Supporting Information

# Facile Synthesis and Evaluation of a Dual-Functioning Furoyl Probe for In-Cell SHAPE.

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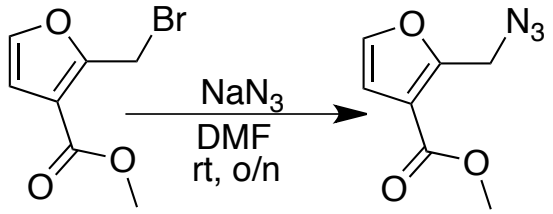
**Supplementary Methods.**

## 1. General

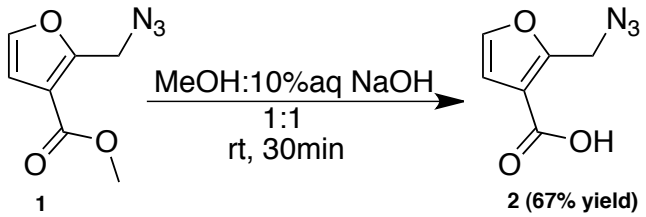
All reagents were purchased from commercial suppliers and were of analytical grade and used without further purification unless otherwise noted. Methyl 2-(bromomethyl)furan-3-carboxylate was purchased from Santa Cruz Biotechnology. Reaction progress was monitored by thin-layer chromatography on EMD 60 F254 plates, visualized with UV light, iodine, ninhydrin, $KMnO_4$, $FeCl_3$, p-anisaldehyde, 2,4-DNP, and bromocresol green stains. Compounds were purified via flash column chromatography using Sorbent Technologies 60 Å 230 x 400 mesh silica gel. Anhydrous solvents acetonitrile (MeCN), dichloromethane (DCM), methanol (MeOH), tetrahydrofuran (THF), dimethylformamide (DMF) were degassed and dried over molecular sieves. Acetone was dried over $MgSO_4$. All reaction vessels were flame dried prior to use. NMR spectra were acquired with Bruker Advanced spectrometers. All spectra were acquired at 298 K. $^1$H-NMR spectra were acquired at 400 MHz and 500 MHz. $^{13}$C-NMR spectra were acquired at 500 MHz. Chemical shifts are reported in ppm relative to residual non-deuterated NMR solvent, and coupling constants (J) are provided in Hz. All NMR spectra was analyzed using MestreNova software. Low and high-resolution electrospray ionization (ESI) mass spectra and Gas Chromatography mass spectra were collected at the University of California-Irvine Mass Spectrometry Facility. IR spectra were acquired from neat samples, unless otherwise noted, with a PerkinElmer Spectrum Two IR Spectrometer.

## 2. Synthesis



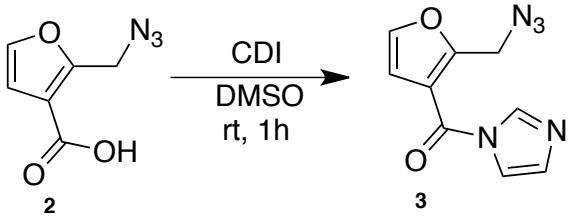

**1 (94% yield)**

**Synthesis of methyl 2-(azidomethyl)furan-3-carboxylate (1).**
To a solution of methyl 2-(bromomethyl)furan-3-carboxylate (0.459mmol, 1eq) in dry DMF (5mL) was added $NaN_3$ (0.918mmol, 2eq). The reaction was left to spin at room temp overnight. Then the reaction was quenched with sat. $NaHCO_3$ and the aqueous layer was extracted with EtOAc. The organics were washed 3x with water and 3x with brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated to yield the product as a brown liquid (0.078g, 94% yield). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.39 (d, *J*=2.5Hz, 1H), 6.71 (d, *J*=2.4Hz, 1H), 4.63 (s, 2H), 3.84 (s, 3H).$^{13}$C NMR (500 MHz, $CDCl_3$) $\delta$ 163.39, 154.61, 142.81, 116.43, 111.05, 51.84, 45.57. HRMS: Theoretical 204.0385 [M+$Na^+$], Observed 204.0393 [M+$Na^+$].




1 2 (67% yield)

**Synthesis of 2-(azidomethyl)furan-3-carboxylic acid (2).**
Methyl 2-(azidomethyl)furan-3-carboxylate (0.293mmol, 1eq) was stirred vigorously in a 1:1 solution of MeOH:10% aq. NaOH (1mL:1mL), and monitored by TLC. Once starting material was gone, the reaction was diluted with water, washed with ether only once, then acidified to pH4 with 2N HCl, and extracted 5 times with EtOAc. The organic layer was dried over $MgSO_4$, filtered, and concentrated to yield product as a tan solid (0.033g, 67% yield). $^1$H NMR (500 MHz, DMSO) δ 13.11 (bs, 1H), 7.83 (d, *J*=1.2Hz, 1H), 6.78 (d, *J*=1.6Hz, 1H), 4.74 (s, 2H).$^{13}$C NMR (500 MHz, DMSO) δ 164.35, 154.41, 144.24, 117.54, 111.59, 45.36. HRMS: Theoretical 212.0048 [M-H+2Na$^+$], Observed 212.0044 [M-H+2Na$^+$].




2 3

**Synthesis of (2-(azidomethyl)furan-3-yl)(1*H*-imidazol-1-yl)methanone (3).**
2-(azidomethyl)furan-3-carboxylic acid (0.623mmol, 1eq) was dissolved in anhydrous DMSO (0.208mL) followed by addition of CDI (0.623mmol, 1eq) to yield a final concentration of 3M. The reaction was allowed to stir at room temp for 1h and then used for cell testing. In order to obtain NMR data for compound **3**, the reaction was run as follows: 2-(azidomethyl)furan-3-carboxylic acid (0.171mmol, 1eq) was dissolved in anhydrous DCM (1mL) followed by addition of CDI (0.171mmol, 1eq) and monitored by TLC. Once starting material was gone, the product was purified by column (95% EtOAc/Hex isochratic) to yield 0.0178g (48% yield) and used for spectroscopic data only. $^1$H NMR (500 MHz, DMSO) δ 8.33 (s, 1H), 8.02 (d, *J*=2Hz, 1H), 7.75 (s, 1H), 7.19 (s, 1H), 7.07 (d, *J*=2Hz, 1H), 4.73 (s, 2H).$^{13}$C NMR (500 MHz, DMSO) δ 160.68, 156.97, 144.77, 138.29, 131.02, 118.24, 117.27, 111.52, 45.59. HRMS: Theoretical 218.0678 [M+Na$^+$], Observed 218.0674 [M+Na$^+$].

**Characterization of ATP deacetylation.**

Radiolabeled ATP was incubated with 1 μL SHAPE reagent for 1 hour at 300 mM final concentration with FAI-$N_3$ (10% final volume) or 100 mM final concentration with 1 M NAI-$N_3$ (10% final volume) in 100 mM HEPES buffer, pH 8.0, containing 6 mM $MgCl_2$ and 100 mM NaCl to a final volume of 10 μL. Reaction was incubated at 37 °C, and then placed on ice. Products were resolved on 25% native polyacrylamide gel. (29:1 acrylamide and bisacrylamide and 1% Tris-borate-EDTA (TBE)) and visualized by phosphorimaging (Typhoon, GE healthcare). ATP supershifts were identified and excised and gel slices were crushed and added to buffer containing a solution of 400 mM KCl and incubated overnight at 4 °C. The following day, ATP conjugates were isolated by spinning down gel slices and isolating the supernatant. For

deacylation reactions, ATP conjugates (1,000 counts per minute, cpm) were added to volume up to 20 μL in ultrapure RNase-free water. The reactions were incubated at appropriate times and temperatures and the reactions were stopped by placing the solutions on ice for immediate loading into the gels. Deacylated products were loaded and resolved on a 25% native polyacrylamide gel as shown above with 10 μL of GLB2. Percent acylation was calculated as the ratio of intensity between ATP and adduct bands. Band intensity were quantified using imageJ.

**Demonstrating SHAPE reactivity with DTT quench.**

Radiolabeled ATP was incubated with each SHAPE electrophile with procedures described above at a final concentration of 300 mM FAI. Following addition of each SHAPE electrophile, the reaction was incubated at 37 °C until the indicated time. An aliquot of reaction was removed and quenched in either an equal volume of Gel Loading Buffer II (Ambion, Inc.), or Gel Loading Buffer II supplanted with 666 mM DTT to a final concentration of 333 mM DTT. For the pre-incubation control, the SHAPE electrophile was added to a solution of Gel Loading Buffer II supplanted with 666 mM DTT. Products were resolved on 28% native polyacrylamide gel and visualized by a gel imager (Typhoon, GE healthcare). Percent acylation was calculated as the intensity from acylated products over the total intensity of ATP signal. Bands intensity were quantified using imageJ.

**Characterization of NAI-$N_3$ and FAI-$N_3$ reactivity with RNA**.

Reverse Transcription primers:

SAM-I (5', ATTTAGGTGACACTATAGTT, 3')

HeLa 18s (5', CCAATTACAGGGCCTCGAAA, 3')

U1 Primer (5', CCCACTACCACAAATTATGCAG, 3')

*In vitro transcription of SAM-I construct.* A 94 nucleotide construct consisting of the sequence for the SAM riboswitch from the metF-metH2 operon of *T. tencongensis* was designed into a plasmid with IDT.[1] One Shot Top 10 chemically competent cells were transformed by SAM-I plasmid and plated on lysogeny broth (LB) supplemented with ampicillin (100 mg/mL) agar plates. A single colony was selected in 3 mL culture and grown overnight. The resulting plasmid was isolated according to conditions using QIAprep Miniprep. Transcription template was prepared by PCR using primers directed against the T7 promoter (5', TAATACGACTCACTATAGGG, 3') and an adaptor sequence for reverse transcription (5', ATTTAGGTGACACTATAGTT, 3'). RNA was transcribed in 40 μL reactions according to conditions using Ribomax Large Scale RNA production systems kit from promega. The transcription reaction was allowed to proceed for three hours at 37 °C. The resulting RNA was treated with 1 μL of RQ1 DNase for 15 min. The resulting RNA was phenol chloroform extracted, and excess nucleotides were removed using a G25 column. Resulting concentrations of RNA was determined through NanoDrop.

*In vitro transcription of U1 construct.* A 164 nucleotide construct for the U1 snRNA was graciously donated to us from the Hertel Lab (UC Irvine).[2] Transcription template was prepared by PCR forward (5', TAATACGACTCACTATAGGGATACTTACCTGGCAGGGGA, 3') and

reverse (5', CAGGGGAAAGCGCGA, 3') primers. RNA was transcribed in 40 μL reactions according to conditions using Ribomax Large Scale RNA production systems kit from promega. The transcription reaction was allowed to proceed for three hours at 37 °C. The resulting RNA was treated with 1 μL of RQ1 DNase for 15 min. The resulting RNA was phenol chloroform extracted, and excess nucleotides were removed using a G25 column. Resulting concentrations of RNA was determined through NanoDrop.

*$^{32}$P End labeling for reverse transcription.* 200 pmol of primer DNA was kinased with Optikinase according to manufacturer's conditions by Affymetrix. The reaction was allowed to proceed for two hours at 37 °C. Reactions were stopped by the addition of equal amounts of Gel Loading Buffer II (Ambion, Inc.). The reactions were loaded onto a 15% denaturing PAGE gel. The band of interest was visualized by gel imager (Typhoon, GE healthcare). The resulting band was excised and eluted overnight in 300 mM KCl. Resulting solution was EtOH precipitated and dissolved to 8,000 cpm/μL for further use in reverse transcription.

*Acylation of RNA in vitro*. In a typical *in vitro* modification protocol, 5 μg total RNA (isolated from HeLa cells) or 2 pmol of *in vitro* transcribed *RNA* was heated in 6 μL metal-free water for 2 min at 95 °C. The RNA was then flash cooled on ice. 3 μL of 3× SHAPE buffer (333 mM HEPES, pH 8.0, 20 mM $MgCl_2$ and 333 mM NaCl) was added, and the RNA was allowed to equilibrate at 37 °C for 5 min. To this mixture, 1 μL of 3 M FAI-$N_3$ or 1 M NAI-$N_3$ in DMSO (+) or DMSO alone (−) was added. The reaction was permitted to continue for 15 min. Reactions were extracted once with acid phenol and chloroform (pH 4.5 ± 0.2 (s.d.)) and twice with chloroform. RNA was ethanol precipitated with 20 μL of 3 M sodium acetate buffer (pH 5.2) and 1 μL of glycogen (20 μg/μL). Pellets were washed twice with 70% ethanol and resuspended in 5 μL RNase-free water.

*Copper free click chemistry of acylated RNA.* In a typical reaction, acylated RNA (1 pmol) was reacted with 100 equivalents of Dibenzocyclooctyne-PEG4-biotin (DBCO-Biotin, sigma) for 1 h at 37 °C in 1x PBS. Reactions were extracted once with acid phenol:chloroform (pH 4.5) and twice with chloroform. RNA was ethanol precipitated with sodium acetate (pH 5.2) and 1 μL glycoblue. Pellets were washed twice with 70% ethanol and resuspended in 17 μL RNase-free water.

*Enrichment of Modified RNA*. To 50 pmol of precipitated and biotinylated RNA (in 700 μL binding buffer: 50 mM Tris-HCl pH 7.0 and 1 mM EDTA) was added with 50 μL of prewashed Dynabeads MyOne C1 beads. The reaction mixture was then mixed at room temperature for 1 h. The beads were collected on a magnetic plate and flowthrough was saved. The beads were then washed three times with 700 μL of Biotin Wash buffer (10 mM Tris-HCl, pH 7.0, 1 mM EDTA, 4 M NaCl, 0.2% Tween). The first wash was saved and combined with the flowthrough for further analysis. Samples were later washed twice with RNase-free water. FAI-$N_3$ adducts undergoing harsher wash conditions was subjected to two 700 μL Biotin wash buffer for 5 min along with two washes with RNase-free water at 70 °C. Samples were eluted twice with 44 μL formamide, 1 μL of 0.5 M EDTA, and 5 μL of 50 mM of free D-Biotin at 95 °C for 5 min. Eluted samples were diluted with 600 μL RNase-free water. All samples were purified using RNA Clean and Concentrator Kit (Zymo). Samples were eluted in 6 μL of RNase-free water and used for subsequent reverse transcription. SAM-I and U1 RNA RT stops were put through biological duplicates. The enrichment factor was calculated by the signal at full-length RT/SHAPE signal at a chosen band, as denoted in the figures.

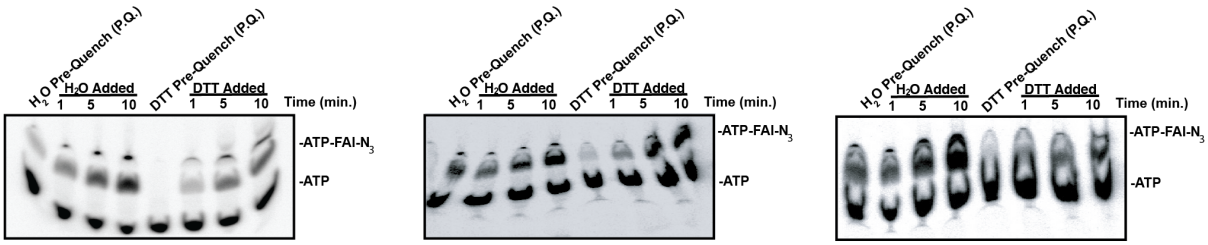
*Dot blot Analysis of Enriched modified RNA.* Hybond N+ membranes (GE) were pre-incubated in 10X SSC. Precipitated biotinylated total RNA was dissolved in 15 μL of RNase free water.

RNA was loaded onto the Hybond membrane and crosslinked using 254 nm ultraviolet light. The membrane was incubated with blocking solution (120 mM NaCl, 16 mM $Na_2HPO_4$, 8 mM $NaH_2PO_4$, 170 mM SDS) for 30 min. To the membrane was added 1 μL streptavidin (Pierce High Sensitivity Streptavidin-HRP) in blocking solution. The membrane was washed twice with wash buffer A (1:10 blocking solution) for 30 min, and twice with wash buffer B (100 mM Tris pH 9.5, 100 mM NaCl, 20 mM $MgCl_2$) for 5 min. Membrane was incubated with Pierce Western blotting substrate (Thermo) and visualized on the ChemiDoc (Biorad) under chemiluminesence hi sensitivity.

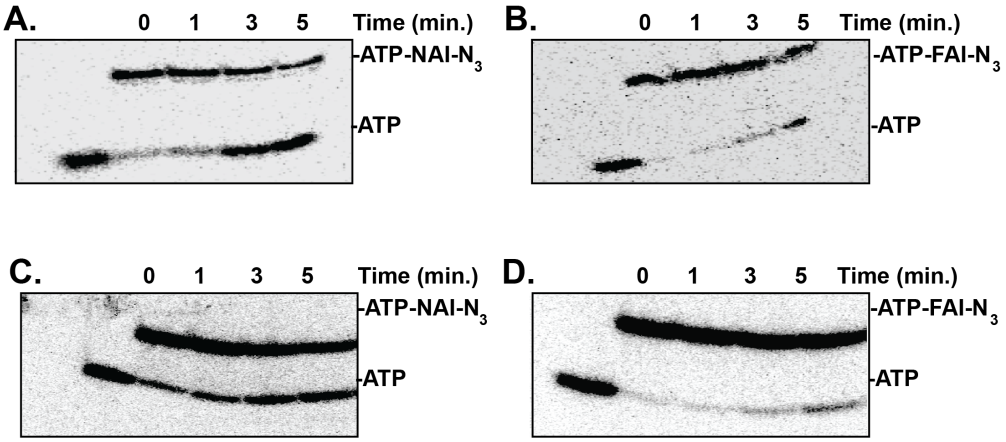
*Acylation of RNA in HeLa cells.* HeLa cells were grown in DMEM (high glucose) culture medium supplemented with 10% FBS, 1% penicillin streptomycin. Cells were washed three times with Dulbecco's phosphate-buffered saline (DPBS) and then scraped and spun down at 1000 r.p.m. for 5 min. Cells (~3-6 x $10^7$) were resuspended in 45 μL DPBS. 5 μL DMSO (-), 10% final concentration or 5 μL 10x electrophilic stock in DMSO (+) was added to the desired final concentration. Cell suspensions were incubated at 37 °C for 10 min. Cells were pelleted by centrifugation at 1000 r.p.m. for 5 min and resuspended in 1 mL Trizol Reagent. RNA was harvested using Trizol Reagent following the manufacturer's instructions.

*Reverse transcription of modified RNA (in vitro and in vivo)*. $^{32}$P-end-labeled DNA primers were annealed to modified RNA by incubating 95 °C for two minutes, then 25 °C for two minutes, and 4 °C for 2 minutes. To the reaction, first strand buffer, DTT, and dNTP's were added. The reaction was preincubated at 52°C for 1 min, then superscript III (2 units/μL final concentration) was added. Extensions were performed for 15 min. To the reaction, sodium hydroxide was added to a final concentration of 400 mM and allowed to react for 5 min at 95 °C. The resulting complementary DNA (cDNA) was snapped cooled on ice, and ethanol precipitated according to above procedures. Purified cDNA was resuspended in 2 μL of nuclease-free water and 2 μL of Gel Loading Buffer II was added. cDNA products were resolved on 10% denaturing polyacrylamide gel, and visualized by a gel imager (Typhoon, GE healthcare).

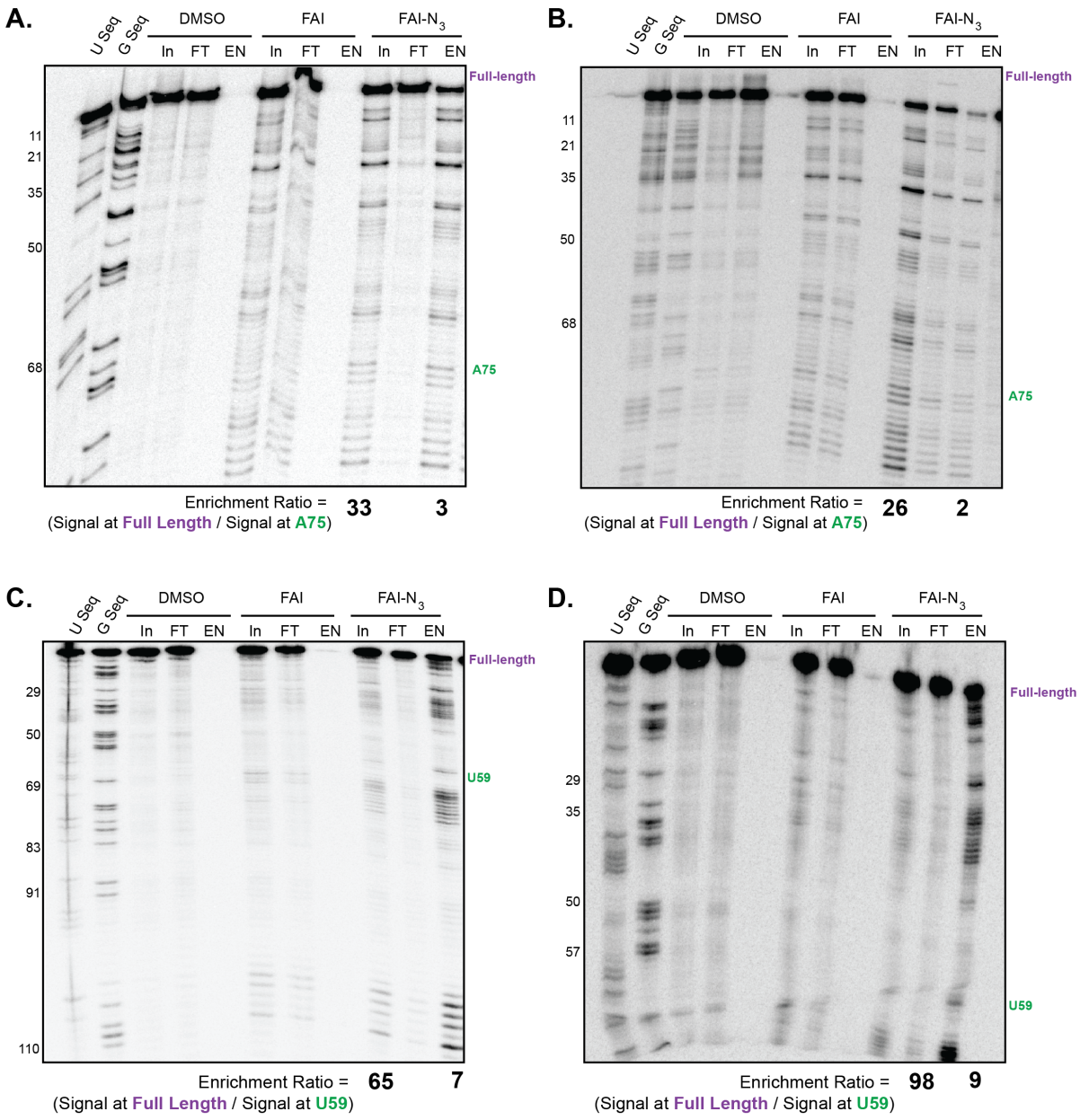
**Supporting Information Figures.**




**Supporting Information Figure 1.** Additional experiments supporting Figure 2 and DTT quenching of acylation. The images are replicates of data that was used for Figure 2 B and C.




**Supporting Information Figure 2.** Additional experiments supporting Figure 2 and Hydrolysis of NAI-$N_3$ and FAI-$N_3$ acylation products. This is in support of Figure 2 D-F. A) Deacylation of NAI-$N_3$ acylation adducts with ATP, REP 1. B) Deacylation of NAI-$N_3$ acylation adducts with ATP, REP 1. C) Deacylation of NAI-$N_3$ acylation adducts with ATP, REP 2. D) Deacylation of NAI-$N_3$ acylation adducts with ATP. REP 2.

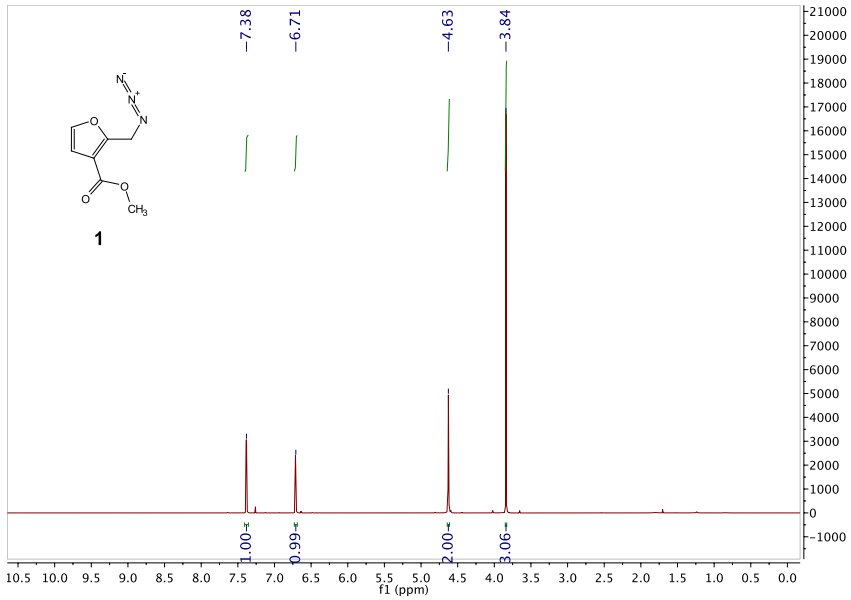



**Supporting Information Figure 3.** Additional experiments supporting Figure 2 and Hydrolysis of NAI-$N_3$ and FAI-$N_3$ acylation products. This is in support of Figure 3 A) Enrichment RT gel for SAM-I, Rep 1. B) Enrichment RT gel for SAM-I, Rep 2. C) Enrichment RT gel for U1, Rep 1. D) Enrichment RT gel for U1, Rep 2.

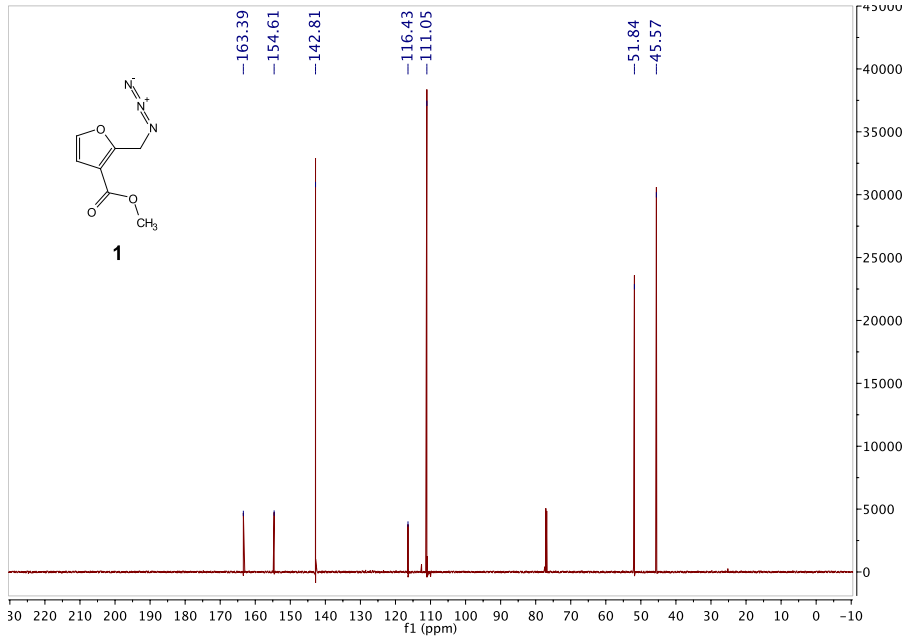
## Spectra

### Methyl 2-(azidomethyl)furan-3-carboxylate (1)

$^{1}$H NMR (500MHz, $CDCl_3$)

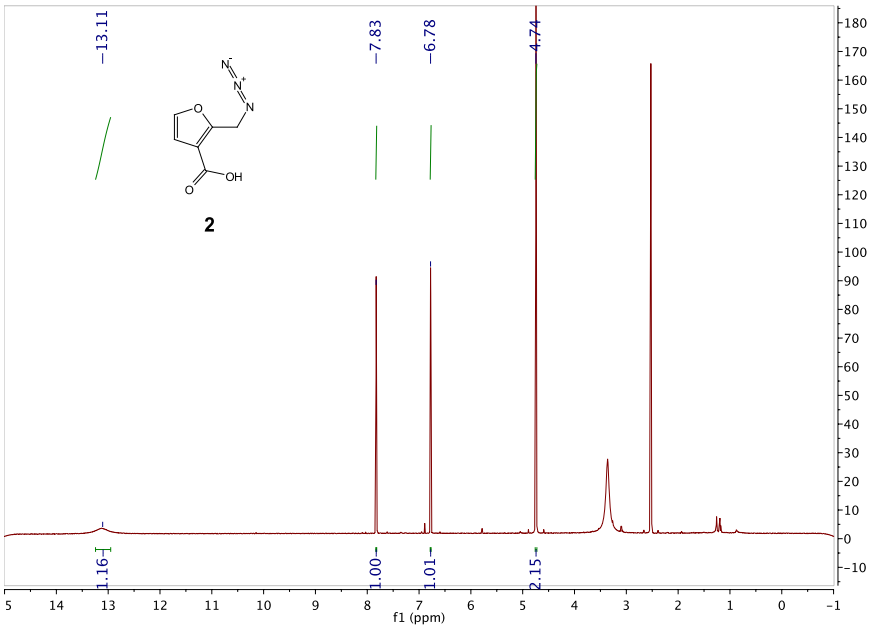



$^{13}$C NMR (500MHz, $CDCl_3$)

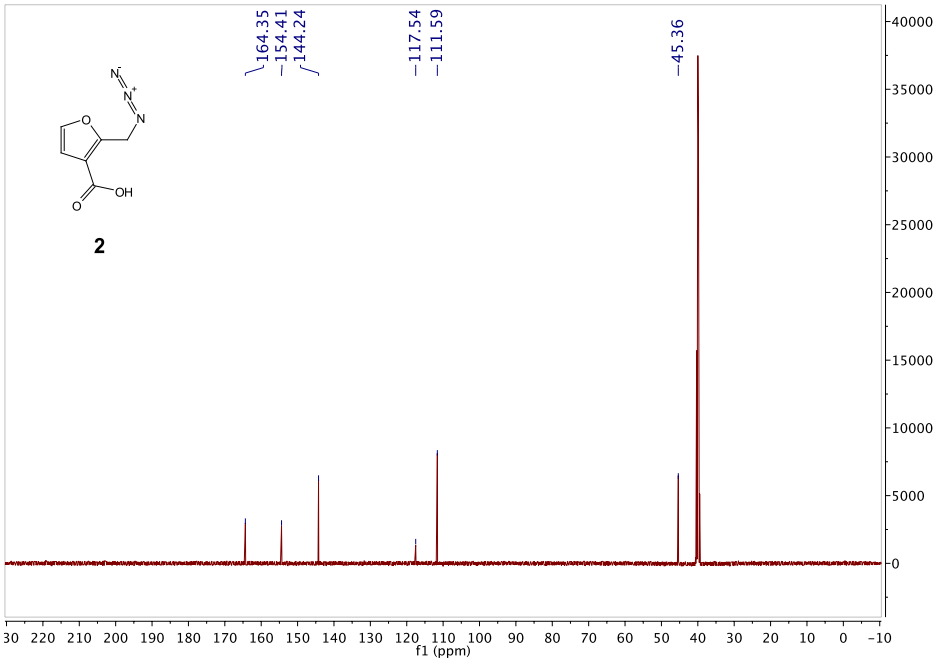



### 2-(azidomethyl)furan-3-carboxylic acid (2)

$^{1}$H NMR (500MHz, DMSO)

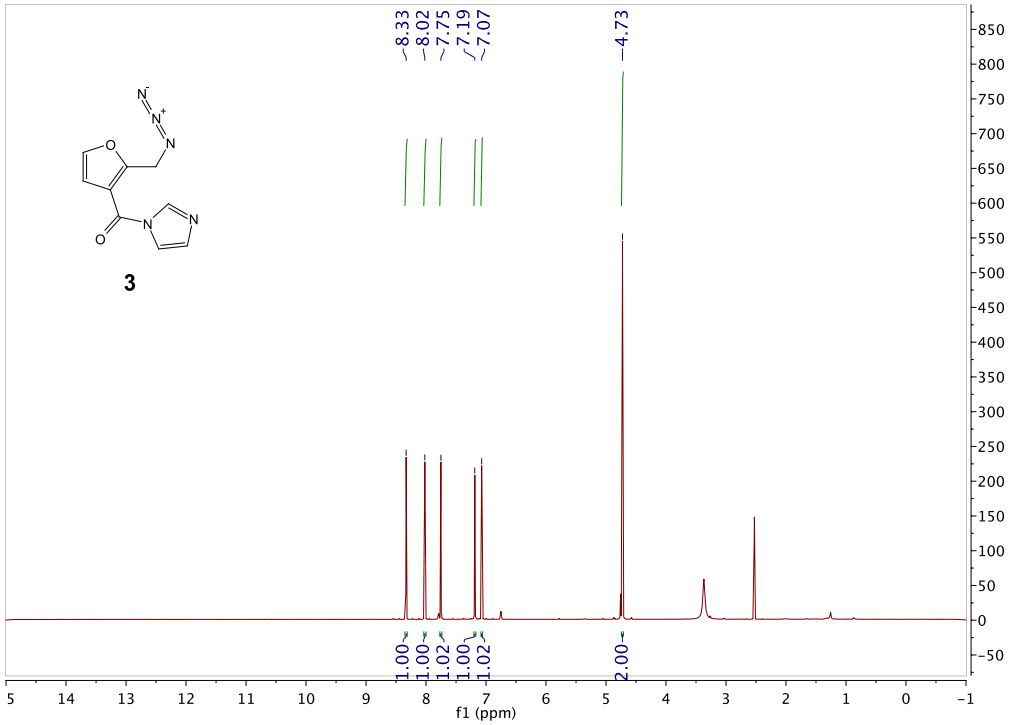



$^{13}$C NMR (500MHz, DMSO)




**(2-(azidomethyl)furan-3-yl)(1*H*-imidazol-1-yl)methanone (3)**

$^{1}$H NMR (500MHz, DMSO)

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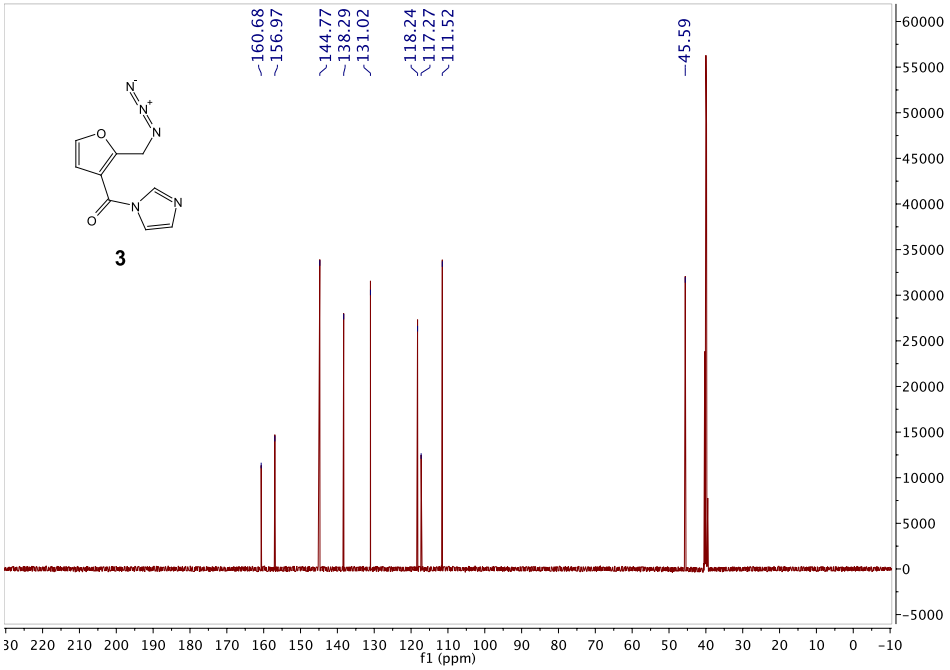
$^{13}$C NMR (500MHz, DMSO)

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**References**

1. Montange, R. K.; Batey, R. T. *Nature* **2006,** *441* (7097), 1172-1175.
2. Bernstein, L. B.; Manser, T.; Weiner, A. M. *Mol. Cell Biol.* **1985**, 5 (9), 2159-2171