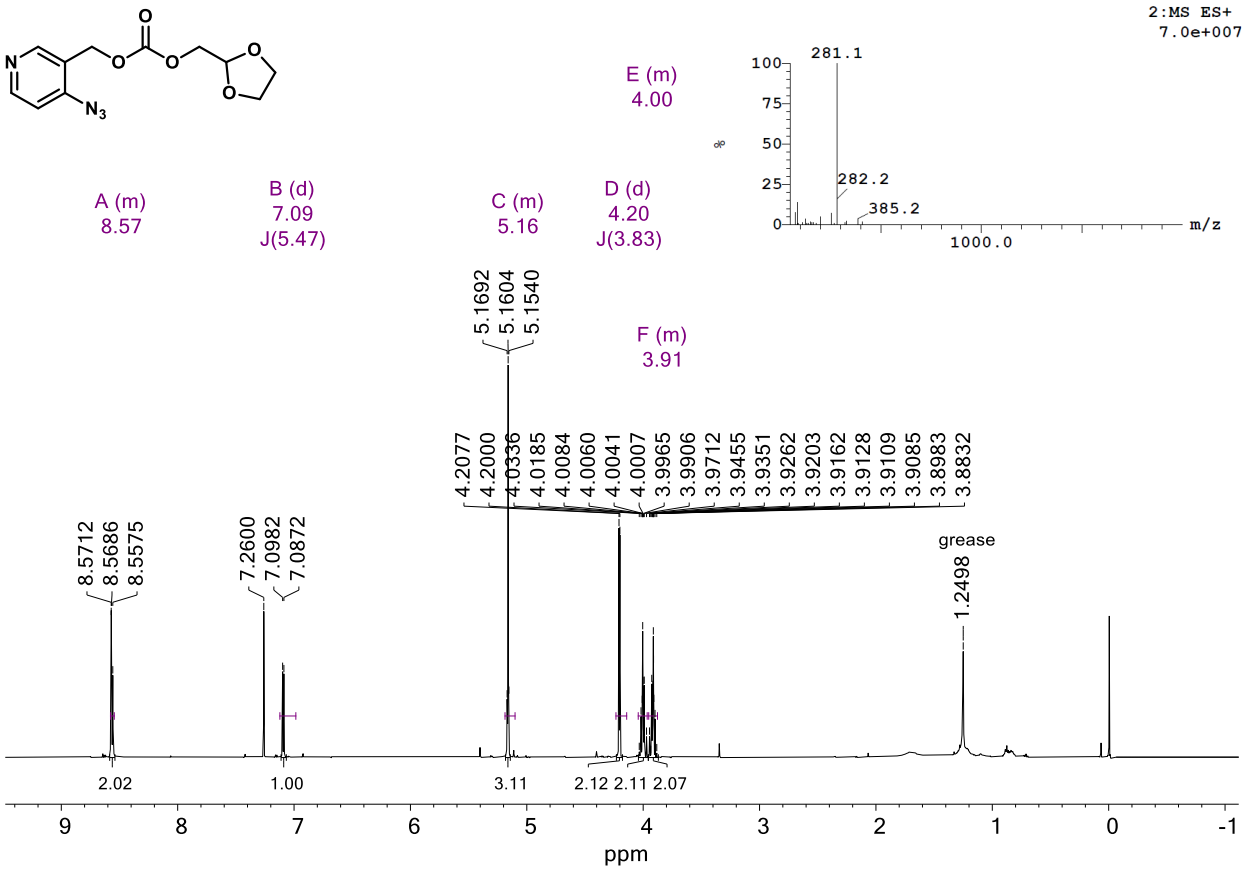

F (s)
130.90
B (s)
148.22
D (s)
137.29
H (s)
121.76
A (s)
154.64
C (s)
141.19
G (s)
126.86
J (s)
76.99
K (s)
20.34
E (s)
137.13
I (s)
117.30
154.6421
148.2200
141.1864
137.2885
137.1332
130.9008
126.8624
121.7646
117.2953
76.9930
20.3371
ppm

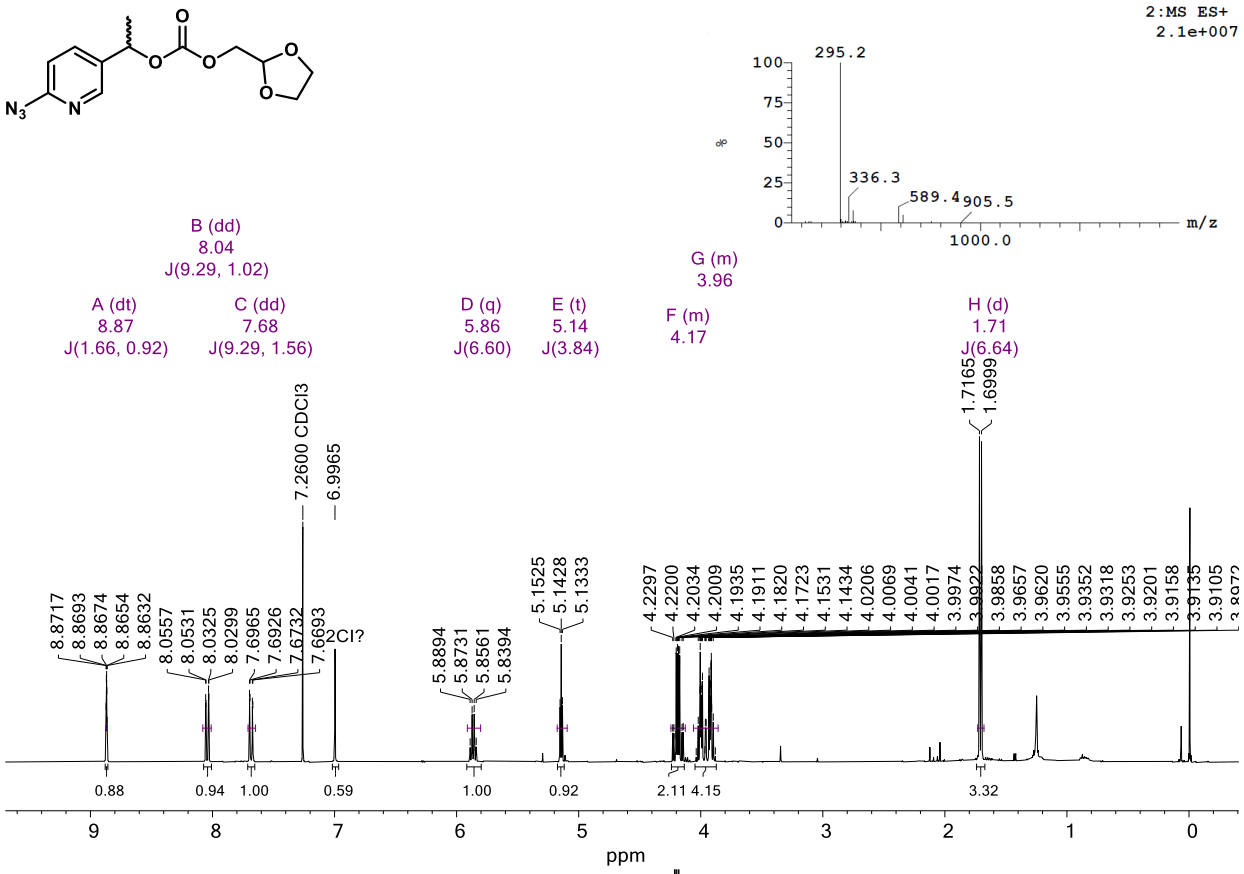

OH
N
N3
2:(Time: 0.44) Combine (6:8-21:23)
2:MS ES+
9.2e+007
165.1
166.1
322.3
413.4
827.8
1000.0
m/z
B (d)
8.46
J(5.42)
A (s)
8.63
C (d)
7.04
J(5.43)
D (q)
5.06
J(6.53)
E (d)
1.51
J(6.57)
8.6312
8.4709
8.4574
7.2600 CDCl3
7.0449
7.0313
5.0889
5.0725
5.0563
5.0399
1.5193
1.5029
1.00 1.01
0.99
1.05
3.33
ppm
B (s)
148.70
A (s)
149.80
D (s)
131.66
E (s)
112.76
F (s)
64.80
G (s)
23.77
C (s)
145.56
149.7964
148.6969
145.5574
131.6572
112.7597
64.8030
23.7699
ppm

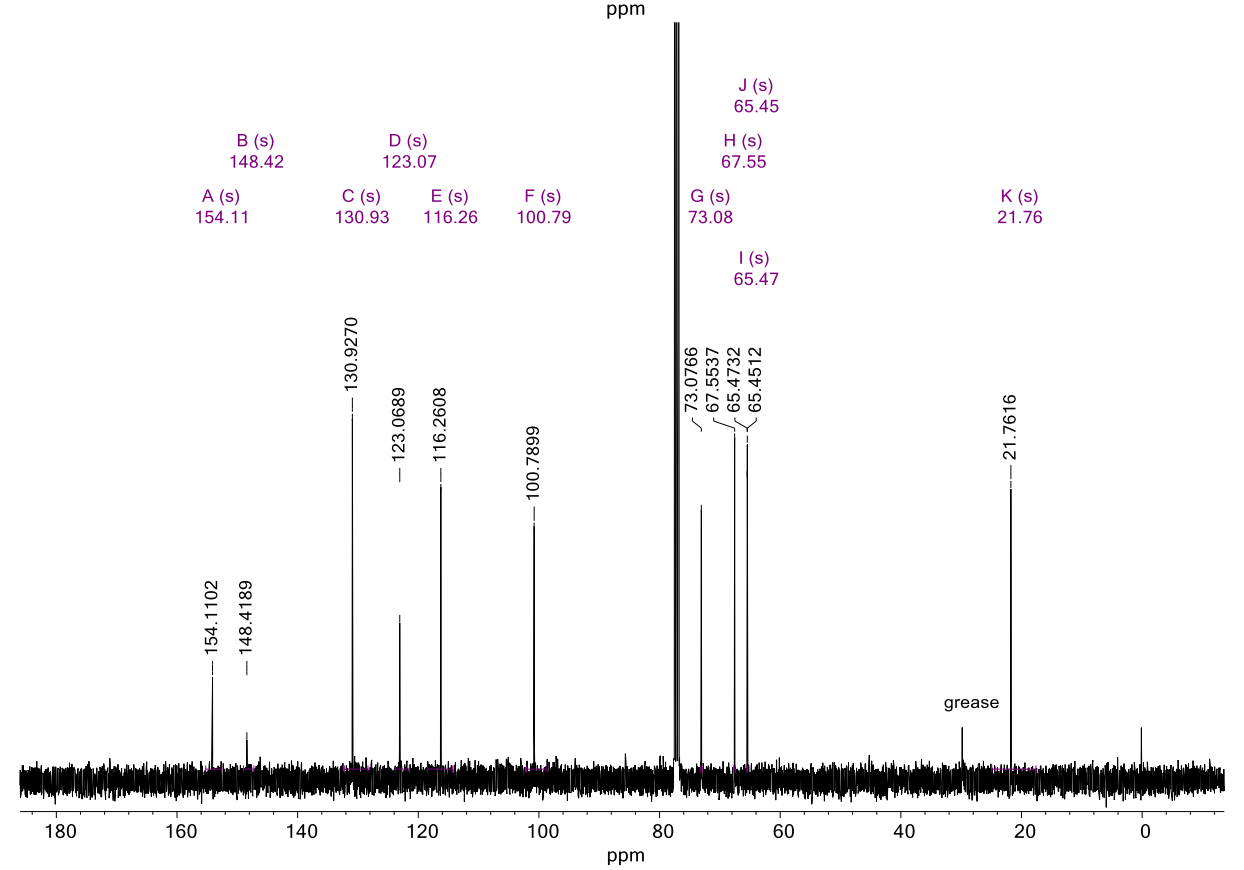

2:MS ES+
5.9e+007
293.1
295.1
296.2
1000.0
m/z
B (d)
8.57
J(5.48)
D (d)
7.11
J(5.46)
A (s)
8.66
C (d)
7.46
J(1.84)
F (q)
6.27
J(6.63)
G (d)
1.73
J(6.65)
E (d)
6.94
J(1.85)
8.6575
8.5728
8.5591
7.4671
7.4624
7.2600 CDCl3
7.1181
7.1044
6.9400
6.9354
6.2906
6.2740
6.2573
6.2407
1.7391
1.7224
EA
1.00 1.02
0.99 1.00 1.11
1.04
3.75
ppm

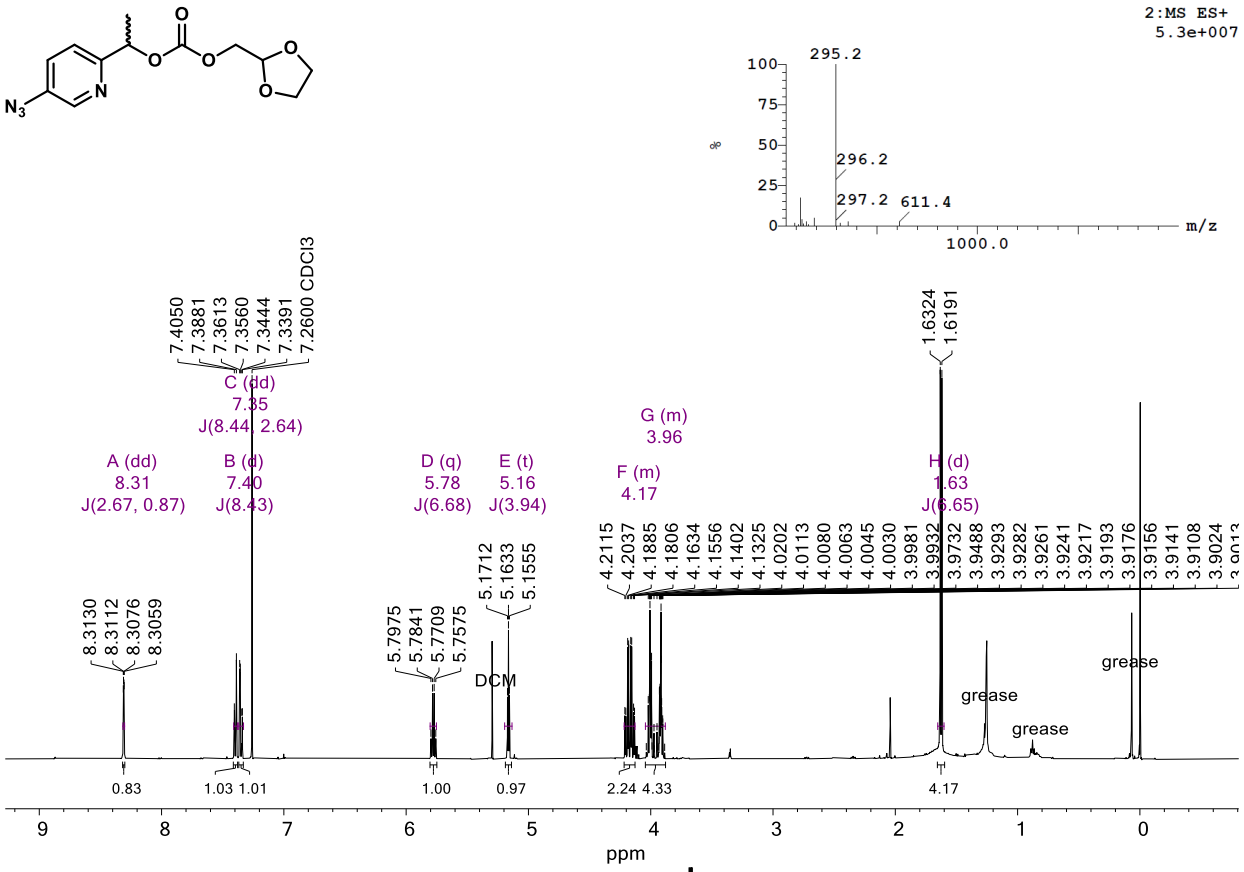

D (s)
146.20
B (s)
148.87
F (s)
128.99
I (s)
113.04
A (s)
151.12
E (s)
133.09
H (s)
120.35
J (s)
71.52
K (s)
20.96
C (s)
147.05
G (s)
126.24
151.1236
148.8690
147.0548
146.1970
133.0948
128.9880
126.2401
120.3456
113.0430
71.5199
20.9634
EA
ppm

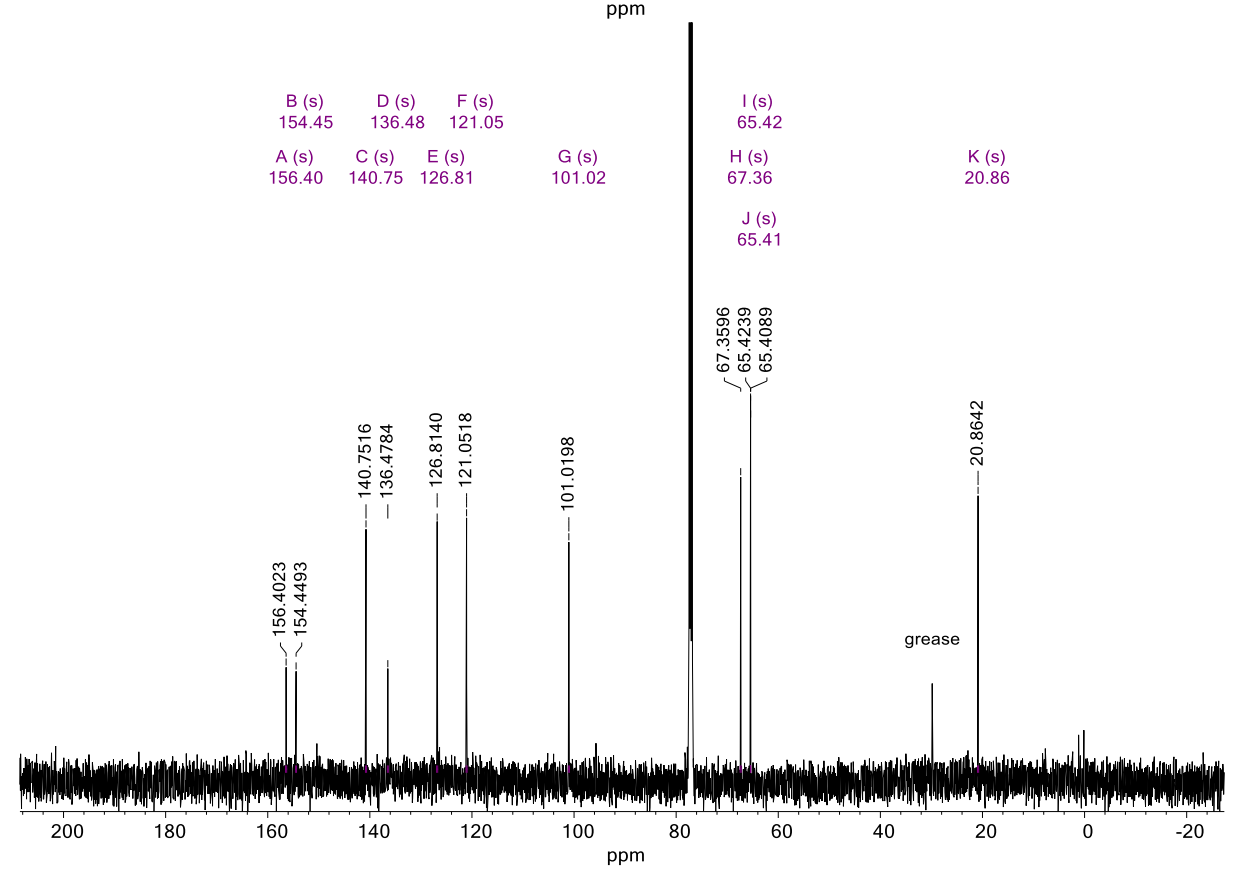

2:MS ES+
6.8e+007
260.1
261.2
m/z
1000.0
B (s)
8.69
A (s)
8.82
D (s)
8.07
E (d)
7.11
J(5.47)
F (q)
6.34
J(6.68)
G (d)
1.79
J(6.68)
C (d)
8.57
J(5.48)
8.8230
8.6921
8.5715
8.5606
8.0685
7.2600 CDCl3
7.1155
7.1045
6.3587
6.3453
6.3320
6.3187
EA
1.7922
1.7788
0.89 0.98
0.91
0.98
1.00
3.39
ppm
F (s)
145.82
D (s)
146.87
B (s)
151.17
A (s)
153.92
G (s)
125.88
H (s)
113.07
I (s)
72.16
J (s)
20.64
C (s)
148.84
E (s)
146.48
153.9240
151.1681
148.8417
146.8660
146.4792
145.8221
125.8767
113.0687
72.1561
20.6357
EA
ppm

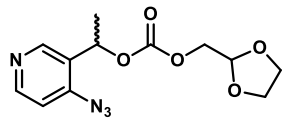

2:MS ES+
7.0e+007
281.1
282.2
385.2
1000.0
m/z
E (m)
4.00
A (m)
8.57
B (d)
7.09
J(5.47)
C (m)
5.16
D (d)
4.20
J(3.83)
F (m)
3.91
grease
2.02
1.00
3.11
2.12 2.11 2.07
ppm

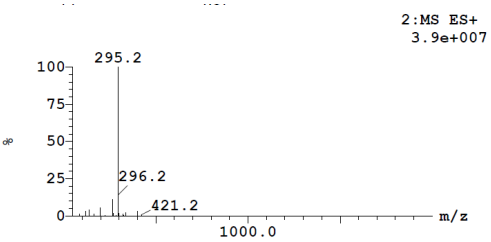

2:MS ES+
2.1e+007
295.2
336.3
589.4
905.5
1000.0
m/z
B (dd)
8.04
J(9.29, 1.02)
A (dt)
8.87
J(1.66, 0.92)
C (dd)
7.68
J(9.29, 1.56)
D (q)
5.86
J(6.60)
E (t)
5.14
J(3.84)
G (m)
3.96
F (m)
4.17
H (d)
1.71
J(6.64)
7.2600 CDCl3
6.9965
2Cl?
ppm

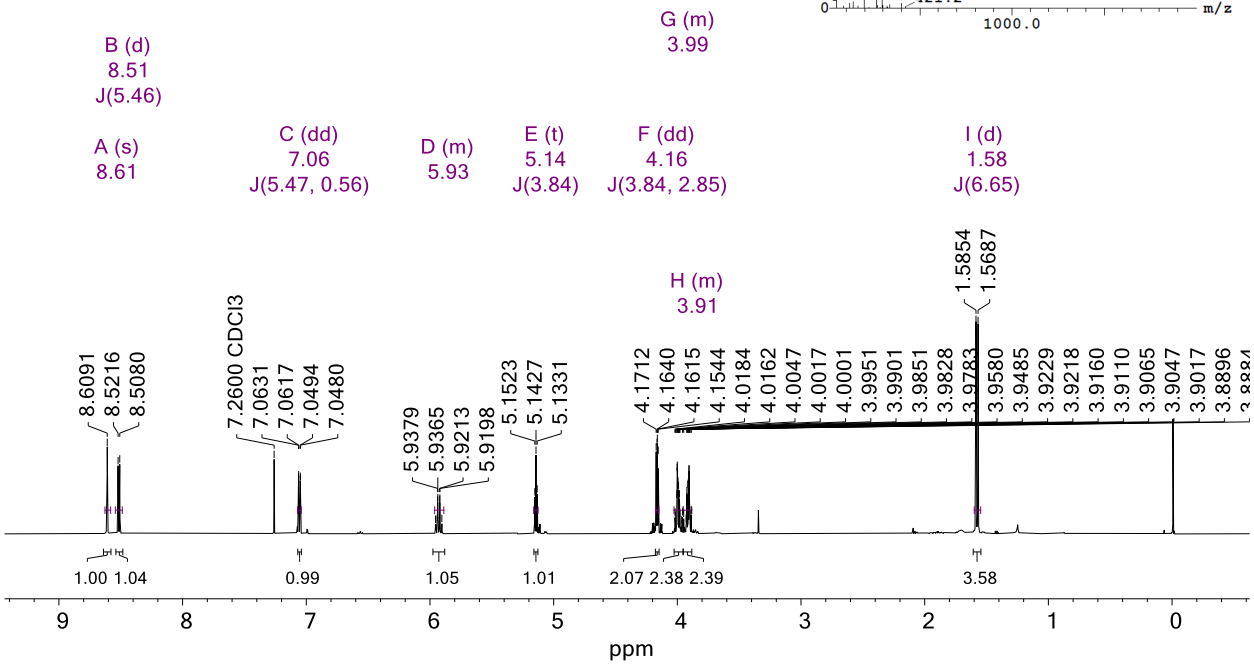

B (s)
148.42
D (s)
123.07
J (s)
65.45
H (s)
67.55
A (s)
154.11
C (s)
130.93
E (s)
116.26
F (s)
100.79
G (s)
73.08
K (s)
21.76
I (s)
65.47
130.9270
123.0689
116.2608
100.7899
73.0766
67.5537
65.4732
65.4512
154.1102
148.4189
21.7616
grease
ppm

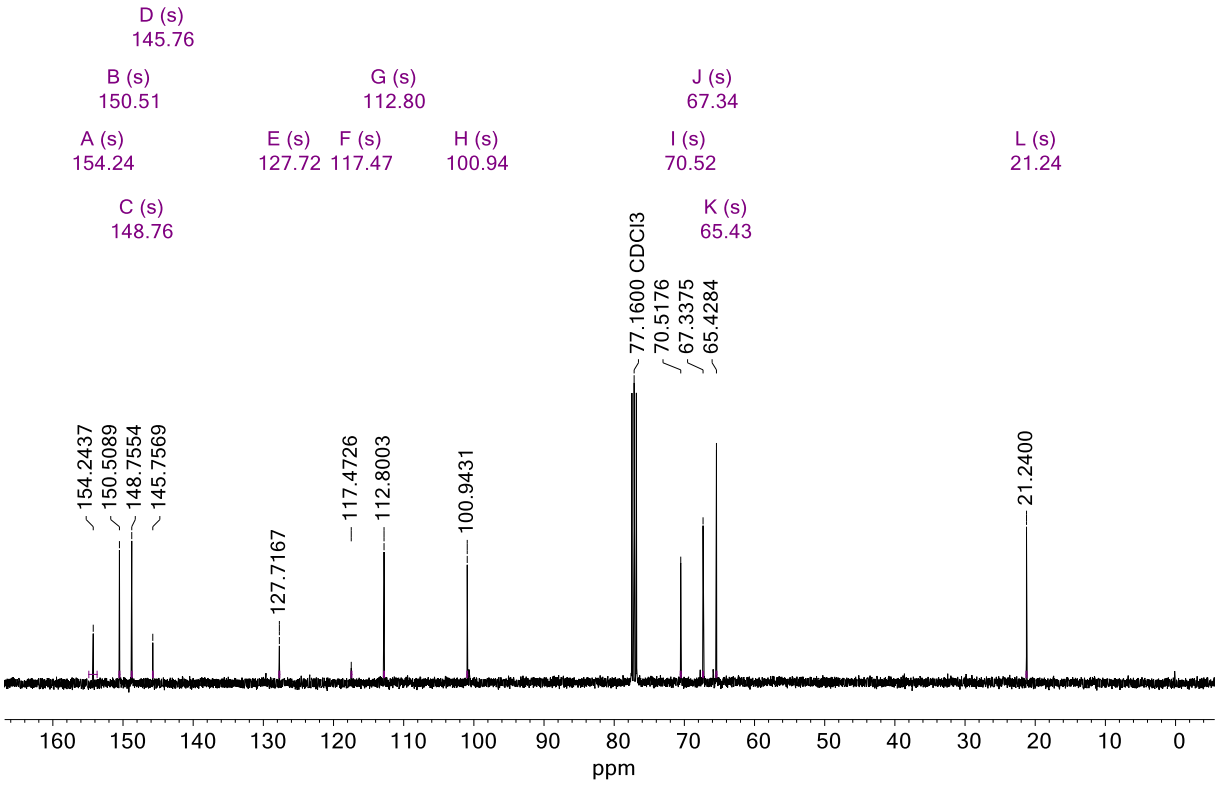

2:MS ES+
5.3e+007
295.2
296.2
297.2
611.4
m/z
1000.0
7.4050
7.3881
7.3613
7.3560
7.3444
7.3391
7.2600 CDCl3
C (dd)
7.35
J(8.44, 2.64)
A (dd)
8.31
J(2.67, 0.87)
B (d)
7.40
J(8.43)
D (q)
5.78
J(6.68)
E (t)
5.16
J(3.94)
G (m)
3.96
F (m)
4.17
H (d)
1.63
J(6.65)
1.6324
1.6191
8.3130
8.3112
8.3076
8.3059
5.7975
5.7841
5.7709
5.7575
5.1712
5.1633
5.1555
DCM
grease
grease
grease
0.83
1.03 1.01
1.00
0.97
2.24 4.33
4.17
ppm

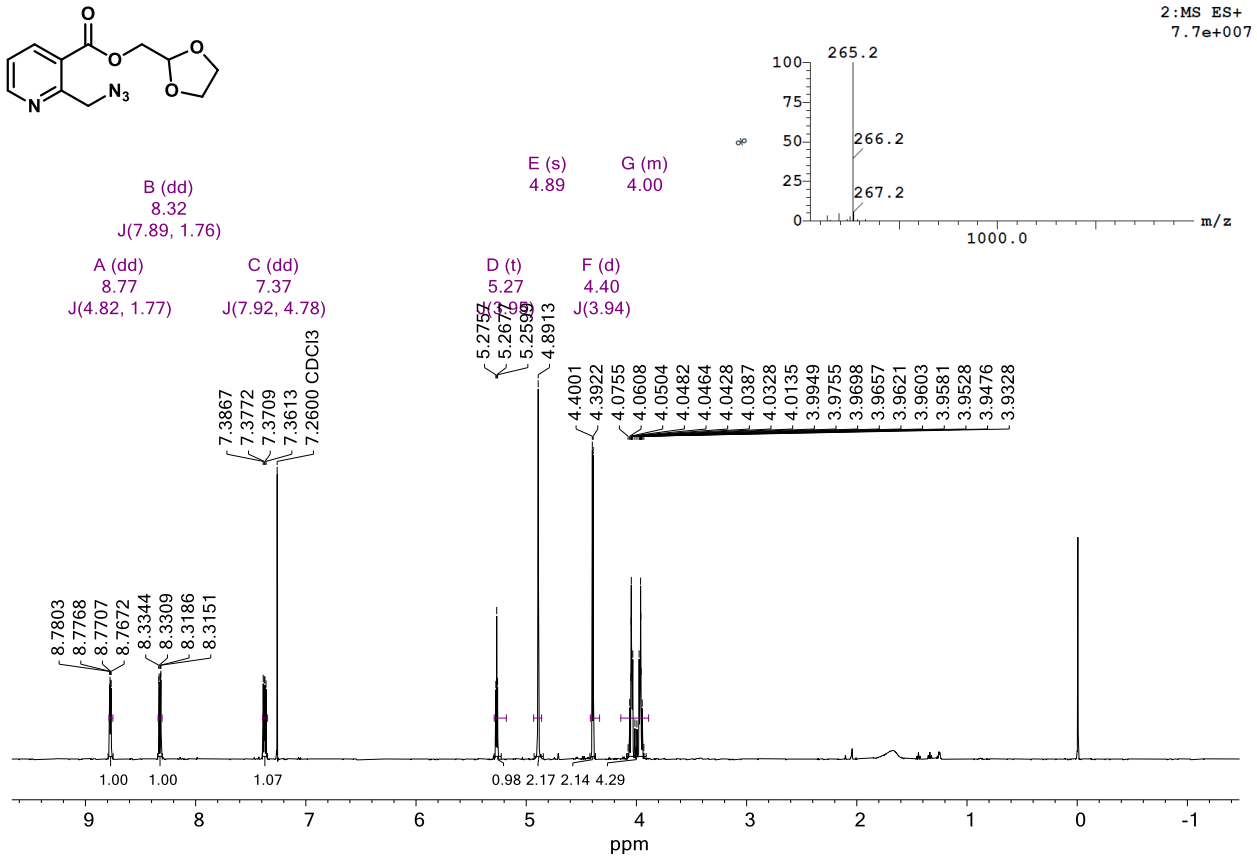

B (s)
154.45
D (s)
136.48
F (s)
121.05
I (s)
65.42
A (s)
156.40
C (s)
140.75
E (s)
126.81
G (s)
101.02
H (s)
67.36
K (s)
20.86
J (s)
65.41
67.3596
65.4239
65.4089
140.7516
136.4784
126.8140
121.0518
101.0198
20.8642
156.4023
154.4493
grease
ppm

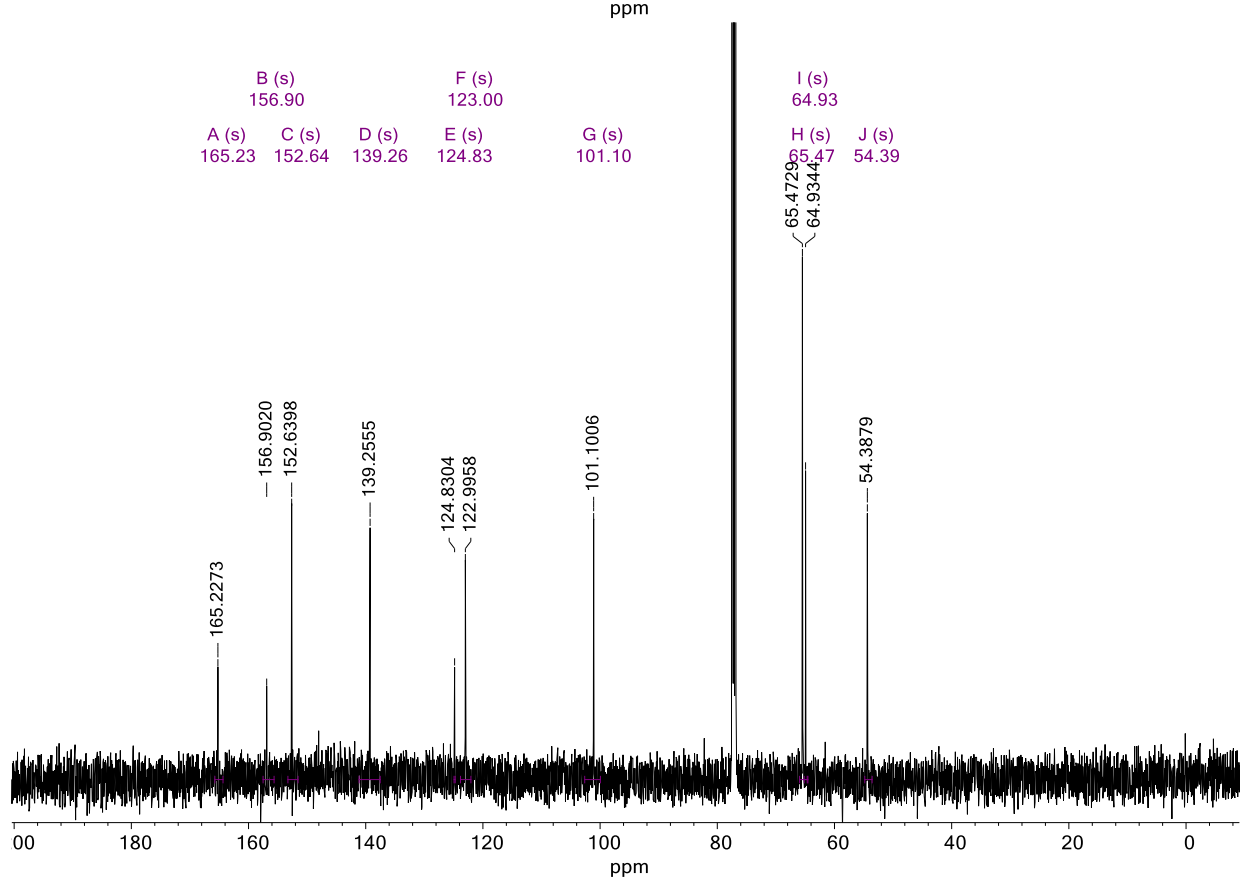


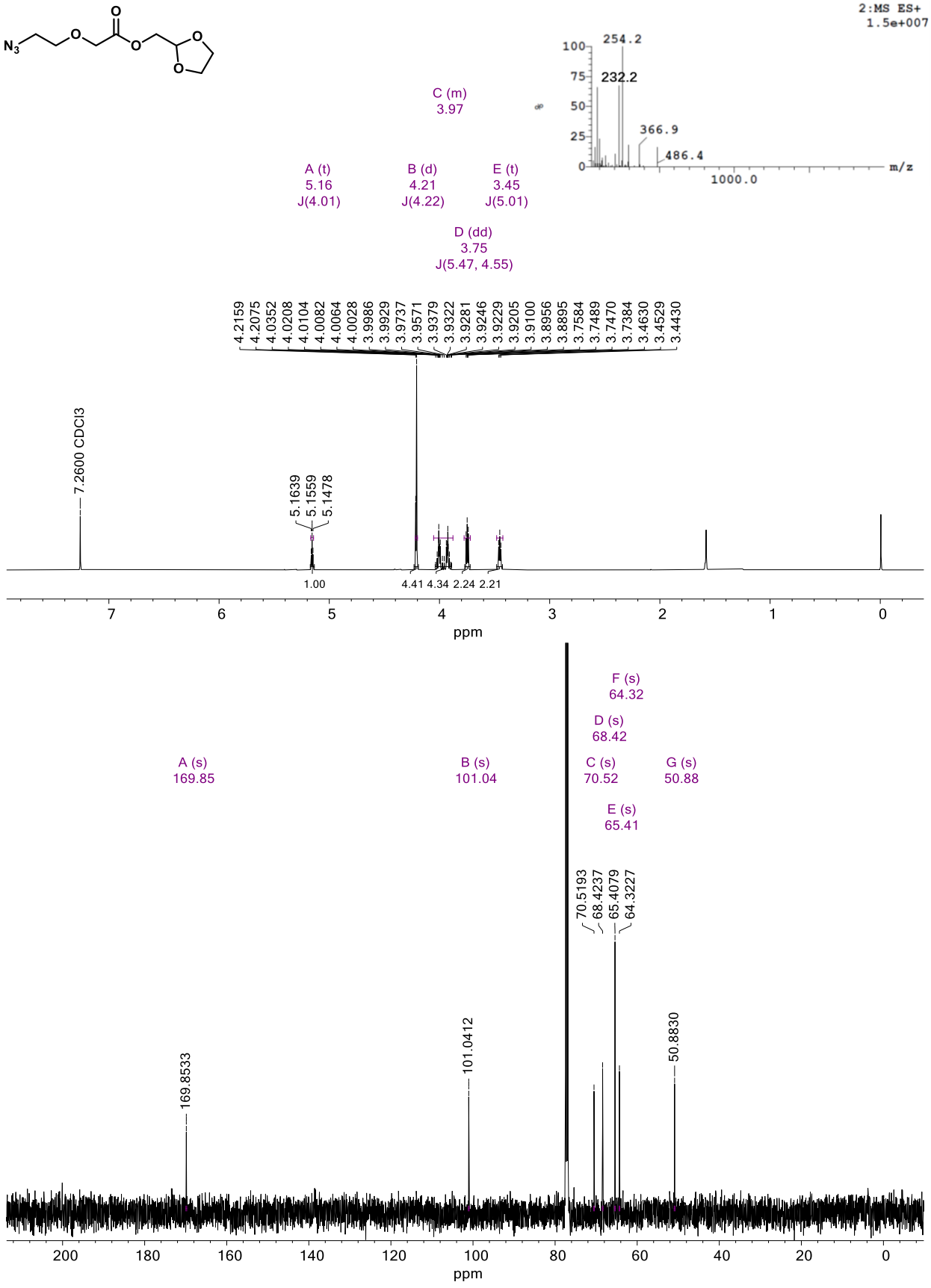

2:MS ES+
3.9e+007
295.2
296.2
421.2
1000.0
m/z


B (d)
8.51
J(5.46)
G (m)
3.99
A (s)
8.61
C (dd)
7.06
J(5.47, 0.56)
D (m)
5.93
E (t)
5.14
J(3.84)
F (dd)
4.16
J(3.84, 2.85)
I (d)
1.58
J(6.65)
H (m)
3.91
8.6091
8.5216
8.5080
7.2600 CDCl3
7.0631
7.0617
7.0494
7.0480
5.9379
5.9365
5.9213
5.9198
5.1523
5.1427
5.1331
4.1712
4.1640
4.1615
4.1544
4.0184
4.0162
4.0047
4.0017
4.0001
3.9951
3.9901
3.9851
3.9828
3.9783
3.9580
3.9485
3.9229
3.9218
3.9160
3.9110
3.9065
3.9047
3.9017
3.8896
1.5854
1.5687
1.00 1.04
0.99
1.05
1.01
2.07 2.38 2.39
3.58
ppm


D (s)
145.76
B (s)
150.51
G (s)
112.80
J (s)
67.34
A (s)
154.24
E (s)
127.72
F (s)
117.47
H (s)
100.94
I (s)
70.52
L (s)
21.24
C (s)
148.76
K (s)
65.43
154.2437
150.5089
148.7554
145.7569
127.7167
117.4726
112.8003
100.9431
77.1600 CDCl3
70.5176
67.3375
65.4284
21.2400
ppm


2:MS ES+
7.7e+007
265.2
266.2
267.2
1000.0
m/z
B (dd)
8.32
J(7.89, 1.76)
E (s)
4.89
G (m)
4.00
A (dd)
8.77
J(4.82, 1.77)
C (dd)
7.37
J(7.92, 4.78)
D (t)
5.27
F (d)
4.40
J(3.94)
7.2600 CDCl3
1.00 1.00 1.07 0.98 2.17 2.14 4.29
ppm


B (s)
156.90
F (s)
123.00
I (s)
64.93
A (s)
165.23
C (s)
152.64
D (s)
139.26
E (s)
124.83
G (s)
101.10
H (s)
65.47
J (s)
54.39
165.2273
156.9020
152.6398
139.2555
124.8304
122.9958
101.1006
65.4729
64.9344
54.3879
ppm


2:MS ES+
1.5e+007
254.2
232.2
366.9
486.4
1000.0
m/z
C (m)
3.97
A (t)
5.16
J(4.01)
B (d)
4.21
J(4.22)
E (t)
3.45
J(5.01)
D (dd)
3.75
J(5.47, 4.55)
7.2600 CDCl3
5.1639
5.1559
5.1478
1.00
4.41 4.34 2.24 2.21
ppm
F (s)
64.32
D (s)
68.42
A (s)
169.85
B (s)
101.04
C (s)
70.52
G (s)
50.88
E (s)
65.41
70.5193
68.4237
65.4079
64.3227
169.8533
101.0412
50.8830
ppm

References

1. Lukasak, B., Morihiro, K. & Deiters, A. Aryl Azides as Phosphine-Activated Switches for Small Molecule Function. *Sci. Rep.* **9**, 1470 (2019).

2. Chen, X. *et al.* Visualizing RNA dynamics in live cells with bright and stable fluorescent RNAs. **37**, 1287–1293 (2019).

3. Spitale, R. C. *et al.* Structural imprints in vivo decode RNA regulatory mechanisms. *Nature* **519**, 486–490 (2015).

4. Park, H. S., Kietrys, A. M. & Kool, E. T. Simple alkanoyl acylating agents for reversible RNA functionalization and control. *Chem. Commun.* **55**, 5135–5138 (2019).

5. Li, Y., Hoskins, J. N., Sreerama, S. G. & Grayson, S. M. MALDI-TOF mass spectral characterization of polymers containing an azide group: Evidence of metastable ions. *Macromolecules* **43**, 6225–6228 (2010).

6. Guillaneuf, Y. *et al.* Radical chain end chemical transformation of SG1-based polystyrenes. *Macromolecules* **43**, 91–100 (2010).

7. Raynaud, J., Absalon, C., Gnanou, Y. & Taton, D. N-heterocyclic carbene-induced zwitterionic ring-opening polymerization of ethylene oxide and direct synthesis of α,ω-difunctionalized poly(ethylene oxide)s and poly(ethylene oxide)-b-poly(ε-caprolactone) block copolymers. *J. Am. Chem. Soc.* **131**, 3201–3209 (2009).

8. Zhang, N., Tomizawa, M. & Casida, J. E. Structural features of azidopyridinyl neonicotinoid probes conferring high affinity and selectivity for mammalian α4β2 and Drosophila nicotinic receptors. *J. Med. Chem.* **45**, 2832–2840 (2002).

9. Busto, E., Gotor-Fernández, V. & Gotor, V. Enantioselective synthesis of 4-(dimethylamino)pyridines through a chemical oxidation-enzymatic reduction sequence. Application in asymmetric catalysis. *Adv. Synth. Catal.* **348**, 2626–2632 (2006).