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Supporting Information

Simple Alkanoyl Acylating Agents for Reversible RNA Functionalization and Control

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Materials and instrumentation

Instrumentation

^{1}H- and ^{13}C-NMR spectra were recorded on Varian Mercury 400 MHz NMR or Varian Inova 300MHz spectrometer. ^{1}H- and ^{13}C-NMR spectra were internally referenced to the residual solvent signal. MALDI-TOF mass spectra was acquired using a Bruker MALDI Microflex LRF instrument using an AnchorChip™ target on 3-hydroxypicolinic acid matrix with addition of diammonium hydrogen citrate and TFA. Mass spectra were analyzed with MestReNova software (v. 11) or FlexAnalysis™ (v. 3.4). MALDI-TOF mass spectrums were corrected with internal standards when applicable. Fluorescence studies were performed on a Fluorolog 3-11 instrument (Jobin Yvon-SPEX). Molecular beacon experiments were performed with Fluoroskan Ascent™ Microplate Fluorometer. High-resolution mass spectrometry analysis was performed by the Vincent Coates Foundation Mass Spectrometry Laboratory at Stanford University. Oligonucleotide concentrations were measured using a NanoDrop One microvolume UV-Vis spectrophotometer. When applicable, molar concentrations were calculated based on Cy5 absorbance.

Reagents

Reagents were purchased from Sigma-Aldrich unless specified otherwise. 6-azido-hexanoic acid was purchased from Chem-Impex. 2-Chloro-1H-imidazole and 1H-1,2,3-triazole were purchased from Combi-Blocks. 7-nitro-1-methyl-1H-benzo [d][1,3]oxazine-2,4-dione and 1H-imidazole-carbonitrile was purchased from AstaTech. 1-Methyl-1H-benzo[d][1,3]oxazine-2,4-dione (NMIA) and 2-Ntiroimidazole was purchased from Oakwood Chemical. TAMRA-DBCO was purchased from Click Chemistry Tools. 4-azidobutanoic acid,[1] 5-azidopentanoic acid,[1] and (2-azidoethoxy)acetic acid[2] were synthesized based on published procedures. 2M NAI-N_3[3] and FAI-N_3[4] solution in DMSO were prepared according to the previously published procedures. Cy5-tRF-3005 (5'-Cy5-AUC CUG CCG ACU ACGCCA-3', RNA) was ordered from Stanford Protein and Nucleic Acid Facility (PAN). Spinach template (5'- TAA TAC GAC TCA CTA TAG GGA GAC GCA ACT GAA TGA AAT GGT GAA GGA CGG GTC CAG GTG TGG CTG CTT CGG CAG TGC AGC TTG TTG AGT AGA GTG TGA GCT CCG TAA CTA GTC GCG TC -3', DNA), Spinach forward primer (5'-TAA TAC GAC TCA CTA TAG GGA GAC-3', DNA), Spinach reverse primer (5'-GAC GCG ACT AGT TAC GGA-3', DNA), tRF-3005 (5'-AUC CUG CCG ACU ACGCCA-3', RNA), tRF-3005-DNA (5'-ATC CTG CCG ACT ACGCCA-3', DNA) were ordered from IDT. The molecular beacon probe (5'-6FAM-CGC GGG CGT AGT CGG CAG GAC GCG-Dabcyl-3', DNA) was purchased from Sigma Aldrich.

Determination of hydrolysis half-lives

Hydrolysis half-lives were determined by NMR spectroscopy as previously reported.[5] Compounds were dissolved in a 1:1 mixture of DMSO-d6 and 50 mM phosphate buffer pH 7 in deuterium oxide, and hydrolysis was monitored over time. Hydrolysis half-lives were approximated by fitting the data to an exponential decay curve.

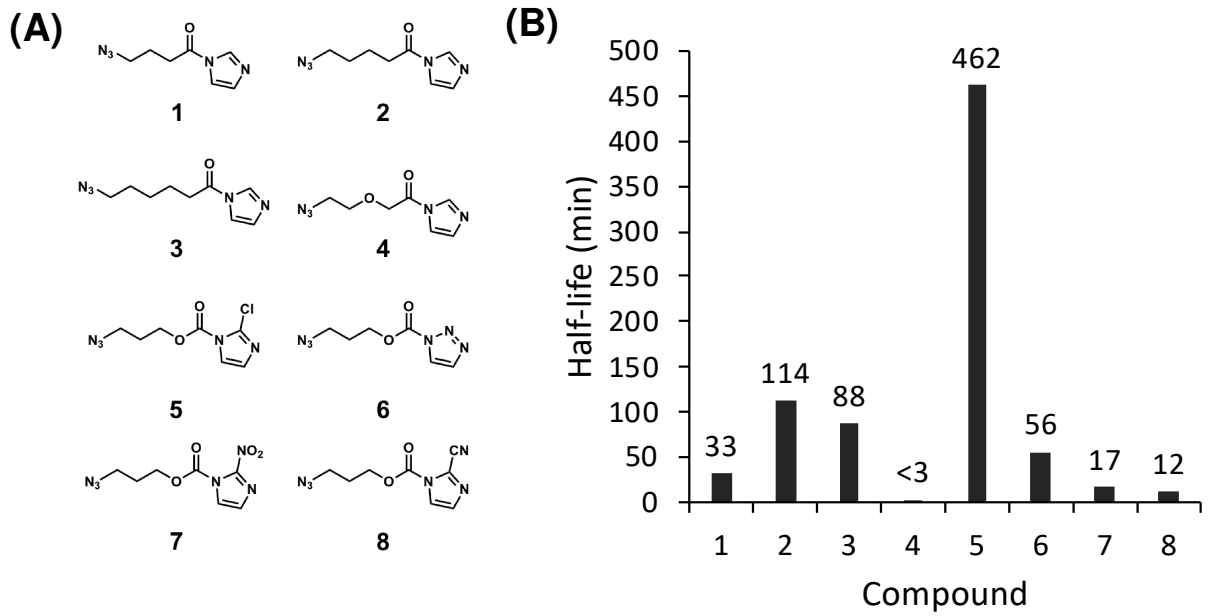


Figure S1. Hydrolysis half-lives of varied azidoalkanoyl imidazoles in aqueous media. (A) Structures of compounds studied. (B) Hydrolysis half-lives of varied acyl imidazoles in 1:1 DMSO : phosphate buffer pH 7. **4** hydrolyzed too rapidly to determine its half-life (half-life < 3min).

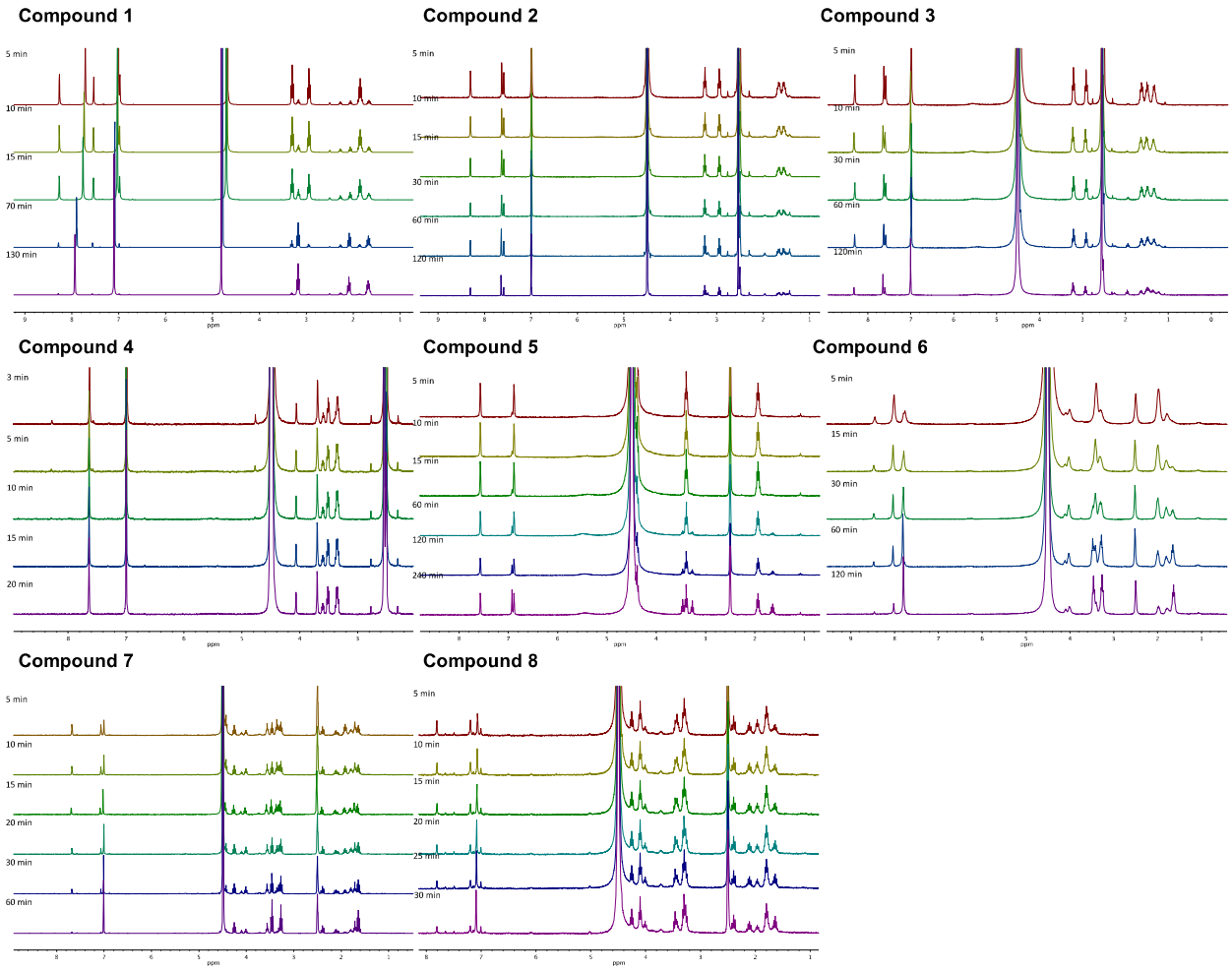


Figure S2. Stacked NMR plots of hydrolysis of the acylimidazoles studied. **4** showed full hydrolysis in <15min.

General RNA polyacylation (cloaking) procedure

In RNase free water, RNA (6 μM) was incubated with 0.8 M acylating probe (2 M stock in dry DMSO) for 4 h at room temperature in a total volume of 100 μL. For **4**, when a higher degree of acylation was desired, RNA diluted in water was pre-chilled to 4 °C before the addition of **4**, and the cloaking was performed at 10 °C for 4 h. The cloaked RNA was then purified via ethanol precipitation. To the reaction mixture, 0.1x v:v of 3 M NaOAc pH 5.2, 1 μL of 20 mg/ml glycogen, and 3.75x v:v of EtOH were added, and the resulting mixture was incubated 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. The supernatant was removed, and the RNA was dried in air for 20 min and dissolved in RNase free water and stored at -20 °C.

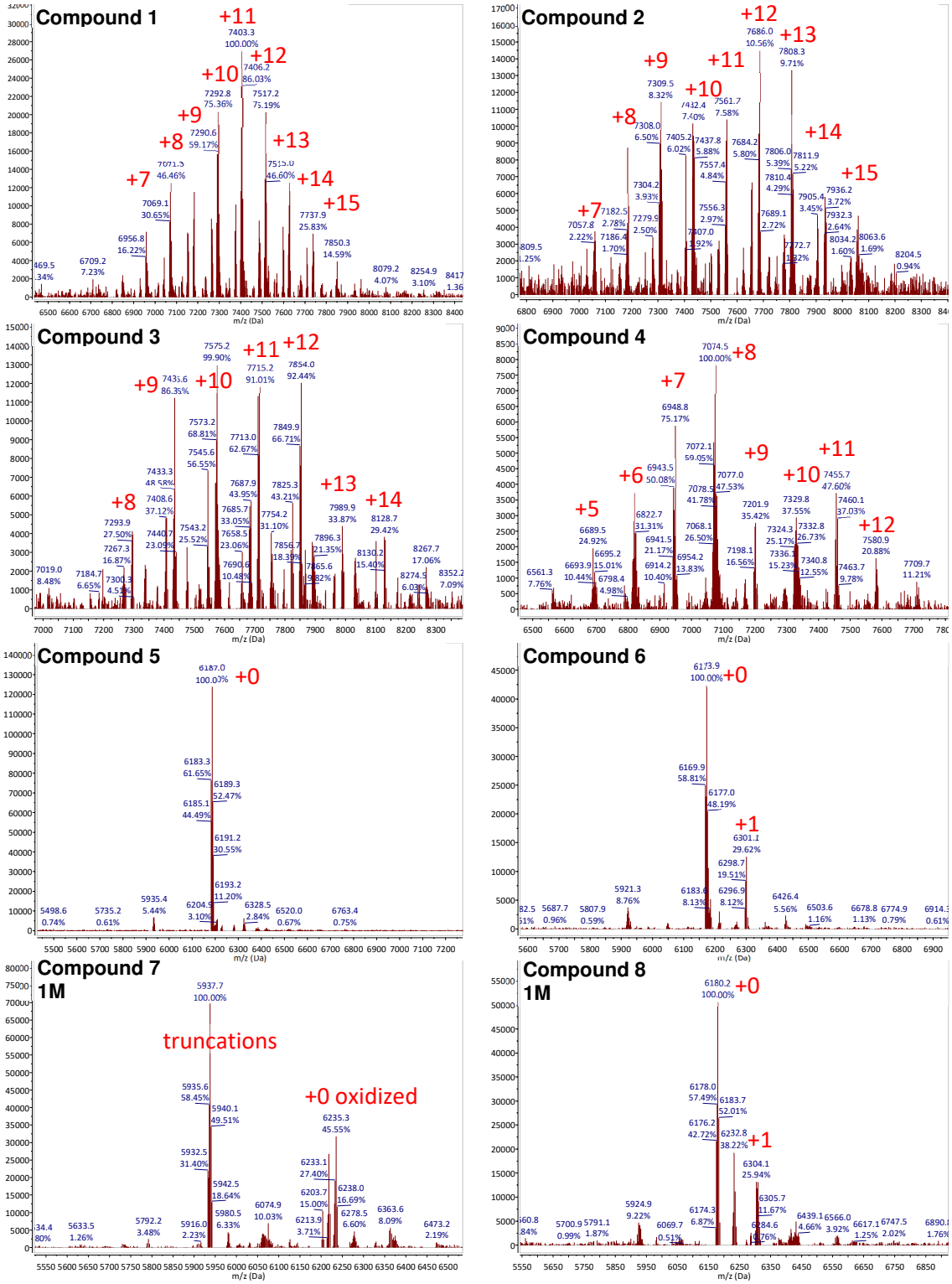


Figure S3. Polyacylation efficiency of acylimidazoles. Cloaking for **7** and **8** was done at 1M. RNA cloaked with **7** were oxidized and showed truncation. Red numbers indicate the number of acyl adducts.

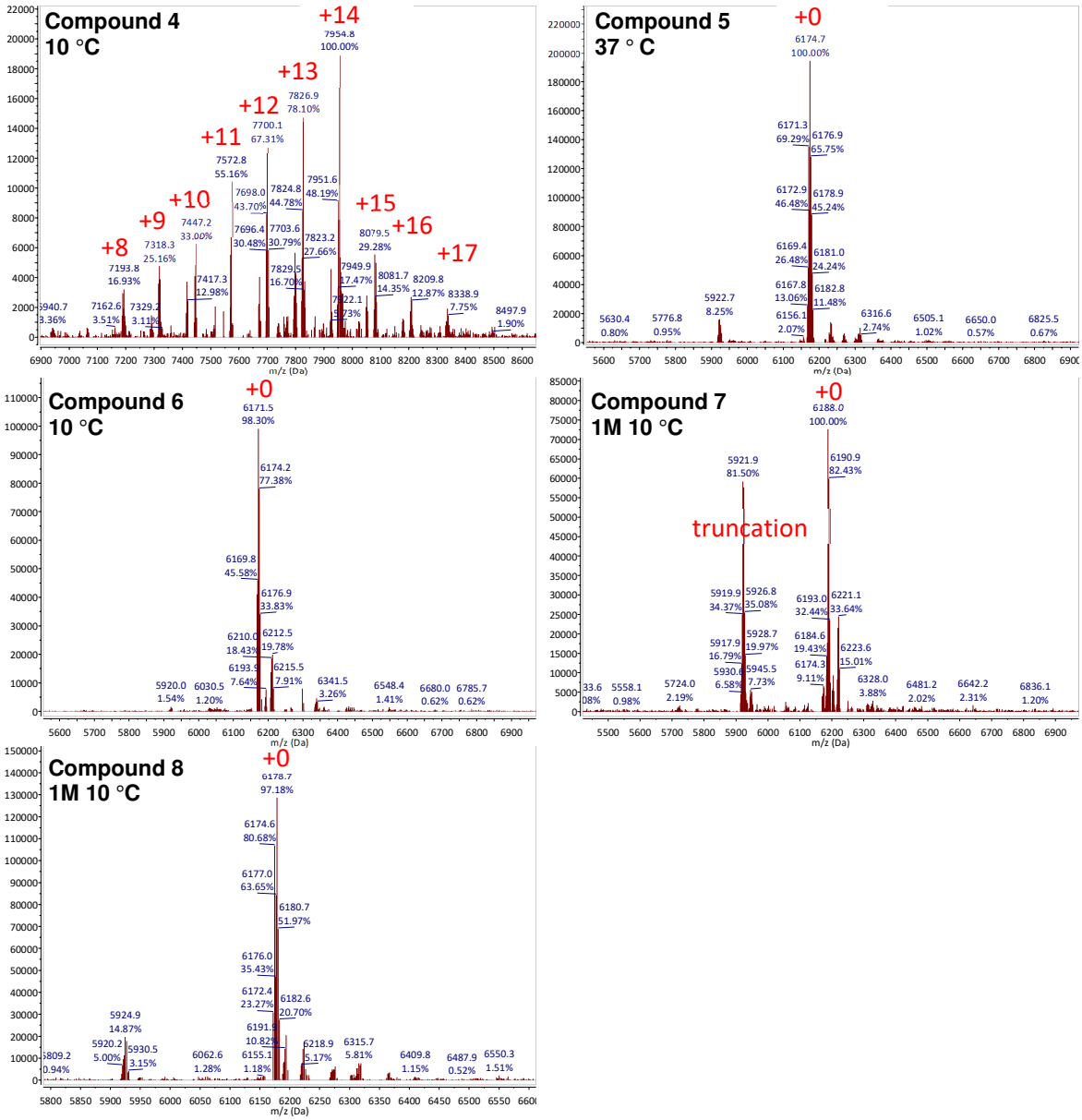


Figure S4. Polyacylation efficiency of acylimidazoles at varied temperatures. **4** showed greater acylation yields at 10 °C (median number of acyl adducts: 12-13) than at room temperature (median number of acyl adducts: 7-8). Red numbers indicate the number of acyl adducts.

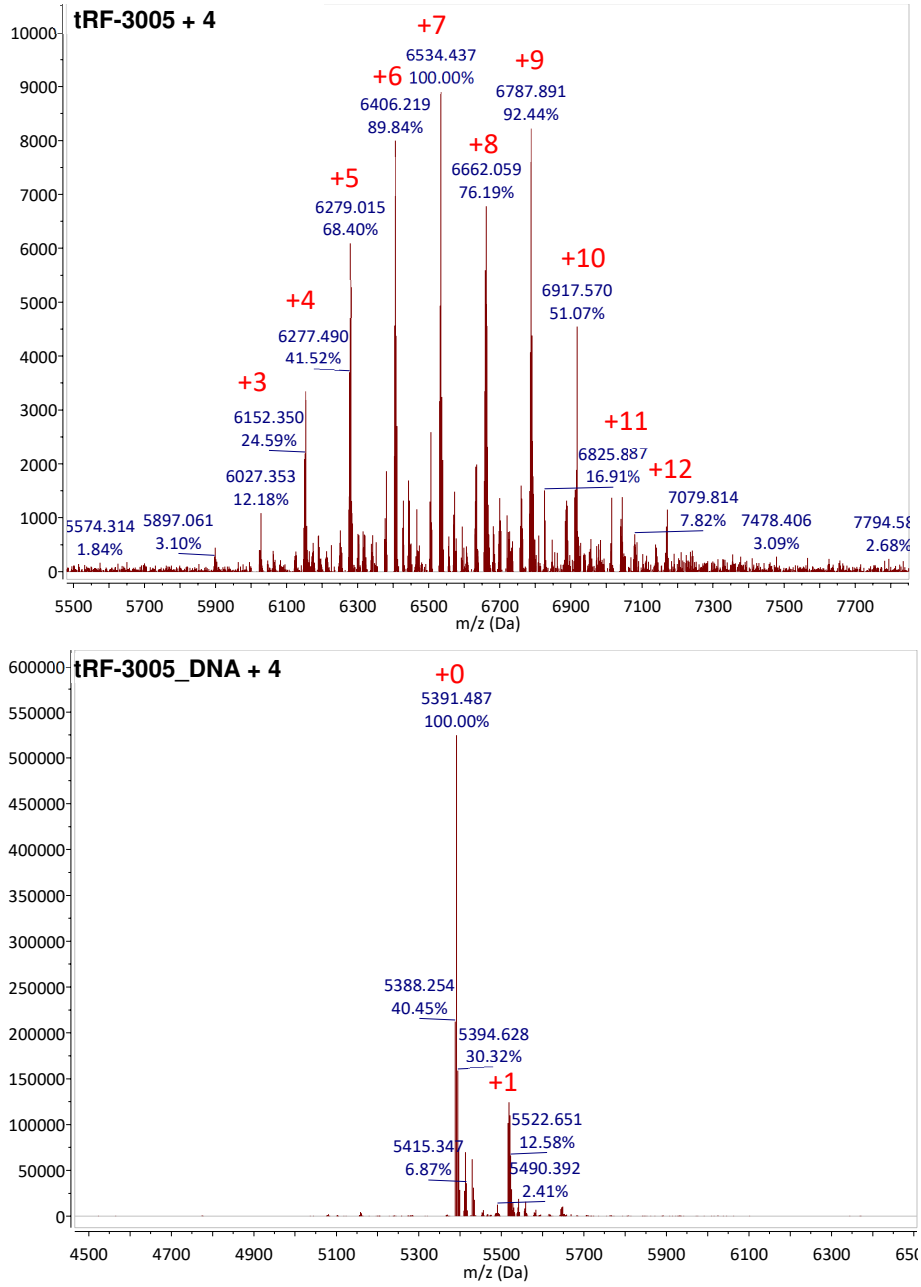


Figure S5. Polyacylation reactivity of **4**, comparing DNA and RNA substrates. DNA and RNA versions of tRF-3005 (6 μM) were treated with 0.35 M **4** at room temperature for 1h. The small amount of acyl adduct observed on the tRF-3005-DNA is presumably at 3'-OH, although reaction at exocyclic amines cannot be ruled out. Red numbers indicate the number of acyl adducts.

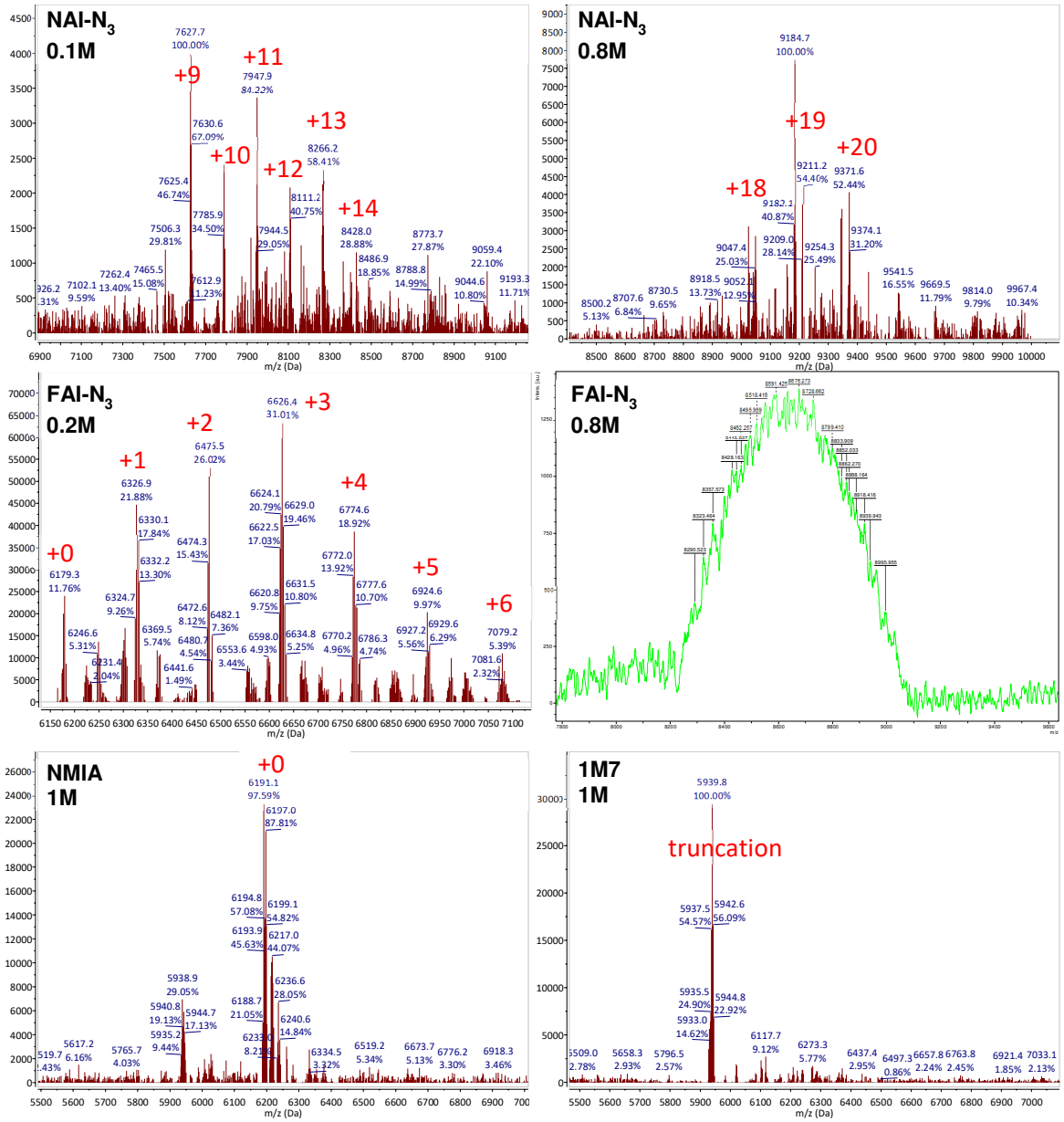


Figure S6. Polyacylation efficiency of published SHAPE reagents. Red numbers indicate the number of acyl adducts. 0.8 M FAI-N_3 treatment caused the RNA to become poorly soluble, and the median modification was estimated to be +16 (MW: 8568 Da) from the median mass (~8600 Da) of the distribution. The data show that reagents NAI-N_3 and FAI-N_3 give high yields of acylation. Reagents NMIA and 1M7 yield no measurable stoichiometric acylation and both show evidence of causing RNA degradation (see peaks at mass 5939 Da).

General RNA deacylation (uncloaking) procedure

The median acylation for RNA was first normalized to approximately 11-12 (except FAI-N_3, for which the median acylation was 3 to obtain a soluble sample). For **4**, the cloaking was performed at 10 °C with 0.8M of **4**. For NAI-N_3, the cloaking was performed with 0.1 M reagent at room temperature. To uncloak, the RNA (1 μM) was incubated in 300 mM Tris-HCl buffer pH 7.5 containing 20% DMSO with 20 mM 2DPPEA for 4 h at 37 °C in a total volume of 100 μl. The RNA was purified via ethanol precipitation procedure identical to that after RNA cloaking.

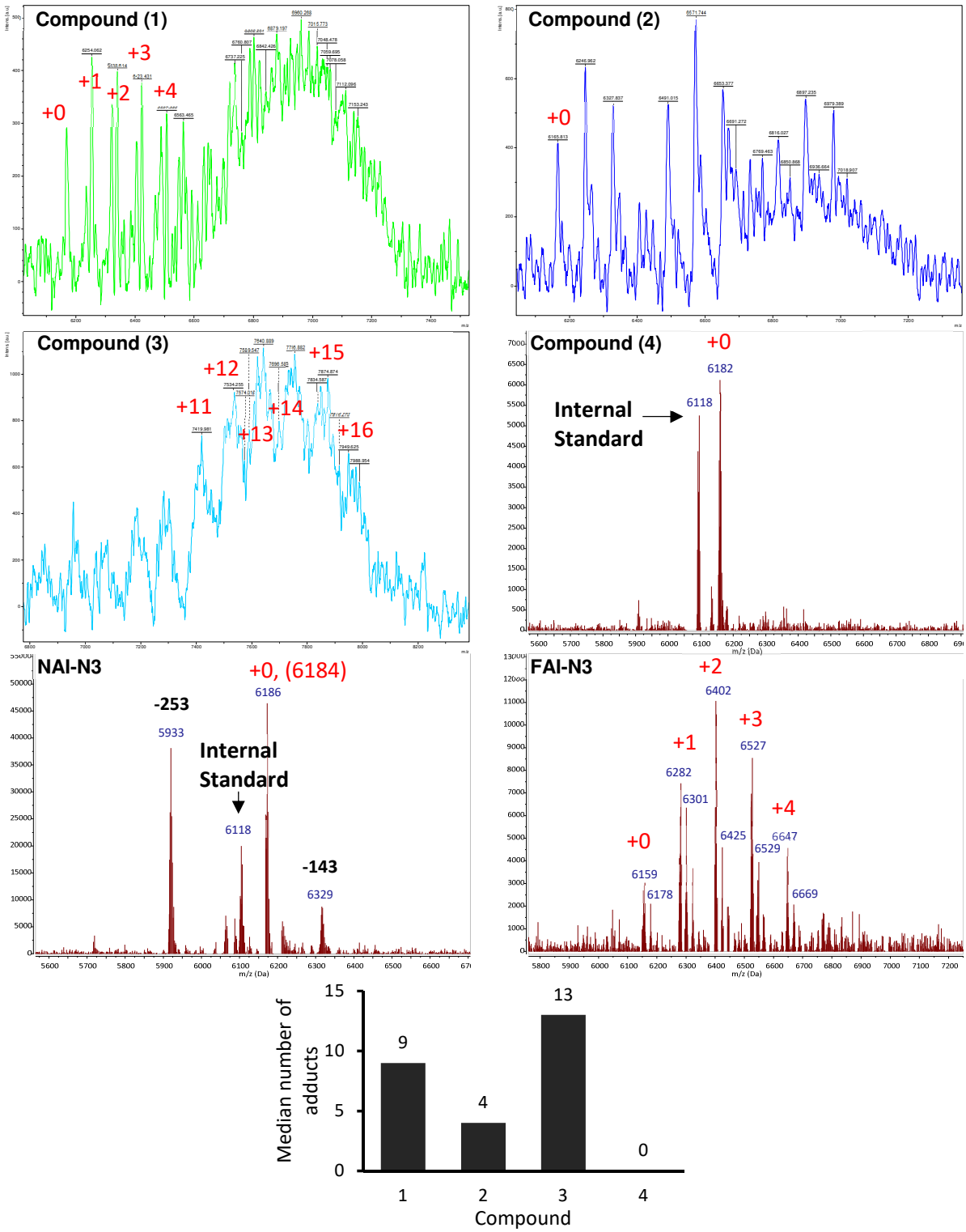


Figure S7. Deacylation (uncloaking) efficiency of acylimidazoles. Cloaked RNA was incubated with 20 mM 2DPPEA at 37 °C for 4 h. The bar graph shows the estimated median number of adducts remaining on the RNA post phosphine treatment. Red numbers indicate the number of amino acyl adducts. Parentheses display the corrected masses from the internal standard (MW: 6118 Da) and the product RNA. The calculated mass of Cy5-tRF-3005 is 6184 Da. **1** and **2** displayed numerous side products that could not be identified.

Molecular beacon assay to detect the effects of polyacylation on hybridization

The median acylation of the cloaked RNA with different compounds was matched to approximately 11-12. According the uncloaking procedure stated above, the uncloaked RNA was generated. On a plate reader, molecular beacon fluorescence signals were recorded at λ_{ex} = 485 nm and λ_{em} = 538 nm at 37 °C. The molecular beacon was added to hybridization buffer (100 mM Tris-HCl, pH 8, 100 mM NaCl, 5 mM $MgCl_2$) to concentration of 80 nM. RNA was added to a final concentration of 100 nM and final volume of 160 µL, and fluorescence signal was recorded for 1 h. The molecular beacon light up signal was calculated by subtracting "dark" molecular beacon fluorescence (no RNA addition) from fluorescence of fully fluorescent beacon in the presence of RNA (~1500 seconds). Then the averaged fluorescence signal was normalized to that of untreated RNA.

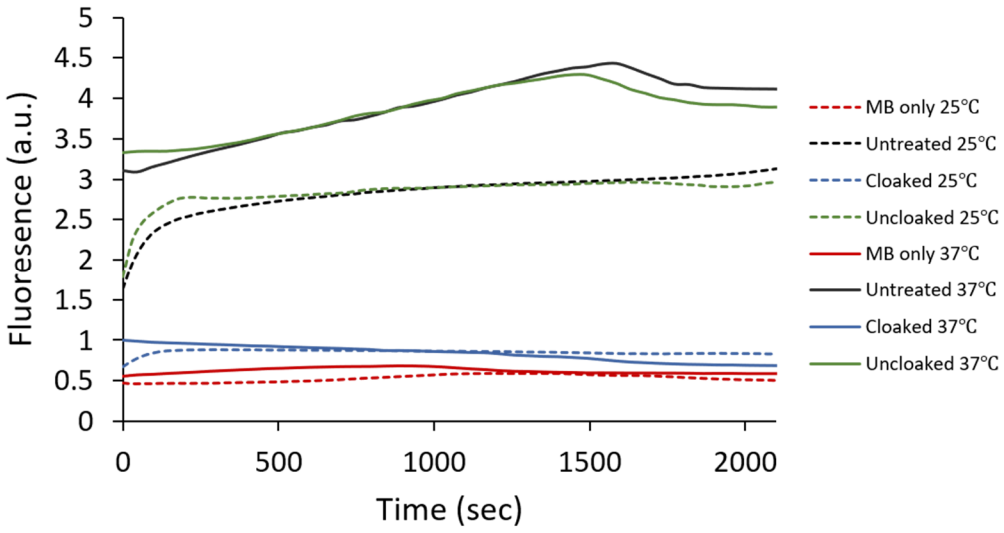


Figure S8. Representative time course of molecular beacon experiment at 25 and 37 °C with **4** treated Cy5-tRF-3005. Black: untreated RNA; blue: cloaked RNA; green: uncloaked RNA; red: no RNA added.

Cloaking and uncloaking of Spinach with 4

Spinach RNA aptamer was transcribed according to the published procedure.[6] For cloaking, 2 μg of transcribed Spinach RNA in 6 μL water was chilled to 4 ˚C. To the chilled RNA solution, 4 μL of 250 mM **4** was added, resulting in 100 mM of **4** and 40% DMSO. The reaction was incubated at 10 ˚C for 1 h. Control reactions were prepared using DMSO instead of **4**. Reactions were quenched by addition of 9 volumes of precipitation solution (0.33M NaOAc, pH 5.2, 0.2 mg/mL glycogen) and 30 volumes of absolute ethanol. RNA was precipitated overnight at -80 ˚C, and then centrifuged (21000 RCF) for 1 h min at 4 ˚C. The pellet was washed with 100 μL 80% ethanol and air dried. The pellets were resuspended in 10 μL RNase free water or stored dry in -20 ˚C. For uncloaking, the cloaked RNA was resuspended in 9 μL 1x PBS pH 7.4, and 1 μL of 50 mM of 2DPPEA was added, resulting in 5 mM final concentration of 2DPPEA. The reaction was incubated at 37 ˚C for 1 h. Control reaction was prepared using DMSO instead of 2DPPEA. The uncloaked RNA was purified via ethanol precipitation procedure identical to one during cloaking. The RNA samples were then analyzed with 12% denaturing polyacrylamide gel electrophoresis (PAGE) in 1xTBE buffer. The gel was stained with SYBR gold and scanned on a Typhoon trio (GE Healthcare).

Spinach folding assay

10 ng/μL of Spinach RNA was incubated with 10 μM DFHBI-1T (Lucerna) in 100 μL of folding buffer (40 mM HEPES, pH 7.4, 100 mM KCl, 5 mM $MgCl_2$) for 1 h at 37 ˚C under protection from light. Fluorescence signals were recorded at λ_{ex} = 450 nm and λ_{em} = 460-600 nm with slit width 3 nm and at 25 ˚C. With the maximal signal at 502 nm, fluorescence fold change of the cloaked/uncloaked RNA signal was calculated by subtracting the background of DFHBI-1T alone in buffer and then dividing by the signal from untreated RNA with DFHBI-1T.

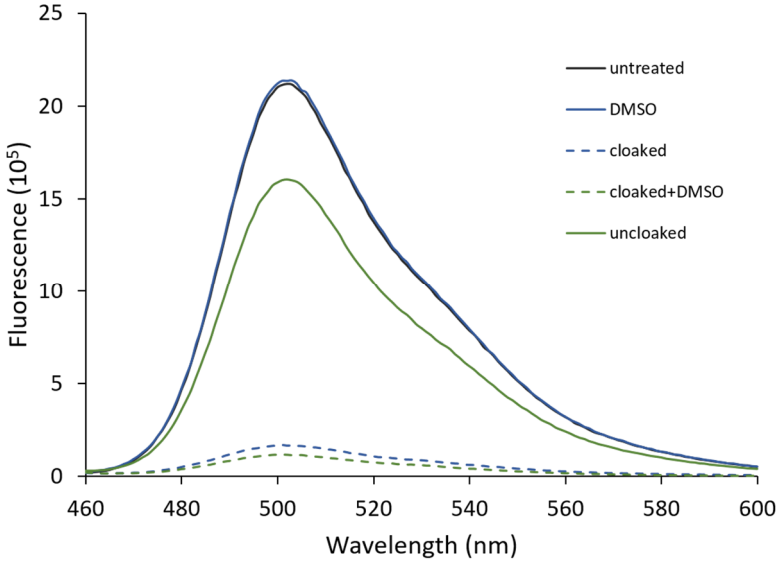


Figure S9. Fluorescence spectra of Spinach RNA after cloaking and uncloaking with **4**. Spinach RNA was treated with 100 mM **4** at 10 ℃ for 1 h. Uncloaking was conducted with 5 mM 2DPPEA at 37 ℃ for 1 h. DMSO: mock DMSO treatment. Cloaked+DMSO: mock DMSO uncloaking treatment. Black: untreated RNA; blue: cloaked or mock cloaked RNA; green: uncloaked or mock uncloaked RNA.

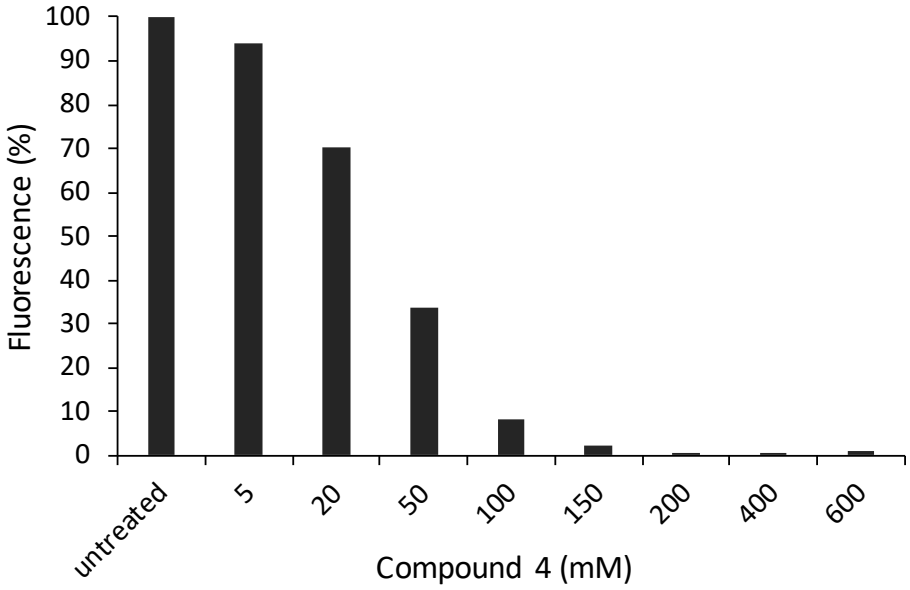


Figure S10. Concentration dependent cloaking of Spinach with **4**, reducing its fluorescence with increasing levels of acylation. 200 ng/μL of Spinach was treated with 5-600 mM **4** at 10 ˚C for 1 h.

Figure S11. PAGE analysis of Spinach RNA cloaked and uncloaked with **4**. The samples were run on 12% denaturing gel and stained with SYBR gold. Lack of strong bands below the main RNA band shows little or no degradation induced by acylation/deacylation.

Fluorescent labeling of RNA (cloak-click procedure)

In RNase free water, tRF-3005 (6 µM) was incubated with 0.35 M **4** (using 2 M stock in dry DMSO) for 1 h at room temperature. The cloaked RNA with median acylation of 7 was then purified via ethanol precipitation as described above. The cloaked RNA (1 µM) was reacted with 5 or 15 µM of TAMRA-DBCO in a total volume of 120 µL in PBS at 37 °C for 2 h. The cloaked-clicked RNA was then purified via ethanol precipitation. For fluorescence measurements, 0.1 µM RNA in PBS buffer pH 7.4 was used at a final volume of 200 µL, and the spectra was obtained with λ_{ex} = 546 nm and λ_{em} = 555-650 nm.

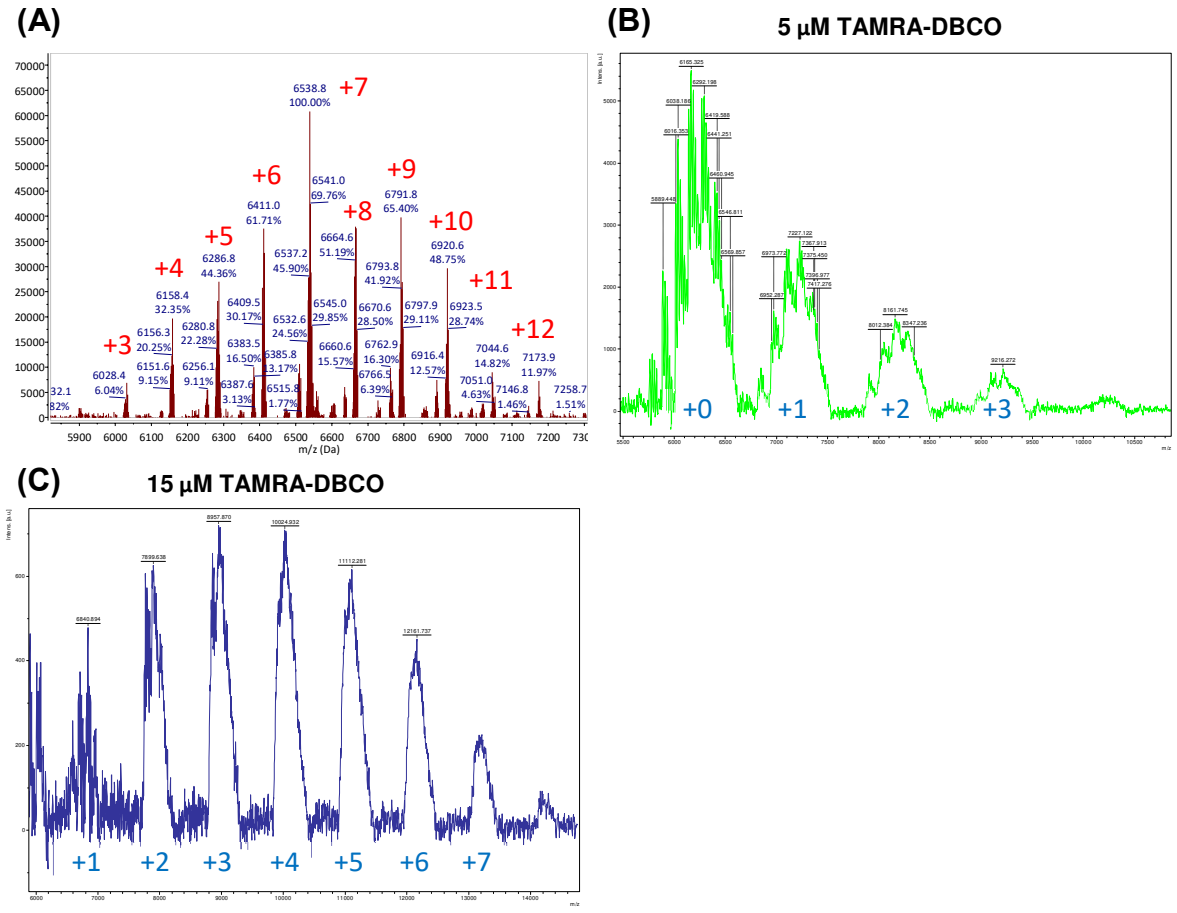


Figure S12. Fluorescence labeling of RNA via cloak-click strategy. (A) Cloaked tRF-3005 (B & C) 5 µM and 15 µM TAMRA-DBCO treatment. Red numbers indicate the number of acyl adducts. Blue numbers indicate the number of TAMRA adducts.

SHAPE analysis of SAM aptamer RNA

The RNA aptamer was transcribed using MEGAshortscript T7 Transcription Kit (Ambion), following the manufacturer's protocol. RNA was transcribed from amplified dsDNA sequence: taatacgactcactatagGGCTTATCAAGAGAGGTGGAGGGACTGGCCCGATGAAACCCGGCAACCAG AAATGGTGCCAATTCCTGCAGCGGAAACGTTGAAAGATGAGCCG. 100 pmole of SAM aptamer RNA was heated in folding buffer (0.06 M MOPS, pH 7.5; 0.1 M KCl; 2.5 mM $MgCl_2$) to 95°C for 2 min and cooled down to 37°C (Δ3°C/min). RNA was incubated at 37°C for next 30 min. To this mixture 0.1 vol. of SHAPE reagent stock solution (NAI-N_3 or compound **4**) or DMSO (control reaction) was added and reaction was incubated for 10 min at 37°C. Reactions were quenched by addition of 9 volumes of precipitation solution (0.33M NaOAc, pH 5.2, 0.2 mg/mL glycogen) and 30 volumes of an ice-cold ethanol. RNA was precipitated overnight at -80˚C, and then centrifuged (21000 RCF) for 40 min at 4˚C. The pellet was washed with 80% ethanol, air dried, and resuspended in 10 µL RNase free water. 4 pmole of RNA was mixed with 6 pmole RT Primer (Thermo Fisher Scientific, NED-CGGCTCATCTTTCAACG) and 0.25 µL dNTP mix (10 mM each, Invitrogen), and incubated for 5 min at 65˚C, then immediately chilled on ice for 2 min. To a final 10 µL volume were added: 2 µL 5x First-Strand Buffer (Invitrogen), 1 µL 0.1 M DTT, 0.5 µL RNaseOUT and 0.25 µL Super Script II (200 U/µl, Invitrogen). The reaction was incubated using the following program: 10 min at 25˚C, 50 min at 42˚C, and 50 min at 52˚C. To the reaction was added 10 µL of loading dye (95% formamide, 5mM EDTA pH 8, 0.05% Bromophenol blue, 0.05% Xylene cyanol FF), the mixture was denatured for 3 min. at 96˚C, and loaded on a denaturing 8% polyacrylamide gel. Products were separated in a gel in 1x TBE (pH 8.3, Sigma Aldrich), 35mA, ~3.5h. The cDNA gel was visualized by fluorescence imaging (Typhoon, GE Healthcare). SHAPE cDNA bands intensity was measured using ImageGauge software and normalized to control. The secondary structure of SAM RNA aptamer was adapted from the published literature.[7]

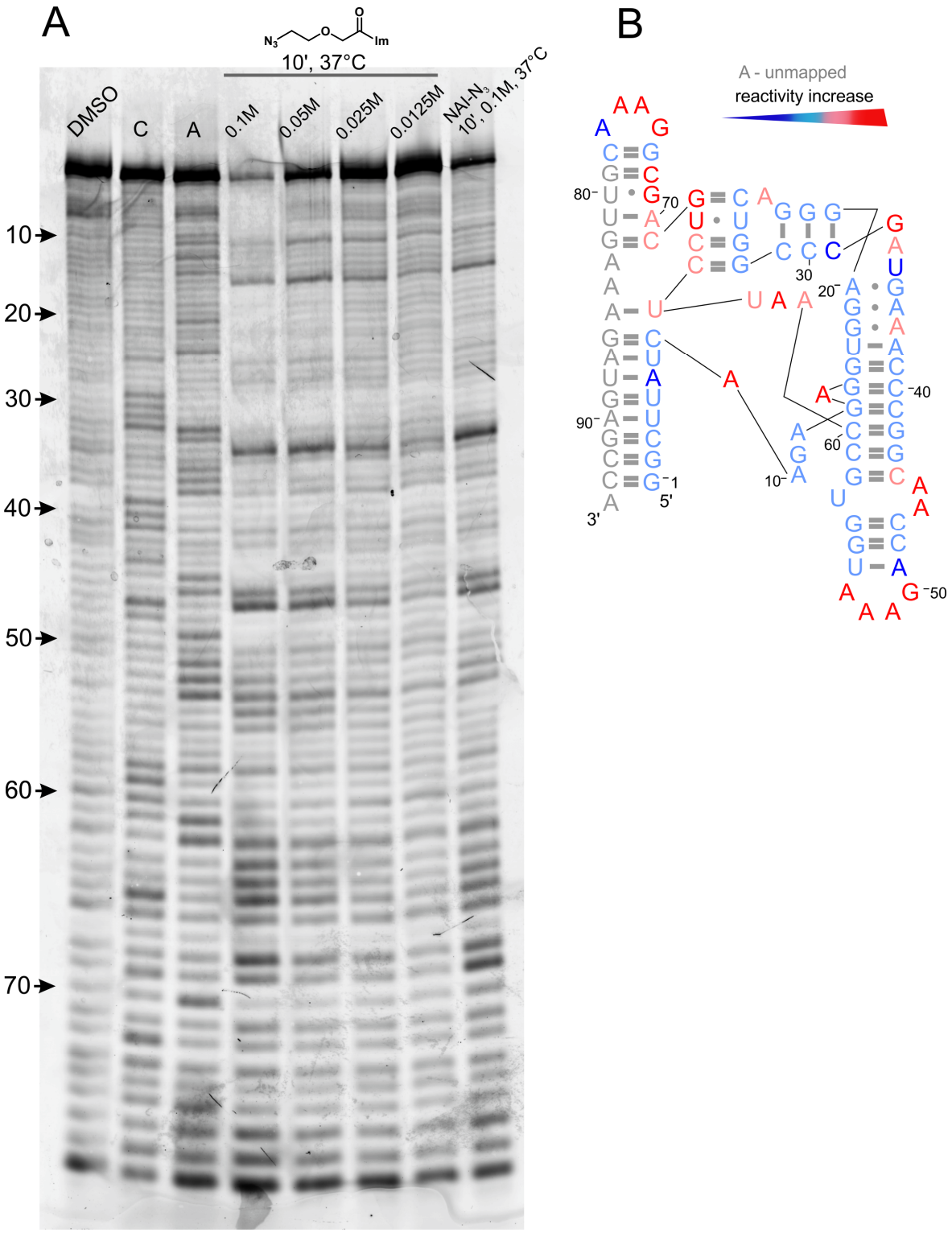
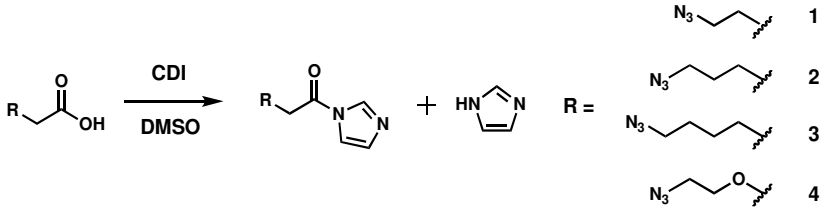


Figure S13. Mapped structure of SAM aptamer using compound **4**. (A) SAM aptamer structure probing patterns generated using the SHAPE compound **4** in increasing concentrations and NAI-N_3. C and A are dideoxy sequencing lanes. In the gel, the positions of selected bases are indicated with arrows. Control (DMSO) lane shows background RNA probing signal. (B) The secondary structure of SAM aptamer RNA with mapped level of reactivity normalized to the control sample.

Synthetic procedures

Synthesis of acyl imidazoles



*General procedure for the preparation of acyl imidazoles (**1**-**4**)*
To a screw cap vial containing carbonyldiimidazole (CDI) (177 mg, 1.1 mmol), the acids (1 mmol) were dissolved in 200 µL anhydrous DMSO and added. The reaction was stirred under Ar at room temperature for 1 h. The total volume was increased to 500 µL with additional dry DMSO to prepare the 2 M stocks. For NMR analysis, the reactions were performed in DMSO-d6.

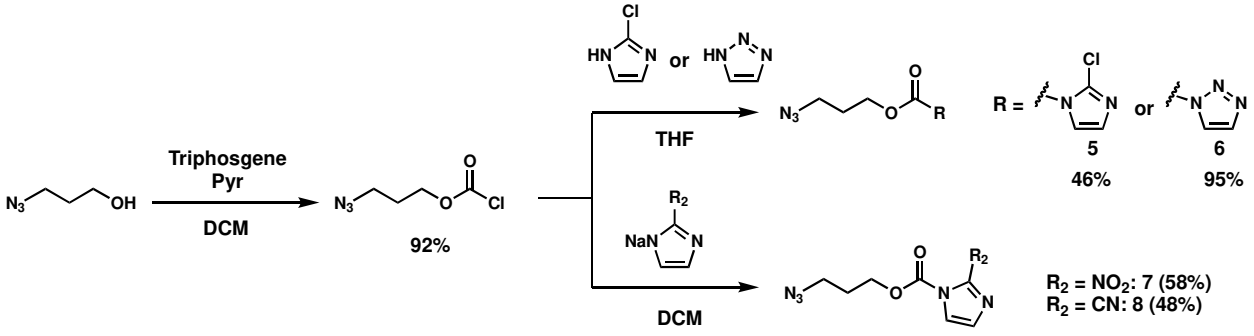
4-azido-1-(1*H*-imidazol-1-yl)butan-1-one (**1**)
^{1}H NMR (400 MHz, DMSO-d_6) δ 8.43 (s, 1H), 7.71 (t, *J* = 1.5 Hz, 1H), 7.07 (dd, *J* = 1.7, 0.9 Hz, 1H), 3.43 (t, *J* = 6.8 Hz, 2H), 3.11 (t, *J* = 7.1 Hz, 2H), 1.91 (p, *J* = 7.0 Hz, 2H).
^{13}C NMR (101 MHz, DMSO-d_6) δ 169.97, 137.03, 130.28, 116.48, 49.82, 31.65, 22.93.
HRMS [+ Scan]; calculated m/z for $C_7H_9N_5O$ [M+H] 180.0880; observed mass 180.0874.

5-azido-1-(1*H*-imidazol-1-yl)pentan-1-one (**2**)
^{1}H NMR (400 MHz, DMSO-d_6) δ 8.42 (s, 1H), 7.71 (t, *J* = 1.6 Hz, 1H), 7.07 (dd, *J* = 1.7, 0.9 Hz, 1H), 3.38 (t, *J* = 6.8 Hz, 2H), 3.07 (t, *J* = 7.1 Hz, 2H), 1.76 – 1.66 (m, 2H), 1.66 – 1.57 (m, 2H).
^{13}C NMR (101 MHz, DMSO-d_6) δ 170.33, 137.03, 130.24, 116.44, 50.28, 33.75, 27.49, 20.70.
HRMS [+ Scan]; calculated m/z for $C_8H_{11}N_5O$ [M+H] 194.1037; observed mass 194.1030.

6-azido-1-(1*H*-imidazol-1-yl)hexan-1-one (**3**)
^{1}H NMR (400 MHz, DMSO-d_6) δ 8.43 (t, *J* = 1.1 Hz, 1H), 7.71 (t, *J* = 1.5 Hz, 1H), 7.06 (dd, *J* = 1.7, 0.8 Hz, 1H), 3.34 (t, *J* = 6.8 Hz, 2H), 3.04 (t, *J* = 7.3 Hz, 2H), 1.67 (p, *J* = 7.4 Hz, 2H), 1.62 – 1.51 (m, 2H), 1.46 – 1.36 (m, 2H).
^{13}C NMR (101 MHz, DMSO-d_6) δ 170.46, 137.04, 130.23, 116.43, 50.53, 34.13, 27.98, 25.46, 23.05.
HRMS [+ Scan]; calculated m/z for $C_9H_{13}N_5O$ [M+H] 208.1193; observed mass 208.1187.

2-(2-azidoethoxy)-1-(1*H*-imidazol-1-yl)ethan-1-one (**4**)
^{1}H NMR (400 MHz, DMSO-d_6) δ 8.38 (s, 1H), 7.69 (t, *J* = 1.2 Hz, 1H), 7.09 (dd, *J* = 1.6, 0.8 Hz, 1H), 4.91 (s, 2H), 3.81 – 3.72 (m, 2H), 3.52 – 3.44 (m, 2H).
^{13}C NMR (101 MHz, DMSO-d_6) δ 167.71, 136.66, 130.19, 116.07, 69.93, 69.12, 50.01.
HRMS [+ Scan]; calculated m/z for $C_7H_9N_5O_2$ [M+H] 196.0829; observed mass 196.0823.

Synthesis of oxycarbonylimidazole derivatives



3-azidopropylcarbonochloridate
3-azidopropanol (50 mg, 360 μmol) and pyridine (30 μL, 380 μmol) was dissolved in 3.5 mL anhydrous DCM and cooled on ice. Triphosgene (107 mg, 360 μmol) was added in one portion, and the reaction was stirred for 1h on ice. The reaction mixture was then filtered through a pad of silica, and the silica was washed with ~7 mL of additional DCM. The filtrate was concentrated *in vacuo* to afford a clear oil (54 mg, 92%).
^{1}H NMR (400 MHz, Chloroform-*d*) δ 4.41 (t, *J* = 6.2 Hz, 2H), 3.46 (t, *J* = 6.5 Hz, 2H), 2.00 (p, *J* = 6.3 Hz, 2H).
^{13}C NMR (101 MHz, Chloroform-*d*) δ 150.78, 68.81, 47.65, 27.97.

*General procedure for oxycarbonyl imidazole derivatives (**5** & **6**)*
3-azidopropylcarbonochloridate (0.75 mmol) was dissolved in 4 mL of anhydrous THF. The imidazole derivative (1.5 mmol) was dissolved in 3.5 mL of THF and added. The reaction was stirred at room temperature overnight under Ar. The volatiles were evaporated, and remaining residues were dissolved in DCM. The imidazole salts were filtered off, and the filtrate was concentrated *in vacuo* to obtain the product.

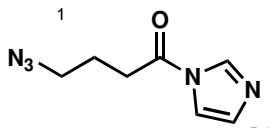
3-azidopropyl 2-chloro-1*H*-imidazole-1-carboxylate (**5**)
Brown oil, 46% yield
^{1}H NMR (300 MHz, DMSO-d_6) δ 7.72 (d, *J* = 1.9 Hz, 1H), 7.02 (d, *J* = 1.9 Hz, 1H), 4.42 (t, *J* = 6.1 Hz, 2H), 3.54 (t, *J* = 6.7 Hz, 2H), 1.99 (p, *J* = 6.4 Hz, 2H).
HRMS [+ Scan]; calculated m/z for $C_7H_8ClN_5O_2$ [M+H] 230.0440; observed mass 230.0432.

3-azidopropyl 1*H*-1,2,3-triazole-1-carboxylate (**6**)
Clear oil, 95% yield
^{1}H NMR (300 MHz, Chloroform-*d*) δ 8.24 (d, *J* = 1.4 Hz, 1H), 7.77 (d, *J* = 1.4 Hz, 1H), 4.66 (t, *J* = 6.2 Hz, 2H), 3.55 (t, *J* = 6.4 Hz, 2H), 2.14 (p, *J* = 6.3 Hz, 2H).
HRMS [+ Scan]; calculated m/z for $C_6H_8N_6O_2$ [M+H] 197.0782; observed mass 197.0775.

*General procedure for oxycarbonyl imidazole derivatives (**7** & **8**)*
The mineral oil from NaH (1.2 mmol) was washed away 3x with hexanes. 8 mL of anhydrous THF was added. Imidazole derivatives (1.2 mmol) were added in one portion and stirred at room temperature for 10 min. 3-azidopropylcarbonochloridate (0.6 mmol) was dissolved in 2 mL of anhydrous THF and added dropwise to the sodium imidazolide mixture. The reaction mixture was further stirred at room temperature for 30 min. The volatiles were evaporated, and remaining residues were dissolved in DCM. The imidazole salts were filtered off, and the filtrate was concentrated *in vacuo* to obtain the product.

3-azidopropyl 2-nitro-1*H*-imidazole-1-carboxylate (**7**)
Yellow oil, ca. 58%
^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.45 (d, *J* = 1.6 Hz, 1H), 7.07 (d, *J* = 1.6 Hz, 1H), 4.55 (t, *J* = 6.1 Hz, 2H), 3.47 (t, *J* = 6.4 Hz, 2H), 2.03 (p, *J* = 6.2 Hz, 2H).
HRMS was attempted, but no result was obtained.

3-azidopropyl 2-cyano-1*H*-imidazole-1-carboxylate (**8**)
Orange oil, ca. 48%
^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.67 (d, *J* = 1.6 Hz, 1H), 7.25 (d, *J* = 1.6 Hz, 1H), 4.63 (t, *J* = 6.1 Hz, 2H), 3.60 (t, *J* = 6.4 Hz, 2H), 2.16 – 2.08 (m, 2H).
HRMS [+ Scan]; calculated m/z for $C_8H_8N_6O_2$ [M+H] 221.0782; observed mass 221.0775.



A (s)
8.43

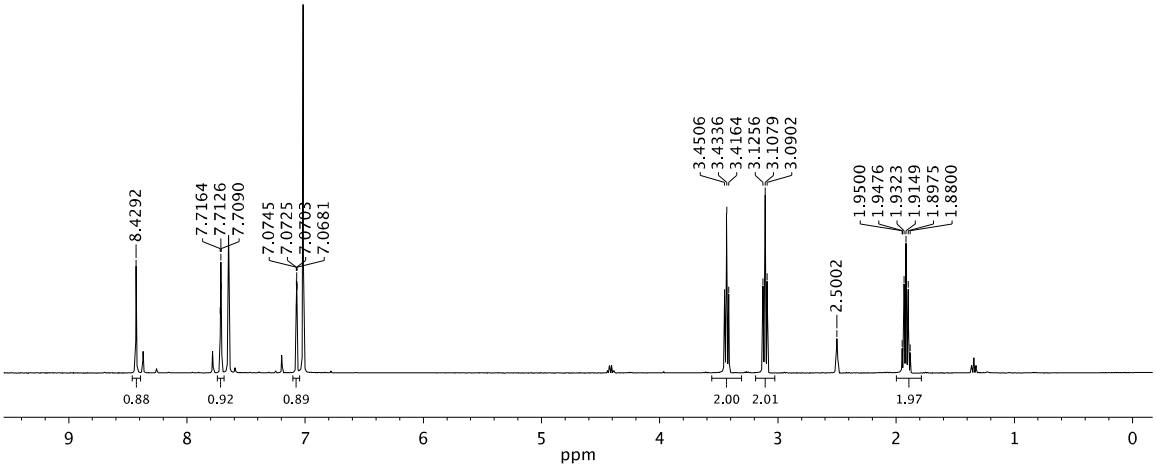
B (t)
7.71
J(1.48)

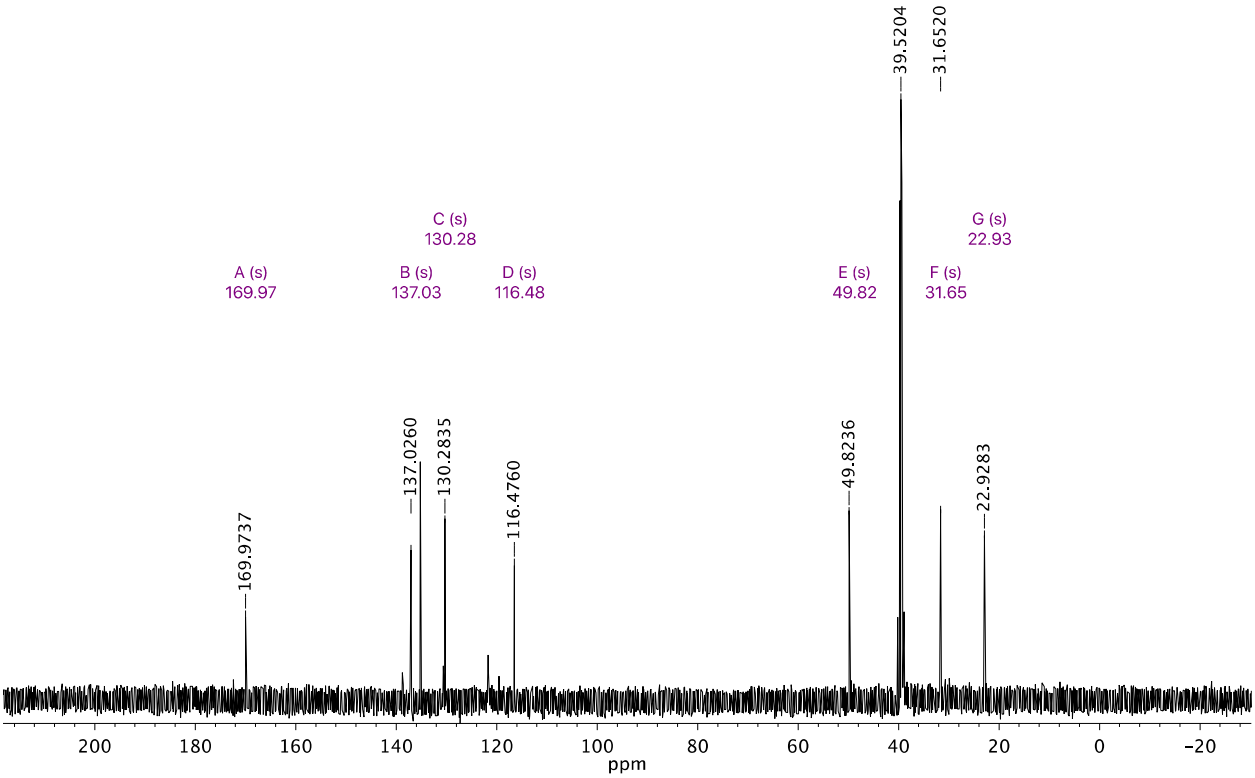
C (dd)
7.07
J(1.72, 0.85)

D (t)
3.43
J(6.84)

E (t)
3.11
J(7.09)

F (p)
1.91
J(7.04)





C (s)
130.28

G (s)
22.93

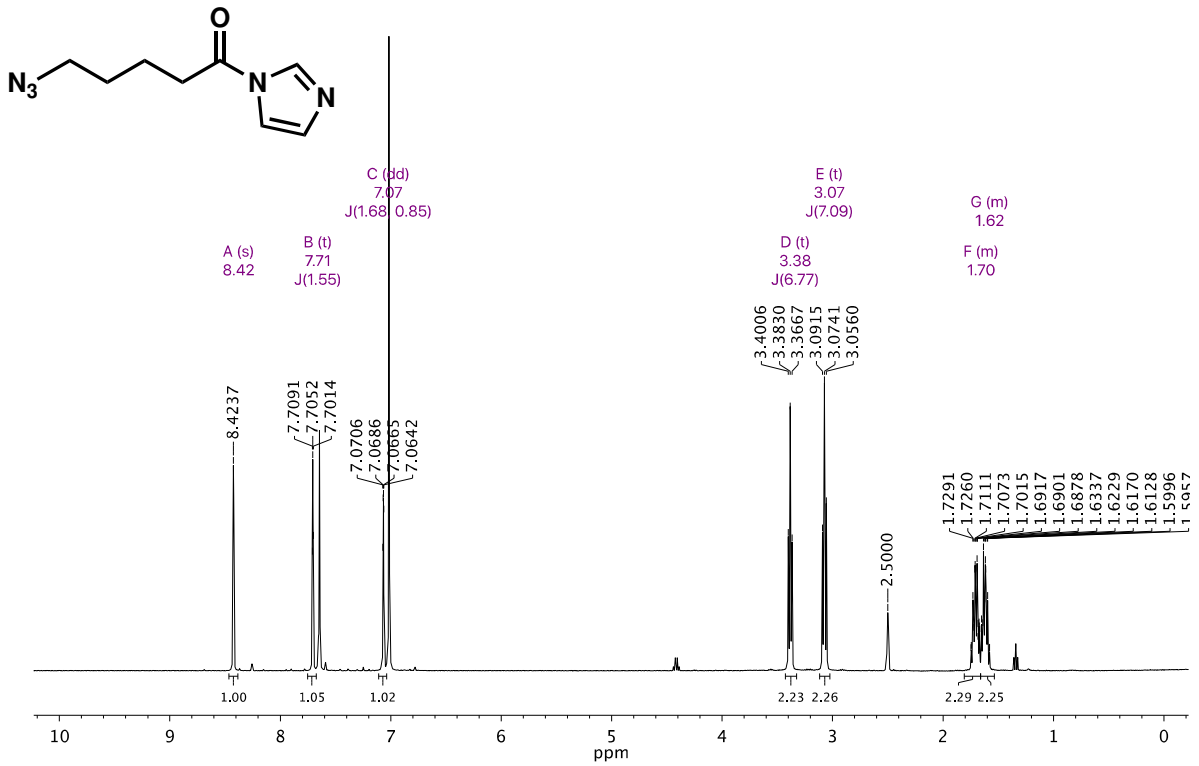
A (s)
169.97

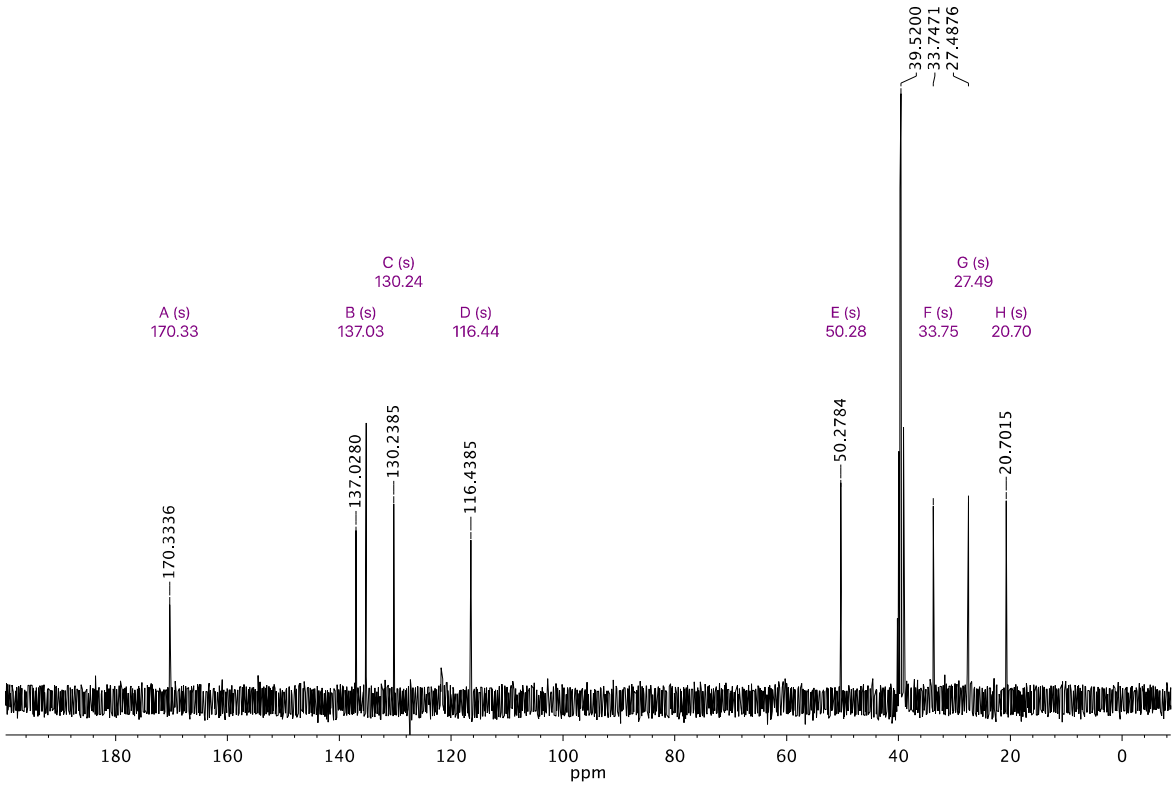
B (s)
137.03

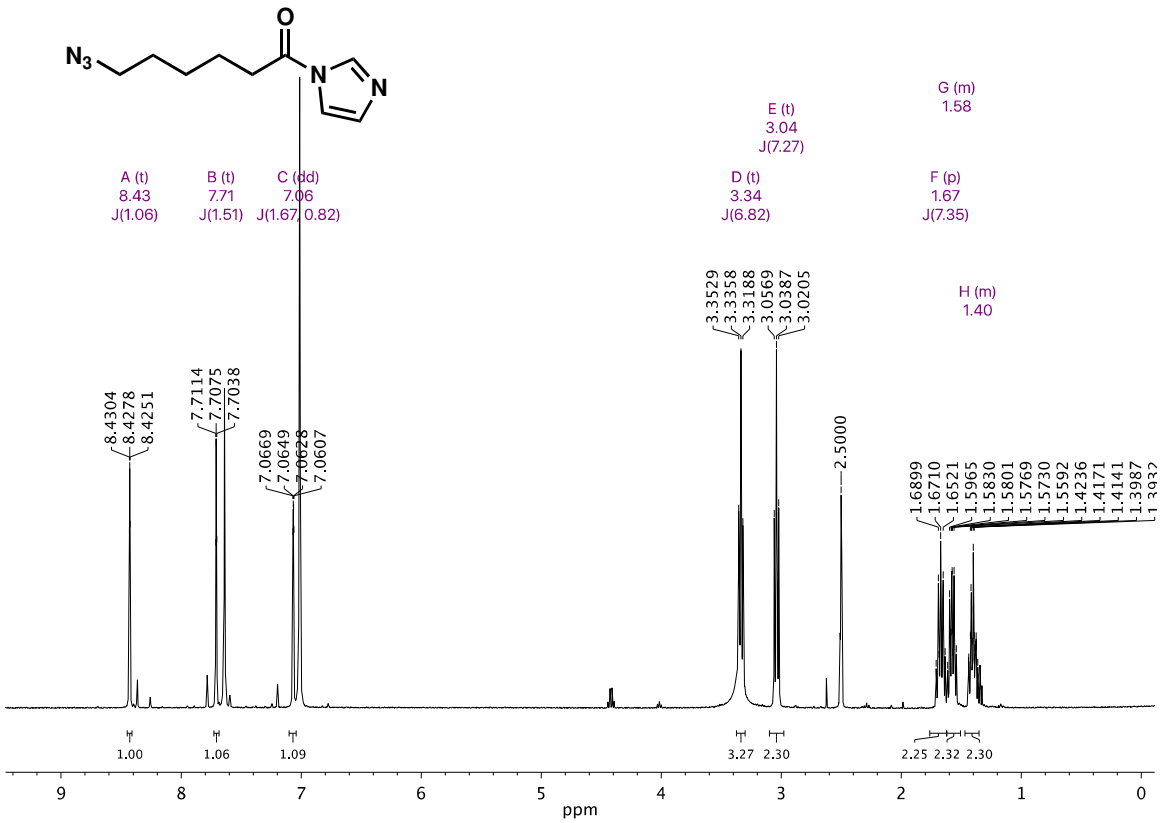
D (s)
116.48

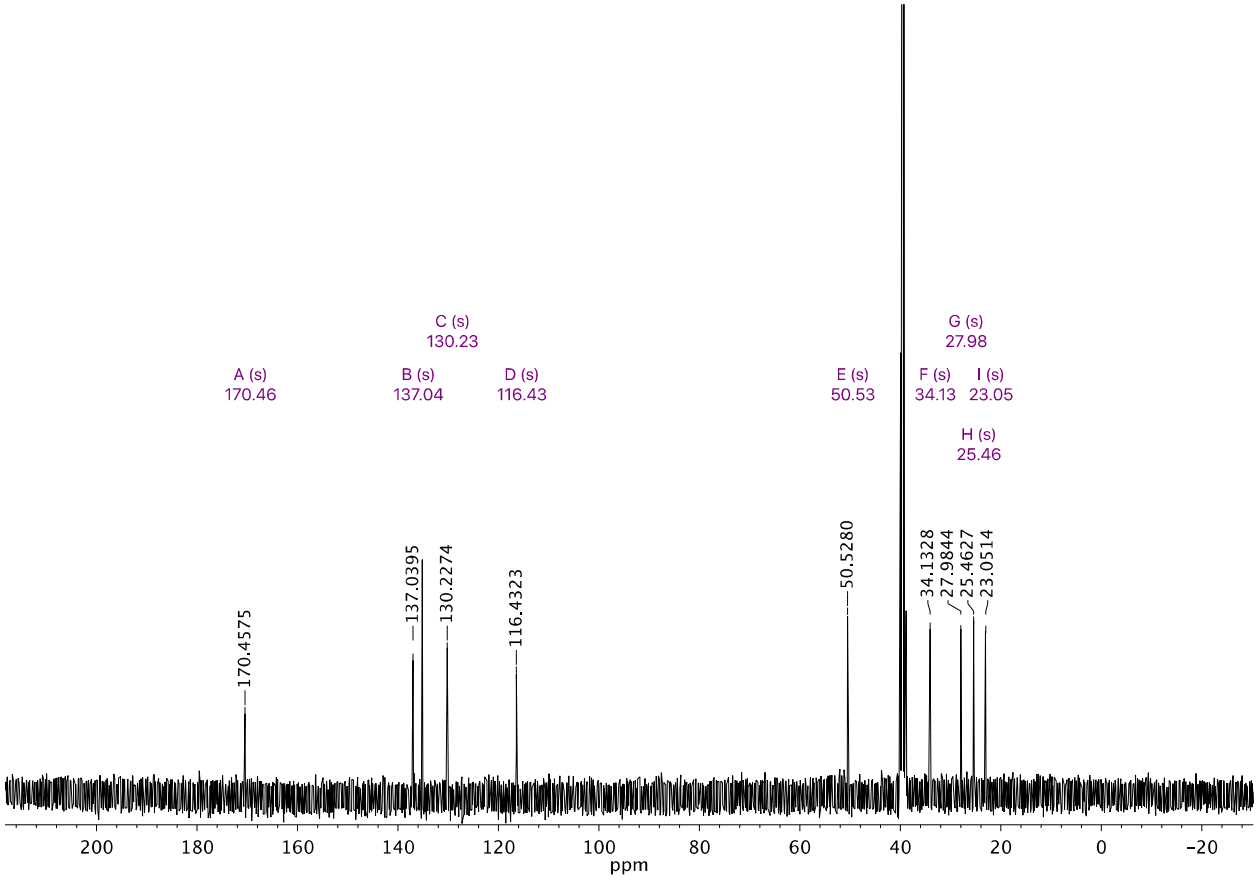
E (s)
49.82

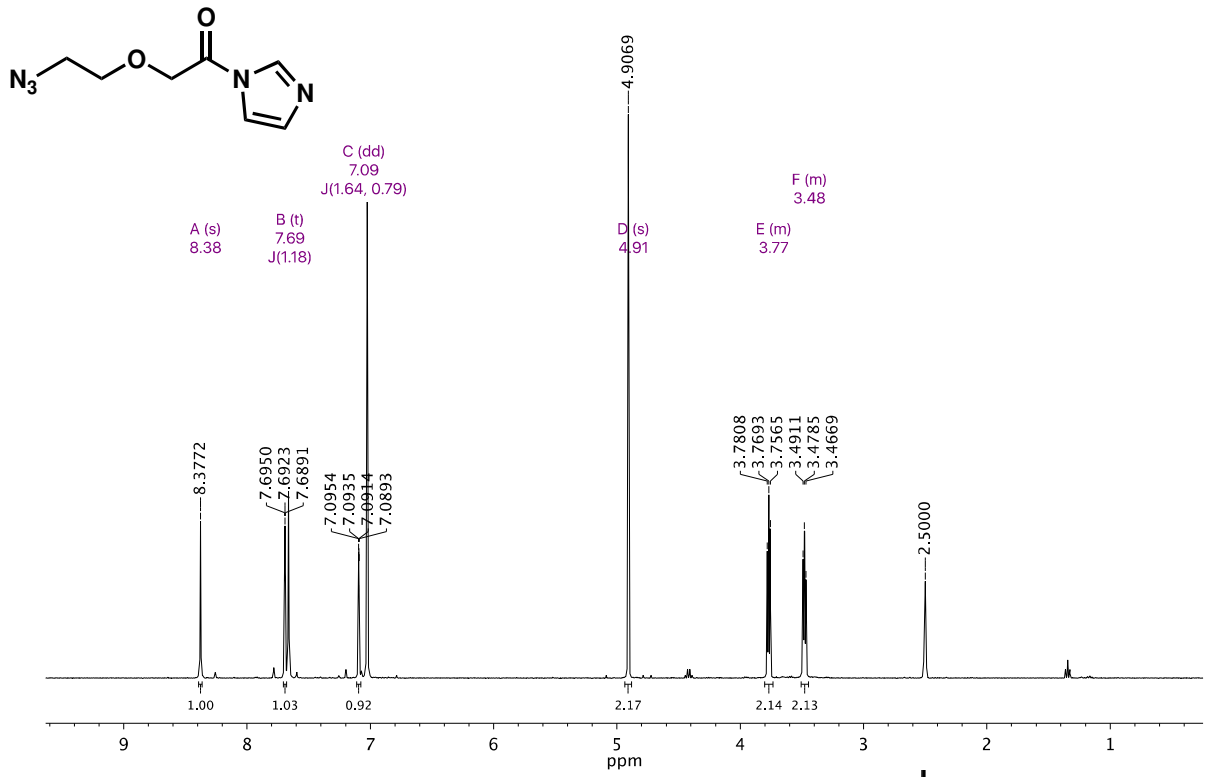
F (s)
31.65

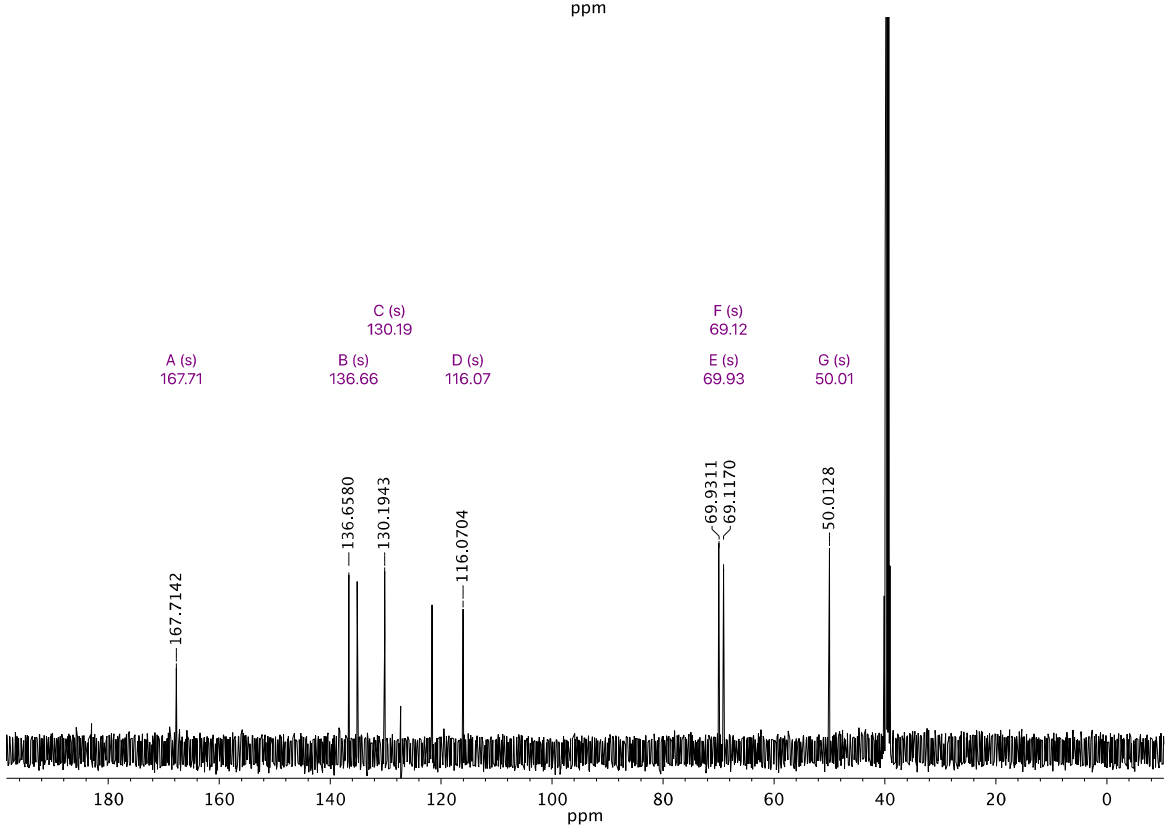

A (s)
8.42
B (t)
7.71
J(1.55)
C (dd)
7.07
J(1.68 0.85)
D (t)
3.38
J(6.77)
E (t)
3.07
J(7.09)
F (m)
1.70
G (m)
1.62
8.4237
7.7091
7.7052
7.7014
7.0706
7.0686
7.0665
7.0642
3.4006
3.3830
3.3667
3.0915
3.0741
3.0560
2.5000
1.7291
1.7260
1.7111
1.7073
1.7015
1.6917
1.6901
1.6878
1.6337
1.6229
1.6170
1.6128
1.5996
1.00
1.05
1.02
2.23
2.26
2.29
2.25
ppm

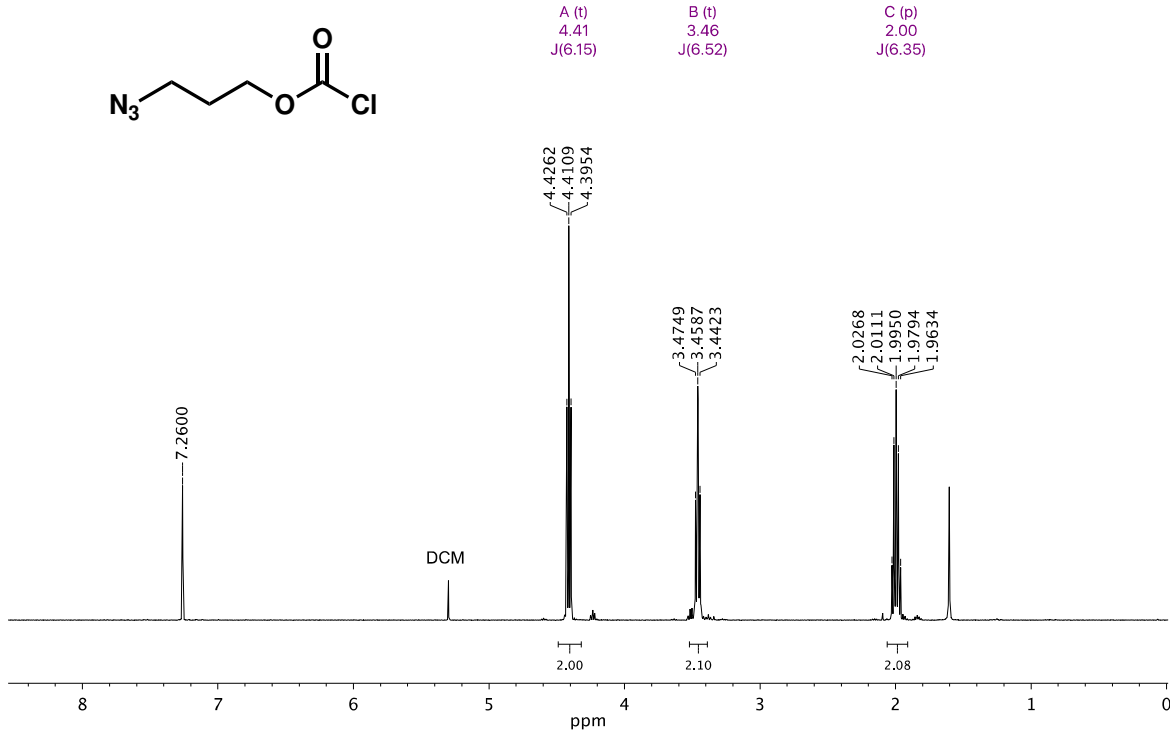

A (s)
170.33
B (s)
137.03
C (s)
130.24
D (s)
116.44
E (s)
50.28
F (s)
33.75
G (s)
27.49
H (s)
20.70
170.3336
137.0280
130.2385
116.4385
50.2784
39.5200
33.7471
27.4876
20.7015
ppm

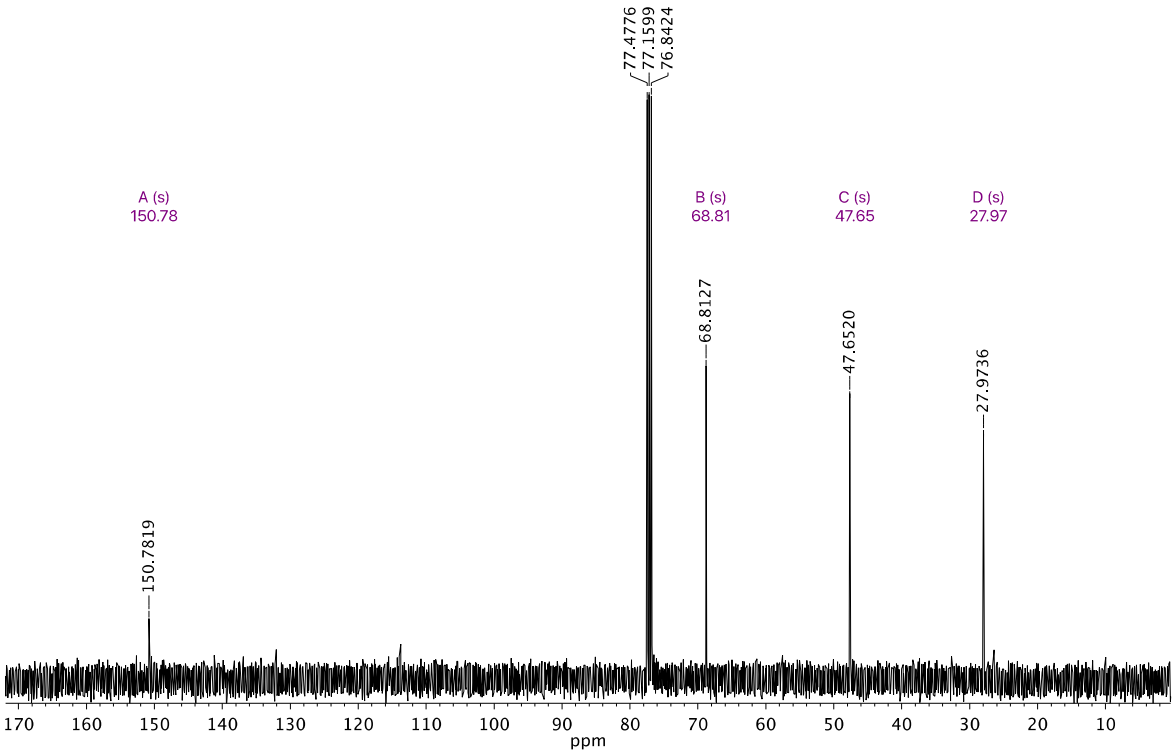

A (t)
8.43
J(1.06)
B (t)
7.71
J(1.51)
C (dd)
7.06
J(1.67, 0.82)
D (t)
3.34
J(6.82)
E (t)
3.04
J(7.27)
F (p)
1.67
J(7.35)
G (m)
1.58
H (m)
1.40
8.4304
8.4278
8.4251
7.7114
7.7075
7.7038
7.0669
7.0649
7.0628
7.0607
3.3529
3.3358
3.3188
3.0569
3.0387
3.0205
2.5000
1.6899
1.6710
1.6521
1.5965
1.5830
1.5801
1.5769
1.5730
1.5592
1.4236
1.4171
1.4141
1.3987
1.00
1.06
1.09
3.27
2.30
2.25
2.32
2.30
ppm

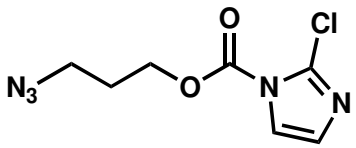

A (s)
170.46
B (s)
137.04
C (s)
130.23
D (s)
116.43
E (s)
50.53
F (s)
34.13
G (s)
27.98
H (s)
25.46
I (s)
23.05
170.4575
137.0395
130.2274
116.4323
50.5280
34.1328
27.9844
25.4627
23.0514
ppm

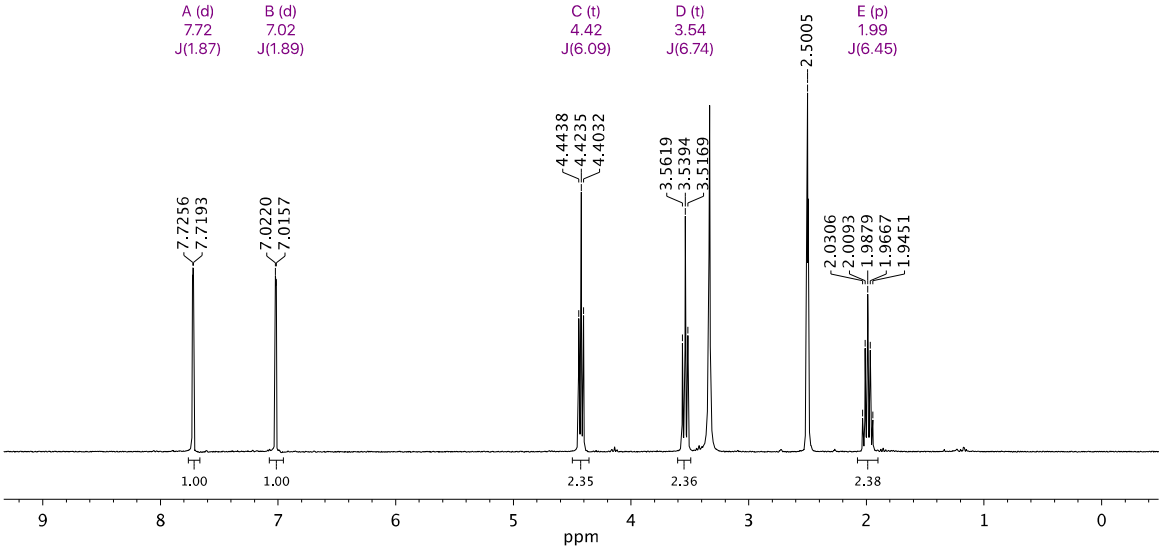

A (s)
8.38
B (t)
7.69
J(1.18)
C (dd)
7.09
J(1.64, 0.79)
D (s)
4.91
E (m)
3.77
F (m)
3.48
8.3772
7.6950
7.6923
7.6891
7.0954
7.0935
7.0914
7.0893
4.9069
3.7808
3.7693
3.7565
3.4911
3.4785
3.4669
2.5000
1.00
1.03
0.92
2.17
2.14
2.13
ppm

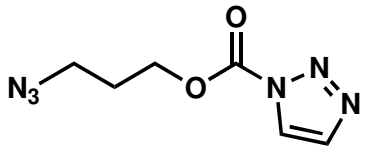

A (s)
167.71
B (s)
136.66
C (s)
130.19
D (s)
116.07
E (s)
69.93
F (s)
69.12
G (s)
50.01
167.7142
136.6580
130.1943
116.0704
69.9311
69.1170
50.0128
ppm

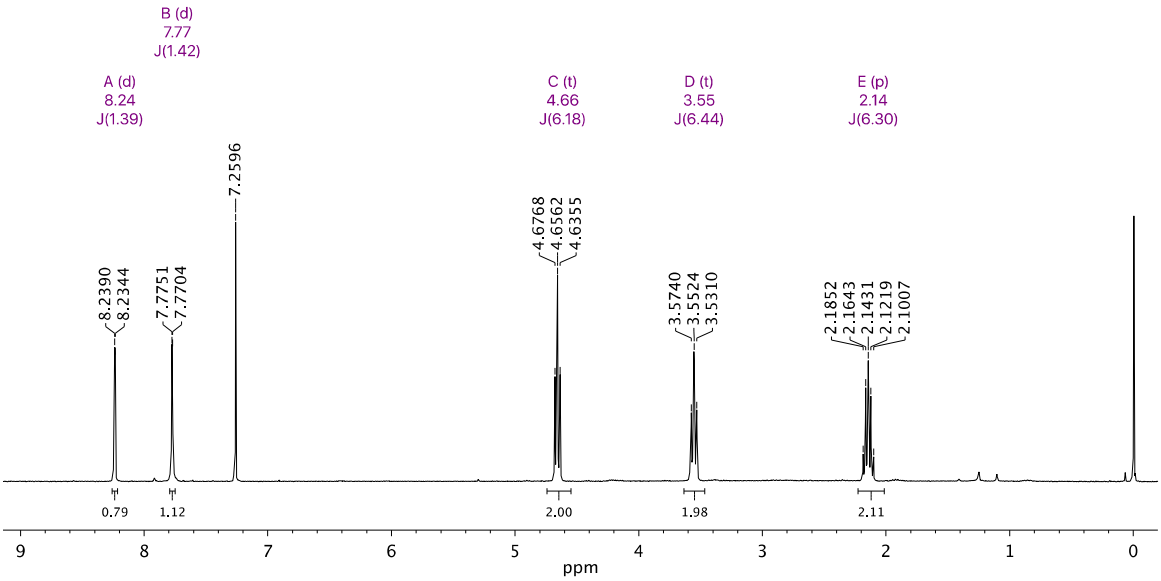

A (t)
4.41
J(6.15)
B (t)
3.46
J(6.52)
C (p)
2.00
J(6.35)
4.4262
4.4109
4.3954
3.4749
3.4587
3.4423
2.0268
2.0111
1.9950
1.9794
1.9634
7.2600
DCM
2.00
2.10
2.08
ppm

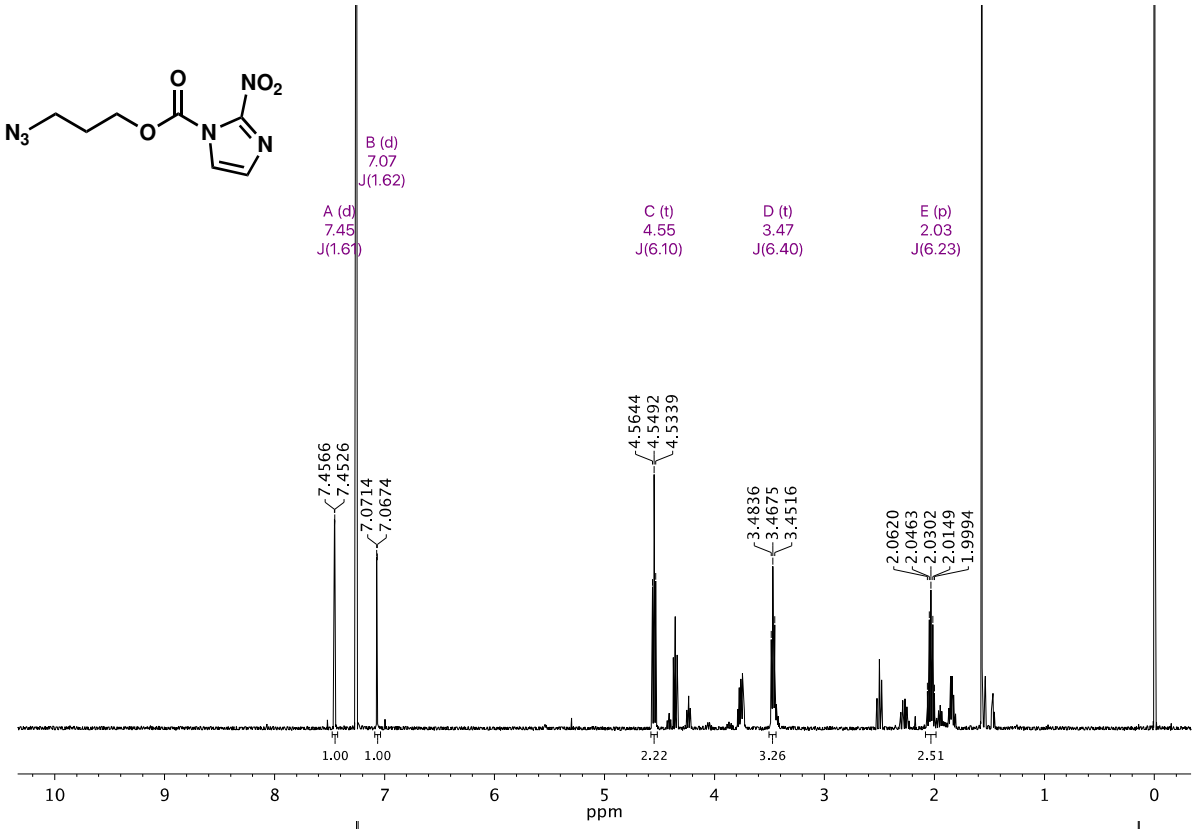

77.4776
77.1599
76.8424
A (s)
150.78
B (s)
68.81
C (s)
47.65
D (s)
27.97
68.8127
47.6520
27.9736
150.7819
ppm

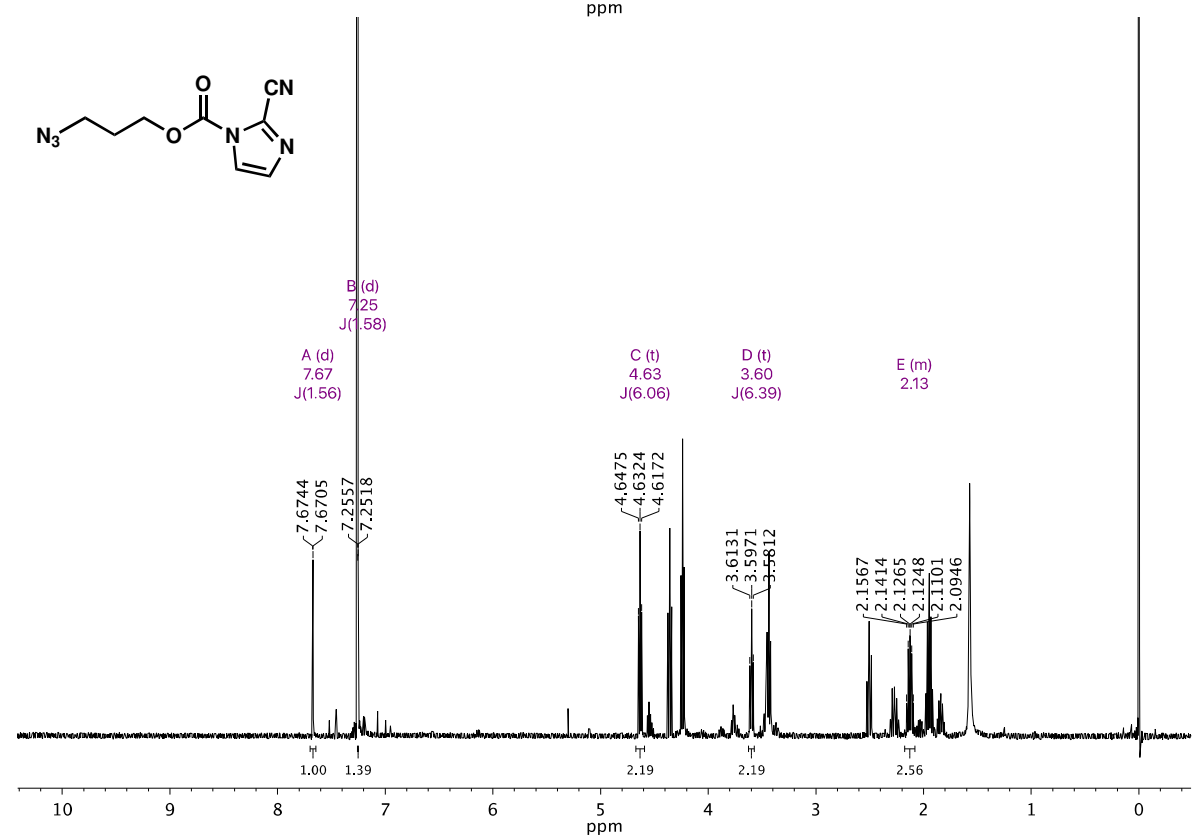

O
Cl
N3
O
N
N


A (d)
7.72
J(1.87)
B (d)
7.02
J(1.89)
C (t)
4.42
J(6.09)
D (t)
3.54
J(6.74)
E (p)
1.99
J(6.45)
7.7256
7.7193
7.0220
7.0157
4.4438
4.4235
4.4032
3.5619
3.5394
3.5169
2.5005
2.0306
2.0093
1.9879
1.9667
1.9451
1.00
1.00
2.35
2.36
2.38
9
8
7
6
5
4
3
2
1
0
ppm

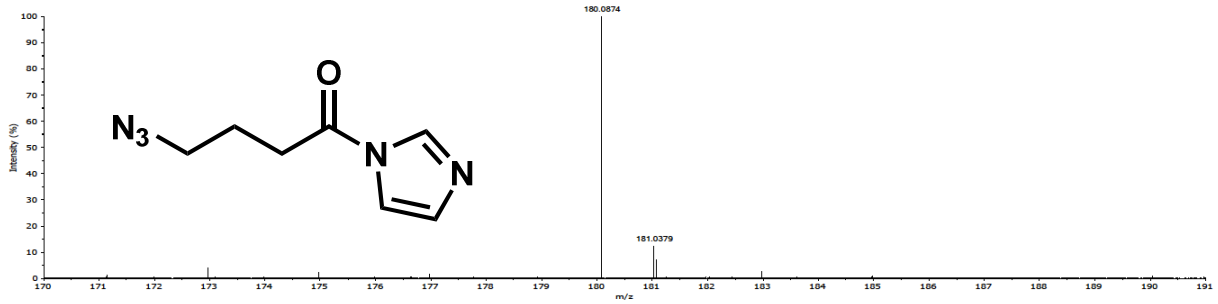

O
N3
O
N
N
N


B (d)
7.77
J(1.42)
A (d)
8.24
J(1.39)
C (t)
4.66
J(6.18)
D (t)
3.55
J(6.44)
E (p)
2.14
J(6.30)
7.2596
8.2390
8.2344
7.7751
7.7704
4.6768
4.6562
4.6355
3.5740
3.5524
3.5310
2.1852
2.1643
2.1431
2.1219
2.1007
0.79
1.12
2.00
1.98
2.11
9
8
7
6
5
4
3
2
1
0
ppm

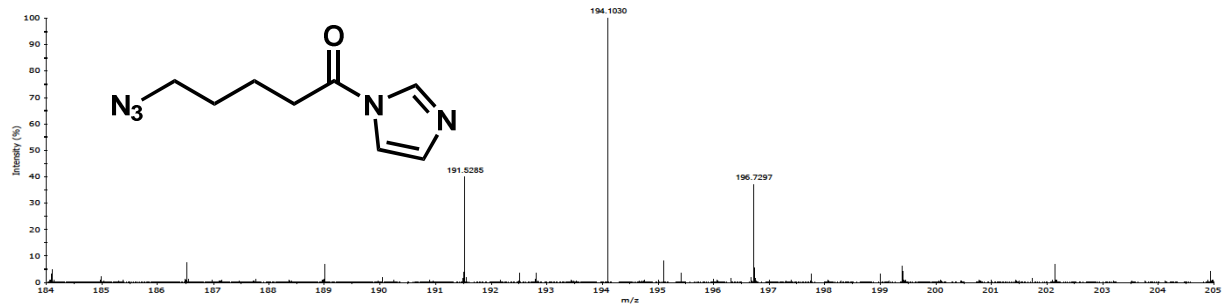

O
NO2
N3
O
N
N
B (d)
7.07
J(1.62)
A (d)
7.45
J(1.61)
C (t)
4.55
J(6.10)
D (t)
3.47
J(6.40)
E (p)
2.03
J(6.23)
7.4566
7.4526
7.0714
7.0674
4.5644
4.5492
4.5339
3.4836
3.4675
3.4516
2.0620
2.0463
2.0302
2.0149
1.9994
1.00
1.00
2.22
3.26
2.51
10
9
8
7
6
5
4
3
2
1
0
ppm


O
CN
N3
O
N
N
B (d)
7.25
J(1.58)
A (d)
7.67
J(1.56)
C (t)
4.63
J(6.06)
D (t)
3.60
J(6.39)
E (m)
2.13
7.6744
7.6705
7.2557
7.2518
4.6475
4.6324
4.6172
3.6131
3.5971
3.5812
2.1567
2.1414
2.1265
2.1248
2.1101
2.0946
1.00
1.39
2.19
2.19
2.56
10
9
8
7
6
5
4
3
2
1
0
ppm

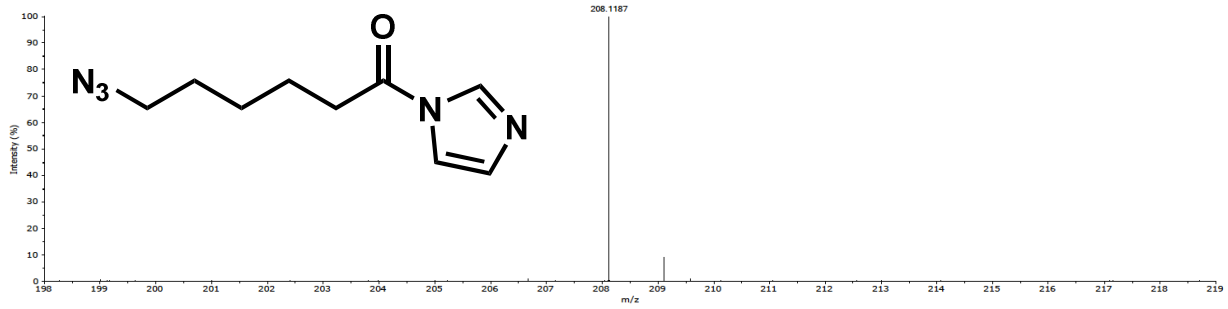
tmcl2108.raw, C7H9N5O [H]+



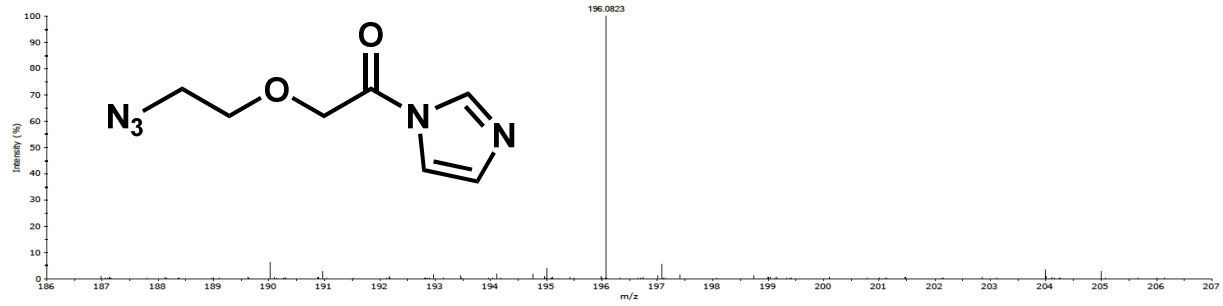
tmcl2102.raw, C8H11N5O [H]+



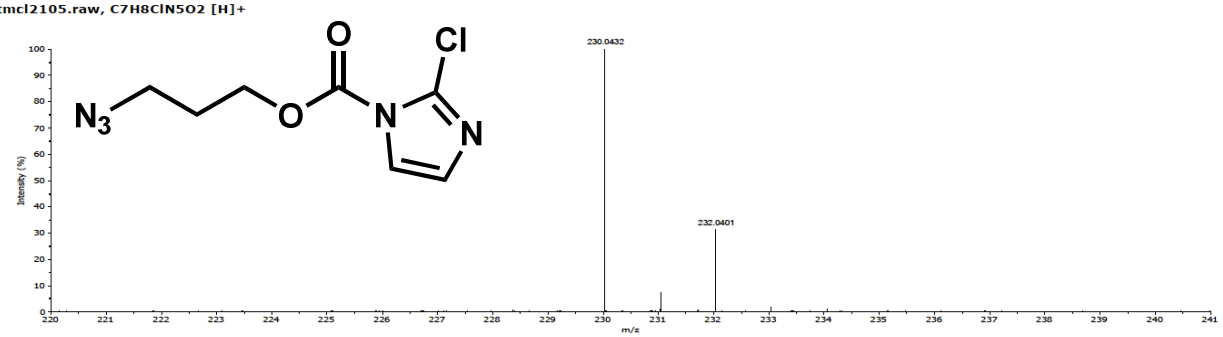
tmcl2109.raw, C9H13N5O [H]+



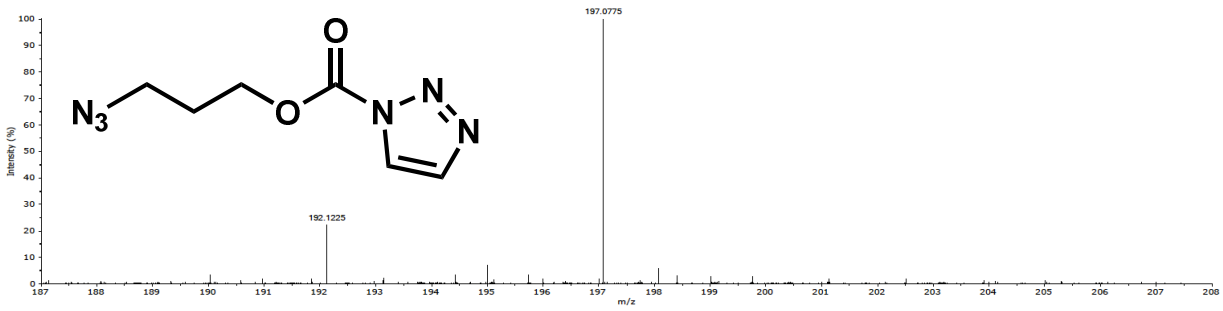
tmcl2110.raw, C7H9N5O2 [H]+



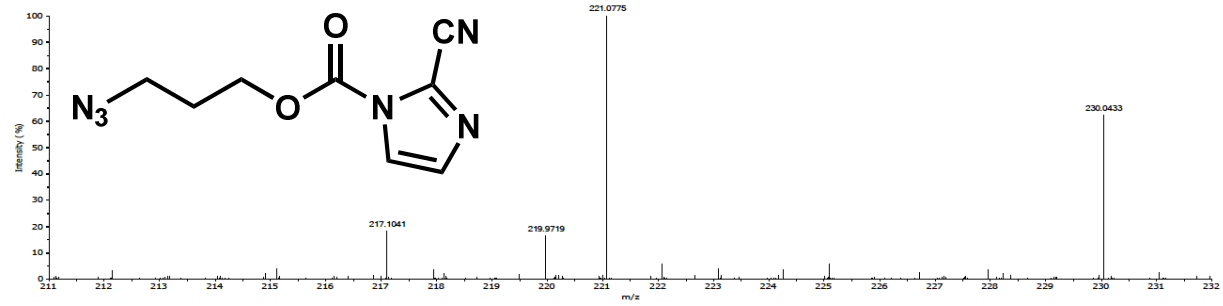
tmcl2105.raw, C7H8ClN5O2 [H]+



tmcl2106.raw, C6H8N6O2 [H]+



tmcl2111.raw, C8H8N6O2 [H]+



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